www.amegroups.com

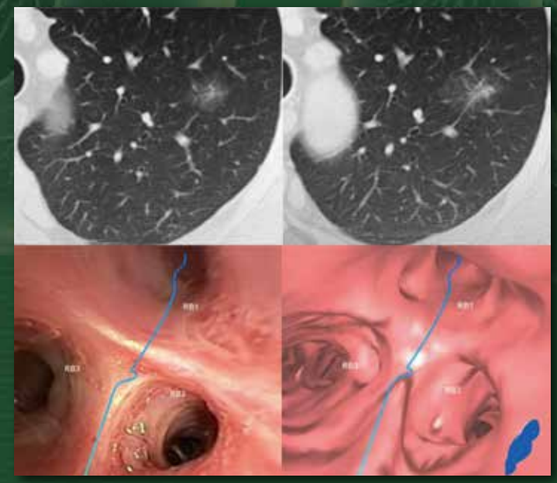
LUNG CANCER

Honorary Editors: Jie He, MD, PhD; Rafael Rosell, MD, PhD; Nanshan Zhong, MD
Editors: Jianxing He, MD, PhD, FACS; Thomas A. D'Amico, MD; Xiuyi Zhi, MD
Associate Editors: Qun Wang, MD; Tristan D. Yan, MD, PhD; Calvin S.H. Ng, MD; Caicun Zhou, MD, PhD; Heather A. Wakelee, MD; Wenhua Liang, MD

Time for personalized medicine

LUNG CANCER

Honorary Editors: Jie He, MD, PhD; Rafael Rosell, MD, PhD; Nanshan Zhong, MD
Editors: Jianxing He, MD, PhD, FACS; Thomas A. D'Amico, MD; Xiuyi Zhi, MD
Associate Editors: Qun Wang, MD; Tristan D. Yan, MD, PhD; Calvin S.H. Ng, MD; Caicun Zhou, MD, PhD; Heather A. Wakelee, MD; Wenhua Liang, MD



www.amegroups.com

AME Publishing Company

Room 604 6/F Hollywood Center, 77-91 Queen's road, Sheung Wan, Hong Kong

Information on this title: www.amepc.org
For more information, contact info@amepc.org

First published 2015
Printed in AME Publishing Company

Jianxing He, Thomas A. D'Amico, Xiuyi Zhi

Lung Cancer

978-988-14027-0-7 (hardback)

Preface

Despite many years of dedicated effort, including basic, translational and clinical research, public education, and health care policy, lung cancer remains the number one cause of death by malignancy in the world, and it is responsible for as many deaths as colon, breast, pancreas and prostate cancers combined in the US. Thus, successful efforts to better understand and better treat lung cancer will have long lasting ramifications, for patient care specifically and for public health globally, as well.

This volume, *Lung Cancer*, is just such an effort. Comprised of contributions by the most accomplished scientists and clinicians internationally, *Lung Cancer* thoroughly examines the spectrum of topics related to better understanding and better treating the world's most lethal malignancy. From the basic science of lung cancer to the most advanced therapeutic techniques, this text provides the reader—student or professional—with a most comprehensive analysis.

It is hoped that in the future the concepts of prevention (tobacco cessation) and screening will dramatically reduce the frequency and mortality of lung cancer. Until then, the efforts of the authors of this text are the best weapons against lung cancer.

Thomas A. D'Amico MD
Gary Hock Endowed Professor of Surgery
Chief, Section of General Thoracic Surgery
Duke Cancer Institute
Duke South, White Zone, Room 3589
Durham, North Carolina 27710
Email: thomas.damico@duke.edu

Lung Cancer
(FIRST EDITION)

Contributors

Honorary Editors

Jie He, MD, PhD
Department of Thoracic Surgical Oncology, Cancer Institute & Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College; National Cancer Center, Beijing 100021, China.

Rafael Rosell, MD, PhD
Breakthrough Cancer Research Unit, Pangaea Biotech, Dexeus University Institute, Catalan Institute of Oncology, Badalona, Spain

Nanshan Zhong, MD
Academician, Chinese Academy of Engineering. The First Affiliated Hospital of Guangzhou Medical University; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease; National clinical research center for respiratory Disease, Guangzhou 510120, China

Editors

Jianxing He, MD, PhD, FACS
Department of Cardiothoracic Surgery, the First Affiliated Hospital of Guangzhou Medical University; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease; National clinical research center for respiratory Disease, Guangzhou 510120, China

Thomas A. D'Amico, MD
Department of Surgery, Duke University Medical Center, Durham, North Carolina, United States

Xiuyi Zhi, MD
Department of Thoracic Surgery, Xuanwu Hospital; Beijing Lung Cancer Center, Capital Medical University, Beijing, China

Associate Editors

Qun Wang, MD, FRCS
Shanghai Zhongshan Hospital of Fudan University, Shanghai, China

Tristan D. Yan, BSc, MBBS, MS, MD, PhD
Department of Cardiothoracic Surgery, Royal Prince Alfred Hospital, University of Sydney, Sydney, Australia; The Collaborative Research (CORE) Group, Macquarie University, Sydney, Australia

Calvin S.H. Ng, BSc(Hons), MBBS(Hons), MD(Res) (Lond), FRCSEd(CTh), FCSHK, FHKAM(Surg), FCCP
Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China

Caicun Zhou, MD, PhD
Department of Oncology, Shanghai Pulmonary Hospital, Affiliated to Tongji University School of Medicine, Shanghai, China

Heather A. Wakelee, MD
Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford Cancer Institute, CA , USA

Wenhua Liang, MD
Department of Thoracic Oncology, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

AUTHORS

David Ball
Peter MacCallum Cancer Centre, and Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Australia

Cristóbal Belda-Iniesta
Thoracic, H&N and Neuro-oncology Unit, CIOCC, GHM, Madrid, Spain

Christine D. Berg
University of Queensland Thoracic Research Centre, School of Medicine, The University of Queensland, Brisbane, Australia; Formerly, Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland, USA

Mark F. Berry
Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA

Andrea Bezjak
Radiation Medicine Program, Princess Margaret Hospital, Toronto, Ontario, Canada; Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada

Fiona Blackhall
Clinical and Experimental Pharmacology Group, Paterson Institute for Cancer Research, University of Manchester, M20 4BX, UK; Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, M20 4BX, UK

Laura Bonanno
Medical Oncology 2, Istituto Oncologico Veneto I.R.C.C.S., Padova, Italy

José I. Borrell
Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, E-08017 Barcelona, Spain

Marc Bos
Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany; Center for Integrated Oncology Cologne/Bonn, Germany

Rayleen V. Bowman
Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Australia; University of Queensland Thoracic Research Centre, School of Medicine, The University of Queensland, Brisbane, Australia

Ross M. Bremner
Center for Thoracic and Esophageal Disease, Heart and Lung Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA

Emmanuel Brian
Thoracic Department, Institut Mutualiste Montsouris, 42 Bd Jourdan, F-75014 Paris, France

Reinhard Buettner
Center for Integrated Oncology Cologne/Bonn, Germany; Institute of Pathology, University Hospital Cologne, Cologne, Germany

Charles A. Butts
Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada

Carlos Camps
Hospital General Universitario de Valencia, Av Tres Cruces, s/n, 46014 Valencia, Spain

Laia Capdevila
Medical Oncology Department, Hospital Universitari Germans Trias i Pujol, Institut Català d'Oncologia-Badalona, Universitat Autònoma de Barcelona, Carretera de Canyet s/n, 08916 Badalona, Barcelona, Spain

Federico Cappuzzo
Istituto Toscano Tumori, Ospedale Civile, Viale Alfieri 36, 57100 Livorno, Italy

Enric Carcereny
Medical Oncology Department, Hospital Universitari Germans Trias i Pujol, Institut Català d'Oncologia-Badalona, Universitat Autònoma de Barcelona, Carretera de Canyet s/n, 08916 Badalona, Barcelona, Spain

Mark Carey
Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA

Javier de Castro Carpeño
Medical Oncology Unit, Department of Translational Oncology, Hospital Universitario La Paz, idiPAZ, Madrid, Spain

Dean A. Fennell
University of Leicester & Leicester University Hospitals, UK

Maria Delgado Roel
Complejo Hospitalario Universitario A Coruna, La Coruna, Spain

Qiuhua Deng
Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Annette G. Dent
Department of Thoracic Medicine, The Prince Charles Hospital, Rode Road, Chermside, Queensland 4032, Australia; The University of Queensland, Brisbane, St Lucia, Queensland 4072, Australia

Harshil D. Dhruv
Cancer and Cell Biology Division, The Translational Genomics Research Institute (TGen), Phoenix, AZ, USA

Caroline Dive
Clinical and Experimental Pharmacology Group, Paterson Institute for Cancer Research, University of Manchester, M20 4BX, UK

Qinglong Dong
Department of Anesthesiology, First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China

Roger Estrada-Tejedor
Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, E-08017 Barcelona, Spain

Michael Fay
Division of Oncology, Royal Brisbane and Women's Hospital, Queensland Health, Brisbane, Australia; School of Medicine, University of Queensland, Brisbane, Australia; Visiting Scientist, Preclinical Molecular Imaging, Eberhard Karls Universität Tübingen, Germany

Enriqueta Felip
Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain

Kwun M. Fong
Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Australia; UQ Thoracic Research Centre, The University of Queensland, Brisbane, Australia

Ludovic Fournel
Thoracic Department, Institut Mutualiste Montsouris, 42 Bd Jourdan, F-75014 Paris, France

Pingfu Fu
Cleveland Clinic, Cleveland, OH, USA

David Gandara
University of California Davis Cancer Center, Sacramento, California, USA

Wen Gao
Shanghai Pulmonary Hospital, Tongji University, Shanghai, China

Masyar Gardizi
Center for Integrated Oncology Cologne/Bonn, Germany; Institute of Pathology, University Hospital Cologne, Cologne, Germany

Pilar Garrido
Medical Oncology Department, IRYCIS, Hospital Universitario Ramón y Cajal, Carretera Colmenar Viejo km 9100, 28034, Madrid, Spain

Ana Christina Garrido-Castro
Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain

Amaya Gasco
IOR, Dexeus University Institute, Barcelona, Spain

Adi F. Gazdar
The Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, Texas, USA; Department of Pathology, University of Texas

Southwestern Medical Center, Dallas, Texas, USA

Matteo Giaj Levra
Department of Oncology, A.O.U San Luigi - Orbassano, University of Turin, Italy

María de los Llanos Gil
Medical Oncology Department, Hospital Universitari Germans Trias i Pujol, Institut Català d'Oncologia-Badalona, Universitat Autònoma de Barcelona, Carretera de Canyet s/n, 08916 Badalona, Barcelona, Spain

Meredith Elana Giuliani
Radiation Medicine Program, Princess Margaret Hospital, Toronto, Ontario, Canada; Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada

Peter Goldstraw
Thoracic Surgery, Royal Brompton Hospital, London, UK; Thoracic Surgery, National Heart and Lung Institute, Imperial College, London, UK

María González Cao
IOR, Dexeus University Institute, Barcelona, Spain

Dominique Gossot
Thoracic Department, Institut Mutualiste Montsouris, 42 Bd Jourdan, F-75014 Paris, France

Ramaswamy Govindan
Division of Oncology, Washington University School of Medicine, St. Louis, MO 63021, USA

Madalina Grigoroiu
Thoracic Department, Institut Mutualiste Montsouris, 42 Bd Jourdan, F-75014 Paris, France

Xiaotong Guo
Department of Thoracic Surgery, Second Affiliated Hospital of Harbin Medical University, Harbin 15000, China

Zhihua Guo
Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Balazs Halmos
Department of Medicine, New York Presbyterian Hospital - Columbia University Medical Center, Columbia College of Physicians and Surgeons, USA

Baohui Han
Shanghai Chest Hospital, Jiaotong University, Shanghai, China

Jennifer M. Hanna
Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA

Jianxing He
Affiliated Hospital of Guangzhou Medical College, Guangzhou, China

Thomas Henzler
Institute of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Florentino Hernando-Trancho
Hospital Clinico Universitario San Carlos, Madrid, Spain

John W. Holloway
Human Development & Health, Southampton, UK; Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

Wenjing Hu
Comprehensive Cancer Centre of Drum Tower Hospital, Medical School of Nanjing University, Nanjing 210008, China

Brett Hughes
Royal Brisbane and Women's Hospital, Herston, Queensland, Australia; The Prince Charles Hospital, Chermside, Queensland, Australia

Landon J. Inge
Center for Thoracic and Esophageal Disease, Heart and Lung Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA

Simona Inghilleri
Laboratory of Biochemistry & Genetics, Division of Pneumology, University and Fondazione IRCCS Policlinico San Matteo, Pavia 27100, Italy

David M. Jablons
UCSF Thoracic Surgery, MU W424, San Francisco, CA 94143-0118, USA

Thierry Jahan
University of California, San Francisco, CA, USA

Gening Jiang
Department of Thoracic Surgery, Shanghai Pulmonary Hospital of Tongji University, Shanghai 200433, China

John D. Mitchell
General Thoracic Surgery, Division of Cardiothoracic Surgery, University of Colorado School of Medicine, Aurora, Colorado, USA

David R. Jones
Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Shiyang Kang
Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Niki Karachaliou
Breakthrough Cancer Research Unit, Pangaea Biotech S.L, Quiron Dexeus University Hospital, Sabino Arana 5-19, 08028 Barcelona, Spain

Yoshihisa Kobayashi
Department of Thoracic Surgery, Aichi Cancer Center Hospital, Nagoya, Japan

Ritsuko Komaki
Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Johannes R. Kratz
Massachusetts General Hospital, GRB 425, Boston, MA 02114, USA

Tunc Lacin
Department of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

David C. L. Lam
Department of Medicine, University of Hong Kong, Hong Kong SAR, China

Stephen Lam
Department of Integrative Oncology, British Columbia Cancer Agency & University of British Columbia, Vancouver, BC V5Z 1L3, Canada

Lorenza Landi
Istituto Toscano Tumori, Ospedale Civile, Viale Alfieri 36, 57100 Livorno, Italy

Eddie W. F. Lau
Centre for Molecular Imaging, Department of Radiology, Peter MacCallum Cancer Centre, University of Melbourne, East Melbourne, Victoria, Australia

Hervé Le Caer
Groupe Français de Pneumo - Cancerologie (GFPC), France; Service de Pneumologie CH - Draguignan, France

Cécile Le Péchoux
Radiation Oncology Department, Thoracic Oncology Unit, Gustave Roussy-Hôpital Universitaire, France

Wai-Kit Lee
Department of Medical Imaging, St. Vincent's Hospital, University of Melbourne, Fitzroy, Victoria, Australia

Peter M. J. Lee
Division of Pulmonary and Critical Care Medicine, Beth Israel Medical Center, New York, NY 10003, USA

Kyungmouk Steve Lee
Division of Interventional Radiology, Weill Cornell Medical College, New York-Presbyterian Hospital, New York, NY 10065, USA

Rom S. Leidner
Departments of Medicine and Biostatistics, University Hospitals - Case Medical Center, Case Western Reserve University, Cleveland, OH, USA

Hervé Lena
Groupe Français de Pneumo - Cancerologie (GFPC), France; Service de Pneumologie CHU - Rennes, France

Nathan A. Pennell
Cleveland Clinic, Cleveland, OH, USA

Kevin Phan
The Collaborative Research (CORE) Group, Macquarie University Hospital, New South Wales, Australia

Rathi N. Pillai
Winship Cancer Institute, Emory University, GA, USA

Christopher M. Poole
Division of Oncology, Royal Brisbane and Women's Hospital, Queensland Health, Brisbane, Australia; Medical Physics, Queensland University of Technology, Brisbane, Australia

Ernesto Pozzi
Policlinico di Monza, University of Pavia, Monza 20025, Italy

Gary Pratt
Division of Oncology, Royal Brisbane and Women's Hospital, Queensland Health, Brisbane, Australia; School of Medicine, University of Queensland, Brisbane, Australia

Bradley B. Pua
Division of Interventional Radiology, Weill Cornell Medical College, New York-Presbyterian Hospital, New York, NY 10065, USA

Raimon Puig de la Bellacasa
Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, E-08017 Barcelona, Spain

Xiaoping Qian
Comprehensive Cancer Centre of Drum Tower Hospital, Medical School of Nanjing University, Nanjing 210008, China

Vanesa Quiroga
Medical Oncology Department, Hospital Universitari Germans Trias i Pujol, Institut Català d'Oncologia-Badalona, Universitat Autònoma de Barcelona, Carretera de Canyet s/n, 08916 Badalona, Barcelona, Spain

Suresh S. Ramalingam
Winship Cancer Institute, Emory University, GA, USA

Jose Luis Ramirez
Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Badalona, Spain; Health Sciences Research Institute Germans Trias i Pujol, Badalona, Spain

Jordi Remon
Hospital de Mataró, Mataró, Spain

Joseph Reza
University of California, San Francisco, CA, USA

Jonathan W. Riess
University of California Davis Cancer Center, Sacramento, California, USA

James R. Rigas
Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA

Rafael Rosell
Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Ctra Canyet s/n, 08916 Badalona, Spain; Breakthrough Cancer Research Unit, Pangaea Biotech S.L, Quiron Dexeus University Hospital, Sabino Arana 5-19, 08028 Barcelona, Spain

Francesc Salvà
Oncology Department, Valld'Hebron University Hospital, Barcelona, Spain

Amanda Sammann
University of California, San Francisco, CA, USA

Belén Sanchez
Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Ctra Canyet s/n, 08916 Badalona, Spain

Jose Miguel Sanchez
Hospital La Princesa, Madrid, Spain

María Sanchez-Ronco
Universidad Alcalá de Henares, Pza. San Diego s/n, 28801 Alcalá de Henares, Madrid, Spain

Irene Sansano
Pangaea Biotech, Dexeus University Institute, Barcelona, Spain; Hospital Vall Hebron, Barcelona, Spain

Laura Saracino
Laboratory of Biochemistry & Genetics, Division of Pneumology, University and Fondazione IRCCS Policlinico San Matteo, Pavia 27100, Italy

Peter Savas
The Peter MaCallum Cancer Centre, East Melbourne, Victoria, Australia

Giorgio Vittorio Scagliotti
University of Torino, Department of Oncology, Torino, Italy

Hans-Ulrich Schildhaus
Center for Integrated Oncology Cologne/Bonn, Germany; Institute of Pathology, University Hospital Cologne, Cologne, Germany

Stefan O. Schoenberg
Institute of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Penelope Schofield
Department of Cancer Experiences Research, Peter MacCallum Cancer Centre, Melbourne, Australia; Sir Peter MacCallum Department of Oncology, (Faculty of Medicine, Dentistry and Health Sciences), School of Behavioural Science, School of Nursing, The University of Melbourne, Parkville, Australia

Oliver Sedlaczek
Department of Diagnostic and Interventional Radiology, Heidelberg University Hospital, Heidelberg, Germany

Lecia V. Sequist
Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA

Rajesh Shah
Department of Thoracic Surgery, University Hospital of South Manchester NHS Foundation Trust, Wythenshawe, M23 9LT, UK

Tawimas Shaipanich
Department of Integrative Oncology, British Columbia Cancer Agency & University of British Columbia, Vancouver, BC V5Z 1L3, Canada

Wenlong Shao
Department of Thoracic Surgery, First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Department of Surgery, Guangzhou Institute of Respiratory Diseases, Guangzhou 510120, China; National Respiratory Disease Clinical Research Center, Guangzhou 510000, China

Xiaoshun Shi
Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Gerard A. Silvestri
Division of Pulmonary and Critical Care Medicine Medical University of South Carolina, Charleston, South Carolina, USA

Heath Devin Skinner
Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Benjamin Solomon
The Peter MaCallum Cancer Centre, East Melbourne, Victoria, Australia

Karin Steinke
Department of Medical Imaging, Royal Brisbane and Women's Hospital, University of Queensland, Herston, Queensland, Australia

Giulia Maria Stella
Laboratory of Biochemistry & Genetics, Division of Pneumology, University and Fondazione IRCCS Policlinico San Matteo, Pavia 27100, Italy

Sonja Sudarski
Institute of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Yvonne Summers
Pulmonary Oncology Unit, University Hospital of South Manchester NHS Foundation Trust, Wythenshawe, M23 9LT, UK; Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, M20 4BX, UK

Jeffrey A. Winkles
Departments of Surgery and Physiology, Center for Vascular and Inflammatory Diseases and the Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD, USA

Jürgen Wolf
Center for Integrated Oncology Cologne/Bonn, Germany; Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany

Xinguo Xiong
Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Lixia Xu
Comprehensive Cancer Centre of Drum Tower Hospital, Medical School of Nanjing University, Nanjing 210008, China

Xin Xu
Department of Cardiothoracic Surgery, The First Hospital Affiliated to Guangzhou Medical College; Guangzhou Institute of Respiratory Disease, State Key Laboratary of Respiratory Disease, Guangzhou 510120, China

Zhiqiang Xu
Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Tristan D. Yan
The Collaborative Research (CORE) group, Sydney, Australia; The University of Sydney, Department of Cardiothoracic Surgery, Royal Prince Alfred Hospital, Sydney, Australia

Ian A. Yang
Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Australia; UQ Thoracic Research Centre, The University of Queensland, Brisbane, Australia

Yue Yang
Beijing Cancer Hospital, Peking University, Beijing, China

Kazuhiro Yasufuku
Division of Thoracic Surgery, Toronto General Hospital, University Health Network, Toronto, ON M5G 2C4, Canada

Patsy Yates
Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Weiqiang Yin
Department of Cardiothoracic Surgery, The First Hospital Affiliated to Guangzhou Medical College; Guangzhou Institute of Respiratory Disease, State Key Laboratary of Respiratory Disease, Guangzhou 510120, China

Rym Zaimi
Thoracic Department, Institut Mutualiste Montsouris, 42 Bd Jourdan, F-75014 Paris, France

Xin Zhang
Department of Throcic Surgery, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China

Yaxiong Zhang
Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Isabella Zhao
Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Xiuyi Zhi
Beijing Lung Cancer Center, Capital Medical University, Beijing, China

Zhihua Zhu
Department of Thoracic Oncology, Cancer Center of Sun Yat-Sen University, Guangzhou 510060, China

Paul V. Zimmerman
Department of Thoracic Medicine, The Prince Charles

Table of Contents

Treatment

Surgery

Medical Oncology

Radiotherapy

Prognosis

Translational Medicine

Molecular biology of lung cancer

Wendy A. Cooper[1,2], David C. L. Lam[3], Sandra A. O'Toole[1,4,5], John D. Minna[6]

[1]Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Camperdown, Sydney, NSW, Australia; [2]School of Medicine, University of Western Sydney, NSW, Australia; [3]Department of Medicine, University of Hong Kong, Hong Kong SAR, China; [4]Kinghorn Cancer Centre and Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW, Australia; [5]Sydney Medical School, University of Sydney, NSW, Australia; [6]Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center at Dallas, Texas, USA

Correspondence to: Associate Professor Wendy A. Cooper. Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Missenden Rd, Camperdown, NSW, Australia. Email: wendy.cooper@sswahs.nsw.gov.au.

Abstract: Lung cancers are characterised by abundant genetic diversity with relatively few recurrent mutations occurring at high frequency. However, the genetic alterations often affect a common group of oncogenic signalling pathways. There have been vast improvements in our understanding of the molecular biology that underpins lung cancer in recent years and this has led to a revolution in the diagnosis and treatment of lung adenocarcinomas (ADC) based on the genotype of an individual's tumour. New technologies are identifying key and potentially targetable genetic aberrations not only in adenocarcinoma but also in squamous cell carcinoma (SCC) of the lung. Lung cancer mutations have been identified in v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), BRAF and the parallel phosphatidylinositol 3-kinase (PI3K) pathway oncogenes and more recently in MEK and HER2 while structural rearrangements in ALK, ROS1 and possibly rearranged during transfection (RET) provide new therapeutic targets. Amplification is another mechanism of activation of oncogenes such as MET in adenocarcinoma, fibroblastgrowth factor receptor 1 (FGFR1) and discoidin domain receptor 2 (DDR2) in SCC. Intriguingly, many of these genetic alternations are associated with smoking status and with particular racial and gender differences, which may provide insight into the mechanisms of carcinogenesis and role of host factors in lung cancer development and progression. The role of tumour suppressor genes is increasingly recognised with aberrations reported in TP53, PTEN, RB1, LKB11 and p16/CDKN2A. Identification of biologically significant genetic alterations in lung cancer that lead to activation of oncogenes and inactivation of tumour suppressor genes has the potential to provide further therapeutic opportunities. It is hoped that these discoveries may make a major contribution to improving outcome for patients with this poor prognosis disease.

Keywords: Lung cancer; mutation; molecular pathology; oncogene; tumour suppressor gene

Submitted Jul 19, 2013. Accepted for publication Aug 01, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.03
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.03

Introduction

The molecular basis of lung cancer is complex and heterogenous. Improvements in our understanding of molecular alterations at multiple levels (genetic, epigenetic, protein expression) and their functional significance have the potential to impact lung cancer diagnosis, prognostication and treatment. Lung cancers develop through a multistep process involving development of multiple genetic and epigenetic alterations, particularly activation of growth promoting pathways and inhibition of tumour suppressor pathways. Greater understanding of the multiple biochemical pathways involved in the molecular pathogenesis of lung cancer is crucial to the development of treatment strategies that can target molecular aberrations and their downstream activated pathways (1). Specific molecular alterations that drive tumour growth and

provide targets for therapy have been best defined in adenocarcinomas (ADC) but there is increasing interest in the molecular landscape of squamous cell carcinoma (SCC) highlighting new potential therapeutic targets. In lung cancer as in other malignancies, tumourigenesis relates to activation of growth promoting proteins [e.g., v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), BRAF, MEK-1, HER2, MET, ALK and rearranged during transfection (RET)] as well as inactivation of tumour suppressor genes [e.g., *P53*, phosphatase with tensin homology (*PTEN*), *LKB-1*] (1). Activation of growth promoting oncogenes can occur by gene amplification or other genetic alterations including point mutations and structural rearrangements leading to uncontrolled signalling through oncogenic pathways. "Oncogene addiction" results when cell survival depends on continued activation of the aberrant signalling (2,3) making them ideal candidates for targeted therapies. Oncogenic driver mutations have been identified in over 50% of lung ADC and are almost always exclusive of other driver mutations (4,5). Signalling pathways regulated by oncogenes and tumour suppressor genes are often interconnected with cross-talk between pathways involved in carcinogenesis. Added to the complexity is the occurrence of mutational evolution of tumours over time during the natural course of disease progression and in response to selection pressure exerted by therapy.

There is great genetic diversity in lung cancer and they harbour among the greatest numbers of genetic aberrations of all tumours (1). Understanding of the molecular biology of lung cancer has been revolutionised by next-generation sequencing technologies that provide a comprehensive means of identifying somatic alterations in entire cancer genomes or exomes. Lung cancers have highly complex genomes with a recent large-scale exome sequencing study of 31 non-small cell lung cancer (NSCLC) identifying 727 mutated genes not previously reported in the literature or in the COSMIC database (6). Genomic studies have confirmed previously well known alterations in lung cancer such as *KRAS*, *EGFR* and *BRAF* and have also identified low frequency but recurrent mutations that are novel in lung cancer (6-8) including potentially targetable alterations in *JAK2*, *ERBB4* (8), *RET* (9-11), fibroblast growth factor receptor 1 (FGFR1) (12) and discoidin domain receptor 2 (*DDR2*) (13). While these studies provide a comprehensive portrait of genetic alterations in lung cancers, the challenge remains of identifying biologically relevant driver mutations from the vast majority of passenger mutations. The relative paucity of high frequency recurrent mutations highlights the heterogeneity and complexity of the molecular biology of lung cancer with common pathways affected by a range of different genetic alterations that poses a challenge for providing personalised medicine.

In this review, we discuss the most commonly altered and most clinically relevant oncogenes and tumour suppressor genes in lung cancer as improved understanding of the molecular pathology of lung cancer is crucial for advancements in treatment strategies.

KRAS

KRAS is part of the *RAS* family of proto-oncogenes (*KRAS*, *NRAS* and *HRAS* occurring in humans) and encodes a G-protein with a critical role in controlling signal transduction pathways which regulate cell proliferation, differentiation and survival (14). Ras proteins are guanosine diphosphate (GDP) bound and inactive in normal quiescent cells. There is a switch to the activated guanosine triphosphate (GTP) bound form following activation of upstream growth factor receptors. The activated Ras-GTP subsequently binds and activates a number of downstream pathways including mitogen-activated protein kinase (MAPK), RAS/RAF/MEK/MAPK pathway and the PI3-K [PI3K/AKT/mammalian target of rapamycin (mTOR)] pathways (15). KRAS plays a critical role in downstream signal transduction induced by a variety of growth factor receptors including EGFR and constitutive activation of the protein circumvents the need for growth factor mediated signalling. Activating mutations alter the GTPase activity of the protein hindering inactivation of the active RAS-GTP to GDP leading to increased signalling through multiple downstream growth promoting pathways (15). The RAS/RAF/MEK/MAPK signal transduction cascade plays a central role in many lung cancers with at least one mutation in the pathway identified in 132 of 188 tumours (7), of which the most common are mutations in *KRAS*.

Activating mutations in the *KRAS* oncogene are the commonest oncogenic alteration in lung ADC occurring in about 25-40% of cases (4,5,7,16-18) while *HRAS* and *NRAS* mutations are very rare (17). Differences in the prevalence of *KRAS* mutations in lung ADC most likely relate to different patient populations as *KRAS* mutations are more common in Western populations compared to Asian populations (19-22) and are more frequent in males and smokers (7,18,22). ADC in never smokers have been

reported to harbour *KRAS* mutations in between 0-15% of cases (16,23). In addition, *KRAS* mutations are very rare or absent in SCCs and small cell cancer (17,24). Comprehensive genomic analysis of 188 SCCs identified only 1 *KRAS* mutation in codon 61 (12). *KRAS* mutations in lung adenocarcinoma consist of single amino acid substitutions in hotspots located mostly in codon 12 but also more rarely in codons 13 and 61 (14,17). The commonest mutations in *KRAS* are G to T transversions (~84%) in smokers while never smokers are more likely to harbour G to A transitions (16).

In keeping with the role of *KRAS* alterations as driver mutations, they do not occur in association with *EGFR* mutations (5,7,21,22), although rare exceptions do occur (18). A meta-analysis has shown *KRAS* mutant tumours are resistant to EGFR tyrosine kinase inhibitors (TKIs) (25), as *KRAS* mutations lead to constitutive activation of pathways downstream of EGFR. There is evidence that different KRAS mutant proteins have differing clinical significance. Interestingly, using data from the BATTLE trial (prospective phase II Biomarker-integrated Approaches of Targeted Therapy for Lung cancer Elimination), either G12C or G12V mutant *KRAS* predicted shorter progression free survival compared to other *KRAS* mutations or wild type *KRAS* (26). Furthermore, different amino acid substitutions were associated with activation of different pathways (PI3-K and MEK with Gly12Asp and Ral with Gly12Cys or mutant Gly12Val) resulting from divergent protein conformations from specific mutations leading to altered ability to associate with downstream protein mediators (26). This highlights that appropriate use of targeted therapies and clinical trial design needs to carefully evaluate the clinical and therapeutic significance of specific genetic alterations in lung cancer. The high frequency of *KRAS* mutations in lung cancer makes it an ideal therapeutic target but unfortunately clinical trials of targeted agents have generally been disappointing.

EGFR

Alterations of *EGFR* are involved in the pathogenesis of many tumours including NSCLC. *EGFR* encodes a transmembrane tyrosine kinase with an extracellular ligand-binding domain and an intracellular component including a tyrosine kinase domain (27). Binding of the ligand epidermal growth factor leads to receptor homo or heterodimerisation with other members of the EGFR family and activation of the tyrosine kinase domain (28,29). Signal transduction stimulated by EGFR occurs through the PI3K/AKT/mTOR, RAS/RAF//MAPK and JAK/STAT signalling pathways (28-30). EGFR is involved in regulation of numerous oncogenic functions such as cell proliferation, survival, differentiation, neovascularisation, invasion and metastasis (29,30). Activating mutations in *EGFR* lead to constitutive tyrosine kinase activation (30,31) and oncogenic transformation of lung epithelial cells *in vitro* (31). A transgenic mouse model with inducible expression of the commonest *EGFR* mutations showed development of multiple lung ADC that were sensitive to small molecule inhibition (32). Other mechanisms of increased EGFR signalling include increased protein expression or increased gene copy number (33,34).

Activating mutations of *EGFR* have been reported in 10-15% of unselected Western patients (5,21,35,36) and 30-40% of Asian populations (19,37,38). Differences in the reported prevalence rates of various mutations may in part relate to different patient populations but also depends on the sensitivity of mutation analysis techniques utilised in different studies. In NSCLC, *EGFR* mutations occur in the first four exons of the intracellular tyrosine kinase domain, most commonly exon 19 in frame deletions (~45%), of which there are over 20 variants, the commonest being delE746-A750. The next commonest *EGFR* mutations are missense mutations, particularly L858R, a single nucleotide point mutation in exon 21 leading to a single amino acid change from leucine to arginine at codon 858 (~40%). However, we found in an Australian population that exon 18 activating mutations constituted 14% of *EGFR* mutations in patients with early stage lung cancer and L858R mutations comprised only 29% of *EGFR* mutations present in this cohort (5). There are also a range of less common mutations including in frame duplications or insertions in exon 20 (~5-10%), of which there are many variants that are often associated with resistance to EGFR TKIs (22,39).

In lung cancer, almost all *EGFR* mutations occur in ADC (19,21,40,41) although they may also be seen in adenosquamous carcinomas. Mutations in *EGFR* are more commonly but not exclusively found in patients who are female, younger and with no history of smoking (7,19,21,22,37,40). *EGFR* mutations occur only very rarely, in histologically well sampled pure SCCs (24,42). However, comprehensive genomic analysis of 188 SCCs identified *EGFR* mutations in 2 cases, both with L861G mutations (12). While *EGFR* mutations are very rare in SCCs, variant-III mutations involving the extracellular domain of EGFR, copy-number gains and protein overexpression are more common in SCCs than in ADCs (43).

Secondary mutations in *EGFR* develop or are clonally selected in patients that develop resistance to EGFR TKIs, the commonest being the T790M activating point mutation in exon 20 which substitutes a "bulkier" methionine for threonine (44) that interferes with binding of reversible TKIs. T790M is found in about 50% of tumours from patients who develop acquired TKI resistance (41,44). Intriguingly, we observed that exon 20 mutations including T790M mutations associated with therapeutic resistance to EGFR TKI were seen in 29% of patients with *EGFR* mutations in a therapy naïve cohort (5). Activation of downstream pathways that bypass EGFR inhibition can also contribute to EGFR-TKI resistance including activation of PI3K pathway through amplification of MET (45).

BRAF

BRAF encodes a serine/threonine protein kinase that is the downstream effector protein of KRAS and activates the MAPK signal transduction pathway involved in regulation of cell proliferation and survival (46). Upon activation, BRAF phosphorylates downstream mediators MEK1 and MEK2 which subsequently activate ERK1 and ERK2, involved in regulation of growth regulating proteins such as c-JUN and ELK1 (14). Activating mutations in *BRAF* lead to increased kinase activity that exhibit transforming activity *in vitro* (46).

While activating *BRAF* mutations are common in melanoma (46), they occur in only about 3% of NSCLC (18,46-50). The mutations in NSCLC differ to those in melanoma and colorectal carcinoma with a lower proportion of V600E mutations that affect the kinase domain of the protein. In lung ADC, V600E mutations in exon 15 account for up to about 50% of *BRAF* mutations followed by G469A in exon 11 and D594G in exon 15 (48,50). Some of the *BRAF* mutations in NSCLC occur in the kinase domain (such as V600E, D594G and L596R) while others occur in the G-loop of the activation domain of the gene (such as G465V and G468A) (46). As *BRAF* and *KRAS* genes are part of the signalling pathway mediated by *EGFR*, it is not surprising that mutations in these genes are almost always mutually exclusive, in keeping with a common downstream pathway to transformation. *BRAF* mutations in lung cancer occur almost always in ADC (48). Non-V600E *BRAF* mutations have been associated with current or former smokers while V600E mutations appear to be more common in female never smokers (48,50). While uncommon, *BRAF* mutations represent an important therapeutic target due to the availability of targeted therapies already in clinical use for melanoma although there is only limited data about the clinical response to this approach in NSCLC (51).

MEK

MEK1 (also known as MAPK1) is a serine-threonine kinase that has an important function as a downstream target of RAS activation. MEK1 activates MAPK2 and MAPK3 downstream of BRAF (14). Rare cases of somatic mutations of *MEK1* have been reported in NSCLC with 2 of 107 lung ADC found to have an activating mutation in exon 2 that did not involve the kinase domain (52). The mutations were exclusive of other driver mutations and were associated with gain of function *in vitro* (52).

MET

The proto-oncogene *MET* located on chromosome 7q21-q31 encodes a membrane tyrosine kinase receptor that is also known as *hepatocyte growth factor receptor* (53). Upon binding of its ligand hepatocyte growth factor, there is receptor homodimerisation, kinase activation and signalling through downstream pathways including RAS/RAF/MEK/MAPK, PI3K/AKT and c-SRC kinase pathways (53). In NSCLC, *MET* is altered by gene amplification in about 1-7% of treatment naive patients (54-57) but in one study amplification was found in 21% of patients (58). Increased *MET* copy number may be more common in SCC than ADC (57) and is mutually exclusive with *KRAS* mutations (56,58). *MET* amplification results in overexpression of MET protein and activation of downstream signal transduction pathways. The oncogenic activity of *MET* has been demonstrated *in vitro* with evidence of gene amplification associated with constitutive receptor phosphorylation, activation of the PI3K/AKT pathway and sensitivity to MET inhibition (45,59). Amplification of *MET* is a known mechanism of secondary EGFR-TKI resistance with this kinase switch occurring in approximately 20% of patients with acquired resistance (45,54,55). In this scenario, *MET* amplification drives and maintains the PI3K/AKT pathway bypassing EGFR blockade by TKIs (45), suggesting concomitant MET inhibition may be a means of overcoming TKI resistance. Mutations of *MET* also occur uncommonly in about 3-5% of ADC (7,56).

HER2

The human epidermal growth factor receptor 2 (*HER2/*

ERBB2) gene encodes a membrane bound receptor tyrosine kinase that is a member of the ERBB family of receptors, along with EGFR. Unlike other ERBB receptors, it does not bind ligand directly but can form heterodimers with other ligand-bound members of the receptor family (60). Activation leads to signalling through a variety of signal transduction pathways including PI3K, MAPK and JAK/STAT pathways (61). Activation of *HER2* occurs in a small proportion of lung cancers with overexpression in approximately 20% of cases, gene amplification in 2% (62) and activating mutations in 1.6-4% of NSCLC (63-65). Activating mutations of *HER2* are exon 20 in frame insertions of 3 to 12 base pairs in length (63). There is *in vivo* evidence of the oncogenic activity of *HER2* with multiple adenosquamous carcinomas developing in a transgenic mouse model expressing mutant *HER2* and exhibiting susceptibility to small molecule inhibition (66). Alterations of *HER2* occur mostly in ADC (63-65) and mutations occur in tumours that are wild-type for *EGFR* and *KRAS* (63,64) and in some studies, are associated with female gender, Asian ethnicity and non-smoking status (63,65), similar to the clinical profile of *EGFR* mutant tumours.

PI3K/AKT/mTOR

The PI3K/AKT/mTOR pathway is an important signal transduction pathway involved in regulation of cell proliferation, survival, differentiation adhesion and motility (67,68). Alterations of this pathway have been implicated in both NSCLC and small cell carcinoma (69,70). The pathway is activated through activation of a variety of membrane tyrosine kinase receptors including EGFR, HER2, insulin-like growth factor receptor, vascular endothelial growth factor receptor and platelet derived growth factor receptor (71,72). Activated receptor tyrosine kinases recruit PI3K to the cell membrane where it phosphorylates PIP2 to PIP3 [phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate]. PIP3 in turn recruits the serine threonine kinase AKT to the membrane where it is phosphorylated by 3-phosphoinositide-dependent kinase 1 (PI3 kinase) and mTOR. mTOR is a serine/threonine kinase that is a downstream target of AKT (72). Activated AKT in turn activates multiple targets including tuberous sclerosis 2 and Bcl-2 associated death promotor leasing to cell proliferation and survival [reviewed in (71)]. There is also interaction with other pathways including RAS/RAF/MEK (Rat sarcoma/rapidly accelerated fibrosarcoma/MAPK or Erk kinase) with RAS having the capacity to directly activate PI3K (72).

The PI3K/AKT/mTOR pathway is frequently deregulated in many tumours including 50-70% of NSCLC (7,71). Significant alterations involving the PI3K pathway were identified in 47% of SCCs in the Cancer Genome Atlas project (12). Pathway activation in lung carcinogenesis occurs through a variety of mechanisms including activating mutations in *EGFR*, *KRAS*, *PI3K* or *AKT* (68,71) as well as *PIK3CA* amplification, or loss of negative regulation by the tumour suppressor gene *PTEN* (72).

The PI3K protein family (phosphatidylinositol 3-kinases) are intracellular lipid kinases and the main catalytic subunit, the p110alpha isoform, is encoded by the *PIK3CA* gene (71). Activating mutations and amplification of *PIK3CA* cause constitutive ligand-independent pathway activation (73,74). *PIK3CA* mutations mostly involve the catalytic domain and have been identified in approximately 1-3% of NSCLCs (7,73,75) and are more common in SCC than ADCs (4,75). Unlike most oncogenic driver mutations, *PIK3CA* mutations may occur in association with *EGFR* or *KRAS* mutations (5,73,75) suggesting they may not represent true driver mutations. However, *in vitro* studies of lung cancer cell lines with *PIK3CA* mutations or copy number gains show increased PI3 kinase activity sensitive to small molecule inhibition (73) and *in vivo* mouse models with *PIK3CA* mutation expression develop numerous ADC, suggesting oncogenic activity (74). *PIK3CA* may also be amplified in NSCLC, especially in SCCs (73,76) and increased copy number of *PIK3CA* has been reported in ~5% of small cell carcinoma cell lines (73). Although rare, PI3K/AKT/mTOR pathway activation can also occur through *AKT* mutations which have been reported in 0.5-2% of NSCLC (5,7,77), particularly SCCs (77).

ALK

Rearrangements of the receptor tyrosine kinase *ALK* resulting most commonly in fusions of the intracellular kinase domain with the amino terminal end of echinoderm microtubule associated protein-like 4 (EML4) occur in a subset of lung cancers (78-80). The rearrangement results from a short inversion in chromosome 2p, whereby in the commonest variant, intron 13 of *EML4* is fused to intron 19 of *ALK* {ALK [inv (2) (p21; p23)]} (79). Numerous variants of *EML4-ALK* fusions have been identified due to differing lengths of *EML4*, the commonest being exons 1-13 of *EML4* joining to exons 20-29 of *ALK* (78,81,82).

More recently, different partner genes have been identified in a small subset of *ALK* rearrangements (<1% of cases) including *KIF5B* (kinesin family member 5b), *TFG* (TRK -fused gene) and *KLC-1* (kinesin light chain1) (83,84). The oncogenic EML4-ALK fusion protein has a constitutively activated kinase and has gain of function activity *in vitro* (80) and *in vivo* mouse models expressing EML4-ALK develop multiple lung ADC that are susceptible to pharmacologic ALK inhibition (85). Activation of ALK is linked to cell proliferation and inhibition of apoptosis mediated through the RAS/RAF/MAPK1, PI3K/AKT and JAK3-STAT3 signalling pathways (82).

ALK rearrangements have been identified in approximately 4% of unselected NSCLC (86) although some studies have found a slightly lower prevalence (5,87). They are more commonly found in ADC from younger patients who are never smokers or light smokers (78,87-91) and almost always occur in ADCs (90). While *ALK* rearrangements are usually mutually exclusive with *EGFR* and *KRAS* mutations (5,87,91,92) cases of coexistent *EGFR* mutations have been reported and provide a mechanism for TKI resistance (78,93-95). While ALK inhibition with the tyrosine kinase inhibitor crizotinib produces profound responses, drug resistance develops with evidence of secondary ALK point mutations and activation of EGFR signalling implicated in some cases (81,93).

ROS1

ROS1 is a proto-oncogene located on chromosome 6q22 which encodes a transmembrane tyrosine kinase receptor that has high homology with ALK in its protein kinase domain (96). ROS1 activation leads to signalling through the PI3K/AKT/mTOR, STAT3 and RAS/MAPK/ERK pathways (96). In 2007, a large scale phosphoproteomic screen for tyrosine kinase activity in lung cancer identified *ROS1* fusion in a NSCLC cell line (1 of 41) and a patient sample (1 of 150) (*SLC34A2-ROS1* and *CD74-ROS1* respectively) (83). Subsequently, a novel *KDELR2-ROS1* in-frame fusion was identified in an adenocarcinoma from a non-smoker using whole genome and transcriptome sequencing (8). In 2 large studies using FISH, *ROS1* rearrangements were found in 18 of 694 ADCs (2.6%) (97) and 13 of 1,116 ADCs (1.2%) (98). A variety of 5' fusion partners have been identified in *ROS1* gene rearrangements (including *FIG*, *KDELR2*, *TPM3*, *SDC4*, *LRIG3*, *EZR*, *SLC34A2* and *CD74*) and it is uncertain what role, if any, the partner plays in the oncogenic function of the fusion kinase (8,83,98). Interestingly, *ROS1* rearrangements appear to be more common in patients who are younger, never smokers or of Asian ethnicity (97) similar to *ALK* rearrangements (90). Furthermore, there is *in vitro* and early clinical evidence that lung cancers with *ROS1* rearrangements are sensitive to kinase inhibitors including the ALK/MET inhibitor crizotinib (97).

RET

RET is located on chromosome 10q11.2 and encodes a receptor tyrosine kinase involved in neural crest development. Alterations of RET have long been known to play a role in papillary and medullary thyroid carcinoma (99) but it was not until recently that activation of RET through chromosomal rearrangement has been identified in a small proportion of lung cancers (9-11). The translocation fuses the functional *RET* kinase domain from exons 12-20 to *KIF5B* (kinesin family 5B gene), that is 10Mb from *RET* on chromosome 10 and encodes a coiled coil domain involved in organelle trafficking (9,10). *KIF5B-RET* fusions have been identified in 1-2% of lung ADC using massively parallel sequencing technologies (10,11) and to date have been found to be mutually exclusive of other driver mutations involving *EGFR*, *KRAS* or *ALK*. In a highly selected cohort of lung ADC from never smokers or light smokers known to be wild type for other driver mutations (*EGFR*, *KRAS*, *ALK*, *HER2*, *BRAF* and *ROS1*), 10 of 159 (6.3%) harboured *RET* rearrangements (11). Similar to *ALK* and *ROS1*, rearrangements of *RET* also appear to be associated with ADC from never smokers (9-11). Importantly, there are several multi-kinase inhibitors that are effective against *RET* and there is *in vitro* evidence that cell lines expressing *KIF5B-RET* fusions are sensitive to RET inhibition (10,11).

FGFR1

Somatic gene amplifications have been found in SCCs in a number of genes including *SOX*, *PDGFRA* (12) and *FGFR1* (12,100). *FGFR1* is a membrane receptor tyrosine kinase that regulates cell proliferation through activation of the MAPK and PI3K pathways (101). Amplification of *FGFR1* has an oncogenic effect on NSCLC cell lines *in vitro* that is sensitive to small molecule inhibition (102). About 20% of SCCs have been shown to harbour *FGFR1* amplifications but the abnormality is uncommon in ADCs (100,102).

DDR2

Recently, a sequencing screen including the entire tyrosine kinome was undertaken in SCCs and mutations were identified in *DDR2* in 3.8% of cases (13). *DDR2* encodes a membrane-bound receptor tyrosine kinase that binds collagen and is involved in regulation of cell proliferation and survival (103). Mutations of *DDR2* are associated with oncogenic activity *in vitro* that is sensitive to inhibition with dasatinib (13).

Tumour suppressor genes

Tumour suppressor genes are crucial negative regulators of normal cell growth. Loss of tumour suppressor gene (TSG) function is an important mechanism of carcinogenesis and requires inactivation of both gene alleles, as outlined in Knudson's two hit hypothesis (104). In one allele, the individual gene is often inactivated by mutation, epigenetic silencing or other aberrations, while the second allele is often inactivated through loss of heterozygosity (LOH) whereby a region of the chromosome is lost by deletion, nonreciprocal translocation or mitotic recombination. In lung cancer, TSGs that are frequently inactivated include TP53, retinoblastoma 1 (*RB1*), serine-threonine kinase 11 (*STK11*), *CDKN2A*, *FHIT*, *RASSF1A* and *PTEN* (1,7,105) and these genes map to chromosomal regions commonly identified in LOH studies. For example, regions frequently exhibiting allelic loss in lung cancer involve known TSGs such as *TP53* (17p13), *RB* (13q12), *p16* (9p21), and *PTEN* (10q22) (105). In a study by Ding *et al.* (7), mutations were identified in several TSGs not previously known to play a significant role in lung adenocarcinoma including the TSG NF1 (involved in neurofibromatosis type 1), that was mutated in 13 tumours and the *TP53* regulator *ATM* in 13 patients.

TP53

TP53 located on chromosome 17p13 encodes a nuclear phosphoprotein of 53 kDa that identifies and binds to regions of damaged DNA (106) and acts as a transcription factor controlling the expression of a multitude of different genes. Damaged DNA or carcinogenic stress induces *TP53* leading to cell cycle arrest by inducing expression of cyclin dependent kinase inhibitors to enable DNA repair or apoptosis. *TP53* inactivation is one of the most significant genetic abnormalities in lung cancer with hemizygous deletion of 17p13, containing the locus of *TP53*, occurring in 90% of small cell carcinomas and about 65% of NSCLC (107). Inactivating mutations in *TP53* (mostly missense mutations within the DNA-binding domain) have been reported in 80-100% of small cell lung carcinomas (108). By contrast, a meta-analysis of *TP53* in over 4,000 NSCLC found alterations by mutation or protein accumulation in only 46.8% of cases (109), more commonly in SCC than ADC and associated with higher tumour stage, grade and male gender. Mutations of *TP53* were found in at least 81% of SCCs that underwent comprehensive genomic analysis as part of The Cancer Genome Atlas (TCGA) project (12). Ding *et al.* (7) found TP53 mutations in 85 of 188 ADC (45%). In NSCLC, *TP53* mutations are associated with a positive smoking history or exposure to environmental tobacco smoke (19,110). The mutational spectrum of different types of *TP53* mutations also differs between smokers and non-smokers with smoking related cancers having a significantly higher frequency of G to T transversions compared to G to C transversions (thought to be induced by polycyclin aromatic hydrocarbons in tobacco smoke) and G to A transitions at CpG dinucleotides more commonly seen in never smokers (110,111). A meta-analysis of 74 studies showed that aberrant p53 detected by protein expression or mutational analysis is an unfavourable prognostic factor in lung NSCLC (112). Genetic alterations of *TP53* have also been associated with treatment resistance (106). *TP53* gene mutations can occur in association with *EGFR* and *KRAS* mutations (19).

PTEN

PTEN encodes a lipid and protein phosphatase on chromosome 10 that inhibits the PI3K/AKT/mTOR signalling pathway by dephosphorylating PI-(3,4,5)-triphosphate (68). Inactivation of the TSG function of *PTEN* leads to unrestricted activation of AKT/protein kinase B independent of ligand binding (68). Mutations of *PTEN* occur only rarely in about 5% of NSCLC (113) being more common in SCC than ADC (10.2% *vs.* 1.7%) and associated with a history of smoking. By contrast, reduced protein expression has been reported in about 75% of NSCLC (114).

LKB1 (STK11)

LKB1 (also known as STK11) is a TSG located on chromosome 19p13 that encodes a serine-threonine kinase

that inhibits mTOR and has been implicated in a range of biological processes including regulation of the cell cycle, chromatin remodelling, cell polarity, and energy metabolism (115,116). Deregulation of mTOR pathway components (not including KRAS mutations) has been reported in 30% of ADCs (7). Germline mutations of *LKB1/STK11* occur in patients with Peutz-Jeghers syndrome (115). In lung cancer, *LKB1* may be inhibited by a variety of somatic mutations or deletions that produce truncated proteins with inactivation of *LKB1* occurring in about 11-30% of lung ADC (7,117-119), making it the third commonest genetic aberration in lung ADC after *TP53* and *KRAS*. *LKB1* inactivation is more common in lung ADC compared to SCCs (117,119). There is some evidence of an association between *LKB1* mutations and a history of smoking (117) in men (118,120) and a correlation with *KRAS* mutations has also been reported (117,118).

The p16^{INK4a}-cyclin D1-CDK4-RB pathway

The p16^{INK4A}/RB pathway regulates cell cycle progression from G1 to S phase. *RB1* is a tumour suppressor gene that encodes RB protein which regulates cell cycle G1/S transition by binding the transcription factor E2F1. *RB1* was the first TSG described in lung cancer (121) and is inactivated in about 90% of small cell lung carcinomas but only about 10-15% of NSCLC (1). In NSCLC, the pathway is mostly switched off through alterations of cyclin D1, CDK4 and the cyclin dependent kinase inhibitor p16 (CDKN2A) (105). p16^{INK4A} inhibits cyclin D1 dependent phosphorylation of RB protein, thereby preventing cell cycle transition through the G1/S checkpoint (122). p16^{INK4A} is inactivated in about 80% of NSCLC (123,124) and was altered in 72% of lung SCCs examined by TCGA, mostly through homozygous deletion, methylation or inactivating mutations (12). In addition, there is overexpression of cyclin D1 through gene amplification or other mechanisms in about 40% of NSCLC (123).

Molecular targeting in NSCLC

The presence of these molecular targets as described above now defines the characteristics of NSCLC, with *EGFR* mutation and *ALK* rearrangements being the most clinically relevant at present (125). The prevalence of these mutations varies in lung cancer arising from patient in different regions (126). Activating *EGFR* mutations were found in up to 20% of Caucasians while in the Asian populations these *EGFR* mutations can be present in up to 40% of patients with NSCLC (127). These ethnic difference in NSCLC properties appears to be not limited to the presence of activating *EGFR* mutations but is also evident in other driver oncogenic mutation profiles (including *ALK*, *KRAS*, *MET etc.*), histology and hence tumour response to targeted therapy treatment (63,126,128). The presence of these driver mutations is generally found to be mutually exclusive to others in the same tumour (126). In lung ADC among Asians, *ALK* rearrangement is seen in up to 7% of patients with lung ADC (79). Lung tumours bearing *EML4-ALK* rearrangement are non-responsive to conventional chemotherapy or EGFR-tyrosine kinase inhibitors but are sensitive to a specific tyrosine kinase inhibitor named crizotinib (129). Based on our current understanding of therapeutic molecular targets of *EGFR* mutation and *ALK* gene rearrangement in NSCLC and the availability of corresponding targeted agents, an algorithm of testing for molecular targets in NSCLC is proposed as in *Figure 1*, which represents a stepwise approach to testing for individual targets, beginning with *EGFR* then, if negative, *ALK* fusion gene or other potential targets if appropriate.

Among NSCLC, adenocarcinoma accounts for up to 80% of histological subtypes (130). There are previous reports of correlations between histological subtypes of ADC demonstrating micropapillary features with presence of activating *EGFR* mutations, leading to the suggestions that the presence of specific mutations in NSCLC actually represent heterogeneity in cancer biology and also response to therapy (131). Given the heterogeneity of lung cancer histology, however, histological subtypes are difficult to be used as the sole reliable marker for guidance to molecular phenotyping and selection of targeted therapy (132,133).

Targeting therapeutic oncogenic mutations like *EGFR* and *ALK* can give dramatic initial treatment response or at least an initial stable clinical disease. The response rate is up to 70% in lung ADC bearing favourable activating *EGFR* mutations (134). The median progression free survival is usually quoted as 9-11 months with different tyrosine kinase inhibitors (135,136), after which most patients with *EGFR* mutations will experience disease progression and drug resistance. A proportion of such drug resistance is attributed to the development of a second mutation, usually T790M at exon 20 (137). It is hard to explain the eventual loss of drug sensitivity in tumours bearing those favourable *EGFR* mutations (exon

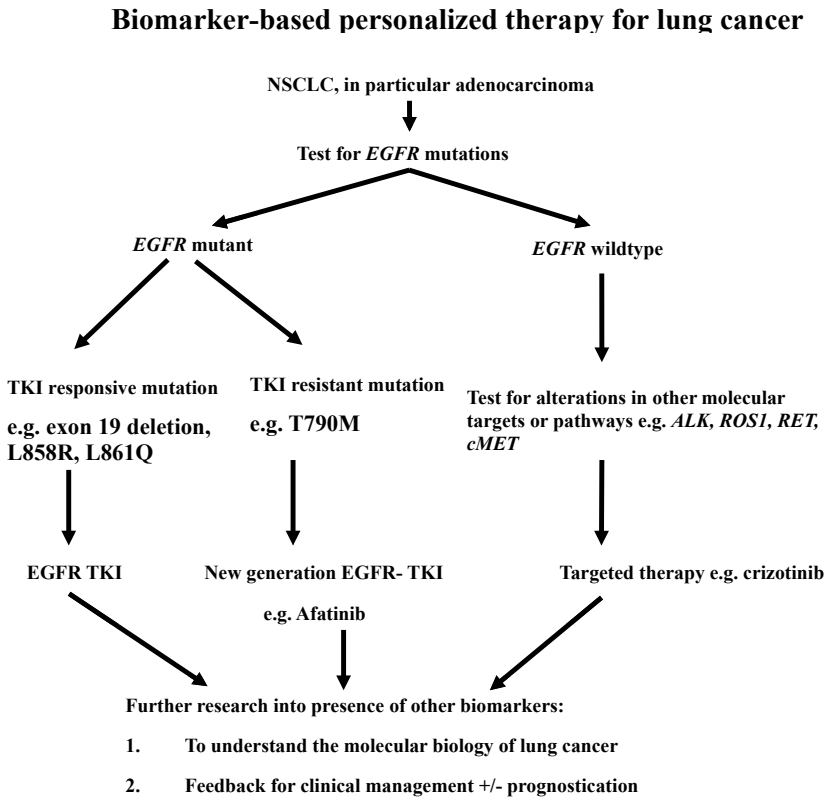


Figure 1 A suggested algorithm for molecular target testing based on understanding of relevant molecular biology in non-small cell lung cancer (NSCLC).

19 deletions and L858R) even without the acquisition of secondary mutations like T790M or the presence of other uncommon or less favourable *EGFR* mutations. This could reflect suboptimal therapeutic targeting and better understanding on the biology of EGFR-related tumour signalling and other oncogenic mutations will improve drug targeting and give patients better prediction of therapeutic response and prognostication.

Conclusions

The identification of driver mutations in *EGFR* and *ALK* heralded a new era of targeted therapy in lung adenocarcinoma and advanced sequencing technologies are providing even more sophisticated insights into the molecular aberrations in oncogenes and tumour suppressor genes underlying lung cancer (12,138-142). These studies have identified a range of potentially targetable genetic aberrations in lung cancer but have also highlighted a troubling complexity and heterogeneity which poses significant challenges for molecular diagnosis and targeted treatment. Greater knowledge of the molecular biology and genomic landscape of lung cancer offers promise for the future. Improvements in outcome from lung cancer will almost certainly require the identification of increasing numbers of ever rarer driver mutations, and diagnostic approaches that can identify multiple therapeutic targets offer significant advantages. However, the identification of driver genomic aberrations also requires the parallel development of effective targeted therapies and for many of these changes (such as *KRAS*) such therapies are not yet available. Resistance to targeted therapeutics is an increasingly recognised issue into which genomic analyses may provide important mechanistic insights underlying future rational therapeutic approaches.

Acknowledgements

Funding sources: Sydney Foundation for Medical Research; Hong Kong SK Yee Medical Foundation; Cancer Institute

NSW Clinical Research Fellowship 10/1/07; Sydney Breast Cancer Foundation; Lifehouse at Royal Prince Alfred Hospital Grant; Lung Cancer SPORE P50CA70907.
Disclosure: WC has received honoraria from Pfizer Oncology. SOT is a member of the Roche Australia Molecular Pathology Advisory Board. The authors declare no other conflicts of interest.

References

1. Larsen JE, Minna JD. Molecular biology of lung cancer: clinical implications. Clin Chest Med 2011;32:703-40.
2. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
3. Weinstein IB. Cancer. Addiction to oncogenes-the Achilles heal of cancer. Science 2002;297:63-4.
4. Sequist LV, Heist RS, Shaw AT, et al. Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. Ann Oncol 2011;22:2616-24.
5. Yip PY, Yu B, Cooper WA, et al. Patterns of DNA mutations and ALK rearrangement in resected node negative lung adenocarcinoma. J Thorac Oncol 2013;8:408-14.
6. Liu P, Morrison C, Wang L, et al. Identification of somatic mutations in non-small cell lung carcinomas using whole exome sequencing. Carcinogenesis 2012;33:1270-6.
7. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455:1069-75.
8. Govindan R, Ding L, Griffith M, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150:1121-34.
9. Ju YS, Lee WC, Shin JY, et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 2012;22:436-45.
10. Kohno T, Ichikawa H, Totoki Y, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med 2012;18:375-7.
11. Lipson D, Capelletti M, Yelensky R, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18:382-4.
12. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.
13. Hammerman PS, Sos ML, Ramos AH, et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. Cancer Discov 2011;1:78-89.
14. Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 2003;3:11-22.
15. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. Nat Rev Mol Cell Biol 2008;9:517-31.
16. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res 2008;14:5731-4.
17. Rodenhuis S, Slebos RJ. Clinical significance of ras oncogene activation in human lung cancer. Cancer Res 1992;52:2665s-9s.
18. Schmid K, Oehl N, Wrba F, et al. EGFR/KRAS/BRAF mutations in primary lung ADC and corresponding locoregional lymph node metastases. Clin Cancer Res 2009;15:4554-60.
19. Kosaka T, Yatabe Y, Endoh H, et al. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919-23.
20. Mao C, Qiu LX, Liao RY, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. Lung cancer 2010;69:272-8.
21. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339-46.
22. Tam IY, Chung LP, Suen WS, et al. Distinct epidermal growth factor receptor and KRAS mutation patterns in non–small cell lung cancer patients with different tobacco exposure and clinicopathologic features. Clin Cancer Res 2006;12:1647-53.
23. Subramanian J, Govindan R. Molecular genetics of lung cancer in people who have never smoked. Lancet Oncol 2008;9:676-82.
24. Rekhtman N, Paik PK, Arcila ME, et al. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. Clin Cancer Res 2012;18:1167-76.
25. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer Lancet Oncol 2008;9:962-72.
26. Ihle NT, Byers LA, Kim ES, et al. Effect of KRAS Oncogene Substitutions on Protein Behavior: Implications

for Signaling and Clinical Outcome. J Natl Cancer Inst 2012;104:228-39.
27. Prenzel N, Fischer OM, Streit S, et al. The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. Endocr Relat Cancer 2001;8:11-31.
28. Scagliotti GV, Selvaggi G, Novello S, et al. The Biology of epidermal growth factor receptor in lung cancer. Clin Cancer Res 2004;10:4227s-32s.
29. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127-37.
30. Sordella R, Bell DW, Haber DA, et al. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004;305:1163-7.
31. Greulich H, Chen TH, Feng W, et al. Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. PLoS Med 2005;2:e313.
32. Ji H, Li D, Chen L, et al. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. Cancer Cell 2006;9:485-95.
33. Dahabreh IJ, Linardou H, Siannis F, et al. Somatic EGFR mutation and gene copy gain as predictive biomarkers for response to tyrosine kinase inhibitors in non–small cell lung cancer. Clin Cancer Res 2010;16:291-303.
34. Okabe T, Okamoto I, Tamura K, et al. Differential constitutive activation of the epidermal growth factor receptor in non–small cell lung cancer cells bearing EGFR gene mutation and amplification. Cancer Res 2007;67:2046-53.
35. Eberhard DA, Giaccone G, Johnson BE. Biomarkers of response to epidermal growth factor receptor inhibitors in non–small-cell lung cancer working group: standardization for use in the clinical trial setting. J Clin Oncol 2008;26:983-94.
36. Russell PA, Barnett SA, Walkiewicz M, et al. Correlation of mutation status and survival with predominant histologic subtype according to the new IASLC/ATS/ERS lung adenocarcinoma classification in stage III (N2) patients. J Thorac Oncol 2013;8:461-8.
37. Tokumo M, Toyooka S, Kiura K, et al. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non–small cell lung cancers. Clin Cancer Res 2005;11:1167-73.
38. Yoshida K, Yatabe Y, Park JY, et al. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. J Thorac Oncol 2007;2:22-8.
39. Yamamoto H, Toyooka S, Mitsudomi T. Impact of EGFR mutation analysis in non-small cell lung cancer. Lung cancer 2009;63:315-21.
40. Marchetti A, Ardizzoni A, Papotti M, et al. Recommendations for the analysis of ALK gene rearrangements in non–small-cell lung cancer: a consensus of the Italian Association of Medical Oncology and the Italian Society of Pathology and Cytopathology. J Thorac Oncol 2013;8:352-8.
41. Wu JY, Wu SG, Yang CH, et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. Clin Cancer Res 2008;14:4877-82.
42. Ohtsuka K, Ohnishi H, Fujiwara M, et al. Abnormalities of epidermal growth factor receptor in lung squamous-cell carcinomas, adenosquamous carcinomas, and large-cell carcinomas: tyrosine kinase domain mutations are not rare in tumors with an adenocarcinoma component. Cancer 2007;109:741-50.
43. Heist RS, Sequist LV, Engelman JA. Genetic changes in squamous cell lung cancer: a review. J Thorac Oncol 2012;7:924-33.
44. Balak MN, Gong Y, Riely GJ, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor–mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 2006;12:6494-501.
45. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
46. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.
47. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 2002;62:6997-7000.
48. Marchetti A, Chen TH, Richards WG, et al. Clinical features and outcome of patients with non–small-cell lung cancer harboring BRAF mutations. J Clin Oncol 2011;29:3574-9.
49. Naoki K, Chen T, Richards W, et al. Missense mutations of the BRAF gene in human lung adenocarcinoma. Cancer Res 2002;62:7001-3.
50. Paik PK, Arcila ME, Fara M, et al. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. J Clin Oncol 2011;29:2046-51.
51. Falchook GS, Long GV, Kurzrock R, et al. Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. Lancet

2012;379:1893-901.
52. Marks JL, Gong Y, Chitale D, et al. Novel MEK1 mutation identified by mutational analysis of epidermal growth factor receptor signalling pathwar genes in lung adenocarcinoma. Cancer Res 2008;68:5524-8.
53. Sadiq AA, Salgia R. MET as a possible target for non–small-cell lung cancer. J Clin Oncol 2013;31:1089-96.
54. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci USA 2007;104:20932-7.
55. Cappuzzo F, Marchetti A, Skokan M, et al. MET increased gene copy number negatively affects survival of surgically resected non–small-cell lung cancer patients. J Clin Oncol 2009;27:1667-74.
56. Onozato R, Kosaka T, Kuwano H, et al. Activtion of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. J Thorac Oncol 2009;4:5-11.
57. Go H, Jeon YK, Park HJ, et al. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol 2010;5:305-13.
58. Beau-Faller M, Ruppert AM, Voegeli AC, et al. MET gene copy number in non-small cell lung cancer: molecular analysis in a targeted tyrosine kinase inhibitor naive cohort. J Thorac Oncol 2008;3:331-9.
59. Lutterbach B, Zeng Q, Davis LJ, et al. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. Cancer Res 2007;67:2081-8.
60. Tzahar E, Waterman H, Chen X, et al. A hierarchical network fo interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol 1996;16:5276-87.
61. Graus-Porta D, Beerli RR, Daly JM, et al. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. EMBO J 1997;16:1647-55.
62. Heinmöller P, Gross C, Beyser K, et al. HER2 status in non-small cell lung cancer: results from patient screening for enrollment to a phase II study of herceptin. Clin Cancer Res 2003;9:5238-43.
63. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005;65:1642-6.
64. Stephens P, Hunter C, Bignell G, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature 2004;431:525-6.
65. Tomizawa K, Suda K, Onozato R, et al. Prognostic and predictive implications of HER2/ERBB2/neu gene mutations in lung cancers. Lung Cancer 2011;74:139-44.
66. Perera SA, Li D, Shimamura T, et al. HER2YVMA drives rapid development of adenosquamous lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy. Proc Natl Acad Sci 2009;106:474-9.
67. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006;7:606-19.
68. Cully M, You H, Levine AJ, et al. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 2006;6:184-92.
69. Brognard J, Clark AS, Ni Y, et al. Akt/Protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. Cancer Res 2001;61:3986-97.
70. Tsurutani J, West KA, Sayyah J, et al. Inhibition of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway but not the MEK/ERK pathway attenuates laminin-mediated small cell lung cancer cellular survival and resistance to imatinib mesylate or chemotherapy. Cancer Res 2005;65:8423-32.
71. Papadimitrakopoulou V. Development of PI3K/AKT/MTOR pathway inhibitors and their application in personalized therapy for non-small-cell lung cancer. J Thorac Oncol 2012;7:1315-26.
72. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase-AKT pathway in human cancer. Nat Rev Cancer 2002;2:489-501.
73. Yamamoto H, Shigematsu H, Nomura M, et al. PIK3CA mutations and copy number gains in human lung cancers. Cancer Res 2008;68:6913-21.
74. Engelman JA, Chen L, Tan X, et al. Effective use of PI3K and MEK inhibitors to treat mutant K-ras G12D and PIK3CA H1047R murine lung cancers. Nat Med 2008;14:1351-6.
75. Kawano O, Sasaki H, Endo K, et al. PIK3CA mutation status in Japanese lung cancer patients. Lung cancer 2006;54:209-15.
76. Ji M, Guan H, Gao C, et al. Highly frequent promoter methylation and PIK3CA amplification in non-small cell lung cancer (NSCLC). BMC Cancer 2011;11:147.
77. Malanga D, Scrima M, De Marco C, et al. Activating E17K mutation in the gene encoding the protein kinase AKT1 in a subset of squamous cell carcinoma of the lung. Cell Cycle 2008;7:665-9.
78. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008;14:4275-83.

79. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
80. Choi YL, Takeuchi K, Soda M, et al. Identification of novel isoform of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Res 2008;68:4971-6.
81. Choi YL, Soda M, Yamashati Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734-9.
82. Shaw AT, Solomon B. Targeting anaplastic lymphoma kinase in lung cancer. Clin Cancer Res 2011;17:2081-6.
83. Rikova K, Guo A, Zeng Q. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190-203.
84. Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 2009;15:3143-9.
85. Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci USA 2008;105:19893-7.
86. Solomon B, Varella-Garcia M, Camidge DR. ALK gene rearrangements. A new therapeutic target in a molecularly defined subset of non-small cell lung cancer. J Thorac Oncol 2009;4:1450-4.
87. Selinger CI, Rogers TM, Russell PA, et al. Testing for ALK rearrangement in lung adenocarcinoma-a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. Mod Pathol 2013;26:1545-53.
88. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the Western population. Clin Cancer Res 2009;15:5216-23.
89. Sakairi Y, Nakajima T, Yasufuku K, et al. EML4-ALK fusion gene assessment using metastatic lymph node samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration. Clin Cancer Res 2010;16:4938-45.
90. Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27:4247-53.
91. Wong DW, Leung EL, So KK, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. Cancer 2009;115:1723-33.
92. Inamura K, Takeuchi K, Togashi Y, et al. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Mod Pathol 2009;22:508-15.
93. Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signalling cause resistance to ALK kinase inhibitors. Cancer Res 2011;71:6051-60.
94. Sholl LM, Weremowicz S, Gray SW, et al. Combined use of ALK immunohistochemistry and FISH for optimal etection of ALK-rearranged lung adenocarcinomas. J Thorac Oncol 2013;8:322-8.
95. Tiseo M, Gelsomino F, Boggiani D, et al. EGFR and EML4-ALK gene mutations in NSCLC: A case report of erlotinib-resistant patient with both concomitant mutations. Lung cancer 2011;71:241-3.
96. Chin LP, Soo RA, Soong R, et al. Targeting ROS1 with anaplastic lymphoma kinase inhibitors: a promising therapeutic strategy for a newly defined molecular subset of non–small-cell lung cancer. J Thorac Oncol 2012;7:1625-30.
97. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-70.
98. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. Nature Med 2012;18:378-81.
99. Wells SA Jr, Santoro M. Targeting the RET pathway in thyroid cancer. Clin Cancer Res 2009;15:7119-23.
100. Tran TN, Selinger CI, Kohonen-Corish MR, et al. Fibroblast Growth Factor Receptor 1 (FGFR1) copy number is an independent prognostic factor in non-small cell lung cancer. Lung Cancer 2013;81:462-7.
101. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010;10:116-29.
102. Dutt A, Ramos AH, Hammerman PS, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 2011;6:e20351.
103. Ikeda K, Wang L, Torres R, et al. Discoidin domain receptor 2 interacts with Src and Shc following its activation by type I collagen. J Biol Chem 2002;277:19206-12.
104. Knudson AG. Antioncogenes and human cancer. Proc Natl Acad Sci USA 1993;90:10914-21.
105. Raso MG, Wistuba II. Molecular pathogenesis of early-stage non-small cell lung cancer and a proposal for tissue banking to facilitate identification of new biomarkers. J Thorac Oncol 2007;2:S128-35.
106. Mogi A, Kuwano H. TP53 mutations in nonsmall cell lung cancer. J Biomed Biotech 2011;2011:583929.
107. Wistuba II, Berry J, Behrens C, et al. Molecular changes in the bronchial epithelium of patients with small cell lung

cancer. Clin Cancer Res 2000;6:2604-10.
108. D'Amico D, Carbone D, Mitsudomi T, et al. High frequency of somatically aquired p53 mutatinos in small-cell lung cancer cell lines and tumors. Oncogene 1992;7:339-46.
109. Tammemagi MC, McLaughlin JR, Bull SB. Meta-analyses of p53 tumor suppressor gene alterations and clinicopathological features in resected lung cancers. Cancer Epidemiol Biomarkers Prev 1999;8:625-34.
110. Husgafvel-Pursiainen K, Boffetta P, Kannio A, et al. p53 mutations and exposure to environmental tobacco mmoke in a multicenter study on lung Cancer. Cancer Res 2000;60:2906-11.
111. Takagi Y, Osada H, Kuroishi T, et al. p53 mutations in non-small-cell lung cancers occurring in individuals without a past history of actve smoking. Br J Cancer 1998;77:1568-72.
112. Steels E, Paesmans M, Berghmans T, et al. Role of p53 as a prognostic factor for survival in lung cancer: a systematic review of the literature with a meta-analysis. Eur Respir J 2001;18:705-19.
113. Jin G, Kim MJ, Jeon HS, et al. PTEN mutations and relationship to EGFR, ERBB2, KRAS, and TP53 mutations in non-small cell lung cancers. Lung cancer 2010;69:279-83.
114. Marsit CJ, Zheng S, Aldape K, et al. PTEN expression in non–small-cell lung cancer: evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. Hum Pathol 2005;36:768-76.
115. Marignani PA. LKB1, the multitasking tumour suppressor kinase. J Clin Pathol 2005;58:15-9.
116. Shaw RJ, Bardeesy N, Manning BD, et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell 2004;6:91-9.
117. Koivunen JP, Kim J, Lee J, et al. Mutations in the LKB1 tumour suppressor are frequently detected in tumours from Caucasion but not Asian lung cancer patients. Br J Cancer 2008;99:245-52.
118. Matsumoto S, Iwakawa R, Takahashi K, et al. Prevalence and specifcity of LKB1 genetic alterations in lung cancers. Oncogene 2007;26:5911-8.
119. Sanchez-Cespedes M, Parrella P, Esteller M, et al. Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. Cancer Res 2002;62:3659-62.
120. Onozato R, Kosaka T, Achiwa H, et al. LKB1 gene mutations in Japanese lung cancer patients. Cancer Sci 2007;98:1747-51.
121. Harbour JW, Lai SL, Whang-Peng J, et al. Abnormalities in structure and epxression of the human retinoblastoma gene in SCLC. Science 1988;241:353-7.
122. Trimarchi JM, Lees JA. Sibling rivalry in the E2F family. Nat Rev Mol Cell Biol 2002;3:11-20.
123. Brambilla E, Moro D, Gazzeri S, et al. Alterations of the expression of Rb, p16(INK4A) and cyclin D1 in non-small cell lung carcinoma and their clinical significance. J Pathol 1999;188:351-60.
124. Otterson GA, Kratzke RA, Coxon A, et al. Absence of p16INK4 protein is restricted to the subset of lung cancer lines that retains wildtype RB. Oncogene 1994;9:3375-8.
125. Pao W, Hutchinson KE. Chipping away at the lung cancer genome. Nat Med 2012;18:349-51.
126. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011;12:175-80.
127. Sriram KB, Larsen JE, Yang IA, et al. Genomic medicine in non-small cell lung cancer: paving the path to personalized care. Respirology 2011;16:257-63.
128. Paik PK, Johnson ML, D'Angelo SP, et al. Driver mutations determine survival in smokers and never-smokers with stage IIIB/IV lung adenocarcinomas. Cancer 2012;118:5840-7.
129. Shaw AT, Solomon B. Targeting anaplastic lymphoma kinase in lung cancer. Clin Cancer Res 2011;17:2081-6.
130. Travis WD, Brambilla E, Riely GJ. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol 2013;31:992-1001.
131. Motoi N, Szoke J, Riely GJ, et al. Lung adenocarcinoma: modification of the 2004 WHO mixed subtype to include the major histologic subtype suggests correlations between papillary and micropapillary adenocarcinoma subtypes, EGFR mutations and gene expression analysis. Am J Surg Pathol 2008;32:810-27.
132. Zakowski MF, Hussain S, Pao W, et al. Morphologic features of adenocarcinoma of the lung predictive of response to the epidermal growth factor receptor kinase inhibitors erlotinib and gefitinib. Arch Pathol Lab Med 2009;133:470-7.
133. Ohtsuka K, Ohnishi H, Fujiwara M, et al. Abnormalities of epidermal growth factor receptor in lung squamous-cell carcinomas, adenosquamous carcinomas, and large-cell carcinomas: tyrosine kinase domain mutations are not rare in tumors with an adenocarcinoma component. Cancer 2007;109:741-50.
134. Mok T, Wu YL, Zhang L. A small step towards personalized medicine for non-small cell lung cancer. Discov Med 2009;8:227-31.
135. Zhou C, Wu YL, Chen G, et al. Erlotinib versus

chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
136. Yang JC, Shih JY, Su WC, et al. Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): a phase 2 trial. Lancet Oncol 2012;13:539-48.
137. Wu JY, Yu CJ, Chang YC, et al. Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. Clin Cancer Res 2011;17:3812-21.
138. Lee W, Jiang Z, Liu J, et al. The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 2010;465:473-7.
139. Pleasance ED, Stephens PJ, O'Meara S, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature 2010;463:184-90.
140. Imielinski M, Berger AH, Hammerman PS, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012; 150:1107-20.
141. Peifer M, Fernández-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012;44:1104-10.
142. Rudin CM, Durinck S, Stawiski EW, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 2012; 44:1111-6.

Cite this article as: Cooper WA, Lam DC, O'Toole SA, Minna JD. Molecular biology of lung cancer. J Thorac Dis 2013;5(S5):S479-S490. doi: 10.3978/j.issn.2072-1439.2013.08.03

The pivotal role of pathology in the management of lung cancer

Morgan R. Davidson[1,2], Adi F. Gazdar[3,4], Belinda E. Clarke[1,5]

[1]University of Queensland Thoracic Research Centre, The Prince Charles Hospital, QLD, Australia; [2]Department of Anatomical Pathology, Royal Brisbane and Women's Hospital, QLD, Australia; [3]The Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, Texas, USA; [4]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas, USA; [5]Department of Anatomical Pathology, The Prince Charles Hospital, QLD, Australia

Correspondence to: Morgan R. Davidson. The Prince Charles Hospital, Rode Road, Chermside, Brisbane QLD 4032, Australia.
Email: morgan.davidson@uqconnect.edu.au.

Abstract: The last decade has seen significant advances in our understanding of lung cancer biology and management. Identification of key driver events in lung carcinogenesis has contributed to the development of targeted lung cancer therapies, heralding the era of personalised medicine for lung cancer. As a result, histological subtyping and molecular testing has become of paramount importance, placing increasing demands on often small diagnostic specimens. This has triggered the review and development of the first structured classification of lung cancer in small biopsy/cytology specimens and a new classification of lung adenocarcinoma from the IASLC/ATS/ERS. These have enhanced the clinical relevance of pathological diagnosis, and emphasise the role of the modern surgical pathologist as an integral member of the multidisciplinary team, playing a crucial role in clinical trials and determining appropriate and timely management for patients with lung cancer.

Keywords: Lung Neoplasms; pathology; non-small-cell lung carcinoma (NSCLC); small cell lung carcinoma (SCLC)

Submitted Jul 31, 2013. Accepted for publication Aug 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.43
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.43

Introduction

Lung cancer remains one of the leading causes of cancer mortality worldwide (1). In Australia in 2009, cancer accounted for 29.8% of all deaths, second only to diseases of the circulatory system, with malignancies of the trachea, bronchus and lungs being the leading cause of cancer related deaths in males (20.1%) and surpassing breast cancer in females (16.5%) (2). Currently, surgical resection with curative intent is the primary treatment for lung cancer, however the vast majority of patients present at an advanced disease stage where medical therapy is the only therapeutic option available. The prognosis for patients diagnosed with lung cancer is poor, with overall five year survival remaining below 15% (3-7). This is partly attributable to relatively ineffective methods for early detection and lack of curative treatment for advanced disease.

However, the last decade has seen rapid development of advanced molecular biology techniques for the study of lung and other cancers, and our understanding and appreciation of the complexity of tumour biology has increased exponentially. It is well established that lung cancer is the result of multiple complex combinations of morphological, molecular and genetic alterations, ultimately leading to a malignant mass of cells bearing the phenotypic 'hallmarks' of cancer (8). Accumulation of multiple molecular transformations ultimately results in an imbalance between tumour suppressor genes (TSG) and tumour promoting oncogenes, providing a cell with the potential to become malignant (9). In particular, the acquisition of somatic mutations in critical oncogenes has emerged as potential 'driver' events in lung carcinogenesis, and has led to the concept of 'oncogene addiction' (10,11). Identification and characterization of such 'driver' events has contributed to

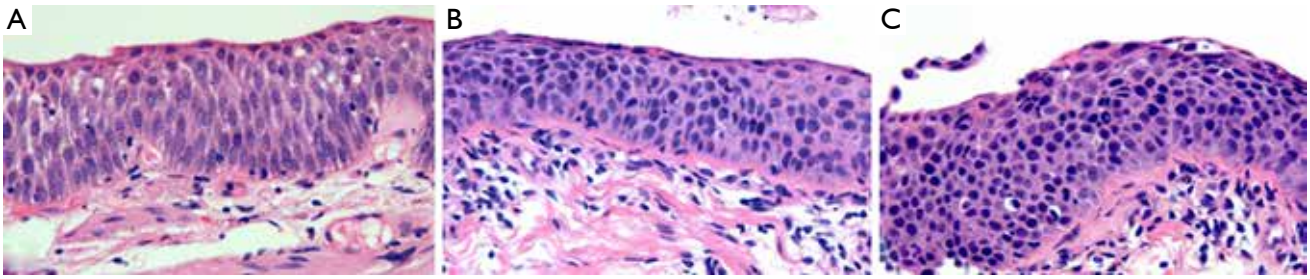


Figure 1 Squamous preneoplasia progresses through mild (A); moderate and severe (B) stages to carcinoma *in situ* (C).

the development of targeted therapies specific to particular subtypes of lung cancer. Improved patient outcomes as a result of these advances requires multidisciplinary planning of diagnosis and treatment and has significantly expanded the role of the surgical pathologist who must not only confirm the diagnosis of malignancy, but also accurately subtype the tumour based on its histology and molecular profile. As the majority of lung cancer is diagnosed on small biopsies or cytology specimens, often obtained by increasingly sophisticated diagnostic procedures, the pathologist must obtain maximal diagnostic yield from these small and valuable tissue samples.

This review will present an overview of recent developments in the histological classification of lung cancer, and address the challenges facing the surgical pathologist in the era of personalized treatment for lung cancer.

Classification of lung cancer

The World Health Organisation (WHO) classification applies to surgically resected malignant tumours of the lung and pleura (12). Primary carcinomas of the lung are traditionally classified as either small cell lung cancer (SCLC) or non small cell lung cancer (NSCLC). NSCLC constitutes approximately 80% of all primary lung cancers with adenocarcinoma, squamous cell carcinoma (SCC) and large cell carcinoma constituting the major histological types (13,14). The recent revision of the lung adenocarcinoma classification by IASLC/ATS/ERS reflects not only histology, but also pathogenesis (preneoplasia) and clinical behaviour, and refines the classification for application to lung cancer diagnosis in small biopsies and cytology specimens.

Preneoplasia

The development and progression of preneoplastic lesions of the lung continue to generate research interest not only to develop methods for early detection but also to increase our understanding of tumour biology. It is well known that lung cancer is the result of multiple complex combinations of morphological, molecular and genetic alterations. Co-localisation of genetic changes within morphologically abnormal epithelial regions has been convincingly demonstrated and there is evidence that a series of key genetic alterations results in progression through increasingly abnormal morphology, eventually leading to invasive lung carcinoma (15-20).

Pulmonary SCC, which typically arises from the bronchial epithelium of the larger, more central airways, progresses through a series of preinvasive neoplastic lesions, from squamous metaplasia, to squamous dysplasia (mild, moderate and severe) and finally carcinoma *in situ* (CIS) (12,21) (*Figure 1A-C*). Multiple molecular alterations contribute to this multistage progression, including loss of heterozygosity at 3p21 (an early event), 9p21, 8p22-24, 5q22 and 17p, deregulation of telomerase activity, p53 mutation and deregulation of cell proliferation (cyclin D1 and E) and apoptosis (Bcl-2) (reviewed by Wistuba and Gazdar 2006; Lantuejoul *et al.* 2009) (22,23).

Conversely, lung adenocarcinomas are predominantly more peripheral tumours, thought to arise from the alveolar or bronchiolar epithelium (pneumocytes or Clara cells) (22). The new IASLC/ATS/ERS classification recognises preinvasive adenocarcinoma lesions to include atypical adenomatous hyperplasia (AAH) and adenocarcinoma *in situ* (AIS) (21) (*Figure 2*). The molecular alterations in these lesions are not as well characterised as their counterparts in squamous carcinogenesis, but it is thought that non-smokers progress through alterations in epidermal growth factor receptor (*EGFR*) signalling whilst smokers progress through alterations in v-Ki-ras2 Kirsten Rat Sarcoma viral oncogene (*KRAS*) signalling pathways (reviewed by Wistuba and Gazdar 2006) (22). Reflecting

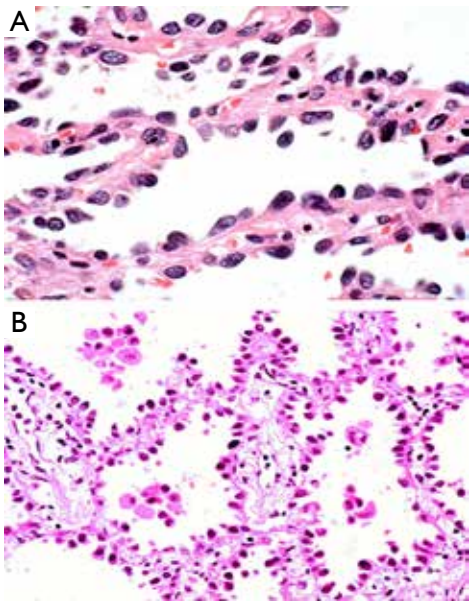


Figure 2 Atypical adenomatous hyperplasia (A) and adenocarcinoma *in situ* (B) are similar histologically and are differentiated on the basis of the overall size of the lesion with a cut-off of 5 mm.

the fact that AIS is a preinvasive lesion, complete resection results in 100% 5-year survival (24-30).

For other tumours of the lung, the preneoplastic processes leading to the development of an invasive tumour are not well defined. Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is thought to be a precursor lesion for carcinoid tumours (12,21,22,31). This is a rare lesion of the distal airways characterised histologically by generalised proliferation of neuroendocrine cells as single cells, small nodules or linear proliferations, either confined to the luminal epithelium or forming extraluminal tumorlets and may be associated with fibrosis (12,22). A definite preneoplastic lesion has not been identified for other neuroendocrine tumours of the lung. However, it has been shown that bronchial epithelium adjacent to SCLC demonstrates genetic alterations, even if morphologically normal (22,32). Therefore it has been proposed that SCLC bypasses the traditional multistage preneoplastic sequence and arises directly from epithelium that demonstrates none or only minimal atypia (22,32).

Why subtype non-small cell lung cancer?

Specific subtypes of NSCLC display varying responses to different chemotherapeutic agents. Key oncogenic 'driver' events in lung adenocarcinomas include mutually exclusive activating mutations of *KRAS* and *EGFR* (33). The discovery of activating mutations in *EGFR* (exons 18-21) led to subsequent development of EGFR tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, revolutionizing the management of patients whose tumours harbor these mutations (33-36). Of note, *KRAS* mutations occur almost exclusively in smokers with adenocarcinoma histology, whilst *EGFR* mutations are associated with never smoking, adenocarcinoma histology, female gender, and Asian ethnicity, with smoking status possibly the strongest clinical predictor of response to EGFR-TKIs (37-40). This is largely a reflection of the major clinicopathological and molecular differences in lung tumours arising in never smokers compared to smokers, supporting the current theory that they are unique diseases (reviewed by Sun *et al.*, 2007) (40).

A proportion of lung adenocarcinomas show translocations involving the *ALK* gene (encoding a tyrosine kinase) and a number of partners (most often *EML4*), resulting in overexpression of the oncogenic *ALK* protein (41-44). EGFR-TKIs (gefitinib and erlotinib) and ALK-TKIs (crizotinib) are now recommended as first line therapy for patients with *EGFR* mutations and *ALK* translocations respectively (45). The IASLC in conjunction with the College of American Pathologists (CAP) and Association for Molecular Pathology (AMP) have published guidelines for *EGFR* mutation and *ALK* translocation testing, recommending that all lung adenocarcinomas, regardless of clinical characteristics, undergo validation molecular testing for *EGFR* mutation and *ALK* translocation (46). Other potential driver 'events' in NSCLC include mutations in *KRAS*, *BRAF*, *HER2* and *FGFR1* (33,47-52). Targeted inhibitors of many of these genes are in various stages of clinical development and may become available in the future as targeted therapies for which additional molecular testing will be required in the diagnostic workup of NSCLC patients.

Not only can molecular biomarkers be used to identify patients that are most likely to benefit from specific targeted molecular therapy, but they can also assist in predicting response to therapeutic agents. Initial studies have shown that levels of thymidylate synthase (TS), the principal enzymatic target of pemetrexed, are high in SCLC and SCC but low in adenocarcinoma (53-55). The observed reduced efficacy of pemetrexed in SCLC and lung SCC compared to adenocarcinoma in clinical trials is likely to be the result of the higher levels of TS (56) and thus it has been suggested

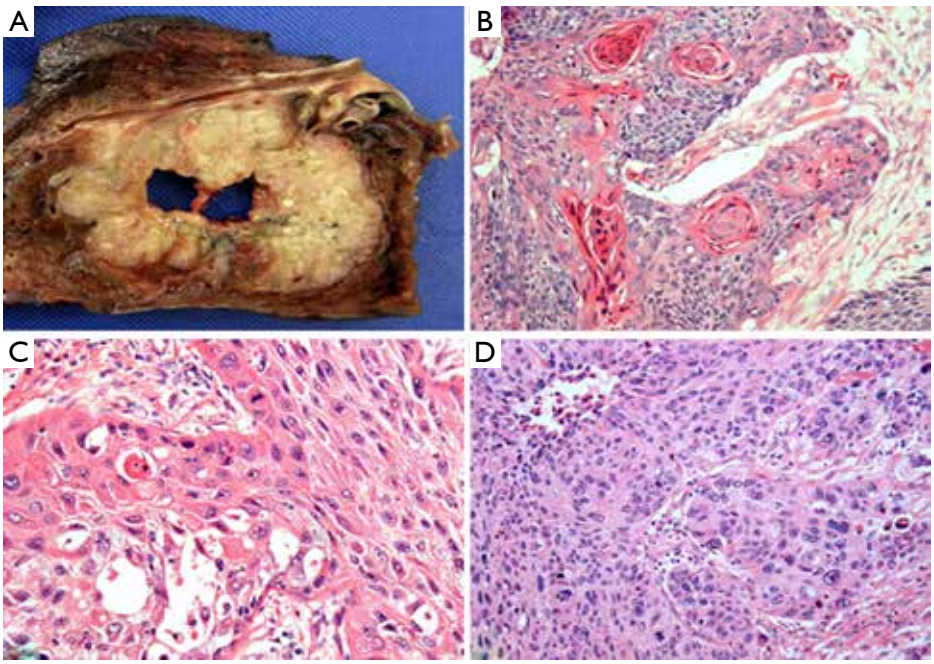


Figure 3 Squamous cell carcinoma typically is a central, often cavitating, malignancy (A); well-differentiated tumours show keratin pearl formation (B); individual cell keratinisation and intercellular bridges are evident at high power; (C) but are less obvious in poorly differentiated examples (D).

that levels of TS expression may be useful as a predictor of response to pemetrexed (55). Similarly, preliminary studies have indicated that ERCC1 protein expression may be a useful biomarker of clinical response to platinum-based chemotherapy (57,58). Despite their potential to assist in personalised targeted therapy for lung cancer patients, the majority of these biomarkers are still at an early stage of development.

Tumour histology of itself may predict response to therapy. Treatment with the vascular endothelial growth factor (VEGF) inhibitor, bevacizumab, has been reported to precipitate life-threatening pulmonary haemorrhage in patients with SCC (59). This has resulted in exclusion of patients whose tumours show squamous differentiation from being treated with this drug or with pemetrexed.

Squamous cell carcinoma

In recent decades the proportion of NSCLC represented by SCC has declined, with current reports estimating that it accounts for approximately 33% worldwide (13). This change is thought to be partly attributable to changes in smoking behaviours. Classically, SCC is a central lung tumour (*Figure 3A*), however, a significant proportion is identified in the periphery (30). The characteristic morphological features of squamous differentiation include intercellular bridges, individual cell keratinisation and squamous pearl formation (14) (*Figure 3B-D*). The current WHO classification includes papillary, clear cell, small cell and basaloid subtypes of SCC (12). Apart from basaloid SCC, these subtypes are descriptive only with no proven clinical or prognostic utility.

Several publications have suggested alternate approaches to the subclassification of SCC. Maeshima *et al.* [2006] found that tumours showing single cell infiltration had worse prognosis than those with large (>6 cells) or small (2-5 cells) invasive tumour cell nests (60). A recent review by Travis [2011] proposed abolition of the small cell descriptor because of confusion with true SCLC, and noted overlap of the small cell variant with the basaloid variant (61). Future subclassification of SCC will require meaningful clinicopathological collaboration to establish relevant predictors of treatment response and prognosis.

Adenocarcinoma

Adenocarcinoma now represents the dominant histological subtype of all lung cancers, and in particular is the most

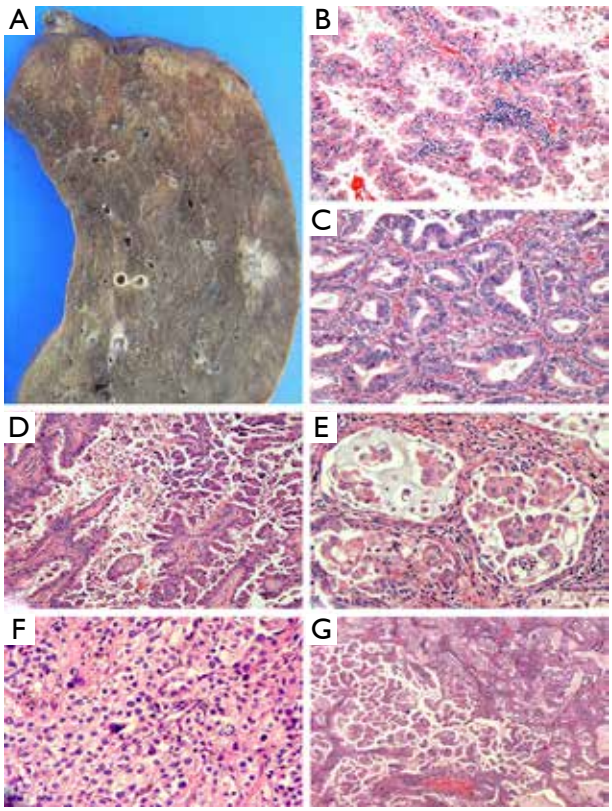


Figure 4 Adenocarcinoma is typically a peripheral lesion (A) showing histological heterogeneity. Architectural patterns include lepidic (B); acinar (C); papillary (D); micropapillary (D,E) and solid (F); the predominant pattern is recorded and lesser patterns are listed as percentages, e.g., in (G), papillary 70%, solid 20%, acinar 10%.

common lung tumour in non-smokers, females and Asian patients (62,63). They predominantly arise peripherally (*Figure 4A*) and are histologically characterised by the presence of glandular differentiation and/or mucin production (12,21). In 2011, the collaborative efforts of the IASLC/ATS/ERS proposed a new subclassification for surgically resected lung adenocarcinoma (21). Of note, the confusing term, bronchioloalveolar carcinoma (BAC), is discarded. The classification introduces the terms AIS (previously BAC) and minimally invasive adenocarcinoma (MIA), both having 5-year survival rates approaching 100% if completely resected (24-30). MIA is defined as a lepidic predominant tumour of less than 3 cm diameter with 5 mm or less of an invasive component (21). Histologically these lesions can be non-mucinous or rarely mucinous and have characteristic radiological appearances (21).

For surgically resected invasive adenocarcinoma, the new IASLC/ATS/ERS classification introduces some important changes reflecting the heterogeneous nature of these tumours. Because the great majority displays mixed histological patterns, it is recommended that the predominant pattern (lepidic, acinar, papillary, micropapillary or solid—see *Figure 4B-F*) be recorded, with all other subpatterns listed in the pathology report with estimated percentages in 5% increments (21) (*Figure 4G*). The micropapillary pattern is included for the first time due to multiple studies reporting an association with poor prognosis in early stage lung adenocarcinoma (21,61,64-66). Clear cell and signet ring are no longer included as histological subtypes of adenocarcinoma, but instead are considered as cytological variants seen in many subtypes of lung cancer; their presence may still be reported (21,61). This algorithm for reporting of lung adenocarcinoma allows for inclusion of small components that may hold prognostic implications (e.g., micropapillary) and may lead toward architectural grading of lung adenocarcinoma (21,61,67).

The IASLC/ATS/ERS classification recognises four adenocarcinoma variants: invasive mucinous (formerly mucinous BAC), colloid, fetal (low or high grade) and enteric (21). Invasive mucinous adenocarcinomas have been classified as an adenocarcinoma variant, distinct from non-mucinous adenocarcinomas, due to the strong association of these tumours with *KRAS* mutations, lack of expression of TTF-1 and frequent multicentricity (21,61). Like their non-mucinous counterparts, mucinous adenocarcinomas can display varying amounts of lepidic, acinar, papillary or micropapillary architectural patterns with abundant mucin production (21,61).

Initial findings from a handful of studies employing the new IASLC/ATS/ERS classification indicate that the proposed histological subtypes may assist in the stratification and identification of patients at risk of poor clinical outcomes. As discussed previously, AIS and MIA have been associated with excellent prognosis (24-30,64,68-70). Intermediate prognosis is associated with the histological subtypes where papillary and acinar patterns predominate, whilst invasive mucinous or colloid variants or presence of predominant solid or micropapillary growth has been associated with poor prognosis (64,68,69).

Large cell carcinoma

LCC represents approximately 3% of all lung cancers (71-73) and is essentially a diagnosis of exclusion, where the

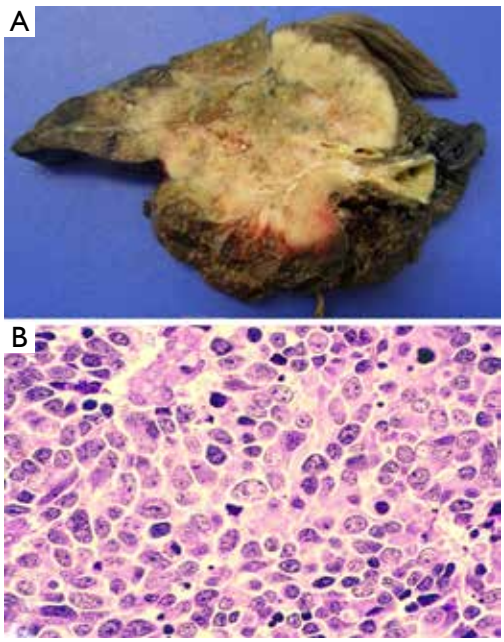


Figure 5 Large cell carcinoma is often large and partially necrotic (A) and comprises patternless sheets of large polygonal cells showing no obvious evidence of histological differentiation (B).

tumour demonstrates no morphological features diagnostic of adenocarcinoma, SCC or SCLC (12,61). These tend to be large, partially necrotic tumours (*Figure 5A*) composed of sheets and nests of large polygonal cells with vesicular nuclei and prominent nucleoli (12) (*Figure 5B*). Although the current WHO classification is based purely on histological appearance, many of these undifferentiated tumours actually show evidence of glandular, squamous or NED when their ultrastructural (electron microscopy), immunophenotypic (IHC) or molecular features are examined (61). A recent review by Travis [2011] suggests that the diagnostic criteria for LCC should remain unchanged, but that pathology reports should comment if the tumour demonstrates evidence of squamous or adenocarcinomatous differentiation with modalities other routine histology (61).

Since LCC is a diagnosis of exclusion, it can only be made on surgical resection specimens as histological assessment of the entire tumour is required to exclude focal differentiation. Therefore, a diagnosis of LCC cannot be made on small biopsies or cytology specimens, and in accordance with the new IASLC/ATS/ERS recommendations, these cases should be classified as NSCLC, not otherwise specified (discussed below) (21,61). Subtypes of LCC recognised by the 2004 WHO classification include large cell neuroendocrine carcinoma (LCNEC), basaloid carcinoma, lymphoepithelioma-like carcinoma, clear cell carcinoma and LCC with rhabdoid phenotype (12). LCNEC is discussed in further detail below.

Neuroendocrine tumours

Neuroendocrine tumours represent approximately 20-25% of all lung cancers (74,75) and form a subset of tumours with common morphological, molecular, immunohistochemical (IHC) and ultrastructural features that distinguish them from other lung tumours (12). The 2004 WHO classification separates neuroendocrine tumours of the lung into four categories: SCLC, LCNEC, typical carcinoid (TC) and atypical carcinoid (AC) tumours (12). Histologically, these tumours demonstrate varying degrees of neuroendocrine morphology, including organoid nesting, pallisading, trabecular growth and rosette-like structures. The major histological features differentiating the four types of neuroendocrine tumours are the presence or absence of necrosis and the mitotic rate (12).

Small cell lung carcinoma

SCLC is a highly aggressive neuroendocrine malignancy that accounts for approximately 12-14% of all lung cancers (71,76). The vast majority of patients have metastatic disease at the time of diagnosis, so surgical resection is rarely an option. Survival rates remain dismal, with only 5-8% of patients surviving 5 years after diagnosis (74,76).

SCLC is an epithelial malignancy comprising small tumour cells (less than the diameter of 3 resting lymphocytes) with distinct cytological features including ill-defined cell borders, scant cytoplasm and finely granular nuclear chromatin without obvious nucleoli (*Figure 6A*; see also *Table 1*) (12). The presence of crush artefact (smearing of nuclear chromatin) and nuclear moulding are common but can be seen with other malignancies (e.g., crush artefact is common in lymphoid infiltrates). The mitotic rate is high (≥11 mitoses per 10 HPF) and there is often extensive necrosis (12). The distinctive histological appearance of SCLC allows for reliable diagnosis in small biopsy and cytology specimens, but for small specimens with significant crush artefact, use of a panel of IHC markers such as a pancytokeratin, neuroendocrine markers (chromogranin, synaptophysin and CD56) and/or TTF-1 and Ki-67 will confirm suspected SCLC (61,77) (*Figure 6B-D*). In cases where cytokeratin stains are negative, it is important to

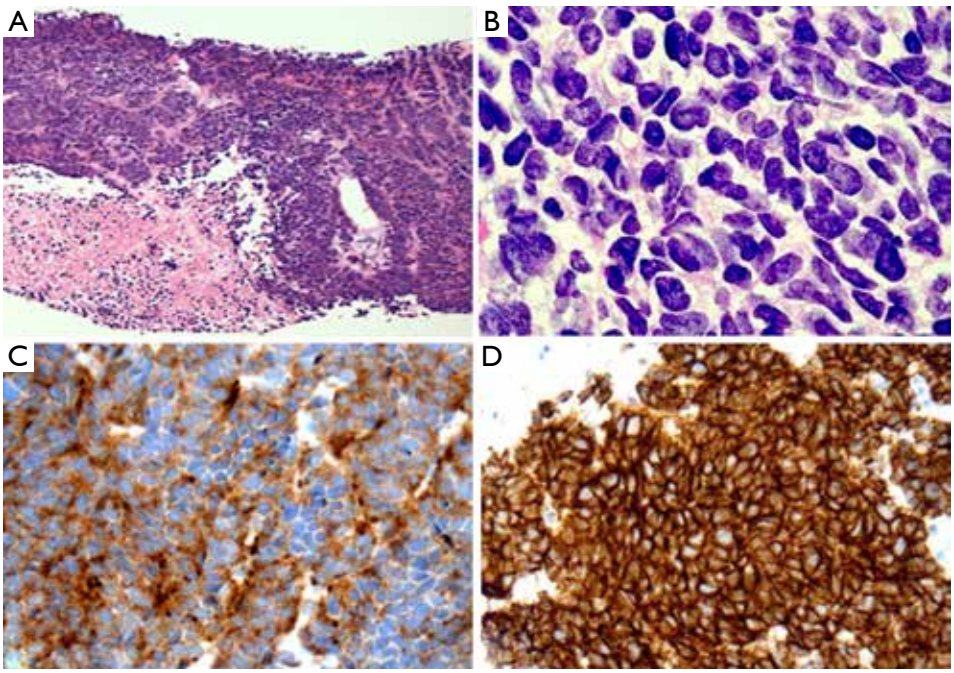


Figure 6 Small cell lung carcinoma in a core biopsy (A,B) showing positive immunoperoxidase staining for synaptophysin (C) and CD56 (D).

Table 1 Summary of histological features differentiating SCLC and LCNEC (12)

Histological feature		SCLC	LCNEC
Cytological features	Size	Small cells (<3 resting lymphocytes)	Large cells with neuroendocrine morphology
	N/C ratio	High (scant cytoplasm)	Low (abundant pink cytoplasm)
	Nuclear chromatin	Finely granular nuclear chromatin	Vesicular, coarse or fine chromatin
	Nucleoli	Absent or inconspicuous	Frequent (not always present)
	Nuclear moulding	Present	Infrequent
	Nuclear smearing	Often	Infrequent
	Cell borders	Indistinct	Distinct
Mitotic rate		≥11 per 10 HPF	≥11 per 10 HPF
Necrosis		Present (large zones)	Present (large zones)
IHC		Can be diagnosed without IHC	Positive staining for ≥1 neuroendocrine marker

SCLC, Small cell lung cancer; LCNEC, Large cell neuroendocrine carcinoma; N/C ratio, nuclear/cytoplasmic ratio; HPF, high power field; IHC, immunohistochemistry.

consider and exclude other diagnoses such as lymphoma, chronic inflammation, small round cell tumour or primitive neuroectodermal tumour (21, 61,78).

The 2004 WHO classification recognises two subtypes of SCLC: pure and combined (12). Combined small cell carcinoma is defined as a classical SCLC with a component showing features of any subtype of NSCLC, most often SCC, adenocarcinoma or LCC (12,61). A threshold for the proportion of non-small cell component is not required when this comprises adenocarcinoma or SCC, but for combined small cell and large cell tumours (SCLC-LC), at least 10% of the tumour must comprise a large cell component (12,61).

Large cell neuroendocrine carcinoma

LCNEC is another highly aggressive neuroendocrine malignancy, where tumour cells demonstrate cytological

features of NSCLC (*Table 1*) but with neuroendocrine architecture (organoid nesting, pallisading, trabecular growth and rosette-like structures) and positive IHC stains for at least one neuroendocrine marker (chromogranin, synaptophysin or CD56) (12). Like SCLC, these tumours often show necrosis and have a high mitotic rate (≥11 mitoses per 10 HPF) (12). They may be pure LCNEC or combined with other types of NSCLC (12).

Making the diagnosis of LCNEC is often challenging due to significant overlap amongst the diagnostic groups (61,77). The differentiation between SCLC and LCNEC is particularly problematic, especially in cytology specimens, since there is overlap in nuclear size and some LCNEC do not contain prominent nucleoli. Currently there is no IHC stain for discrimination between SCLC and LCNEC and the distinction is based solely on cytological features (detailed in *Table 1*). Differentiating a LCNEC tumour from other NSCLC is based on the presence of neuroendocrine morphology and positive IHC for at least one neuroendocrine marker (12). However, up to 20% of NSCLC (adenocarcinoma, SCC and LCC) with no obvious neuroendocrine morphology demonstrate positive IHC staining for neuroendocrine markers (12,61). Currently, these tumours are classified as their NSCLC subtype but with neuroendocrine differentiation (NED) (i.e., NSCLC-NED) (12). The clinical significance of IHC evidence of NED without neuroendocrine morphology in NSCLC remains unclear and further research is required.

Carcinoid tumours

Carcinoid tumours comprise 1-2% of all lung tumours (74,75) and represent the most common lung tumour in children (79). Two subtypes of carcinoid tumour are recognised: typical carcinoid (TC) and AC (12). Both demonstrate morphological growth patterns indicative of NED (organoid, trabecular, insular, pallisading, ribbon, rosette-like structures) (12). The diagnostic criteria for differentiating TC and AC are mitotic rate and the presence or absence of necrosis. TC has <2 mitoses per 10 HPF and no necrosis. Conversely, AC show necrosis (usually focal or punctate) and/or 2-10 mitoses per 10 HPF (12).

Other NSCLC subtypes

Adenosquamous carcinoma

Adenosquamous carcinoma accounts for less than 5% of all lung cancers (80-82) and is defined as a NSCLC comprising at least 10% of both squamous and glandular differentiation (12). Similar to LCC, this diagnosis should be based on histology and not immunophenotype. However, further guidelines and definitions for characterisation using immunohistochemistry are likely in upcoming revisions of lung cancer classification. Adenosquamous carcinoma can only be diagnosed with certainty on surgical resections, however the diagnosis can be suspected in small biopsy or cytology specimens showing features of both squamous and glandular differentiation (21).

Sarcomatoid carcinoma

Sarcomatoid carcinoma is a poorly differentiated NSCLC that demonstrates morphological features of sarcoma or sarcoma-like (spindle and/or giant cells) differentiation, and represents approximately 1% of all lung cancers (12). The 2004 WHO classification recognises five subtypes, including pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma (12). These highly aggressive tumours are believed to represent epithelial malignancies that have undergone divergent differentiation (12,83-87). Because of the heterogeneity of these tumours, they should not be diagnosed on small biopsy or cytology. Instead, the IASLC/ATS/ERS classification recommends using the diagnosis "poorly differentiated NSCLC with spindle and/or giant cell carcinoma" (21). Of note, the new IASLC/ATS/ERS classification now recognises fetal adenocarcinoma as a adenocarcinoma variant and not as an epithelial pattern of pulmonary blastoma (21,61,88).

Carcinomas of salivary gland type

Salivary gland tumours arising from bronchial glands are rare, representing less than 1% of all lung cancers (21). The 2004 WHO classification recognises three subtypes, including mucoepidermoid carcinoma, adenoid cystic carcinoma and epithelial-myoepithelial carcinoma (12).

Other primary tumours of the lung

As in any organ or tissue, primary tumours of the lung can arise from any cell type and are not purely derived from epithelial cells. Amongst the "non-epithelial" lung tumours, the 2004 WHO classification identifies broad groups including mesenchymal, lymphoproliferative and miscellaneous tumours (e.g., melanoma, germ cell

tumours) (12). Detailed descriptions of these tumour types can be found in the 2004 WHO classification (12) and are beyond the scope of this review.

Diagnostic challenges

Patients with multiple lung tumours can present a diagnostic challenge for surgical pathologists. Discriminating between true independent primary lung cancers and a primary tumour with satellite lesions or intrapulmonary metastases is of critical importance, as the clinical management and prognosis varies significantly. Over 30 years ago, multiple primary lung cancers were defined as either synchronous (detected or diagnosed simultaneously) or metachronous (when there is a time interval between detection or diagnosis of two separate primary lesions) (89-91). To aid in diagnosis of metachronous tumours, the more common occurrence, the IASLC/ATS/ERS classification for adenocarcinomas notes the importance of not just reporting the predominant pattern of adenocarcinoma, but of detailed reporting of percentages of the various histological patterns in 5% increments (21). This allows for better comparison of subsequent adenocarcinomas, particularly if slides from the original primary tumour are not available for review (21).

Comprehensive histological and cytological examination of multiple tumours can distinguish primary from metastatic lung cancers in the majority of cases (92). But despite careful histological examination and IHC profiling, for a proportion of cases with multiple lung tumours, definitive distinction between multiple primary lung cancers and metastatic lung cancer may be impossible (89,90,93). Detailed clinical history and multidisciplinary case review is imperative to assist diagnosis. Genetic studies have demonstrated unique molecular phenotypes for multiple tumours with similar histology, suggesting that in the future molecular analysis of these tumours may provide greater diagnostic accuracy (90,91).

Differentiating metastases to the lung from primary lung cancers poses another diagnostic challenge for surgical pathologists, especially in small biopsy and cytology specimens. When initial histological examination of the specimen does not clearly indicate a primary lung malignancy, metastatic disease must be considered (61). Specific subtypes of lung cancer can be difficult to distinguish from metastatic disease, such as enteric differentiation in lung adenocarcinoma which shares morphologic and IHC features with colorectal adenocarcinoma (21,94). However, due to the heterogeneity of lung tumours, areas of more typical pulmonary differentiation should be evident (21). For example, lepidic growth favours primary adenocarcinoma (21) but rare cases of metastatic adenocarcinoma may show this pattern of spread (personal observation).

Differentiation of pulmonary SCC from metastatic head and neck SCC (HNSCC) presents a unique diagnostic challenge, as they demonstrate similar morphology and can occur in the same patient (95,96) due to similar aetiologies and risk factors (97,98). Recently, p16 has been investigated as a potential IHC marker for differentiating lung SCC from HNSCC with negative staining favouring lung SCC and positive staining favouring extrapulmonary SCC (99). However, a proportion of primary lung SCC stains positively for p16 (100) and may reflect the limited association of HPV infection with the development of lung cancer, with reports of HPV prevalence ranging from 5-22% (101,102). Currently, there is no reliable IHC marker for differentiating lung SCC from HNSCC (99,103,104). In cases where a metastatic lesion may be suspected, provision of detailed clinical history (i.e., history of previous malignancy and site) cannot be over emphasised, as it will guide detailed morphological and IHC assessment, and avoid wastage of valuable tissue on multiple IHC stains.

Small biopsies and cytology—the reality of non-small cell lung cancer diagnosis

The WHO classification of lung tumours was developed and designed for histological diagnosis and staging of surgically resected lung tumours. However, the vast majority of patients present with either locally advanced or metastatic disease and do not proceed to surgical resection, so the diagnosis of lung cancer is confirmed using small biopsies and/or cytology. With the advent of subtype-specific and targeted molecular therapies for lung cancer, the need for accurate histological classification and guided molecular characterisation is placing increasing demands on the surgical pathologist to do more with less tissue.

The recently published IASLC/ATS/ERS classification has, for the first time, provided a clinically focused and relevant classification applicable to small biopsies and cytology specimens. Of particular clinical importance is the necessity to differentiate between adenocarcinoma and SCC as this will guide subsequent molecular testing and therapeutic management. If a tumour demonstrates distinct histological features of adenocarcinoma or SCC then the standard diagnostic terms should be used (21,61). For poorly differentiated carcinomas, the IASLC/ATS/ERS advise the

use of a limited IHC panel (discussed below) to differentiate adenocarcinoma and SCC, effectively reducing the use of the term NSCLC-NOS (not otherwise specified) whilst preserving tissue for molecular testing.

Of course, alternative diagnoses must be considered in the assessment of small biopsies/cytology specimens and provision of relevant clinical history from treating clinicians is essential in this process. Not only potentially benign conditions but other malignancies of the lung, both primary and metastatic, need to be considered. Both pathologists and treating clinicians need to be aware of inherent tumour heterogeneity and recognise that small biopsies/cytology specimens represent only a small sampling of the entire tumour; indeed, definitive diagnosis can only be made on surgical resection specimens for a subset of NSCLC (MIA, LCC, adenosquamous carcinoma). For small biopsy/cytology specimens, the IASLC/ATS/ERS suggests classification as NSCLC, with a description of the morphological features seen and whether a particular diagnosis is favoured, e.g., NSCLC with neuroendocrine morphology (positive neuroendocrine markers)—possible LCNEC (21).

Limiting use of NSCLC-NOS

No longer can tumours of the lung be simply classified as NSCLC or SCLC. There are now strong clinical indications driving the need for surgical pathologists to further subtype NSCLC, in particular to differentiate adenocarcinoma from SCC even on small biopsies/cytology specimens. In contrast to previous WHO classifications, the IASLC/ATS/ERS now recommend limiting use of the term NSCLC-NOS. All available clinical material must be utilised, and correlation carried out between cytology and histology specimens (21). Indeed, Sigel *et al.* [2011] reported a NSCLC-NOS diagnosis rate of 4% when cytology and small biopsies were correlated, reduced from 11% for cytology alone and 6% for biopsies alone (105).

For tumours in which differentiation is not evident on histological or cytological examination, a limited panel of histochemical and IHC markers is required (*Figure 7A-D*). The most widely used adenocarcinoma markers include mucin (periodic acid Schiff with diastase or mucicarmine), TTF-1 (thyroid transcription factor 1) and napsin-A, and for SCC the favoured markers are p63 and CK5/6. Of these, TTF-1 and p63 demonstrate the greatest sensitivity for their respective NSCLC subtypes (21,106-111). Alternative IHC markers such as CK7 (adenocarcinoma) and 34βE12 (squamous cell carcinoma) can be considered for indeterminate cases but have less sensitivity and specificity and tend not to be included in routine IHC panels (108,110-113). There has been recent interest in p40 (ΔNp63), a relatively new IHC marker for SCC. Current IHC stains for p63 detect all isoforms of the p63 gene, while p40 specifically detects non-transactivating or truncated forms of p63, resulting in increased specificity for SCC regardless of organ site (107,114-120).

Numerous diagnostic IHC algorithms for differentiating lung adenocarcinoma and SCC have been discussed in the literature, most including TTF-1 and p63 with or without a third or fourth stain. For example, Rekhtman and colleagues [2011] recently reported 100% accuracy in small biopsy specimens (diagnosis confirmed at surgical resection) with use of TTF-1 and p63 as a first line panel and addition of CK5/6 for equivocal cases (106). The IASLC/ATS/ERS advise the use of a single adenocarcinoma marker (TTF-1) and SCC marker (p63) with or without mucin stain, which should allow for differentiation of the majority of NSCLC (*Table 2*) (21,61). Where the IHC profile favours adenocarcinoma (TTF-1 positive and/or mucin positive, p63 negative), the tumour should be classified: "NSCLC, favour adenocarcinoma" (21,61). When the IHC profile favours SCC (p63 positive, TTF-1 negative, mucin negative), the tumour should be classified: "NSCLC, favour squamous cell carcinoma" (21,61). Only when there is no morphological or IHC evidence of clear lineage differentiation should the tumour be classified as NSCLC-NOS. In the hands of experienced pathologists and cytopathologists and judicial use of IHC stains, the IASLC/ATS/ERS estimate that less than 5% of NSCLC cases should be classified as NSCLC-NOS (21,61).

Molecular testing in NSCLC

The development of targeted molecular therapy for pulmonary adenocarcinoma has not only driven review of the classification and guidelines for NSCLC diagnosis, but also brought significant implications with regards to tissue sampling and processing. These increasingly small diagnostic biopsy and cytology specimens are no longer required purely for confirmation of malignancy and tumour subtyping, but there must be sufficient tumour tissue available for molecular testing to complete the pathological diagnostic assessment. Factors that need to be considered range from specimen collection, tissue processing in the laboratory, requests for molecular testing, provision of sufficient samples

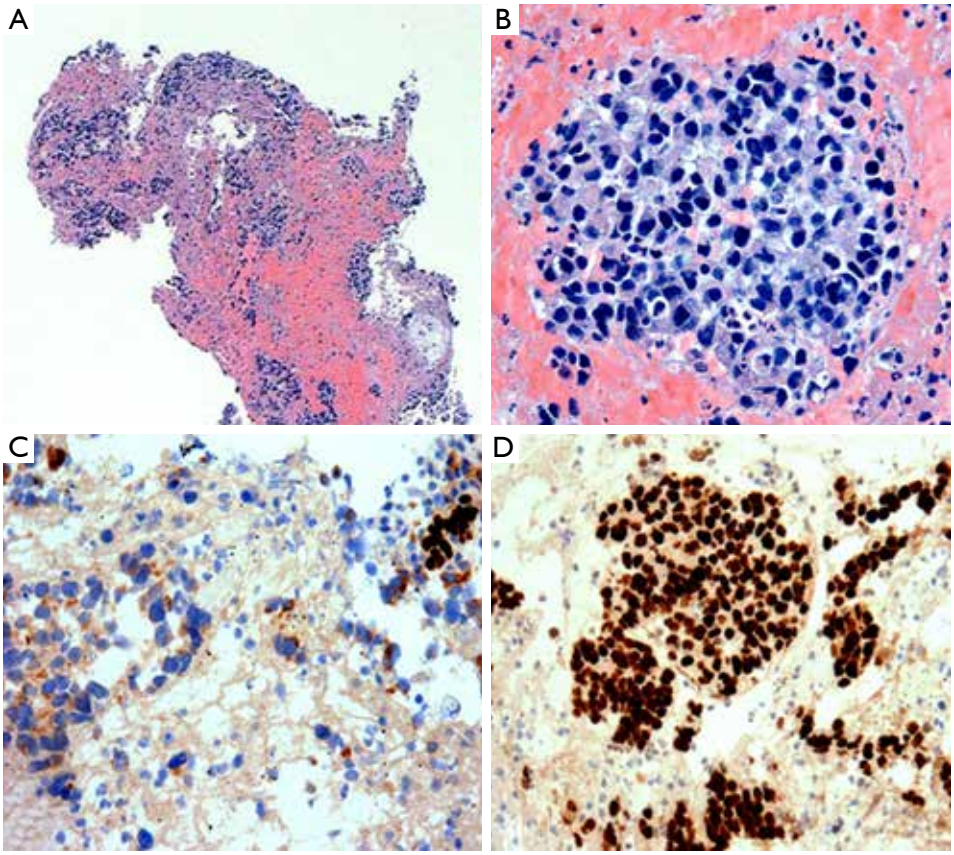


Figure 7 Immunohistochemistry on an undifferentiated non-small cell carcinoma in a bronchial biopsy (A,B) favours a diagnosis of adenocarcinoma. Staining for p63 (C) is negative (note the positive internal control comprising benign basal bronchial epithelial cells) while there is strong TTF-1 positivity (D).

Table 2 Summary of immunohistochemical stains for small biopsy/cytology diagnosis (21)

Small biopsy/cytology diagnosis	Adenocarcinoma markers		Squamous cell carcinoma markers	
	TTF-1	+/– Mucin	p63/p40	+/– CK5/6
NSCLC—favour adenocarcinoma	+	+	–	–
	+	+	p63 weakly +	–
NSCLC—favour squamous cell carcinoma	–	–	+	+/–
NSCLC—NOS	–	–	–	

NSCLC, Non small cell lung cancer; NOS, not otherwise specified.

to the molecular laboratory and timely communication of results to the treating clinicians. Therefore the surgical pathologist must engage with the multidisciplinary team to develop strategic guidelines that will ensure that a complete histological and molecular diagnosis is provided for the patient (61).

In order to maximise diagnostic yield, it is crucial to optimise not only the amount but also the adequacy of the tissue sampled. Although the choice of procedure and sampling method will largely be guided by the lesion itself (i.e., size, location), patient factors (e.g., comorbidities) and available resources, pathologists should encourage collection of both cytology and biopsy specimens (if possible) as this can aid in diagnostic accuracy. Initially developed for non-ultrasound guided needle aspirates, rapid on site evaluation (ROSE) of specimen adequacy can be performed by trained

cytopathologists and cytotechnologists, not only to confirm sampling of the target lesion but to ensure sufficient sample is collected (121,122). For various reasons, ROSE is not currently available in every institution (123). Interestingly, Alsharif *et al.* [2010] have shown that telepathology can be successfully used to assess adequacy of FNA (fine needle aspiration) specimens (120).

Once the specimen is collected, it is imperative that adequate clinical history is provided to ensure that the specimen is processed in a manner which will provide adequate diagnostic sections as well as preserving tissue for molecular testing. The IASLC/ATS/ERS guidelines strongly encourage surgical pathologists to minimise that amount of tissue used for diagnosis, in particular by limiting the number of first line IHC stains (discussed above) (21). Another suggested strategy is "reflex cutting" of paraffin blocks and preservation of unstained sections in order to avoid unnecessary loss of tissue during facing, although there is a small risk that DNA or epitope quality may degrade if not used shortly after sectioning (124,125).

Currently, activating mutations of *EGFR* are the best established and most widely used molecular biomarker in NSCLC. Additional molecular biomarkers are available, but with the exception of *ALK* translocations, are not recommended by the IASLC/CAP/AMP for routine testing (46). Fluorescence in situ hybridisation (FISH) remains the recommended method for clinical testing of *ALK* translocations, however the IASLC/CAP/AMP suggest that *ALK* IHC could be considered as a method for screening patients prior to formal *ALK* FISH testing (46).

Evolution of rapid and accurate *EGFR* mutation testing provides important data for clinical application. Traditionally the gold standard for *EGFR* mutation testing required direct sequencing of extracted tumour DNA, a time consuming methodology with low sensitivity (high levels of tumour DNA required). Newer validated methods for *EGFR* mutation testing provide increased sensitivity (fewer tumour cells required), improved turnaround times and allow for testing on a greater variety of clinical samples. Current techniques for *EGFR* mutation testing can be screening (detect all mutations including novel mutations, i.e., sequencing) or targeted methods (detect known mutations only). Of course, both approaches have their unique advantages and disadvantages (reviewed by Ellison *et al.*, 2013) (126), and the available tests will vary amongst institutions. Therefore surgical pathologists must be aware of the available tests and specific tissue requirements for their local molecular laboratory.

Once a diagnosis of lung adenocarcinoma has been made, a decision must be made as to who is required to order the appropriate molecular testing. Implementation of "reflex" molecular testing initiated by the surgical pathologist (analogous to HER2 testing of invasive breast carcinoma) is a topic of debate and varies across institutions and government jurisdictions. The surgical pathologist may not be aware if the patient is a surgical candidate and that a more representative tissue specimen may follow. Conversely, delaying molecular testing may potentially contribute to delays in initiation of therapy. For patients diagnosed as NSCLC-NOS on small biopsy/cytology, the IASLC/ATS/ERS recommend biomarker testing (*EGFR* and *ALK*) (21) with discussion at multidisciplinary meetings to plan further testing and management.

Evolving role of the surgical pathologist

No longer is making a tissue diagnosis of pulmonary malignancy and the distinction between SCLC and NSCLC the only role of the surgical pathologist. Their contribution to lung cancer diagnosis, management and research is dynamic and continually evolving. The advent of personalised medicine for lung cancer has brought with it novel challenges and driven significant change. Of note is the first structured classification of lung cancer in small biopsy and cytology specimens developed by the IASLC/ATS/ERS, and a new classification of lung adenocarcinoma. Both have enhanced the clinical relevance of pathological diagnosis, allowing surgical pathologists to interact closely with clinicians to ensure that new concepts are understood and applied in the clinical setting.

With personalised medicine has come the development and clinical application of molecular testing in lung cancer. As the majority of patients never progress to surgical resection, the diagnostic small biopsy or cytology specimen has become a precious resource from which the surgical pathologist must aim to maximise diagnostic yield. Surgical pathologists have become the guardian for these limited precious samples, evaluating specimen adequacy, ensuring that appropriate processing techniques are applied, selecting suitable slides or blocks, enriching the tumour proportion by microdissection if required, and interpreting and providing timely results to the multidisciplinary team.

Furthermore, it is becoming increasingly important that surgical pathologists be involved in clinical trials and

basic research to assist in the attainment of pathologically and clinically meaningful data. Where feasible, the surgical pathologist can also assist in the collection for research (with patient consent) tissue that is not required for clinical decision making. In this way, the modern surgical pathologist becomes an integral member of the multidisciplinary team, playing a crucial role in clinical trials and determining appropriate and timely management for patients with lung cancer.

Conclusions

During the last decade, through significant advances in our understanding of the complexity of lung tumour biology, we have finally entered the era of personalised medicine for lung cancer. No longer is basic tissue diagnosis and cancer staging alone central to determining treatment options for lung cancer, but histological subtyping and molecular testing have become of paramount importance. The surgical pathologist has become the guardian of the small biopsy/cytology specimen, a limited and precious resource from which diagnostic yield must be maximised.

Acknowledgements

Partial funding support for this work came from SPORE (P50CA70907) grant from the NCI, USA (AFG).
Disclosure: The authors declare no conflict of interest.

References

1. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Australian Bureau of Statistics. Causes of Death Australia 2009. Australian Government, ABS. 2011. http://www.abs.gov.au/AUSSTATS/abs@.nsf/Latestproducts/3303.0Contents2009?opendocument&tabname=Summary&prodno=3303.0&issue=2009&num=&view=. Accessed 26 February 2012.
3. Naruke T, Tsuchiya R, Kondo H, et al. Implications of staging in lung cancer. Chest 1997;112:242S-8S.
4. Nesbitt JC, Putnam JB Jr, Walsh GL, et al. Survival in early-stage non-small cell lung cancer. Ann Thorac Surg 1995;60:466-72.
5. Australian Bureau of Statistics. Causes of Death, Australia, 2004. Australian Government, ABS. 2006. http://www.abs.gov.au/Ausstats/abs@.nsf/b06660592430724fca2568b5007b8619/c67a858ba00cb846ca2568a9001393c6!OpenDocument. Accessed 2 October 2006.
6. Jemal A, Clegg LX, Ward E, et al. Annual report to the nation on the status of cancer, 1975-2001, with a special feature regarding survival. Cancer 2004;101:3-27.
7. Australian Institute of Health and Welfare (AIHW) Cancer Australia (CA) and Australian Association of Cancer Registries (AACR). Cancer survival and prevalence in Australia: cancers diagnosed from 1982 to 2004. Canberra: AIHW2008. Report No.: Cancer Series. No. 42: Cat. No. 38.
8. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.
9. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759-67.
10. Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heal of cancer. Science 2002;297:63-4.
11. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011;12:175-80.
12. Travis WD, Brambilla E, Muller-Hermelink HK, et al. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. WHO/IASLC classification of lung and pleural tumours. Lyon: IARC Press, 2004.
13. Fong KM, Bowman RV, Fielding D, et al. Queensland integrated lung cancer outcomes project (qilcop): initial accrual and preliminary data from the first 30 months. Respirology 2003;8 Suppl:A53.
14. Kumar V, Abbas A, Aster J. Robbins Basic Pathology. 9th ed. Philadelphia, PA: Elsevier/Saunders, 2013.
15. Auerbach O, Forman JB, Gere JB, et al. Changes in the bronchial epithelium in relation to smoking and cancer of the lung; a report of progress. N Engl J Med 1957;256:97-104.
16. Auerbach O, Stout AP, Hammond EC, et al. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N Engl J Med 1961;265:253-67.
17. Zochbauer-Muller S, Gazdar AF, Minna JD. Molecular pathogenesis of lung cancer. Annu Rev Physiol 2002;64:681-708.
18. Niklinski J, Niklinska W, Chyczewski L, et al. Molecular genetic abnormalities in premalignant lung lesions: biological and clinical implications. Eur J Cancer Prev 2001;10:213-26.
19. Wistuba, II, Gazdar AF. Characteristic genetic alterations in lung cancer. Methods Mol Med 2003;74:3-28.
20. Wistuba, II, Behrens C, Milchgrub S, et al. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. Oncogene 1999;18:643-50.
21. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic

society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85.
22. Wistuba, II, Gazdar AF. Lung cancer preneoplasia. Annu Rev Pathol 2006;1:331-48.
23. Lantuéjoul S, Salameire D, Salon C, et al. Pulmonary preneoplasia--sequential molecular carcinogenetic events. Histopathology 2009;54:43-54.
24. Sakurai H, Dobashi Y, Mizutani E, et al. Bronchioloalveolar carcinoma of the lung 3 centimeters or less in diameter: a prognostic assessment. Ann Thorac Surg 2004;78:1728-33.
25. Vazquez M, Carter D, Brambilla E, et al. Solitary and multiple resected adenocarcinomas after CT screening for lung cancer: histopathologic features and their prognostic implications. Lung Cancer 2009;64:148-54.
26. Yamato Y, Tsuchida M, Watanabe T, et al. Early results of a prospective study of limited resection for bronchioloalveolar adenocarcinoma of the lung. Ann Thorac Surg 2001;71:971-4.
27. Yoshida J, Nagai K, Yokose T, et al. Limited resection trial for pulmonary ground-glass opacity nodules: fifty-case experience. J Thorac Cardiovasc Surg 2005;129:991-6.
28. Koike T, Togashi K, Shirato T, et al. Limited resection for noninvasive bronchioloalveolar carcinoma diagnosed by intraoperative pathologic examination. Ann Thorac Surg 2009;88:1106-11.
29. Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Cancer 1995;75:2844-52.
30. Tomashefski JF Jr, Connors AF Jr, Rosenthal ES, et al. Peripheral vs central squamous cell carcinoma of the lung. A comparison of clinical features, histopathology, and survival. Arch Pathol Lab Med 1990;114:468-74.
31. Davies SJ, Gosney JR, Hansell DM, et al. Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia: an under-recognised spectrum of disease. Thorax 2007;62:248-52.
32. Wistuba, II, Berry J, Behrens C, et al. Molecular changes in the bronchial epithelium of patients with small cell lung cancer. Clin Cancer Res 2000;6:2604-10.
33. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med 2008;359:1367-80.
34. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
35. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
36. Marks JL, Broderick S, Zhou Q, et al. Prognostic and therapeutic implications of EGFR and KRAS mutations in resected lung adenocarcinoma. J Thorac Oncol 2008;3:111-6.
37. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339-46.
38. Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. Int J Cancer 2006;118:257-62.
39. Pham D, Kris MG, Riely GJ, et al. Use of cigarette-smoking history to estimate the likelihood of mutations in epidermal growth factor receptor gene exons 19 and 21 in lung adenocarcinomas. J Clin Oncol 2006;24:1700-4.
40. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers--a different disease. Nat Rev Cancer 2007;7:778-90.
41. Palmer RH, Vernersson E, Grabbe C, et al. Anaplastic lymphoma kinase: signalling in development and disease. Biochem J 2009;420:345-61.
42. Horn L, Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. J Clin Oncol 2009;27:4232-5.
43. Camidge DR, Kono SA, Flacco A, et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. Clin Cancer Res 2010;16:5581-90.
44. Yi ES, Boland JM, Maleszewski JJ, et al. Correlation of IHC and FISH for ALK gene rearrangement in non-small cell lung carcinoma: IHC score algorithm for FISH. J Thorac Oncol 2011;6:459-65.
45. Moreira AL, Thornton RH. Personalized medicine for non-small-cell lung cancer: implications of recent advances in tissue acquisition for molecular and histologic testing. Clinical lung cancer 2012;13:334-9.
46. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013;8:823-59.
47. Suda K, Tomizawa K, Mitsudomi T. Biological and clinical significance of KRAS mutations in lung cancer: an oncogenic driver that contrasts with EGFR mutation. Cancer Metastasis Rev 2010;29:49-60.
48. Roberts PJ, Stinchcombe TE, Der CJ, et al. Personalized medicine in non-small-cell lung cancer: is KRAS a useful

marker in selecting patients for epidermal growth factor receptor-targeted therapy? J Clin Oncol 2010;28:4769-77.
49. Vakiani E, Solit DB. KRAS and BRAF: drug targets and predictive biomarkers. J Pathol 2011;223:219-29.
50. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005;65:1642-6.
51. Buttitta F, Barassi F, Fresu G, et al. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. Int J Cancer 2006;119:2586-91.
52. Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2:62ra93.
53. Monica V, Scagliotti GV, Ceppi P, et al. Differential Thymidylate Synthase Expression in Different Variants of Large-Cell Carcinoma of the Lung. Clin Cancer Res 2009;15:7547-52.
54. Ceppi P, Volante M, Saviozzi S, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 2006;107:1589-96.
55. Nicolson MC, Fennell DA, Ferry D, et al. Thymidylate Synthase Expression and Outcome of Patients Receiving Pemetrexed for Advanced Nonsquamous Non-Small-Cell Lung Cancer in a Prospective Blinded Assessment Phase II Clinical Trial. J Thorac Oncol 2013;8:930-9.
56. Socinski MA, Smit EF, Lorigan P, et al. Phase III study of pemetrexed plus carboplatin compared with etoposide plus carboplatin in chemotherapy-naive patients with extensive-stage small-cell lung cancer. J Clin Oncol 2009;27:4787-92.
57. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983-91.
58. Custodio AB, Gonzalez-Larriba JL, Bobokova J, et al. Prognostic and predictive markers of benefit from adjuvant chemotherapy in early-stage non-small cell lung cancer. J Thorac Oncol 2009;4:891-910.
59. Johnson DH, Fehrenbacher L, Novotny WF, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 2004;22:2184-91.
60. Maeshima AM, Maeshima A, Asamura H, et al. Histologic prognostic factors for small-sized squamous cell carcinomas of the peripheral lung. Lung Cancer 2006;52:53-8.
61. Travis WD. Pathology of lung cancer. Clin Chest Med 2011;32:669-92.
62. Cagle PT, Allen TC, Dacic S, et al. Revolution in lung cancer: new challenges for the surgical pathologist. Arch Pathol Lab Med 2011;135:110-6.
63. Charloux A, Quoix E, Wolkove N, et al. The increasing incidence of lung adenocarcinoma: reality or artefact? A review of the epidemiology of lung adenocarcinoma. Int J Epidemiol 1997;26:14-23.
64. Yoshizawa A, Motoi N, Riely GJ, et al. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Mod Pathol 2011;24:653-64.
65. Miyoshi T, Satoh Y, Okumura S, et al. Early-stage lung adenocarcinomas with a micropapillary pattern, a distinct pathologic marker for a significantly poor prognosis. Am J Surg Pathol 2003;27:101-9.
66. Tsutsumida H, Nomoto M, Goto M, et al. A micropapillary pattern is predictive of a poor prognosis in lung adenocarcinoma, and reduced surfactant apoprotein A expression in the micropapillary pattern is an excellent indicator of a poor prognosis. Mod Pathol 2007;20:638-47.
67. Sica G, Yoshizawa A, Sima CS, et al. A grading system of lung adenocarcinomas based on histologic pattern is predictive of disease recurrence in stage I tumors. Am J Surg Pathol 2010;34:1155-62.
68. Yoshizawa A, Sumiyoshi S, Sonobe M, et al. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. J Thorac Oncol 2013;8:52-61.
69. Yanagawa N, Shiono S, Abiko M, et al. New IASLC/ATS/ERS classification and invasive tumor size are predictive of disease recurrence in stage I lung adenocarcinoma. J Thorac Oncol 2013;8:612-8.
70. Russell PA, Wainer Z, Wright GM, et al. Does lung adenocarcinoma subtype predict patient survival?: A clinicopathologic study based on the new International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society international multidisciplinary lung adenocarcinoma classification. J Thorac Oncol 2011;6:1496-504.
71. Howlader N NA, Krapcho M, Garshell J, et al. eds. SEER Cancer Statistics Review, 1975-2010, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data

submission, posted to the SEER web site, April 2013.
72. Sun Z, Aubry MC, Deschamps C, et al. Histologic grade is an independent prognostic factor for survival in non-small cell lung cancer: an analysis of 5018 hospital- and 712 population-based cases. J Thorac Cardiovasc Surg 2006;131:1014-20.
73. Sawabata N, Asamura H, Goya T, et al. Japanese Lung Cancer Registry Study: first prospective enrollment of a large number of surgical and nonsurgical cases in 2002. J Thorac Oncol 2010;5:1369-75.
74. Gustafsson BI, Kidd M, Chan A, et al. Bronchopulmonary neuroendocrine tumors. Cancer 2008;113:5-21.
75. Rekhtman N. Neuroendocrine tumors of the lung: an update. Arch Pathol Lab Med 2010;134:1628-38.
76. Australia AIoHaWC. Lung cancer in Australia: an overview. Canberra: AIHW2011. Report No.: Cancer series no. 64. Cat. no. CAN 58. .
77. Fan Z, Schraeder R. The changing pathology of lung cancer. Surg Oncol Clin N Am 2011;20:637-53.
78. Travis WD, Brambilla E, Noguchi M, et al. Diagnosis of Lung Cancer in Small Biopsies and Cytology: Implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society Classification. Arch Pathol Lab Med 2013;137:668-84.
79. Lack EE, Harris GB, Eraklis AJ, et al. Primary bronchial tumors in childhood. A clinicopathologic study of six cases. Cancer 1983;51:492-7.
80. Fitzgibbons PL, Kern WH. Adenosquamous carcinoma of the lung: a clinical and pathologic study of seven cases. Hum Pathol 1985;16:463-6.
81. Naunheim KS, Taylor JR, Skosey C, et al. Adenosquamous lung carcinoma: clinical characteristics, treatment, and prognosis. Ann Thorac Surg 1987;44:462-6.
82. Ben Y, Yu H, Wang Z, et al. Adenosquamous lung carcinoma: clinical characteristics, surgical treament and prognosis. Chin Med Sci J 2000;15:238-40.
83. Dacic S, Finkelstein SD, Sasatomi E, et al. Molecular pathogenesis of pulmonary carcinosarcoma as determined by microdissection-based allelotyping. Am J Surg Pathol 2002;26:510-6.
84. Nappi O, Glasner SD, Swanson PE, et al. Biphasic and monophasic sarcomatoid carcinomas of the lung. A reappraisal of 'carcinosarcomas' and 'spindle-cell carcinomas'. Am J Clin Pathol 1994;102:331-40.
85. Thompson L, Chang B, Barsky SH. Monoclonal origins of malignant mixed tumors (carcinosarcomas). Evidence for a divergent histogenesis. Am J Surg Pathol 1996;20:277-85.
86. Nakajima M, Kasai T, Hashimoto H, et al. Sarcomatoid carcinoma of the lung: a clinicopathologic study of 37 cases. Cancer 1999;86:608-16.
87. Ro JY, Chen JL, Lee JS, et al. Sarcomatoid carcinoma of the lung. Immunohistochemical and ultrastructural studies of 14 cases. Cancer 1992;69:376-86.
88. Travis WD. Sarcomatoid neoplasms of the lung and pleura. Arch Pathol Lab Med 2010;134:1645-58.
89. Martini N, Melamed MR. Multiple primary lung cancers. J Thorac Cardiovasc Surg 1975;70:606-12.
90. Huang J, Behrens C, Wistuba I, et al. Molecular analysis of synchronous and metachronous tumors of the lung: impact on management and prognosis. Ann Diagn Pathol 2001;5:321-9.
91. Hiroshima K, Toyozaki T, Kohno H, et al. Synchronous and metachronous lung carcinomas: molecular evidence for multicentricity. Pathol Int 1998;48:869-76.
92. Girard N, Deshpande C, Lau C, et al. Comprehensive histologic assessment helps to differentiate multiple lung primary nonsmall cell carcinomas from metastases. Am J Surg Pathol 2009;33:1752-64.
93. Neugut AI, Sherr D, Robinson E, et al. Differences in histology between first and second primary lung cancer. Cancer Epidemiol Biomarkers Prev 1992;1:109-12.
94. Inamura K, Satoh Y, Okumura S, et al. Pulmonary adenocarcinomas with enteric differentiation: histologic and immunohistochemical characteristics compared with metastatic colorectal cancers and usual pulmonary adenocarcinomas. Am J Surg Pathol 2005;29:660-5.
95. Leong PP, Rezai B, Koch WM, et al. Distinguishing second primary tumors from lung metastases in patients with head and neck squamous cell carcinoma. J Natl Cancer Inst 1998;90:972-7.
96. Geurts TW, Nederlof PM, van den Brekel MW, et al. Pulmonary squamous cell carcinoma following head and neck squamous cell carcinoma: metastasis or second primary? Clin Cancer Res 2005;11:6608-14.
97. Day TA, Davis BK, Gillespie MB, et al. Oral cancer treatment. Curr Treat Options Oncol 2003;4:27-41.
98. Keith RL, Miller YE. Lung cancer: genetics of risk and advances in chemoprevention. Curr Opin Pulm Med 2005;11:265-71.
99. Pereira TC, Share SM, Magalhaes AV, et al. Can we tell the site of origin of metastatic squamous cell carcinoma? An immunohistochemical tissue microarray study of 194 cases. Appl Immunohistochem Mol Morphol 2011;19:10-4.
100. Doxtader EE, Katzenstein AL. The relationship between p16 expression and high-risk human papillomavirus

infection in squamous cell carcinomas from sites other than uterine cervix: a study of 137 cases. Hum Pathol 2012;43:327-32.
101. Fong KM, Kida Y, Zimmerman PV, et al. Loss of heterozygosity frequently affects chromosome 17q in non-small cell lung cancer. Cancer Res 1995;55:4268-72.
102. Klein F, Amin Kotb WF, Petersen I. Incidence of human papilloma virus in lung cancer. Lung Cancer 2009;65:13-8.
103. Suo Z, Holm R, Nesland JM. Squamous cell carcinomas. An immunohistochemical study of cytokeratins and involucrin in primary and metastatic tumours. Histopathology 1993;23:45-54.
104. Kaufmann O, Fietze E, Mengs J, et al. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am J Clin Pathol 2001;116:823-30.
105. Sigel CS, Moreira AL, Travis WD, et al. Subtyping of non-small cell lung carcinoma: a comparison of small biopsy and cytology specimens. J Thorac Oncol 2011;6:1849-56.
106. Rekhtman N, Ang DC, Sima CS, et al. Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. Mod Pathol 2011;24:1348-59.
107. Righi L, Graziano P, Fornari A, et al. Immunohistochemical subtyping of nonsmall cell lung cancer not otherwise specified in fine-needle aspiration cytology: a retrospective study of 103 cases with surgical correlation. Cancer 2011;117:3416-23.
108. Mukhopadhyay S. Utility of small biopsies for diagnosis of lung nodules: doing more with less. Mod Pathol 2012;25 Suppl 1:S43-57.
109. Bishop JA, Sharma R, Illei PB. Napsin A and thyroid transcription factor-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. Hum Pathol 2010;41:20-5.
110. Nicholson AG, Gonzalez D, Shah P, et al. Refining the diagnosis and EGFR status of non-small cell lung carcinoma in biopsy and cytologic material, using a panel of mucin staining, TTF-1, cytokeratin 5/6, and P63, and EGFR mutation analysis. J Thorac Oncol 2010;5:436-41.
111. Loo PS, Thomas SC, Nicolson MC, et al. Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens. J Thorac Oncol 2010;5:442-7.
112. Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6. Am J Surg Pathol 2011;35:15-25.
113. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol 2000;13:962-72.
114. Pelosi G, Rossi G, Cavazza A, et al. ΔNp63 (p40) Distribution Inside Lung Cancer: A Driver Biomarker Approach to Tumor Characterization. Int J Surg Pathol 2013;21:229-39.
115. Candi E, Dinsdale D, Rufini A, et al. TAp63 and DeltaNp63 in cancer and epidermal development. Cell Cycle 2007;6:274-85.
116. Bishop JA, Teruya-Feldstein J, Westra WH, et al. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol 2012;25:405-15.
117. Pelosi G, Fabbri A, Bianchi F, et al. ΔNp63 (p40) and thyroid transcription factor-1 immunoreactivity on small biopsies or cellblocks for typing non-small cell lung cancer: a novel two-hit, sparing-material approach. J Thorac Oncol 2012;7:281-90.
118. Yang X, Lu H, Yan B, et al. ΔNp63 versatilely regulates a Broad NF-kappaB gene program and promotes squamous epithelial proliferation, migration, and inflammation. Cancer Res 2011;71:3688-700.
119. Saintigny P, El-Naggar AK, Papadimitrakopoulou V, et al. DeltaNp63 overexpression, alone and in combination with other biomarkers, predicts the development of oral cancer in patients with leukoplakia. Clin Cancer Res 2009;15:6284-91.
120. Alsharif M, Carlo-Demovich J, Massey C, et al. Telecytopathology for immediate evaluation of fine-needle aspiration specimens. Cancer Cytopathol 2010;118:119-26.
121. Bulman W, Saqi A, Powell CA. Acquisition and processing of endobronchial ultrasound-guided transbronchial needle aspiration specimens in the era of targeted lung cancer chemotherapy. Am J Respir Crit Care Med 2012;185:606-11.
122. Wallace WA, Rassl DM. Accuracy of cell typing in nonsmall cell lung cancer by EBUS/EUS-FNA cytological samples. Eur Respir J 2011;38:911-7.
123. Layfield LJ, Bentz JS, Gopez EV. Immediate on-site interpretation of fine-needle aspiration smears: a cost and compensation analysis. Cancer 2001;93:319-22.
124. Travis WD, Rekhtman N. Pathological diagnosis and classification of lung cancer in small biopsies and cytology: strategic management of tissue for molecular testing. Semin Respir Crit Care Med 2011;32:22-31.
125. Thunnissen E, Kerr KM, Herth FJ, et al. The challenge of NSCLC diagnosis and predictive analysis on small

samples. Practical approach of a working group. Lung cancer 2012;76:1-18.
126. Ellison G, Zhu G, Moulis A, et al. EGFR mutation testing in lung cancer: a review of available methods and their use for analysis of tumour tissue and cytology samples. J Clin Pathol 2013;66:79-89.

Cite this article as: Davidson MR, Gazdar AF, Clarke BE. The pivotal role of pathology in the management of lung cancer. J Thorac Dis 2013;5(S5):S463-S478. doi: 10.3978/j.issn.2072-1439.2013.08.43

Towards optimal pathologic staging of resectable non-small cell lung cancer

Raymond U. Osarogiagbon[1], Gail E. Darling[2]

[1]Thoracic Oncology Research Group, Multidisciplinary Thoracic Oncology Program, Baptist Cancer Center, Memphis, TN, USA; [2]Toronto General Hospital, University Health Network, University of Toronto, Toronto, Canada

Correspondence to: Raymond U. Osarogiagbon, MBBS, FACP. Thoracic Oncology Research (ThOR) Group, 80 Humphreys Drive, Suite 220, Memphis, TN 38120, USA. Email: rosarogi@bmg.md.

Abstract: Pathologic nodal staging is the most accurate means of determining prognosis of patients with resectable non-small cell lung cancer (NSCLC), but confusion prevails about the optimal pre-operative and surgical lymph node examination procedures for candidates of curative-intent resection. The landmark American College of Surgeons Oncology Group Z0030 trial revealed no difference in the survival of patients with clinical T1 or T2, N0 or N1 (hilar node-negative), M0 NSCLC who either had a fastidious, pre-defined systematic hilar and mediastinal lymph node sampling procedure, or who received a complete mediastinal lymph node dissection. We place the results of this major trial into a contemporary clinical practice context, and discuss problems associated with apparent misunderstanding of the lessons from this trial, especially in light of evidence of prevailing sub-optimal nodal examination practices. We also discuss evolving knowledge about the origin of the quality gap in pathologic nodal staging and the emerging literature on corrective interventions.

Keywords: Lymph node examination; quality improvement; surgery; survival

Submitted Jun 21, 2013. Accepted for publication Oct 10, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.04
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.04

Accurate staging is essential to the appropriate treatment of cancer. After histologic confirmation of a diagnosis of lung cancer come the questions: 'what is the prognosis?', 'what are the best treatment options?', 'how likely is treatment to be successful?', 'will chemotherapy be necessary?' The answer to each of these questions requires knowledge of the stage of the cancer. The tumor, node, and metastasis (TNM) system, our current means of staging lung cancer, serves many functions. It is the language with which we communicate the extent of a patient's cancer across time and space, provides prognostic information, guides selection among treatment alternatives, and is a key aspect in selecting patients for clinical trials.

Advances in technology have improved the accuracy of clinical staging. Clinical staging incorporates all non-invasive radiologic tests such as computerized tomography (CT), positron emission tomography (PET), magnetic resonance imaging, and bone scans (1,2). In the surgical resection population, in which distant metastasis has usually been ruled out, the most difficult staging problem is the accurate determination of nodal metastasis status. Radiologic determination of the size and extent of the primary tumor is fairly accurate, although delineating the T3-T4 border, i.e., determining whether a tumor that seems to extend to major mediastinal structures is actually invasive (T4) or merely abutting (T3), can sometimes only be resolved at thoracotomy. However, nodal status is the most important determinant of survival in the lung cancer patient who does not have distant metastatic disease, and the question of lymph node metastasis is less easily resolved by radiologic tests (1,3). Invasive clinical staging of mediastinal lymph nodes may be accomplished by

Table 1 Comparison of 5-year survival rates by clinical and pathologic staging in the International Association for the Study of Lung Cancer staging project cohort. Modified from ref (12)

	5-year survival rate (%)	
	AJCC 6	AJCC 7
IA		
Clinical	50	50
Pathologic	73	73
IB		
Clinical	40	43
Pathologic	54	58
IIA		
Clinical	24	36
Pathologic	48	46
IIB		
Clinical	25	25
Pathologic	38	36
IIIA		
Clinical	18	19
Pathologic	25	24

transbronchial needle aspiration, endobronchial ultrasound guidance, endoscopic ultrasound guidance, mediastinoscopy, video-assisted mediastinal lymphadenectomy, transcervical extended mediastinal lymphadenectomy or video-assisted thoracoscopy (2,4,5).

However, clinical staging tests have their sensitivity, specificity and accuracy limitations. The positive predictive value (PPV) for CT ranges from 0.16 to 0.88 and the negative predictive value (NPV) ranges from 0.54-0.83 (1). Specifically, normal sized lymph nodes by CT criteria may harbor metastatic disease and enlarged lymph nodes may be enlarged because of benign processes such as postobstructive pneumonia, histoplasmosis, and sarcoidosis. The likelihood of an enlarged mediastinal node being histologically positive is only 60% whereas 20% of normal sized nodes may harbor metastasis (6). Similarly, PET-positive nodes may have increased metabolic activity because of an inflammatory process whereas histologically positive nodes may be negative on PET because of low metabolic activity or low burden of disease. Although PET performs better than CT, with a PPV ranging from 0.40 to 1.00 and a NPV ranging from 0.71-1.00, the false-negative rate is approximately 20% for normal sized nodes. Conversely, enlarged nodes that are PET positive are falsely positive 15-25% of the time (1). Invasive tests have limits imposed by the reach of the instrument and the degree of effort applied by the operator, or what Frank Detterbeck has described as the 'thoroughness of execution' (7).

Recent studies have demonstrated the value of combining clinical staging tests in the pre-operative work up of patients (8,9). For this reason current staging guidelines, including Cancer Care Ontario's Program in Evidence Based Care Practice Guidelines, recommend invasive mediastinal staging in the presence of either enlarged nodes on CT or "hot" nodes on PET to rule out false-positive imaging tests. These guidelines also recommend invasive mediastinal staging even with a negative CT and PET for high risk tumors (defined as central, large, T3/T4, or adenocarcinoma) (10).

For all the advances in clinical staging options, the most accurate determination of stage in patients who are able to undergo surgical resection comes from examination of the resection material obtained at thoracotomy (pathologic staging) (11). Comparison of the 5-year survival rates in groups of patients who are staged by clinical and pathologic means reveals a 5-23% higher survival in patients with pathologic stage I, II, and IIIA over those with the identical clinical stage (*Table 1*) (12). This difference is independent of the combination of descriptors used to assign aggregate stage, and is probably partly explained by the 'Will Rogers phenomenon', in which improved staging accuracy leads to more accurate assignment of low risk patients into low risk groups and upstaging of seemingly low risk patients with subtle metastatic disease into higher risk categories, thereby improving the aggregate outcomes of the higher risk cohorts (13). Pathologic staging is therefore our most accurate prognostic tool in lung cancer.

However, current pathologic staging of lung cancer remains insufficiently discriminatory of future patient outcomes. For example, the 5-year survival of patients with resected stage IA non-small cell lung cancer (NSCLC) is 73%, meaning the mortality rate of the lowest risk cohort is 27% (*Table 1*) (12). Although lymph node metastasis is our most powerful prognostic determinant in the surgical resection population, the 5-year survival of patients with pathologic N0 NSCLC is 56%, meaning that 44% of patients with apparently low risk disease die within 5 years (14). Are these poor results solely due to the biologic aggressiveness of lung cancer (or the frailty of the lung cancer patient), or do they reflect other problems such as limitations of the TNM staging system as a prognostic tool, or, very importantly-because of the opportunity for corrective intervention-poor application of the prognostic tool?

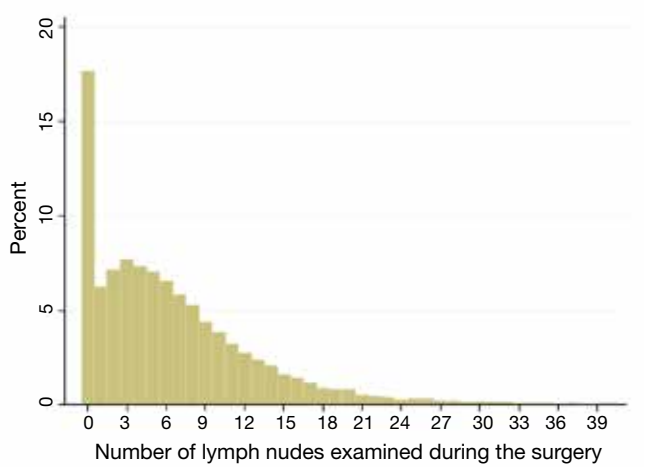


Figure 1 Number of lymph nodes examined after surgical resection of 'lymph node negative' non-small cell lung cancer. US Surveillance, Epidemiology and End Results Database 1998 to 2009. Obtained from ref (15).

Determining the stage-relevant characteristics of the primary tumor (its size and extent of direct invasion) is relatively straightforward for the pathologist. In the surgical resection population, distant metastasis usually being inevident, the most important pathologic staging problem is determining lymph node metastasis status. This requires the collaborative efforts of the surgeon (to retrieve the hilar and mediastinal lymph nodes, and to accurately communicate the provenance of all lymph node specimens to the pathologist for accurate mapping) and the pathologist (to examine all lymph nodes in the resection specimen, both those directly provided by the surgeon and those indirectly provided within the lung resection material). There is compelling evidence that this collaborative effort frequently breaks down, to the detriment of patients.

At one extreme, 13% of all curative-intent resections (and 18% of resections for 'node-negative disease') have no lymph nodes examined (15). The survival of patients with pathologically ambiguous nodal stage (pNX) approximates very closely to that of patients with pN1, not pN0 disease (when pN0 is defined as actually having at least one examined lymph node), suggesting that a significant proportion have missed lymph node metastasis (15). Secondly, 40-50% of all curative lung cancer resections in large North American databases have no mediastinal lymph nodes examined (16,17). Indeed, 63% of resections for mediastinal node negative (pN0 or pN1) disease in the US Surveillance, Epidemiology, and End Results (SEER) database from 1998 to 2009 had no mediastinal lymph nodes examined, leading to a 14% survival deficit (17). To put this survival impact in perspective, the estimated absolute survival benefit of post-operative adjuvant chemotherapy is about 5.4% (18). This problem is not unique to the US (19).

Furthermore, and more subtly, most patients with pathologic N0 disease cluster at the low end of the total lymph node number spectrum, with a median lymph node count of 6 in the US (*Figure 1*) (20). Patients with fewer than 6 lymph nodes have a significantly worse survival than matched patients with greater than 6 lymph nodes despite ostensibly having the same pathologic stage (21,22). Hence the recommendation in the 7th edition of the AJCC/UICC staging guidelines for examination of at least 6 lymph nodes and 3 nodal stations (23). However, this recommendation is probably insufficiently stringent because of evidence of sequential improvement in survival of patients with pathologic N0 disease with increasing number of lymph nodes examined, with the optimal number being 'greater than 10' and possibly as high as 18 to 21 (20,24-26). It is therefore unsettling that fewer than 15% of all pN0 lung cancer resections in large US databases have examination of greater than 10 lymph nodes. Even in patients with lymph node metastasis, there is prognostic value to the number of lymph nodes examined, both in helping determine the absolute number of lymph nodes with metastasis and in determining the ratio of positive and negative lymph nodes (27-32).

The etiology of suboptimal nodal examination has been the subject of recent investigation. Conceptually, it appears reasonable to separate the origin of the problem into three sites: events during the surgical operation (such as the hilar and mediastinal lymph node harvest), events during the transfer of specimens from the operating room to the pathology laboratory, and events during the pathology examination. Clearly, when surgeons do not harvest hilar and mediastinal lymph nodes, pathologists have no access to material for a thorough staging examination. Therefore, the solution to the problem of non-examination of mediastinal lymph nodes might be best achieved by focusing on intraoperative events. However, surgeons frequently complain that the specimens they submit are not completely examined. This assertion may be supported by 'before and after' intervention studies in which use of pre-labeled specimen collection kits improves the quality of pathologic staging, with a reversion to pre-intervention levels during the intervention phase in cases when the kit is inadvertently unavailable (33).

It therefore seems plausible that the communication between surgeons and pathologists during the transfer of specimens needs to be improved. Solutions might range from prevention of specimen loss in transit (34), to improved labeling of specimens in order to improve the ability of pathologists to determine the source and nature of submitted materials (35). Both of these factors (loss of specimens in transit, and inadequate specimen labeling) may impair the pathologic examination and lymph node mapping. The foregoing notwithstanding, the gross dissection of lung resection specimens for intrapulmonary lymph nodes may be an opportunity for pathology-centered quality improvement (36). For example, 10% of patients with one or more lymph nodes examined have no N1 lymph nodes, meaning that but for the mediastinal lymph nodes provided by the surgeon, there would have been no nodes examined in the resection specimen (37). Pathologists not infrequently omit the pathologic nodal stage in the report summary, or make errors in stage attribution, such as labeling N1 disease as N2 and vice-versa. This combination occurred in 33% of pathology reports in one city-wide audit of lung resection pathology reports (38). The very existence of the 12-18% pNX population is the clearest illustration of the possibility of concurrent glitches in intraoperative and pathology processes.

All of this naturally raises the question: what is the optimal surgical resection and pathologic staging procedure? We shall not engage the debate about the extent of resection and whether, or not, sublobar resection is oncologically sound in lobectomy candidates, a topic that remains the subject of ongoing clinical trials in North America (Cancer and Leukemia Group B 140503, clinicaltrials.gov #00499330) and Japan (Japan Clinical Oncology Group 0802/West Japan Oncology Group 4607L); Nor shall we address the looming controversy about the appropriateness of lobar resection in patients with low grade lesions such as adenocarcinoma in-situ, minimally invasive adenocarcinoma and ground glass opacity (39); Nor shall we discuss the definition of an oncologically complete resection for lung cancer, a topic of much interest which has been provocatively addressed in the recent past (40). Our focus is primarily on the lymph node staging problem.

The optimal surgical lymph node staging procedure has been partially clarified by the landmark American College of Surgery Oncology Group Z0030 trial which compared the long-term survival of patients with clinical T1-2, N0-1 NSCLC who underwent a fastidious, pre-specified systematic sampling procedure versus a more extensive mediastinal nodal dissection (41). Although 4% of patients in the extensive dissection arm had lymph node metastasis that had been missed by the systematic sampling procedure, there was no difference in recurrence free- or overall survival between the two groups. Early data analyses from this trial established the safety of mediastinal lymph node dissection in both academic and community care settings (42). It also revealed that surgeons' attention to the mediastinal lymph node harvest procedure provides a much higher lymph node yield than usually obtained—a median of 18 additional lymph nodes were collected in the mediastinal lymph node dissection arm (two-thirds of which were N2 lymph nodes), 6 or more nodes were examined from a minimum of 3 nodal stations in >99% of patients, and a minimum of 10 lymph nodes were examined from at least 3 nodal stations in 90% of patients (43). Most importantly, ACOSOG Z0030 definitively established the adequacy of systematic sampling as an oncologically sound mediastinal lymph node staging procedure in patients with relatively low risk early stage NSCLC and is now oft-cited in support of a pathologic staging strategy short of formal mediastinal nodal dissection (44).

However, it is important that we interpret Z0030 in the right context. First, the eligibility criteria specifically excluded patients with cT3 and T4 tumors, and those with hilar or mediastinal lymph node metastasis on frozen section analysis of the lymph nodes collected after the rigorous systematic nodal sampling procedure. Therefore, the results of this trial must not be misinterpreted as proof of equivalency between the two nodal dissection procedures in higher risk patients, such as those with clinically more advanced disease, because the results may be dissimilar in these patients. Secondly, this trial cannot be cited in support of the idea that noninvasive staging (with CT and PET) is a substitute for surgical mediastinal lymph node staging. It must be emphasized that all patients in Z0030 received a fastidious nodal sampling procedure, which included sampling of lymph nodes from stations 2R, 4R, 7 and 10R for right-sided tumors and stations 5, 6, 7 and 10L for left sided tumors regardless of lymph node size or metabolic activity. The randomization to cessation of further nodal dissection versus complete mediastinal lymph node dissection was performed only after establishment of histologic node negativity in stations 2-10, and the survival analysis included only patients who met the stringent quality criteria for the nodal sampling procedure. Z0030 cannot be used to justify a strategy of either no mediastinal nodal sampling (which is the experience of a large proportion of

patients who undergo resection in US databases) (16,17) or random sampling (the experience of the vast majority of all others) (45).

A prior study by Wu *et al.* corroborates the veracity of the above observations (46). In this study, 532 patients with clinical stage I, II or III NSCLC were randomized to either mediastinal lymph node dissection or to a nodal sampling procedure that was much less thorough than Z0030, requiring hilar nodal dissection, routine harvesting of station 7 and inspection of stations 1-9 with only removal of 'nodes with suspected cancer metastasis (diameter >1 cm or hard)'. They reported improved survival in favor of node dissection with a median survival of 43 months compared to 32 months for sampling (P=0.0001). In contrast to Z0030, patients had no cytological or histological assessment of lymph nodes prior to randomization and resection, suggesting that if pre-resection systematic lymph node sampling has not been performed, survival is improved by mediastinal lymph node dissection (46).

In one community-based series, only 8% of patients who had lung resection over the course of a 4-year time span met criteria for a less stringent definition of systematic sampling than was performed in Z0030 (45). This study highlighted the loose use of terminology by surgeons: in the 45% of resections in which the surgeon reported having performed a 'mediastinal lymph node dissection', objective review of the pathology report suggested that none met the Z0030 mediastinal nodal dissection criteria, 9% were better classified as systematic sampling, 50% had random sampling and 42% had no mediastinal lymph nodes examined. It would be an unfortunate misunderstanding of the state of the evidence for the results of Z0030 to be used to justify such practice.

A less obvious side-bar to the discordance between surgeon procedure claims and the results of pathology report-based audits of the quality of nodal examination is the contribution of pathology practice. Despite the consensus statement that pathologists should 'examine all lymph nodes in the lung resection specimen' (47), re-examination of lung resection specimens after completion of routine pathology examination reveals that 137% more intrapulmonary lymph nodes (and 165% more lymph nodes with metastasis) can be retrieved from discarded lung specimens than the number retrieved during the routine examination (36). Indeed, up to 12% of patients said to have pN0 disease on routine examination, had identifiable lymph node metastasis by hematoxylin and eosin staining of discarded lymph nodes. Using fastidious intrapulmonary nodal retrieval procedures, a median of 11 N1 lymph nodes were retrieved from lobar lung resection specimens, up from a pre-intervention median of 3 N1 nodes (36). Interestingly, this is greater than the median of 5 to 6 N1 lymph nodes examined in the ACOSOG Z0030 trial, even though per study protocol surgeons helped retrieve nodes from stations 10-13 (43). This suggests that the opportunity for quality improvement in routine pathology examination of lung resection specimens exists across different types of institutions. This opportunity might be greater in routine practice because of the expectation most surgeons have that nodes within the resection specimen would be retrieved by gross dissection in the pathology laboratory.

It is incumbent on the surgeon to provide adequate N2 nodes through systematic sampling or mediastinal lymph node dissection, but also to harvest N1 nodes including stations 10 and 11. Recent data demonstrated significant upstaging with respect to N1 nodes in open compared to VATS lobectomy suggesting that surgeons were not harvesting the hilar zone nodes when performing VATS lobectomy (48). Clearly, the pathologist cannot examine nodes that are left in the chest. Optimal pathologic nodal staging requires the collaborative actions of surgeons, members of the operating room team, specimen handlers, the pathology laboratory team and the pathologist. A chain of actions is required for optimal pathologic staging of curatively resected lung cancer. Like all chains, it is only as strong as its weakest link. Effective interventions to correct the prevailing quality deficit in staging must encompass the full spectrum of potential sites of quality breakdown, from the surgical operation to the posting of the final pathology report.

Interventions in which pre-labeled specimen collection kits have been combined with fastidious gross dissection of the lung resection specimen demonstrate early promise in rectifying the quality deficit. Studies of these interventions suggest that the proportion of patients found to have nodal metastasis increases significantly, with strong trends towards significant upward aggregate stage migration (49). Unfortunately, these studies do not yet provide data on the survival impact of these quality improvement measures (50-52). Despite the paucity of data on survival impact and cost-effectiveness of these corrective interventions, it seems prudent to narrow or eliminate the quality gap in pathologic nodal staging, given its well-documented adverse impact on patient survival.

It is also important to emphasize that the results of Z0030 should be applied to patients with relatively early

clinical stage NSCLC. These results cannot automatically be extrapolated to patients with more advanced disease. In addition, we propose that systematic sampling must be performed at least as rigorously as in Z0030 in order to provide sufficient quality pathologic staging for patients who undergo staging by that strategy. Calling a procedure 'systematic sampling' or 'mediastinal lymph node dissection' does not necessarily make it so. The definitions must be based on the actual lymph nodes retrieved from specific stations, all of which must be clearly labeled for, and examined by, the pathologist.

In conclusion, there is a great need to heighten general awareness of the prevalence and severity of the quality gap between optimal, recommended, nodal staging of resectable lung cancer, and actual practice. This awareness campaign must be sponsored and supported by all the clinical professional groups with influence over the problem, including associations of surgeons, pathologists, medical oncologists and radiation oncologists, and their various guidelines-making bodies. Research into the evaluation and implementation of corrective solutions must be supported by funding agencies, in order to provide clear evidence with which healthcare policymakers can develop incentives that will ultimately facilitate the elimination of this major quality of care deficit.

Acknowledgements

Disclosure: Dr. Osarogiagbon has filed a patent application for a surgical lymph node specimen collection kit; Dr. Darling has declared no conflict of interest.

References

1. Silvestri GA, Gould MK, Margolis ML, et al. Noninvasive staging of non-small cell lung cancer: ACCP evidenced-based clinical practice guidelines (2nd edition). Chest 2007;132:178S-201S.
2. De Leyn P, Lardinois D, Van Schil PE, et al. ESTS guidelines for preoperative lymph node staging for non-small cell lung cancer. Eur J Cardiothorac Surg 2007;32:1-8.
3. Herth FJ, Eberhardt R, Krasnik M, et al. Endobronchial ultrasound-guided transbronchial needle aspiration of lymph nodes in the radiologically and positron emission tomography-normal mediastinum in patients with lung cancer. Chest 2008;133:887-91.
4. Detterbeck FC, Jantz MA, Wallace M, et al. Invasive mediastinal staging of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 2007;132:202S-220S.
5. Kramer H, Groen HJ. Current concepts in the mediastinal lymph node staging of nonsmall cell lung cancer. Ann Surg 2003;238:180-8.
6. Gould MK, Kuschner WG, Rydzak CE, et al. Test performance of positron emission tomography and computed tomography for mediastinal staging in patients with non-small-cell lung cancer: a meta-analysis. Ann Intern Med 2003;139:879-92.
7. Detterbeck F, Puchalski J, Rubinowitz A, et al. Classification of the thoroughness of mediastinal staging of lung cancer. Chest 2010;137:436-42.
8. Tournoy KG, De Ryck F, Vanwalleghem LR, et al. Endoscopic ultrasound reduces surgical mediastinal staging in lung cancer: a randomized trial. Am J Respir Crit Care Med 2008;177:531-5.
9. Annema JT, van Meerbeeck JP, Rintoul RC, et al. Mediastinoscopy vs endosonography for mediastinal nodal staging of lung cancer: a randomized trial. JAMA 2010;304:2245-52.
10. Darling G, Dickie J, Malthaner R, et al. Invasive mediastinal staging of non small cell lung cancer. Available online: http://www.cancercare.on.ca/toolbox/qualityguidelines/clin-program/surgery-ebs/t. Accessed on 6.17.13.
11. D'Cunha J, Herndon JE 2nd, Herzan DL, et al. Poor correspondence between clinical and pathologic staging in stage 1 non-small cell lung cancer: results from CALGB 9761, a prospective trial. Lung Cancer 2005;48:241-6.
12. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
13. Feinstein AR, Sosin DM, Wells CK. The Will Rogers phenomenon. Stage migration and new diagnostic techniques as a source of misleading statistics for survival in cancer. N Engl J Med 1985;312:1604-8.
14. Rusch VW, Crowley J, Giroux DJ, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the N descriptors in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2007;2:603-12.
15. Osarogiagbon RU, Yu X. Nonexamination of Lymph Nodes and Survival After Resection of Non-Small Cell Lung Cancer. Ann Thorac Surg 2013;96:1178-89.

16. Little AG, Rusch VW, Bonner JA, et al. Patterns of surgical care of lung cancer patients. Ann Thorac Surg 2005;80:2051-6; discussion 2056.
17. Osarogiagbon RU, Yu X. Mediastinal lymph node examination and survival in resected early-stage non-small-cell lung cancer in the surveillance, epidemiology, and end results database. J Thorac Oncol 2012;7:1798-806.
18. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
19. Verhagen AF, Schoenmakers MC, Barendregt W, et al. Completeness of lung cancer surgery: is mediastinal dissection common practice? Eur J Cardiothorac Surg 2012;41:834-8.
20. Osarogiagbon RU, Ogbata OU, Yu X. The number of lymph nodes associated with maximal reduction of long term mortality risk in pathologic node-negative non-small cell lung cancer. Ann Thorac Surg. In press, accepted 9.16.13.
21. Gajra A, Newman N, Gamble GP, et al. Effect of number of lymph nodes sampled on outcome in patients with stage I non-small-cell lung cancer. J Clin Oncol 2003;21:1029-34.
22. Osarogiagbon RU, Yu X. Comparative survival of resected node-negative non-small cell lung cancer with and without lymph node examination in the SEER database. J Thorac Oncol 2012;7:abstr 131.
23. Goldstraw P. eds. International Association for the Study of Lung Cancer Staging Handbook in Thoracic Oncology. Orange Park, FL: Editorial Rx Press, 2009.
24. Ludwig MS, Goodman M, Miller DL, et al. Postoperative survival and the number of lymph nodes sampled during resection of node-negative non-small cell lung cancer. Chest 2005;128:1545-50.
25. Ou SH, Zell JA. Prognostic significance of the number of lymph nodes removed at lobectomy in stage IA non-small cell lung cancer. J Thorac Oncol 2008;3:880-6.
26. Varlotto JM, Recht A, Nikolov M, et al. Extent of lymphadenectomy and outcome for patients with stage I nonsmall cell lung cancer. Cancer 2009;115:851-8.
27. Fukui T, Mori S, Yokoi K, et al. Significance of the number of positive lymph nodes in resected non-small cell lung cancer. J Thorac Oncol 2006;1:120-5.
28. Lee JG, Lee CY, Park IK, et al. Number of metastatic lymph nodes in resected non-small cell lung cancer predicts patient survival. Ann Thorac Surg 2008;85:211-5.
29. Jonnalagadda S, Smith C, Mhango G, et al. The number of lymph node metastases as a prognostic factor in patients with N1 non-small cell lung cancer. Chest 2011;140:433-40.
30. Wei S, Asamura H, Kawachi R, et al. Which is the better prognostic factor for resected non-small cell lung cancer: the number of metastatic lymph nodes or the currently used nodal stage classification? J Thorac Oncol 2011;6:310-8.
31. Jonnalagadda S, Arcinega J, Smith C, et al. Validation of the lymph node ratio as a prognostic factor in patients with N1 nonsmall cell lung cancer. Cancer 2011;117:4724-31.
32. Nwogu CE, Groman A, Fahey D, et al. Number of lymph nodes and metastatic lymph node ratio are associated with survival in lung cancer. Ann Thorac Surg 2012;93:1614-9; discussion 1619-20.
33. Osarogiagbon RU, Miller LE, Ramirez RA, et al. Use of a surgical specimen-collection kit to improve mediastinal lymph-node examination of resectable lung cancer. J Thorac Oncol 2012;7:1276-82.
34. Makary MA, Epstein J, Pronovost PJ, et al. Surgical specimen identification errors: a new measure of quality in surgical care. Surgery 2007;141:450-5.
35. Molnar TF. A new device for the identification of lymph nodes at lung cancer surgery. Eur J Cardiothorac Surg 2007;31:311-2.
36. Ramirez RA, Wang CG, Miller LE, et al. Incomplete intrapulmonary lymph node retrieval after routine pathologic examination of resected lung cancer. J Clin Oncol 2012;30:2823-8.
37. Allen JW, Farooq A, O'Brien TF, et al. Quality of surgical resection for nonsmall cell lung cancer in a US metropolitan area. Cancer 2011;117:134-42.
38. Farooq A, Osarogiagbon RU, Allen JW, et al. Accuracy and comprehensiveness of pathology reportage after lung cancer resection. J Clin Oncol 2009;27:abstr 6523.
39. Nakao M, Yoshida J, Goto K, et al. Long-term outcomes of 50 cases of limited-resection trial for pulmonary ground-glass opacity nodules. J Thorac Oncol 2012;7:1563-6.
40. Rami-Porta R, Wittekind C, Goldstraw P, et al. Complete resection in lung cancer surgery: proposed definition. Lung Cancer 2005;49:25-33.
41. Darling GE, Allen MS, Decker PA, et al. Randomized trial of mediastinal lymph node sampling versus complete lymphadenectomy during pulmonary resection in the patient with N0 or N1 (less than hilar) non-small cell carcinoma: results of the American College of Surgery Oncology Group Z0030 Trial. J Thorac Cardiovasc Surg 2011;141:662-70.
42. Allen MS, Darling GE, Pechet TT, et al. Morbidity and

mortality of major pulmonary resections in patients with early-stage lung cancer: initial results of the randomized, prospective ACOSOG Z0030 trial. Ann Thorac Surg 2006;81:1013-9; discussion 1019-20.
43. Darling GE, Allen MS, Decker PA, et al. Number of lymph nodes harvested from a mediastinal lymphadenectomy: results of the randomized, prospective American College of Surgeons Oncology Group Z0030 trial. Chest 2011;139:1124-9.
44. Murthy SC. Less is more… (more or less…). J Thorac Cardiovasc Surg 2011;141:670-2.
45. Osarogiagbon RU, Allen JW, Farooq A, et al. Objective review of mediastinal lymph node examination in a lung cancer resection cohort. J Thorac Oncol 2012;7:390-6.
46. Wu Yl, Huang ZF, Wang SY, et al. A randomized trial of systematic nodal dissection in resectable non-small cell lung cancer. Lung Cancer 2002;36:1-6.
47. Association of Directors of Anatomic and Surgical Pathology. Recommendations for processing and reporting of lymph node specimens submitted for evaluation of metastatic disease. Am J Clin Pathol 2001;115:799-801.
48. Boffa DJ, Kosinski AS, Paul S, et al. Lymph node evaluation by open or video-assisted approaches in 11,500 anatomic lung cancer resections. Ann Thorac Surg 2012;94:347-53; discussion 353.
49. Osarogiagbon RU, Ramirez RA, Wang CG, et al. Dual intervention to improve pathologic staging of resectable lung cancer. Ann Thorac Surg 2013;96:1975-81.
50. Brzezniak C, Giaccone G. Intrapulmonary lymph node retrieval: unclear benefit for aggressive pathologic dissection. Transl Lung Cancer Res 2012;1:230-3.
51. Osarogiagbon RU, Miller LE, Wang CG, et al. Response to editorial titled 'Intrapulmonary lymph node retrieval: unclear benefit for aggressive pathologic dissection'. Transl Lung Cancer Res 2013;2:E33-E36.
52. Riquet M, Mordant P. Incomplete intrapulmonary lymph node retrieval after routine pathologic examination of resected lung cancer. Transl Lung Cancer Res 2013;2:1-2.

Cite this article as: Osarogiagbon RU, Darling GE. Towards optimal pathologic staging of resectable non-small cell lung cancer. Transl Lung Cancer Res 2013;2(5):364-371. doi: 10.3978/j.issn.2218-6751.2013.10.04

Molecular determinants of lung cancer metastasis to the central nervous system

Timothy G. Whitsett[1], Landon J. Inge[2], Harshil D. Dhruv[1], Philip Y. Cheung[1], Glen J. Weiss[1,3], Ross M. Bremner[2], Jeffrey A. Winkles[4], Nhan L. Tran[1]

[1]Cancer and Cell Biology Division, The Translational Genomics Research Institute (TGen), Phoenix, AZ, USA; [2]Center for Thoracic and Esophageal Disease, Heart and Lung Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA; [3]Medical Oncology, Cancer Treatment Centers of America, Goodyear, AZ, USA; [4]Departments of Surgery and Physiology, Center for Vascular and Inflammatory Diseases and the Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD, USA

Correspondence to: Nhan L. Tran. The Translational Genomics Research Institute, 445 N 5th St. Phoenix, AZ 85004, USA. Email: ntran@tgen.org.

Abstract: Lung cancer remains the leading cause of cancer-related mortality worldwide. The propensity for metastasis to the central nervous system (CNS) is a major clinical hurdle contributing to the low five-year survival rate of advanced disease. CNS metastases significantly outnumber primary brain tumors and carry a dismal prognosis in part due to the inability of therapeutic agents to cross the blood brain barrier. Standard treatment using radiation has been largely ineffective in improving mortality, suggesting the need for new agents targeting the critical metastatic drivers. The genetic and molecular events governing CNS metastasis from the lung are poorly understood at this time. This review highlights genetic events associated with CNS dissemination from the lung and molecular mechanisms associated with CNS metastasis. *In vivo* model systems that faithfully recapitulate escape from the lung and colonization of the CNS are described as tools for understanding the metastatic phenotype and for testing new therapeutic agents. A deeper understanding of the mechanisms of lung cancer metastasis to the CNS is needed to elucidate novel therapeutic avenues towards the improvement of the mortality associated with advanced stage lung cancer.

Keywords: Lung cancer; CNS metastases; molecular determinants

Submitted Feb 25, 2013. Accepted for publication Mar 29, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.12
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.12

Introduction to lung cancer and CNS metastasis

In 2013, lung cancer is expected to affect 246,000 people and result in 164,000 deaths in the USA (1). Worldwide, lung cancer kills close to 1.5 million people per year (2). The five-year survival rate for advanced stage lung cancer is less than 10% (3). Advanced stage lung tumors are the most likely tumor type to disseminate to the central nervous system (CNS). There have been estimates that 50% of the patients diagnosed with either small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC), the two major histological types of lung cancer, will develop metastatic brain lesions. Interestingly, the differing histological subtypes of lung cancer disseminate to the CNS at different rates (*Table 1*). For SCLC, more than 10% of patients present clinically with CNS involvement (10). The overall survival for patients diagnosed with metastatic CNS involvement is dismal, usually ranging from 3-6 months. Thus, improved clinical management of advanced lung cancer patients necessitates an understanding and therapeutic interventions towards metastatic disease, particularly in the brain.

Metastatic brain lesions outnumber primary brain tumors more than 10:1, with 50% of all CNS metastases arising from lung cancer (11,12). CNS metastases carry a clinical burden of morbidity and mortality, but also acute neurological deficits, cognitive impairment and seizures (12,13). Strikingly, the incidence of CNS metastases

Table 1 Reported incidence of CNS metastasis from primary lung cancers by histologic subtype

Histologic subtype	Incidence of CNS metastasis	References
Small cell lung cancer	13.5-59%	(4-7)
Adenocarcinoma	6.6-43%	(8,9)
Squamous cell carcinoma	5.2-13%	(8,9)
Large cell carcinoma	8.3%	(8)
Undifferentiated	41.0%	(9)
NSCLC-NOS	7.4%	(8)

appears to be on the rise, due to factors ranging from an aging population, increased CNS screening after cognitive warning signs, and improvements in treatment of systemic disease (12). The brain presents a unique challenge to therapeutic interventions given that the blood brain barrier (BBB) restricts access to many therapeutic compounds, especially bulky antibodies. Therapeutic interventions derived from understanding the molecular processes of CNS metastases will have to overcome hurdles not faced in many of the other disseminated tumor sites.

Clinical management of primary lung cancer with CNS metastasis

Clinical management of metastatic lung cancer continues to be a significant challenge. Specifically, the majority of lung cancer patients (~50%) are diagnosed with local and/or distant metastasis, which has a median survival of 7-11 months (14-16). The brain is a common site for metastasis in NSCLC patients, present in 25-30% of patients at diagnosis and the majority (40-50%) of patients will develop brain metastases during the course of their disease (14-16). The presence of brain metastases comes with a dismal patient outcome; overall survival for these patients is 2 months with palliative steroid treatment (14-16). While platinum-based therapies have positive benefits for metastatic NSCLC at other sites, application of these therapies for metastatic NSCLC brain lesions is limited, due to the inefficient transport of therapeutics across the BBB (16). Instead, radiation is the treatment of choice for metastatic NSCLC brain lesions, provided radiation therapy is compatible with the chosen systemic therapy. However, even with radiation, survival remains poor, with median survival at 7.6 months (16). Alternatively, a small subset of metastatic NSCLC patients (7%) is found to have a solitary brain lesion either at initial diagnosis or at recurrence (14,15,17). The course of treatment differs for these patients, with primary treatment focused on the brain metastases. Evidence indicates that localized therapy for the brain lesion, in the form of surgical resection of the brain metastasis with whole brain radiation, followed by standard treatment of the primary NSCLC lesion (surgery, surgery with adjuvant chemo/radiation), can improve survival for these patients, increasing median survival time from 2 to 7-27 months (14,15,17). More recently, targeted therapy for adenocarcinoma patients with epidermal growth factor receptor (EGFR) mutations has been shown to control metastatic NSCLC within the brain (16,18), suggesting that targeted therapies may have value in combating CNS metastasis. With such a severe mortality rate, there is an urgent clinical need to understand the mechanisms that govern lung cancer metastasis to the brain so we can identify therapeutic vulnerabilities.

Genetic mechanisms associated with CNS metastasis from the lung

Hanahan and Weinberg characterized genomic instability and mutations as one of the enabling characteristics of cells that facilitates the acquisition of hallmarks of cancer (19). In the course of carcinogenesis, cells acquire several genetic alterations, such as mutations, gene deletions, copy-number aberrations or chromosomal rearrangements, that are associated with the transition from a pre-neoplastic lesion to an invasive tumor and finally progression to the metastatic state (20,21). Even though lung cancer is the most frequent primary site that metastasizes to brain (22), very little is known about the genetic aberrations associated with CNS metastasis from the lung. The next section summarizes the available data on genetic aberrations of matched primary and CNS metastatic lung cancer specimens, and these findings are summarized in *Table 2*.

Somatic gene mutations and CNS metastasis

In the majority of the lung cancer studies, somatic mutations (including EGFR, KRAS, TP53, and many others) were identified on primary lung tumors to understand the genetic basis of the disease (35-41). The genetic landscapes of lung tumor subtypes are now being surveyed by next-generation sequencing towards understanding driver mutations (42-45). However, very few studies have interrogated matched primary and metastatic tumor specimens to correlate the metastatic potential of tumors with somatic mutations.

Table 2 Genetic and chromosomal aberrations associated with CNS metastasis

Locus	Alteration	References
EGFR	Mutation	(23-25)
EGFR	Copy number gain	(26)
KRAS	Mutation	(25,27-29)
Ch2q	Loss	(30,31)
Ch4q12-q32	Loss	(32)
Ch5q35	Amplification	(33)
Ch10q23	Amplification	(33)
Ch11p15	Imbalance	(34)
Ch17q23-24	Amplification	(33)
Ch18q	Loss	(30,31)
Ch22q	Loss	(30,31)

Additionally, investigations of matched primary and metastatic tumor specimens have focused primarily on the mutational status of only EGFR or KRAS. In regard to the EGFR studies, a recent review by Burel-Vandenbos *et al.* thoroughly summarized EGFR mutation status in lung cancer brain metastasis (23). In East Asian cohorts, known to have a higher prevalence of EGFR mutations, activating mutations were found in 44-63% of brain metastases. In Caucasian cohorts, with a low overall prevalence of EGFR mutations, activating mutations were found 0-2% of brain metastases. Eichler and colleagues demonstrated that patients with brain metastasis were more likely to have primary tumors with EGFR mutations (24). Few patient-matched primary and brain metastatic tumor sets have been explored for EGFR mutation status. Four studies with small sample sizes have suggested a discordant EGFR mutation rate between primary and brain metastatic tumors between 0 and 32% (23). There are reported instances of EGFR mutations in CNS metastatic tumors not seen in the patient-matched primary tumor (25), however the impact of technical detection limits of the mutations remains a question.

There is very little data available on the mutation status of KRAS in primary lung cancer with corresponding brain metastasis. Cortot *et al.* reported that 2 out of 13 patients with brain metastasis demonstrated KRAS mutation at codon 12 (G12C) (27). Of the two patients with KRAS mutation, one patient demonstrated KRAS mutation in both primary and corresponding brain metastasis while the other patient demonstrated gain of KRAS mutation in metastatic lesions. However, they were not able to verify the gain of mutation determined using direct sequencing in one patient, using mutant-enriched PCR. In a similar study, Kalikaki *et al.* showed gain of KRAS mutation at codon 12 (G12S) in one metastatic brain tumor sample as compared to corresponding primary lung tumor sample out of two analyzed matched specimens (25). Matsumoto *et al.* found a KRAS mutation in 2 out of 19 metastatic tumors at codon 12 (G12C) (28). However, they didn't have matched primary lung tumor available for KRAS mutation analysis. Finally, a recent study by Munfus-McCray and co-workers found that 23.5% of analyzed metastatic lung tumors with KRAS mutation metastasize to brain (29).

In summary, available data do not establish any clear correlation between EGFR and KRAS mutation status of primary lung tumors and their propensity to metastasize to the CNS. Additional studies are needed to further investigate the link between gene mutations in primary tumors and their potential for CNS dissemination.

Chromosomal imbalances associated with CNS metastasis

Despite the advent of next-generation sequencing and array-based comparative genomic hybridization (aCGH), few studies have been conducted examining genomic aberrations associated with brain metastasis from the lung. In one of the first studies of its kind Shiseki *et al.* investigated 22 brain metastases and 23 early-stage, primary lung tumors from 43 patients (10 matched primary and brain metastasis samples) for allelic losses at 40 loci in 10 chromosomes using restriction fragment length polymorphism (RFLP) (30,31). They demonstrated that in brain metastasis, a significant ($P<0.05$) incidence of allelic losses (>60%) was observed at loci on chromosomes 2q, 18q, and 22q. Takahashi *et al.* investigated 8 primary lung tumors, their 14 corresponding metastases and 8 corresponding normal lung tissues using SNP array analysis (34). In 5 primary lung tumors and their 7 corresponding brain metastasis, a majority (≥81%) of allelic imbalances were similar between primary and matched metastasis. Allelic imbalance at 11p15 was most frequently observed when the genetic imbalance only occurred in the metastatic lesion. In a recent study, Lee and co-workers investigated 18 primary NSCLC and their corresponding brain metastasis for copy number alterations using molecular inversion probe (MIP) technology (33). Using comparative MIP analysis they found that amplification of chromosomal regions 5q35, 10q23, and 17q23-24 in primary lung adenocarcinomas was significantly associated with development of early brain

metastasis.

Sun *et al.* investigated EGFR copy number variations in NSCLC primary tumors and corresponding brain metastasis using fluorescence *in situ* hybridization (FISH) analysis and demonstrated high frequency of gain in EGFR copy number was present in NSCLC primary (62%) and brain metastases (64%) (26). Further, a relatively high level of concordance (84%) for EGFR copy number status was observed between primary tumor and corresponding brain metastasis. Conversely, 9 cases (16% of total) demonstrated discordance between EGFR copy number status between primary tumor and corresponding brain metastasis; in 6 of these, brain metastasis sites had a gain in copy number.

Wrage *et al.* discovered that lung cancer patients in a bone marrow positive group (patients tested positive for disseminated tumor cells in bone marrow) show loss in 4q12-q32 as compared to lung cancer patients in a bone marrow negative group using aCGH, signifying a role of 4q deletion in metastasis (32). Additionally, they performed FISH analysis for 4q21 on tissue microarray with 36 brain metastases and demonstrated that 39% of samples show one allele loss of 4q, whereas gains were only found in 6% of tumors (32). Their comprehensive FISH analysis on 43 primary lung tumor and 35 brain metastasis tumors showed significant loss of 4q21 in brain metastases as compared to primary lung tumors. This data would suggest that a metastasis suppressor gene(s) for lung cancer metastasis to brain could be present on chromosome 4q.

In summary, while multiple genomic aberrations have been reported for lung cancer metastasis to the brain, there is little concordance and few data sets. Next-generation sequencing projects employing larger sample sizes of patient-matched primary and brain metastasis may identify new genomic aberrations driving brain metastasis that can be exploited both for patient prognosis and to guide treatment options.

Molecular mechanisms associated with CNS metastasis

Despite the frequency of CNS metastasis from primary lung tumors, the molecular mechanisms governing this complex process are not well understood. Here we will discuss genes and/or signatures that have been associated with CNS metastases from studies using human clinical samples or *mouse* models of tumor growth and metastasis.

Identification of genes associated with CNS metastasis using clinical specimens

Kargi *et al.* examined 30 NSCLC cases, including 15 excised brain metastases and determined that CD44 protein was significantly inversely related to metastatic potential (46). CD44 isoforms play a role in cell adhesion and are dysregulated in a number of tumor types (47). Kikuchi and colleagues performed expression profiles on 16 metastatic brain foci compared to 37 primary NSCLC tumors and 244 genes showed significantly differential expression between brain metastasis and primary lung tumors (48). Several cytoskeletal protein genes and genes associated with cell movement were differentially up-regulation in the metastases including metallothionein 2A (MT2A), fascin homolog 3 (FSCN3), microtubule-associated protein 7 (MAP7) and CXCL13. Grinberg-Rashi *et al.* analyzed 142 NSCLC tumors and found that N-cadherin, kinesin family member C1 (KIFC1) and bromodomain PHD finger transcription factor (BPTF/FALZ) expression was predictive of brain metastasis (49). N-Cadherin was over-expressed in brain metastasis. This protein has been associated with cells undergoing epithelial-to-mesenchymal transition, and cell invasiveness (50). In addition, a recent report showed that loss of E-cadherin expression was significantly associated with brain metastasis (51). NSCLC patients who developed brain metastasis during follow-up compared to NSCLC patients with no evidence of brain metastasis displayed low E-cadherin expression. Finally, KIF1C, a kinesin family member known to be associated with cell movement (52), was also over-expressed in brain metastasis, while FALZ, a transcription factor with chromatin remodeling properties (53), was under-expressed in brain metastasis.

Another family of genes with strong associations to tumor cell migration and invasion are the chemokine receptors. Chemokine receptors and their cognate ligands are up-regulated in a number of cancers and have been demonstrated to play vital, non-redundant roles in cancer metastasis from multiple primary tumors [reviewed in (54)]. Thus, chemokine receptors have been examined for a role in lung metastasis to the CNS. In 32 patients with solitary brain metastasis from NSCLC, 90% of primary tumors and 100% of brain metastases expressed CXCR4, significantly higher than NSCLC without distant metastases or primary brain tumors (55). Another chemokine receptor associated with lung cancer and metastatic spread is CX3CR1. Protein expression of CX3CR1 was elevated in NSCLC compared

to SCLC (56). While CX3CR1 positivity was significantly associated with number of metastatic sites, paradoxically CX3CR1-negative lung adenocarcinomas were more likely to have disseminated to the brain. The studies summarized above indicate that metastatic colonization of the CNS from lung is a complex process that employs dysregulation of a number of genes known to play a role in cell migration and invasion.

The role of constitutive receptor tyrosine kinase activation in cancer biology is well established. A significant amount of research in lung cancer has focused on the ERBB family members, especially EGFR. The dysregulation of this receptor through mutation or amplification is a known driver of some lung cancers, and serves as a frontline therapeutic target. The role of the ERBB receptors in brain metastasis is less appreciated. It is known the ERBB2 expression in breast cancer is associated with worse prognosis and brain metastasis (57-59). A report by Sun and colleagues examined the protein expression patterns of EGFR, ERBB2, ERBB3, and their ligands in 50 NSCLC primary tumors and corresponding brain metastases (26). The metastases displayed significantly higher protein expression of EGF and amphiregulin in the nucleus. The phosphorylation of EGFR and ERBB3 was elevated in the membrane of the brain metastases compared to primary tumors. In contrast, transforming growth factor-alpha and neuregulin demonstrated significantly higher expression in primary tumors compared to brain metastases. Thus, ERBB family members and ligands are differentially expressed in primary tumors versus brain metastases. In another study, the phosphorylation status of 128 signaling proteins was examined in 42 brain metastases from breast and NSCLC patients by reverse phase protein microarray. The NSCLC metastases exhibited elevated relative levels of the EGFR/ERK network (60). The breast cancer metastases showed higher activation of the ERBB2/IGFR-Akt network compared to lung cancer metastases. Thus, there appears to be a role for EGFR in the brain metastatic phenotype of lung tumors, and this pathway is under investigation as a therapeutic target.

Another receptor tyrosine kinase associated with tumor cell invasion and metastasis is the hepatocyte growth factor receptor (c-MET). c-MET and its ligand, hepatocyte growth factor (HGF), have been associated with tumor progression and metastasis in many solid tumor types (61). The protein expression of c-MET was observed in ~30% of adenocarcinomas and c-MET gene amplification is seen in 10% of adenocarcinomas (62). Increased activity of c-MET can occur via oncogenic activation of KRAS (63), while gene amplification of c-MET is often related to resistance to EGFR-tyrosine kinase inhibitors (TKIs). The expression of c-MET and/or HGF has been associated with therapeutic resistance against EGFR-TKIs (64-66), cisplatin (67), and radiation (68). Expression of c-MET was more common in poorly differentiated adenocarcinomas compared to well-differentiated tumors (62). Benedettini *et al.* demonstrated that c-MET expression and phosphorylation were associated with the development of brain metastasis, and enriched in brain metastases compared to patient-matched primary tumors (69). Thus, the HGF/c-MET pathway may offer unique therapeutic vulnerabilities against brain metastases.

In SCLC, the most highly aggressive lung cancer subtype with strong predilection for metastasis to the brain, placental growth factor (PLGF) and vascular endothelial growth factor receptor 1 (VEGFR1) expression levels were recently associated with brain metastasis (70). Elevated serum levels of PLGF were detected in SCLC patients with brain metastasis compared to SCLC patients without brain metastasis. This elevated expression of PLGF was also seen in the brain metastasis tissue. PLGF triggered VEGFR1 signaling and promoted SCLC cell trans-endothelial migration *in vitro*. Depletion of PLGF via shRNA technology inhibited brain metastasis in an *in vivo* model system. Thus, the VEGF member PLGF may play a role in the invasive nature of SCLC.

Identification of genes associated with CNS metastasis using mouse models

Transgenic mouse technology has become a powerful tool for investigating the contribution of a gene or genes in development and disease pathology, particularly cancer. Manipulating expression of genes involved in human NSCLC with conditional alleles or transgenes has led to a variety of genetically-engineered mouse models (GEMM) (71). Several GEMMs have been developed carrying reported mutated oncogenes of NSCLC (e.g., $EGFR^{L858R}$, T790M, $ERBB2^{YVMA}$, EML4-ALK chimera, $PIK3CA^{H1074R}$, $KRAS^{G12V}$, c-MET), in the presence or absence of deletion of tumor suppressors such as TP53, P16 or LKB1 (71). Several of these models have shown the capacity for metastatic spread into lymph nodes, the surrounding chest cavity and even into distal organs such as bone (72). Despite these aggressive model systems, metastatic colonization of the CNS has remained elusive.

The lack of CNS metastasis may be accounted for by the shortened survival times of these aggressive models, or the lack of SCLC models, the lung histologic subtype most likely to present with CNS metastasis. It will be interesting to see whether expression of SCC- or SCLC-specific oncogene mutations will result in GEMMs of lung cancer cell dissemination to the brain.

While the development of GEMMs with reproducible brain metastasis has proven elusive to date as outlined above, a number of cell line models (both syngeneic and xenograft) have been generated with resultant CNS metastatic features. These models have used multiple injection routes including inter-cardiac injection and lung orthotopic injection. Two groups have reported the ability of A549 lung cancer cells to colonize the brain when injected in the bloodstream or orthotopically implanted into the lungs of immune-deficient mice (73,74). Another cell line with reported brain metastatic phenotype is NCI-H250, a SCLC model (70). H250 cells injected into the internal carotid artery presented with brain metastasis in 5 of 18 mice, with a suppression of PLGF activity completely abrogating brain metastasis. One drawback of using human cell lines in the mouse model is the lack of complete immune response. To overcome this challenge, syngeneic models have been generated using Lewis lung carcinoma (LLC) cells. These cells have the ability to produce metastatic tumors from orthotopic injection to multiple organs, including a low incidence of brain metastasis (75). Injection of the LLC cells into the internal carotid artery could also produce brain metastatic lesions (76).

Multiple labs have generated a number of site-specific metastatic models across multiple cancer types. *In vivo* selection of lung tumor cells with brain colonizing potential, followed by extraction/expansion in culture, and re-introduction *in vivo* has the ability to produce cell subclones with enhanced 'brain seeking' potential. In 2004, Yoshimasu *et al.* described an *in vivo* model of CNS metastasis using the SCC cell line EBC-1 (77). Ventricular injection of parental EBC-1 cells produced low incidence of both brain and bone metastasis. Extraction of these metastatic cells and repeated *in vivo* selection produced EBC-1 subclones with enhanced potential to colonize the brain or bone. The highly brain metastatic subclone of EBC-1 cells expressed significantly higher expression of integrin alpha-3 compared to EBC-1 parental cells or EBC-1 cells metastatic to the bone (77). Suppression of integrin alpha-3/beta-1 significantly diminished brain metastasis using the *in vivo* model. ADAM9, a member of the "a disintegrin and metalloprotease" family has also been associated with brain metastasis from NSCLC. The ADAM family members regulate cell-cell and cell-matrix interactions (78,79). ADAM9 mRNA was highly expressed in EBC-1 brain metastatic lines compared to EBC-1 parental and bone metastatic lines (73). Over-expression of ADAM9 in A549 cells enhanced micro-metastatic foci in the brain. Another SCC cell line capable of brain metastasis *in vivo* is HARA (80). Again, *in vivo* selection after cardiac injection resulting in brain lesions produced a subclone with enhanced brain metastatic potential. This *in vivo* model has been used to begin to understand the recruitment and interplay between astrocytes and metastatic cells in metastatic growth (81).

The Massague lab has generated a number of site-specific metastatic models across multiple cancer types. In H2030 and PC9 cells (adenocarcinoma cell lines driven by KRAS and EGFR mutations, respectively), inter-cardiac injection of these cells, extraction and expansion in culture of brain lesions, and multiple rounds of *in vivo* selection produced cells that seeded the brain 100% of the time (82). One feature of this model is the ability to orthotopically implant cells into the lungs with resultant brain metastasis. The brain seeker lines compared to the parental cell lines displayed enriched activity of the WNT/TCF pathway. Genes in this pathway associated with metastasis were lymphoid enhancer-binding factor 1 (LEF1), homeobox B9 (HOXB9), and bone morphogenetic protein 4 (BMP4). The knockdown of LEF1 or HOXB9 significantly decreased the ability of the brain seeker lines to form metastases. Huang and colleagues used the PC9 brain seeker line to test a toxin directed at EGFR and urokinase receptor (uPAR) (83). They found that immunotoxin administration prolonged mouse survival.

Role of microRNAs in brain metastasis

miRNA biomarkers associated with CNS metastasis

MicroRNAs (miRNAs) are noncoding endogenous RNA species that regulate gene expression at the post-transcriptional level [reviewed in (84)]. Dysregulation of miRNAs has been linked to the development and progression of multiple cancer types, but the role of miRNAs in CNS metastasis remains an emerging area of research. The stability of miRNAs in tissues and fluids makes them attractive candidates for use in predictive and prognostic markers (85,86). Multiple groups have explored

Table 3 Selected molecular targets with potential therapeutic agents

Gene	Drugs	References
EGFR	Cetuximab, erlotinib, gefitinib, afatinib, dacomitinib	(93)
ERBB2	Afatinib, dacomitinib, lapatinib	(93)
N-cadherin	ADH-1	(94)
VEGFR1	Vandetanib, sorafenib, sunitinib, axitinib, cabozantinib, pazopanib	(95)
CX3CR1	F1 hCX3CL1 analog	(96)
MET	Onartuzumab, tivantinib	(93)
CXCR4	Plerixafor	(95)

miRNA expression as biomarkers to stratify patients or identify CNS metastasis. Lu *et al.* (87) profiled miRNAs extracted from surgically resected Stage I lung tumors in search of signatures predictive of recurrence and relapse-free survival. Ten miRNAs were found to be differentially expressed in samples from patients who subsequently developed brain metastases: miR-1, -29c, -30d, -145*, -148a*, -187, -218, -375, -450b-3p, and -708*. Teplyuk *et al.* explored miRNA signatures in cerebrospinal fluid (CSF) to detect and distinguish CNS malignancies (88). Members of the miR-200 family (including miR-141 and miR-200a/b/c) were highly expressed in the CSF of patients with metastases from breast or lung but not in other pathologic conditions. Recently, Arora *et al.* demonstrated that miR-328 and miR-330-3p expression significantly distinguished seven patients with brain metastasis from six patients without brain metastasis (89). Thus, miRNA profiles may help to identify lung cancers more likely to metastasize to the brain.

CNS metastasis mechanisms associated with miRNA function

There are now efforts towards understanding the mechanistic role(s) of miRNAs in the brain metastatic phenotype. For instance, miR-378 has been reported to promote survival, invasion and migration in A549 cells through MMP2, MMP9 and VEGF (90). Arora *et al.* showed that overexpression of miR-328 increases NSCLC migration via regulation of PRKCA, a member of the VEGF-IL1 family (89). Cheng *et al.* have elucidated a PRKCA-dependent mechanism through which IL1-beta induces uPA expression and cellular migration in A549 NSCLC cells (91). Others report that miR-146a exerts a mechanistically similar metastasis-suppressing function (92). Overexpression of miR-146a inhibits the degradation of β-catenin and acts to suppress hnRNPC, which in turn reduces expression of uPA, uPAR, MT1-MMP and MMP1. Both increased expression of β-catenin and suppression of hnRNPC served to inhibit invasion and migration. The role of miRNAs in lung cancer metastasis to the CNS is just beginning to be understood, and may offer novel therapeutic targets against advanced lung cancer.

Therapeutic opportunities against CNS metastasis

There is an urgent need to improve the clinical outcomes for patients who present with or develop CNS metastasis from primary lung tumors. With improved systemic treatment of primary lung lesions and enhanced imaging modalities, the incidence or detection of CNS metastasis will continue to increase. Current clinical regimens of surgery plus radiation (for solitary brain lesion) or radiation for multi-focal lesion have demonstrated infrequent clinical success. Selected targets that have been associated with CNS metastasis with known pharmacologic inhibitors are listed in *Table 3*. Demonstrated clinical benefits using EGFR-targeted therapies towards mutant EGFR expressing CNS lesions (97) gives credence to improved therapeutics with enhanced understanding of the genetics and molecular mechanisms of the CNS phenotype. The targeting of other ERBB family members may also prove to be a viable strategy for combating CNS metastases from lung or breast primary tumors (26). CNS lesions arising from mutant KRAS-expressing primary lesions may be susceptible to combined targeting of MAPK and PI3K signaling or SRC inhibitors. The demonstration that c-MET expression is associated with CNS metastatic events opens the possibility of exploiting c-MET inhibitors (98,99) in this setting. It is therefore crucial to further understand the driver events of CNS metastasis through the collection of patient-

matched primary and metastatic lesions for investigation as well as continued development of *in vivo* models capable of faithfully recapitulating the CNS metastatic phenotype from primary lung tumors. It will also be critical to test the ability of these agents to cross the BBB to affect the desired target.

Conclusions

Lung cancer continues to be a leading cause of cancer-related mortality throughout the world. The five-year survival rate for advanced, especially metastatic, disease is dismal. The colonization of the brain as a metastatic site contributes to the mortality of this disease, resulting in a dramatically reduced survival expectation. A thorough understanding of the genetics and molecular mechanisms that govern CNS metastasis from the lung are far from complete at this time. The emergence of next-generation sequencing along with the collection of patient-matched primary and CNS metastatic lesions offers a path forward towards a more complete understanding of the metastatic process and novel therapeutic avenues. Targeted approaches as seen with EGFR-targeted therapeutics positively affecting patient outcome with CNS metastasis offers hope that a full understanding of the CNS metastatic process will lead to better therapeutics and improved patient survival.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
2. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
3. Heist RS, Engelman JA. SnapShot: non-small cell lung cancer. Cancer Cell 2012;21:448.e2.
4. Lekic M, Kovac V, Triller N, et al. Outcome of small cell lung cancer (SCLC) patients with brain metastases in a routine clinical setting. Radiol Oncol 2012;46:54-9.
5. Nakazawa K, Kurishima K, Tamura T, et al. Specific organ metastases and survival in small cell lung cancer. Oncol Lett 2012;4:617-20.
6. Ramlov A, Tietze A, Khalil AA, et al. Prophylactic cranial irradiation in patients with small cell lung cancer. A retrospective study of recurrence, survival and morbidity. Lung Cancer 2012;77:561-6.
7. Arriagada R, Le Chevalier T, Rivière A, et al. Patterns of failure after prophylactic cranial irradiation in small-cell lung cancer: analysis of 505 randomized patients. Ann Oncol 2002;13:748-54.
8. Hubbs JL, Boyd JA, Hollis D, et al. Factors associated with the development of brain metastases: analysis of 975 patients with early stage nonsmall cell lung cancer. Cancer 2010;116:5038-46.
9. Mujoomdar A, Austin JH, Malhotra R, et al. Clinical predictors of metastatic disease to the brain from non-small cell lung carcinoma: primary tumor size, cell type, and lymph node metastases. Radiology 2007;242:882-8.
10. Castrucci WA, Knisely JP. An update on the treatment of CNS metastases in small cell lung cancer. Cancer J 2008;14:138-46.
11. Weil RJ, Palmieri DC, Bronder JL, et al. Breast cancer metastasis to the central nervous system. Am J Pathol 2005;167:913-20.
12. Gril B, Evans L, Palmieri D, et al. Translational research in brain metastasis is identifying molecular pathways that may lead to the development of new therapeutic strategies. Eur J Cancer 2010;46:1204-10.
13. Rahmathulla G, Toms SA, Weil RJ. The molecular biology of brain metastasis. J Oncol 2012;2012:723541.
14. Pfannschmidt J, Dienemann H. Surgical treatment of oligometastatic non-small cell lung cancer. Lung Cancer 2010;69:251-8.
15. Villaruz LC, Kubicek GJ, Socinski MA. Management of non-small cell lung cancer with oligometastasis. Curr Oncol Rep 2012;14:333-41.
16. Yamanaka R. Medical management of brain metastases from lung cancer (Review). Oncol Rep 2009;22:1269-76.
17. Villarreal-Garza C, de la Mata D, Zavala DG, et al. Aggressive treatment of primary tumor in patients with non-small-cell lung cancer and exclusively brain metastases. Clin Lung Cancer 2013;14:6-13.
18. Kim JE, Lee DH, Choi Y, et al. Epidermal growth factor receptor tyrosine kinase inhibitors as a first-line therapy for never-smokers with adenocarcinoma of the lung having asymptomatic synchronous brain metastasis. Lung Cancer 2009;65:351-4.
19. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
20. Qin LX. Chromosomal aberrations related to metastasis of human solid tumors. World J Gastroenterol 2002;8:769-76.
21. Nguyen DX, Massagué J. Genetic determinants of cancer

metastasis. Nat Rev Genet 2007;8:341-52.
22. Petersen I, Hidalgo A, Petersen S, et al. Chromosomal imbalances in brain metastases of solid tumors. Brain Pathol 2000;10:395-401.
23. Burel-Vandenbos F, Ambrosetti D, Coutts M, et al. EGFR mutation status in brain metastases of non-small cell lung carcinoma. J Neurooncol 2013;111:1-10.
24. Eichler AF, Kahle KT, Wang DL, et al. EGFR mutation status and survival after diagnosis of brain metastasis in nonsmall cell lung cancer. Neuro Oncol 2010;12:1193-9.
25. Kalikaki A, Koutsopoulos A, Trypaki M, et al. Comparison of EGFR and K-RAS gene status between primary tumours and corresponding metastases in NSCLC. Br J Cancer 2008;99:923-9.
26. Sun M, Behrens C, Feng L, et al. HER family receptor abnormalities in lung cancer brain metastases and corresponding primary tumors. Clin Cancer Res 2009;15:4829-37.
27. Cortot AB, Italiano A, Burel-Vandenbos F, et al. KRAS mutation status in primary nonsmall cell lung cancer and matched metastases. Cancer 2010;116:2682-7.
28. Matsumoto S, Takahashi K, Iwakawa R, et al. Frequent EGFR mutations in brain metastases of lung adenocarcinoma. Int J Cancer 2006;119:1491-4.
29. Munfus-McCray D, Harada S, Adams C, et al. EGFR and KRAS mutations in metastatic lung adenocarcinomas. Hum Pathol 2011;42:1447-53.
30. Shiseki M, Kohno T, Nishikawa R, et al. Frequent allelic losses on chromosomes 2q, 18q, and 22q in advanced non-small cell lung carcinoma. Cancer Res 1994;54:5643-8.
31. Shiseki M, Kohno T, Adachi J, et al. Comparative allelotype of early and advanced stage non-small cell lung carcinomas. Genes Chromosomes Cancer 1996;17:71-7.
32. Wrage M, Ruosaari S, Eijk PP, et al. Genomic profiles associated with early micrometastasis in lung cancer: relevance of 4q deletion. Clin Cancer Res 2009;15:1566-74.
33. Lee HW, Seol HJ, Choi YL, et al. Genomic copy number alterations associated with the early brain metastasis of non-small cell lung cancer. Int J Oncol 2012;41:2013-20.
34. Takahashi K, Kohno T, Matsumoto S, et al. Clonal and parallel evolution of primary lung cancers and their metastases revealed by molecular dissection of cancer cells. Clin Cancer Res 2007;13:111-20.
35. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 2002;62:6997-7000.
36. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455:1069-75.
37. Naoki K, Chen TH, Richards WG, et al. Missense mutations of the BRAF gene in human lung adenocarcinoma. Cancer Res 2002;62:7001-3.
38. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
39. Sanchez-Cespedes M, Parrella P, Esteller M, et al. Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. Cancer Res 2002;62:3659-62.
40. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005;65:1642-6.
41. Stephens P, Hunter C, Bignell G, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature 2004;431:525-6.
42. Liu J, Lee W, Jiang Z, et al. Genome and transcriptome sequencing of lung cancers reveal diverse mutational and splicing events. Genome Res 2012;22:2315-27.
43. Govindan R, Ding L, Griffith M, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150:1121-34.
44. Seo JS, Ju YS, Lee WC, et al. The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res 2012;22:2109-19.
45. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.
46. Kargi HA, Kuyucuoğlu MF, Alakavuklar M, et al. CD44 expression in metastatic and non-metastatic non-small cell lung cancers. Cancer Lett 1997;119:27-30.
47. Jothy S. CD44 and its partners in metastasis. Clin Exp Metastasis 2003;20:195-201.
48. Kikuchi T, Daigo Y, Ishikawa N, et al. Expression profiles of metastatic brain tumor from lung adenocarcinomas on cDNA microarray. Int J Oncol 2006;28:799-805.
49. Grinberg-Rashi H, Ofek E, Perelman M, et al. The expression of three genes in primary non-small cell lung cancer is associated with metastatic spread to the brain. Clin Cancer Res 2009;15:1755-61.
50. Chunhacha P, Sriuranpong V, Chanvorachote P. Epithelial-mesenchymal transition mediates anoikis resistance and enhances invasion in pleural effusion-derived human lung cancer cells. Oncol Lett 2013;5:1043-7.
51. Yoo JY, Yang SH, Lee JE, et al. E-cadherin as a predictive marker of brain metastasis in non-small-cell lung cancer, and its regulation by pioglitazone in a preclinical model. J Neurooncol 2012;109:219-27.

52. Theisen U, Straube E, Straube A. Directional persistence of migrating cells requires Kif1C-mediated stabilization of trailing adhesions. Dev Cell 2012;23:1153-66.
53. Jones MH, Hamana N, Shimane M. Identification and characterization of BPTF, a novel bromodomain transcription factor. Genomics 2000;63:35-9.
54. Kakinuma T, Hwang ST. Chemokines, chemokine receptors, and cancer metastasis. J Leukoc Biol 2006;79:639-51.
55. Chen G, Wang Z, Liu XY, et al. High-level CXCR4 expression correlates with brain-specific metastasis of non-small cell lung cancer. World J Surg 2011;35:56-61.
56. Mauri FA, Pinato DJ, Trivedi P, et al. Isogeneic comparison of primary and metastatic lung cancer identifies CX3CR1 as a molecular determinant of site-specific metastatic diffusion. Oncol Rep 2012;28:647-53.
57. Bravo Marques JM. Treatment of brain metastases in patients with HER2+ breast cancer. Adv Ther 2009;26 Suppl 1:S18-26.
58. Lin NU, Winer EP. Brain metastases: the HER2 paradigm. Clin Cancer Res 2007;13:1648-55.
59. Cheng X, Hung MC. Breast cancer brain metastases. Cancer Metastasis Rev 2007;26:635-43.
60. Improta G, Zupa A, Fillmore H, et al. Protein pathway activation mapping of brain metastasis from lung and breast cancers reveals organ type specific drug target activation. J Proteome Res 2011;10:3089-97.
61. Stella GM, Benvenuti S, Comoglio PM. Targeting the MET oncogene in cancer and metastases. Expert Opin Investig Drugs 2010;19:1381-94.
62. Tsuta K, Kozu Y, Mimae T, et al. c-MET/phospho-MET protein expression and MET gene copy number in non-small cell lung carcinomas. J Thorac Oncol 2012;7:331-9.
63. Yang Y, Wislez M, Fujimoto N, et al. A selective small molecule inhibitor of c-Met, PHA-665752, reverses lung premalignancy induced by mutant K-ras. Mol Cancer Ther 2008;7:952-60.
64. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
65. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932-7.
66. Yano S, Wang W, Li Q, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res 2008;68:9479-87.
67. Chen JT, Huang CY, Chiang YY, et al. HGF increases cisplatin resistance via down-regulation of AIF in lung cancer cells. Am J Respir Cell Mol Biol 2008;38:559-65.
68. Bhardwaj V, Zhan Y, Cortez MA, et al. C-Met inhibitor MK-8003 radiosensitizes c-Met-expressing non-small-cell lung cancer cells with radiation-induced c-Met-expression. J Thorac Oncol 2012;7:1211-7.
69. Benedettini E, Sholl LM, Peyton M, et al. Met activation in non-small cell lung cancer is associated with de novo resistance to EGFR inhibitors and the development of brain metastasis. Am J Pathol 2010;177:415-23.
70. Li B, Wang C, Zhang Y, et al. Elevated PLGF contributes to small-cell lung cancer brain metastasis. Oncogene 2013;32:2952-62.
71. de Seranno S, Meuwissen R. Progress and applications of mouse models for human lung cancer. Eur Respir J 2010;35:426-43.
72. Gandhi L, McNamara KL, Li D, et al. Sunitinib prolongs survival in genetically engineered mouse models of multistep lung carcinogenesis. Cancer Prev Res (Phila) 2009;2:330-7.
73. Shintani Y, Higashiyama S, Ohta M, et al. Overexpression of ADAM9 in non-small cell lung cancer correlates with brain metastasis. Cancer Res 2004;64:4190-6.
74. Yi BR, Kim SU, Kim YB, et al. Antitumor effects of genetically engineered stem cells expressing yeast cytosine deaminase in lung cancer brain metastases via their tumor-tropic properties. Oncol Rep 2012;27:1823-8.
75. Rashidi B, Yang M, Jiang P, et al. A highly metastatic Lewis lung carcinoma orthotopic green fluorescent protein model. Clin Exp Metastasis 2000;18:57-60.
76. Zhang Z, Hatori T, Nonaka H. An experimental model of brain metastasis of lung carcinoma. Neuropathology 2008;28:24-8.
77. Yoshimasu T, Sakurai T, Oura S, et al. Increased expression of integrin alpha3beta1 in highly brain metastatic subclone of a human non-small cell lung cancer cell line. Cancer Sci 2004;95:142-8.
78. Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. Mol Aspects Med 2008;29:258-89.
79. Rocks N, Paulissen G, El Hour M, et al. Emerging roles of ADAM and ADAMTS metalloproteinases in cancer. Biochimie 2008;90:369-79.
80. Noda M, Seike T, Fujita K, et al. The role of immune cells in brain metastasis of lung cancer cells and neuron-tumor cell interaction. Ross Fiziol Zh Im I M Sechenova 2009;95:1386-96.
81. Seike T, Fujita K, Yamakawa Y, et al. Interaction between

lung cancer cells and astrocytes via specific inflammatory cytokines in the microenvironment of brain metastasis. Clin Exp Metastasis 2011;28:13-25.
82. Nguyen DX, Chiang AC, Zhang XH, et al. WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. Cell 2009;138:51-62.
83. Huang J, Li YM, Massague J, et al. Intracerebral infusion of the bispecific targeted toxin DTATEGF in a mouse xenograft model of a human metastatic non-small cell lung cancer. J Neurooncol 2012;109:229-38.
84. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 2011;12:99-110.
85. Zen K, Zhang CY. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med Res Rev 2012;32:326-48.
86. Hui A, How C, Ito E, et al. Micro-RNAs as diagnostic or prognostic markers in human epithelial malignancies. BMC Cancer 2011;11:500.
87. Lu Y, Govindan R, Wang L, et al. MicroRNA profiling and prediction of recurrence/relapse-free survival in stage I lung cancer. Carcinogenesis 2012;33:1046-54.
88. Teplyuk NM, Mollenhauer B, Gabriely G, et al. MicroRNAs in cerebrospinal fluid identify glioblastoma and metastatic brain cancers and reflect disease activity. Neuro Oncol 2012;14:689-700.
89. Arora S, Ranade AR, Tran NL, et al. MicroRNA-328 is associated with (non-small) cell lung cancer (NSCLC) brain metastasis and mediates NSCLC migration. Int J Cancer 2011;129:2621-31.
90. Chen LT, Xu SD, Xu H, et al. MicroRNA-378 is associated with non-small cell lung cancer brain metastasis by promoting cell migration, invasion and tumor angiogenesis. Med Oncol 2012;29:1673-80.
91. Cheng CY, Hsieh HL, Sun CC, et al. IL-1 beta induces urokinase-plasminogen activator expression and cell migration through PKC alpha, JNK1/2, and NF-kappaB in A549 cells. J Cell Physiol 2009;219:183-93.
92. Hwang SJ, Seol HJ, Park YM, et al. MicroRNA-146a suppresses metastatic activity in brain metastasis. Mol Cells 2012;34:329-34.
93. Hirsch FR, Janne PA, Eberhardt WE, et al. Epidermal growth factor receptor inhibition in lung cancer: status 2012. J Thorac Oncol 2013;8:373-84.
94. Blaschuk OW. Discovery and development of N-cadherin antagonists. Cell Tissue Res 2012;348:309-13.
95. Uy GL, Rettig MP, Cashen AF. Plerixafor, a CXCR4 antagonist for the mobilization of hematopoietic stem cells. Expert Opin Biol Ther 2008;8:1797-804.
96. Dorgham K, Ghadiri A, Hermand P, et al. An engineered CX3CR1 antagonist endowed with anti-inflammatory activity. J Leukoc Biol 2009;86:903-11.
97. Namba Y, Kijima T, Yokota S, et al. Gefitinib in patients with brain metastases from non-small-cell lung cancer: review of 15 clinical cases. Clin Lung Cancer 2004;6:123-8.
98. Liu X, Newton RC, Scherle PA. Development of c-MET pathway inhibitors. Expert Opin Investig Drugs 2011;20:1225-41.
99. Belalcazar A, Azana D, Perez CA, et al. Targeting the Met pathway in lung cancer. Expert Rev Anticancer Ther 2012;12:519-28.

Cite this article as: Whitsett TG, Inge LJ, Dhruv HD, Cheung PY, Weiss GJ, Bremner RM, Winkles JA, Tran NL. Molecular determinants of lung cancer metastasis to the central nervous system. Transl Lung Cancer Res 2013;2(4):273-283. doi: 10.3978/j.issn.2218-6751.2013.03.12

Genetic susceptibility to lung cancer and co-morbidities

Ian A. Yang[1,2], John W. Holloway[3,4], Kwun M. Fong[1,2]

[1]Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Australia; [2]UQ Thoracic Research Centre, The University of Queensland, Brisbane, Australia; [3]Human Development & Health, Southampton, UK; [4]Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

Correspondence to: Dr. Ian A. Yang. Department of Thoracic Medicine, The Prince Charles Hospital, Rode Rd, Chermside, Brisbane QLD 4032, Australia. Email: Ian_Yang@health.qld.gov.au.

Abstract: Lung cancer is a leading cause of cancer death and disease burden in many countries. Understanding of the biological pathways involved in lung cancer aetiology is required to identify key biomolecules that could be of significant clinical value, either as predictive, prognostic or diagnostic markers, or as targets for the development of novel therapies to treat this disease, in addition to smoking avoidance strategies. Genome-wide association studies (GWAS) have enabled significant progress in the past 5 years in investigating genetic susceptibility to lung cancer. Large scale, multi-cohort GWAS of mainly Caucasian, smoking, populations have identified strong associations for lung cancer mapped to chromosomal regions 15q [nicotinic acetylcholine receptor (nAChR) subunits: *CHRNA3*, *CHRNA5*], 5p (*TERT-CLPTM1L* locus) and 6p (*BAT3-MSH5*). Some studies in Asian populations of smokers have found similar risk loci, whereas GWAS in never smoking Asian females have identified associations in other chromosomal regions, e.g., 3q (*TP63*), that are distinct from smoking-related lung cancer risk loci. GWAS of smoking behaviour have identified risk loci for smoking quantity at 15q (similar genes to lung cancer susceptibility: *CHRNA3*, *CHRNA5*) and 19q (*CYP2A6*). Other genes have been mapped for smoking initiation and smoking cessation. In chronic obstructive pulmonary disease (COPD), which is a known risk factor for lung cancer, GWAS in large cohorts have also found *CHRNA3* and *CHRNA5* single nucleotide polymorphisms (SNPs) mapping at 15q as risk loci, as well as other regions at 4q31 (*HHIP*), 4q24 (*FAM13A*) and 5q (*HTR4*). The overlap in risk loci between lung cancer, smoking behaviour and COPD may be due to the effects of nicotine addiction; however, more work needs to be undertaken to explore the potential direct effects of nicotine and its metabolites in gene-environment interaction in these phenotypes. Goals of future genetic susceptibility studies of lung cancer should focus on refining the strongest risk loci in a wide range of populations with lung cancer, and integrating other clinical and biomarker information, in order to achieve the aim of personalised therapy for lung cancer.

Keywords: Lung cancer; genetics; pulmonary disease; chronic obstructive; genome-wide association study (GWAS)

Submitted Aug 01, 2013. Accepted for publication Aug 02, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.06
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.06

Introduction

Lung cancer is a leading cause of cancer death and disease burden in many countries (1,2). Despite the great progress made in several areas of oncology, the treatment and outcome of lung cancer has not improved significantly. Understanding of the biological pathways involved in lung cancer aetiology is required to identify key biomolecules that could be of significant clinical value, either as predictive, prognostic or diagnostic markers, or as targets for the development of novel therapies to treat this disease, in addition to smoking avoidance strategies.

Lung cancers, like all human tumours, are caused by abnormalities in DNA sequence or expression. Lung cancer is comprised of two main histologic subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC).

NSCLC can now be classified by 'driver' mutations that occur in multiple oncogenes (3-5). However, much interest remains in the genetic susceptibility to lung cancer related to single nucleotide polymorphisms (SNPs) in the germline (6,7), independent of somatic mutations that develop in the tumour.

This review will explore the latest concepts of genetic susceptibility to lung cancer. First, principles of study designs for genetic susceptibility will be outlined, particularly genome-wide association studies (GWAS), which have provide significant progress in the past 5 or more years in the field of lung cancer genetics. Next, GWAS reported for lung cancer will be described, together with candidate susceptibility genes discovered to date. Finally, GWAS for susceptibility to cigarette smoking behaviour and chronic obstructive pulmonary disease (COPD) will be summarised, as related genetic markers for the most common cause of lung cancer (cigarette smoking) and a chronic disease risk factor for lung cancer (COPD).

Genetic susceptibility to lung cancer–study designs

The heritability of lung cancer susceptibility has been clearly established in numerous studies, including analyses of familial risk (8) and segregation analyses (9). However genetic influence on lung cancer is moderate at best. For example, using the 9.6 million subject Swedish Family-Cancer database, Czene *et al*. estimated heritability at 8% (10) and in twin studies and a higher concordance for monozygotic than for dizygotic twins has been noted (11). With tobacco smoking being by far the strongest environmental cause, it is possible that the heritable effects of genes governing smoking behaviour, [given the high heritability of smoking habits, ~0.5 in twin studies (12)], rather those determining individual susceptibility to carcinogenesis may play a more important role. However Lorenzo Bermejo *et al*. estimated the relative risk of lung cancer attributable to smoking according to the extent to which smokers transmit their smoking habits to the offspring (heritability of smoking), the prevalence of smoking in the general population, and the risk of lung cancer for smokers compared with non-smokers (13). They showed that the relative risk of lung cancer for the offspring of lung cancer patients attributable to smoking was 1.19 when published data on smoking practice were modelled suggesting familial cases of lung cancer cannot be attributed to shared smoking habits.

Given that there are apparent genetic determinants of lung cancer, there are a number of alternative study approaches that can be utilised to determine the genetic determinants of disease susceptibility.

Linkage analysis involves proposing a model to explain the inheritance pattern of phenotypes and genotypes observed in a pedigree (14). Linkage is evident when a gene that produces a phenotypic trait and its surrounding markers are coinherited. In contrast, those markers not associated with the anomalous phenotype of interest will be randomly distributed among affected family members as a result of the independent assortment of chromosomes and crossing over during meiosis. *Association studies* do not examine inheritance patterns of alleles; rather, they are case-control studies based on a comparison of allele frequencies between groups of affected and unaffected individuals from a population. A particular allele is said to be associated with the trait if it occurs at a significantly higher frequency among affected individuals as compared with those in the control group. The odds ratio of the trait in individuals is then assessed as the ratio of the frequency of the allele in the affected population compared with the unaffected population. The greatest problem in association studies is the selection of a suitable control group to compare with the affected population group. Genome-wide linkage analysis in family cohorts resulted in the identification of highly penetrant, low-frequency susceptibility genes for cancers, such as *BRCA1* and *BRCA2* for breast cancer and *APC* for colorectal cancer.

For lung cancer, several studies have attempted to identify susceptibility loci using a genome-wide linkage approach. However, while a few genetic loci that potentially harbour susceptibility genes have been identified, e.g., linkage of lung, laryngeal, and pharyngeal cancer in families to a region on chromosome 6q23-25 (15), no causal gene has been identified and, as with subsequent GWAS (see below), there is considerable overlap between the result for lung cancer and those for COPD, and lung function (16).

GWAS

Advances in array-based SNP genotyping technologies and haplotype mapping of the human genome (17) have presented the possibility of simultaneously determining millions of SNPs throughout the genome of an individual and this has allowed extension of association study design to allow hypothesis independent assessment of association across the genome. GWAS have revolutionized the study of genetic factors in complex common disease (18,19).

For more than 200 phenotypes, from common diseases to physiological measurements such as height and BMI and biological measurements such as circulating lipid levels, GWAS have provided compelling statistical associations for over hundreds of different loci in the human genome (www.gwascentral.org/). There are now clearly established approaches for GWAS including stringent genotype calling, quality control, population stratification (genomic controls) and statistical techniques (20). Due to the large number of statistical tests undertaken, carefully controlling for multiple testing using Bonferroni or false discovery rate (FDR) corrections is essential. A cluster of P-values below the 1% FDR from SNPs in one chromosomal location is defined as the region of 'maximal association' and is the first candidate gene region to examine further with analysis of secondary outcome measures, gene database searches, fine mapping to find the causal locus and replication in other cohorts/populations. It is unlikely that the SNP showing the strongest association will be the causal locus, as SNPs are chosen to provide maximal coverage of other variation in that region of the genome and not on biological function. Once a candidate region or gene is identified, gene expression can be compared between a selection of cases and controls and within individuals of different genotypes to provide further evidence for the genes involvement in disease. If linkage disequilibrium prevents the identification of a specific gene in a haplotype block then it may be necessary to utilize different racial and ethnic populations to hone in on the causative candidate gene that accounts for the genetic signal in GWAS (21).

Valuable insights into lung cancer susceptibility genes have been identified using the GWAS approach; however, the loci identified account for an extremely small proportion of the familial risk. The finding that loci identified through GWAS studies for common disease fail to account for all heritability of these conditions (termed missing heritability) is a subject of much discussion. There are a number of possible reasons that may account for this observation. These include gene-gene interaction, gene-environment interaction, and other types of genetic variation such as rare variants and structural variation and epigenetic heritability. In the future, the analysis of genome-wide copy number variation and/or rare variants through exome- or whole-genome sequencing, as is being applied to other complex diseases, may identify further loci responsible for inherited susceptibility to lung cancer (22). However one approach is to utilise novel analytical approaches to identify weakly associated variants whose effect may be lost in the GWAS approach due to the stringent significance level after multiple comparison correction. For example, utilisation of a less stringent multiple correction followed by gene pathway analysis can highlight genes involved in common biological pathways in the 'grey area' of SNPs whose association with disease status lies below the conventional level of genome-wide significance. Using a similar approach Zhang *et al*. performed a two-stage pathway analysis in GWAS of lung cancer in Han Chinese using gene set enrichment analysis (GSEA) method. Four pathways (achPathway, metPathway, At1rPathway and rac1Pathway) were consistently significant and may provide new insights into the etiology of lung cancer (23).

GWAS of lung cancer

GWAS of susceptibility to lung cancer in smokers

A number of GWAS have now been performed in a range of populations, to test genetic influences in susceptibility to lung cancer (*Table 1*).

An initial, relatively small GWAS was reported in an Italian population, showing association with SNPs in the *KLF6* gene, but not in replication cohorts (24). Another relatively small study of patients with familial lung cancer found associations with SNPs at chromosomal region 15q (27). Larger scale GWAS have since been performed in Caucasian populations, with replication cohorts (25,26,28-30,32) and meta-analyses (31,32,42). These GWAS have found statistically significant associations with SNPs, particularly in chromosomal regions 15q, 5p and 6p (*Table 1*). GWAS have also been undertaken in Asian populations with lung cancer (35-37,39,41), identifying some similar SNPs as detected in the studies of Caucasian populations, but also finding other SNPs conferring lung cancer risk distinctly in Asian populations (44).

In many of these studies, the observed associations with key SNPs were independent of smoking status or smoking history (25,26,29,32), although in some studies, SNPs (e.g., on 15q) were related to smoking behaviour (28). In studies of smokers, population attributable risk (PAR) for lung cancer for these SNPs, where calculated, were modest, ranging between 14% (26) and 18% (28), compared to the overwhelming PAR of >80% from tobacco smoking.

GWAS in specific lung cancer populations

GWAS have also been undertaken in never smokers,

Table 1 GWAS of lung cancer *vs.* controls

Study	Lung cancer cases (discovery set)*	Controls (discovery set)	Arrays [nos. of SNPs]	Chromosomal regions and main associated genes
Spinola 2007 (24)	335 smokers	338 smokers	Affymetrix [116,204]	10p KLF6
Amos 2008 (25)	1,154 smokers	1,137 smokers	Illumina [317,498]	15q CHRNA3
Hung 2008 (26)	1,989 smokers	2,625 smokers	Illumina [317,139]	15q CHRNA3, CHRNA5
Liu 2008 (27)	194 with familial lung cancer	219 smokers and non-smokers	Affymetrix [500,568 and 906,703]	15q various genes
Thorgeirsson 2008 (28)	1,024 smokers	32,244 controls	Illumina [306,207]	15q CHRNA3
McKay 2008 (29)	3,259 smokers	4,159 smokers	Illumina [315,194]	5p TERT-CLPTM1L, 15q CHRNA3
Wang 2008 (30)	1,952 smokers	1,438 smokers	Illumina [511,919]	5p CLPTM1L, 6p BAT3-MSH5, 15q CHRNA3
Broderick 2009 (31)	1,978 smokers, and meta-analysis	1,438 smokers, and meta-analysis	Meta-analysis	5p TERT-CLPTM1L, 6p BAT3-MSH5, TNXB, 15q CHRNA3
Landi 2009 (32)	5,739 smokers	5,848 smokers	Illumina [515,922]	5p TERT-CLPTM1L, 15q CHRNA3
Hsiung 2010 (33)	584 cases (never smoking females with lung adenocarcinoma)	585 controls (never smoking females)	Illumina [610,901]	5p15 TERT-CLPTM1L
Li 2010 (34)	377 never smokers	377 never smokers	Illumina [373,397 and 592,532]	13q31.3 GPC5
Miki 2010 (35)	1,004 with lung adenocarcinoma	1,900 controls	Illumina [610,901]	3q28 TP63, 5p15 TERT
Yoon 2010 (36)	621 cases (smokers and never smokers)	1,541 controls (smokers and never smokers)	Affymetrix [500,568]	3q29 C3orf21, 5p TERT-CLPTM1L
Hu 2011 (37)	2,331 cases (smokers and never smokers)	3,077 controls (smokers and never smokers)	Affymetrix [906,703]	3q28 TP63, 5p15 TERT-CLPTM1L, 13q12 MIPEP-TNFRSF19, 22q12 MTMR3-HORMAD2-LIF
Ahn 2012 (38)	446 never smokers	497 healthy controls	Affymetrix [906,703]	18p11 FAM38B
Dong 2012 (39)	833 cases with SCC	3,094 controls	Affymetrix [906,703]	12q23 SLC17A8-NR1H4
Lan 2012 (40)	5,510 never-smoking female lung cancer cases	4,544 controls	Various	3q28 TP63, 5p15, 6p21 HLA, 6q22 ROS1, DCBLD1, 10q25 VTI1A, 17q24 BPTF
Shiraishi 2012 (41)	1,722 cases (smokers and never smokers)	5,846 controls (smokers and never smokers)	Illumina [709,857]	3q28 TP63, 5p15 TERT, 6p21 BTNL2, 17q24 BPTF
Timofeeva 2012 (42)	Meta-analysis: 14,900 cases (smokers and never smokers)	29,485 controls (smokers and never smokers)	Various	5p15, 6p21, 15q25 for NSCLC; 9p21 for SCC
Kim 2013 (43)	285 female never smokers with lung cancer	1,455 controls	Affymetrix [440,794]	2p16 NRXN1

* In Tables 1 to 3, discovery study details have been included, and replication study samples sizes have not been included. For details, see http://www.genome.gov/gwastudies.

and also to address other lung cancer-related questions. A GWAS of lung cancer in never smokers in the USA, with replication cohorts, identified an association with SNPs at the 13q region, in the *GPC5* gene (34). Studies have also examined genome-wide association in never smokers in Asian populations (38,40,43), finding new susceptibility loci that were different to the loci found for Caucasian populations. SNPs in the *TERT* gene on 5p15 were associated with lung adenocarcinoma as a specific lung cancer histology (32), whereas SNPs at 9p21 are associated with risk for lung squamous cell carcinoma (SCC) (42). Genome-environment interaction was tested in a GWAS of lung cancer risk and self-reported asbestos exposure (45). Whilst this pilot study was not sufficiently powered to find significant differences, a suggested gene-asbestos exposure interaction was seen for SNPs in *C7orf54* on 7q32. In addition, a number of GWAS have identified SNPs that predict response to chemotherapy in patients with SCLC (46) and NSCLC (47), and other GWAS have explored SNPs related to prognosis and survival in patients with lung cancer [e.g., (48)].

Candidate genes for lung cancer susceptibility from GWAS

The discovery and replication studies from the GWAS (*Table 1*), and other replication studies since, have identified emerging patterns in candidate genes for lung cancer susceptibility [reviewed in (6,7,42)]. Consistent candidate genes for Caucasian smoking populations have been the neuronal nicotinic acetylcholine receptor (nAChR) subunits (cholinergic receptor, nicotinic, alpha 3 and 5: *CHRNA3* and *CHRNA5*) at 15q25. Neuronal nAChRs are activated by acetylcholine or nicotine, and comprise subunits (pentamers) of α and β subunits. In the lungs, nAChRs are expressed in neurones, and also non-neuronal cells, including bronchial epithelial cells and lung cancer cells. Whilst SNPs in nAChRs may alter risk of lung cancer through smoking behaviour, these SNPs could also regulate direct effects of nicotine, through anti-apoptotic and proliferative effects, or effects of nicotine-derived carcinogens in tobacco smoke (6,7,49-51).

On the 5p15 locus, SNPs in the *TERT* and *CLPTM1L* genes have been associated in a number of GWAS of lung cancer and other cancers (52). The *TERT* gene encodes human telomerase reverse transcriptase, which is important in the maintenance of telomere length. *CLPTM1L* (cleft lip and palate transmembrane protein 1-like protein) may induce apoptosis in lung cells (29). The identified SNPs in *TERT* have generally been intronic, and in linkage disequilibrium with SNPs in *CLPTM1L*. On 6p21, *BAT3* and *MSH5* have emerged as signals in a number of GWAS. *BAT3* (renamed *BAG6*, BCL2-associated athanogene 6) encodes a nuclear protein involved in DNA damage-induced apoptosis and modulation of p53 in response to genotoxic stress (7). The *MSH5* [mutS homolog 5 (E. coli)] gene is involved in DNA mismatch repair.

Whilst the 5p15 SNPs have demonstrated replication in both Caucasian and Asian populations, this has not been the case for the 15q SNPs (40,53). This discordance may represent differences in lung cancer aetiology between smoking and never smoking populations (particularly where indoor air pollution from biomass fuels may be the predominant carcinogen). In the never smokers, especially in Asian female populations, other candidate genes emerge, for example, that are involved in receptor tyrosine kinase activity [*ROS1*, c-ros oncogene 1, receptor tyrosine kinase (40)], vesicle transport [*VTI1A*, vesicle transport through interaction with t-SNAREs 1A (40)] and cell adhesion [*NRXN1*, neurexin 1 (43)]. Unexpectedly, the transcription factor TP63 (tumor protein p63) is also a candidate marker in the never smoking populations. *TP63* encodes a protein which is often used as an immunohistochemical marker of squamous cell carcinoma, a cancer strongly associated with tobacco smoking (54,55).

Many of these associations are novel for lung cancer susceptibility, and were not detected in previous candidate gene studies of lung cancer, which focused on metabolising enzymes [e.g., *CYP1A1* (56-58)], oxidative stress pathways and other DNA repair mechanisms (7).

GWAS and lung cancer pharmacogenetics

The GWAS approach is now being extended to examine other related phenotypes in lung cancer. For example, Han *et al*. recently undertook a GWAS of survival in small-cell lung cancer patients treated with irinotecan plus cisplatin chemotherapy, and identified candidate SNPs that may be predictive of the clinical outcome (59).

GWAS of smoking behaviour

Of relevance to lung cancer aetiology, GWAS of smoking behaviour have been performed in large population cohorts (*Table 2*), and have focused on smoking initiation, smoking quantity (cigarettes per day) and success of smoking cessation. The interest for lung cancer susceptibility is not

Table 2 GWAS of smoking behaviour (selected studies)

Study	Subjects	Arrays (nos. of SNPs)	Chromosomal regions and main associated genes
Liu 2010 (60)	41,150 from 20 cohorts	Various (>500,000)	15q CHRNA3 and CHRNA5 with smoking quantity
Tobacco and Genetics Consortium 2010 (61)	74,053 from 16 cohorts	Various (>500,000)	10q25 various genes,15q CHRNA3, 19q EGLN2 with smoking quantity; 11p BDNF with smoking initiation; 9q DBH with smoking cessation
Thorgeirsson 2010 (62)	31,266 and 46,481 subjects from cohorts	Various (>500,000)	8p CHRNB3, 15q CHRNA3 and CHRNA5, 19q CYP2A6 with smoking quantity
Siedlinski 2011 (63)	3,441 ever-smoking patients with COPD	Illumina (>500,000)	15q CHRNA3 and CHRNA5, 19q CYP2A6 with smoking quantity; 2q21 intergenic region, 6p21 HLA with smoking initiation; 9q DBH with smoking cessation

only for causation from smoking, but also for SNPs that are common to both smoking behaviour and lung cancer, that could have direct biological effects.

Smoking onset

Onset of smoking has been associated with the 11p region, with *BDNF* (brain-derived neurotrophic factor), a neurotropin, identified as a possible candidate gene (61). Other regions identified with age of smoking initiation in a study of patients with COPD were 6p21 (HLA) and 2q21 (intergenic region) (63).

Smoking quantity

The intensity of smoking (smoking quantity) has been consistently associated in GWAS with SNPs in the 15q25 region (60-65), at the loci of nAChR genes (especially *CHRNA3* and *CHRNA5*). Other loci include 19q (*CYP2A6*, a cytochrome P450 nicotine metabolising enzyme) (61-63, 66) and 8p (*CHRNB3*, a nAChR subunit) (62).

Smoking cessation

A range of biopsychosocial factors influence an individual's ability to abstain from smoking (67). Genetic factors for smoking cessation identified from GWAS have centred around SNPs in the 9q region, including *DBH* (dopamine beta-hydroxylase) (61,63), which is involved in the metabolism of dopamine.

GWAS of COPD

Lung cancer and COPD frequent coexist in at-risk smokers. Epidemiological evidence supports an association between the presence of COPD and increased risk of developing lung cancer. Common mechanisms for susceptibility to lung cancer and COPD, in addition to tobacco smoke, may involve biological processes such as inflammation, epithelial-mesenchymal transition, abnormal repair, oxidative stress, and cell proliferation. In addition, genomic and epigenomic changes are likely to alter biological pathways, leading to susceptibility to lung cancer and COPD (68,69). Therefore, studying genetic influences in COPD could yield greater insight into the shared pathogenesis of lung cancer and COPD. Importantly, genetic epidemiological principles should be considered when designing and interpreting studies of COPD and lung cancer, because of the shared aetiological and possibly genetic factors (70).

GWAS of susceptibility to COPD

A number of GWAS have been performed for COPD (*Table 3*), albeit a smaller number of studies than the lung cancer GWAS to date.

GWAS have so far been undertaken in the Framingham cohort (71); Bergen (Norway) COPD Cohort (with replication in other cohorts) (72); combined Bergen cohort with National Emphysema Treatment Trial (NETT) and Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study subjects (73); combined four cohorts [ECLIPSE, Normative Aging Study, Bergen (Norway) COPD Cohort and COPD Gene] (74); and a combination of 15 cohorts (75).

Candidate genes for COPD from GWAS

Several regions significantly associated with COPD have

Table 3 GWAS of COPD *vs.* controls

Study	COPD cases (discovery)	Controls (discovery)	Arrays (nos. of SNPs)	Chromosomal regions and main associated genes
Wilk 2009 (71)	7,691 Framingham study participants, plus replication cohort		Affymetrix (550,000)	4q31 HHIP
Pillai 2009 (72)	823 COPD	810 smokers	Illumina (561,466)	4q31 HHIP, 15q CHRNA3, CHRNA5
Cho 2010 (73)	2,940 COPD	1,380 smokers	Various (>500,000)	4q24 FAM13A
Cho 2012 (74)	3,499 COPD	1,922 controls	Illumina (>500,000)	4q24 FAM13A, 19q13 RAB4B, EGLN2, CYP2A6
Wilk 2012 (75)	3,368 COPD, plus replication cohort	29,507 controls	Various (>500,000)–meta-analysis	5q HTR4; 15q AGPHD1, IREB2, CHRNA5, CHRNA3

emerged from the COPD GWAS to date (*Table 3*). At the 15q locus (similar to the GWAS for lung cancer and for smoking behaviour), SNPs in the nAChR subunit genes (*CHRNA5*, *CHRNA3*) were associated with COPD (72,75), possibly indicating a link with smoking intensity as an aetiological factor, although direct effects should also be considered (76). Similarly, association at 19q13 may be related to smoking behaviour [e.g., *CYP2A6* (74)].

Other novel associations have been found for COPD in these GWAS. At 4q31, hedgehog interacting protein (*HHIP*) has been identified as a candidate in two studies (71,72). *HHIP* encodes a membrane glycoprotein that is an inhibitor of hedgehog signalling, which is involved in development processes. Gene expression studies in BEAS-2B bronchial epithelial cell lines implicate HHIP in extracellular matrix and cell proliferation (77). At 4q24, *FAM13A* (family with sequence similarity 13, member A) has been detected in two GWAS (73,74), and also in a genetic association in which SNPs were related to both COPD and lung cancer, indicating a possible shared genetic pathway (78). *FAM13A* contains a Rho GTPase-activating protein-binding domain, inhibits signal transduction and responds to hypoxia; however, its full function in the lung remains to be determined. At the 5q region, *HTR4* [5-hydroxytryptamine (serotonin) receptor 4] was associated with COPD in smokers; its function in airways disease may involve regulation of cytokine release (75).

Summary and clinical implications

Main findings to date from GWAS

Whilst lung cancer is predominantly caused by cigarette smoking, a genetic component to susceptibility is well-recognised in epidemiological studies. GWAS have now been completed in lung cancer and the related phenotypes of smoking behaviour and COPD. Large scale, multi-cohort GWAS of lung cancer in mainly Caucasian, smoking populations have identified strong associations for lung cancer mapped to chromosomal regions 15q (*CHRNA3*, *CHRNA5*), 5p (*TERT-CLPTM1L* locus) and 6p (*BAT3-MSH5*). Some studies in Asian populations of smokers have found similar risk loci, whereas other GWAS, particularly in never smoking Asian females, have identified associations in other chromosomal regions that are distinct from the smoking-related genetic loci. GWAS of smoking behaviour have identified risk loci for smoking quantity at 15q (similar genes to lung cancer susceptibility: *CHRNA3*, *CHRNA5*) and also at 19q (*CYP2A6*). Other genes have been mapped for smoking initiation and smoking cessation. In COPD, GWAS in large cohorts have also found NAChR SNPs mapping at 15q as risk loci, as well as other regions at 4q31 (*HHIP*), 4q24 (*FAM13A*) and 5q (*HTR4*). The overlap in risk loci between lung cancer, smoking behaviour and COPD may be due to the effects of nicotine addiction; However, more work needs to be undertaken to explore the potential direct effects of nicotine and its metabolites in gene-environment interaction in these phenotypes.

Applications and future directions

From the evidence presented to date, GWAS have been useful not only in addressing genetic influences in lung cancer susceptibility, but also gene-environment interaction in terms of smoking as causation, as well as COPD as a risk factor for lung cancer. The translation of findings from the lung cancer and related GWAS could in the future enable profiling of an individual's risk of lung cancer, biomarkers for diagnosis, and markers for prognosis and therapy.

The challenge now will be to combine genomics (79), epigenomics (80), transcriptomics (81-83) and proteomics profiling to improve the management of patients with lung cancer and related comorbidities (68). Future studies should include DNA sequencing of lung tumours and lung tissue, and network analysis of genomic information and clinical phenotypes (3,84,85). Goals of future genetic susceptibility studies of lung cancer should now be focused on refining the strongest risk loci in a wide range of populations with lung cancer, and integrating other clinical and biomarker information, in order to achieve the aim of personalised therapy for lung cancer (4), through enhanced diagnosis, prognosis, prevention and management.

Acknowledgements

This work was supported by Asthma, Allergy & Inflammation Research (AAIR) (JWH), NHMRC Career Development Fellowship (IY), NHMRC Practitioner Fellowship (KF), and project grants from NHMRC, Queensland Health and Medical Research, The Prince Charles Hospital Foundation, and Cancer Council Queensland.
Disclosure: The authors declare no conflict of interest.

References

1. Thun MJ, Lally CA, Flannery JT, et al. Cigarette smoking and changes in the histopathology of lung cancer. J Natl Cancer Inst 1997;89:1580-6.
2. Mathers C, Vos T. The burden of disease and injury in australia-summary report. Canberra: Australian Institute of Health and Welfare, 1999.
3. Sriram KB, Larsen JE, Yang IA, et al. Genomic medicine in non-small cell lung cancer: paving the path to personalized care. Respirology 2011;16:257-63.
4. Fong KM, Yang IA, Bowman RV. Personalized medicine for lung cancer. Lung Cancer Management 2012;1:83-6.
5. Sriram KB, Tan ME, Savarimuthu SM, et al. Screening for activating EGFR mutations in surgically resected nonsmall cell lung cancer. Eur Respir J 2011;38:903-10.
6. Brennan P, Hainaut P, Boffetta P. Genetics of lung-cancer susceptibility. Lancet Oncol 2011;12:399-408.
7. Marshall AL, Christiani DC. Genetic susceptibility to lung cancer--light at the end of the tunnel? Carcinogenesis 2013;34:487-502.
8. Jonsson S, Thorsteinsdottir U, Gudbjartsson DF, et al. Familial risk of lung carcinoma in the Icelandic population. JAMA 2004;292:2977-83.
9. Sellers TA, Chen PL, Potter JD, et al. Segregation analysis of smoking-associated malignancies: evidence for Mendelian inheritance. Am J Med Genet 1994;52:308-14.
10. Czene K, Lichtenstein P, Hemminki K. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. Int J Cancer 2002;99:260-6.
11. Braun MM, Caporaso NE, Page WF, et al. A cohort study of twins and cancer. Cancer Epidemiol Biomarkers Prev 1995;4:469-73.
12. Tyndale RF. Genetics of alcohol and tobacco use in humans. Ann Med 2003;35:94-121.
13. Lorenzo Bermejo J, Hemminki K. Familial lung cancer and aggregation of smoking habits: a simulation of the effect of shared environmental factors on the familial risk of cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1738-40.
14. Peddle L, Rahman P. Genetic epidemiology of complex phenotypes. Methods Mol Biol 2009;473:187-201.
15. Bailey-Wilson JE, Amos CI, Pinney SM, et al. A major lung cancer susceptibility locus maps to chromosome 6q23-25. Am J Hum Genet 2004;75:460-74.
16. Schwartz AG, Ruckdeschel JC. Familial lung cancer: genetic susceptibility and relationship to chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006;173:16-22.
17. International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437:1299-320.
18. Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. Science 2008;322:881-8.
19. McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008;9:356-69.
20. Amos CI. Successful design and conduct of genome-wide association studies. Hum Mol Genet 2007;16 Spec No. 2:R220-5.
21. Lam AC, Powell J, Wei WH, et al. A combined strategy for quantitative trait loci detection by genome-wide association. BMC Proc 2009;3 Suppl 1:S6.
22. Frazer KA, Murray SS, Schork NJ, et al. Human genetic variation and its contribution to complex traits. Nat Rev Genet 2009;10:241-51.
23. Zhang R, Zhao Y, Chu M, et al. Pathway analysis for genome-wide association study of lung cancer in Han Chinese population. PLoS One 2013;8:e57763.
24. Spinola M, Leoni VP, Galvan A, et al. Genome-wide single nucleotide polymorphism analysis of lung cancer risk detects the KLF6 gene. Cancer Lett 2007;251:311-6.

25. Amos CI, Wu X, Broderick P, et al. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nat Genet 2008;40:616-22.
26. Hung RJ, McKay JD, Gaborieau V, et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 2008;452:633-7.
27. Liu P, Vikis HG, Wang D, et al. Familial aggregation of common sequence variants on 15q24-25.1 in lung cancer. J Natl Cancer Inst 2008;100:1326-30.
28. Thorgeirsson TE, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature 2008;452:638-42.
29. McKay JD, Hung RJ, Gaborieau V, et al. Lung cancer susceptibility locus at 5p15.33. Nat Genet 2008;40:1404-6.
30. Wang Y, Broderick P, Webb E, et al. Common 5p15.33 and 6p21.33 variants influence lung cancer risk. Nat Genet 2008;40:1407-9.
31. Broderick P, Wang Y, Vijayakrishnan J, et al. Deciphering the impact of common genetic variation on lung cancer risk: a genome-wide association study. Cancer Res 2009;69:6633-41.
32. Landi MT, Chatterjee N, Yu K, et al. A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. Am J Hum Genet 2009;85:679-91.
33. Hsiung CA, Lan Q, Hong YC, et al. The 5p15.33 locus is associated with risk of lung adenocarcinoma in never-smoking females in Asia. PLoS Genet 2010;6:e1001051.
34. Li Y, Sheu CC, Ye Y, et al. Genetic variants and risk of lung cancer in never smokers: a genome-wide association study. Lancet Oncol 2010;11:321-30.
35. Miki D, Kubo M, Takahashi A, et al. Variation in Tp63 Is Associated with Lung Adenocarcinoma Susceptibility in Japanese and Korean Populations. Nat Genet 2010;42:893-6.
36. Yoon KA, Park JH, Han J, et al. A genome-wide association study reveals susceptibility variants for non-small cell lung cancer in the Korean population. Hum Mol Genet 2010;19:4948-54.
37. Hu Z, Wu C, Shi Y, et al. A genome-wide association study identifies two new lung cancer susceptibility loci at 13q12.12 and 22q12.2 in Han Chinese. Nat Genet 2011;43:792-6.
38. Ahn MJ, Won HH, Lee J, et al. The 18p11.22 locus is associated with never smoker non-small cell lung cancer susceptibility in Korean populations. Hum Genet 2012;131:365-72.
39. Dong J, Jin G, Wu C, et al. Genome-wide association study identifies a novel susceptibility locus at 12q23.1 for lung squamous cell carcinoma in han chinese. PLoS Genet 2013;9:e1003190.
40. Lan Q, Hsiung CA, Matsuo K, et al. Genome-wide association analysis identifies new lung cancer susceptibility loci in never-smoking women in Asia. Nat Genet 2012;44:1330-5.
41. Shiraishi K, Kunitoh H, Daigo Y, et al. A genome-wide association study identifies two new susceptibility loci for lung adenocarcinoma in the Japanese population. Nat Genet 2012;44:900-3.
42. Timofeeva MN, Hung RJ, Rafnar T, et al. Influence of common genetic variation on lung cancer risk: meta-analysis of 14 900 cases and 29 485 controls. Hum Mol Genet 2012;21:4980-95.
43. Kim JH, Park K, Yim SH, et al. Genome-wide association study of lung cancer in korean non-smoking women. J Korean Med Sci 2013;28:840-7.
44. Dong J, Hu Z, Wu C, et al. Association analyses identify multiple new lung cancer susceptibility loci and their interactions with smoking in the Chinese population. Nat Genet 2012;44:895-9.
45. Wei S, Wang LE, McHugh MK, et al. Genome-wide gene-environment interaction analysis for asbestos exposure in lung cancer susceptibility. Carcinogenesis 2012;33:1531-7.
46. Wu C, Xu B, Yuan P, et al. Genome-wide examination of genetic variants associated with response to platinum-based chemotherapy in patients with small-cell lung cancer. Pharmacogenet Genomics 2010;20:389-95.
47. Wu X, Ye Y, Rosell R, et al. Genome-wide association study of survival in non-small cell lung cancer patients receiving platinum-based chemotherapy. J Natl Cancer Inst 2011;103:817-25.
48. Lee Y, Yoon KA, Joo J, et al. Prognostic implications of genetic variants in advanced non-small cell lung cancer: a genome-wide association study. Carcinogenesis 2013;34:307-13.
49. West KA, Brognard J, Clark AS, et al. Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. J Clin Invest 2003;111:81-90.
50. Tournier JM, Birembaut P. Nicotinic acetylcholine receptors and predisposition to lung cancer. Curr Opin Oncol 2011;23:83-7.
51. Al-Wadei HA, Al-Wadei MH, Schuller HM. Cooperative regulation of non-small cell lung carcinoma by nicotinic and beta-adrenergic receptors: a novel target for intervention. PLoS One 2012;7:e29915.

52. Mocellin S, Verdi D, Pooley KA, et al. Telomerase reverse transcriptase locus polymorphisms and cancer risk: a field synopsis and meta-analysis. J Natl Cancer Inst 2012;104:840-54.
53. Truong T, Hung RJ, Amos CI, et al. Replication of lung cancer susceptibility loci at chromosomes 15q25, 5p15, and 6p21: a pooled analysis from the International Lung Cancer Consortium. J Natl Cancer Inst 2010;102:959-71.
54. Khayyata S, Yun S, Pasha T, et al. Value of P63 and CK5/6 in distinguishing squamous cell carcinoma from adenocarcinoma in lung fine-needle aspiration specimens. Diagn Cytopathol 2009;37:178-83.
55. Zhang P, Han Y, Huang L, et al. Expression and Clinical Significance of TTF-1 and p63 in NSCLC. Zhongguo Fei Ai Za Zhi 2009;12:995-9.
56. Larsen JE, Colosimo ML, Yang IA, et al. Risk of non-small cell lung cancer and the cytochrome P4501A1 Ile462Val polymorphism. Cancer Causes Control 2005;16:579-85.
57. Larsen JE, Colosimo ML, Yang IA, et al. CYP1A1 Ile462Val and MPO G-463A interact to increase risk of adenocarcinoma but not squamous cell carcinoma of the lung. Carcinogenesis 2006;27:525-32.
58. Wright CM, Larsen JE, Colosimo ML, et al. Genetic association study of CYP1A1 polymorphisms identifies risk haplotypes in nonsmall cell lung cancer. Eur Respir J 2010;35:152-9.
59. Han JY, Lee YS, Shin ES, et al. A genome-wide association study of survival in small-cell lung cancer patients treated with irinotecan plus cisplatin chemotherapy. Pharmacogenomics J 2014;14:20-7.
60. Liu JZ, Tozzi F, Waterworth DM, et al. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. Nat Genet 2010;42:436-40.
61. Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat Genet 2010;42:441-7.
62. Thorgeirsson TE, Gudbjartsson DF, Surakka I, et al. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. Nat Genet 2010;42:448-53.
63. Siedlinski M, Cho MH, Bakke P, et al. Genome-wide association study of smoking behaviours in patients with COPD. Thorax 2011;66:894-902.
64. Saccone NL, Culverhouse RC, Schwantes-An TH, et al. Multiple independent loci at chromosome 15q25.1 affect smoking quantity: a meta-analysis and comparison with lung cancer and COPD. PLoS Genet 2010;6:e1001053.
65. David SP, Hamidovic A, Chen GK, et al. Genome-wide meta-analyses of smoking behaviors in African Americans. Transl Psychiatry 2012;2:e119.
66. Kumasaka N, Aoki M, Okada Y, et al. Haplotypes with copy number and single nucleotide polymorphisms in CYP2A6 locus are associated with smoking quantity in a Japanese population. PLoS One 2012;7:e44507.
67. Fung PR, Snape-Jenkinson SL, Godfrey MT, et al. Effectiveness of hospital-based smoking cessation. Chest 2005;128:216-23.
68. Yang IA, Relan V, Wright CM, et al. Common pathogenic mechanisms and pathways in the development of COPD and lung cancer. Expert Opin Ther Targets 2011;15:439-56.
69. Houghton AM. Mechanistic links between COPD and lung cancer. Nat Rev Cancer 2013;13:233-45.
70. El-Zein RA, Young RP, Hopkins RJ, et al. Genetic predisposition to chronic obstructive pulmonary disease and/or lung cancer: important considerations when evaluating risk. Cancer Prev Res (Phila) 2012;5:522-7.
71. Wilk JB, Chen TH, Gottlieb DJ, et al. A genome-wide association study of pulmonary function measures in the Framingham Heart Study. PLoS Genet 2009;5:e1000429.
72. Pillai SG, Ge D, Zhu G, et al. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. PLoS Genet 2009;5:e1000421.
73. Cho MH, Boutaoui N, Klanderman BJ, et al. Variants in FAM13A are associated with chronic obstructive pulmonary disease. Nat Genet 2010;42:200-2.
74. Cho MH, Castaldi PJ, Wan ES, et al. A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. Hum Mol Genet 2012;21:947-57.
75. Wilk JB, Shrine NR, Loehr LR, et al. Genome-wide association studies identify CHRNA5/3 and HTR4 in the development of airflow obstruction. Am J Respir Crit Care Med 2012;186:622-32.
76. Bossé Y. Updates on the COPD gene list. Int J Chron Obstruct Pulmon Dis 2012;7:607-31.
77. Zhou X, Qiu W, Sathirapongsasuti JF, et al. Gene expression analysis uncovers novel hedgehog interacting protein (HHIP) effects in human bronchial epithelial cells. Genomics 2013;101:263-72.
78. Young RP, Hopkins RJ, Hay BA, et al. FAM13A locus in COPD is independently associated with lung cancer - evidence of a molecular genetic link between COPD and lung cancer. Appl Clin Genet 2010;4:1-10.
79. Sriram KB, Larsen JE, Savarimuthu Francis SM, et al. Array-comparative genomic hybridization reveals loss of SOCS6 is associated with poor prognosis in primary lung squamous cell carcinoma. PLoS One 2012;7:e30398.

80. Davidson MR, Larsen JE, Yang IA, et al. Microrna-218 Is Deleted and Downregulated in Lung Squamous Cell Carcinoma. PLoS ONE 2010;5:e12560.
81. Larsen JE, Pavey SJ, Bowman R, et al. Gene expression of lung squamous cell carcinoma reflects mode of lymph node involvement. Eur Respir J 2007;30:21-5.
82. Larsen JE, Pavey SJ, Passmore LH, et al. Expression profiling defines a recurrence signature in lung squamous cell carcinoma. Carcinogenesis 2007;28:760-6.
83. Larsen JE, Pavey SJ, Passmore LH, et al. Gene expression signature predicts recurrence in lung adenocarcinoma. Clin Cancer Res 2007;13:2946-54.
84. Daniels M, Goh F, Wright CM, et al. Whole genome sequencing for lung cancer. J Thorac Dis 2012;4:155-63.
85. Silverman EK. Perspective: how can genetics help? Nature 2012;489:S7.

Cite this article as: Yang IA, Holloway JW, Fong KM. Genetic susceptibility to lung cancer and co-morbidities. J Thorac Dis 2013;5(S5):S454-S462. doi: 10.3978/j.issn.2072-1439.2013.08.06

Non-neuronal cholinergic system in airways and lung cancer susceptibility

Laura Saracino[1], Michele Zorzetto[1], Simona Inghilleri[1], Ernesto Pozzi[2], Giulia Maria Stella[1]

[1]Laboratory of Biochemistry & Genetics, Division of Pneumology, University and Fondazione IRCCS Policlinico San Matteo, Pavia 27100, Italy; [2]Policlinico di Monza, University of Pavia, Monza 20025, Italy

Correspondence to: Giulia Maria Stella. Laboratory of Biochemistry & Genetics, Division of Pneumology, University and Fondazione IRCCS Policlinico San Matteo, Pavia 27100, Italy. Email: g.stella@smatteo.pv.it.

Abstract: In the airway tract acetylcholine (ACh) is known to be the mediator of the parasympathetic nervous system. However ACh is also synthesized by a large variety of non-neuronal cells. Strongest expression is documented in neuroendocrine and in epithelial cells (ciliated, basal and secretory elements). Growing evidence suggests that a cell-type specific Ach expression and release do exist and act with local autoparacrine loop in the non-neuronal airway compartment. Here we review the molecular mechanism by which Ach is involved in regulating various aspects of innate mucosal defense, including mucociliary clearance, regulation of macrophage activation as well as in promoting epithelial cells proliferation and conferring susceptibility to lung carcinoma onset. Importantly this non-neuronal cholinergic machinery is differently regulated than the neuronal one and could be specifically therapeutically targeted.

Keywords: Cholinergic system; non-neuronal compartment; innate defense; cancer susceptibility; actionable target

Submitted Jun 04, 2013. Accepted for publication Jun 05, 2013.
doi: 10.3978/j.issn.2218-6751.2013.06.01
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.06.01

Introduction

Acetylcholine (ACh) is one of the main regulators of airway function and one of the most powerful known bronchoconstrictors and stimulators of secretion. However it is also involved in regulating less acute mechanisms, such as airways remodeling which takes place in pathologic settings and in response to immunomodulation (1). As a consequence, pharmacological manipulation of cholinergic signaling—mostly inhibitory—is a key option among treatments of common lung diseases, such as chronic obstructive pulmonary disease (COPD) and asthma. On the other hand, it is now well documented that various non-neuronal cells are capable of ACh synthesis and release (e.g., keratinocytes, lymphocytes, placental trophoblast and endothelial cells) and that non-neuronal ACh is also present in the airway epithelium where it is believed to regulate cell proliferation (2) (*Table 1*).

On the airway surface, at least twelve types of epithelial cells can be identified whereas other five types can be found in the airway glands; among them differentiating or intermediate elements can be identified too. The most important function of the respiratory epithelium is mediated by the ciliated cells which provide the driving force for mucociliary clearance, namely the cleaning of the airway surface from inhaled particles by transporting a mucous layer towards the larynx. Ciliated cells constitute form 32% to 55% of tracheal epithelial cells (3). They feature columnary structure in the trachea and large bronchi (approximately 20 μm long and 7 μm wide) whereas decrease in height towards small bronchi and bronchioli. They also structure microvilli that protrude into the lumen. Notably on their surface compartment is located the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which—when structurally altered in consequence to point mutations affecting the corresponding coding gene—is

Table 1 Spectrum of biological effects mediated by Ach at airways level

Target cells	Effects	
	Stimulus	Inibition
Epithelial cells	Proliferation	
	Mucous secretion	
	IL-8 release	
	Frequency of ciliary beat	
	MAPKs activation	
Mucous glands	Secretion	
Neutrophils, Magrophages, T lymphocytes	Inflammation	Inflammation
	T cell differentiation	IL-8, TNFa, co-stimulatory molecules inibition
	LT-B4 release	
	Chemotaxis	
Fibroblasts	Proliferation	Myofibroblasts differentiation Fibronectin release
	IL-8, MMP2 release	
	Collagen synthesis	
Smooth muscolar cells	Contraction	
	Collagen proteins expression	
	Mitosis	

MAPK, mitogen-activated protein kinase; LT- B4, leukotriene B4; MMP2, matrix metallopeptidase 2 (gelatin A).

causally linked to cystic fibrosis onset. Submucosal glands producing the carbohydrate-rich glycoproteins ('mucins') and lipids of the mucous layer are known as 'non-ciliated cells'. Classical goblet cells account for 9% in the human trachea and are almost absent from human distal bronchioli (3). Smaller airways contain cells with protruding apical region harboring few microvilli, abundant smooth or rough endoplasmic reticulum, and secretory granules smaller than those found in goblet cells: in distal airways they identified the Clara cells. Basal cells account approximately for 30% of tracheal epithelial cells and are found in larger airways only. Finally, there are—rather infrequent—specialized epithelial cells that are capable of producing conspicuous amounts of acetylcholine; among these there are neuroendocrine cells that origin from different precursors from those of other epithelial cells. They generally occur solitarily in the airway epithelium, or clustered as neuroepithelial bodies (NEBs) preferentially located at bronchial branching (4). They contain, in their basal compartment, dense core granules, mainly composed by bioactive amines and neuropeptides. NEBs prevail in embryo and during the neonatal period and, by functioning as oxygen sensors, are correlated with lung maturation (5). The oxygen sensor function, however, has not been completely established yet; however more recent evidence suggests that NEBs are myelinated vagal afferents belonging to the subpopulation of the myelinated mechano-sensitive vagal airway receptors (6). The role of solitary pulmonary neuroendocrine cells is still partially unclear, probably they are involved in fetal and newborn lung development including regulation of branching morphogenesis, cellular growth and maturation. It has been demonstrated that, in adult mice, they are associated with stem cell niches in proximal and distal airways, and it has been proposed that they contribute to the protection of stem cells from environmental agents thus promoting stem cell renewal (7). Finally, an equally infrequent cell type is represented by the so-called 'brush cells' (characterized by an apical brush of microvilli) (8). Tracheobronchial brush cells express components of the taste-signaling cascade and are hence considered chemosensory cells. It has also been proposed that they may sense bacterial colonization and are useful to initiate defense mechanisms (9).

ACh synthesis and recycling

Cholinergic nerve fibers

Acetylcholine is synthesized in the axoplasm by choline

acetyltransferase (ChAT) from choline—taken up from the extracellular space—and acetyl coenzyme A (acetyl-CoA) which is produced in mitochondria. This uptake represents the rate-limiting step in neuronal ACh synthesis and it is realized through a high-affinity choline transporter (CHT1) (10). Once generated in the axoplasm, the ACh is stocked into small synaptic vesicles. The process is mediated by the vesicular acetylcholine transporter (VAChT), a twelve transmembrane domain protein which acts as a H^+/ACh exchanger. Inside each vesicle there are up to 10,000 molecules of ACh bound in a matrix enriched with proteoglycan SV2. The depolarization of the nerve terminal triggers exocytotic release of ACh from the vesicles into the extracellular space; once there, ACh is able to interact with two classes of cholinergic receptors: (I) metabotropic muscarinic receptors (MR): G protein-coupled receptors with seven transmembrane domains, having five different isoforms (M1-M5); (II) ionotropic nicotinic acetylcholine receptors (nAChR) which are cationic channels having two ACh binding sites and structured as hetero- or homopentamers (11,12). The action of ACh terminates quite rapidly being spatially limited by cleavage into acetate and choline via the acetylcholine-esterase (AChE). This efficient enzyme is synthesized by cholinergic neurons themselves and guarantees equilibrium between ACh production and its degrading capacity. Choline is thus taken up again at the nerve terminal via CHT1, and a new cycle of ACh synthesis and release is to begin (1).

Non-neuronal cells

Non-neuronal ACh synthesis system identifies a philogenetically old process which is detectable also in bacteria and plants (1,13). Indeed some of the enzymes and transporters of cholinergic neurons have evolved relatively recently and cannot be found in the non-neuronal cholinergic system. Each cell includes an uptake mechanism for choline which represents a necessary element for the synthesis of plasma membrane lipids, in particular phosphatidylcholine. There are a number of plasma membrane choline transporters, but only few cholinergic non-neuronal cells are capable of expressing the high-affinity choline transporter CHT1. An alternative way for ACh synthesis is provided by carnitine acetyltransferase (CarAT) which, although in principle less efficient than ChAT, plays a key role in ACh synthesis in skeletal muscle fibres (14). VAChT and vesicular storage mechanisms for ACh, in non-neuronal cholinergic cells, are still unclear, but it is widely accepted that they do not imply exocytosis. In fact, there is evidence of ACh release via plasma membrane-bound polyspecific organic cation transporters (OCTs) (15). These electrogenic transporters are bidirectional and their driving forces are represented by substrate concentration and membrane potential. A proteolipid known as 'mediatophore' is also involved in the release of ACh, either directly from the cytoplasm or by forming the fusion pore between the synaptic vesicle and the plasma membrane. It is part of the vacuolar H^+-ATPase (V-ATPase, V_0 subunit c) that is predominantly targeted to acidic organelles such as lysosomes, endosomes and secretory vesicles (16,17). V-ATPase complex is localized to the plasma membrane in human lung microvascular endothelial cells, so that 'mediatophore' could mediate ACh release from these cells (18). Once released, ACh can be cleaved by esterases which are less specific than AChE; among them the most important is butyrylcholinesterase (BChE) (19).

Choline transporters in the airway epithelium

It has been demonstrated that several systems of choline uptake and transport can be detected, featuring a cell-specific distribution. For instance, the high-affinity choline transporter CHT1, known from the nervous system, is localized to the apical membrane of the ciliated cells in the rat trachea (20). This finding has been validated by *in situ*-hybridization, Western blotting of abraded tracheal epithelium, and RT-PCR of tracheal epithelium obtained by laser-assisted microdissection which have led to the identification of a molecule featuring the same biochemical properties and immunophenotype of CHT1. Overall these data put in evidence the existence of high affinity uptake of choline from the airway lining fluid into ciliated cells via a transport system that has been originally thought to be specific for neurons; nevertheless it remains still unclear how other epithelial cholinergic cells, lacking CHT1, are capable of ACh synthesis. In fact, airway epithelial cells express additional choline transport systems, which can work alone or in parallel with CHT1 in specific cell types. For example, A549 cells (human lung adenocarcinoma cells) co-express, in addition to CHT1, a sodium-independent choline transport system, that relies on a transmembrane H^+ gradient and which is sensitive to amiloride (21).

Apart from CHT1, choline transporters can be classified into two large families: choline-specific transporter-like proteins (CTL family) and polyspecific organic cation transporters (OCT family); members of both families are

expressed in the lung. CTL1, the most relevant member of the CTL family, detectable by Western blotting in human lung extracts, is expressed in A549 cells and participates predominantly to choline uptake in this cell line (22). Among the polyspecific OCT family members, OCT1 and OCT2 (but not OCT3) do transport choline: OCT1 is expressed in the mouse, rat and human bronchial epithelium, and immunohistochemical stain has showed a predominant localization in the apical membrane of ciliated cells (23). OCT1 expression, in the airway epithelium, is cell type-specific: rat studies confirmed that OCT1-immunoreactivity is selective for ciliated cells but absent in secretory, brush and basal cells (23).

On the other side, OCT2 is expressed in human, but not in mouse, bronchial epithelium. In human bronchi, OCT2-immunoreactivity is predominant in the luminal membrane of ciliated cells, rarely found in basal cell membranes, and absent from goblet cells (23). In OCT1/OCT2 double-knockout mice, tracheal epithelial ACh content is quite elevated instead of being, as expected, reduced; thus, despite OCT1 and OCT2 are capable of choline translocation across the plasma membrane, they are not crucial for providing choline for epithelial ACh synthesis (23). In conclusion, there is a multiplicity of choline uptake systems in airway epithelial cells with a cell type-specific distribution and a distinct apical *vs.* basolateral polarization.

ACh synthesis in the airway epithelium

Notwithstanding the presence of choline acetyltransferase enzyme (ChAT—responsible for the production of Ach—has been undoubtedly documented), the real identity of the ACh synthesizing enzyme in the individual airway epithelial cell types is nowadays not completely known. First, it is important to underline that there is a great diversity among ChAT variants, although they are all encoded by the same gene. These differences are so marked that the various ChAT variants react with different antisera. The mammalian ChAT gene contains three non-coding exons (termed R-, M- and N-exon in mouse and rat models) and, depending on species, 15-16 coding exons. The sequence encoding for VAChT is inserted between the two not transduced exons R- and N-. This peculiar gene structure codifying for ChAT and VAChT is known as 'cholinergic gene locus' (24). Multiple transcripts derive from alternative splicing processes; a numbers of these variants can be found in mouse and rat models, and at least six are known in humans. In the central nervous system, all of these variants are expressed with the M-type ChAT-mRNA usually dominating, while in the bronchial epithelium, expression of non-coding exons M- (in rat), N- and S- (in monkey) has been identified. Different ChAT protein variants can also result from alternative splicing in the coding region. For example, in central nervous system, in addition to the 69 kDa (cChAT), a protein deriving from the removal of 6-9 coding exons can be identified: this form is prevalent in peripheral autonomic neurons (pChAT); whereas, in the rat tracheal epithelium, only the complete form of the enzyme has been detected. These findings have been confirmed by immunohistochemistry studies, that have documented the presence of cChAT in all epithelial cell types of trachea; in more distal airways, cChAT-immunolabelling of ciliated and secretory cells was generally less intense than in the trachea, whereas endocrine cells and brush cells were particularly cChAT-immunolabelled (25,26). Within tracheal ciliated cells, a more intense labeling of the apical cytoplasmatic region was registered: this evidence suggest an earlier ACh synthesis in trachea then in distal bronchi. In these cells cChAT is located close to the high-affinity uptake system for choline CHT1. The latter allows the concentration of the entire ACh synthesizing machinery at the apical side of the ciliated cell thus suggesting its luminal release. These data altogether point to a rather homogenous expression of a single variant of ChAT (ChAT translated from M-type ChAT m-RNA) in various airway epithelial cell types. There are, however, several data on protein level that indicate a more complicated situation. In particular, in bronchial epithelial extracts, ChAT-labeling of human bronchial epithelium with an antibody that recognized 54 and 41 kDa proteins has been reported (27). These controversial findings cannot be entirely justified through a possible cross reaction with a closely related or even unrelated protein, but, in this regard, several detailed studies are still in progress.

Mechanisms of ACh release in the airway epithelium

In neurons, VAChT shuffles ACh from the axoplasm into synaptic vesicles. The particular 'cholinergic gene locus' plays a key role in orchestrating the coordinate expression of ChAT and VAChT thus balancing production and release of ACh. In the airway epithelium, VAChT labeling has been demonstrated by immunohistochemistry in trachea and bronchial neuroepithelial and secretory cells, as well. Human small cell lung carcinoma cell lines, derived

from airway neuroendocrine cells, express VAChT along with ChAT, and ACh release from these cells is sensitive to vesamicol (a VAChT inhibitor) (28). Ciliated cells, however, seem to utilize a non-vesicular ACh release mechanism. OCT1 and OCT2 are localized at the apical membrane of ciliated airway epithelial cells while OCT3 is concentrated at the basolateral membrane of several cell types. This distribution permit to hypothesize that direction (release or uptake) of ACh is determined by concentration gradient and membrane potential. The apical localization of OCT1 and OCT2 in airway ciliated cell suggests a complete cycle of ACh synthesis, release and reuptake of choline between the ciliated cell and the luminal airway lining fluid.

On the other hand, the role of OCT3 is less clear: probably, OCT3 requires expression of additional proteins to serve as an ACh transporter (23). These polyspecific transporters are the target of a number of drugs which either compete with transport of other cations or block transport without being transported themselves. Very important for airway pharmacology, nicotine and corticosteroids (corticosterone, fluticasone, and budesonide) could block ACh release by OCT1 and OCT2 *in vitro*. Consequently, inhibition of non-neuronal ACh release is a non-genomic effect of corticosteroids that clearly discriminates non-neuronal from neuronal cholinergic mechanisms in the airways (29). Within respect to 'mediatophore', its occurrence and distribution in the airway epithelium has not been deeply investigated yet. Altogether, the currently available data suggest that ACh release in the respiratory epithelium can occur through: (I) vesicular basal release by neuroendocrine and possibly brush cells; (II) vesicular luminal release by secretory cells or (III) apical concentration- and membrane potential-driven transmembrane release from the cytoplasm of ciliated cells.

Mechanisms of ACh degradation in the airway epithelium

As discussed above, in the nervous system the cholinergic signaling finishes at short distances from the site of ACh release since ACh is divided into acetate and choline through the action of the AChE. A number of other esterases, among which BChE, also coexist. The high speed of AChE effect allows some considerations. First it should be noted that the amount of the ACh generated in the airways is much less than that one produced at nervous system level and that the intraluminal ACh release takes place mainly through transmembrane mechanisms and not by exocytosis. As a consequence several doubt emerged regarding the real extracellular effect of that ACh which is released by the non-neuronal cholinergic system localized in the airways. Besides it is coincevable that ACh could display intracellular effects, mainly mediated by intracytoplasmic receptors. On the other hand, ACh degradation capacity in the airways is lower than that in the nervous system, since it is mainly mediated by the BChE enzyme. These data are coherent with a potential paracrine/autocrine loop of ACh on epithelial cells (1,30). Indeed immunohistochemical studies confirm that ACh activity prevails in the nervous fibers among smooth muscles, whereas BChE is directly detectable into smooth muscle cells. Thus, although the mechanisms of ACh degradation in the airways has not been yet completely elucidated, preliminary data allow to hypothesize that, in this setting, lower concentration of released ACh might act through a reverberating loop on cells themselves.

Targets and functions of non-neuronal cholinergic system

The effects of the ACh released through the non-neuronal system can be divided on the bases of the site of release itself, luminal or basal, thus displaying specific effects on the target cells.

Luminal side

When released on the luminal side, ACh can reach a limited number of cells, among which epithelial cells, macrophages and many elements of the immunitary system. Both epithelial cells and macrophages present muscarinic and nicotinic receptors that could interact with the released ACh. In particular the epithelial cells express M1 and M3 receptors and the α and β subunits of the nAChR receptor (31). Through the binding of these receptors, the ACh regulates proliferation of epithelial cells, mucous secretion, and the release of GM-CSF and IL-8, which stimulates ciliary beat. Macrophages express the isoform 3 of the muscarinic receptor and several subunits of the nicotinic one, among which the α9/α10 units (32). M3 receptor activation induces *in vitro* the release of pro-inflammatory mediators, whereas the stimulation of the the nAChR suppresses macrophages activation with a more general anti-inflammatory effect. It should be noted that, due to their localization, macrophages cannot be reached by the ACh produced by the neuronal cholinergic system

and thus identify a specific target of the ACh derived from non-neuronal system and released from the luminal side of epithelial cells.

Latero-basal side

A clear distinction among neuronal and non-neuronal derived ACh is really difficult at cellular basal side. In mouse models, epithelial cells at tracheal level can release ACh following serotonin stimulation thus directly inducing bronchoconstriction; this effect is susceptible to atropine, an inhibitor of muscarinic receptors (33). Few data are currently available on the effect of non-neuronal ACh on smooth muscle cells. It is conceivable that ACh could reach structures close to the epithelium or could be caught by the epithelium itself. Fibroblasts localized in the sub-epithelial lining represent a selective and specific ACh target (34). Below the basal lamina, several immune system and nervous cells can be found. Sensitive and vagal neurons express several nAChR subunits and are so connected to the epithelial cells to be sensitive to inhaled nicotine (35). Their stimulation induces local release of neuropeptides that, by activating local defense response, causes local irritation and provoke cough reflex.

Relevance of non-neuronal cholinergic system in pathogenesis and therapy of airways diseases

Deregulation of muscarinic receptors is a frequent feature of airway diseases such as asthma and COPD, and the use of muscarinic antagonists represents an important strategy in pharmacological treatment of COPD. The contribution of non-neuronal ACh in patho-mechanisms is still partially unclear. A number of data, however, suggests that epithelial ACh may be increased in airway inflammatory diseases, thus contributing to activation of immune cells and to bronchoconstriction. On the contrary, total airway ACh content is reduced in patients affected by cystic fibrosis, and expression of the non-neuronal ACh synthesis and release machinery is down-regulated in acute allergic airway inflammation. The stimulatory effect of ACh on epithelial cell proliferation, the presence of nAChR on airway epithelial cells and the association between use of tobacco and lung cancer clearly suggest the presence of a possible association of the intrinsic epithelial cholinergic system with the development of lung cancer. Small cell lung cancer cells (originating from neuroendocrine cells of the epithelium) and squamous carcinoma cells synthesize and release ACh, and this acts as an autocrine growth factor, operating both via muscarinic M3 and nAChR receptors. Most recently, it has been demonstrated that a variation in a region of 15q25.1 containing nAChR genes coding for subunits α3, α5, β4 is correlated with an increase in lung cancer risk. Nicotinic effects on cell proliferation are summarized in *Figure 1*. In summary, non-neuronal ACh release is involved in plastic changes and in the activation of the immune response, in ongoing chronic inflammatory airways disease. Furthermore, there is growing evidence that disturbances of this system directly contribute to the development of lung cancer, through stimulation of pro-mitotic activity. In light of the aforementioned evidences, it is reasonable to consider airways non-neuronal cholinergic system as a potential pharmacological target in the treatment of inflammatory and proliferative lung diseases.

Cholinergic system and lung cancer

Lung cancer is the number one cause of death for solid tumors in Western world and smoking habit is associated to its development in the vast majority of cases. Although the primary mechanism of smoking-induced carcinogenesis is related to smoke's carcinogens, recent data show that nicotine and nitrosamines bind to nicotinic acetylcholine receptor (nAChR) on lung cancer cells to stimulate tumor growth and inhibit apoptotic death program. Mechanisms of cholinergic signal activation in lung cancer are represented in *Figure 2*. These experimental observations are consequent to the demonstration that cholinergic system is not only confined in nervous system but, as demonstrated above, is ubiquitous and specifically, present in airways epithelial cells and lung cancer cells. Thus the stimulatory effect of ACh on epithelial cell proliferation, the presence of nACh on airway epithelial cells as well as the exposure to tobacco smoking create a strong rationale to deeper investigate the possible link between non neuronal cholinergic system and susceptibility to lung cancer development. For instance it is well known that small cell lung cancer (SCLC) (36)—which originates from neural crest—and squamous cell carcinoma (SCC) are able to synthetize and produce ACh, which in turn acts as autocrine growth factor, by linking both M3 and nACh receptors (37). nAChR subunit α3β2 mediates smoking related toxic effects through the activation p21, Bcl-2, NF-κB e STAT-1 signaling; the 7α subunit nAChR is involved in damage on cheratinocytes on the oral cavity through the activation of MAP kinases and JAK/STAT pathway (38). Gene expression

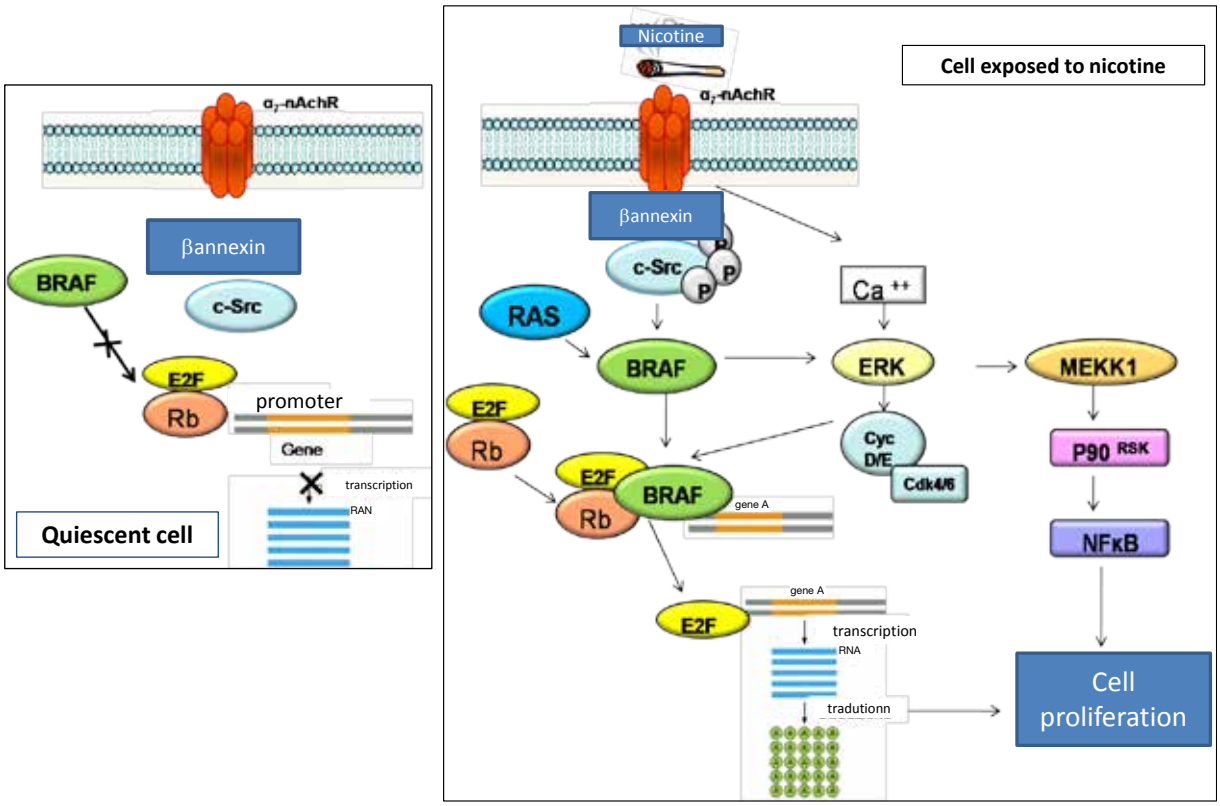


Figure 1 Nicotine: activation of cell proliferation. In quiescent cell there is the absence of the association between β-annexin, src and nAchR. Rb is dephoshorylated and binds the transcription factor E2F which, as a consequence, cannot be active on cell proliferation. The binding of nicotine to its receptor nAcR induces formation of a holigomeric complex between β-annexin, src and nAchR. The latter activates MAPKs activation and in detail the binding of BRAF and the complex RB-E2F. Undetermined mitogenic stimuli (*e.g.*, cyclins) can enable the E2F transcription factor to act on gene promoters and lead the cell to the phase S of the cell cycle. The increase of intracellular calcium levels which is consequent to nicotine binding to the nAchR promote the activation of ERK signaling. As a consequence EKK1 activates the transcription factor NFκB which sustains cell proliferation.

profiling analyses have been thus addressed to evaluate how tobacco and nicotine can affect receptor expression: in both cases a 7α nAChR-mediated over regulation of cell growth factor and proliferation can be found.

It should be remembered that several allelic variant exist for the vast majority of genes (about 80% of human genome). Genetic polymorphism can be defined as a genetic variation that occurs in more than 1% of a population, whereas a genetic mutation identifies a variation which occur in less than 0.1% of a population. Several types of polymorphisms do exist: the more frequent are single nucleotide polymorphisms (SNPs) which are classified based on their position in the context of the genes: (I) cSNP which are localized in codifying exons and are thus able to induce a variation in the aminoacidic sequence of the protein; (II) pSNPs or peripheral polymorphisms which affect regulatory regions (e.g., promoters, enhancers), introns (splicing regions) and could determine interference which the expressione levels and the structure of the proteins; (III) rSNPs or random polymorphisms, which are detected in intergenic regions (which represent 98.5% of the whole genome) and which have no direct effect on gene expression but could be relevant due to their diagnostic potential according to *linkage disequilibrium* phenomenon. Indeed SNPs analysis is becoming of great relevance in predictive oncology with the aim to stratify patients based for their risk of cancer onset based on the presence of certain SNPs.

From this perspective, recent studies on lung cancer susceptibility have drawn researchers' attention on the SNPs of the gene encoding for the nAChR. Interestingly genome-wide sequencing analyses have provided evidences

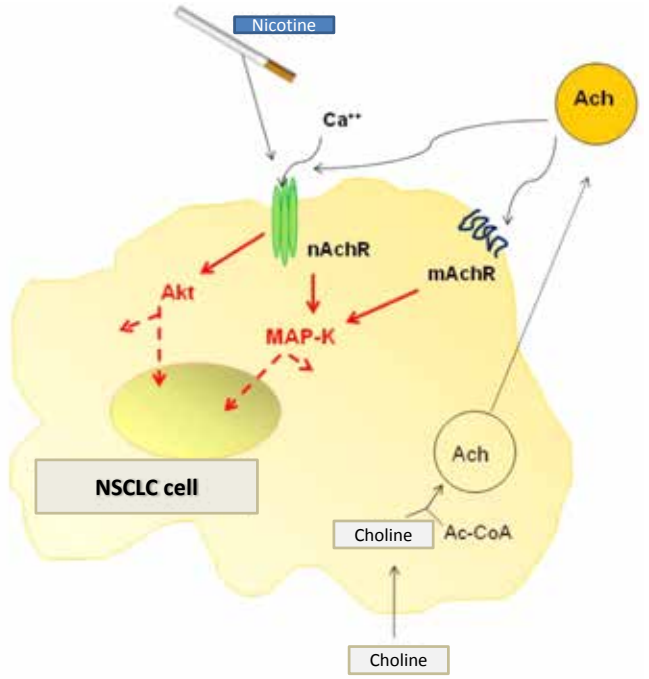


Figure 2 Cholinergic signal activation in lung cancer. The binding of nicotine—from cigarette smoking—to its receptor nAchR can induce the activation of several intracellular trasnducers which promote cell proliferation (MAPK e Akt). Endogenous Ach, which is overexpressed, activates the same mediators by acting on both nicotinic and muscarinic receptors. Thus inhibitors of muscarinic receptors cooperate in sustaining the inhibition of cell proliferation related to both the exposure of exogenous nicotine and endogenous Ach as well.

of a significant association between NSCLC risk, smoking behavior and polymorphisms on 15q21 locus, containing genes encoding for nAChR α3, α5 (*CHRNA5*, *CHRNA3* genes) and β4 (*CHRNB4* gene) subunits (39-41). In particular a non-synonymous substitution (D398N, substitution of aspartic acid with asparagine in position 398) encoding for a highly conserved region of the receptor (M2 domain) represents one of the most powerful markers of disease risk. That haplotype has been identified among Europeans and Nord Americans, whereas it is really rare among Asiatic and African populations (data from *HapMap database*, website at www.hapmap.ncbi.nlm.nih.gov). The high SNPs frequency in the 15q21 gene (the rs8034191 e rs1051730 SNPs are detectable in about 50% of Europe population) makes the study of the genes localized in that locus very relevant also in a public health perspective and identifies *CHRNA3* e *CHRNA5* very promising actionable targets. It has been also documented that SNPs in that genetic regions are related to nicotine dependence; consequently the correlation between lung cancer onset and smoking habit has been also investigated. As expected the risk for disease inset is higher among smokers than in never smokers whereas no correlation has been found within respect to neoplastic histotypes. It is conceivable that 15q21 polymorphisms although not playing a causal role in inducing tumor development (no direct pathogenetic role), might be related to the induction of smoking habit, which, in turn, is the most relevant risk factor for lung cancer. Thus, genetic variability of the long arm of chromosome 15 is directly related to nicotine dependence and consequently, might expose to the risk of smoke-related disease, among which the most important is lung cancer (39).

Overall these findings show how genetic interindividual variability plays a central role in the pathogenesis of complex or polygenic diseases, among which cancer, by modulating the mechanisms by which each subject reacts to external stimuli (e.g., nicotine exposure by regulating ligand-receptor affinity) and by affecting inclination towards given environmental stimuli (e.g., persistence of smoking habit despite its predictable consequences). From this perspective, nAChRs are becoming interesting targets both in lung cancer screening and in molecular tailored therapy. It has been demonstrated that in SCC the cholinergic signaling is up-regulated and in this scenario, nicotine exposure can activate different oncogenic pathways, being responsible for tumor spread and neoplastic angiogenesis. Thus pharmacological inhibition of cholinergic receptors—both nicotinic and muscarinic—might be a promising tool to limit basal and nicotine-stimulated tumor growth.

Molecular mechanisms of cholinergic signal in lung cancer

According to what has been previously described, correlation between lung cancer onset and nicotine depends on two different mechanisms: (I) inter-individual genetic variability (polymorphisms in the locus 15q21) which is responsible for a higher susceptibility or predisposition to the onset of lung cancer, mainly due to an increased nicotine dependence; (II) the proliferative and anti-apoptotic effect on neoplastic cells played directly by nicotine through cholinergic receptors activation. Historically the first study which has shown a nicotine effect different from neuronal signaling is that of Schuller and coll (42). The authors demonstrated a proliferative effect mediated by nicotine

in a series of lung cancer cell lines, through the increased release of growth factors (VEGF, HGF, TGF-β, PDGF, TGF-α) and their receptors (VEGFR, MET, EGFR). In detail, nicotine induced EGFR transactivation through the increase of intacellular calcium which is responsible for the activation of several kinases downstream EGFR. It has been reported that in gastric cancer nicotine is able to induced the overexpression of VEGFR through the activation of COX-2; the latter induce an increase in neoplastic angiogenic and invasion capacity which involves some elements of extracellular matrix, such as metalloproteases (MMP2 and MMP-9) and enzymes responsible for plasminogen activation cascade. In lung adenocarcinoma cell line A549 the exposure to nicotine promotes inhibition of phosphorylation of the protein phosphatase 1 (PP1) which in turn, induces the deregulation of protein p27 Kip1. The latter plays inhibitory effects on the cyclin-dependent kinase 1 which determines cell cycle progression. Moreover nicotine is responsible for an increased NSCLC cell proliferation through the expression of fibronection and α5β1-integrin and the activation of ERK and PI3K-mTOR signaling. More recent studies have demonstrated that the nAChR is the main mediator of proliferative effects of nicotine of transformed cells. Consequently the 7α subunit identifies a novel druggable target of NSCLC therapeutic approach. Growing evidence demonstrates similar effects of the subunit 7α nAChR in SCLC and mesothelioma as well. The activation of the nicotinic receptor induces an increase in cell proliferation and survival mediated by the MEKK-1, ERK1/2 e $p90^{RSK}$ kinases; notably in A549 cells the nicotine-induced activation of the MPAKs occurs in a 7α nAChR-mediated manner (43). Moreover biological effects of nicotine on transformed cells involves other mediators and transcription factors such as, NF-κB, Src, Akt, HIF-1 and the lipo-oxygenase cascade, as well (44). Nicotine improves cancer (NSCLC, SCLC, breast and ovary cancer) cells survival by inducing avoidance of apoptosis mediated by a number of stimuli (e.g., radiation, chemotherapy agents, oxidative stress). Besides nicotine promotes tumor progression and spreading since it can induce angiogenesis and arteriogenesis; tissue hypoxia and ischemia themselves induce overexpression and sensitization of endothelial cells to the 7α subunit of nAChR (45). Thus the nAChR plays a crucial role in the complex molecular network which is responsible for tumor progression orchestrated by angiogenic processes.

It has been recently reported in lung tumor samples (SCC) the presence of high levels of α5 e β3 nAChR mRNA, in association to high levels of ACh consequent to an increase of ChAT due to low expression of the cholinesterase enzyme. These findings demonstrate that in lung cancer the cholinergic signal is aberrantly activated, with increased receptors levels and lower levels of their inhibitors. Similar results have been reported *in vitro* through the exposure to nicotine of lung cancer cell line (H520) and by measuring the receptor activity levels. The results demonstrated that NSCLC expresses the ACh signaling and that both ACh and nicotine may activate the cascade thus promoting tumor growth. In such setting, cell exposure to cigarette smoking represents on one hand a relevant stimulus to cancer cell proliferation and on the other a novel actionable target. From this perspective it has been shown that pharmacologic block of M3 muscarinic receptor with darifenacin can inhibits *in vitro* MAPs-mediated cell proliferation induced by activation of muscarinic and nicotinic receptors (46) MAPKs activation is the key point of the signaling cascade activated by the two receptor families and the blockade of the M3 receptors could represent ideally a novel potentally targetable axis in lung cancer therapy platform.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Kummer W, Lips KS, Pfeil U. The epithelial cholinergic system of the airways. Histochem Cell Biol 2008;130:219-34.
2. Wessler I, Kirkpatrick CJ, Racké K. Non-neuronal acetylcholine, a locally acting molecule, widely distributed in biological systems: expression and function in humans. Pharmacol Ther 1998;77:59-79.
3. Sleigh MA. Ciliary function in mucus transport. Chest 1981;80:791-5.
4. Gwilt CR, Donnelly LE, Rogers DF. The non-neuronal cholinergic system in the airways: an unappreciated regulatory role in pulmonary inflammation? Pharmacol Ther 2007;115:208-22.
5. Cho T, Chan W, Cutz E. Distribution and frequency of neuro-epithelial bodies in post-natal rabbit lung: quantitative study with monoclonal antibody against serotonin. Cell Tissue Res 1989;255:353-62.
6. Cutz E, Yeger H, Pan J. Pulmonary neuroendocrine cell system in pediatric lung disease-recent advances. Pediatr

Dev Pathol 2007;10:419-35.
7. De Proost I, Pintelon I, Brouns I, et al. Functional live cell imaging of the pulmonary neuroepithelial body microenvironment. Am J Respir Cell Mol Biol 2008;39:180-9.
8. Reid L, Meyrick B, Antony VB, et al. The mysterious pulmonary brush cell: a cell in search of a function. Am J Respir Crit Care Med 2005;172:136-9.
9. Krasteva G, Kummer W. "Tasting" the airway lining fluid. Histochem Cell Biol 2012;138:365-83.
10. Pfeil U, Lips KS, Eberling L, et al. Expression of the high-affinity choline transporter, CHT1, in the rat trachea. Am J Respir Cell Mol Biol 2003;28:473-7.
11. Racké K, Matthiesen S. The airway cholinergic system: physiology and pharmacology. Pulm Pharmacol Ther 2004;17:181-98.
12. Barnes PJ. Distribution of receptor targets in the lung. Proc Am Thorac Soc 2004;1:345-51.
13. Wessler I, Kirkpatrick CJ. Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. Br J Pharmacol 2008;154:1558-71.
14. Ramsay RR, Naismith JH. A snapshot of carnitine acetyltransferase. Trends Biochem Sci 2003;28:343-6.
15. Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. Pharm Res 2007;24:1227-51.
16. Falk-Vairant J, Meunier FM, Lesbats B, et al. Cell lines expressing an acetylcholine release mechanism; correction of a release-deficient cell by mediatophore transfection. J Neurosci Res 1996;45:195-201.
17. Dunant Y, Cordeiro JM, Gonçalves PP. Exocytosis, mediatophore, and vesicular $Ca2^{+}/H^{+}$ antiport in rapid neurotransmission. Ann N Y Acad Sci 2009;1152:100-12.
18. Malo M, Israël M. Expression of the acetylcholine release mechanism in various cells and reconstruction of the release mechanism in non-releasing cells. Life Sci 2003;72:2029-38.
19. Fukami T, Yokoi T. The emerging role of human esterases. Drug Metab Pharmacokinet 2012;27:466-77.
20. Pfeil U, Lips KS, Eberling L, et al. Expression of the high-affinity choline transporter, CHT1, in the rat trachea. Am J Respir Cell Mol Biol 2003;28:473-7.
21. Fisher AB, Dodia C, Chander A, et al. Transport of choline by plasma membrane vesicles from lung-derived epithelial cells. Am J Physiol 1992;263:C1250-7.
22. Wang T, Li J, Chen F, et al. Choline transporters in human lung adenocarcinoma: expression and functional implications. Acta Biochim Biophys Sin (Shanghai) 2007;39:668-74.
23. Lips KS, Volk C, Schmitt BM, et al. Polyspecific cation transporters mediate luminal release of acetylcholine from bronchial epithelium. Am J Respir Cell Mol Biol 2005;33:79-88.
24. Eiden LE. The cholinergic gene locus. J Neurochem 1998;70:2227-40.
25. Reinheimer T, Bernedo P, Klapproth H, et al. Acetylcholine in isolated airways of rat, guinea pig, and human: species differences in role of airway mucosa. Am J Physiol 1996;270:L722-8.
26. Wessler I, Michel-Schmidt R, Brochhausen C, et al. Subcellular distribution of choline acetyltransferase by immunogold electron microscopy in non-neuronal cells: placenta, airways and murine embryonic stem cells. Life Sci 2012;91:977-80.
27. Klapproth H, Reinheimer T, Metzen J, et al. Non-neuronal acetylcholine, a signalling molecule synthezised by surface cells of rat and man. Naunyn Schmiedebergs Arch Pharmacol 1997;355:515-23.
28. Song P, Sekhon HS, Jia Y, et al. Acetylcholine is synthesized by and acts as an autocrine growth factor for small cell lung carcinoma. Cancer Res 2003;63:214-21.
29. Horvath G, Mendes ES, Schmid N, et al. The effect of corticosteroids on the disposal of long-acting beta2-agonists by airway smooth muscle cells. J Allergy Clin Immunol 2007;120:1103-9.
30. Proskocil BJ, Sekhon HS, Jia Y, et al. Acetylcholine is an autocrine or paracrine hormone synthesized and secreted by airway bronchial epithelial cells. Endocrinology 2004;145:2498-506.
31. Moulton BC, Fryer AD. Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD. Br J Pharmacol 2011;163:44-52.
32. Koarai A, Traves SL, Fenwick PS, et al. Expression of muscarinic receptors by human macrophages. Eur Respir J 2012;39:698-704.
33. Kummer W, Wiegand S, Akinci S, et al. Role of acetylcholine and polyspecific cation transporters in serotonin-induced bronchoconstriction in the mouse. Respir Res 2006;7:65.
34. Pieper MP, Chaudhary NI, Park JE. Acetylcholine-induced proliferation of fibroblasts and myofibroblasts in vitro is inhibited by tiotropium bromide. Life Sci 2007;80:2270-3.
35. Dehkordi O, Kc P, Balan KV, et al. Airway-related vagal preganglionic neurons express multiple nicotinic

acetylcholine receptor subunits. Auton Neurosci 2006;128:53-63.
36. Song P, Sekhon HS, Jia Y, et al. Acetylcholine is synthesized by and acts as an autocrine growth factor for small cell lung carcinoma. Cancer Res 2003;63:214-21.
37. Song P, Spindel ER. Basic and clinical aspects of non-neuronal acetylcholine: expression of non-neuronal acetylcholine in lung cancer provides a new target for cancer therapy. J Pharmacol Sci 2008;106:180-5.
38. Egleton RD, Brown KC, Dasgupta P. Nicotinic acetylcholine receptors in cancer: multiple roles in proliferation and inhibition of apoptosis. Trends Pharmacol Sci 2008;29:151-8.
39. Amos CI, Wu X, Broderick P, et al. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nat Genet 2008;40:616-22.
40. Hung RJ, McKay JD, Gaborieau V, et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 2008;452:633-7.
41. Thorgeirsson TE, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature 2008;452:638-42.
42. Schuller HM, Orloff M. Tobacco-specific carcinogenic nitrosamines. Ligands for nicotinic acetylcholine receptors in human lung cancer cells. Biochem Pharmacol 1998;55:1377-84.
43. Zheng Y, Ritzenthaler JD, Roman J, et al. Nicotine stimulates human lung cancer cell growth by inducing fibronectin expression. Am J Respir Cell Mol Biol 2007;37:681-90.
44. Zhang Q, Tang X, Zhang ZF, et al. Nicotine induces hypoxia-inducible factor-1alpha expression in human lung cancer cells via nicotinic acetylcholine receptor-mediated signaling pathways. Clin Cancer Res 2007;13:4686-94.
45. Grozio A, Paleari L, Catassi A, et al. Natural agents targeting the alpha7-nicotinic-receptor in NSCLC: a promising prospective in anti-cancer drug development. Int J Cancer 2008;122:1911-5.
46. Song P, Sekhon HS, Fu XW, et al. Activated cholinergic signaling provides a target in squamous cell lung carcinoma. Cancer Res 2008;68:4693-700.

Cite this article as: Saracino L, Zorzetto M, Inghilleri S, Pozzi E, Stella GM. Non-neuronal cholinergic system in airways and lung cancer susceptibility. Transl Lung Cancer Res 2013;2(4):284-294. doi: 10.3978/j.issn.2218-6751.2013.06.01

Diagnostic bronchoscopy--current and future perspectives

Steven Leong[1], Tawimas Shaipanich[2], Stephen Lam[2], Kazuhiro Yasufuku[3]

[1]Department of Thoracic Medicine, University of Queensland Thoracic Research Centre, The Prince Charles Hospital, Queensland, Australia 4032; [2]Department of Integrative Oncology, British Columbia Cancer Agency & University of British Columbia, Vancouver, BC V5Z 1L3, Canada; [3]Division of Thoracic Surgery, Toronto General Hospital, University Health Network, Toronto, ON M5G 2C4, Canada

Correspondence to: Dr. Steven Leong. Department of Thoracic Medicine, The Prince Charles Hospital, Rode Rd, Chermside, Brisbane, Queensland 4032, Australia. Email: steven_leong@health.qld.gov.au.

Abstract: Lung cancer is the leading cause of cancer-related mortality worldwide. Standard bronchoscopy has limited ability to accurately localise and biopsy pulmonary lesions that cannot be directly visualised. The field of advanced diagnostic bronchoscopy is rapidly evolving due to advances in electronics and miniaturisation. Bronchoscopes with smaller outer working diameters, coupled with miniature radial and convex ultrasound probes, allow accurate central and peripheral pulmonary lesion localisation and biopsy while at the same time avoiding vascular structures. Increases in computational processing power allow three-dimensional reconstruction of computed tomographic raw data to enable virtual bronchoscopy (VB), providing the bronchoscopist with a preview of the bronchoscopy prior to the procedure. Navigational bronchoscopy enables targeting of peripheral pulmonary lesions (PPLs) via a "roadmap", similar to in-car global positioning systems. Analysis of lesions on a cellular level is now possible with techniques such as optical coherence tomography (OCT) and confocal microscopy (CM). All these tools will hopefully allow earlier and safer lung cancer diagnosis and in turn better patient outcomes. This article describes these new bronchoscopic techniques and reviews the relevant literature.

Keywords: Lung cancer; bronchoscopy; diagnosis

Submitted Jul 27, 2013. Accepted for publication Sep 11, 2013.
doi: 10.3978/j.issn.2072-1439.2013.09.08
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.09.08

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Overall 5-year survival is poor at 15% with little improvement over the last twenty years (1). Lung cancer presenting at a late stage is largely responsible for this poor survival; only 20% are potentially curative with surgical resection. Anti-smoking campaigns and government legislation will reduce lung cancer burden in younger generations but a significant proportion of the older generation who have smoked in the past remain at risk.

Innovative bronchoscopic techniques diagnose lung cancer earlier and more accurately to improve patient outcomes. Narrow band imaging (NBI) and autofluorescence bronchoscopy (AFB) detect pre-invasive malignancy. Endobronchial ultrasound (EBUS) and Electromagnetic Navigation Bronchoscopy (ENB) are safer alternatives to mediastinoscopy and computed tomography guided transthoracic needle aspiration (CT-TTNA). Emerging technologies such as optical coherence tomography (OCT) and confocal microscopy (CM) allow lesional assessment on a cellular level.

This article describes these technologies and explains how they enhance lung cancer diagnosis and staging.

Standard bronchoscopy

Gustav Killian invented the rigid bronchoscope in 1898. In 1967 Ikeda pioneered the flexible bronchoscope, and video bronchoscopes became mainstream in the early 1980's. Despite quickly becoming the cornerstone of lung cancer diagnosis, the shortcomings of white light bronchoscopy (WLB) are immediately obvious. Due to the

branching nature of the bronchial tree, the airway diameter rapidly reduces the more distal one advances towards lung periphery. A standard 5.9 mm bronchoscope can only access the 4-5th generation bronchi with visualization of the next 1-2 generations, covering only 1/3 of the approximately 23-generation bronchial tree. The majority of lesions visualized on CT are therefore beyond direct bronchoscopic vision. Aberrations in bronchial mucosa, such as angiogenic squamous dysplasia (ASD) or squamous cell carcinoma (SqCC) in situ (CIS) are indistinct and easily missed when lit by white light (2). Similarly extra-bronchial structures such as mediastinal lymph nodes cannot be visualized, and blind needle aspiration (cTBNA) is possible for a limited number of nodal stations (3).

Meta-analysis by Rivera from studies between 1970-2001 found the sensitivity and specificity for *bronchoscopically visible* lesions was 88% and 100% respectively (4). Diagnostic accuracy for *bronchoscopically invisible* lesions under fluroscopic guidance varied from 36% to 88%, dependent on biopsy method (transbronchial biopsy *vs.* cytology brush *vs.* bronchoalveolar lavage), the number of samples taken, and lesion size. Yield was most affected by lesion size-sensitivity for peripheral pulmonary lesions (PPLs) >2 cm was 63%, decreasing to 34% for PPLs <2 cm. Whilst CT-TTNA is undoubtably more accurate than WLB (pooled sensitivity 0.9; 95% confidence interval 0.88-0.91) its complication rate is higher (5).

Thus, WLB is effective at diagnosing bronchoscopically *visible* lesions, but is limited in diagnosing bronchoscopically *invisible* lesions, in situ tumours, and for mediastinal staging of lung cancer.

Endobronchial/mucosal lesions

AFB

AFB takes advantage of endogenous fluorophores in bronchial tissue to inform about metabolic state and biochemical composition of tissues. Normal bronchial tissue fluoresces strongly in green when illuminated by violet or blue light, however as the epithelium becomes dysplastic, progresses to *in-situ* carcinoma and finally to invasive cancer, the amount of green autofluorescence decreases and red fluorescence also decreases although to a lesser degree. These abnormal areas contrast sharply with normal mucosa.

Two meta-analyses have studied the value of AFB combined with WLB versus WLB alone for detection of intraepithelial neoplasia and invasive lung cancer. The first (14 studies, n=1,358) demonstrated a pooled sensitivity and specificity of AFB + WLB of 0.9 and 0.56, compared to 0.66 and 0.69 for WLB alone (6). The second (21 studies, n=3,266) showed the relative sensitivity on a per lesion basis of AFB + WLB versus WLB alone to detect CIS and invasive cancer was 2.04 and 1.15 respectively (2). AFB + WLB is less specific than WLB alone because false positives are common with AFB due to inflammation, mucous gland hyperplasia and inter-observer error, however specificity and inter- and intra-observer variation can be improved to 80% by combining the quantitative red/green fluorescence ratio (R/G) with bronchoscopic findings (7).

NBI

NBI visualizes bronchial mucosa with blue light (415 nanometers) and green light (540 nanometers) to accentuate superficial capillaries and deeper submucosal vessels respectively, while at the same time reducing light scatter from other wavelengths seen with white light. NBI detects the characteristic abnormal angiogenesis associated with dysplastic lesions. Most of the data detailing the benefits of NBI are from gastroenterological studies and head and neck cancers, however pulmonologists are increasingly using NBI to detect early stage bronchial mucosal lesions.

In 2003 Shibuya *et al.* studied 48 patients with sputum cytology suspicious or positive for malignancy (8). These patients underwent WLB and AFB, with suspicious areas subsequently interrogated with NBI of differing wavelengths and biopsied for histology. Dotted vessel diameter seen on NBI-B1 (400-430 nm) most closely agreed with pathological examination of ASD vessel diameter.

Shibuya *et al.* also studied differing NBI characteristics of ASD, CIS, micro-invasive tumour, and invasive SqCC (9). By identifying tortuous vessel networks, dotted vessels, and spiral and screw type vessels, the authors could confidently differentiate between the different stages of multistep carcinogenesis of SqCC.

NBI has a higher specificity and equivalent sensitivity to AFB. Herth *et al.* evaluated diagnostic yields of NBI alone, and combined with AFB and WLB, in 62 patients referred for airway cancer screening (10). All abnormal lesions underwent forceps biopsy. NBI was less sensitive but more specific than AFB, when compared to WLB. There was no benefit in combining AFI and NBI, a finding confirmed in a more recent study (11). NBI detects dysplasia or malignancy in 23% of patients with normal WLB (12). NBI after WLB

led to a change in therapeutic decisions in approximately 10% of patients (13). It is uncertain how NBI compares to high definition WLB (which provides substantially higher resolution images than conventional WLB) for detection of early bronchial mucosal abnormalities.

The natural history of pre-invasive malignancy remains uncertain and treatment at this early stage has not been shown to improve survival however it is likely that at least some of these lesions, if left untreated, will progress to invasive carcinoma. AFB and NBI have higher sensitivity and specificity for detecting mucosal lesions compared to WLB, and diagnostic bronchoscopists should be familiar with their use.

OCT

OCT provides cellular imaging at and below the tissue surface (14-16). It was developed in the 1990's for ophthalmic applications but has since been used to assess vessel structure, atherosclerotic plaque, and more recently, bronchial wall structure.

Light is emitted by an imaging catheter and the interference pattern between reflected light and light backscattered from the tissue at different depths is collected and analyzed by an interferometer. These patterns are then recombined and decoded, forming a high resolution cross-sectional image. Contact between instrument and tissue is unnecessary and intravenous contrast, dyes or radiation are not needed. OCT resolution is 20 times higher than ultrasound and can be displayed on a monitor in real time. OCT imaging depth is 2-3 mm, and axial and lateral resolution varies between 5-30 micrometers depending on the scanning conditions.

Tsuboi *et al.* compared OCT images to histological findings of bronchial lesions (14) and found that on OCT, normal bronchial mucosa appears homogeneous whereas the submucosal layer is reflective due to extracellular matrix; A gap is visible between the submucosa and smooth muscle layer, and underlying cartilage shows much scattering. Alveoli have a uniform bronchial wall appearance and air-containing alveoli can be clearly differentiated. Infiltrating cancers, on the other hand, show unevenly distributed high backscattering areas and loss of layer structure and glandular tissue. Lam *et al.* demonstrated that quantitative measurement of epithelial thickness could differentiate between invasive carcinoma and CIS (P=0.004), and also between dysplasia and metaplasia or hyperplasia (P=0.002). Basement membrane remained intact with CIS, but became disrupted with invasive cancer (15). Certain OCT characteristics can potentially differentiate SqCC from adenocarcinoma without the need for biopsy (17).

Despite these promising pilot studies, it remains to be seen if and how OCT will add value to our current diagnostic approaches. Possible uses for OCT include: distinguishing benign from malignant central and peripheral lesions, differentiating CIS from mincr-invasive cancer; and improving bronchoscopic sampling of PPLs. The performance characteristics and limitations of OCT need to be defined before the technique becomes mainstream.

PPLs

EBUS radial probe (RP-EBUS)

Advances in electronic miniaturization allow a 360-degree viewing ultrasound to fit into a 1.4 mm probe that can be passed through the working channel of a standard bronchoscope. This so-called RP-EBUS allows localization of peripheral lesions and depth assessment of endobronchial lesions. At standard frequency of 20 MHz, the spatial resolution is less than 1 mm and penetration depth is 4-5 cm.

RP-EBUS has two main uses.

Localisation of PPLs

By placing the ultrasound probe into lung periphery one can characterize the tissue densities surrounding the probe. Normal air-filled alveoli have a homogeneous "snow-storm" appearance. If the probe is within a solid lesion however the interface between the mass and surrounding aerated lung is represented by a bright line, confirming the probe is within the target.

The use of a guide sheath (GS) in combination with RP-EBUS was introduced in 2004 (18). The GS is a catheter that fits over the RP-EBUS, leaving only the distal ultrasound probe exposed. The RP-EBUS/GS are advanced together through the working channel into the target subsegment until the lesion of interest is localized. Advancing and retracting the RP-EBUS/GS defines the lesion's proximal and distal extents. Once the desired biopsy site is established, the GS is left in situ and the RP-EBUS is removed and replaced by pre-measured biopsy tools, ensuring samples are taken from the desired location. An additional theoretical benefit of GS is tamponade of biopsy-related bleeding (*Figure 1*).

Perhaps the best evidence favouring RP-EBUS/GS over fluoroscopy guided transbronchial lung biopsy

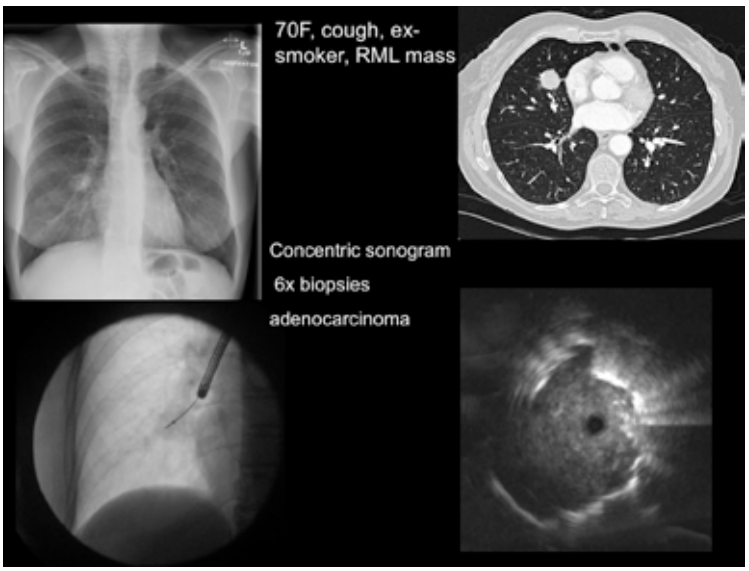


Figure 1 Example of RP-EBUS being used to diagnose a peripheral pulmonary lesion. Note the well demarcated outline of the lesion and the concentric nature of the image in the bottom right panel.

(TBLBx) for PPLs was published in 2005 by Paone (19). 221 patients with PPLs were randomly assigned to either procedure (97 RP-EBUS/GS, 124 TBLBx) and those without a diagnosis underwent more invasive investigation. Sensitivity for lung cancer was 0.79 and 0.55 (P=0.004), and accuracy was 0.85 and 0.69 (P=0.007) in RP-EBUS/GS and TBLBx groups respectively, with the majority of the benefit evident in lesions <3 cm [sensitivity of RP-EBUS/GS *vs.* TBLBx for <3 cm, 71% (47-95%) *vs.* 23% (3-43%), P value <0.001].

A systematic review and meta-analysis of RP-EBUS/GS for PPL diagnosis (16 studies n=1,420 patients) showed point sensitivity for diagnosis of lung cancer was 0.73, however there was significant study heterogeneity. Complication rates varied between 0-7.4%, with the most common being minor bleeding. Pooled pneumothorax rate was 1.0%, and rate of intercostal drainage was only 0.4% (20).

Most RP-EBUS/GS data originate from centres with bronchoscopic expertise and hence may not reflect "real-world" results. Roth *et al.* performed a prospective randomised cohort study of RP-EBUS/GS for PPLs by 29 different physicians practicing at community hospitals in Western Norway between June 2005 and January 2008 (21). With a cancer prevalence of 71.5%, the sensitivity for malignancy in the RP-EBUS/GS group was 36% compared with 43.7% in the non-EBUS group. If there was a bronchus leading directly to the lesion, the diagnostic sensitivity was considerably higher (62.2%). This study may provide a more realistic view of what is achievable with RP-EBUS/GS in a community hospital and demonstrates that EBUS is useful in confirming lesion location, but not in guiding the bronchoscopist to the lesion.

The only consistent predictor of success is probe location in relation to the lesion; If the probe is surrounded by tumour (concentric ultrasound image) the diagnostic yield is much higher than if the probe is adjacent to (eccentric image) or not associated with the target lesion (18,22-24). Although Kurimoto found yield was independent of lesion size (18), subsequent studies recognise that higher diagnostic yields are achieved from larger lesions (20).

Few studies have compared RP-EBUS/GS to the non-invasive gold standard of CT-TTNA. In 2008 Fielding described a prospective series of RP-EBUS/GS and compared this to a retrospective review of CT-TTNA during the same period. Diagnostic sensitivity for RP-EBUS/GS was only 35% for lesions touching the visceral pleura, compared to 74% for lesions not touching the visceral pleural. While overall pneumothorax rates were 1% and 28% in EBUS GS and CT groups respectively, the CT-TTNA pneumothorax rate was only 2.6% for lesions in contact with the visceral pleura (25). These results suggest that CT-TTNA rather than RP-EBUS/GS should be the first line investigation for pleural-based lesions.

Depth of invasion of endobronchial lesions

RP-EBUS with a surrounding inflatable balloon can clearly define tracheal and bronchial wall layers, making it an excellent tool to assess tumour invasion. Ultrasonographic assessment of tumour depth correlates well with histopathologic findings (26) and this measurement determines appropriate therapy; Tumours that invade through the cartilage layer require radiotherapy or surgery, whereas those with an intact cartilage layer can be treated endoscopically.

RP-EBUS can also determine whether centrally located tumours adjacent to the trachea are *invading* the trachea (clinical T4 stage) or simply *adjacent to and compressing but not invading* the trachea (clinical T1a-3 depending on lesion size). Herth *et al.* studied 131 consecutive patients with central thoracic malignancies potentially involving the central airways (27). All patients underwent chest CT followed by WLB and RP-EBUS, with subsequent surgical evaluation and radiology results blinded from the bronchoscopists and surgeons. CT reported 77% of lesions were invading airways, but RP-EBUS showed invasion in only 47% of cases. When using surgical assessment as the gold standard, RP-EBUS had a specificity of 100%, sensitivity of 89%, and accuracy of 94%, for assessing tumour invasion.

ENB

ENB is a relatively new bronchoscopic technique with both diagnostic and therapeutic applications. ENB is a two-stage process: pre-procedure planning, and the actual procedure itself. DICOM data is uploaded to a planning computer via network or compact disk. The planning screen consists of four windows, each of which can display axial, sagittal or coronal views, as well as a virtual bronchoscopic animation and three-dimensional bronchial tree. The bronchoscopist outlines the target and then places waypoints along bronchi that lead to this lesion. The planned path can then be viewed via virtual bronchoscopic animation, allowing the operator to see precisely which sequence of airways lead to the lesion.

Selective cannulation of bronchi is possible with a specialized cannula housed in an extended working channel (EWC), passed together through the working channel. The proximal end houses a "steering wheel" that allows deflection of the distal tip in one of eight directions; the distal end contains a "locatable guide" (LG), whose position is tracked through an electromagnetic field encompassing the patient's chest with the assistance of three location pads placed on the patient's chest. The computer provides instructions on how and when to turn the "steering wheel" and advance the catheter to reach each waypoint and finally the target lesion (*Figure 2*). Once the LG is in close proximity and aligned to the target lesion, the EWC is left in place and the LG is removed and replaced with biopsy instruments.

Most of the published ENB literature is case series of patients with PPLs. The overall diagnostic yield for ENB alone is highly variable and ranges from 59% to 77.3% (28-34). The only randomised controlled trial compared (31) EBUS RP, ENB, or a combined approach (ENB to navigate to the lesion and RP-EBUS to confirm lesion localization) to diagnose one hundred and twenty PPLs. Diagnostic yield was 69%, 59% and 88% for RP-EBUS, ENB, and combined ENB/RP-EBUS groups respectively, suggesting that highest diagnostic yield may be achieved via combined procedures that utilize the strengths of each modality.

The majority of ENB publications are non-consecutive cohorts that do not describe selection/inclusion criteria, and (apart from Eberhardt *et al.*) do not randomize patients to competing modalities. ENB has never been compared to CT-TTNA and thus it is uncertain where ENB fits into the diagnostic algorithm. Due to its high cost and considerable pre-procedure planning (both to obtain DICOM images of recommended parameters, as well as pathway planning) ENB is only likely to become mainstream if consumable prices fall and high level evidence demonstrates diagnostic equivalence to CT-TTNA and/or additional benefit above RP-EBUS. Based on Eberhardt's study, ENB and RP-EBUS may have complimentary roles, however this combined approach would likely increase cost and procedure time.

Virtual bronchoscopy (VB)/ultrathin bronchoscopy (UB)

VB aims to address the inability of RP-EBUS to guide the bronchoscopist to the target lesion. Traditionally, the bronchoscopist views two-dimensional axial, coronal, and sagittal CT views, and mentally reconstructs a three-dimensional image of the bronchial tree before plotting a path to the target lesion. VB allows CT reconstruction of the bronchial tree allowing "virtual" bronchoscopic animation enabling more accurate procedure planning. An example of this was shown in *Figure 3*.

For maximum utility, VB should be coupled with pathway planning software (for example, "Lungpoint", Broncus Medical Inc, CA, USA) and UB; Newer scopes

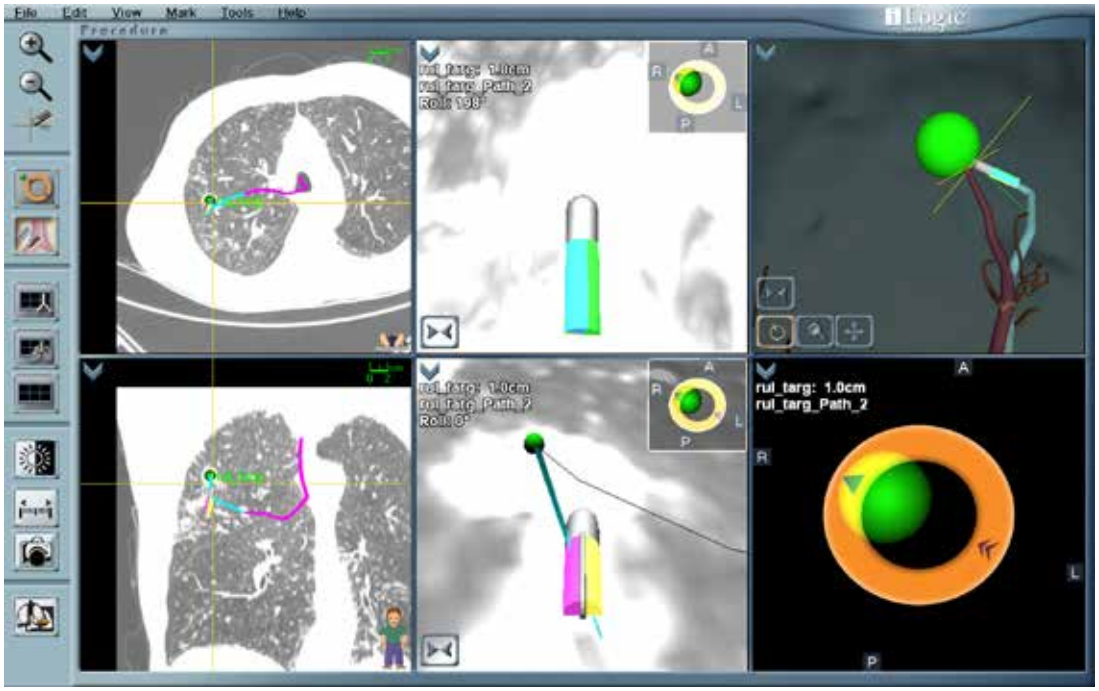


Figure 2 ENB procedure screen showing 6 viewports. Top right = dynamic 3D map; bottom right = tip view (green sphere = target); bottom centre-local view; top centre = maximal image projection view.

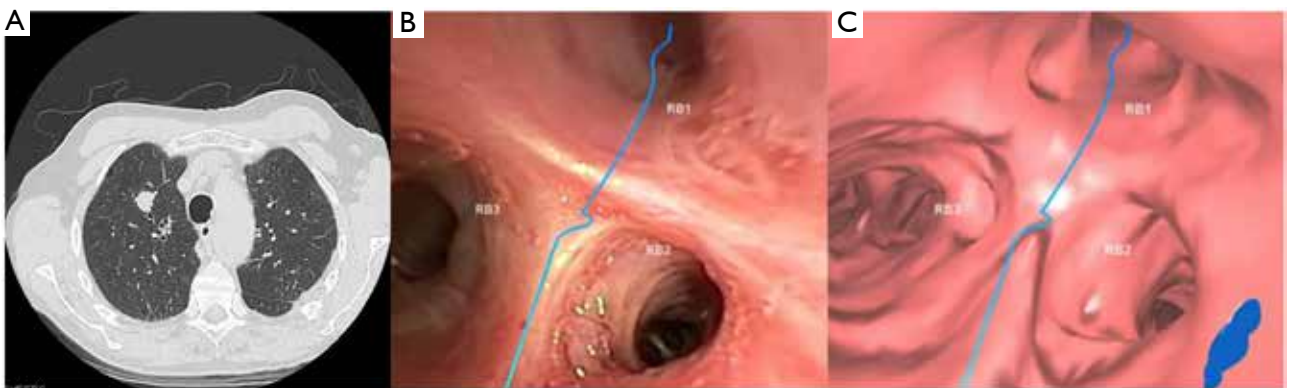


Figure 3. An example of virtual bronchoscopy. (A) Lesion in right upper lobe; (B) Real time virtual image overlay to guide the pathway to the tumour; (C) Virtual bronchoscopic image.

have external diameters of only 2.8 mm (35), and allow direct visualization up to the 9th generation bronchus. A direct consequence of UB however is a smaller working channel and hence smaller biopsy samples.

UB was initially used by Asano to perform barium marking before thoracoscopic surgery for PPLs (36). The UB could be guided under direct vision to a median sixth generation bronchi (range 4th-9th generation) and markers could be placed to a median distance of 4 mm from the lesion (within 10 mm in 27 of 31 lesions). A separate study whereby patients underwent both WLB and UB in the same procedure demonstrated diagnostic rates of 54.3% and 60% respectively, and when both were combined the yield increased further to 62.8% (37). UB was able to obtain diagnostic material in 59.3% of the patients who had negative rapid cytology on WLB.

Asahina *et al.* assessed the utility of combining VB with RP-EBUS/GS in 29 patients with PPLs ≤30 mm. 80% of

lesions were visualized ultrasonographically and diagnostic sensitivities were 44.4% for lesions <20 mm, and 91.7% for lesions ≥20 mm (38). In a randomised trial of 199 patients with PPLs ≤30 mm undergoing RP-EBUS/GS with and without VB (VBNA *vs.* non-VBNA groups) the VBNA group demonstrated higher diagnostic yield (80.4% *vs.* 67%, P=0.032), shorter procedure time (24.0 *vs.* 26.2 mins, P=0.016), and shorter navigation time (8.1 *vs.* 9.8 mins, P=0.045) (39).

Recently though a randomised controlled multicentre trial of UB with and without VB for PPLs found no difference in diagnostic yield. 350 patients were randomised and yields were 67.1% *vs.* 59.9% for VNBA *vs.* non-VBNA groups respectively. Subgroup analysis showed improved yield in the VBNA group for right upper lobe lesions, lesions invisible on CXR, and lesions in the peripheral third of the lung field. It could be argued that VB is of little benefit to highly experienced operators, however it still may be of significant assistance in those less familiar with bronchial anatomy (40).

UB/VB has three major shortcomings. Firstly, the ability to obtain sufficient tissue for molecular analysis with UB is unknown but presumably reduced as biopsy forceps size may affect biopsy size and quality. Secondly, VB quality is dependent on CT source data and recommended DICOM parameters may be unavailable. Thirdly, VB systems rely on a skilled second operator to manipulate the VB image to the same orientation as the real-time bronchoscopic image; without this, the risk of disorientation is high (41).

Complications of guided bronchoscopic techniques

The biggest advantage of bronchoscopic methods over CT-TTNA for biopsy of PPLs is the lower complication rate. In a meta-analysis of RP-EBUS (16 studies, n=1,420), complication rates varied between 0-7.4%. The pooled pneumothorax rate was 1.0% and the pooled rate of intercostal catheter drainage was 0.4%. No patients had bleeding requiring intervention, and no deaths were reported.

In contrast, data from 15,865 adults who underwent CT-TTNA from the 2006 Healthcare Cost and Utilisation Project's State Ambulatory Surgery Databases and State Inpatient Databases for California, Florida, Michigan and New York, demonstrated significantly higher complication rates: pneumothorax rate was 15% (95% CI: 14.0-16.0%) and 6.6% of all biopsies (95% CI: 6-7.2%) required chest tube insertion. Furthermore, the population most likely to have a PPL requiring investigation (60-69 years old smokers, those with COPD) was also the most likely to suffer from procedural complications. 1% of procedures were associated with hemorrhage with 17.8% of this required blood transfusion.

Conclusion: PPLs

Guided bronchoscopic methods (EBUS GS/RP, ENB, VB/UB) have higher diagnostic sensitivity than TBLBx, but slightly lower sensitivity than CT-TTNA. the biggest advantage with a bronchoscopic approach is the lower complication rate; the diagnostic yield of each guided bronchoscopic technique is similar (42). Each technique has advantages and disadvantages and will ultimately depend on availability, local expertise, and lesion location. Only a large scale multicentre randomised trial directly comparing guided bronchoscopy to CT-TTNA will verify the merits of each procedure and determine when each procedure should be used. The weakness of contemporary data is that they are case series of non-consecutive patients where the selection criteria are not explicitly outlined and comparator groups are not used. Even when comparator studies are performed, the results are dependent on operator expertise so that data are not necessarily generalisable.

Peribronchial/peritracheal/mediastinal lesions

Convex probe EBUS guided transbronchial needle aspiration (CP-EBUS TBNA)

Peribronchial and mediastinal lesions are accessible by conventional TBNA (cTBNA) however intimate anatomical knowledge is required to ensure safety and adequate diagnostic yield. Accurate assessment of an abnormal mediastinum is vital in lung cancer staging to guide best treatment. For several years, the gold standard for mediastinal staging has been surgical mediastinoscopy however this requires significant cost including hospital admission, general anaesthesia, and has associated morbidity and mortality (43). CP-EBUS TBNA addresses many of the shortcomings of cTBNA and surgical mediastinoscopy. Its rapid widespread adoption is due to its excellent utility, ease of use, ability to perform as a day case under light anaesthesia, and excellent patient satisfaction (44). Furthermore, the most recent American College of Chest Physician guidelines regarding staging of lung cancer recommend using EBUS TBNA, EUS FNA, or a combined approach over surgical staging as the best first test for

investigating radiologically suspicious mediastinal lymph nodes (grade 1C) (45).

An integrated CP-EBUS TBNA scope/aspirating needle combination allows ultrasonic, real-time visualization of the needle inside the target lesion. The dedicated scope has a 6.9 mm outer diameter and 2 mm instrument channel whose distal end houses a CP-EBUS with flex of 120 degrees upward to 90 degrees downward. An inflatable balloon is sometimes applied over the probe to improve ultrasound signal, particularly in regions where the probe cannot be flexed against the bronchus wall. Vision is through a 30 degree oblique forward viewing fibre-optic lens with an 80 degree viewing angle. The dedicated 21 or 22 G needle is advanced to the distal end of the working channel and secured proximally onto the bronchoscope. Once the target is identified ultrasonically and doppler excludes overlying vessels, the needle is plunged into the lesion. The central stylet is moved back and forth to clear bronchial debris, and 8 to 10 aspirations are taken.

Several studies have proven the utility of CP-EBUS TBNA for mediastinal staging of lung cancer. In one study 163 lymph nodes were sampled in 105 patients. CP-EBUS TBNA correctly predicted lymph node stage with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of 94.6%, 100%, 100%, 89.5%, and 96.3% respectively (46). A series of CP-EBUS TBNA of 572 lymph nodes from 502 patients demonstrated a sensitivity, specificity, and PPV were 94%, 100%, and 100% respectively with surgical staging as the gold standard (47). The same group performed CP-EBUS TBNA on NSCLC patients with a radiologically normal mediastinum, before undergoing surgical staging. For the detection of malignancy, sensitivity, specificity, and NPV was 92.3%, 100%, and 96.3% respectively, with no complications; performing CP-EBUS TBNA on NSCLC patients with a radiologically normal mediastinum avoided unnecessary surgical exploration in one of six patients (48).

Two large systematic reviews/meta-analyses confirm these findings and cement the utility of CP-EBUS TBNA in the staging of lung cancer. In the first (20 studies), sensitivity ranged between 85-100% and NPV ranged from 11-97.4%, with no serious complications reported (49). In the second (11 studies, P=1,299), sensitivity and specificity was 0.93 and 1.00 respectively; For the subgroup selected based on CT or PET findings sensitivity increased to 0.94 compared to only 0.76 without CT or PET. Only two patients had complications (0.15%) (50).

Yasufuku *et al.* performed one of the few prospective studies directly comparing CP-EBUS TBNA to mediastinoscopy for staging of lung cancer (51). Patients with confirmed or suspected NSCLC requiring mediastinoscopy as part of their staging investigations were eligible for inclusion. 153 patients underwent CP-EBUS TBNA followed by mediastinoscopy, with the operator blinded to the rapid on-site examination results; If lymph node involvement was present, patients proceeded onto surgical resection and these results were used as gold standard. Sensitivity, NPV and diagnostic accuracy for CP-EBUS TBNA and mediastinoscopy was 81%, 91%, 93% and 79%, 90%, 93% respectively, with no significant differences between the two in yielding true pathologic N stage (P=0.78).

Combined mediastinal staging with CP-EBUS TBNA and EUS-FNA (so called "medical staging") provides more complete staging allowing additional access to stations 8 and 9. Medical staging seems superior to either staging method alone (52-54). A randomized controlled study showed that medical staging had a higher sensitivity for nodal metastases and was associated with fewer unnecessary thoracotomies when compared to mediastinoscopy (55). Both EUS-FNA and CP-EBUS TBNA can be performed in the one sitting using a single bronchoscope without compromising efficacy, and presumably saving time and money (56,57).

CP-EBUS TBNA equipment, technique and specimen preparation are critical. Diagnostic yield does not differ between 21 and 22 G aspirating needles, but the former results in fewer needle passes (58), better preserves histological structure, with the trade-off being more blood contamination (59). Although suction is traditionally applied to aspirate samples, a randomised controlled trial found no difference in specimen adequacy, quality or diagnosis between specimens with and without suction (60). Three aspiration passes per lymph node is diagnostically optimal with additional passes offering minimal benefit when no rapid onsite evaluation (ROSE) is available (61). The "tissue coagulum clot" method, which involves pushing the specimen onto a pre-cut piece of filter paper with the needle moved in a circular motion to build a cone shaped coagulum of clot and tissue, may increase amounts of diagnostic material when compared to conventional saline needle rinse (62). The use of mini-forceps-transbronchial needle forceps with a beveled end to facilitate penetration through the bronchus wall and jaws that can be opened under ultrasound guidance-is an alternate method to achieve larger volume samples and pilot studies confirm its

safety and efficacy (63).

Histological subtyping from CP-EBUS TBNA reflects true histology. In one retrospective study, 88 patients who underwent CP-EBUS TBNA had these results compared to core biopsies and/or follow up surgery. Sensitivity, specificity, PPV, and NPV for CP-EBUS TBNA were 85%, 100%, 100%, and 89.7% respectively (64). A more recent study of 92 patients with NSCLC demonstrated a 76% agreement between needle aspirate and biopsy for subtyping (kappa =0.52), with agreement increasing if cell blocks were available (96%, kappa 0.91 *vs.* 69%, kappa 0.39 respectively) (65).

Mutational analysis is also possible from CP-EBUS TBNA samples. 154 out of 156 cases were successfully analysed (98.7%) for EGFR mutations using the PCR clamp technique on cell-pellets derived from needle-washed solution (66). Garcia-Olive and colleagues showed EGFR analysis was possible in 72.2% of patients undergoing CP-EBUS TBNA with metastatic nodal specimens (67). In a different study analysis for EGFR and KRAS sequences using COLD-PCR was achieved in 95.5% and 98.4% respectively of samples (68). A UK group found that 88% of their CP-EBUS TBNA samples were adequate for mutational analysis using the Scorpion ARMS kit (69).

The actual false negative rate for CP-EBUS TBNA is a matter of debate; whilst specificity is unequivocally acceptable, sensitivity and NPV is more important when staging cancer. In 109 patients who underwent CP-EBUS TBNA of PET-avid N2 and N3 lymph nodes, 32 patients were tumour negative by CP-EBUS TBNA but subsequent biopsy in 19 showed malignancy in 7, four due to sampling error, and three due to detection error (70). Thus, in the setting of a high pre-test probability of nodal metastasis and no malignant cells on CP-EBUS TBNA, surgical biopsy should be used for confirmation (71).

Aside from mediastinal staging for NSCLC, CP-EBUS TBNA can diagnose central parenchymal lesions not visible on WLB, avoiding procedures associated with higher complication rates such as CT guided TTNA or mediastinoscopy (72-74).

Sarcoidosis can be reliably detected on CP-EBUS TBNA specimens. The combination of CP-EBUS TBNA and ROSE has high diagnostic accuracy, good interobserver agreement, and can inform the bronchoscopist of whether additional passes are necessary (75). Diagnostic accuracy of CP-EBUS TBNA is significantly higher than transbronchial biopsy or bronchoalveolar lavage (91.4% CP-EBUS TBNA, 65.7% BAL, 40% TBLBx) in Stage I sarcoid disease, although the three modalities have equivalent diagnostic rates in Stage II sarcoid disease (76). A systematic review and meta-analysis of CP-EBUS TBNA for sarcoidosis (15 studies, n=533) found a pooled diagnostic accuracy of 79% with only five minor complications reported, however significant study heterogeneity and publication bias were identified (77).

The diagnosis of sarcoidosis on CP-EBUS TBNA, however, should be tempered by the patient's pre-test probability of having this condition; in a case series of 1,275 patients undergoing CP-EBUS TBNA, granulomatous inflammation was found in 154 (12.1%) patients of whom 12 (7.8%) had a concurrent diagnosis of cancer, although no patient had both granulomatous inflammation and malignancy within the same lymph node (78). Patients with a high pre-test probability of malignancy but only granulomatous inflammation from CP-EBUS TBNA samples should be considered for additional sampling or close radiological follow up to ensure a benignity (79).

Cost effectiveness

Economic analyses validate the economic viability of CP-EBUS TBNA compared to cTBNA and mediastinoscopic staging. In a retrospective cohort of 294 patients with thoracic lymphadenopathy from a University Hospital, 37 patients underwent cTBNA and 257 had CP-EBUS TBNA. 90% of the CP-EBUS TBNA group was diagnostic compared to 62.2% of the cTBNA group; a higher proportion in the cTBNA group needed additional surgical procedures such as mediastinoscopy, video-assisted thoracic surgery (VATS), or an open thoracotomy. The mean savings with CP-EBUS TBNA was $1,071.09 per patient (80). Improved cost efficacy was also found in a health technology assessment involving hospitals from the United Kingdom, Belgium, and the Netherlands (81). Study patients were randomized to either surgical staging alone, or CP-EBUS TBNA/EUS-FNA followed by surgical staging if negative. The 6-month cost of the former group was £10,459 per patient compared to £9,713 per patient with the latter approach, a saving of £746 per patient mainly through reducing mediastinoscopies and unnecessary thoracotomies.

Learning curve/training

Proficiency in CP-EBUS TBNA improves with experience, however the number of procedures required for proficiency is uncertain (82,83). A cusum (cumulative sum control chart) analysis determined that learning curve duration was highly variable, even for experienced bronchoscopists, with one

operator almost immediately gaining competence, whilst another still on the learning curve after 100 procedures (84).

Trainees performing CP-EBUS TBNA increase procedure time, amount of sedation used, and complication rates (85). Obtaining proficiency using an CP-EBUS TBNA simulator before performing real procedures may address some of these issues; In one study, simulator training was equivalent to 15-25 "on-the-job" procedures in terms of procedure time and percentage of lymph nodes successfully identified (86,87). With the number of trainees wanting to acquire this new skill, coupled with the increasing focus on efficiency and reduction in complications, the EBUS simulator may become an increasingly valuable asset.

ROSE

ROSE of needle aspirates is thought to be beneficial in CP-EBUS TBNA but results have been varied. Potential advantages of ROSE for CP-EBUS TBNA include: quicker diagnosis, shorter procedure time, fewer needle passes per lymph node, and as a consequence reduction in complications.

In a prospective study, 120 patients suspected of having lung cancer with mediastinal adenopathy ≥10 mm were randomized to CP-EBUS TBNA with or without ROSE. In the ROSE group, the decision to make additional passes/procedures was based on ROSE findings at the operator's discretion; In the non-ROSE group, the target lesion underwent a minimum of three punctures, and additional punctures or bronchoscopic procedures were performed if the examiner deemed it necessary. There were significantly fewer punctures of the target lesion in the ROSE group (mean 2.2 punctures *vs.* 3.1 punctures, P<0.001) and significantly greater additional procedures in the non-ROSE group (57% non-ROSE *vs.* 11% ROSE). The mean bronchoscopy time, sensitivity and diagnostic accuracy did not differ between the groups.

ROSE results, however, need to be interpreted with caution as false negatives can occur (88) and concordance between staging and final pathological diagnosis is not perfect. On-site adequacy criteria have been proposed to reduce the risk of false negative specimens (89) but have not been prospectively validated.

CP-EBUS TBNA complications

CP-EBUS TBNA is a very safe procedure. Data from the prospectively enrolled American College of Chest Physicians Quality Improvement Registry, Evaluation, and Education (AQuIRE database) included 1,317 patients from 6 hospitals who underwent CP-EBUS TBNA for lymph node sampling (90). 19 patients (1.44%) had a complication with one patient dying from bleeding. Only TBLBx was associated with increased risk on multivariate analysis. Pneumothorax occurred in seven patients and TBLBx was the only variable associated with increased risk; [2.7% TBLBx had pneumothorax *vs.* 0.2% of those who did not (P=0.001)]. Factors associated with escalation in care included age >70, deep sedation or general anaesthesia, and inpatient status. There were no differences in complication rates between hospitals and outcomes were not associated with procedural volume. ROSE reduced the rate of subsequent TBLBx (P=0.006).

A questionnaire about CP-EBUS TBNA sent to 520 Japan Society for Respiratory Endoscopy-accredited facilities aimed to determine the rate of complications in Japan (40). Of 7,345 CP-EBUS TBNA performed in 210 facilities, 90 complications occurred (1.23%) with hemorrhage being the most frequent complication (0.68%), followed by infection (0.19%) and pneumothorax (0.03%). Only one death occurred (0.01%), the cause of which was cerebral infarction. Equipment related complications were common, with breakage of the ultrasound bronchoscope and puncture needle in 1.33% and 0.2% respectively. These rates are slightly greater than those reported in two meta-analyses (0% and 0.15%) (49,50). Individual case reports detail infectious complications (91-96), needle breakage (97), intramural hematoma (98), and pneumothorax.

Conclusion: peribronchial/peritracheal lesions including mediastinal lymphadenopathy

CP-EBUS TBNA has revolutionised mediastinal staging of lung cancer with sensitivity approaching mediastinoscopy associated with few complications. The widespread adoption of CP-EBUS TBNA internationally by surgeons and physicians is a tribute to its utility, usefulness, simplicity and safety; It is one of the few diagnostic techniques that has truly revolutionized lung cancer diagnostics.

Conclusions

Innovative bronchoscopic techniques are allowing lung cancer to be diagnosed earlier and more accurately in an increasingly non-invasive fashion. Peripheral lesions are targeted by RP-EBUS/GS, ENB, and UB/

VB; mucosal lesions can be identified with NBI and AFB; OCT allows cellular analysis without the need for biopsy; and central peribronchial lesions can be accurately localized and sampled with EBUS TBNA. Whilst the role of established techniques like RP-EBUS/GS, CP-EBUS TBNA, NBI and AFB are well established, data regarding emerging techniques such as ENB and OCT are immature and require further study to establish their utility.

The field of interventional diagnostic pulmonology is rapidly advancing, with the aim of safer, less invasive and more accurate modalities to identify and diagnose lung cancer earlier. Coupled with newer therapeutics such as stereotactic body radiation and targeted therapeutic agents, it is hoped that lung cancer mortality will no longer be the most common cause of cancer related mortality worldwide.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Thun MJ, Ries LA, et al. Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control. J Natl Cancer Inst 2008;100:1672-94.
2. Sun J, Garfield DH, Lam B, et al. The value of autofluorescence bronchoscopy combined with white light bronchoscopy compared with white light alone in the diagnosis of intraepithelial neoplasia and invasive lung cancer: a meta-analysis. J Thorac Oncol 2011;6:1336-44.
3. Holty JE, Kuschner WG, Gould MK. Accuracy of transbronchial needle aspiration for mediastinal staging of non-small cell lung cancer: a meta-analysis. Thorax 2005;60:949-55.
4. Rivera MP, Mehta AC, Wahidi MM. Establishing the diagnosis of lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e142S-65S.
5. Wiener RS, Schwartz LM, Woloshin S, et al. Population-based risk for complications after transthoracic needle lung biopsy of a pulmonary nodule: an analysis of discharge records. Ann Intern Med 2011;155:137-44.
6. Chen W, Gao X, Tian Q, et al. A comparison of autofluorescence bronchoscopy and white light bronchoscopy in detection of lung cancer and preneoplastic lesions: a meta-analysis. Lung Cancer 2011;73:183-8.
7. Lee P, van den Berg RM, Lam S, et al. Color fluorescence ratio for detection of bronchial dysplasia and carcinoma in situ. Clin Cancer Res 2009;15:4700-5.
8. Shibuya K, Hoshino H, Chiyo M, et al. High magnification bronchovideoscopy combined with narrow band imaging could detect capillary loops of angiogenic squamous dysplasia in heavy smokers at high risk for lung cancer. Thorax 2003;58:989-95.
9. Shibuya K, Nakajima T, Fujiwara T, et al. Narrow band imaging with high-resolution bronchovideoscopy: a new approach for visualizing angiogenesis in squamous cell carcinoma of the lung. Lung Cancer 2010;69:194-202.
10. Herth FJ, Eberhardt R, Anantham D, et al. Narrow-band imaging bronchoscopy increases the specificity of bronchoscopic early lung cancer detection. J Thorac Oncol 2009;4:1060-5.
11. Zaric B, Perin B, Becker HD, et al. Combination of narrow band imaging (NBI) and autofluorescence imaging (AFI) videobronchoscopy in endoscopic assessment of lung cancer extension. Med Oncol 2012;29:1638-42.
12. Vincent BD, Fraig M, Silvestri GA. A pilot study of narrow-band imaging compared to white light bronchoscopy for evaluation of normal airways and premalignant and malignant airways disease. Chest 2007;131:1794-9.
13. Zaric B, Becker HD, Perin B, et al. Narrow band imaging videobronchoscopy improves assessment of lung cancer extension and influences therapeutic strategy. Jpn J Clin Oncol 2009;39:657-63.
14. Tsuboi M, Hayashi A, Ikeda N, et al. Optical coherence tomography in the diagnosis of bronchial lesions. Lung Cancer 2005;49:387-94.
15. Lam S, Standish B, Baldwin C, et al. In vivo optical coherence tomography imaging of preinvasive bronchial lesions. Clin Cancer Res 2008;14:2006-11.
16. Tearney GJ, Brezinski ME, Bouma BE, et al. In vivo endoscopic optical biopsy with optical coherence tomography. Science 1997;276:2037-9.
17. Hariri LP, Applegate MB, Mino-Kenudson M, et al. Volumetric optical frequency domain imaging of pulmonary pathology with precise correlation to histopathology. Chest 2013;143:64-74.
18. Kurimoto N, Miyazawa T, Okimasa S, et al. Endobronchial ultrasonography using a guide sheath increases the ability to diagnose peripheral pulmonary lesions endoscopically. Chest 2004;126:959-65.
19. Paone G, Nicastri E, Lucantoni G, et al. Endobronchial

ultrasound-driven biopsy in the diagnosis of peripheral lung lesions. Chest 2005;128:3551-7.
20. Steinfort DP, Khor YH, Manser RL, et al. Radial probe endobronchial ultrasound for the diagnosis of peripheral lung cancer: systematic review and meta-analysis. Eur Respir J 2011;37:902-10.
21. Roth K, Eagan TM, Andreassen AH, et al. A randomised trial of endobronchial ultrasound guided sampling in peripheral lung lesions. Lung Cancer 2011;74:219-25.
22. Yamada N, Yamazaki K, Kurimoto N, et al. Factors related to diagnostic yield of transbronchial biopsy using endobronchial ultrasonography with a guide sheath in small peripheral pulmonary lesions. Chest 2007;132:603-8.
23. Huang CT, Ho CC, Tsai YJ, et al. Factors influencing visibility and diagnostic yield of transbronchial biopsy using endobronchial ultrasound in peripheral pulmonary lesions. Respirology 2009;14:859-64.
24. Shirakawa T, Imamura F, Hamamoto J, et al. Usefulness of endobronchial ultrasonography for transbronchial lung biopsies of peripheral lung lesions. Respiration 2004;71:260-8.
25. Fielding DI, Robinson PJ, Kurimoto N. Biopsy site selection for endobronchial ultrasound guide-sheath transbronchial biopsy of peripheral lung lesions. Intern Med J 2008;38:77-84.
26. Kurimoto N, Murayama M, Yoshioka S, et al. Assessment of usefulness of endobronchial ultrasonography in determination of depth of tracheobronchial tumor invasion. Chest 1999;115:1500-6.
27. Herth F, Ernst A, Schulz M, et al. Endobronchial ultrasound reliably differentiates between airway infiltration and compression by tumor. Chest 2003;123:458-62.
28. Makris D, Scherpereel A, Leroy S, et al. Electromagnetic navigation diagnostic bronchoscopy for small peripheral lung lesions. Eur Respir J 2007;29:1187-92.
29. Lamprecht B, Porsch P, Pirich C, et al. Electromagnetic navigation bronchoscopy in combination with PET-CT and rapid on-site cytopathologic examination for diagnosis of peripheral lung lesions. Lung 2009;187:55-9.
30. Seijo LM, de Torres JP, Lozano MD, et al. Diagnostic yield of electromagnetic navigation bronchoscopy is highly dependent on the presence of a Bronchus sign on CT imaging: results from a prospective study. Chest 2010;138:1316-21.
31. Eberhardt R, Anantham D, Ernst A, et al. Multimodality bronchoscopic diagnosis of peripheral lung lesions: a randomized controlled trial. Am J Respir Crit Care Med 2007;176:36-41.
32. Eberhardt R, Morgan RK, Ernst A, et al. Comparison of suction catheter versus forceps biopsy for sampling of solitary pulmonary nodules guided by electromagnetic navigational bronchoscopy. Respiration 2010;79:54-60.
33. Mahajan AK, Patel SB, Hogarth DK. Electromagnetic navigational bronchoscopy: an effective and safe approach to diagnosing peripheral lung lesions unreachable by conventional bronchscopy. Chest Meeting Abstracts 2008;134:98002.
34. Wilson DS, Bartlett RJ. Improved diagnostic yield of bronchoscopy in a community practice: combination of electromagnetic navigation system and rapid on-site evaluation. J Bronchol 2007;14:227-32.
35. Dolina MY, Cornish DC, Merritt SA, et al. Interbronchoscopist variability in endobronchial path selection: a simulation study. Chest 2008;133:897-905.
36. Asano F, Shindoh J, Shigemitsu K, et al. Ultrathin bronchoscopic barium marking with virtual bronchoscopic navigation for fluoroscopy-assisted thoracoscopic surgery. Chest 2004;126:1687-93.
37. Yamamoto S, Ueno K, Imamura F, et al. Usefulness of ultrathin bronchoscopy in diagnosis of lung cancer. Lung Cancer 2004;46:43-8.
38. Asahina H, Yamazaki K, Onodera Y, et al. Transbronchial biopsy using endobronchial ultrasonography with a guide sheath and virtual bronchoscopic navigation. Chest 2005;128:1761-5.
39. Ishida T, Asano F, Yamazaki K, et al. Virtual bronchoscopic navigation combined with endobronchial ultrasound to diagnose small peripheral pulmonary lesions: a randomised trial. Thorax 2011;66:1072-7.
40. Asano F, Aoe M, Ohsaki Y, et al. Complications associated with endobronchial ultrasound-guided transbronchial needle aspiration: a nationwide survey by the Japan Society for Respiratory Endoscopy. Respir Res 2013;14:50.
41. Asano F, Matsuno Y, Tsuzuku A, et al. Diagnosis of peripheral pulmonary lesions using a bronchoscope insertion guidance system combined with endobronchial ultrasonography with a guide sheath. Lung Cancer 2008;60:366-73.
42. Wang Memoli JS, Nietert PJ, Silvestri GA. Meta-analysis of guided bronchoscopy for the evaluation of the pulmonary nodule. Chest 2012;142:385-93.
43. Lemaire A, Nikolic I, Petersen T, et al. Nine-year single center experience with cervical mediastinoscopy: complications and false negative rate. Ann Thorac Surg 2006;82:1185-9; discussion 1189-90.

44. Steinfort DP, Irving LB. Patient satisfaction during endobronchial ultrasound-guided transbronchial needle aspiration performed under conscious sedation. Respir Care 2010;55:702-6.
45. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for staging non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e211S-50S.
46. Yasufuku K, Chiyo M, Koh E, et al. Endobronchial ultrasound guided transbronchial needle aspiration for staging of lung cancer. Lung Cancer 2005;50:347-54.
47. Herth FJ, Eberhardt R, Vilmann P, et al. Real-time endobronchial ultrasound guided transbronchial needle aspiration for sampling mediastinal lymph nodes. Thorax 2006;61:795-8.
48. Herth FJ, Ernst A, Eberhardt R, et al. Endobronchial ultrasound-guided transbronchial needle aspiration of lymph nodes in the radiologically normal mediastinum. Eur Respir J 2006;28:910-4.
49. Varela-Lema L, Fernández-Villar A, Ruano-Ravina A. Effectiveness and safety of endobronchial ultrasound-transbronchial needle aspiration: a systematic review. Eur Respir J 2009;33:1156-64.
50. Gu P, Zhao YZ, Jiang LY, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for staging of lung cancer: a systematic review and meta-analysis. Eur J Cancer 2009;45:1389-96.
51. Yasufuku K, Pierre A, Darling G, et al. A prospective controlled trial of endobronchial ultrasound-guided transbronchial needle aspiration compared with mediastinoscopy for mediastinal lymph node staging of lung cancer. J Thorac Cardiovasc Surg 2011;142:1393-400.e1.
52. Rintoul RC, Skwarski KM, Murchison JT, et al. Endobronchial and endoscopic ultrasound-guided real-time fine-needle aspiration for mediastinal staging. Eur Respir J 2005;25:416-21.
53. Wallace MB, Pascual JM, Raimondo M, et al. Minimally invasive endoscopic staging of suspected lung cancer. JAMA 2008;299:540-6.
54. Zhang R, Ying K, Shi L, et al. Combined endobronchial and endoscopic ultrasound-guided fine needle aspiration for mediastinal lymph node staging of lung cancer: a meta-analysis. Eur J Cancer 2013;49:1860-7.
55. Annema JT, van Meerbeeck JP, Rintoul RC, et al. Mediastinoscopy vs endosonography for mediastinal nodal staging of lung cancer: a randomized trial. JAMA 2010;304:2245-52.
56. Hwangbo B, Lee GK, Lee HS, et al. Transbronchial and transesophageal fine-needle aspiration using an ultrasound bronchoscope in mediastinal staging of potentially operable lung cancer. Chest 2010;138:795-802.
57. Herth FJ, Krasnik M, Kahn N, et al. Combined endoscopic-endobronchial ultrasound-guided fine-needle aspiration of mediastinal lymph nodes through a single bronchoscope in 150 patients with suspected lung cancer. Chest 2010;138:790-4.
58. Yarmus LB, Akulian J, Lechtzin N, et al. Comparison of 21-gauge and 22-gauge aspiration needle in endobronchial ultrasound-guided transbronchial needle aspiration: results of the American College of Chest Physicians Quality Improvement Registry, Education, and Evaluation Registry. Chest 2013;143:1036-43.
59. Nakajima T, Yasufuku K, Takahashi R, et al. Comparison of 21-gauge and 22-gauge aspiration needle during endobronchial ultrasound-guided transbronchial needle aspiration. Respirology 2011;16:90-4.
60. Casal RF, Staerkel GA, Ost D, et al. Randomized clinical trial of endobronchial ultrasound needle biopsy with and without aspiration. Chest 2012;142:568-73.
61. Lee HS, Lee GK, Lee HS, et al. Real-time endobronchial ultrasound-guided transbronchial needle aspiration in mediastinal staging of non-small cell lung cancer: how many aspirations per target lymph node station? Chest 2008;134:368-74.
62. Yung RC, Otell S, Illei P, et al. Improvement of cellularity on cell block preparations using the so-called tissue coagulum clot method during endobronchial ultrasound-guided transbronchial fine-needle aspiration. Cancer Cytopathol 2012;120:185-95.
63. Herth FJ, Schuler H, Gompelmann D, et al. Endobronchial ultrasound-guided lymph node biopsy with transbronchial needle forceps: a pilot study. Eur Respir J 2012;39:373-7.
64. Feller-Kopman D, Yung RC, Burroughs F, et al. Cytology of endobronchial ultrasound-guided transbronchial needle aspiration: a retrospective study with histology correlation. Cancer 2009;117:482-90.
65. Tournoy KG, Carprieaux M, Deschepper E, et al. Are EUS-FNA and EBUS-TBNA specimens reliable for subtyping non-small cell lung cancer? Lung Cancer 2012;76:46-50.
66. Nakajima T, Yasufuku K, Nakagawara A, et al. Multigene mutation analysis of metastatic lymph nodes in non-small cell lung cancer diagnosed by endobronchial ultrasound-guided transbronchial needle aspiration. Chest

2011;140:1319-24.
67. Garcia-Olivé I, Monsó E, Andreo F, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for identifying EGFR mutations. Eur Respir J 2010;35:391-5.
68. Santis G, Angell R, Nickless G, et al. Screening for EGFR and KRAS mutations in endobronchial ultrasound derived transbronchial needle aspirates in non-small cell lung cancer using COLD-PCR. PLoS One 2011;6:e25191.
69. Esterbrook G, Anathhanam S, Plant PK. Adequacy of endobronchial ultrasound transbronchial needle aspiration samples in the subtyping of non-small cell lung cancer. Lung Cancer 2013;80:30-4.
70. Rintoul RC, Tournoy KG, El Daly H, et al. EBUS-TBNA for the clarification of PET positive intra-thoracic lymph nodes-an international multi-centre experience. J Thorac Oncol 2009;4:44-8.
71. Andrade RS, Odell DD, D'Cunha J, et al. Endobronchial ultrasonography (EBUS)--its role in staging of non-small cell lung cancer and who should do it? J Thorac Cardiovasc Surg 2012;144:S9-13.
72. Tournoy KG, Rintoul RC, van Meerbeeck JP, et al. EBUS-TBNA for the diagnosis of central parenchymal lung lesions not visible at routine bronchoscopy. Lung Cancer 2009;63:45-9.
73. Fielding D, Bashirzadeh F, Nguyen P, et al. Review of the role of EBUS-TBNA for the pulmonologist, including lung cancer staging. Zhongguo Fei Ai Za Zhi 2010;13:410-7.
74. Bhatti HA, Bajwa A, Bhatti JA, et al. Diagnostic yield of EBUS-TBNA for the evaluation of centrally located peribronchial pulmonary lesions. J Bronchology Interv Pulmonol 2013;20:107-12.
75. Plit ML, Havryk AP, Hodgson A, et al. Rapid cytological analysis of endobronchial ultrasound-guided aspirates in sarcoidosis. Pharmacogenomics J 2014;14:20-7.
76. Nakajima T, Yasufuku K, Kurosu K, et al. The role of EBUS-TBNA for the diagnosis of sarcoidosis--comparisons with other bronchoscopic diagnostic modalities. Respir Med 2009;103:1796-800.
77. Agarwal R, Srinivasan A, Aggarwal AN, et al. Efficacy and safety of convex probe EBUS-TBNA in sarcoidosis: a systematic review and meta-analysis. Respir Med 2012;106:883-92.
78. DePew ZS, Gonsalves WI, Roden AC, et al. Granulomatous inflammation detected by endobronchial ultrasound-guided transbronchial needle aspiration in patients with a concurrent diagnosis of cancer a clinical conundrum. J Bronchology Interv Pulmonol 2012;19:176-81.
79. Chee A, Tremblay A. EBUS, granulomas, and cancer: what does this represent? J Bronchology Interv Pulmonol 2012;19:171-3.
80. Grove DA, Bechara RI, Josephs JS, et al. Comparative cost analysis of endobronchial ultrasound-guided and blind TBNA in the evaluation of hilar and mediastinal lymphadenopathy. J Bronchology Interv Pulmonol 2012;19:182-7.
81. Sharples LD, Jackson C, Wheaton E, et al. Clinical effectiveness and cost-effectiveness of endobronchial and endoscopic ultrasound relative to surgical staging in potentially resectable lung cancer: results from the ASTER randomised controlled trial. Health Technol Assess 2012;16:1-75, iii-iv.
82. Groth SS, Whitson BA, D'Cunha J, et al. Endobronchial ultrasound-guided fine-needle aspiration of mediastinal lymph nodes: a single institution's early learning curve. Ann Thorac Surg 2008;86:1104-9; discussion 1109-10.
83. Bizekis CS, Santo TJ, Parker KL, et al. Initial experience with endobronchial ultrasound in an academic thoracic surgery program. Clin Lung Cancer 2010;11:25-9.
84. Kemp SV, El Batrawy SH, Harrison RN, et al. Learning curves for endobronchial ultrasound using cusum analysis. Thorax 2010;65:534-8.
85. Stather DR, Maceachern P, Chee A, et al. Trainee impact on advanced diagnostic bronchoscopy: an analysis of 607 consecutive procedures in an interventional pulmonary practice. Respirology 2013;18:179-84.
86. Stather DR, MacEachern P, Chee A, et al. Evaluation of clinical endobronchial ultrasound skills following clinical versus simulation training. Respirology 2012;17:291-9.
87. Stather DR, Maceachern P, Rimmer K, et al. Assessment and learning curve evaluation of endobronchial ultrasound skills following simulation and clinical training. Respirology 2011;16:698-704.
88. Nakajima T, Yasufuku K, Saegusa F, et al. Rapid on-site cytologic evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for nodal staging in patients with lung cancer. Ann Thorac Surg 2013;95:1695-9.
89. Nayak A, Sugrue C, Koenig S, et al. Endobronchial ultrasound-guided transbronchial needle aspirate (EBUS-TBNA): a proposal for on-site adequacy criteria. Diagn Cytopathol 2012;40:128-37.
90. Eapen GA, Shah AM, Lei X, et al. Complications, consequences, and practice patterns of endobronchial ultrasound-guided transbronchial needle aspiration: Results of the AQuIRE registry. Chest 2013;143:1044-53.

91. Oguri T, Imai N, Imaizumi K, et al. Febrile complications after endobronchial ultrasound-guided transbronchial needle aspiration for intra-pulmonary mass lesions of lung cancer--a series of 3 cases. Respir Investig 2012;50:162-5.
92. Huang CT, Chen CY, Ho CC, et al. A rare constellation of empyema, lung abscess, and mediastinal abscess as a complication of endobronchial ultrasound-guided transbronchial needle aspiration. Eur J Cardiothorac Surg 2011;40:264-5.
93. Steinfort DP, Johnson DF, Irving LB. Incidence of bacteraemia following endobronchial ultrasound-guided transbronchial needle aspiration. Eur Respir J 2010;36:28-32.
94. Parker KL, Bizekis CS, Zervos MD. Severe mediastinal infection with abscess formation after endobronchial ultrasound-guided transbrochial needle aspiration. Ann Thorac Surg 2010;89:1271-2.
95. Steinfort DP, Johnson DF, Irving LB. Infective complications from endobronchial ultrasound-transbronchial needle aspiration. Eur Respir J 2009;34:524-5; author reply 525.
96. Haas AR. Infectious complications from full extension endobronchial ultrasound transbronchial needle aspiration. Eur Respir J 2009;33:935-8.
97. Ozgül MA, Cetinkaya E, Tutar N, et al. An unusual complication of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration (EBUS-TBNA): the needle breakage. Ann Thorac Cardiovasc Surg 2013. [Epub ahead of print].
98. Botana-Rial M, Núñez-Delgado M, Pallarés-Sanmartín A, et al. Intramural hematoma of the pulmonary artery and hemopneumomediastinum after endobronchial ultrasound-guided transbronchial needle aspiration. Respiration 2012;83:353-6.

Cite this article as: Leong S, Shaipanich T, Lam S, Yasufuku K. Diagnostic bronchoscopy--current and future perspectives. J Thorac Dis 2013;5(S5):S498-S510. doi: 10.3978/j.issn.2072-1439.2013.09.08

Modern diagnostic and therapeutic interventional radiology in lung cancer

Wai-Kit Lee[1], Eddie W. F. Lau[2], Kwang Chin[3], Oliver Sedlaczek[4], Karin Steinke[5]

[1]Department of Medical Imaging, St. Vincent's Hospital, University of Melbourne, Fitzroy, Victoria, Australia; [2]Centre for Molecular Imaging, Department of Radiology, Peter MacCallum Cancer Centre, University of Melbourne, East Melbourne, Victoria, Australia; [3]Department of Radiology, Peter MacCallum Cancer Centre, University of Melbourne, East Melbourne, Victoria, Australia; [4]Department of Diagnostic and Interventional Radiology, Heidelberg University Hospital, Heidelberg, Germany; [5]Department of Medical Imaging, Royal Brisbane and Women's Hospital, University of Queensland, Herston, Queensland, Australia

Correspondence to: Wai-Kit Lee. Department of Medical Imaging, St. Vincent's Hospital, University of Melbourne, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia. Email: leewk33@hotmail.com.

Abstract: Imaging has an important role in the multidisciplinary management of primary lung cancer. This article reviews the current state-of-the-art imaging modalities used for the evaluation, staging and post-treatment follow-up and surveillance of lung cancers, and image-guided percutaneous techniques for biopsy to confirm the diagnosis and for local therapy in non-surgical candidates.

Keywords: Lung neoplasms; computed tomography (CT); positron emission tomography (PET)/CT; magnetic resonance (MR) ; biopsy; ablation

Submitted Jun 27, 2013. Accepted for publication Jul 16, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.27
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.27

Introduction

Imaging has an important role in the multidisciplinary management of primary lung cancer, and is necessary to establish the diagnosis; localise, characterise and stage the tumour; map relevant nodal, vascular and bronchial anatomy for treatment planning; and for surveillance of treatment efficacy and development of metachronous tumours. Image-guided treatment of primary lung cancers can be performed in select cases. This article reviews the imaging modalities currently used for the evaluation of lung cancers, and discusses image-guided percutaneous interventional techniques for histopathologic diagnosis and local tumour treatment. Lung cancer screening is beyond the scope of this article.

Imaging modalities

Computed tomography (CT) is the imaging modality of choice for the initial evaluation of suspected or proven lung cancers. Positron emission tomography (PET)/CT is the most accurate imaging modality for the staging of primary lung cancers. Magnetic resonance (MR) imaging is useful for evaluation of superior sulcus (Pancoast) tumours and suspected malignant invasion of the chest wall, mediastinum or spine. The current recommended imaging required for lung cancer staging is CT scan of the thorax and PET/CT from skull base to mid-thigh (1).

Computed tomography

Advanced CT scanners permit a high-resolution, comprehensive evaluation of the entire chest in a single breath-hold lasting several seconds with an improved radiation dose profile to generate an isotropic dataset that allows detailed anatomical assessment as well as functional assessment of lung cancers. Radiation dose reduction is achieved by utilising automatic tube current modulation and iterative reconstruction techniques, which enable a CT examination to be performed either at a reduced dose with a similar image quality or at the same dose with improved image quality (2,3). Improved detection of small lung tumours is achieved by rapid acquisition and new visualisation

techniques. Rapid acquisition reduces respiratory and cardiac motion artefacts that allow more accurate depiction of lung nodules, especially in the lung bases and in the para-cardiac lung. New visualisation techniques, such as maximum intensity projection, volume rendering, stereographic display and computer-aided detection, enhance lung cancer detection and enable the reader to differentiate small lung nodules from vessels (4). Isotropic dataset acquisition permits easy multiplanar reconstructions, including high-resolution angiograms and three-dimensional reconstruction of vascular and bronchial anatomy, for surgical or percutaneous interventional planning.

Staging

Despite recent advances in CT technology, lung cancer staging with CT remains suboptimal but is routinely performed because it remains excellent for local staging of T1 and T2 tumours, is able to delineate T3 and T4 tumours, guides selection of the most appropriate lymph nodes and the invasive technique for nodal sampling, and allows triage of patients to non-surgical therapy when unequivocal distant metastases are present. Limitations of CT for staging include accurate detection of early mediastinal and chest wall invasion, mediastinal staging and detection of small extrathoracic metastases. With regards to local tumour extent, differentiation between absent, minimal and gross T3 and T4 disease is of critical clinical significance as it determines whether the tumour is completely resectable and the surgical approach (5). The utility of thin-section CT has not significantly improved the detection of malignant invasion of the parietal pleura. One study of 90 patients using a 4-detector CT showed sensitivity, specificity and accuracy of 42%, 100% and 83%, respectively, for the detection of chest wall invasion (6). Another small study using a 4-detector CT showed the use of multiplanar reconstructions can improve the sensitivity, specificity and accuracy of CT to 86%, 96% and 95%, respectively, for the detection of chest wall invasion (7). With regards to malignant nodal involvement, a recent meta-analysis showed a pooled sensitivity and specificity of 55% and 81%, respectively, for the detection of malignant mediastinal lymph nodes when a widely accepted definition of normal-sized lymph nodes of a short-axis diameter of ≤1 cm on a transverse CT scan image is used to differentiate benign from malignant lymph nodes (8). These results show that nodal size criterion alone is insufficient for the accurate detection of nodal metastases because metastases to normal-sized lymph nodes are missed and enlarged lymph nodes can be reactive or hyperplastic in aetiology. Recent studies suggest that evaluation of nodal morphology and CT enhancement pattern can improve the accuracy of CT for the detection of nodal metastases in lung cancer (9,10). With regards to distant metastases, CT is inferior to PET/CT for detection of extrapulmonary metastases with an accuracy of 88% compared to 97% with PET/CT (11). To our knowledge, there has been no study that examined the accuracy of CT for lung cancer staging using CT scanners with more than 16-detector rows.

Post-treatment evaluation and surveillance

There is currently no consensus on the optimal follow-up and surveillance programme for patients with proven lung cancers. CT has been recommended for the routine evaluation and surveillance of patients who have undergone therapy with curative-intent for non-small cell lung cancer (NSCLC), but routine imaging surveillance is not recommended in asymptomatic patients with advanced lung cancer who are not undergoing therapy (12-14). CT evaluation of response to treatment is usually dependent on morphologic changes in tumour and nodes. However, morphology is not a good indicator of early response to treatment and a positive response can be manifested as a delayed reduction in size or paradoxical increase in size (15). CT can be effective for post-treatment surveillance with one study showing CT detected 93% of new lung cancers and 61% of recurrences in a cohort of over 1,000 patients after resection of early-stage NSCLC (16).

New developments

Recent advances in CT technology have allowed investigation of novel methods for the evaluation of lung cancers including nodule volumetry, nodule perfusion analysis, dual-energy applications and computer-aided detection. Quantitative analysis of lung nodules by assessment of a nodule's volume can be performed using semi-automated or automated segmentation tools that allow assessment of nodule stability or progression over time. The rate of growth of a nodule is a predictor of the likelihood of malignancy, and volumetric analysis can be used to predict tumour response to treatment (17). CT perfusion analysis of nodules may allow better characterisation of the nodule in order to determine likelihood of malignancy as well as earlier determination of treatment response compared

to morphologic change in size (18,19). The likelihood of malignancy is considered low when contrast uptake is below 30 Hounsfield units (HU) (20). Dual-energy CT is a technique that allows differentiation of iodine from other materials, such as soft tissue and bone, due to iodine's stronger photoelectric absorption (21). This method allows visualisation of the degree and pattern of enhancement within a mass following contrast-enhanced CT. One study showed that the degree of enhancement within a pulmonary nodule can be used to differentiate benign from malignant tumours with a sensitivity, specificity and accuracy of 92%, 70% and 82%, respectively (22). In another study, moderate correlation was found between the maximum iodine attenuation and SUV_{max} in thoracic nodes in patients with NSCLC, but poor correlation in those patients with small cell lung cancer (23). The authors suggest that moderate correlation in NSCLC could be explained by moderate specificity of PET for determination of malignant nodes, and the difference in correlation seen with NSCLC compared to SCLC due to differing tumour biology such as angiogenic ability.

Positron emission tomography

Solitary pulmonary nodule

PET with F-18 deoxyglucose (FDG) is a useful technique for the characterisation of pulmonary nodules to distinguish between benign and malignant lesions. Two meta-analyses showed the sensitivity and specificity of FDG-PET for the diagnosis of malignant pulmonary nodules were about 96% and 80%, respectively (24,25). The significance of a PET-positive result is dependent on the clinical context and the prevalence of granulomatous and infectious disease, which are recognised causes of false positive PET results. False negative results can occur in small (<10 mm) nodules due to partial volume effect or the effect of respiratory blurring, or in some subtypes of lung malignancy with a low intrinsic FDG avidity, such as adenocarcinoma in situ. On a practical level, a PET-positive study often implies that biopsy or intervention is warranted to obtain pathological confirmation (26), while a PET-negative study may allow conservative approach and avoidance of unnecessary invasive procedures (27). There is evidence that the use of FDG-PET is cost-effective in the management of solitary pulmonary nodules (28,29).

Staging

PET is the most accurate imaging modality for the assessment of nodal and distant metastases from lung cancer, which is vital for treatment planning. PET has been found to be more accurate than CT in the staging of mediastinal nodal disease in many clinicopathological studies, including two meta-analyses that showed the sensitivity and specificity of PET was 79-85% and 90-91%, respectively, compared to 60-61% and 77-79% for CT (30,31). The accuracy of nodal assessment is further increased with PET/CT, which has an excellent negative predictive value of 91% in the mediastinal assessment of early-stage disease (32,33). Despite the high accuracy of PET/CT in nodal staging, there remains a significant false positive rate that is more common with larger (>1 cm) nodes, which is often due to reactive or granulomatous nodal disease (34). With the increased availability of minimally invasive mediastinal nodal sampling procedures, such as endobronchial ultrasound and endoscopic ultrasound, it is imperative to obtain pathological confirmation of PET-positive nodes before denying surgery to patients with potentially curable disease (35,36). PET is the imaging modality of choice in the assessment for distant metastases of lung cancer due to its whole body imaging capability and high tumour to background contrast which allows superior detection of both osseous and soft tissue metastases (37-39). There is a significant incidence of unrecognised distant metastatic disease when staging with conventional CT and bone scintigraphy. One study showed distant metastases were only identified with PET in 7.5%, 18% and 24% of stage I, stage II and stage III disease, respectively (40). Up to 20% of patients who are thought to be operable when staged with conventional imaging are found to be inoperable following PET and, therefore, PET is considered essential prior to curative treatment to avoid unnecessary futile surgical intervention (41,42). PET/CT has been shown to be superior to standalone PET or CT in the detection of distant disease mainly due to the ability of PET/CT to obtain anatomical correlation to reduce false positive PET interpretation of physiologic uptake in normal structures (43). A recent meta-analysis showed PET/CT was significantly superior to PET, MR imaging and bone scintigraphy for the detection of bony metastases with a pooled PET/CT sensitivity and specificity of 92% and 98%, respectively (44). Given the high background FDG uptake in the brain, FDG-PET is not the optimal imaging modality for the exclusion of cerebral metastases, which should be evaluated by MR imaging when clinically indicated (45). The availability of both functional and structural information on PET/CT also facilitates the selection of stage critical lesions for biopsy to allow pathological confirmation. The use of

PET and PET/CT is cost-effective in the staging of NSCLC (46-48) with a recent randomised clinical trial showing cost savings of 899 Euro per patient and 4,495 Euro per avoided thoracotomy (49). There is also a strong correlation between PET-stage and survival in both surgical and radical radiotherapy candidates which suggests that PET provides prognostically significant information (50,51).

Radiotherapy planning

FDG-PET and PET/CT have been found to have a critical role in patient selection and target volume definition in patients with locoregionally advanced NSCLC considered for curative or radical radiotherapy. Radical radiotherapy is given with curative intent to non-surgical patients with gross locoregional tumour that can be encompassed by high-dose radiation in the absence of distant disease (52). A number of prospective studies investigating the utility of PET in the staging of potential candidates for radical radiotherapy found 25-30% of the patients were unsuitable for radical treatment owing to the presence of more advanced disease that was not shown on conventional imaging (53-55). PET-assisted radiotherapy treatment volume contouring has been found to be more accurate and significantly different from conventional treatment volumes, and a change in radiation volume was found in more than 30% of the patients (53,56,57). Survival benefit has also been shown with PET/CT-assisted radical radiotherapy. In one study, the 4-year survival of stage IIIA patients managed with PET/CT-assisted radical radiotherapy is 32%, which is superior to outcome with CT-assisted radical radiotherapy (58).

Post-treatment evaluation

A prospective study of 73 patients comparing FDG-PET with CT for the assessment of response following radical radiotherapy and chemoradiation of NSCLC showed significantly more complete responders on PET (34 patients) than CT (10 patients). PET response was more predictive of survival duration than CT response, and is the only prognostic factor associated with survival duration on multifactor analysis (59). A more recent paper also reported a high metabolic tumour volume post definitive treatment for NSCLC was an independent poor prognostic factor (60). FDG-PET has also been found to provide prognostically significant response assessment in NSCLC patients undergoing induction chemotherapy. In a prospective study involving 31 patients with stage III unresectable disease, complete response on PET was more accurate than response on CT, and PET showed superior correlation with longer time to progression and overall survival (61). The ability of FDG-PET to provide superior prognostic information has also been reported in the setting of induction chemotherapy prior to surgical resection or chemoradiation, and there may be a role of PET in treatment selection and planning (62,63).

Magnetic resonance imaging

Modern MR techniques have overcome the principal problem of magnetic field inhomogeneities due to the numerous air-soft tissue interfaces when imaging the lung as well as artefacts associated with cardiac and respiratory movement to produce diagnostic images. The utility of MR for the diagnosis, staging, radiotherapy planning and post-treatment evaluation in lung tumours is under-utilised and has been investigated at only a few centres. The sensitivity, specificity, positive predictive value and negative predictive value of MR for the detection of lung carcinoma and non-calcified lesions greater than 5 mm are close to 100% (64). Therefore, MR can potentially be used for lung cancer screening, but to our knowledge, there are no prospective trials investigating the utility of MR for this purpose (65,66). MR imaging with diffusion-weighted imaging (DWI) can be used to predict benignity of pulmonary lesions. One prospective study of 66 patients showed DWI had a sensitivity, specificity and positive predictive value of 95%, 73% and 87%, respectively, for the diagnosis of a malignant lesion (67).

In current clinical practice, MR imaging is primarily used for the assessment of suspected chest wall or mediastinal invasion by lung cancer due to the superior soft tissue contrast resolution of MR. Comparative studies between MR and FDG-PET/CT have shown the two techniques to be equivalent for staging NSCLC (68-70). The strength of MR is in the detection of cerebral and hepatic metastases, while PET/CT is better at nodal staging. A recent prospective study showed MR imaging with DWI was superior to PET/CT for the detection and nodal assessment of NSCLC (71). MR also allows differentiation of viable tumour from necrotic tumour and atelectasis, and is helpful in radiotherapy planning (72). Post-treatment tumour response has been investigated using MR techniques such as magnetization transfer, blood oxygen level dependent MR, and perfusion and diffusion imaging. Dynamic contrast-enhanced perfusion allows

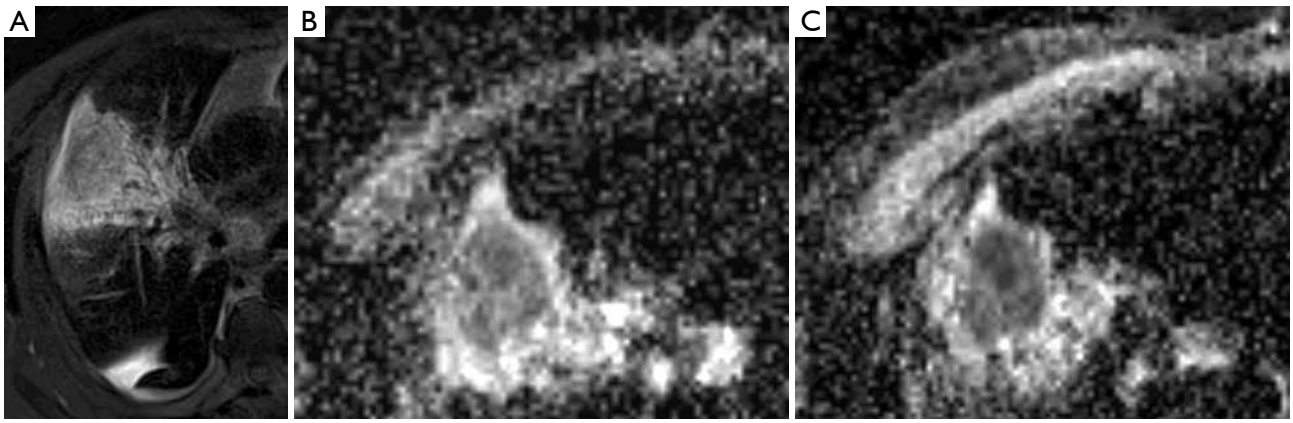


Figure 1 A 71-year-old man with Stage III NSCLC in the right upper lobe. (A) Axial T2-weighted MR image shows a heterogeneous T2 hyperintense mass with surrounding atelectasis; (B,C) axial ADC diffusion images before (B) and 24 hours after (C) starting chemotherapy show reduced ADC signal intensity in tumour following treatment.

assessment of tumour angiogenesis, which has the potential to predict chemotherapy response in NSCLC patients, but its use is unproven for monitoring of anti-angiogenic therapy (73). MR perfusion can also be used for prediction of postoperative lung function (74). With regards to DWI, the apparent diffusion coefficient (ADC) measures the magnitude of diffusion of water molecules within tissue. Cell swelling or shrinkage is reflected in changes in ADC values. In general, an initial decrease in tumour ADC measurement performed within 30 days of treatment (*Figure 1*), and an increase in tumour ADC measurement 30 days following treatment were found to be predictive of a positive outcome, and our own unpublished data support similar findings (75,76).

Image-guided percutaneous interventions

Image-guided percutaneous biopsy can be performed for confirmation of diagnosis and treatment planning. In non-surgical candidates, image-guided percutaneous therapy can be performed with curative-intent or for palliation, and these techniques include cryoablation, radiofrequency ablation and microwave ablation.

Percutaneous biopsy

Percutaneous needle core biopsy is a minimally invasive procedure that can be used to confirm the diagnosis of lung malignancy. The most common complications are pneumothorax and bleeding. A higher risk of pneumothorax has been reported to occur with biopsy of smaller lesions and deeper lesions. Biopsy of lesions less than 2 cm in size is associated with an 11 times greater risk of pneumothorax than lesions greater than 4 cm, and this may be explained by the prolonged procedure time required to successfully biopsy smaller lesions (77). This study also showed that the risk of a pneumothorax is negligible for lesions abutting the pleura because the needle does not need to cross aerated lung, but there is a seven-fold increase in the rate of pneumothorax for biopsy of lesions less than 2 cm from pleura and a four-fold rate for lesions greater than 2 cm (77). Hence, the authors advocated a longer oblique needle path for biopsy of sub-pleural nodules to minimise pneumothorax risk, but a different study suggested that a smaller needle-to-pleura angle increases the risk of a pneumothorax (78). Other potential factors for the higher risk of a pneumothorax when lesions less than 2 cm from the pleura are biopsied include multiple punctures and difficulty anchoring a heavy hub cutting needle. A higher risk of pneumothorax following biopsy in patients with obstructive pulmonary disease has been reported in some studies (78,79), but other studies did not find this association (77,80,81). Factors that were not associated with an increased risk of pneumothorax include biopsy of cavitary lesions, biopsy needle size and patient positioning following biopsy (77). Bleeding is the second most common complication of percutaneous lung biopsy, and the two main predisposing factors were lesion size and distance of lesion from the pleural surface. A six-fold increase in bleeding was shown for lesions less than 2 cm in size compared to those greater than 4 cm in size, and lesions greater than 2 cm from the pleura have a ten-fold bleeding risk compared to those abutting the pleural surface

(77,82). The presence of a pleural effusion on the side of the biopsy was associated with a decreased risk of bleeding and was found to be an independent risk factor for bleeding following biopsy (77).

Cryoablation

Cryoablation is a percutaneous minimally invasive technique used for the treatment of lung tumours in non-surgical candidates. Cryoablation causes coagulative necrosis of tumour cells and its vasculature. During cryoablation, a 2 to 3 cm rim of normal tissue surrounding the lesion should be ablated to achieve a margin of safety. The cryoablation probe is introduced into the tumour and cooled to –40 ℃ for about 10 minutes, then thawed for 8 minutes and then cooled once again for a further 10 minutes. Ice formation around the probe disrupts cell membrane function and enzymes, and creates a relative hypertonic extracellular environment causing intracellular dehydration by osmosis. The rapid return of water into the cell during the thawing process causes cell lysis. Direct damage to small (<3 mm) vessel walls and vessel stasis supplying the tumour may also play a role in tumour destruction (83). There is limited long-term outcome data for lung tumours treated with cryoablation. A Japanese series of 20 patients with 35 treated lesions followed for a median of 28 months showed a local recurrence rate of 20% (84). Another study investigated patients with stage I NSCLC who were unsuitable for standard surgical resection, and showed a 3-year survival rate of 77%, 88% and 87% when treated with cryoablation, radiofrequency ablation (RFA) or sublobar resection, respectively (85). Although cryoablation is comparable to other ablative techniques for the management of non-surgical candidates with lung cancer, longer term follow-up data are required to determine its role in the management of lung cancers.

Radiofrequency ablation

RFA is a technique that involves the placement of an electrode into tissue to cause focal destruction with thermal energy, which is generated by friction secondary to oscillating tissue ions that occur when an alternating electric current in the frequency of 460-500 kHz (radio waves) is applied. Heating tissue to 50 ℃ for at least 5 minutes causes cells to undergo coagulative necrosis. Therapeutic RFA aims to heat tissues to 60-100 ℃, which leads to near instant cell death through protein denaturation (86). Pulmonary lesions are ideal for RFA because air in lung parenchyma surrounding the lesion provides thermal insulation to allow concentration of the applied radiofrequency (RF) energy within the lesion. Non-surgical candidates or patients who refuse surgery are potential candidates for RFA and the decision to treat with RFA should ideally be made in consultation with the interdisciplinary pulmonary tumour board. The most suitable lesions for RFA are less than 3 cm in size, and patients with stage I NSCLC are ideal candidates. Reported long-term survival rates after RFA of stage I NSCLC at 1, 2, 3, 4 and 5 years are 78%, 57%, 36%, 27% and 27%, respectively (87). RFA in combination with conventional radiotherapy has been used to treat inoperable lung tumours because hypoxic cells in the centre of tumour respond poorly to radiotherapy alone. A study showed cumulative survival rates of 50% and 39% at 2 and 5 years, respectively, following combined RFA and conventional radiotherapy for stage I NSCLC (88), and dual therapy achieves better local tumour control and patient survival compared to radiotherapy alone (89). Furthermore, RFA can be used to treat small slow growing pulmonary metastases (90) and to palliate patients with larger tumours that cause chest pain, dyspnoea, cough or haemoptysis (87). RFA of larger tumours often requires multiple overlapping ablations to ensure satisfactory tumour coverage, and recurrence rates tend to be higher in larger tumours than with smaller tumours (87,89).

Microwave ablation

Microwave ablation (MWA) represents the most recent addition to the growing armamentarium of minimally invasive thermal ablation therapies for the nonsurgical treatment of lung malignancies (91). Microwaves are electromagnetic waves with a frequency range that extends from 300 MHz to 300 GHz. However, microwave generators for clinical use operate at frequencies of 915 MHz or 2.45 GHz (92). Microwaves agitate water molecules, which are small electric dipoles, in the target tissue, and they spin between 2 and 5 billion times per second in an attempt to follow the rapidly alternating electric field (92,93). This leads to heat generation through friction. Conduction and convection allow further tissue heating beyond the directly agitated water molecules (94). Temperatures thus generated, usually in excess of 100 ℃, lead to almost instantaneous irreversible cell damage. The centrifugally growing coagulation necrosis around the active tip of the microwave antenna is spherically shaped. It should ideally encompass the target tumour and

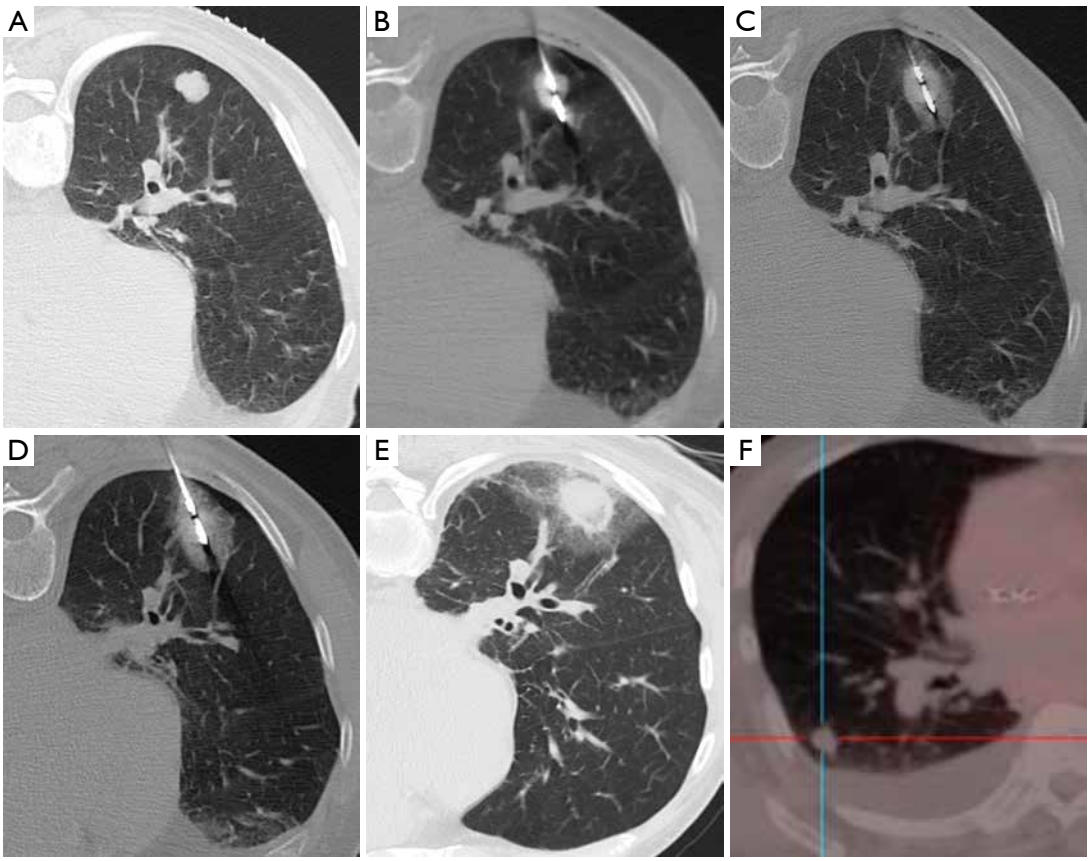


Figure 2 A 70-year-old man with cardiomyopathy and new solitary melanoma metastasis to the right lower lobe. (A) Prone axial CT image shows a lobulated 1.6 cm tumour; (B) the feed point of the microwave antenna is positioned within the centre of the tumour; (C,D) at 5 minutes following the start of ablation (C), there is ground glass opacity forming mainly on the far edge of the tumour, and at 10 minutes (D), a 3-10 mm circumferential rim of ground glass opacity has formed around the tumour; (E) at 3 hours following ablation, there is marked (>1 cm) circumferential ground glass opacity around the tumour; (F) axial FDG-PET/CT image 6 months following MWA shows complete lack of FDG uptake at the site of tumour, indicating eradication of tumour. New cardiac-related pleural effusion is present.

a circumferentially surrounding safety margin (*Figure 2*). This safety margin, ideally at least 6 mm in thickness, is necessary to destroy tumour cell nests and satellite foci in the immediate periphery of the tumour not perceivable on cross-sectional imaging. A smaller safety margin carries a higher risk for local recurrence (95).

Advantages of MWA over RFA

There are several advantages of microwave over RF energy. RF heating requires an electrical conduction path and is, therefore, less effective in areas of low electrical conductivity and high baseline impedance, such as lung parenchyma. This results in heating of the target tissue only immediately adjacent to the RF electrode (96,97). Microwaves are capable of propagating through many types of tissue and effectively heating them, even those with low electrical conductivity, high impedance or low thermal conductivity (98). Unlike RF and laser, microwaves can penetrate through the charred or desiccated tissues that build up around all hyperthermic ablation applicators which result in limited power delivery for non-microwave energy systems (99). Multiple microwave antennae can be simultaneously powered to maximise the ablation volume when placed in close proximity to each other, or when widely spaced, to simultaneously ablate several tumours, such as multiple pulmonary metastases (95). Larger and more homogeneous ablation volumes can be achieved with MWA because the rapid heating with MWA results in less susceptibility to heat sink effect (92). Further advantages

of MWA over RFA include no requirement for grounding pads which avoids potential pad site burns, and implanted cardiac devices are less prone to malfunction (100,101).

Detailed histopathological assessment of microwave ablated lesions has confirmed the concentric layered ablation zones post RFA described by Clasen *et al.* (102). The central inner necrosis is surrounded by an intermediate zone of equally irreversibly destroyed tissue corresponding to the safety margin strived for. The outer zone of ground glass opacity encompasses a haemorrhagic ring, which in turn is surrounded by oedema and a lymphocytic infiltrate (103). In these outermost layers, there was only partial thermal cell destruction seen with RFA (102). Vital histochemical nicotinamide adenine dinucleotide staining of resected lung tumours which have undergone intra-operative MWA immediately prior to resection confirmed cellular death (because of a lack of mitochondrial enzymatic activity) in a much larger ablation zone (104). No viable cells could be detected within five of the six ablation zones; uniform cellular death was shown to extend through sharp well-demarcated transition zones separating viable and nonviable ablated cells (104).

Patient selection and method

Patients selected for MWA are usually deemed medically inoperable. Exclusion criteria for MWA include uncontrolled primary tumour, radiologic evidence of lymph node metastases, extrathoracic spread, infiltration of the chest wall, mediastinal structures, main bronchi or main pulmonary arteries, sepsis and irreversible coagulopathy (94,105). Patients who have undergone prior surgery (including pneumonectomy), chemotherapy or radiotherapy are usually not excluded. Some patients may have resectable tumours, but have declined surgery. The patient is positioned in the CT scanner that allows the shortest and safest access route to the tumour. Crossing of fissures should be avoided whenever possible. The skin is prepped and draped as usual, and local anaesthetic is infiltrated along the needle tract. A short cut into the skin allows for a smooth passage of the antenna. Under visual guidance, preferably CT fluoroscopy, the antenna is advanced in a stepwise manner into the tumour. Calcified pleural plaques and cartilage should be avoided because the fragile microwave antenna is at risk of fracture if forced through rigid tissue (106). Conscious sedation or general anaesthesia can be used with no difference in complications and outcome between the two modalities (107). Single ablation duration depends on tumour size, location and power capacity of the generator, but usually does not exceed 20 minutes. It is advisable to perform a limited CT scan during the ablation cycle to identify any displacement of the antenna from its original position or early complications. Only a few centres routinely administer prophylactic antibiotics for the procedure (108). Immediate post-procedural recovery includes continuous monitoring for pulse and oxygen saturation, and blood pressure measurements taken every 15-20 minutes. Other observations are performed as per the hospital's policy. A baseline chest radiograph is performed 3 to 4 hours following the ablation. In most centres, patients are hospitalised after the procedure and discharged the following day provided no complications have occurred. In principle, the procedure can be performed on an outpatient basis, but it is recommended to observe them overnight because of the potential for delayed complications.

Complications

Complications resulting from ablation procedures should be classified in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) (109). The complication rate from MWA varies and can be expected to be higher in patients who have poor underlying pulmonary reserve. Pneumothorax is the most common immediate complication with a reported incidence of up to 43% (110), but less than one-third of these patients require a chest tube (105,110,111). Post-ablation syndrome, defined as a constellation of productive cough with or without minor haemoptysis, residual soreness in the treated area, and fever occurring several days after ablation, is reported in 2% of cases (105). Small pleural effusions not requiring thoracentesis occur in around a quarter of ablations. Cavitary changes are reported in up to 43% of ablated tumours, 14% of which display air-fluid levels that usually involute spontaneously (105). Infective complications (abscess, pneumonia) are rare (105,110). Chest wall emphysema occurs in approximately 20% of cases (unpublished author's experience) and is usually concurrent with a pneumothorax (*Figure 3*). Ablated tumours abutting the visceral pleura result in pleural thickening in over one-third of cases (105), and prolonged pleural retractions occur in a small proportion of these cases. Up to 15% of patients require hospitalisation after MWA primarily due to pneumothoraces (105).

There is scarce comparable long-term outcome data for the effectiveness of MWA in lung cancer owing to the relatively

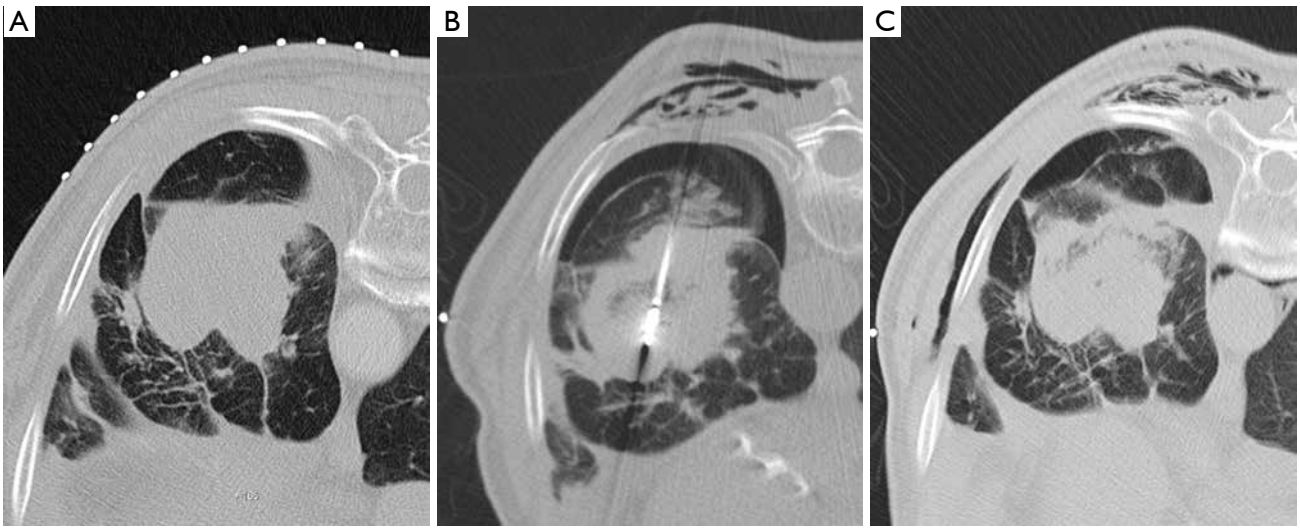


Figure 3 A 72-year-old man with incomplete response to external beam radiation to left lower lobe NSCLC, who presented for salvage MWA. He had an intractable cough throughout the procedure. (A) Prone axial CT image shows a 5.5 cm mass before MWA; (B) prone axial CT during the procedure shows a small pneumothorax and surgical emphysema developing around the antenna entry site; (C) prone axial CT at the end of the procedure shows the pneumothorax has remained similar in size, but there is increased surgical emphysema.

recent addition of MWA to the armamentarium of minimally invasive hyperthermal treatment modalities; different MWA protocols that use different ablation systems operating at different frequencies with different shaft cooling mechanisms, different antennae size and different active tip lengths; and heterogeneous patient population treated, including treatment-naïve primary lung cancer, locally recurrent primary lung cancer following prior therapy, synchronous or metachronous lung cancers, and pulmonary metastases. Two recent publications using the same MWA device and with similar protocols showed promising short to mid-term results. In a homogeneous patient population of early stage NSCLC, the local control rate was 88% and 75% at a median follow-up of 6 months and 1 year, respectively (110,111).

Post-treatment evaluation

CT is the imaging modality of choice for follow-up despite additional radiation exposure. A common post-ablation surveillance protocol is to perform a CT study the day following ablation to assess for complications, and this study can be used as a reference for comparison with subsequent studies, which are performed at 3 , 6 and 12 months during the first year, and at 6-monthly intervals thereafter (110). A thin (<5 mm) symmetric circumferential rim of peripheral enhancement is seen up to 6 months following ablation and is considered a sign of benign reactive enhancement. Irregular focal soft-tissue enhancement of >15 HU is, however, considered to be a sign of residual or recurrent disease (105). The initial size of the ablation zone is supposed to be much larger than the treated tumour as it encompasses the surrounding safety margin. Continuous shrinkage thereafter should occur and this usually leaves a small focus of atelectasis (*Figure 4*) or scar. FDG-PET imaging is considered to be more sensitive for detecting early tumour recurrence. The specificity, however, is low in the early post-ablation period. Performing FDG-PET scans sooner than 6 months following ablation should be discouraged to ensure a low false-positive rate (112,113). In addition to the FDG uptake values, the pattern of FDG uptake is also indicative of ablation success or failure (113). Modified response evaluation criteria in solid tumours (RECIST) criteria, which incorporate both the CT and FDG-PET appearances of the lesion following ablation, are considered the most appropriate tool for follow-up assessment (114).

Conclusions

The current challenge for imaging is to exploit the advantages of each imaging modality and integrate them into a clinically useful algorithm. At present, CT and PET/CT are recommended for lung cancer staging, and MR imaging is used for evaluation of suspected T3 and T4 disease. A few recent studies suggest that MR is equivalent to FDG-

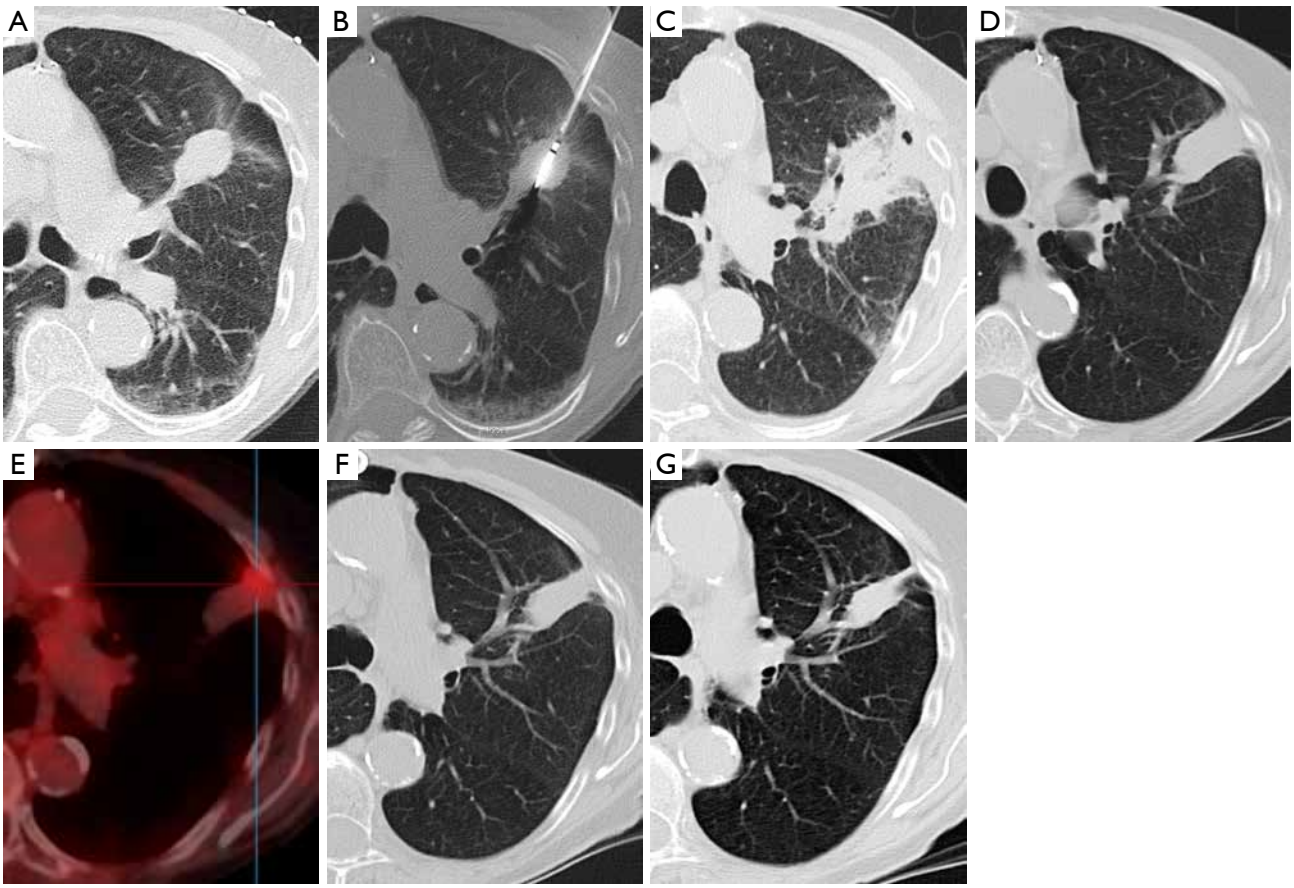


Figure 4 A 75-year-old ex-smoker with prior RFA of biopsy-proven left upper lobe NSCLC. FDG-PET 3 months following RFA was equivocal for residual tumour and repeat treatment with CT-guided MWA was performed. (A) Axial CT image shows a 3 cm left upper lobe mass; (B) axial CT shows microwave antenna is positioned within the centre of the mass; (C) axial CT 24 hours following MWA shows ablation site encompasses target lesion. There is a focal pleural effusion with small cavity, and surrounding atelectasis and ground glass opacity changes; (D,E) axial CT (D) and axial fused FDG-PET/CT (E) 6 months following ablation show resolution of the ground glass opacity and shrinking of the ablation volume, but a residual broad-based pleural contact remain. FDG-PET/CT shows lack of FDG-avidity of the ablated lesion but mild sub-pleural FDG uptake, likely inflammatory; (F,G) axial CT 12 months (F) and 24 months (G) following ablation show further gradual shrinking of the ablation volume and narrowing of the pleural contact.

PET/CT for staging NSCLC. New developments in CT, PET/CT and MR have the potential to provide improved anatomical and functional assessment of lung cancers that result in more individualized and targeted therapy. Cryoablation, RFA and MWA are promising powerful percutaneous techniques for curative-intent therapy or localised palliation of lung cancer, but available short- to mid-term data suggest MWA to be superior to RFA. However, more mid- and long-term data are required to assess for survival and cancer-free outcome following such therapies.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Ravenel JG, Mohammed TL, Movsas B, et al. ACR Appropriateness Criteria® noninvasive clinical staging of bronchogenic carcinoma. J Thorac Imaging 2010;25:W107-11.
2. Willemink MJ, Leiner T, de Jong PA, et al. Iterative reconstruction techniques for computed tomography part

2: initial results in dose reduction and image quality. Eur Radiol 2013;23:1632-42.
3. Vardhanabhuti V, Loader RJ, Mitchell GR, et al. Image quality assessment of standard- and low-dose chest CT using filtered back projection, adaptive statistical iterative reconstruction, and novel model-based iterative reconstruction algorithms. AJR Am J Roentgenol 2013;200:545-52.
4. Walsh SL, Nair A, Hansell DM. Post-processing applications in thoracic computed tomography. Clin Radiol 2013;68:433-48.
5. Stoelben E, Ludwig C. Chest wall resection for lung cancer: indications and techniques. Eur J Cardiothorac Surg 2009;35:450-6.
6. Bandi V, Lunn W, Ernst A, et al. Ultrasound vs. CT in detecting chest wall invasion by tumor: a prospective study. Chest 2008;133:881-6.
7. Higashino T, Ohno Y, Takenaka D, et al. Thin-section multiplanar reformats from multidetector-row CT data: utility for assessment of regional tumor extent in non-small cell lung cancer. Eur J Radiol 2005;56:48-55.
8. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for staging non-small cell lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e211S-50S.
9. Volterrani L, Mazzei MA, Banchi B, et al. MSCT multi-criteria: a novel approach in assessment of mediastinal lymph node metastases in non-small cell lung cancer. Eur J Radiol 2011;79:459-66.
10. Takahashi Y, Takashima S, Hakucho T, et al. Diagnosis of regional node metastases in lung cancer with computer-aided 3D measurement of the volume and CT-attenuation values of lymph nodes. Acad Radiol 2013;20:740-5.
11. De Wever W, Vankan Y, Stroobants S, et al. Detection of extrapulmonary lesions with integrated PET/CT in the staging of lung cancer. Eur Respir J 2007;29:995-1002.
12. Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. J Clin Oncol 2004;22:330-53.
13. Crinò L, Weder W, van Meerbeeck J, et al. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v103-15.
14. Colt HG, Murgu SD, Korst RJ, et al. Follow-up and surveillance of the patient with lung cancer after curative-intent therapy: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e437S-54S.
15. Junker K, Thomas M, Schulmann K, et al. Tumour regression in non-small-cell lung cancer following neoadjuvant therapy. Histological assessment. J Cancer Res Clin Oncol 1997;123:469-77.
16. Lou F, Huang J, Sima CS, et al. Patterns of recurrence and second primary lung cancer in early-stage lung cancer survivors followed with routine computed tomography surveillance. J Thorac Cardiovasc Surg 2013;145:75-81; discussion 81-2.
17. Revel MP, Merlin A, Peyrard S, et al. Software volumetric evaluation of doubling times for differentiating benign versus malignant pulmonary nodules. AJR Am J Roentgenol 2006;187:135-42.
18. Fraioli F, Anzidei M, Zaccagna F, et al. Whole-tumor perfusion CT in patients with advanced lung adenocarcinoma treated with conventional and antiangiogenetic chemotherapy: initial experience. Radiology 2011;259:574-82.
19. Tacelli N, Santangelo T, Scherpereel A, et al. Perfusion CT allows prediction of therapy response in non-small cell lung cancer treated with conventional and anti-angiogenic chemotherapy. Eur Radiol 2013;23:2127-36.
20. Yi CA, Lee KS, Kim EA, et al. Solitary pulmonary nodules: dynamic enhanced multi-detector row CT study and comparison with vascular endothelial growth factor and microvessel density. Radiology 2004;233:191-9.
21. Johnson TR, Krauss B, Sedlmair M, et al. Material differentiation by dual energy CT: initial experience. Eur Radiol 2007;17:1510-7.
22. Chae EJ, Song JW, Seo JB, et al. Clinical utility of dual-energy CT in the evaluation of solitary pulmonary nodules: initial experience. Radiology 2008;249:671-81.
23. Schmid-Bindert G, Henzler T, Chu TQ, et al. Functional imaging of lung cancer using dual energy CT: how does iodine related attenuation correlate with standardized uptake value of 18FDG-PET-CT? Eur Radiol 2012;22:93-103.
24. Gould MK, Maclean CC, Kuschner WG, et al. Accuracy of positron emission tomography for diagnosis of pulmonary nodules and mass lesions: a meta-analysis. JAMA 2001;285:914-24.
25. Hellwig D, Ukena D, Paulsen F, et al. Meta-analysis of the efficacy of positron emission tomography with F-18-fluorodeoxyglucose in lung tumors. Basis for discussion of the German Consensus Conference on PET in Oncology

2000. Pneumologie 2001;55:367-77.
26. Gould MK, Sanders GD, Barnett PG, et al. Cost-effectiveness of alternative management strategies for patients with solitary pulmonary nodules. Ann Intern Med 2003;138:724-35.
27. Hashimoto Y, Tsujikawa T, Kondo C, et al. Accuracy of PET for diagnosis of solid pulmonary lesions with 18F-FDG uptake below the standardized uptake value of 2.5. J Nucl Med 2006;47:426-31.
28. Keith CJ, Miles KA, Griffiths MR, et al. Solitary pulmonary nodules: accuracy and cost-effectiveness of sodium iodide FDG-PET using Australian data. Eur J Nucl Med Mol Imaging 2002;29:1016-23.
29. Lejeune C, Al Zahouri K, Woronoff-Lemsi MC, et al. Use of a decision analysis model to assess the medicoeconomic implications of FDG PET imaging in diagnosing a solitary pulmonary nodule. Eur J Health Econ 2005;6:203-14.
30. Dwamena BA, Sonnad SS, Angobaldo JO, et al. Metastases from non-small cell lung cancer: mediastinal staging in the 1990s--meta-analytic comparison of PET and CT. Radiology 1999;213:530-6.
31. Gould MK, Kuschner WG, Rydzak CE, et al. Test performance of positron emission tomography and computed tomography for mediastinal staging in patients with non-small-cell lung cancer: a meta-analysis. Ann Intern Med 2003;139:879-92.
32. Shim SS, Lee KS, Kim BT, et al. Non-small cell lung cancer: prospective comparison of integrated FDG PET/CT and CT alone for preoperative staging. Radiology 2005;236:1011-9.
33. Yang W, Fu Z, Yu J, et al. Value of PET/CT versus enhanced CT for locoregional lymph nodes in non-small cell lung cancer. Lung Cancer 2008;61:35-43.
34. Al-Sarraf N, Gately K, Lucey J, et al. Lymph node staging by means of positron emission tomography is less accurate in non-small cell lung cancer patients with enlarged lymph nodes: analysis of 1,145 lymph nodes. Lung Cancer 2008;60:62-8.
35. Eloubeidi MA, Cerfolio RJ, Chen VK, et al. Endoscopic ultrasound-guided fine needle aspiration of mediastinal lymph node in patients with suspected lung cancer after positron emission tomography and computed tomography scans. Ann Thorac Surg 2005;79:263-8.
36. Herth FJ, Ernst A, Eberhardt R, et al. Endobronchial ultrasound-guided transbronchial needle aspiration of lymph nodes in the radiologically normal mediastinum. Eur Respir J 2006;28:910-4.
37. Pieterman RM, van Putten JW, Meuzelaar JJ, et al. Preoperative staging of non-small-cell lung cancer with positron-emission tomography. N Engl J Med 2000;343:254-61.
38. Yun M, Kim W, Alnafisi N, et al. 18F-FDG PET in characterizing adrenal lesions detected on CT or MRI. J Nucl Med 2001;42:1795-9.
39. Cheran SK, Herndon JE 2nd, Patz EF Jr. Comparison of whole-body FDG-PET to bone scan for detection of bone metastases in patients with a new diagnosis of lung cancer. Lung Cancer 2004;44:317-25.
40. MacManus MP, Hicks RJ, Matthews JP, et al. High rate of detection of unsuspected distant metastases by pet in apparent stage III non-small-cell lung cancer: implications for radical radiation therapy. Int J Radiat Oncol Biol Phys 2001;50:287-93.
41. van Tinteren H, Hoekstra OS, Smit EF, et al. Effectiveness of positron emission tomography in the preoperative assessment of patients with suspected non-small-cell lung cancer: the PLUS multicentre randomised trial. Lancet 2002;359:1388-93.
42. Fischer B, Lassen U, Mortensen J, et al. Preoperative staging of lung cancer with combined PET-CT. N Engl J Med 2009;361:32-9.
43. Lardinois D, Weder W, Hany TF, et al. Staging of non-small-cell lung cancer with integrated positron-emission tomography and computed tomography. N Engl J Med 2003;348:2500-7.
44. Qu X, Huang X, Yan W, et al. A meta-analysis of 18FDG-PET-CT, 18FDG-PET, MRI and bone scintigraphy for diagnosis of bone metastases in patients with lung cancer. Eur J Radiol 2012;81:1007-15.
45. Ettinger DS, Akerley W, Bepler G, et al. Non-small cell lung cancer. J Natl Compr Canc Netw 2010;8:740-801.
46. Dietlein M, Weber K, Gandjour A, et al. Cost-effectiveness of FDG-PET for the management of potentially operable non-small cell lung cancer: priority for a PET-based strategy after nodal-negative CT results. Eur J Nucl Med 2000;27:1598-609.
47. Farjah F, Flum DR, Ramsey SD, et al. Multi-modality mediastinal staging for lung cancer among medicare beneficiaries. J Thorac Oncol 2009;4:355-63.
48. Schreyögg J, Weller J, Stargardt T, et al. Cost-effectiveness of hybrid PET/CT for staging of non-small cell lung cancer. J Nucl Med 2010;51:1668-75.
49. Søgaard R, Fischer BM, Mortensen J, et al. Preoperative staging of lung cancer with PET/CT: cost-effectiveness evaluation alongside a randomized controlled trial. Eur J Nucl Med Mol Imaging 2011;38:802-9.

50. Dunagan D, Chin R Jr, McCain T, et al. Staging by positron emission tomography predicts survival in patients with non-small cell lung cancer. Chest 2001;119:333-9.
51. MacManus MR, Hicks R, Fisher R, et al. FDG-PET-detected extracranial metastasis in patients with non-small cell lung cancer undergoing staging for surgery or radical radiotherapy—survival correlates with metastatic disease burden. Acta Oncol 2003;42:48-54.
52. De Ruysscher D, Nestle U, Jeraj R, et al. PET scans in radiotherapy planning of lung cancer. Lung Cancer 2012;75:141-5.
53. Mac Manus MP, Hicks RJ, Ball DL, et al. F-18 fluorodeoxyglucose positron emission tomography staging in radical radiotherapy candidates with nonsmall cell lung carcinoma: powerful correlation with survival and high impact on treatment. Cancer 2001;92:886-95.
54. Eschmann SM, Friedel G, Paulsen F, et al. FDG PET for staging of advanced non-small cell lung cancer prior to neoadjuvant radio-chemotherapy. Eur J Nucl Med Mol Imaging 2002;29:804-8.
55. Kolodziejczyk M, Kepka L, Dziuk M, et al. Impact of [18F]fluorodeoxyglucose PET-CT staging on treatment planning in radiotherapy incorporating elective nodal irradiation for non-small-cell lung cancer: a prospective study. Int J Radiat Oncol Biol Phys 2011;80:1008-14.
56. Deniaud-Alexandre E, Touboul E, Lerouge D, et al. Impact of computed tomography and 18F-deoxyglucose coincidence detection emission tomography image fusion for optimization of conformal radiotherapy in non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2005;63:1432-41.
57. Pommier P, Touboul E, Chabaud S, et al. Impact of (18) F-FDG PET on treatment strategy and 3D radiotherapy planning in non-small cell lung cancer: A prospective multicenter study. AJR Am J Roentgenol 2010;195:350-5.
58. Mac Manus MP, Everitt S, Bayne M, et al. The use of fused PET/CT images for patient selection and radical radiotherapy target volume definition in patients with non-small cell lung cancer: results of a prospective study with mature survival data. Radiother Oncol 2013;106:292-8.
59. Mac Manus MP, Hicks RJ, Matthews JP, et al. Positron emission tomography is superior to computed tomography scanning for response-assessment after radical radiotherapy or chemoradiotherapy in patients with non-small-cell lung cancer. J Clin Oncol 2003;21:1285-92.
60. Lee P, Bazan JG, Lavori PW, et al. Metabolic tumor volume is an independent prognostic factor in patients treated definitively for non-small-cell lung cancer. Clin Lung Cancer 2012;13:52-8.
61. Decoster L, Schallier D, Everaert H, et al. Complete metabolic tumour response, assessed by 18-fluorodeoxyglucose positron emission tomography (18FDG-PET), after induction chemotherapy predicts a favourable outcome in patients with locally advanced non-small cell lung cancer (NSCLC). Lung Cancer 2008;62:55-61.
62. Pöttgen C, Levegrün S, Theegarten D, et al. Value of 18F-fluoro-2-deoxy-D-glucose-positron emission tomography/computed tomography in non-small-cell lung cancer for prediction of pathologic response and times to relapse after neoadjuvant chemoradiotherapy. Clin Cancer Res 2006;12:97-106.
63. Eschmann SM, Friedel G, Paulsen F, et al. 18F-FDG PET for assessment of therapy response and preoperative re-evaluation after neoadjuvant radio-chemotherapy in stage III non-small cell lung cancer. Eur J Nucl Med Mol Imaging 2007;34:463-71.
64. Biederer J, Hintze C, Fabel M. MRI of pulmonary nodules: technique and diagnostic value. Cancer Imaging 2008;8:125-30.
65. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395-409.
66. Wu NY, Cheng HC, Ko JS, et al. Magnetic resonance imaging for lung cancer detection: experience in a population of more than 10,000 healthy individuals. BMC Cancer 2011;11:242.
67. Gümüştaş S, Inan N, Akansel G, et al. Differentiation of malignant and benign lung lesions with diffusion-weighted MR imaging. Radiol Oncol 2012;46:106-13.
68. Yi CA, Shin KM, Lee KS, et al. Non-small cell lung cancer staging: efficacy comparison of integrated PET/CT versus 3.0-T whole-body MR imaging. Radiology 2008;248:632-42.
69. Ohno Y, Koyama H, Onishi Y, et al. Non-small cell lung cancer: whole-body MR examination for M-stage assessment--utility for whole-body diffusion-weighted imaging compared with integrated FDG PET/CT. Radiology 2008;248:643-54.
70. Sommer G, Wiese M, Winter L, et al. Preoperative staging of non-small-cell lung cancer: comparison of whole-body diffusion-weighted magnetic resonance imaging and 18F-fluorodeoxyglucose-positron emission tomography/computed tomography. Eur Radiol 2012;22:2859-67.
71. Usuda K, Zhao XT, Sagawa M, et al. Diffusion-weighted

imaging is superior to positron emission tomography in the detection and nodal assessment of lung cancers. Ann Thorac Surg 2011;91:1689-95.
72. Yang RM, Li L, Wei XH, et al. Differentiation of central lung cancer from atelectasis: comparison of diffusion-weighted MRI with PET/CT. PLoS One 2013;8:e60279.
73. Ohno Y, Nogami M, Higashino T, et al. Prognostic value of dynamic MR imaging for non-small-cell lung cancer patients after chemoradiotherapy. J Magn Reson Imaging 2005;21:775-83.
74. Iwasawa T, Saito K, Ogawa N, et al. Prediction of postoperative pulmonary function using perfusion magnetic resonance imaging of the lung. J Magn Reson Imaging 2002;15:685-92.
75. Koh DM, Scurr E, Collins D, et al. Predicting response of colorectal hepatic metastasis: value of pretreatment apparent diffusion coefficients. AJR Am J Roentgenol 2007;188:1001-8.
76. Sedlaczek O. Alteration of MR-DWI/ADC before and 24 hours after induction of chemotherapy in patients with NSCLC. Presented at 3rd World Congress of Thoracic Imaging, Seoul, 2013.
77. Yeow KM, Su IH, Pan KT, et al. Risk factors of pneumothorax and bleeding: multivariate analysis of 660 CT-guided coaxial cutting needle lung biopsies. Chest 2004;126:748-54.
78. Saji H, Nakamura H, Tsuchida T, et al. The incidence and the risk of pneumothorax and chest tube placement after percutaneous CT-guided lung biopsy: the angle of the needle trajectory is a novel predictor. Chest 2002;121:1521-6.
79. García-Río F, Pino JM, Casadevall J, et al. Use of spirometry to predict risk of pneumothorax in CT-guided needle biopsy of the lung. J Comput Assist Tomogr 1996;20:20-3.
80. Anderson CL, Crespo JC, Lie TH. Risk of pneumothorax not increased by obstructive lung disease in percutaneous needle biopsy. Chest 1994;105:1705-8.
81. Moore EH. Technical aspects of needle aspiration lung biopsy: a personal perspective. Radiology 1998;208:303-18.
82. Fish GD, Stanley JH, Miller KS, et al. Postbiopsy pneumothorax: estimating the risk by chest radiography and pulmonary function tests. AJR Am J Roentgenol 1988;150:71-4.
83. Hoffmann NE, Bischof JC. The cryobiology of cryosurgical injury. Urology 2002;60:40-9.
84. Kawamura M, Izumi Y, Tsukada N, et al. Percutaneous cryoablation of small pulmonary malignant tumors under computed tomographic guidance with local anesthesia for nonsurgical candidates. J Thorac Cardiovasc Surg 2006;131:1007-13.
85. Zemlyak A, Moore WH, Bilfinger TV. Comparison of survival after sublobar resections and ablative therapies for stage I non-small cell lung cancer. J Am Coll Surg 2010;211:68-72.
86. Gazelle GS, Goldberg SN, Solbiati L, et al. Tumor ablation with radio-frequency energy. Radiology 2000;217:633-46.
87. Simon CJ, Dupuy DE, DiPetrillo TA, et al. Pulmonary radiofrequency ablation: long-term safety and efficacy in 153 patients. Radiology 2007;243:268-75.
88. Dupuy DE, DiPetrillo T, Gandhi S, et al. Radiofrequency ablation followed by conventional radiotherapy for medically inoperable stage I non-small cell lung cancer. Chest 2006;129:738-45.
89. Grieco CA, Simon CJ, Mayo-Smith WW, et al. Percutaneous image-guided thermal ablation and radiation therapy: outcomes of combined treatment for 41 patients with inoperable stage I/II non-small-cell lung cancer. J Vasc Interv Radiol 2006;17:1117-24.
90. Steinke K, Glenn D, King J, et al. Percutaneous imaging-guided radiofrequency ablation in patients with colorectal pulmonary metastases: 1-year follow-up. Ann Surg Oncol 2004;11:207-12.
91. Wasser EJ, Dupuy DE. Microwave ablation in the treatment of primary lung tumors. Semin Respir Crit Care Med 2008;29:384-94.
92. Simon CJ, Dupuy DE, Mayo-Smith WW. Microwave ablation: principles and applications. Radiographics 2005;25 Suppl 1:S69-83.
93. Carrafiello G, Laganà D, Mangini M, et al. Microwave tumors ablation: principles, clinical applications and review of preliminary experiences. Int J Surg 2008;6 Suppl 1:S65-9.
94. Vogl TJ, Naguib NN, Gruber-Rouh T, et al. Microwave ablation therapy: clinical utility in treatment of pulmonary metastases. Radiology 2011;261:643-51.
95. Haemmerich D, Lee FT Jr. Multiple applicator approaches for radiofrequency and microwave ablation. Int J Hyperthermia 2005;21:93-106.
96. Schramm W, Yang D, Haemmerich D. Contribution of direct heating, thermal conduction and perfusion during radiofrequency and microwave ablation. Conf Proc IEEE Eng Med Biol Soc 2006;1:5013-6.
97. Brace CL. Radiofrequency and microwave ablation of the liver, lung, kidney, and bone: what are the differences?

Curr Probl Diagn Radiol 2009;38:135-43.
98. Lubner MG, Brace CL, Hinshaw JL, et al. Microwave tumor ablation: mechanism of action, clinical results, and devices. J Vasc Interv Radiol 2010;21:S192-203.
99. Skinner MG, Iizuka MN, Kolios MC, et al. A theoretical comparison of energy sources--microwave, ultrasound and laser--for interstitial thermal therapy. Phys Med Biol 1998;43:3535-47.
100. Steinke K, Gananadha S, King J, et al. Dispersive pad site burns with modern radiofrequency ablation equipment. Surg Laparosc Endosc Percutan Tech 2003;13:366-71.
101. Skonieczki BD, Wells C, Wasser EJ, et al. Radiofrequency and microwave tumor ablation in patients with implanted cardiac devices: is it safe? Eur J Radiol 2011;79:343-6.
102. Clasen S, Krober SM, Kosan B, et al. Pathomorphologic evaluation of pulmonary radiofrequency ablation: proof of cell death is characterized by DNA fragmentation and apoptotic bodies. Cancer 2008;113:3121-9.
103. Crocetti L, Bozzi E, Faviana P, et al. Thermal ablation of lung tissue: in vivo experimental comparison of microwave and radiofrequency. Cardiovasc Intervent Radiol 2010;33:818-27.
104. Wolf FJ, Aswad B, Ng T, et al. Intraoperative microwave ablation of pulmonary malignancies with tumor permittivity feedback control: ablation and resection study in 10 consecutive patients. Radiology 2012;262:353-60.
105. Wolf FJ, Grand DJ, Machan JT, et al. Microwave ablation of lung malignancies: effectiveness, CT findings, and safety in 50 patients. Radiology 2008;247:871-9.
106. Danaher LA, Steinke K. Hot tips on hot tips: technical problems with percutaneous insertion of a microwave antenna through rigid tissue. J Med Imaging Radiat Oncol 2013;57:57-60.
107. Hoffmann RT, Jakobs TF, Lubienski A, et al. Percutaneous radiofrequency ablation of pulmonary tumors--is there a difference between treatment under general anaesthesia and under conscious sedation? Eur J Radiol 2006;59:168-74.
108. Carrafiello G, Mangini M, Fontana F, et al. Complications of microwave and radiofrequency lung ablation: personal experience and review of the literature. Radiol Med 2012;117:201-13.
109. Retrieved May 20, 2013. Available online: http://evs.nci.nih.gov/ftp1/CTCAE/About.html
110. Little MW, Chung D, Boardman P, et al. Microwave ablation of pulmonary malignancies using a novel high-energy antenna system. Cardiovasc Intervent Radiol 2013;36:460-5.
111. Liu H, Steinke K. High-powered percutaneous microwave ablation of stage I medically inoperable non-small cell lung cancer: a preliminary study. J Med Imaging Radiat Oncol 2013;57:466-74.
112. Yoo DC, Dupuy DE, Hillman SL, et al. Radiofrequency ablation of medically inoperable stage IA non-small cell lung cancer: are early posttreatment PET findings predictive of treatment outcome? AJR Am J Roentgenol 2011;197:334-40.
113. Singnurkar A, Solomon SB, Gönen M, et al. 18F-FDG PET/CT for the prediction and detection of local recurrence after radiofrequency ablation of malignant lung lesions. J Nucl Med 2010;51:1833-40.
114. Herrera LJ, Fernando HC, Perry Y, et al. Radiofrequency ablation of pulmonary malignant tumors in nonsurgical candidates. J Thorac Cardiovasc Surg 2003;125:929-37.

Cite this article as: Lee WK, Lau EW, Chin K, Sedlaczek O, Steinke K. Modern diagnostic and therapeutic interventional radiology in lung cancer. J Thorac Dis 2013;5(S5):S511-S523. doi: 10.3978/j.issn.2072-1439.2013.07.27

Screening for lung cancer with low-dose computed tomography: a review of current status

Henry M. Marshall[1,2], Rayleen V. Bowman[1,2], Ian A. Yang[1,2], Kwun M. Fong[1,2], Christine D. Berg[2,3]

[1]Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Australia; [2]University of Queensland Thoracic Research Centre, School of Medicine, The University of Queensland, Brisbane, Australia; [3]Formerly, Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland, USA

Correspondence to: Dr. Henry M. Marshall. Department of Thoracic Medicine, The Prince Charles Hospital, Rode Rd, Chermside, 4032, Australia. Email: Henry_Marshall@health.qld.gov.au.

Abstract: Screening using low-dose computed tomography (CT) represents an exciting new development in the struggle to improve outcomes for people with lung cancer. Randomised controlled evidence demonstrating a 20% relative lung cancer mortality benefit has led to endorsement of screening by several expert bodies in the US and funding by healthcare providers. Despite this pivotal result, many questions remain regarding technical and logistical aspects of screening, cost-effectiveness and generalizability to other settings. This review discusses the rationale behind screening, the results of on-going trials, potential harms of screening and current knowledge gaps.

Keywords: Lung neoplasms/mortality; mass screening tomography; helical computed; early detection of cancer/methods

Submitted Aug 28, 2013. Accepted for publication Sep 10, 2013.
doi: 10.3978/j.issn.2072-1439.2013.09.06
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.09.06

Introduction

The rationale for lung cancer screening

Lung cancer caused an estimated 1.4 million deaths in 2008 (1), and is the leading cause of cancer death worldwide. Incidence and mortality closely follow smoking trends with a time-lag of twenty years. This explains why death rates are falling or plateauing in countries such as the US, yet rising in others such as China (2,3). Lung cancer carries a poor prognosis with reported overall five year survival between 8 and 16% in Europe and the USA, and between 6% and 32% in China (4-6).

Currently 25-30% of patients present with localised, potentially curable disease. Five year survival for those with pathological stage IA non-small cell lung cancer (NSCLC) is 73% whereas metastatic disease has a dismal prognosis (13% 5-year survival) (7,8).

Given that lung cancer has a detectable pre-clinical phase, effective treatment, especially surgery, with effective and potentially cost-effective applicable screening methods, it would seem to fulfil the criteria for screening first described by Wilson and Jungner (9) (Box 1). Although early screening studies using plain chest radiography (CXR) had methodological drawbacks (11), it is generally accepted that CXR screening does not confer a mortality benefit, a conclusion reinforced by the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial (12). In contrast, computed tomography (CT) is a far more sensitive imaging modality which has been studied for its potential utility in lung cancer screening over the past 25 years. Recently, the National Lung Screening Trial (13) showed that low-dose CT (LDCT) screening reduced lung cancer mortality by 20% compared with CXR screening. This was the first demonstration in a randomized clinical trial of a mortality reduction with screening. In response to these findings several expert bodies in the USA issued guidelines for screening high-risk populations and the US Preventive Services Task Force has awarded a Grade B draft recommendation (14-17).

Condition
- The condition should be an important health problem.
- There should be a recognisable latent or early symptomatic stage.
- The natural history of the condition, including development from latent to declared disease should be adequately understood.

Test
- There should be a suitable test or examination.
- The test should be acceptable to the population.

Treatment
- There should be an accepted treatment for patients with recognised disease.

Screening program
- There should be an agreed policy on whom to treat as patients.
- Facilities for diagnosis and treatment should be available.
- The cost of case-findings (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
- Case-findings should be a continuing process and not a 'once and for all' project.

Box 1 Principles of early disease detection [adapted from Wilson and Jungner (9)].

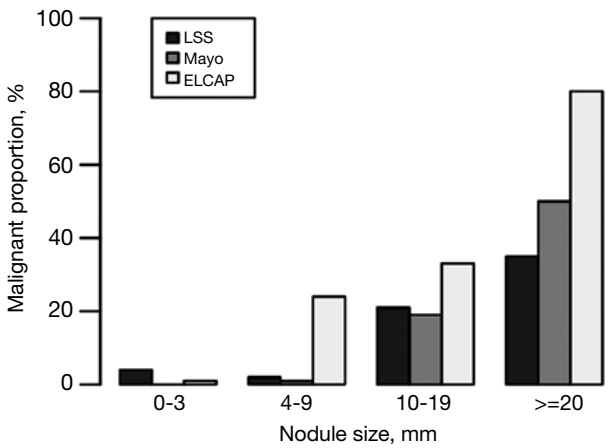


Figure 1 Nodule size correlates to risk of malignancy*. LSS, Lung Screening Study; Mayo, Mayo Clinic Study; ELCAP, Early Lung Cancer Action Project. *Cut-off sizes were slightly different between studies (29-31).

LDCT screening--practical issues and technical considerations

One of the most important issues confronting those who wish to consider implementation of LDCT screening in high-risk populations is the problem of the high rate of positive examinations, primarily pulmonary nodules.

Nodule detection

Pulmonary nodules can be defined as rounded or irregular opacities, well or poorly defined, measuring up to 3 cm in diameter (18). There is inherent subjectivity in identifying nodules, reflected in inter- and intra-reader variability, even amongst experienced radiologists (19,20).

A considerable proportion of nodules may be missed at first reading and identified retrospectively at later scans (21). Nodule detection may be increased by using a second reader (22), image formatting, e.g., to maximum intensity projections (MIPs) (23-25) or by using computer aided detection (CAD) software as a "second reader" (26-28).

Nodule assessment

Nodules are best classified in four important ways: size, attenuation, presence/absence of calcification and, once a follow-up scan has been obtained, interval growth rate.

Size

Nodule size is the most important predictor for malignancy (*Figure 1*) (29-31). Detailed analysis of baseline NLST results found the positive predictive values (PPV) for malignancy increased significantly from 1.7% for nodules 7-10 mm in diameter to 11.9%, 29.7% and 41.3% for those 11-20, 21-30 and >30 mm diameter respectively (32). However even very small nodules (micronodules) have some risk of malignancy, e.g., 3 of 230 nodules <5 mm diameter (1.3%) at baseline scan followed for one year (33).

Attenuation

Certain calcification patterns and intra-nodular fat reliably

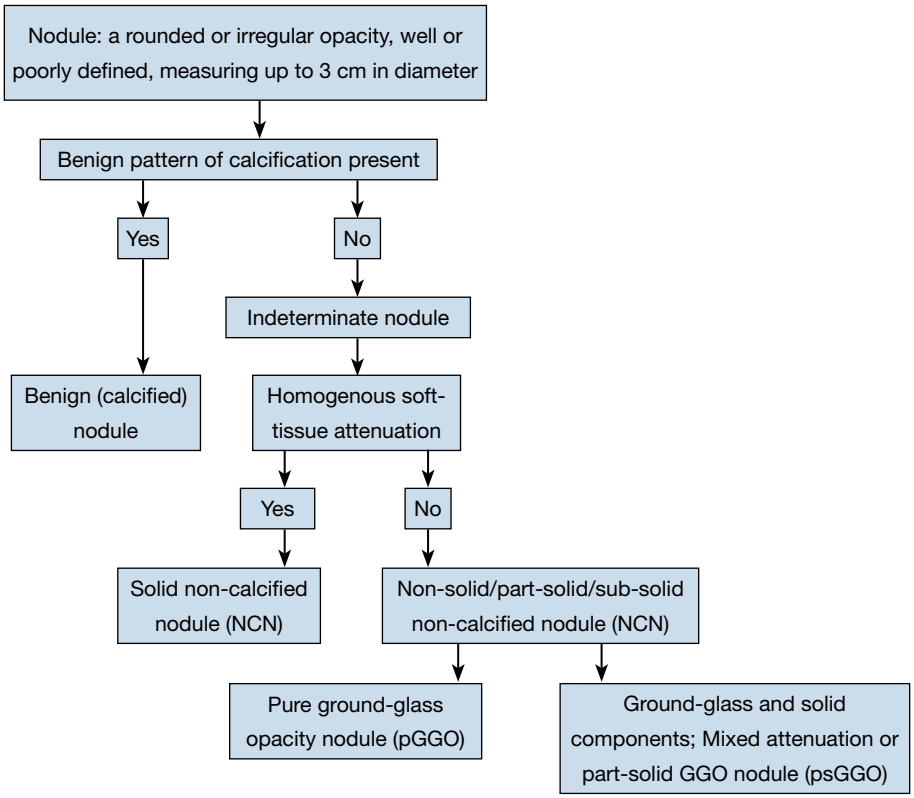


Figure 2 Classification of nodules detected by LDCT screening.

indicate benignity (34), however, many nodules are too small to resolve internal features and are simply classified as 'non-calcified' nodules (NCNs). NCNs are common and detected in 25-50% of LDCT scans.

The majority of NCNs are of 'solid' (soft-tissue) radiological attenuation. The remainder are classified as non-solid nodules (NSNs) and subdivided into pure ground-glass (pGGO) or mixed (part-solid) attenuation nodules (solid and ground-glass components; psGGO). Synonyms vary between studies (*Figure 2*). The significance of GGOs is contentious as discussed below.

Ground glass opacities

The ELCAP study reported positive findings in 233/1,000 baseline scans. 19% of lesions were pGGO or psGGO (prevalence 4.4%; slice thickness 10 mm). Twenty-seven cancers were detected. After adjusting for size, the malignancy rate was 63% for psGGO, 32% for solid nodules and 13% for pGGOs (35). Other studies highlight the importance of a new or increasing solid component within NSNs, a finding highly suggestive of lung cancer (36-38). More recent studies demonstrate many NSNs spontaneously resolve. Felix (39) reported 75 GGOs in 37/280 patients (prevalence 13%; slice thickness 0.75 mm). The population was atypical for screening studies as over half had a history of lung or head and neck cancer. Approximately half the GGOs were present at baseline and half disappeared over a median 29 months follow-up. No morphological features allowed reliable discrimination between resolving and non-resolving GGOs. Kwon (40) reported 69 pGGO and 117 psGGO mostly detected by screening in 186 patients (total screenees not reported; slice thickness 5 mm). After 3 months, 45% regressed or disappeared. Malignant and benign lesions were similar in size (average 15-16 mm). Only 27% (33/122) were malignant but this may reflect a short follow-up time (mean 8.6 months; 64 lesions were still under active follow-up at publication). A second Korean study (41) identified 126 NSNs >5 mm diameter in 93 of 16,777 (0.5%) asymptomatic screenees. Forty-four had never smoked. 70% of NSNs were transient. Younger age, detection at a follow-up scan, blood eosinophilia, multiple lesions, larger solid component and ill-defined border independently predicted transiency. Mario (42) reported 76 NSNs retrospectively identified in 56/1,866 baseline screening

scans in a high-risk screening cohort (prevalence 3%; slice thickness 0.75 mm) and followed for 50±7.3 months. Only 13 nodules were prospectively identified. 40 of 48 pGGOs (83%) resolved, decreased in size or remained stable. 16 of 28 psGGOs (57%) resolved or remained stable. Overall, 74% NSNs resolved, decreased in size or remained stable and 26% progressed. One psGGO (2%) was confirmed as lung adenocarcinoma.

In summary, perhaps as many as 50-70% of NSNs detected on modern thin-slice CT scans are transient but predicting which will persist is currently beyond our ability. The data suggest that a substantial difference in NSN prevalence between Western and Asian populations is unlikely. In view of slower growth rates for non-solid tumours (37,43) active surveillance for >2 years may be prudent for non-resolving NSNs (44).

Growth rate

Once a follow-up scan is obtained, assessment of growth can be made. Generally, absence of growth in a solid nodule over a 2 year period makes malignancy unlikely (45), although a contemporary review found the underpinning data (based on CXR studies from the 1950s) less than compelling (46).

Growth is best assessed by CT. For example, assuming exponential growth, a 5 mm diameter nodule with a volume doubling time (VDT) of 460 days will only increase to 6 mm diameter after one year and 7.2 mm after two years-changes which may not be measurable on CXR but which can be appreciated on CT. However, reproducible measurement is difficult: the 95% confidence intervals (CIs) for inter-reader measurements of nodules with a mean diameter of 8.5 mm were ± 1.73 mm in one study (47). Semi-automated volumetric measurement using computer software may be more reproducible and accurate (48,49) and is the basis of nodule management in the NELSON trial (47-50). However even this is subject to error, e.g., with smaller nodules, in the presence of motion artefact (51), nodules attached to other structures and NSNs (52).

There are limited long-term data supporting the two year stability guideline for sub-centimetre NCNs; In an Irish study (53) 83 subjects with NCNs <10 mm stable over two years were imaged again at seven years. Virtually all nodules remained unchanged at the seven-year CT, however one 3 mm GGO grew to 15 mm in four years and was subsequently diagnosed as (what was previously called) bronchioloalveolar cell carcinoma. Thus ideally, the two year stability guideline suggested by CXR studies should be validated in larger, contemporary CT datasets.

The importance of baseline size and interval growth is shown in data from the NELSON study (54). 891 solid nodules 5-10 mm diameter were followed for one year. 743 nodules, all with smooth margins and/or attached to fissures, pleura, or vessels (contact length ≥50% of nodule diameter) were benign and excluded from multivariate analysis. Spiculated, irregular or lobulated nodules were analysed further. 10 of 69 (14.5%) nodules with spiculated or irregular margins and 6 of 168 (3.6%) nodules with lobulated margins were malignant. At baseline the only characteristic that predicted malignancy was volume ≥130 mm^3 (OR 6.3; 95% CI: 1.7 to 23.0). At 3-months, baseline volume and VDT <400 days were significant (OR 4.9; 95% CI: 1.2 to 20.1 and OR 15.6; 95% CI: 4.5 to 53.5, respectively); At one year only VDT was predictive (OR 213.3; 95% CI: 18.7 to 2,430.9). Very few nodules showed change in margin or shape over 12 months, so these features were unable to distinguish malignant from benign nodules (55).

Other morphological features

Diederich (56), in a study of 133 consecutive resolving nodules, found the demographic and morphologic features of resolving and non-resolving nodules overlapped so greatly that none could be used to predict outcome over two years' follow-up.

Features of benignity noted by Takashima after two years follow-up (72 nodules ≤10 mm diameter including 25 cancers) were polygonal shape, subpleural location, solid attenuation and elongation (higher long-axis-to-short-axis diameter ratio) (57). Long-term analysis of 234 similar nodules (perifissural with any of the following features: polygonal shape, long-axis-to-short-axis diameter ratio >1.78, peripheral location, vascular attachment) detected in 98/146 consecutive screenees found the nodules were multiple in half the subjects, ranged from 1-13 mm diameter, were mostly triangular or oval (86%), inferior to the carina (84%) and had a septal connection (73%) (58). 139 screenees were accounted for after 7.5 years, and none of the perifissural nodules had developed into cancers. These types of nodules most probably represent intrapulmonary lymph nodes, however histopathologic confirmation was not performed in either study (57,58).

The difficulty in predicting which nodules might be malignant is highlighted by low PPV in screening studies; with a cancer prevalence of 1-2% the PPV of a nodule designated by the radiologist as 'suspicious' or large in size or with VDT <400 days actually being malignant was only

Table 1 Comparison of nodule management protocols for three leading LDCT studies

		Nodule characteristics (attenuation, diameter, volume)	Recommended action	Interval findings	Recommended action
Small	NLST	<4 mm d_{max}	12 m LDCT		
	NEL	<50 mm^3 without benign characteristics			
	IE	Solid/ part-solid <5 mm d_{mean}; non-solid: any size			
Intermediate	NLST	Solid 4-10 mm d_{max}	3-6 m LDCT (may vary up to 24 m according to level of suspicion)	No growth†	→ 12 m LDCT
				Growth <7 mm	→ 3-6 m LDCT or refer to pulmonologist
				Growth ≥7 mm	→ Refer to pulmonologist
		Pure GGO 4-10 mm d_{max}	6-12 m LDCT	As per solid 4-10 mm nodules	
	NEL	Solid: 50-500 mm^3; Solid, pleural based: 5-10 mm Mixed: GGO component: ≥8 mm d_{mean} or solid component: 50-500 mm^3 Pure GGO: ≥8 mm d_{mean}	3 m LDCT	Growth††	→ Refer to pulmonologist
	IE	Solid/ part-solid 5-15 mm d_{mean}	3 m LDCT (preferred option) or Antibiotics & 3 m LDCT if infection possible or	Growth†††	→ Biopsy
			PET scan if solid/solid component >10 mm	PET scan negative	→ 3 m LDCT
Large	NLST	Solid >10 mm d_{max} Other suspicious finding	Refer to pulmonologist		
	NEL	Solid: >500 mm^3; Solid, pleural based: >10 mm Mixed, solid component: >500 mm^3			
	IE	Solid/ Mixed >15 mm d_{mean}			

Key: NLST-NLST, NEL-NELSON, IE-I-ELCAP; m, month; d_{mean}, mean of maximal diameter and width viewed on same CT slice; d_{max}, maximal diameter on axial CT slice; PET, Positron-emission tomography; GGO, ground glass opacity attenuation nodule; Definitions of growth minimum significant change: †, >10% increase in diameter; ††, ≥25% increase in volume after at least a 3 months interval; †††, Minimum change in nodule diameter/solid component of part-solid nodules to define significant growth: for nodules <5 mm in diameter, ≥50%; for nodules 5-9 mm in diameter ≥30%; for nodules >10 mm in diameter ≥20%. Adapted from NLST (60) NELSON (52), I-ELCAP (61).

around 35% in two studies (50,59).

Nodule management protocols

LDCT nodule management protocols reflect the association of size and growth with malignancy. The protocols from the three largest studies, NLST, NELSON and I-ELCAP are summarized in *Table 1* (52,60,61). These protocols have been applied to 26,722, 7,557 and 31,567 LDCT screenees respectively although I-ELCAP has no control arm. Size category definitions vary slightly, but in general terms 'micronodules' (usually less than 4-5 mm diameter) are followed after 12 months, large

nodules (>10-15 mm diameter) are sent for immediate investigation and medium size nodules are followed-up to determine growth. Most studies use linear measurements of nodule size but the NELSON study uses volumetric measurement (50). Retrospective analysis of I-ELCAP data suggested the threshold to define a 'positive' baseline scan may be too inclusive; increasing the threshold to 7-8 mm (mean of maximal diameter and width) may reduce the false positive rate and subsequent work-up by 50-68% but at the cost of diagnostic delay for 5-6% of true positive cases (62). To date, only the NLST protocol has been proven to reduce lung cancer mortality.

Non-nodule (incidental) findings (IFs)

Non-cancer IFs such as coronary artery calcification (CAC), emphysema, and thyroid nodules are common but rates vary widely depending on study definitions and recording protocols. A NELSON substudy (n=1,929) found an IF rate of 81%. Six percent of participants received follow-up but only 1% had clinically important findings arguing against systematically searching for IFs (63). A Canadian study (n=4,073) found IFs in 19%; Approximately half would have required follow-up and 0.8% immediate action (64).

LDCT screening may be an opportunity to screen for other conditions which can be detected on chest CT such as CAC, chronic obstructive pulmonary disease (COPD) and osteoporosis (65,66). This may increase cost-effectiveness and provide better global outcomes but is currently untested. Radiologist-detected emphysema on CT scans appears to confer an independent increased risk of lung cancer (OR 2.1) (67) and may have the potential to help determine screening frequency following baseline scan (68) (i.e., more frequent screening for those with visually-detected emphysema), but this hypothesis remains to be tested.

CAC, a risk marker for cardiac events (69) is potentially the most important IF. Worldwide, smoking is estimated to cause 0.8 million deaths from acute heart attacks annually (70). The ELCAP investigators found varying degrees of CAC in 64% of 4,250 screenees (71). They developed a simple visual scoring system which was able to stratify cardiovascular death risk in a second cohort of 8,782 screenees followed for a median of six years (72). The NELSON study reported higher hazard ratios for all-cause mortality with increasing CAC in 958 participants followed for 21 months (73). However these findings do not appear to be reflected in NLST data where approximately 75% of all deaths were from non-lung cancer causes (13). Cardiovascular illness accounted for 486/1,865 (26.1%) deaths in the LDCT group and 470/1,991 (23.6%) in the CXR group. The 6.7% reduction in all-cause mortality in the LDCT group lost statistical significance when lung cancer deaths were removed from the comparison (3.2%, P=0.28) indicating that reduced lung cancer mortality was largely responsible for the reduction in all-cause mortality (13). Clinically significant IFs were identified in 7.5% of all scans and although details of CAC prevalence and follow-up are not yet reported, it seems unlikely that identification of CAC on LDCT screening made a significant impact on cardiovascular mortality in this study.

Thus IFs are common but mostly of little significance. Exhaustive investigation of IFs will increase the costs of screening through downstream investigation and follow-up, and should be accounted for in cost analyses. Further analysis of CAC and possibly other conditions in screening studies is warranted.

Screening by LDCT-effectiveness

Observational studies

The earliest LDCT screening studies were observational cohort studies from the USA and Japan (*Table 2*). CT appeared to be 3-4 times more sensitive than CXR in the ELCAP study, and the majority of tumours were stage I. Entry criteria were varied. Studies recruiting younger participants (<50 years old) and never-smokers had lower prevalence and/or incidence rates. For example, in a Japanese study (75) in which the majority of screenees had never smoked, cancer prevalence was only 0.4% compared to ELCAP 2.7% (31). These results underline the importance of recruiting a high-risk population. Subsequently, most studies follow the ELCAP strategy recruiting older persons with extensive smoking histories. Risk stratification is an area of current research interest and is discussed later.

Although very promising, these studies lacked control groups to allow estimation of mortality benefit. Survival, as a surrogate endpoint of effectiveness, is subject to several biases and cannot therefore be used to prove screening efficacy (*Box 2*). To add to the debate, studies modelling mortality benefit markedly diverged in their conclusions (78-80).

Randomised controlled trials

The randomised control trials of LDCT screening are summarized in Table 3. Two trials, the NLST (USA) and NELSON (Holland/Belgium), have adequate statistical

Table 2 Results from selected observational LDCT lung cancer screening studies

	Year	n	Cohort characteristics	Additional tests	Cancer prevalence %	Cancer incidence %	Stage I tumours %	5-year survival
ELCAP (31)	1992	1,000	>60 yr old; >10 PY smoking	CXR	2.7	0.6	85	65%
ALCA (74)	1993-95	1,611	40-75 yr old; 14% non-smokers; 16% <50 yr old	Sputum cytology, CXR	0.87	0.28	82	70%
Matsumoto Research Centre (75)	1996-98	5,480	40-74 yr old; 54% never smokers; 10% <50 yr old	Sputum cytology	0.41	0.23-0.56	83	83%
Mayo Clinic (76)	1999	1,520	>50 yr old; >20 PY smoking	–	1.9	2	56	–
I-ELCAP (77)	1993-2005	31,560	>40 yr old; 16% never smokers	–	1.3	0.3	85	80% (10 yr)

Bias in screening studies

- This box describes the three most important survival biases in screening studies. Survival cannot be used as a robust endpoint as, without a control group, there is no way of determining the relative contribution of each bias. Relative mortality between the intervention (screened) and control group is the best endpoint to use.

Lead-time bias

- Survival is measured from time of diagnosis to time of death. CT is more sensitive than CXR and will therefore detect smaller tumours earlier. Even though there may be no benefit in terms of reducing mortality, survival will appear longer for CT detected tumours as the diagnosis was simply made earlier.

Length bias

- Screening tends to detect slower-growing tumours and miss more aggressive ones. Rapidly-growing and aggressive tumours are more likely to grow and metastasise in the between-scan interval, and thus be missed whereas slowly growing tumours have a longer preclinical phase and are more likely to be detected by screening. As screening selects for less aggressive tumours, outcomes are more favourable thus survival may appear better in the screened group.

Overdiagnosis bias (pseudodisease)

- The detection of tumours which are never destined to cause morbidity; the patient dies from competing causes 'with' the cancer rather than 'from' it. In the absence of screening the cancer would never have been diagnosed in the lifetime of the person. Most of these tumours will be slow-growing or indolent. People at risk of lung cancer have a high risk of dying from other causes because of the shared risk factors of smoking and older age. Overdiagnosis bias makes screening appear to be more successful than it really is but essentially has detected non-lethal disease. This is a major problem in prostate cancer screening where, for example, as many as 60% of cases detected by prostate-specific antigen screening may be overdiagnosed (10). Individuals with overdiagnosed cancer undergo investigation and treatment with no hope of living longer. This futile management exposes patients to unnecessary harms and diverts finite health resources from other areas. Overdiagnosis in lung cancer screening has yet to be quantified (see text).

Box 2 Survival bias in screening studies.

Table 3 Randomized clinical trials of lung cancer screening using low-dose computed tomography; Target group and screening schedule

Study	Age range	Smoking history	Participants (baseline), n			Screening schedule (years)**	Control group	Total period of follow up	Years of recruitment	Completion/ expected completion
			LDCT arm	Control arm	Total					
NLST, USA (13,81)	55-74	Current or ex-smokers >30 PY, quit <15 yr	26,722	26,732	53,454	0,1,2	CXR	5	2002-4	2009
LSS, USA (pilot study) (82)	55-74	Current or ex-smokers >30 PY, quit <10 yr	1,660	1,658	3,318	0	CXR	1	2000	2001
DANTE, Italy (83)	60-74 (men only)	Current or ex-smokers >20 PY	1,276	1,196	2,472	0,1,2,3,4	Annual clinic review†	4	2001-6	2010
Dépiscan, France (pilot study) (84)	50-75	Current or ex-smokers >15 PY, quit <15 yr	336	285	765	0,1,2,3	CXR	2	2002-4	2004
NELSON, The Netherlands and Belgium (50,85,86)	50-74	Current or ex-smokers >15 PY, quit <10 yr	7,915	7,907	15,822	0,1,3	Usual care (no intervention)	10	2003-6	2015
DLCST, Denmark (87)	50-70	Current or ex-smokers >20 PY, quit <10 yr	2,052	2,052	4,104	0,1,2,3,4	Usual care (no intervention)	10	2004-6	2014
ITALUNG, Italy (88)	55-69	Current or ex-smokers >20 PY, quit <10 yr	1,613	1,593	3,206	0,1,2,3	Usual care (no intervention)	4	2004-6	–
MILD, Italy (89)	49-75	Current or ex-smokers >20 PY, quit <10yr	1,190 annual; 1,186 biennial	1,723	4,099	Annual or biennial for 10 years	Usual care (no intervention)	10	2005-onwards	Ongoing-started 2005
LUSI, Germany (90)	50-69	Current or ex-smokers >15 PY, quit <10yr	2,029	2,023	4,052	0,1,2,3,4	Usual care (no intervention)	5	2007-onwards	Ongoing-started 2007
UKLS (pilot study), United Kingdom (91,92)	50-75	5% risk of developing lung cancer in 5 years (Liverpool Lung Project risk model)	2,000*	2,000*	4,000	0	Usual care (no intervention)	10	2011-onwards	Ongoing-started 2011

*Planned recruitment; PY, Pack years (cigarettes per day/20× duration of smoking in years); CXR, chest radiograph; **Screening schedule indicates which year the scans are performed with '0' indicating baseline scan; †, all participants received CXR + sputum cytology at baseline.

Table 4 Factors affecting screening cost-effectiveness

Population	Screening intervention	Nodule management	Clinical
Disease prevalence in the target population (determined by risk, e.g., age, smoking history)	True-positive rate	Definition and rate of 'positive' scan results	Stage distribution of detected disease
Uptake of screening	False-positive rate	Nodule follow-up algorithm	Treatment costs
Adherence to screening	Over-diagnosis rate	Invasive diagnostic procedure rate	Investigation and treatment of incidental findings
	Screening frequency (interval between scans)	Adverse event rate	
	Screening duration (years)	Cost of diagnostic work-up	
	Lung cancer mortality reduction		
	Effectiveness of smoking cessation program		
	Radiation exposure		
	Cost of screening scan		

power to detect a reduction in lung cancer mortality. The smaller European studies are planning a meta-analysis (93). All European studies (except for Depiscan and DANTE) randomised LDCT screening against no screening, the current standard of care.

The most important RCT result to date is from the NLST study (13). This landmark study randomised 53,454 high risk volunteers to three rounds of screening by CXR or LDCT (baseline, year 1 and year 2) and followed up for a median of 6.5 years. Eligibility criteria included: current or former smokers with ≥30 pack year smoking history (quit no more than 15 years previously); No history of lung or other cancer in the past five years; No current symptoms suggesting lung cancer; No chest CT in the previous 18 months. The study demonstrated a relative reduction in lung cancer-specific mortality of 20.0% in the LDCT arm (95% CI: 6.8 to 26.7; P=0.004).

Despite this positive result, several issues remain particularly generalizability and cost-effectiveness. The NLST authors stated their data alone are 'insufficient' to fully inform lung-cancer screening recommendations (13) and the Position Statement from the International Association for the Study of Lung Cancer (IASLC) Task Force on CT Screening reminds us that screening benefit, costs and potential harms must be defined in a 'cultural context', i.e., positive results seen in USA studies may not translate directly to other countries or healthcare systems (94). Additionally, the negative effects of screening and knowledge gaps, discussed below, must be considered.

Screening adherence

Good adherence is important to the success of mass screening. NLST reported 95% adherence across all three screening scans and NELSON reported 97% at year two. Long-term observational studies report 80% adherence at year five and 86% at year seven (76,95). How this will translate to the 'real world' is not known.

Downstream healthcare use

Positive scans and incidental findings require clinical and radiological follow-up. Healthcare use may rise in the first six months following screening but return to baseline levels 6-12 months after screening and appears independent of result (i.e., negative, indeterminate or suspicious findings) (96). Although this study found doctor visits increased by 50%, in absolute terms this only meant one extra visit per participant (96).

Cost-effectiveness

Cost-effectiveness, a fundamental requirement of screening implementation, remains to be addressed. It depends on a complex mix of factors which vary from program to program and country to country (*Table 4*). Estimates vary

widely depending on the underlying assumptions and models used, making conclusions difficult to draw (97). Using NLST data, Goulart estimated that if 75% of the eligible US population underwent screening, the cost to avoid one lung cancer death would be $240,000 (98). McMahon's analysis paid particular attention to a model combining screening and smoking-cessation (99). The estimated cost per Quality Adjusted Life Year (QALY) in a cohort of 50 years old could be below $75,000/QALY if quit rates could be doubled from the background rate. From a health insurance perspective cost estimates were highly favourable (100); screening high-risk 50-64 years old would cost $1 per insured member per month, and the cost per life-year saved would be below $19,000.

To date, heterogeneous modelling methodologies and underlying assumptions have led to highly conflicting cost-effectiveness estimates. The final analysis from NLST has yet to be reported in a peer-reviewed format and is eagerly awaited. Preliminary data (101) suggest that it will be cost-effective with an Incremental Cost Effectiveness Ratio (ICER) of $72,900 US per QALY.

Negative effects of screening

Screening for any disease has risks and benefits. The balance helps determine overall effectiveness and acceptability of the screening program. The main negative effects are discussed below.

Radiation

It is generally accepted that ionising radiation is a cause of cancer without a lower "dose" threshold, although the absolute level of risk is debated (102,103). Minimising radiation dose according to the ALARA principle ('as low as reasonably acceptable') (104) is particularly important when screening asymptomatic, healthy subjects. CT radiation dose is determined by many factors including tube current, tube voltage, the use of filters and scan length (Z-axis). In screening studies, the most common way to limit dose is to adjust tube current (milliamperes, mA) (105) according to patient weight. This can degrade image quality as image noise (grainy mottling) is inversely proportional to the square root of the radiation dose. Fortunately the inherently high contrast between air-filled lung parenchyma and soft tissue lesions means pulmonary nodules are well-visualised. The mean effective dose from screening CT scans can be reduced from 8 mSv (standard CT chest) to approximately 1.5 mSv without significant deterioration in resolution or image quality (13,106,107). Although the lower radiation dose results in more noise it has been shown to provide adequate diagnostic pictures and is thus the current standard for screening (108-110). Total radiation dose can be further limited by restricting the scope of follow-up CTs to a region of interest surrounding the nodule(s) in question rather than covering the entire chest, so-called 'limited' LDCT (111).

Smoking appears to interact synergistically with ionising radiation. In absolute terms the risk of cancer from LDCT is small, perhaps only an excess lifetime risk of 0.85% (95% CI: 0.28% to 2.2%) for the worst case scenario of a 50-year-old female smoker receiving 25 annual LDCT scans. This compares to a 17% risk of developing lung cancer (112). Berrington de Gonzalez estimated the cumulative risk of excess death from lung cancer from LDCT screening in 50-year-old smokers to be 2 per 10,000 men screened and 5 per 10,000 women screened. Additionally an estimated 3 cases of breast cancer per 10,000 women screened may occur (113). The NLST estimated the number needed to screen (NNS) to prevent one death from lung cancer was 320, equating to a rate of 30 fewer deaths per 10,000 screenees (13) a larger benefit than the radiation harm particularly as the cancers induced occur after a delay of many years and the lives saved are over the short term. Estimates from the ITALUNG RCT reached similar conclusions with an estimated 1.1 excess deaths per 10,000 screenees compared to approximately 15-100 lives saved per 10,000 screenees (women and men respectively) assuming a 20% mortality reduction from screening (114). Thus the radiation risk-benefit ratio of LDCT screening appears quite favourable in older populations of smokers.

Adverse events

Adverse events may result from investigation of LDCT findings. As 25-50% of screenees may have one or more nodules detected, a potentially large reservoir of patients at risk exists. In the NLST the cumulative chance of a positive screening scan was 39.1%.

Despite guidelines (115), significant variation in pulmonary nodule biopsy rates (14.7 to 36.2 per 100,000 adults) and complication rates have been found between hospitals in the USA (116). The risk of haemorrhage and pneumothorax requiring intercostal catheter drainage (ICC) were 1.0% and 6.6% respectively. Complications were associated with an increased length of stay and risk

of respiratory failure. Those at highest risk were smokers, persons aged 60 to 69 years, and those with COPD, i.e., the types of patients targeted for screening. LDCT screening study adverse event rates may be slightly higher than the above study but this probably reflects more rigorous, prospective reporting. There appears to be no standard way of defining or reporting adverse event data which makes some studies difficult to compare directly. 'Number of events per 10,000 scans' may be a useful metric to allow cross-study comparison.

A study of 4,782 participants (117) screened using the I-ELCAP protocol reported a biopsy rate of 2.6% (n=127) including 110 percutaneous CT-guided fine-needle aspiration biopsies (CT-FNA). 13% of CT-FNAs were complicated by a moderate-to-large pneumothorax requiring ICC or hospitalization. Overall 16% of biopsies were for benign disease (117). Using a volumetric-based protocol, NELSON reported the surgical diagnostic procedure rate as 1.2% in round one and 0.8% in round two; 32/92 (35%) and 13/61 (21%) procedures in each round were for benign disease. Very few CT-FNAs were performed: 5/13 CT-FNA in round one and 3/3 FNA in round 2 showed benign disease. Across both rounds bronchoscopy diagnosed cancer in 111/247 (45%) procedures-a lower than expected figure likely reflecting peripheral tumour location. Complication rates were not reported (50).

The PLuSS study (118) screened 3,642 participants using an in-house protocol. 82 (2.3%) underwent surgical procedures (thoracotomy or VATS), twenty-eight of whom (34%) had benign disease. The study investigators cited "an apparent community bias toward aggressive intervention" for indeterminate lung nodules.

At baseline, 27.3% in the NLST LDCT group had a positive scan result (13). 155/7,191 participants had a percutaneous diagnostic procedure (CT-FNA in 120) and 297 (4.1% of positive scans) had a diagnostic surgical procedure (thoracotomy, thoracoscopy, mediastinoscopy or mediastinotomy) including 197 thoracotomies. Across all three screening rounds (75,126 screenings), 164/673 (24%) of surgical procedures in the LDCT group resulted in a non-cancer diagnosis. 191/673 (29%) of participants whose most invasive diagnostic procedure was surgical experienced at least one complication; in 80 (12%) this was classified as major. Only 14 of 99 (14%) participants who underwent a needle biopsy as their most invasive diagnostic procedure experienced one or more complication and none were major. 16 participants (10 with lung cancer) died within 60 days of an invasive diagnostic procedure, but it is not known whether death resulted directly from the diagnostic procedure. Put differently, 33 per 10,000 screenees suffered major complications during any diagnostic evaluation, but complications following bronchoscopy or needle biopsy were low, 1.5 and 0.7 per 10,000 screenees respectively; the frequency of death occurring within 2 months of a diagnostic evaluation was 8 per 10,000 (16). I-ELCAP has not reported its rates of diagnostic procedures or complications.

CT-FNA appears safe with a complication rate of 13-14% and good concordance of biopsy result with resected pathological specimens histology (119). Bronchoscopy on the other hand, although safe, may have a lower yield for small, peripheral cancers detected by screening, although newer techniques such as endobronchial ultrasound and electromagnetic navigation may be able to improve yield (120,121). Surgical procedures have major complication rates of 12% but around 20-35% of cases are ultimately diagnosed with benign disease. This has an impact on cost-effectiveness.

Although ultimately the decision to resect an indeterminate nodule is a clinical one, given the high proportion of reported benign disease detected by screening, a positive tissue diagnosis prior to surgical resection is desirable. As demonstrated by the NELSON study, definite growth over a three month interval was due to benign disease in up to one third of cases. To date most studies have been run from expert tertiary centres where CT-FNA is available as the initial diagnostic procedure for small peripheral lesions. It is likely that strict governance and quality assurance will be needed to keep unnecessary biopsies and resections to a minimum.

Lung-preserving surgery

As reviewed by Blasburg *et al.*, evolving surgical technique, the recognition of good prognosis for small tumours, especially with a high GGO component, and the on-going risk of subsequent tumours, has turned attention to 'lung preserving' surgery (anatomical segmentectomy and wedge resection) as an alternative to lobar resection for small tumours (122). Two randomized controlled trials which will hopefully be able to answer this important question are currently recruiting [CALGB 140503 and JCOG0802/WJOG4607L (123)].

Quality of life (QoL)

Three studies have reported generic health-related QoL (HRQoL), anxiety and lung-cancer specific distress data from approximately 2,500 screening participants (124-126). All found some transient negative psychological effects for participants who received an indeterminate or suspicious screening result. These effects subsided fairly rapidly such that there were no significant differences in HRQoL between baseline and 12-24 months follow-up. The NELSON study reported that half the participants found waiting for their baseline CT scan results 'discomforting', but that an indeterminate result at the second round of screening had no impact on HRQoL. This suggests that minimizing the waiting time for test results is beneficial and that participants soon accept that an indeterminate scan result does not necessarily warrant high anxiety (124,127).

Smoking cessation

Smoking cessation is important not only for future risk reduction in participants without cancer, but may also improve the prognosis of those diagnosed with early stage lung cancer (128). Screening for lung cancer may be a "teachable moment" increasing motivation to quit, particularly if the participant receives an abnormal CT scan report (129-131). As successful smoking cessation programs may also make screening more cost-effective (99), and smoking cessation assistance 'adds value' to screening in several ways, it should be a core component of any lung cancer screening program.

Knowledge gaps

Despite the positive result from NLST, screening outside of a research trial should be conducted in a controlled environment with careful risk assessment prior to recommending screening and careful analysis of all outcomes to ensure quality. Two international workshops have considered the current state of evidence and future directions for research. Areas that need addressing were highlighted including: (I) how to optimise identification of high-risk individuals; (II) Screening protocols (e.g., screen interval, number of screening rounds); (III) Definition of a positive screen result; (IV) Management of indeterminate nodules; (V) Diagnostic and therapeutic interventions for suspicious nodules; (VI) Integrated smoking cessation programs; (VII) The role of early detection biomarkers in individual lung cancer risk assessment; (VIII) The rate of overdiagnosis. Important steps will be to standardise equipment and image quality, nodule analysis and interpretation, and participant follow-up and outcome reporting (93,132). Some of these areas are discussed below.

Overdiagnosis

Overdiagnosis is difficult to ascertain (see Box 2 for definition). It was estimated at 13% in the NLST-the relative difference between 1,060 cancers detected in LDCT arm and 941 cancers detected in control arm (13). However this figure has been criticised as an underestimate (133) on the basis that the appropriate denominator should be the number of lung cancers detected in the control group *during the screening period* (n=470), not at the end of follow-up (n=941), making overdiagnosis closer to 25%, a figure similar to that estimated by the Mayo LDCT study on the basis of VDT (37). However even this figure may be an underestimate if the CXR screening arm is also subject to overdiagnosis (133). Against this, subset analysis of the PLCO cohort who met NLST eligibility criteria (n=30,321) found similar numbers of lung cancer cases in the CXR and the non-screened arms (518 *vs.* 520 cancers respectively after 6 years' follow-up) (12). It is likely that only the European trials comparing screening to usual care (i.e., no screening) will be able to give a true estimate of overdiagnosis (90). This question therefore remains unanswered at present.

Screening interval and length of follow-up

The appropriate screening interval should provide a favourable ratio between disease control and screening costs (134). The MILD trial recently published their findings from a three-arm RCT of observation *vs.* annual *vs.* biennial screening in 4,099 participants (89). Stage distribution and resection rates were similar in the two LDCT arms. The cumulative 5-year lung cancer incidence was highest in the annual LDCT group compared to biennial and control groups (620/100,000 *vs.* 457 and 311 respectively, P=0.036). Adherence to the screening protocol was >95% in each LDCT arm but median duration of follow-up was only 4.4 years. Recruitment fell significantly short of the planned 10,000 participants meaning the study was underpowered to detect mortality differences. Also, differences in characteristics of screened and non-screened groups (such as smoking status, smoking intensity and lung function) raise doubts about the adequacy of randomization (135). Long-term follow-up results

from this study may be more informative. The NELSON study, in which participants are screened at Year 1 (baseline), Year 2 and Year 4, i.e., a two-year gap between the second and third scan, could also inform on optimal screen interval when Year 4 results are reported. As previously mentioned, data gathered at baseline scan (i.e., presence of radiographic emphysema) may be useful in determining risk and thus optimal screening interval (68). Regarding duration of screening, the NLST LDCT arm detected 649 cancers after a positive screening test (270 at baseline and 168 and 211 at years 1 and 2 respectively) and 367 in participants who either missed the screening or were diagnosed after completing the trial screening phase (median follow-up 6.5 years). This suggests that cancer detection rates (i.e., cancer risk) do not drop significantly over time and that on-going screening may be required. Accordingly, current guidelines suggest annual screening until the age of 74 (14,16) or 79 (15).

Recruitment

Recruitment strategies have varied between studies, most commonly direct mailing and/or media releases, but some used general practitioner referral (84,88). Smokers, by definition are less risk averse than non-smokers, at least in terms of their health. The decision to enter a screening trial is a complex balance of factors including acceptability of screening methods, risk perception, altruism, and self-interest (136). Inevitably, volunteers in any trial are self-selected and contribute to the 'healthy volunteer' effect. This may result in overly optimistic outcomes (e.g., better screening compliance, higher smoking cessation rates) or overly pessimistic outcomes (e.g., lower effectiveness as lower-risk individuals benefit less from screening).

Both the NLST and NELSON studies found some differences between their study populations and eligible general population; Participants were younger and less likely to be current smokers and had higher education levels (a proxy for socio-economic status). These differences were considered minor, meaning that a significant healthy volunteer effect was unlikely (81,137).

Risk stratification

Risk stratification has been applied at a basic level with most studies adopting the ELCAP strategy of screening older persons with a smoking history. Although age and tobacco smoke exposure account for the vast majority of lung cancer risk it is well recognised that other risk factors such as family history, socioeconomic status, occupational exposure and COPD contribute (138). Further risk stratification using other readily available information may be able to improve screening efficiency by excluding lower risk participants (139). Various models have been proposed, the largest derived from PLCO Trial data and recently updated (140,141). A retrospective analysis of this model applied to the PLCO dataset found that it was more efficient in comparison to the standard age- and smoking-based NLST entry criteria improving sensitivity from 71% to 83% ($P<0.001$), positive predictive value from 3.4% to 4.0% ($P=0.01$), and maintaining specificity (63% each). Use of the risk model to select screenees would have missed 41.3% fewer lung cancers (141). Prospective evaluation of another risk model is being undertaken by the UK Lung Cancer Screening Trial (142). Risk stratification may enhance screening effectiveness and cost-effectiveness by increasing lung cancer prevalence and incidence and reducing false-positive scan results. Although risk stratification makes intuitive sense it has not been proven experimentally, thus screening guideline recommendations diverge [recommend use of published risk model (15), informal risk assessment (14), no recommendation (16)].

Screening implementation

Generalization of findings from tightly controlled trial situations to large-scale mass screening programs require uniform standards and high quality control in order to be able to accurately track and assess nodules over time (132). Lung cancer screening is more than simple provision of a CT service; It is as a long-term commitment requiring extensive infrastructure to allow for invitation and recruitment; quality improvement; workforce/facility capacity for screening, diagnosis and treatment; health professional training; participant information and support. On-going evaluation and monitoring of the program is essential to ensure high standards of care are met and delivered in a consistent and acceptable way (134,143).

Future research

Minimally invasive, inexpensive tests to identify individuals at highest risk of lung cancer most likely to benefit from screening or to distinguish benign from malignant screen-detected nodules would represent major advances in lung cancer screening. Promising new technologies in this regard include analysis of blood for circulating microRNAs and

exhaled breath for volatile organic compounds (144-146). Most recent LDCT screening studies included biomarker collection in their protocols, so we can expect exciting new insights into these areas in the near future.

Conclusions

The results of the landmark NLST have proven the long-held belief that screening for lung cancer can save lives. Understandably, as a new intervention, many questions remain making generalizability to non-US settings difficult. Over the next few years, further analysis of NLST data and maturation of other important trials will be able to fill these knowledge gaps allowing the lung cancer community to evolve and refine the way we screen.

Acknowledgements

We thank the study volunteers and staff of The Prince Charles Hospital for their involvement in our research program and the Queensland Lung Cancer Screening Study research team.

Funding: This work was supported by National Health and Medical Research Council (NHMRC) project grants, NHMRC Practitioner Fellowship (KF), NHMRC Career Development Award (IY), Cancer Council Queensland Senior Research Fellowship (KF), Cancer Council Queensland project grants, Queensland Smart State project grants, Office of Health and Medical Research (OHMR) project grants, The Prince Charles Hospital Foundation, NHMRC Postgraduate Medical Scholarship (HM), University of Queensland PhD Scholarship (HM).

Disclosure: The authors declare no conflict of interest.

References

1. Ferlay J, Shin H, Bray F, et al. GLOBOCAN 2008. Available online: http://globocan.iarc.fr
2. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
3. Gu D, Kelly TN, Wu X, et al. Mortality attributable to smoking in China. N Engl J Med 2009;360:150-9.
4. Sant M, Allemani C, Santaquilani M, et al. EUROCARE-4. Survival of cancer patients diagnosed in 1995-1999. Results and commentary. Eur J Cancer 2009;45:931-91.
5. Howlader N, Noone AM, Krapcho M, et al. SEER cancer statistics review, 1975-2008. Available online: http://seer.cancer.gov/csr/1975_2008/
6. Sankaranarayanan R, Swaminathan R, Brenner H, et al. Cancer survival in Africa, Asia, and Central America: a population-based study. Lancet Oncol 2010;11:165-73.
7. Scott WJ, Howington J, Feigenberg S, et al. Treatment of non-small cell lung cancer stage I and stage II*. Chest 2007;132:234S-242S.
8. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
9. Wilson JMG, Jungner G. eds. Principles and practice of screening for disease. Geneva: World Health Organization, 1968.
10. Welch HG, Black WC. Overdiagnosis in Cancer. J Natl Cancer Inst 2010;102:605-13.
11. Manser RL, Irving LB, Stone C, et al. Screening for lung cancer. Cochrane Cochrane Database Syst Rev 2004;(1):CD001991.
12. Oken MM, Hocking WG, Kvale PA, et al. Screening by chest radiograph and lung cancer mortality. JAMA 2011;306:1865-73.
13. National Lung Screening Trial Research Team. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395-409.
14. Wood DE, Eapen GA, Ettinger DS, et al. Lung cancer screening. J Natl Compr Canc Netw 2012;10:240-65.
15. Jaklitsch MT, Jacobson FL, Austin JH, et al. The American Association for Thoracic Surgery guidelines for lung cancer screening using low-dose computed tomography scans for lung cancer survivors and other high-risk groups. J Thorac Cardiovasc Surg 2012;144:33-8.
16. Bach PB, Mirkin JN, Oliver TK, et al. Benefits and harms of CT screening for lung cancer: a systematic review. JAMA 2012;307:2418-29.
17. Force UPST. US preventive services task force. Draft recommendation statement. Available online: http://www.uspreventiveservicestaskforce.org/draftrec.htm
18. Hansell DM, Bankier A, MacMahon H, et al. Fleischner society: glossary of terms for thoracic imaging. Radiology 2008;246:697-722.
19. Armato SG, Roberts RY, Kocherginsky M, et al. Assessment of radiologist performance in the detection

of lung nodules: dependence on the definition of "truth". Acad Radiol 2009;16:28-38.
20. Gierada DS, Pilgram TK, Ford M, et al. Lung cancer: interobserver agreement on interpretation of pulmonary findings at low-dose CT screening. Radiology 2008;246:265-72.
21. Swensen SJ, Jett JR, Sloan JA, et al. Screening for lung cancer with low-dose spiral computed tomography. Am J Respir Crit Care Med 2002;165:508-13.
22. Wormanns D, Ludwig K, Beyer F, et al. Detection of pulmonary nodules at multirow-detector CT: effectiveness of double reading to improve sensitivity at standard-dose and low-dose chest CT. Eur Radiol 2005;15:14-22.
23. Jankowski A, Martinelli T, Timsit JF, et al. Pulmonary nodule detection on MDCT images: evaluation of diagnostic performance using thin axial images, maximum intensity projections, and computer-assisted detection. Eur Radiol 2007;17:3148-56.
24. Coakley FV, Cohen MD, Johnson MS, et al. Maximum intensity projection images in the detection of simulated pulmonary nodules by spiral CT. Br J Radiol 1998;71:135-40.
25. Kawel N, Seifert B, Luetolf M, et al. Effect of slab thickness on the CT detection of pulmonary nodules: use of sliding thin-slab maximum intensity projection and volume rendering. AJR Am J Roentgenol 2009;192:1324-9.
26. Armato SG 3rd, Li F, Giger ML, et al. Lung cancer: performance of automated lung nodule detection applied to cancers missed in a CT screening program. Radiology 2002;225:685-92.
27. Yuan R, Vos PM, Cooperberg PL. Computer-aided detection in screening CT for pulmonary nodules. AJR Am J Roentgenol 2006;186:1280-7.
28. Saba L, Caddeo G, Mallarini G. Computer-aided detection of pulmonary nodules in computed tomography: analysis and review of the literature. J Comput Assist Tomogr 2007;31:611-9.
29. Midthun DE, Swensen SJ, Jett JR, et al. Evaluation of nodules detected by screening for lung cancer with low dose spiral computed tomography. Lung Cancer 2003;41:S40.
30. Gohagan J, Marcus P, Fagerstrom R, et al. Baseline findings of a randomized feasibility trial of lung cancer screening with spiral CT scan vs chest radiograph: the Lung Screening Study of the National Cancer Institute. Chest 2004;126:114-21.
31. Henschke CI, McCauley DI, Yankelevitz DF, et al. Early Lung Cancer Action Project: overall design and findings from baseline screening. Lancet 1999;354:99-105.
32. The National Lung Screening Trial Research Team. Results of initial low-dose computed tomographic screening for lung cancer. N Engl J Med 2013;368:1980-91.
33. Bellomi M, Veronesi G, Rampinelli C, et al. Evolution of lung nodules < or =5 mm detected with low-dose CT in asymptomatic smokers. Br J Radiol 2007;80:708-12.
34. Erasmus JJ, Connolly JE, McAdams HP, et al. Solitary pulmonary nodules: part I. morphologic evaluation for differentiation of benign and malignant lesions. Radiographics 2000;20:43-58.
35. Henschke CI, Yankelevitz DF, Mirtcheva R, et al. CT screening for lung cancer: frequency and significance of part-solid and nonsolid nodules. Am J Roentgenol 2002;178:1053-7.
36. Kakinuma R, Ohmatsu H, Kaneko M, et al. Progression of focal pure ground-glass opacity detected by low-dose helical computed tomography screening for lung cancer. J Comput Assist Tomogr 2004;28:17-23.
37. Lindell RM, Hartman TE, Swensen SJ, et al. Five-year lung cancer screening experience: CT appearance, growth rate, location, and histologic features of 61 lung cancers. Radiology 2007;242:555-62.
38. Aoki T, Nakata H, Watanabe H, et al. Evolution of peripheral lung adenocarcinomas: CT findings correlated with histology and tumor doubling time. AJR Am J Roentgenol 2000;174:763-8.
39. Felix L, Serra-Tosio G, Lantuejoul S, et al. CT characteristics of resolving ground-glass opacities in a lung cancer screening programme. Eur J Radiol 2011;77:410-6.
40. Oh JY, Kwon SY, Yoon HI, et al. Clinical significance of a solitary ground-glass opacity (GGO) lesion of the lung detected by chest CT. Lung Cancer 2007;55:67-73.
41. Lee SM, Park C, Goo J, et al. Transient part-solid nodules detected at screening thin-section CT for lung cancer: comparison with persistent part-solid nodules. Radiology 2010;255:242-51.
42. Mario M, Nicola S, Carmelinda M, et al. Long-term surveillance of ground-glass nodules: evidence from the mild trial. J Thorac Oncol 2012;7:1541-6.
43. Hasegawa M, Sone S, Takashima S, et al. Growth rate of small lung cancers detected on mass CT screening. Br J

Radiol 2000;73:1252-9.
44. Godoy MC, Naidich DP. Overview and strategic management of subsolid pulmonary nodules. J Thorac Imaging 2012;27:240-8.
45. MacMahon H, Austin JH, Gamsu G, et al. Guidelines for management of small pulmonary nodules detected on CT scans: a statement from the Fleischner Society. Radiology 2005 237:395-400.
46. Yankelevitz DF, Henschke CI. Does 2-year stability imply that pulmonary nodules are benign? AJR Am J Roentgenol 1997;168:325-8.
47. Revel MP, Bissery A, Bienvenu M, et al. Are two-dimensional CT measurements of small noncalcified pulmonary nodules reliable? Radiology 2004;231:453-8.
48. Revel MP, Lefort C, Bissery A, et al. Pulmonary nodules: preliminary experience with three-dimensional evaluation. Radiology 2004;231:459-66.
49. Yankelevitz DF, Reeves AP, Kostis WJ, et al. Small pulmonary nodules: volumetrically determined growth rates based on CT evaluation. Radiology 2000;217:251-6.
50. van Klaveren RJ, Oudkerk M, Prokop M, et al. Management of lung nodules detected by volume CT scanning. N Engl J Med 2009;361:2221-9.
51. Kostis WJ, Yankelevitz D, Reeves A, et al. Small pulmonary nodules: reproducibility of three-dimensional volumetric measurement and estimation of time to follow-up CT. Radiology 2004;231:446-52.
52. Xu DM, Gietema H, de Koning H, et al. Nodule management protocol of the NELSON randomised lung cancer screening trial. Lung Cancer 2006;54:177-84.
53. Slattery MM, Foley C, Kenny D, et al. Long-term follow-up of non-calcified pulmonary nodules (<10 mm) identified during low-dose CT screening for lung cancer. Eur Radiol 2012;22:1923-8.
54. Xu DM, van der Zaag-Loonen HJ, Oudkerk M, et al. Smooth or attached solid indeterminate nodules detected at baseline CT screening in the NELSON study: cancer risk during 1 year of follow-up. Radiology 2009;250:264-72.
55. Xu DM, van Klaveren RJ, de Bock GH, et al. Role of baseline nodule density and changes in density and nodule features in the discrimination between benign and malignant solid indeterminate pulmonary nodules. Eur J Radiol 2009;70:492-8.
56. Diederich S, Hansen J, Wormanns D. Resolving small pulmonary nodules: CT features. Eur Radiol 2005;15:2064-9.
57. Takashima S, Sone S, Li F, et al. Small solitary pulmonary nodules (1 cm) detected at population-based CT screening for lung cancer: reliable high-resolution CT features of benign lesions. AJR Am J Roentgenol 2003;180:955-64.
58. Ahn MI, Gleeson TG, Chan IH, et al. Perifissural nodules seen at CT screening for lung cancer. Radiology 2010;254:949-56.
59. Markowitz SB, Miller A, Miller J, et al. Ability of low-dose helical CT to distinguish between benign and malignant noncalcified lung nodules. Chest 2007;131:1028-34.
60. ACRIN. ACRIN Protocol. Available online: http://www.acrin.org/Portals/0/Protocols/6654/Protocol-ACRIN%206654%20Amendment%2010,%2011.1.04.pdf
61. International Early Lung Cancer Action Program. Enrollment and screening protocol. Available online: www.ielcap.org/professionals/docs/ielcap.pdf
62. Henschke CI, Yip R, Yankelevitz DF, et al. Definition of a positive test result in computed tomography screening for lung cancer: a cohort study. Ann Intern Med 2013;158:246-52.
63. van de Wiel JC, Wang Y, Xu DM, et al. Neglectable benefit of searching for incidental findings in the Dutch-Belgian lung cancer screening trial (NELSON) using low-dose multidetector CT. Eur Radiol 2007;17:1474-82.
64. Kucharczyk MJ, Menezes RJ, McGregor A, et al. Assessing the impact of incidental findings in a lung cancer screening study by using low-dose computed tomography. Can Assoc Radiol J 2011;62:141-5.
65. Mets OM, de Jong PA, Prokop M. Computed tomographic screening for lung cancer: an opportunity to evaluate other diseases. JAMA 2012;308:1433-4.
66. Patel AR, Wedzicha J, Hurst J. Reduced lung-cancer mortality with CT screening. N Engl J Med 2011;365:2035; author reply 2037-8.
67. Smith BM, Pinto L, Ezer N, et al. Emphysema detected on computed tomography and risk of lung cancer: a systematic review and meta-analysis. Lung Cancer 2012;77:58-63.
68. Maisonneuve P, Bagnardi V, Bellomi M, et al. Lung cancer risk prediction to select smokers for screening CT--a model based on the Italian COSMOS trial. Cancer Prev Res (Phila) 2011;4:1778-89.

69. Waugh N, Black C, Walker S, et al. The effectiveness and cost-effectiveness of computed tomography screening for coronary artery disease: systematic review. Health Technol Assess 2006;10:iii-iv, ix-x, 1-41.
70. Jha P. Avoidable global cancer deaths and total deaths from smoking. Nat Rev Cancer 2009;9:655-64.
71. Shemesh J, Henschke CI, Farooqi A, et al. Frequency of coronary artery calcification on low-dose computed tomography screening for lung cancer. Clin Imaging 2006;30:181-5.
72. Shemesh J, Henschke CI, Shaham D, et al. Ordinal scoring of coronary artery calcifications on low-dose CT scans of the chest is predictive of death from cardiovascular disease. Radiology 2010;257:541-8.
73. Jacobs PC, Gondrie MJ, van der Graaf Y, et al. Coronary artery calcium can predict all-cause mortality and cardiovascular events on low-dose ct screening for lung cancer. AJR Am J Roentgenol 2012;198:505-11.
74. Sobue T, Moriyama N, Kaneko M, et al. Screening for lung cancer with low-dose helical computed tomography: anti-lung cancer association project. J Clin Oncol 2002;20:911-20.
75. Sone S, Nakayama T, Honda T, et al. Long-term follow-up study of a population-based 1996-1998 mass screening programme for lung cancer using mobile low-dose spiral computed tomography. Lung Cancer 2007;58:329-41.
76. Swensen SJ, Jett JR, Hartman TE, et al. CT screening for lung cancer: five-year prospective experience. Radiology 2005;235:259-65.
77. The International Early Lung Cancer Action Program I, Henschke CI, Yankelevitz D, et al. Survival of patients with stage I lung cancer detected on CT screening. N Engl J Med 2006;355:1763-71.
78. Bach PB, Jett JR, Pastorino U, et al. Computed tomography screening and lung cancer outcomes. JAMA 2007;297:953-61.
79. Patz EF Jr, Swensen SJ, Herndon JE. Estimate of lung cancer mortality from low-dose spiral computed tomography screening trials: implications for current mass screening recommendations. J Clin Oncol 2004;22:2202-6.
80. Foy M, Yip R, Chen X, et al. Modeling the mortality reduction due to computed tomography screening for lung cancer. Cancer 2011;117:2703-8.
81. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Baseline characteristics of participants in the randomized national lung screening trial. J Natl Cancer Inst 2010;102:1771-9.
82. Gohagan JK, Marcus PM, Fagerstrom RM, et al. Final results of the lung screening study, a randomized feasibility study of spiral CT versus chest X-ray screening for lung cancer. Lung Cancer 2005;47:9-15.
83. Infante M, Cavuto S, Lutman FR, et al. A randomized study of lung cancer screening with spiral computed tomography: three-year results from the DANTE trial. Am J Respir Crit Care Med 2009;180:445-53.
84. Blanchon T, Brechot JM, Grenier PA, et al. Baseline results of the Depiscan study: a French randomized pilot trial of lung cancer screening comparing low dose CT scan (LDCT) and chest X-ray (CXR). Lung Cancer 2007;58:50-8.
85. van Iersel CA, de Koning HJ, Draisma G, et al. Risk-based selection from the general population in a screening trial: selection criteria, recruitment and power for the Dutch-Belgian randomised lung cancer multi-slice CT screening trial (NELSON). Int J Cancer 2007;120:868-74.
86. Baecke E, de Koning HJ, Otto SJ, et al. Limited contamination in the Dutch-Belgian randomized lung cancer screening trial (NELSON). Lung Cancer 2010;69:66-70.
87. Pedersen JH, Ashraf H, Dirksen A, et al. The Danish randomized lung cancer CT screening trial--overall design and results of the prevalence round. J Thorac Oncol 2009;4:608-14.
88. Lopes Pegna A, Picozzi G, Mascalchi M, et al. Design, recruitment and baseline results of the ITALUNG trial for lung cancer screening with low-dose CT. Lung Cancer 2009;64:34-40.
89. Pastorino U, Rossi M, Rosato V, et al. Annual or biennial CT screening versus observation in heavy smokers: 5-year results of the MILD trial. Eur J Cancer Prev 2012;21:308-15.
90. Becker N, Motsch E, Gross ML, et al. Randomized study on early detection of lung cancer with MSCT in Germany: study design and results of the first screening round. Journal of cancer research and clinical oncology 2012;138:1475-86.
91. Field JK, Baldwin D, Brain K, et al. CT screening for lung cancer in the UK: position statement by UKLS investigators following the NLST report. Thorax 2011;66:736-7.
92. NIHR. Health technology assessment programme. Available online: http://www.hta.ac.uk/2382
93. Italian lung cancer CT screening trial workshop. International workshop on randomized lung cancer

screening trials; Position statement, Pisa, March 3-4. 2011. Available online: www.studio-sesto.com/ons/.../pisa_position_statement_english.pdf
94. International Association for the Study of Lung Cancer. IASLC task force on CT screening: position statement. 2011 [cited 01/12/2011]. Available online: http://iaslc.org/policies/statement-on-ct-screening/
95. Veronesi G, Maisonneuve P, Spaggiari L, et al. Long-term outcomes of a pilot CT screening for lung cancer. Ecancermedicalscience 2010;4:186.
96. Byrne MM, Koru-Sengul T, Zhao W, et al. Healthcare use after screening for lung cancer. Cancer 2010;116:4793-9.
97. Black C, Bagust A, Boland A, et al. The clinical effectiveness and cost-effectiveness of computed tomography screening for lung cancer: systematic reviews. Health Technol Assess 2006;10:iii-iv, ix-x, 1-90.
98. Goulart BHL, Bensink ME, Mummy DG, et al. Lung cancer screening with low-dose computed tomography: costs, national expenditures, and cost-effectiveness. J Natl Compr Canc Netw 2012;10:267-75.
99. McMahon PM, Kong CY, Bouzan C, et al. Cost-effectiveness of computed tomography screening for lung cancer in the United States. J Thorac Oncol 2011;6:1841-8.
100. Pyenson BS, Sander MS, Jiang Y, et al. An actuarial analysis shows that offering lung cancer screening as an insurance benefit would save lives at relatively low cost. Health Aff (Millwood) 2012;31:770-9.
101. Black WC. Cost Effectiveness of CT Screening in the National Lung Screening Trial, in 2nd Joint Meeting of the National Cancer Institute Board of Scientific Advisors & National Cancer Advisory Board 2013: Bethseda (Accessed 19 Aug 2013, at http://videocast.nih.gov/launch.asp?18018).
102. Berrington de González A, Darby S. Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries. Lancet 2004;363:345-51.
103. Herzog P, Rieger CT. Risk of cancer from diagnostic X-rays. Lancet 2004;363:340-41.
104. ICRP. Recommendations of the ICRP. Annals of the ICRP 1977;1:3.
105. Kubo T, Lin P-JP, Stiller W, et al. Radiation dose reduction in chest CT: a review. AJR Am J Roentgenol 2008;190:335-43.
106. Marshall H, Smith I, Keir B, et al. Radiation dose during lung cancer screening with low-dose computed tomography: how low is low dose? J Thorac Oncol 2010;6.
107. Mascalchi M, Mazzoni LN, Falchini M, et al. Dose exposure in the ITALUNG trial of lung cancer screening with low-dose CT. Br J Radiol 2012;85:1134-9.
108. Takahashi M, Maguire WM, Ashtari M, et al. Low-dose spiral computed tomography of the thorax: comparison with the standard-dose technique. Invest Radiol 1998;33:68-73.
109. Mayo JR, Hartman TE, Lee KS, et al. CT of the chest: minimal tube current required for good image quality with the least radiation dose. AJR Am J Roentgenol 1995;164:603-7.
110. Diederich S, Lenzen H, Windmann R, et al. Pulmonary nodules: experimental and clinical studies at low-dose CT. Radiology 1999;213:289-98.
111. Marshall HM, Bowman RV, Crossin J, et al. Queensland lung cancer screening study: rationale, design and methods. Intern Med J 2013;43:174-82.
112. Brenner DJ. Radiation risks potentially associated with low-dose CT screening of adult smokers for lung cancer. Radiology 2004;231:440-5.
113. Berrington de González A, Kim KP, Berg CD. Low-dose lung computed tomography screening before age 55: estimates of the mortality reduction required to outweigh the radiation-induced cancer risk. J Med Screen 2008;15:153-8.
114. Mascalchi M, Belli G, Zappa M, et al. Risk-benefit analysis of X-ray exposure associated with lung cancer screening in the Italung-CT trial. AJR Am J Roentgenol 2006;187:421-9.
115. Eisenberg RL, Bankier A, Boiselle P. Compliance with Fleischner Society guidelines for management of small lung nodules: a survey of 834 radiologists. Radiology 2010;255:218-24.
116. Wiener RS, Schwartz LM, Woloshin S, et al. Population-based risk for complications after transthoracic needle lung biopsy of a pulmonary nodule: an analysis of discharge records. Ann Intern Med 2011;155:137-44.
117. Wagnetz U, Menezes RJ, Boerner S, et al. CT screening for lung cancer: implication of lung biopsy recommendations. AJR Am J Roentgenol 2012;198:351-8.
118. Wilson DO, Weissfeld JL, Fuhrman CR, et al. The Pittsburgh Lung Screening Study (PLuSS): outcomes within 3 years of a first computed tomography scan. Am J Respir Crit Care Med 2008;178:956-61.
119. Vazquez MF, Koizumi JH, Henschke CI, et al. Reliability of cytologic diagnosis of early lung cancer. Cancer 2007;111:252-8.

120. Leong S, Ju H, Marshall H, et al. Electromagnetic navigation bronchoscopy: a descriptive analysis. J Thorac Dis 2012;4:173-85.
121. Steinfort DP, Khor YH, Manser RL, et al. Radial probe endobronchial ultrasound for the diagnosis of peripheral lung cancer: systematic review and meta-analysis. Eur Respir J 2011;37:902-10.
122. Blasberg JD, Pass HI, Donington JS. Sublobar resection: a movement from the Lung Cancer Study Group. J Thorac Oncol 2010;5:1583-93.
123. Nakamura K, Saji H, Nakajima R, et al. A phase III randomized trial of lobectomy versus limited resection for small-sized peripheral non-small cell lung cancer (JCOG0802/WJOG4607L). Jpn J Clin Oncol 2010;40:271-4.
124. van den Bergh KA, Essink-Bot ML, Borsboom GJ, et al. Long-term effects of lung cancer computed tomography screening on health-related quality of life: the NELSON trial. Eur Respir J 2011;38:154-61.
125. Vierikko T, Kivisto S, Jarvenpaa R, et al. Psychological impact of computed tomography screening for lung cancer and occupational pulmonary disease among asbestos-exposed workers. Eur J Cancer Prev 2009;18:203-6.
126. Byrne MM, Weissfeld J, Roberts MS. Anxiety, fear of cancer, and perceived risk of cancer following lung cancer screening. Med Decis Making 2008;28:917-25.
127. van den Bergh KA, Essink-Bot ML, Bunge EM, et al. Impact of computed tomography screening for lung cancer on participants in a randomized controlled trial (NELSON trial). Cancer 2008;113:396-404.
128. Parsons A, Daley A, Begh R, et al. Influence of smoking cessation after diagnosis of early stage lung cancer on prognosis: systematic review of observational studies with meta-analysis. BMJ 2010;340:b5569.
129. Ostroff JS, Buckshee N, Mancuso CA, et al. Smoking cessation following CT screening for early detection of lung cancer. Prev Med 2001;33:613-21.
130. Cox LS, Clark MM, Jett JR, et al. Change in smoking status after spiral chest computed tomography scan screening. Cancer 2003;98:2495-501.
131. Styn MA, Land SR, Perkins KA, et al. Smoking behavior 1 year after computed tomography screening for lung cancer: effect of physician referral for abnormal CT findings. Cancer Epidemiol Biomarkers Prev 2009;18:3484-9.
132. Field JK, Smith RA, Aberle DR, et al. International Association for the Study of Lung Cancer Computed Tomography Screening Workshop 2011 report. J Thorac Oncol 2012;7:10-9.
133. Bach PB. Reduced lung-cancer mortality with CT screening. N Engl J Med 2011;365:2036; author reply 2037-8.
134. Strong K, Wald N, Miller A, et al. Current concepts in screening for noncommunicable disease: World Health Organization Consultation Group Report on methodology of noncommunicable disease screening. J Med Screen 2005;12:12-9.
135. Humphrey LL, Deffebach M, Pappas M, et al. Screening for lung cancer with low-dose computed tomography: a systematic review to update the U.S. preventive services task force recommendation. Ann Intern Med 2013;159:411-20.
136. Patel D, Akporobaro A, Chinyanganya N, et al. Attitudes to participation in a lung cancer screening trial: a qualitative study. Thorax 2012;67:418-25.
137. van der Aalst CM, van Iersel CA, van Klaveren RJ, et al. Generalisability of the results of the Dutch-Belgian randomised controlled lung cancer CT screening trial (NELSON): does self-selection play a role? Lung Cancer 2012;77:51-7.
138. Youlden DR, Cramb SM, Baade PD. The international epidemiology of lung cancer: geographical distribution and secular trends. J Thorac Oncol 2008;3:819-31.
139. Marshall H, Bowman R, Crossin J, et al. The Queensland Lung Cancer Screening Study: risk stratification using participant data and lung function tests can significantly increase screening effectiveness, Journal of Thoracic Oncology 4th Australian Lung Cancer Conference (ALCC), Adelaide, 2012.
140. Tammemagi CM, Pinsky PF, Caporaso NE, et al. Lung cancer risk prediction: Prostate, Lung, Colorectal And Ovarian Cancer Screening Trial models and validation. J Natl Cancer Inst 2011;103:1058-68.
141. Tammemägi MC, Katki HA, Hocking WG, et al. Selection criteria for lung-cancer screening. N Engl J Med 2013;368:728-36.
142. Cassidy A, Myles JP, van Tongeren M, et al. The LLP risk model: an individual risk prediction model for lung cancer. Br J Cancer 2008;98:270-6.
143. Australian Population Health Development Principal Committee Screening Subcommittee, Population based screening framework. Commonwealth of Australia, Canberra, 2008.
144. Bianchi F, Nicassio F, Marzi M, et al. A serum circulating miRNA diagnostic test to identify asymptomatic high-

risk individuals with early stage lung cancer. EMBO Mol Med 2011;3:495-503.
145. Hennessey PT, Sanford T, Choudhary A, et al. Serum microRNA biomarkers for detection of non-small cell lung cancer. PloS One 2012;7:e32307.
146. Peled N, Hakim M, Bunn PA Jr, et al. Non-invasive breath analysis of pulmonary nodules. J Thorac Oncol 2012;7:1528-33.

Cite this article as: Marshall HM, Bowman RV, Yang IA, Fong KM, Berg CD. Screening for lung cancer with low-dose computed tomography: a review of current status. J Thorac Dis 2013;5(S5):S524-S539. doi: 10.3978/j.issn.2072-1439.2013.09.06

Exhaled breath analysis for lung cancer

Annette G. Dent[1,2], Tom G. Sutedja[3], Paul V. Zimmerman[1,2]

[1]Department of Thoracic Medicine, The Prince Charles Hospital, Rode Road, Chermside, Queensland 4032, Australia; [2]The University of Queensland, Brisbane, St Lucia, Queensland 4072, Australia; [3]Department of Pulmonary Medicine, Division of Thoracic Oncology, Vrije Universiteit Academic Medical Center, de Boelelaan 117, 1181 HV Amsterdam, The Netherlands

Correspondence to: Annette G. Dent. Respiratory Investigation Unit, Department of Thoracic Medicine, The Prince Charles Hospital, Rode Road, Chermside, QLD 4032, Australia. Email: annette_dent@health.qld.gov.au.

Abstract: Early diagnosis of lung cancer results in improved survival compared to diagnosis with more advanced disease. Early disease is not reliably indicated by symptoms. Because investigations such as bronchoscopy and needle biopsy have associated risks and substantial costs, they are not suitable for population screening. Hence new easily applicable tests, which can be used to screen individuals at risk, are required. Biomarker testing in exhaled breath samples is a simple, relatively inexpensive, non-invasive approach. Exhaled breath contains volatile and non-volatile organic compounds produced as end-products of metabolic processes and the composition of such compounds varies between healthy subjects and subjects with lung cancer. Many studies have analysed the patterns of these compounds in exhaled breath. In addition studies have also reported that the exhaled breath condensate (EBC) can reveal gene mutations or DNA abnormalities in patients with lung cancer. This review has summarised the scientific evidence demonstrating that lung cancer has distinct chemical profiles in exhaled breath and characteristic genetic changes in EBC. It is not yet possible to accurately identify individuals with lung cancer in at risk populations by any of these techniques. However, analysis of both volatile organic compounds in exhaled breath and of EBC have great potential to become clinically useful diagnostic and screening tools for early stage lung cancer detection.

Keywords: Lung cancer; biomarkers; volatile organic compounds; exhaled breath

Submitted Aug 09, 2013. Accepted for publication Aug 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.44
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.44

Lung cancer is the most common cause of cancer deaths worldwide, accounting for 1.37 million deaths in 2008 (1,2). Early diagnosis (Stage I) is associated with far better survival (67% 5-year survival rate) than later stage disease (Stage III, 23% 5-year survival rate) (3). Symptoms alone cannot be relied upon to indicate the presence of lung cancer as they often do not appear until the cancer is relatively advanced. New techniques to detect disease earlier in high risk populations of asymptomatic individuals would be expected to significantly improve survival. The aim of this review was to examine the scientific evidence relating result of the analyses of exhaled breath and exhaled breath condensate (EBC) to the presence of lung cancer.

Background

As early as Roman times, the smell of a person's breath has assisted physicians with the diagnosis of a disease, e.g., uncontrolled diabetes was associated with a sweet, acetone odour; liver failure produced a fish-like smell; and renal failure was identified by a urine-like smell (4).

McCulloch (5) demonstrated that dogs could be trained to detect lung cancer and breast cancer in subjects with various stages of disease with almost 100% accuracy, merely by smelling the subject's breath. These observations suggest that there are biomarkers in exhaled breath that are potentially useful for diagnosing disease.

Over the last 40 years there have been many studies aiming to characterise these biomarkers. In 1971, Pauling *et al.* (6), using a gas chromatograph (GC), measured 250 different compounds in human breath samples. Since then Phillips measured 1,259 compounds in normal subjects in 1997 (7), and over 3,000 compounds in 1999 (8).

Analysis of exhaled breath

The compounds in exhaled breath may be useful indicators of a disease process in the lung. They have been classified as:

I Inorganic compounds, e.g., carbon dioxide, oxygen, and nitric oxide;

II Non-volatile compounds measured in EBC, e.g., isoprostanes, cytokines, leukotrienes and hydrogen peroxide (4).

III Volatile organic compounds (VOCs) which can be divided into different classes, e.g., saturated hydrocarbons (ethane, pentane, aldehydes), unsaturated hydrocarbons (isoprene), oxygen containing (acetone), sulphur containing (ethyl mercaptane, dimethylsulfide) and nitrogen containing (dimethylamine, ammonia) (9). The most commonly identified VOCs are isoprene, acetone, ethanol, methanol, other alcohols and alkanes (8).

VOCs

Little is known about the genesis of exhaled breath VOCs. Some are thought to be endogenous, that is produced by the body as end-products of metabolic pathways, e.g., isoprene, an unsaturated hydrocarbon, formed along the mevalonic acid pathway of cholesterol synthesis (10); acetone, an oxygen containing compound produced from glucose metabolism; And saturated hydrocarbons or alkanes such as ethane and pentane produced from oxygen free radical-mediated lipid peroxidation of fatty acid components of cell membranes (11). The latter compounds are thought to be markers of oxidative stress. VOCs are also components of exogenous contaminants from the external environment that have been inhaled and absorbed through the lungs or skin. Apart from lung excretion, VOCs can be catabolized and excreted through the liver or kidney (12).

There have been studies published on the VOCs detected and measured in various respiratory disease states including asthma, COPD, cystic fibrosis, and lung cancer.

Table 1 summarizes the VOCs detected in the different lung diseases. It appears that there is not one VOC that is diagnostic for a condition but rather a combination of VOCs (15,22).

Exhaled breath VOC analysers

GC and mass spectrometry (GC-MS)

Because of the low concentrations of VOCs (parts per billion, ppb) (14,16) in exhaled breath, sensitive and highly accurate GCs and mass spectrometers have been utilized. *Table 2* summarizes some of the published results of GC/mass spectrometric analysis of VOCs in exhaled breath. GCs and mass spectrometers have limited application in a clinical setting because of their expense, difficulty of use, and the need for highly experienced analysts to operate them and interpret the results.

Portable/inexpensive devices

Several technologies, more portable and relatively inexpensive, have been developed and adapted to analyze exhaled breath samples. These include ion mobility spectrometers, and electronic nose instruments such as the Cyranose 320, the quartz microbalance, colorimeters, and gold particle nanosensors. *Table 3* summarises published sensitivity and specificity results of electronic nose devices in the analysis of VOCs in exhaled breath of control and lung cancer subjects.

Ion mobility spectrometry (IMS)

The principle of the IMS system is a 550 MBq ^{63}Ni β-radiation ionising source (Ni) which breaks down analytes from exhaled breath into ions. The ions separate and travel down a chamber at speeds that are related to their size, mass and geometry hitting a Faraday plate at the end of the chamber. As each ion hits the plate an electrical signal is generated which, when combined, produce an ion spectrum which is a fingerprint for the exhaled breath. Westhoff *et al.* (31) in 2009 was able to discriminate between 32 patients with lung cancer and 54 healthy subjects including both non-smokers and smokers in the group with 100% accuracy.

Electronic noses

Advances in technology have produced small, portable

Table 1 VOCs detected in lung diseases

Author	Disease	Significant VOCs identified
Phillips 2003 (13)	Lung Cancer	butane; 3-methyl tridecane; 7-methyl tridecane; 4-methyl octane; 3-methyl hexane; heptane; 2-methyl hexane, pentane; 5-methyl decane
Machado 2005 (14)	Lung Cancer	isobutane; methanol; ethanol; acetone; pentane; isoprene; isopropanol; dimethylsulfide; carbon disulfide; benzene; toluene
Poli 2005 (15)	Lung Cancer (NSCLC)	2-methyl pentane; pentane; ethyl benzene; xylenes (total); trimethyl benzene; toluene; benzene; decane; octane; penta methyl heptane
Barker 2006 (16)	Cystic Fibrosis	ethane; propane; pentane*#; methanol$^{\theta}$; ethanol; 2-propanol#; acetone; isoprene$^{\theta}$; benzene; toluene; dimethyl sulfide#$^{\theta}$; limonene
Dragonieri 2007 (17)	Asthma	4 methyl octane; 2,4-dimethyl heptane; isopropanol; toluene; isoprene; alkane; acetic acid; acetone; 2,6,11-trimethyl dodecane; 3,7-dimethyl undecane; 2,3-dimethyl heptane
Chen 2007 (18)	Lung cancer	styrene; decane; isoprene; benzene; undecane; 1-hexene; hexanol; propyl benzene; 1,2,4-trimethyl benzene; heptanal; methyl cyclopentane
Peng 2010 (19)	Lung, breast, colon, prostate cancer	16 compounds identified that varied in abundance between healthy groups and cancer groups-1-methyl-4-(1-methyl)benzene; toluene; dodecane; 3,3-dimethyl pentane; 2,3,4-trimethyl hexane; 1,1'-(1-butenylidene) bis benzene; 1,3-dimethyl benzene;1-iodo nonane; (1,1-dimethylethyl thio) acetic acid; 4-(4-propylcyclohexyl)-4'-cyano[1,1'-biphenyl]4-yl ester benzoic acid; 2 amino-5-isopropyl-8-methyl-1-azulenecarbonitrile; 5-(2-methylpropyl) nonane; 2,3,4-trimethyl decane; 6-ethyl-3-octyl ester 2 trifluromethyl benzoic acid; p-xylene; and 2,2-dimethyldecane
Fuchs 2010 (20)	Lung cancer	Aldehydes-butanal; formaldehyde; acetaldehyde; pentanal; hexanal; octanal; nonanal
Wang 2012 (21)	Lung cancer	Adenocarcinoma-2,4,6-trimethyloctane; 2-methyldodecane; 2-tridecanone; 2-pentadecanone; 8-methy lheptadecane; 2-heptadecanone; nonadecane; eicosane; squamous-methanoic acid; 2-nonanone; 2-pentadecanone; nonadecane; eicosane; SCC-2-decanone; 2-hendecanone; 2-methylnaphthaline; 2-tridecanone; 2-pentadecanone; 2,6-dimethylnaphthaline; 1- heptadecanol; 2-heptadecanone; nonadecane; eicosane

VOCs, volatile organic compounds. #sig diff from control group; *difference with antibiotics, body mass, Pseudomonas infection; $^{\theta}$difference with diabetics.

array type devices to detect and identify chemicals in gaseous samples. They are designed to respond to the mix of compounds in the sample rather than identify individual compounds. The principle behind the devices is that the VOCs adsorb onto a sensor producing a change in conductivity, color or oscillation of a crystal. Output is usually a pattern representing the mix of VOCs.

Quartz microbalance

The quartz microbalance is an 8 sensor array of oscillating quartz crystals coated with varied metalloporphyrins to which VOCs adsorb, changing the mass of the sensors and their oscillation frequency. The change in the oscillation frequency is recorded for each sensor.

Di Natale *et al.* (32) used the quartz microbalance to demonstrate a 90.3% accuracy in discriminating between subjects with lung cancer (n=42), healthy volunteers (n=18) and post-surgery lung cancer patients (n=9, two tested pre and post-surgery). All lung cancer subjects were correctly identified, and overlap was reported between the healthy control group and the post-surgery group. In 2009 D'Amico *et al.* (29) demonstrated 85% sensitivity and 100% specificity in discriminating lung cancer from healthy non-smokers and 93% sensitivity and 79% specificity when compared to subjects with other lung diseases.

Cyranose 320

The Cyranose 320 is a portable analyzer with 32 built-in carbon-black polymer composite chemiresistors in an array format. The polymer matrix adsorbs VOCs in exhaled breath, and swells causing an increase in electrical resistance. Each polymer chemoresistor has different properties which

Table 2 Summary of gas chromatograph/mass spectrometry studies on VOCs in exhaled breath

Author	Analysis method	Patient numbers	Results
Gordon 1985 (23)	GC-MS	Lung cancer 12; controls 17	Acetone; methyl ethyl ketone; n-propanol major difference between cancer and controls
Phillips 2003 (13)	GC-MS	Primary lung cancer 67; metastatic lung cancer 15; no evidence of lung cancer 91; healthy volunteers 41	Sensitivity 90% (60/67), specificity 83% (34/41); Cross validation using one-out-jacknife procedure yielded sensitivity 85% (57/67) and specificity 81% (33/41); In patients with metastatic cancer-sensitivity =67% (10/15); smokers/ex-smokers did not affect sensitivity histology and TNM staging showed no affects
Poli 2005 (15)	GC-MS Biovac sampler	NSCLC (pre + post-surgery) 36 (24); healthy smoker35; healthy non-smoker 50; COPD (mild to moderate) 25	NSCLC vs. COPD-increased 2 methyl pentane + isoprene; NSCLC vs. control smokers-decreased benzene, heptane, toluene; control vs. COPD-decreased 2 methyl pentane, benzene, and toluene and increased styrene; control smokers-increased in almost all VOCs; NSCLC vs. COPD-sig diff in isoprene, 2 methyl pentane, ethyl benzene; styrene smokers vs. COPD-sig diff; NSCLC vs. controls-sig diff in most VOCs; only isoprene and decane decreased post-surgery
Barker 2006 (16)	GC-MS	CF (stable) 15; CF (in hospital IV) 5; controls 20	CF: increased pentane output, decreased dimethyl sulphide (DMS) prod, increased 2-propanol uptake; Δ pentane higher in IV vs. stable, malnutrition, or pseudomonas, DMS lower in CF. Diabetic showed increased isoprene, less DMS and methanol, FEV1%Pred correlated with toluene
Phillips 2007 (22)	GC-MS	Lung cancer 193 (128 pred set, 65 test set); control 211 (141 pred set, 70 test set); post-surgery 80	Prediction of lung cancer: sensitivity 85%, specificity 80%, NS diff between stages; Test module: +ve for primary lung cancer (45/45)
Chen 2007 (18)	Solid phase microextraction (SPME), GC on cell culture and exhaled breath	Lung cancer 29; control 13; chronic bronchitis 7	Lung cancer sensitivity 86%; Control specificity 69%, chronic bronchitis specificity 71%, PPV 80.6 and NPV 78%
Bajtarevic 2009 (25)	PTR-MS + SPME GC-MS	Lung cancer 220 (68 smokers/ 129 ex-smokers/ 23 never smokers); healthy volunteers 441 (84 smokers /86 ex-smokers/ 221 never smokers)	Isoprene, acetone, methanol lower in lung cancer compared to healthy controls (PTR-MS), 100% specificity, Sensitivity Set A: 52% for 2-butanone; benzaldehyde. 2,3-butanedione; 1-propanol. Add. compounds for set B: Sensitivity =71% 3-hydroxy- 2-butanone; 3-butyn-2-ol; 2-methyl-butane; 2-methyl-2-butene; acetophenone; 1-cyclopentene; methyl propyl sulphide; tetramethyl-urea; n-pentanal; 1-methyl-1,3-cyclopentadiene; 2,3-dimethyl-2-butanol. Add. compounds for set C: sensitivity = 80% 1,2,3,4-tetrahydro-isoquinoline; 3,7-dimethyl-undecane; cyclobutyl-benzene; butyl acetate; ethylenimine; n-undecane (80% sensitivity)
Fuchs 2010 (20)	SPME + GC-MS	Lung cancer 12; healthy smokers 12; healthy never smokers 12	Pentanal; hexanal; octanal and nonanal conc higher in lung cancer than controls; NS diff between SCLC and NSCLC; hexanal higher in SCLC than NSCLC; pentanal—sensitivity 75%, specificity 95.8%

Table 2 (*continued*)

Table 2 (*continued*)

Author	Analysis method	Patient numbers	Results
Wang 2012 (21)	SPME, GC on cell culture and exhaled breath	Lung cancer 85; lung benign disease 70 (including pulmonitis, pulmonary tuberculosis, asthma and so on); healthy people 88	Sig diff with AUC >0.6 and P<0.01 in 8-hexylpentadecane; 3,7-dimethylpentadecane; 8-methylheptadecane; 2-pentadecanone; 5-(1-methyl-)propylnonane between adenocarcinoma and squamous. 96.5% of lung cancer patients were correctly classified with lung cancer; 34.3% benign incorrectly classified as healthy; 33.3% late stage lung cancer classified as early stage lung cancer
Peled 2012 (26)	SPME + GC-MS	72 subjects with pulmonary nodules–19 benign + 53 cancer	1-octene sig diff (P=0.0486) between benign and cancer. No sig diff between early and late stage disease and histology subtypes

GC-MS, gas chromatograph and mass spectrometry; SPME, solid phase microextraction; PTR-MS, proton transfer reaction mass spectrometry; NSCLC, non-small cell lung carcinoma; SCLC, small cell lung cancer; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; FEV1 % Pred, forced expiratory volume in one second, percent predicted; NS diff, no significant difference; Sig difference, significant difference; +ve, positive; pred, predicted; AUC, Area Under the Curve (AUC) of the receiver operating characteristic curve (ROC).

Table 3 Electronic nose results

Author	Analysis method	Lung cancer subjects (n)	Control subjects (n)	Sensitivity %	Specificity %
Machado 2005 (14)	Cyranose 320	14	19 alpha-1-antitrypsin deficiency, 6 chronic pulmonary beryllium disease, 20 healthy	71.4	91.9
Mazzone 2007 (27)	Colorimetric	49 NSCLC	18 COPD, 15 IPF, 20 PAH, 20 sarcoidosis, 21 healthy controls	73.3	72.4
Dragonieri 2008 (28)	Cyranose 320	10	10 COPD, 10 healthy		
Peng 2010 (19)	Nanosensor array with gold nano-particles	30	26 colon cancer, 22 breast cancer, 18 prostate, 22 healthy controls		
D'Amico 2010 (29)	Quartz Microbalance	28	36 control, 29 other lung diseases	85, 92.8	100, 78.6
Mazzone 2012 (30)	Colorimetric	92 NSCLC	67 lung cancer screening group, 70 indeterminate nodules (mean diameter 11 mm)	70	86
Peled 2012 (26)	Nanosensor array with single wall carbon nanotubes + gold nano-particles	53 malignant nodules	19 benign nodules	86	96

NSCLC, non-small cell lung carcinoma; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; PAH, pulmonary arterial hypertension.

absorb VOCs to varying degrees producing a differential response across the array. The combined results from the sensors produce a sample 'smellprint'.

The statistical algorithms for the analysis of patterns include Principal Component Analysis (PCA) to reduce the initial data set from the 32 sensors to a set of 4 principal components that capture the greatest variance of the data. Canonical Discriminate Analysis (CDA) using the factors that demonstrated a significant difference between the patients groups is used to create a model that maximizes the ratio of between-group distance to within-group distance. Wilks' lambda with a P value <0.05 is utilized to demonstrate a statistically significant difference between patient groups. The distance between group means is quantified with a number called Mahalanobis distance (M-distance). M-distance >3 indicates a high probability of difference between groups (P<0.1). Accuracy or capacity of the prediction model to completely distinguish all members of a clinical group is assessed using the "leave one-out method". The cross validated value (CVV) is the percentage of participants that were correctly classified to a particular participant group.

Machado *et al.* (14) demonstrated a difference between the exhaled breath VOC profile of a lung cancer group (n=14) and a control group (n=20) with M-distance of 3.25 and cross validated accuracy (CVA) of 71.6%. However there was no difference between stages or between histopathology subtypes of the lung cancer. When the model created from the training set was validated with a new group of lung cancer subjects (n=14) and control subjects (n=62) the electronic nose was able to discriminate between the two groups with sensitivity 71.4% and specificity 91.9%.

In 2008 Dragonieri *et al.* (28) also found that it was possible to use the Cyranose 320 to distinguish patients with lung cancer (n=10) and COPD (n=10) with accuracy of 85% (M-distance 3.73), and healthy control subjects (n=10), 90%, (M-distance 2.96).

Colorimetry

The colorimetric analyzer has dots impregnated with chemically sensitive compounds, e.g., metalloporphyrins on a cartridge. Each dot is sensitive to a broad range of VOCs but with varying sensitivity. Adsorption of VOCs to the dots causes them to change color. The cartridges are scanned before and after exposure to the sample, and the change in color of the spots is measured and converted to a number.

Two studies published by Mazzone *et al.* (27,30) used a colorimetric analyzer to compare the exhaled breath VOCs of subjects with lung cancer and control groups. The first, in 2007, (27) described a model discriminating between VOCs of subjects with lung cancer and control subjects [IPF, PAH, COPD, sarcoidosis, healthy subjects (smokers and non-smokers)] with an error rate of 14.1%. Using an independent validation set of subjects the sensitivity of the model in diagnosing lung cancer was 73.3% and specificity 72.4%. These results were not influenced by the patients' smoking history (P=0.87), histology (P=0.49), cancer stage (P=0.79) or size of the tumour (P=0.69). In 2012, Mazzone *et al.* (30) found that a combination of the breath profile and clinical risk factors including age, sex, smoking status and COPD improved the accuracy of the model in discriminating between patients with and without lung cancer [Area Under the Curve (AUC) of the receiver operating characteristic curve (ROC) 0.811]. The same model showed a difference between exhaled breath of subjects with squamous and adenocarcinomas (AUC =0.864), early (I and II) and late stage disease (III and IV) (AUC 0.784) and survival <12 months compared to survival >12 months (AUC 0.770).

Gold particle nanosensor

Peng *et al.* (19) developed a nanosensor array with 14 gold nanoparticle electrodes overlaid with a mixture of compounds including dodecanethiol, 4-methoxy-toluenethiol, hexanethiol, 11-mercapto-1-undecanol, decanethiol, octadecanethiol, tert-dodecanethiol, 1-butanethiol, 2-ethyl-hexanethiol, 3-methyl-1-butanethiol, 2-mercaptobenzoxazole, 11-mercapto-1-undecanol, 2-mercapto-benzyl alcohol, and 3-methyl-1-butanethiol. When exposed to a breath sample the 14 sensors undergo a reversible change in resistance and are analyzed using principal component and cluster analysis. The 2010 study demonstrated a difference between patterns of healthy subjects and patients with lung, colon and breast cancers, but an overlap was found with patients with prostate cancer. Distinct patient groups were identified in one plot when patient groups were analyzed together.

The study also investigated the VOC composition of exhaled breath for each patient group using a GC-MS. They identified a total of 16 VOCs that varied in concentration between healthy and patient groups; six for lung cancer, six for colon cancer, five for breast cancer, and four in prostate cancer. However, there was overlap in abundance of compounds for each of the cancer groups. Peled *et al.* (26) used a tailor-made chemical nanoarray of 18 cross-reactive sensors, 2 based

Table 4 Canine detection of cancer

Author	Sample Type	Cancer type	Cancer subjects (n)	Control subjects (n)	Sensitivity %	Specificity %
Williams 1989 (33)	Tissue	Melanoma	1	0		
Church 2001 (34)	Tissue	Basal cell carcinoma	1	0		
Willis 2004 (35)	Urine	Bladder	9	54	41% detection rate	
Pickel 2004 (36)	Body	Malignant melanoma	7	0	82	100
McCulloch 2006 (5)	Breath	Lung and breast	55, 31	83	99, 88	99, 98
Horvath 2008 (37)	Tissue	Ovarian	20	5	100	98
Horvath 2010 (38)	Tissue plasma	Ovarian	40	200	100, 100	95, 98
Sonoda 2010 (39)	Breath	Colo-rectal	33	132	91	99
Ehmann 2011 (40)	Breath	Lung	60	160	71	93
Cornu 2011 (41)	Urine	Prostate	33	33	91	91
Buszewski 2012 (42)	Breath	Lung	29	44	82	82

on random networks of single-wall carbon nanotubes, and 16 based on spherical gold nanoparticles. The accuracy of the nanoarray in discriminating between malignant and benign disease was 88% with an AUC of 0.986. It was also able to discriminate between early and late disease (accuracy 88% AUC 0.961) and adenocarcinoma and squamous (accuracy 88% AUC 0.974).

Canine detection

Dogs have a highly developed sense of smell with a detection threshold at several parts per trillion. *Table 4* summarizes the findings of canine detection of cancer.

The premise that dogs may be able to detect cancer by smell was first described in 1989 by Williams (33) who reported the case of a patient's dog showing an interest in one mole (later identified as melanoma) but not others. In 2001, Church (34) also described the case of a dog sniffing a skin lesion later identified as a basal cell carcinoma. In each case the dogs showed no further interest after the lesions were excised.

The first published research study in 2004 by Willis *et al.* (35) demonstrated that dogs were able to be trained to detect bladder cancer (1 positive sample in 7) by smelling urine samples. Dogs were successful in 22 out 54 cases (41%) compared with 14% expected by chance. Sonoda *et al.* (39) trained a dog to detect colorectal cancer in exhaled breath of 33 patients with sensitivity and specificity of 91% and 99%. In 2010 Horvath *et al.* (38) demonstrated that dogs were able to detect ovarian cancer from tissue and plasma with sensitivity and specificity of 100% and 95%, and 100% and 98% respectively. The dogs were also able to detect early cancer and discriminate between ovarian and other gynaecological malignancies such as endometrial, cervical and vulvar carcinomas. The first study to use dogs to detect lung cancer was by McCulloch *et al.* (5) in 2006. He trained five dogs to identify exhaled breath samples of subjects with lung cancer and breast cancer. The sensitivity of the canine detection technique for biopsy-confirmed lung cancer (n=55) was 99%, with 99% specificity, while in breast cancer (n=31) the sensitivity was 88% and specificity 98%, with equal accuracy scored by all dogs. The results were different to those of Ehmann *et al.* (40) who showed that dogs were able to identify lung cancer with sensitivity 71% and specificity 93%. Buszewski *et al.* (42) compared the chromatographic VOC content of exhaled breath samples of lung cancer subjects (n=29) with canine recognition of lung cancer, and found positive correlations of r=0.85 and 0.97 for ethyl acetate and 2-pentanone while acetonitrile, propanal and 1-propanol were negatively correlated with the dogs' response to breath samples (r=–0.78, –0.87 and –0.98). He concluded that dogs are probably discriminating between breath samples based on a specific breath odour but it is still unknown what odour or mix of compounds dogs detect.

Although canine scent detection by trained dogs seemed relatively simple and inexpensive, apart from high quality studies performed (43), relatively few published data in general or lung cancer in particular have been reported.

EBC analysis

EBC consists of approximately 99% water vapor (44)

as well as a small fraction of respiratory airway lining fluid droplets (45). EBC collection is a simple, safe, non-invasive technique for investigating inflammation and oxidative stress. The EBC can be collected by the subject breathing through a tube inserted in either a metal tube cooled to 0 degrees (R Tube) or through a condenser (Ecoscreen). They breathe tidally for 10-20 minutes through the system and at the end of the time the condensate is collected and analyzed. Studies have shown elevated levels of inflammatory and oxidative stress biomarkers such as hydrogen peroxide, leukotrienes, isoprostanes, hydrogen ions, prostaglandins, and nitrogen oxides (46) in EBC of patients with asthma, COPD, bronchiectasis and cystic fibrosis (47). Studies by Carpagnano *et al.* (48-53) and Gessner *et al.* (54) demonstrated they were able to detect human DNA in EBC. A *p53* gene mutation (54) was found in 36% of NSCLC subjects while none of the control subjects showed the mutation. Carpagnano *et al.* (51) demonstrated that microsatellite alterations in EBC-DNA were more frequent in subjects with NSCLC (89%) compared with healthy control subjects (35%). A recent publication from Carpagnano *et al.* (48) demonstrated a significant difference (P<0.001) in EBC matrix metalloproteinase 9 (MMP-9) concentrations between NSCLC and control subjects with transudative pleural effusion (25 *vs.* 2.6 ng/mL). The authors also described positive correlations between MMP-9 concentration and pack years smoking history (r=0.8, P<0.0001) and stage of lung cancer (r=0.6, P<0.01). However there was no correlation with the histopathological type of lung cancer.

Methodological limitations

The main limitation of exhaled breath analysis is the lack of recommended guidelines in the sampling of exhaled breath. As seen in *Table 5* there have been many techniques used. The methods vary on whether the inhaled room air is filtered (14,19), or unfiltered (5,27-30,40); the period of tidal breathing prior to collecting breath samples (0-12 minutes); the technique used to collect the breath sample, e.g., tidal breath sample (27,29,30,40), vital capacity (5,14,28), or alveolar breath sample (19); and what system was used to collect the sample, one (14,27-30), two bag sampling system (19) or cylindrical polypropylene sampling tubes (5,40). There are also many variations on the statistical analysis. Finally, there has been no comparison between different equipment.

Applying VOCs analysis in lung cancer

Post lung cancer resection results

Phillips *et al.* (22) used GC-MS to analyze the exhaled breath of untreated lung cancer subjects and healthy controls from which he developed a mathematical model to discriminate between the two subject groups. The model was tested on 80 post-surgical patients and found that it was positive in 77/80 subjects indicating that the VOC profile was unchanged post-surgical resection. These findings suggested that the VOCs do not come from the tumor itself but rather from other tissues. Poli *et al.* (15) selected thirteen VOCs using GC-MS that discriminated between lung cancer subjects and control groups. He retested 26 subjects 15-30 days post-surgery and found that two of the thirteen previously identified VOCs, isoprene and decane, altered significantly. When retested at 1 month (n=21), Poli *et al.* (55) showed that isoprene continued to be significantly decreased from pre-surgery levels while at 3 years (n=10), 5 VOCs were significantly different; isoprene and benzene were decreased while pentane, toluene and ethylene benzene levels were increased. When compared to control subjects, post-surgical subjects at three years had no significant difference in levels of benzene, heptanes and octanes. These findings again suggest that the exhaled breath VOCs measured in lung cancer subjects are not produced by the tumor.

Validation studies

Machado (14), Phillips (22), and Mazzone (27) have performed validation studies using an independent group of subjects to test the model developed from their training set of subjects. See *Table 6* for summary of results. Machado *et al.* (14) tested the exhaled breath of lung cancer subjects, healthy control subjects and subjects with other respiratory conditions including asthma, COPD and pulmonary hypertension with the Cyranose 320 analyzer. He used support vector machine (SVM) analysis, a learning algorithm, to discriminate between exhaled breath samples of different subject groups. The developed model was accurate in 85% of the subjects. The analyzer had a sensitivity of 71.4%, a specificity of 91.9%, a positive predicted value (PPV) for lung cancer of 66.6% and a 93.4% negative predictive value (NPV). In 2007, Phillips (22) published a study using fuzzy logic to develop a model of breath biomarkers for lung cancer. He tested this model using GC-MS on the exhaled breath of 135 subjects, 65 lung cancer subjects and 70 healthy control subjects.

Table 5 Summary of methodological and statistical differences between different techniques of VOC analysis

Author	Analysis method	Filtered/ unfiltered room air	Tidal breathing time prior to sample collection (minutes)	Breath collection tidal/ VC/ alveolar	Sample collection system	Statistics
Machado 2005 (14)	Cyranose 320	Filtered	0	1 EVC	1 bag	PCA, CDA, SVM
McCulloch 2006 (5)	Canine	Unfiltered	0	3-5 full exhalations	Cylindrical polypropylene organic vapor sampling tubes	Sensitivity/specificity Fisher 2-sided exact test
Mazzone 2007 (27)	Colorimetric	Unfiltered	12	Tidal breath sample	1 bag	Random forest method
Dragonieri 2008 (28)	Cyranose 320	Unfiltered	5	1 EVC	1 bag	PCA, CDA
Peng 2010 (19)	Nano-sensor array with gold nanoparticles	Filtered	3-5 min TLC breathing	Alveolar	2 bags	PCA
D'Amico 2010 (29)	Quartz Micro-balance	2 bag system collecting end-tidal breath	Deep breaths to fill 4 L bag	Tidal	1 bag	Partial least squares discriminate analysis
Mazzone 2012 (30)	Colorimetric	Unfiltered	5	Tidal breathing	1 bag	Logistic regression model with backwards step down variable selection
Ehmann 2012 (40)	Canine	Unfiltered	5 full exhaled breaths	Tidal	Cylindrical glass tubes with polyproy-lene fleece and silicone oil	Fishers exact test, Wilcoxon's test, Kruskal-Wallis, Holm's method
Peled 2012 (26)	Nano-sensor array with single wall carbon nanotubes + gold nano-particles	Filtered	3-5 min TLC breathing	Alveolar	2 bags	Wilcoxon test, discriminate factor analysis, leave one out cross validation

VC, vital capacity; EVC, exhaled vital capacity; TLC, total lung capacity; L, litre; min, minute; PCA, principal component analysis; CDA, canonical discriminant analysis; SVM, support vector machine analysis.

Table 6 Validation of developed test models

Author	Device	Sensitivity (%)	Specificity (%)	PPV	NPV	AUC	Accuracy (%)
Machado(14)	Cyranose	71.4	91.9	66.6	93.4		85
Phillips (22)	GC-MS	84.6	80.0			0.88	
Mazzone (27)	Colorimetric	73.3	72.4				

PPV, positive predictive value; NPV, negative predictive value; AUC, Area Under the Curve (AUC) of the receiver operating characteristic curve (ROC).

It had 84.6% sensitivity, 80% specificity and 0.88 AUC. Mazzone (27) when using the colorimetric analyzer produced lower sensitivity and specificity values (73.3%, 72.4%) when testing his model on an independent group of subjects with lung cancer, sarcoidosis, IPF, PAH, COPD and healthy subjects. However, the sensitivity was 100% and specificity 60% when a group of subjects (n=29) with indeterminate pulmonary nodules <30 mm was tested.

Factors which may confound VOC analysis results

Age

Using GC-MS Phillips *et al.* in 2000 (56) investigated the effect of age on alkane contours in 102 normal subjects. He found that there was a statistical significant difference between age groups 9-31 and 46-89. This was different to Dragonieri *et al.* (17), Mazzone *et al.* (27), and Wehinger *et al's.* (57) findings who used Cyranose 320, colorimetry and proton transfer reaction MS. Dragonieri *et al.* demonstrated that there was no significant difference in smellprints between age groups <45 and >45 years in 20 normal subjects. Mazzone *et al.* also demonstrated no difference in results (27) with age (P=0.96). Wehinger *et al.* using PTR-MS for VOC 31 (believed to be formaldehyde) and 43 (believed to be isopropanol) showed no differences with age. Peng *et al.* (19) again found no difference with age using the gold particle nanosensor.

Airway calibre

Lazar *et al.* (58) found with the Cyranose 320 that the exhaled breath profile in 10 asthmatics was altered by nebulisation of methacholine and isotonic saline but was not altered by the airway calibre.

Smoking

Gordon *et al.* (59) demonstrated with GC-MS in 5 smokers and 5 non-smokers that cigarette smoke affected the volatile organic composition of their exhaled breath. However, the level of the measured VOCs returned to an approximate baseline after 15 minutes. As part of a study of lung cancer and healthy controls using the Cyranose 320, the exhaled breath profiles of current and non-smokers in both healthy subjects and those with disease were found by Machado *et al.* (14) not to be different and he concluded that the difference between the subject groups was most likely due to the disease process and not to smoking. This was supported by Mazzone *et al.* (27) whose colorimetry results were not affected by the patients' smoking history, (current, former or non-smoker) (P=0.87). Peng *et al.* (19) also found that the subjects' smoking habits did not affect the results using the gold particle nanosensor. Phillips *et al.* (22,60) used GC-MS to analyze the exhaled breath of subjects with lung cancer and subjects with a smoking history and a negative CT for lung cancer and developed a model to discriminate between the two patient groups. The accuracy of the model was tested on an independent group of subjects. On evaluating the ROC curves for current and former smokers he found no difference between the two groups. Fens *et al.* (61) findings in a COPD and asthma study using the Cyranose 320 also showed no difference in breathprints between current and ex-smokers (P=0.16).

Lung function severity

Machado *et al.* (14) used the Cyranose 320 in his breath analysis and indicated that the severity of lung dysfunction did not affect the clustering of samples.

Gender

Several studies have showed that gender has no effect on the profile (19,27,57).

Conclusions

This review has demonstrated a consistent association between patterns of VOCs in exhaled breath, and genetic markers in EBC, and the presence of lung cancer.

Historically, canine detection of lung cancer was reported to be highly sensitive and specific but it still requires further validation and replication in larger trials to establish its accuracy. Studies examining exhaled breath using GC and mass spectrometers have identified individual chemical compounds associated with lung cancer and confirmed that there is not one VOC but rather a combination of VOCs that are either increased or decreased in concentration. These techniques have limited applicability in the clinical setting because of their expense, difficulty of use, and the need for highly experienced analysts to operate and interpret the results. Electronic noses and related instruments are simpler, cheaper and easier to use, facilitating their utilization in the clinical setting. These instruments employ different technologies to identify VOC patterns. Studies

using a variety of these instruments, sampling techniques, and different statistical analyses have consistently discriminated between groups of patients with lung cancer and control subjects. No combination of the instruments, methodologies or statistical analysis has yet been shown to reliably predict which patients in an at risk population are likely to have lung cancer. There will obviously need to be some consensus regarding the most appropriate instruments, collection techniques and statistical methods to optimise the accuracy of the identification of at risk patients for lung cancer. Further validation of the models developed to discriminate between lung cancer and control groups will also be required to determine the sensitivity and specificity of the techniques. With these limitations, exhaled breath analysis does hold great promise because of its simplicity and low cost as a new screening and diagnostic technique in lung cancer.

EBC, a technique which can allow quantification of genetic markers, also shows promise, but does not at this stage have an established place in the screening of lung cancer primarily because it requires a sophisticated genetics laboratory to analyze the samples.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Center MM, DeSantis C, et al. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev 2010;19:1893-907.
2. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
3. Mountain CF. Revisions in the international system for staging lung cancer. Chest 1997;111:1710-7.
4. Buszewski B, Kesy M, Ligor T, et al. Human exhaled air analytics: biomarkers of diseases. Biomed Chromatogr 2007;21:553-66.
5. McCulloch M, Jezierski T, Broffman M, et al. Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. Integr Cancer Ther 2006;5:30-9.
6. Pauling L, Robinson AB, Teranishi R, et al. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. Proc Natl Acad Sci U S A 1971;68:2374-6.
7. Phillips M. Method for the collection and assay of volatile organic compounds in breath. Anal Biochem 1997;247:272-8.
8. Phillips M, Gleeson K, Hughes JM, et al. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. Lancet 1999;353:1930-3.
9. Mazzone PJ. Analysis of volatile organic compounds in the exhaled breath for the diagnosis of lung cancer. J Thorac Oncol 2008;3:774-80.
10. Stone BG, Besse TJ, Duane WC, et al. Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men. Lipids 1993;28:705-8.
11. Kneepkens CM, Lepage G, Roy CC. The potential of the hydrocarbon breath test as a measure of lipid peroxidation. Free Radic Biol Med 1994;17:127-60.
12. Phillips M, Herrera J, Krishnan S, et al. Variation in volatile organic compounds in the breath of normal humans. J Chromatogr B Biomed Sci Appl 1999;729:75-88.
13. Phillips M, Cataneo RN, Cummin AR, et al. Detection of lung cancer with volatile markers in the breath. Chest 2003;123:2115-23.
14. Machado RF, Laskowski D, Deffenderfer O, et al. Detection of lung cancer by sensor array analyses of exhaled breath. Am J Respir Crit Care Med 2005;171:1286-91.
15. Poli D, Carbognani P, Corradi M, et al. Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-term follow-up study. Respir Res 2005;6:71.
16. Barker M, Hengst M, Schmid J, et al. Volatile organic compounds in the exhaled breath of young patients with cystic fibrosis. Eur Respir J 2006;27:929-36.
17. Dragonieri S, Schot R, Mertens BJ, et al. An electronic nose in the discrimination of patients with asthma and controls. J Allergy Clin Immunol 2007;120:856-62.
18. Chen X, Xu F, Wang Y, et al. A study of the volatile organic compounds exhaled by lung cancer cells in vitro for breath diagnosis. Cancer 2007;110:835-44.
19. Peng G, Hakim M, Broza YY, et al. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. Br J Cancer 2010;103:542-51.
20. Fuchs P, Loeseken C, Schubert JK, et al. Breath gas aldehydes as biomarkers of lung cancer. Int J Cancer 2010;126:2663-70.
21. Wang Y, Hu Y, Wang D, et al. The analysis of volatile organic compounds biomarkers for lung cancer in exhaled breath, tissues and cell lines. Cancer Biomark 2012;11:129-37.
22. Phillips M, Altorki N, Austin JH, et al. Prediction of lung cancer using volatile biomarkers in breath. Cancer Biomark 2007;3:95-109.

23. Gordon SM, Szidon JP, Krotoszynski BK, et al. Volatile organic compounds in exhaled air from patients with lung cancer. Clin Chem 1985;31:1278-82.
24. Ellis JR, Gleeson FV. Lung cancer screening. Br J Radiol 2001;74:478-85.
25. Bajtarevic A, Ager C, Pienz M, et al. Noninvasive detection of lung cancer by analysis of exhaled breath. BMC Cancer 2009;9:348.
26. Peled N, Hakim M, Bunn PA Jr, et al. Non-invasive breath analysis of pulmonary nodules. J Thorac Oncol 2012;7:1528-33.
27. Mazzone PJ, Hammel J, Dweik R, et al. Diagnosis of lung cancer by the analysis of exhaled breath with a colorimetric sensor array. Thorax 2007;62:565-8.
28. Dragonieri S, Annema JT, Schot R, et al. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. Lung Cancer 2009;64:166-70.
29. D'Amico A, Pennazza G, Santonico M. et al. An investigation on electronic nose diagnosis of lung cancer. Lung Cancer 2010;68:170-6.
30. Mazzone PJ, Wang XF, Xu Y, et al. Exhaled breath analysis with a colorimetric sensor array for the identification and characterization of lung cancer. J Thorac Oncol 2012;7:137-42.
31. Westhoff M, Litterst P, Freitag L, et al. Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. Thorax 2009;64:744-8.
32. Di Natale C, Macagnano A, Martinelli E, et al. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. Biosens Bioelectron 2003;18:1209-18.
33. Williams H, Pembroke A. Sniffer dogs in the melanoma clinic? Lancet 1989;1:734.
34. Church J, Williams H. Another sniffer dog for the clinic? Lancet 2001;358:930.
35. Willis CM, Church SM, Guest CM, et al. Olfactory detection of human bladder cancer by dogs: proof of principle study. BMJ 2004;329:712.
36. Pickel D. Evidence for canine olfactory detection of melanoma. Appl Anim Behav Sci 2004;89:107-16.
37. Horvath G, Jarverud GA, Jarverud S, et al. Human ovarian carcinomas detected by specific odor. Integr Cancer Ther 2008;7:76-80.
38. Horvath G, Andersson H, Paulsson G. Characteristic odour in the blood reveals ovarian carcinoma. BMC Cancer 2010;10:643.
39. Sonoda H, Kohnoe S, Yamazato T, et al. Colorectal cancer screening with odour material by canine scent detection. Gut 2011;60:814-9.
40. Ehmann R, Boedeker E, Friedrich U, et al. Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. Eur Respir J 2012;39:669-76.
41. Cornu JN, Cancel-Tassin G, Ondet V, et al. Olfactory detection of prostate cancer by dogs sniffing urine: a step forward in early diagnosis. Eur Urol 2011;59:197-201.
42. Buszewski B, Ligor T, Jezierski T, et al. Identification of volatile lung cancer markers by gas chromatography-mass spectrometry: comparison with discrimination by canines. Anal Bioanal Chem 2012;404:141-6.
43. McCulloch M, Turner K, Broffman M. Lung cancer detection by canine scent: will there be a lab in the lab? Eur Respir J 2012;39:511-2.
44. Effros RM, Hoagland KW, Bosbous M, et al. Dilution of respiratory solutes in exhaled condensates. Am J Respir Crit Care Med 2002;165:663-9.
45. Effros RM, Dunning MB 3rd, Biller J, et al. The promise and perils of exhaled breath condensates. Am J Physiol Lung Cell Mol Physiol 2004;287:L1073-80.
46. Montuschi P. Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications. Ther Adv Respir Dis 2007;1:5-23.
47. Grob NM, Aytekin M, Dweik RA. Biomarkers in exhaled breath condensate: a review of collection, processing and analysis. J Breath Res 2008;2:037004.
48. Carpagnano GE, Costantino E, Palladino GP, et al. Microsatellite alterations and cell-free DNA analysis: could they increase the cytology sensitivity in the diagnosis of malignant pleural effusion? Rejuvenation Res 2012;15:265-73.
49. Carpagnano GE, Foschino-Barbaro MP, Mule G, et al. 3p microsatellite alterations in exhaled breath condensate from patients with non-small cell lung cancer. Am J Respir Crit Care Med 2005;172:738-44.
50. Carpagnano GE, Foschino-Barbaro MP, Resta O, et al. Endothelin-1 is increased in the breath condensate of patients with non-small-cell lung cancer. Oncology 2004;66:180-4.
51. Carpagnano GE, Foschino-Barbaro MP, Spanevello A, et al. 3p microsatellite signature in exhaled breath condensate and tumor tissue of patients with lung cancer. Am J Respir Crit Care Med 2008;177:337-41.
52. Carpagnano GE, Palladino GP, Martinelli D, et al. Exhaled matrix metalloproteinase-9 in lung cancer. Rejuvenation Res 2012;15:359-65.
53. Carpagnano GE, Spanevello A, Carpagnano F, et al. Prognostic value of exhaled microsatellite alterations at 3p

in NSCLC patients. Lung Cancer 2009;64:334-40.
54. Gessner C, Kuhn H, Toepfer K, et al. Detection of p53 gene mutations in exhaled breath condensate of non-small cell lung cancer patients. Lung Cancer 2004;43:215-22.
55. Poli D, Goldoni M, Caglieri A, et al. Breath analysis in non small cell lung cancer patients after surgical tumour resection. Acta Biomed 2008;79 Suppl 1:64-72.
56. Phillips M, Cataneo RN, Greenberg J, et al. Effect of age on the breath methylated alkane contour, a display of apparent new markers of oxidative stress. J Lab Clin Med 2000;136:243-9.
57. Wehinger A. Lung cancer detection by proton transfer reaction mass-spectrometric analysis of human breath gas. Int J Mass Spectrom 2007;265:49-59.
58. Lazar Z, Fens N, van der Maten J, et al. Electronic nose breathprints are independent of acute changes in airway caliber in asthma. Sensors (Basel) 2010;10:9127-38.
59. Gordon SM, Wallace LA, Brinkman MC, et al. Volatile organic compounds as breath biomarkers for active and passive smoking. Environ Health Perspect 2002;110:689-98.
60. Phillips M, Altorki N, Austin JH, et al. Detection of lung cancer using weighted digital analysis of breath biomarkers. Clin Chim Acta 2008;393:76-84.
61. Fens N, Zwinderman AH, van der Schee MP, et al. Exhaled breath profiling enables discrimination of chronic obstructive pulmonary disease and asthma. Am J Respir Crit Care Med 2009;180:1076-82.

Cite this article as: Dent AG, Sutedja TG, Zimmerman PV. Exhaled breath analysis for lung cancer. J Thorac Dis 2013;5(S5):S540-S550. doi: 10.3978/j.issn.2072-1439.2013.08.44

Interventional pulmonology approaches in the diagnosis and treatment of early stage non small cell lung cancer

Ryu Peter Hambrook Tofts*, Peter M. J. Lee*, Arthur Wai Sung

Division of Pulmonary and Critical Care Medicine, Beth Israel Medical Center, New York, NY 10003, USA

*Drs Tofts and Lee contributed equally to the work of this manuscript.

Correspondence to: Arthur Wai Sung, M.D. Division of Pulmonary and Critical Care Medicine, Stanford University School of Medicine, 300 Pasteur Drive, H3143, Stanford, CA 94305, USA. Email: arthursung@aol.com.

Abstract: Lung cancer management is complex and requires a multi-disciplinary approach to provide comprehensive care. Interventional pulmonology (IP) is an evolving field that utilizes minimally invasive modalities for the initial diagnosis and staging of suspected lung cancers. Endobronchial ultrasound guided sampling of mediastinal lymph nodes for staging and detection of driver mutations is instrumental for prognosis and treatment of early and later stage lung cancers. Advances in navigational bronchoscopy allow for histological sampling of suspicious peripheral lesions with minimal complication rates, as well as assisting with fiducial marker placements for stereotactic radiation therapy. Furthermore, IP can also offer palliation for inoperable cancers and those with late stage diseases. As the trend towards early lung cancer detection with low dose computed tomography is developing, it is paramount for the pulmonary physician with expertise in lung nodule management, minimally invasive sampling and staging to integrate into the paradigm of multi-specialty care.

Keywords: Bronchoscopy; gene expression profiling; interdisciplinary communication; TNM staging; radial probe-EBUS (RP-EBUS); virtual bronchoscopic navigation (VBN); electromagnetic navigational bronchoscopy (ENB™)

Submitted Jun 19, 2013. Accepted for publication Sep 24, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.11
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.11

Background of interventional pulmonology (IP)

At the dawn of the 20th century, respiratory physicians largely provided medical care in sanatoriums tending to patients infected with tuberculosis. TB was the second leading cause of death in that era behind pneumonias and influenza infections (1,2). Advances in antimicrobials, including isoniazid and penicillin, led to significant improvement in outcomes. During the subsequent decades, tobacco smoking became widespread in the United States. It was not until the mid-20th century that lung cancer was strongly linked to smoking with epidemiological data (3). Since then, pulmonologists have managed the myriad of lung ailments consequent to tobacco addiction, such as emphysema. As lung cancer became the leading cause of cancer deaths in both men and women, the pulmonologist's role in lung cancer care has evolved. Smoking cessation remains the most important role a pulmonologist assumes to prevent lung cancers; as one fifth of U.S. population are smokers, and among the economically disadvantaged, the number increases to about forty percent (4). On the other hand, the management role of pulmonologists in lung cancer has also evolved from diagnosis of late stage lung cancers and risk stratification for surgery, to management of early stage disease with personalized approach in a multidisciplinary setting.

Interventional pulmonology concentrates on the use and development of diagnostic and therapeutic endobronchial techniques (5-7). The IP armatorium consists of (and is not limited to) rigid bronchoscopy, endobronchial laser

Table 1 Current interventional and advanced diagnostic modalities for managing malignant neoplasia of the lung

Diagnostic:
Endobronchial US
Radial probe
EBUS-TBNA
Narrow band imaging
Electromagnetic navigation
Navigational bronchoscopy
Confocal microendoscopy
Therapeutic:
Extrinsic compression:
Stent
Intrinsic obstruction:
Microdebridement
Rigid bronchoscopy coring
Argon plasma coagulation (APC)
Laser (Nd:YAG, CO_2)
Electrocautery
Cryotherapy
Photodynamic therapy (PDT)
HDR-brachytherapy
Peripheral tumor:
Fiducial marker placement to assist stereotactic body radiation therapy (SBRT) for inoperable patients with resectable stage I lung CA
Fiducial marker placement for localization for surgical managament

Abbreviations: EBUS, Endobronchial ultrasound; TBNA, Transbronchial needle aspiration; Nd:YAG, neodymium-doped yttrium aluminum garnet; CO_2, Carbon Dioxide

therapy, electrocautery, cryotherapy brachytherapy and endobronchial or tracheal stent placement and the advanced diagnostic techniques available to pulmonologists such as endobronchial ultrasound (EBUS) and navigational bronchoscopy (NB) (*Table 1*).

It is important to bear in mind that the field of IP depends on close-working and complementary relationships with the thoracic radiologist, radiation oncologist, and the thoracic surgeon as part of a multidisciplinary team. This review will cover diagnostic and therapeutic techniques that are being used in the management of early stage lung cancer.

Bronchoscopic early detection of malignancy

Lung cancer is the most lethal of solid tumors. Up to 85% are attributed largely to heavy smoking. Furthermore, despite smoking cessation many are still at risk for several years since their last cigarette (8). In the last decade evidence in favor of lung cancer screening with low-dose computed tomography (LDCT) has been shown to be superior to chest X-ray (CXR) (8).

Henscke *et al.* demonstrated in a prospective observational study of 31,567 asymptomatic patients, low dose CT screening resulted in the diagnosis of lung cancer in 484 patients, 85% of whom had Stage I disease, and who after treatment had a 10-year survival rate of 88% (95% CI, 88-95%) (9). The National Cancer Institute-Sponsored Lung Screening Trial that followed supported its findings. The NSLT was a randomized control trial in which 53,454 patients were randomized to three years of annual low dose CT screening versus plain chest X-ray (8). After three years the NSLT investigators had achieved their primary objective, which was a 20% relative reduction in mortality from lung cancer. The corresponding number needed to screen (NNS) to prevent 1 death after 1 year of screening is 320. Although questions remain about trial design, generalizability, applicability and cost-effectiveness of LDCT in the community, the goal of detecting early stage lung cancer with concomitant reduction in cancer specific mortality has become achievable.

As a result of NLST, the U.S. Preventive Services Task Force has recently updated its original 2004 recommendations regarding lung cancer screening. Its main recommendation, based on the NLST, provides LDCT screening to high risk individuals with at least a 30 pack-year smoking history between the ages of 55-79 years. The prospective efficacy of these recommendations will depend on the ability of the pulmonologist to meet the demands of accurate and timely diagnosis, proper risk stratifications, tissue sampling when appropriate, and familiarity thoracic oncologists' needs beyond tumor types, i.e., molecular profiling.

Early detection of malignancies arising from the central airways by bronchoscopy has also been evaluated over the past decade. Squamous cell carcinomas of the central airways have shown to develop through several stages from metaplasia, dysplasia, carcinoma in-situ (CIS) and advanced invasion (10). The cellular transformation of bronchial carcinomas has been described as a spectrum of lesions from basal layer hyperplasia, metaplasia, dysplasia, and CIS (11).

CIS is characterized by involvement of the entire epithelium with marked cytologic atypia. Bronchoscopic follow-up by autofluorescence bronchoscopy (AFB) and biopsy data among patients with high suspicion for lung cancer either from positive sputum cytology or prior upper respiratory cancers has shown that severe dysplastic lesions were more likely to progress towards CIS and further invasive cancer (12,13). Furthermore, Bota *et al.* showed 75% of CIS lesions, which persisted at 3 months required therapy (13). Currently the American College of Chest Physicians (ACCP) recommendations regarding known CIS and high-grade dysplastic lesions suggest performing follow-up white-light bronchoscopy (WLB) to rule out endobronchial lesions with use of AFB if available (14). However, relatively higher false-positive rates and suboptimal specificity of AFB, likely due to failure to distinguish inflammatory airways from dysplasia, limit its potential to preclude the need for unnecessary biopsies (15).

Narrow band imaging (NBI)

NBI utilizes narrow wavelengths of blue (400-430 & 420-470 nm; B1 & B2, respectively) and green (560-590 nm) light to enhance visualization of abnormal collections of submucosal capillaries (16,17). This strategy has been shown to be highly effective in differentiating normal mucosa from highly vascular precancerous lesions such as angiogenic squamous dysplasia (ASD). ASD is characterized by abnormal collections of microvessels projecting into dysplastic cells within the bronchial mucosa (18). NBI has shown improved sensitivity in detecting ASD not readily seen with WLB or AFB (19-21). Diagnostic yield was similar between NBI and AFB without any increased false-positives suggesting that NBI may serve as an alternative tool in early lung cancer detection. NBI's unique ability to detect early angiogenesis undetectable by AFB with high specificity (85-90%) and negative predictive value (>90%) has the potential for influencing therapeutic decision-making (22). Currently, the ACCP recommends NBI or AFB, when available, be used to delineate tumor margins in patients who are candidates for early lung cancer resection (23).

Lung cancer diagnosis and staging

The solitary pulmonary nodule (SPN)

The solitary pulmonary nodule, commonly detected incidentally, is among the top reasons for referral to a pulmonologist. Concurrently, as the work on early lung cancer screening evolves, it behooves the pulmonary specialist to become an expert in the management of the solitary lung nodule.

The current ACCP guidelines recommend, depending on patient and SPN features, observation, tissue biopsy and direct surgical excision. The role for pulmonologists for biopsies will be expanding with advancing bronchoscopic techniques. Currently, with more peripheral lesions where tissue biopsy is indicated, the guidelines recommend trans-thoracic needle biopsy (TTNB) as the preferred modality as the diagnostic yield is slightly below 90% (23-25). Traditional transbronchial biopsy with bronchoscopy has a diagnostic yield of only 14-63% (26). In 2012, Wang *et al.* published a meta-analysis of 39 pooled studies (n=patients >3,000) of all available guided-techniques (discussed below) that demonstrated a pooled diagnostic yield of 70% (25). While this is improved compared to standard bronchoscopy, it still remains below the diagnostic yield of TTNB. Concurrently, Wang reported a pneumothorax rate in TTNB of 25% (15% requiring a chest tube) versus less than 2% (less than 1% requiring a chest tube) in bronchoscopic techniques. The three main techniques used in this field of specialized bronchoscopy are radial probe-EBUS (RP-EBUS), virtual bronchoscopic navigation (VBN) and electromagnetic navigational bronchoscopy (ENB™) (5).

Radial probe EBUS (RP-EBUS)

RP-EBUS uses ultrasound to take samples sequentially. It allows excellent visualization of the bronchial walls and a tumor in situ. It is also the only technology that allows for real-time confirmation that the target lesion has been reached which translates to improved yield over conventional transbronchial biopsy with or without fluoroscopy (27-31). In a prospective cohort study of 131 patients, RP-EBUS demonstrated better sensitivity and accuracy (89% and 100%, respectively) at detecting bronchial wall invasion over CT (75% and 51%, respectively) (32). RP-EBUS uses a flexible bronchoscope to access the bronchiole closest to the nodule, and then a miniaturized radial probe and sheath are passed through the working channel until the nodule is visualized. The probe is removed leaving the sheath in position, then biopsy forceps is inserted through the guide sheath and the nodule sampled (*Figure 1*). Steinfort *et al.* showed in a meta-analysis of 13 studies and 1,090 patients, that RP-EBUS in SPNs

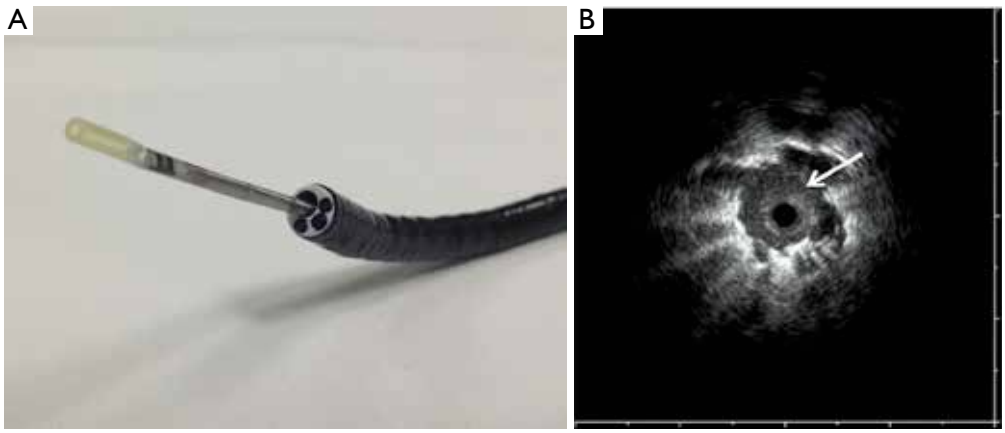


Figure 1 (A) Radial Probe 20-mHz Endobronchial Ultrasound fitted into a therapeutic bronchoscopy channel; (B) radial EBUS image of a peripheral lesion (arrow) (Courtesy Olympus Endoscopy USA).

had a point specificity of 1.00 (95% CI, 0.99-1.0) and point sensitivity of 0.73 (95% CI, 0.70-0.76) (33). Eberthardt *et al.* in a randomized control trial showed that RP-EBUS with a guide sheath alone had a diagnostic yield of 69% (34). This increased to 88% when combined with electromagnetic navigational bronchoscopy (ENB™); a diagnostic yield comparable to TTNB and SPNs with a mean diameter of 25 mm.

Electromagnetic navigational bronchoscopy (ENB™)

ENB™ is a technology developed for access to peripheral nodules beyond the reach of conventional bronchoscopes. Akin to global positioning systems (GPS), ENB™ is able to provide real-time orientation of a proprietary sensor probe by utilizing an electromagnetic field generated by a board underneath the patient (16,35). Pre-procedural planning involves importing the patient's CT data and assigning anatomical landmarks including the target lesion. The sensor probe (1 mm diameter × 8 mm long) is loaded into a flexible catheter, and then passed through the working channel of a standard bronchoscope. Guidance is provided by a matched virtual bronchoscopy image aside the real-time video bronchoscopy overlain with pre-determined pathway markers. Once the bronchoscope is wedged into the segment of interest the flexible catheter with the sensor probe is advanced until the target lesion is reached. At this point the sensor probe is retracted leaving the flexible catheter in place to act as an extended working channel (*Figure 2*). The diagnostic yield of ENB™ alone is reported to range from 59-74% (36-38). While early studies postulated that target lesion size might be significant, recently it is believed that CT-body divergence (a measure of image data registration accuracy) may determine navigational success (36,38). Other factors related to local anatomy and distance influence overall success (39,40). The presence of a bronchus sign significantly improved success to 79% in series of 51 patients by Seijo and colleagues (39).

Recently, a randomized-controlled trial by Asano and colleagues demonstrated virtual bronchoscopy navigational guidance (VBN, a computerized guidance system without electromagnetic correlation) with an ultrathin scope significantly improves diagnostic yield in the right upper lobe, peripheral third and lesions invisible on chest X-ray (41). However, the main limitation of this technique is the lack of real-time confirmation that a nodule has been reached. Addition of radial EBUS has shown to overcome this by increasing diagnostic yield to 88-93% (42).

Lung cancer staging with convex-EBUS (EBUS-TBNA)

Staging and confirmation of nodal status is central to the diagnosis and management of non-small cell lung cancer (NSCLC) (*Figure 3*). The treatment of choice of stage I and II disease is surgical resection in operable candidates, whereas combined chemo and radiotherapy is indicated for patients with Stage III disease and above (43). In academic settings, a multimodality approach can be considered for functional IIIA patients to undergo surgical resection after neoadjuvant therapy (23). Mediastinal nodal metastases are detected non-invasively with CT and/or positron emission

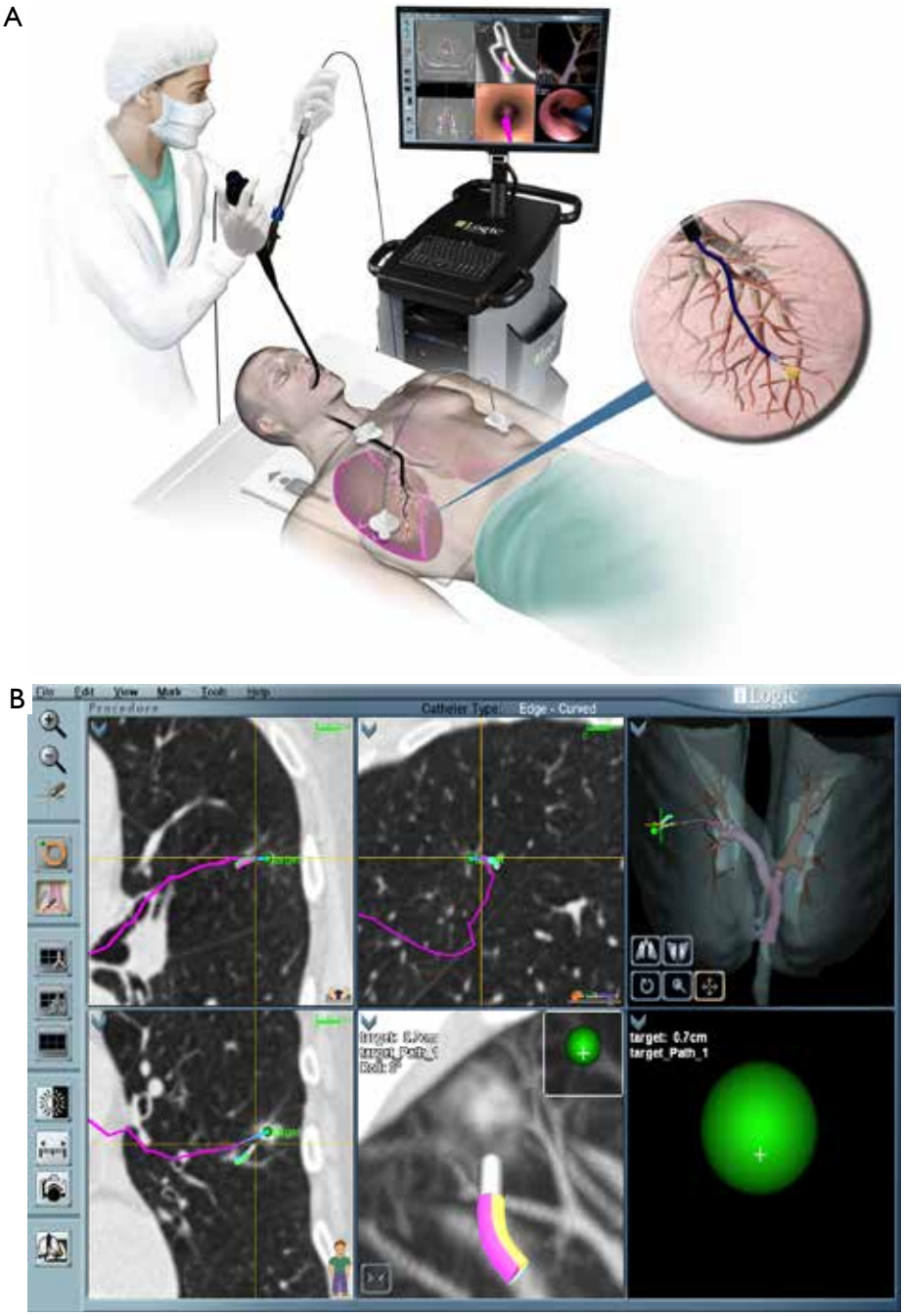


Figure 2 (A) Electromagnetic Navigation Bronchoscopy (reproduced with permission from SuperDimension® ENB™); (B) screen capture of a procedure in process. The route (pink line) to the lesion is predetermined by analysis of CT chest images. The target (green sphere) is represented and distance to target is continuously updated. (courtesy Joe Cicenia, M.D., Cleveland Clinic Foundation).

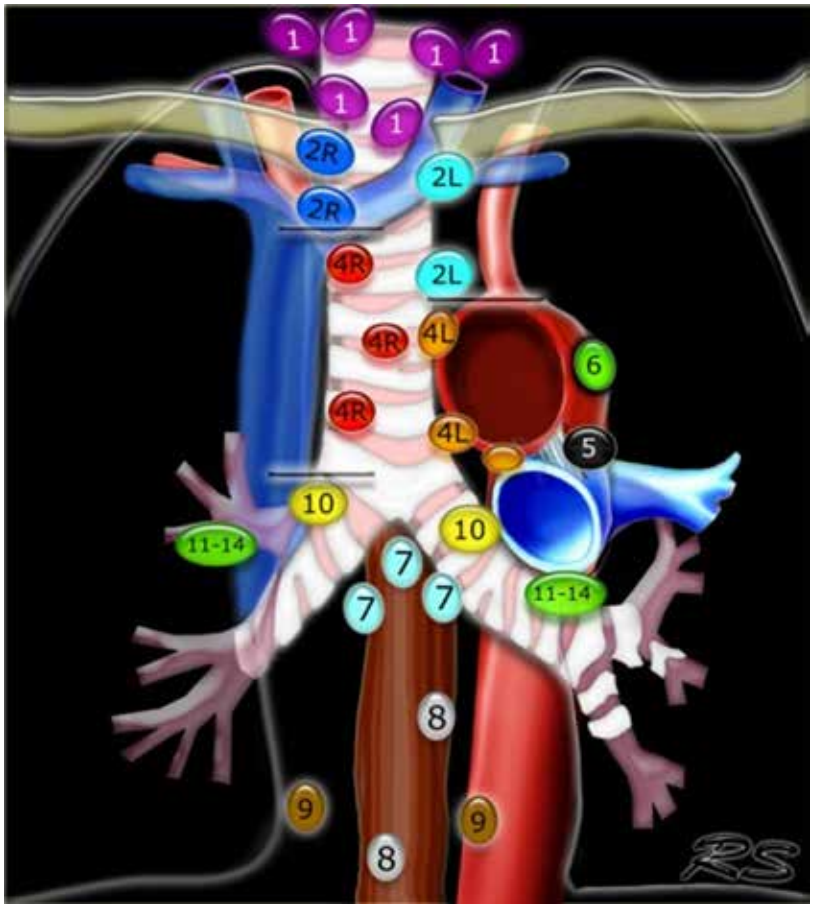


Figure 3 Regional lymph node classification for lung cancer staging based on the IASLC staging 2009 (43). (courtesy of Dr Robin Smithius www.radiologyassistant.nl).

tomography (PET) scanning followed by tissue sampling of any pathologic nodes. Depending on population prevalence, non-invasive imaging alone is inadequate as histological staging is obligatory to prognosticate and stratify management options (44,45). For example, in geographic regions where histoplasmosis infection is endemic, granulomatous infection can lead to PET-avidity in the mediastinum and lung lesions. Conversely, Altorki *et al.* conducted a retrospective review of 224 patients identified with clinical stage I NSCLC by CT and PET scan. At resection they found that 6.5% of clinical T1 patients had occult N2 disease (46).

Surgical mediastinoscopy is the gold standard for confirming CT/PET negative mediastinal metastases with a sensitivity of 78%. However, it has some limitations. Convex EBUS is a bronchoscopic technique that compliments mediastinoscopy (23) (*Table 2*). EBUS is minimally invasive, performed under conscious sedation or with general anesthesia in the outpatient setting, Lymph node sampling occurs under direct real-time ultrasound guidance with the convex probe EBUS (CP-EBUS) allowing a much greater diagnostic yield over blind sampling (47,48) (*Figure 4*). In a prospective cohort study of 108 patients, CP-EBUS-TBNA successfully sampled 163 mediastinal lymph nodes and demonstrated sensitivity and specificity of 94.6% and 100%, respectively, and a diagnostic accuracy of 96% (49). Several systematic reviews confirm equivalent sensitivity for EBUS-TNA to Mediastinoscopy (23,50-52). Yasufuku performed a prospective controlled comparison of EBUS-TBNA and mediastinoscopy in 153 patients with potentially resectable NSCLC (prevalence of N2/N3 disease 35%). They found sensitivities for mediastinoscopy and EBUS-TBNA were

Table 2 Accessibility of lymph node stations with the various biopsying techniques

Lymph node station	Mediastinoscopy	EBUS†	EUS‡	EUS-EBUS
1-2: Highest mediastinal	+	+		+
3: Prevascular + retrotracheal			+	+
4: Upper paratracheal	+	+		+
4: Lower paratracheal	+	+		+
4: Subaortic (AP window)			+	+
5-6: Para-aortic				
7: Subcarinal (anterior)	+	+	+	+
7: Subcarinal (posterior)		+	+	+
8: Paraesophageal			+	+
9: Pulmonary ligament			+	+
10: Hilar		+		+
11: Interlobular		+		+
12: Lobular		+		+
13: Segmental		+		+
14: Subsegmental		+		+

EBUS†, convex probe endobronchial ultrasound; EUS‡, esophageal ulrasound.

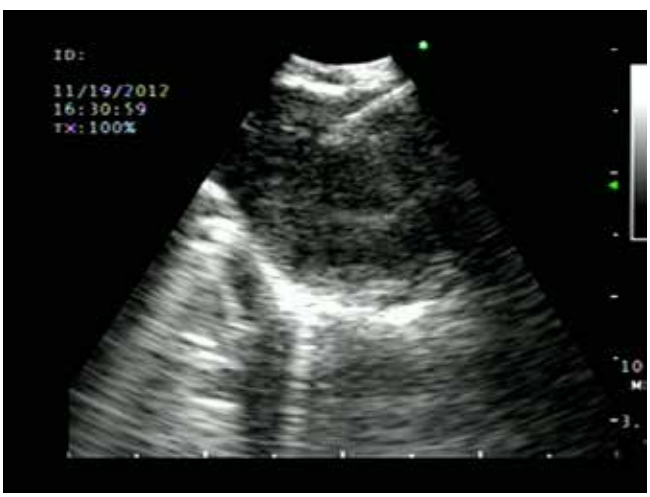


Figure 4 EBUS TBNA—Ultrasound image of real-time needle aspiration of homogenous echogenic lymph node measuring 1.5 cm.

79% and 85%, respectively, with comparable specificity (100%) and no significant differences in detecting true pathological N stage (McNemar test P=0.78) (53). In most community hospitals surgical mediastinoscopy remains the only available mediastinal staging technique. Many of the aforementioned studies were conducted at tertiary referral centers and it is uncertain how generalizable the results are.

EBUS combined with EUS/with mediastinoscopy

EBUS-TBNA has the ability to access most of the mediastinum (the anterior and superior), however the presence of paraesophageal, inferior and posterior mediastinal lymph nodes may require combined EBUS with endoscopic ultrasound guided fine needle aspiration (EUS-FNA) (*Table 2*). The combination improves the diagnostic yield compared to either procedure alone (48,54). Annema *et al.* in 2010 performed a multi-center randomized control trial in 241 patients with resectable NSCLC comparing mediastinoscopy alone with combined endosonography (EBUS-TBNA and/or EUS-FNA) approach followed by mediastinoscopy if no nodal metastases were found (55). The sensitivities of surgical staging compared to endosonography alone were 79% and 85%, respectively (P=0.47). Sensitivity improved to 94% if endosonography was followed by mediastinoscopy (P=0.02). This combined endosonographic and surgical approach resulted in greater sensitivity and fewer unnecessary thoracotomies.

Adequacy and techniques for molecular profiling

There have been dramatic advances in our understanding of the molecular makeup of NSCLCs, particularly in non-smokers or smokers with lower cumulative dose. Driver

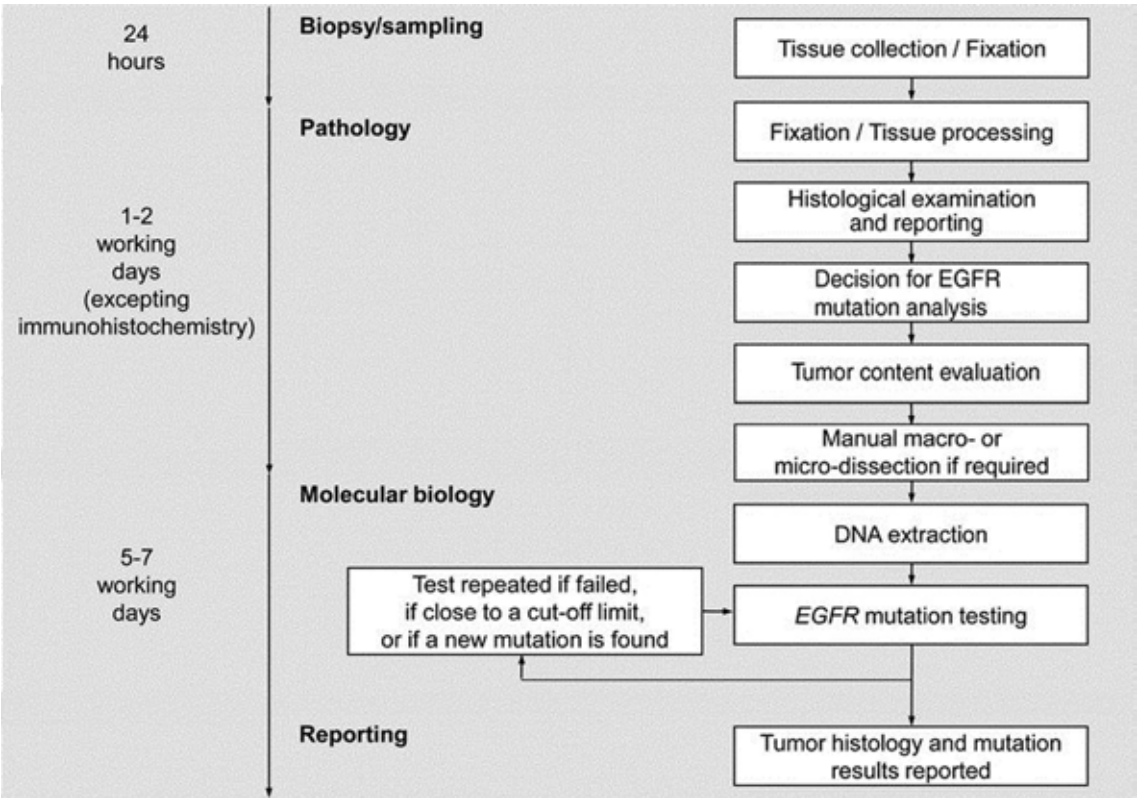


Figure 5 Sample preparation, Pathology and EGFR mutation analysis flow diagram suggested by the European EGFR workshop. *Reproduced with permission from Journal of Thoracic Oncology 2010* (69).

mutations in NSCLC that can be targeted have caused a shift away from a uniform therapeutic approach to a more personalized approach (56-60). In this era of personalized medicine, there is a need to provide high quality biopsy samples not only for pathologic diagnosis but also for the detailed molecular analyses that are becoming important to patient care. Initial studies in small populations suggested that EBUS-TBNA samples can be used for molecular analysis; EGFR, K-ras, p53 and EML4-ALK mutations (61,62). Navani *et al.* conducted a large multicenter study of 774 patients and confirmed these results. While the appropriate triaging of small biopsy specimens for cytologic, pathologic, and molecular analysis is vital there are as yet no guidelines for managing EBUS-TBNA samples. It is extremely important that the bronchoscopist obtaining samples do so in a manner that optimizes the diagnostic yield from molecular analyses (63,64). Rapid on-site cytologic evaluation (ROSE) of EBUS-TBNA has been shown to increase sensitivity from 80-88% without any added time to the procedure. Where available, ROSE allows repeated sampling of confirmed high-yield sites for triaging of specimens to cytologic diagnosis, immunohistochemisty or molecular analysis with clear communication of these goals to pathologist (61,65-68). In addition to ROSE, a few specialist centers are examining what additional procedural steps can be taken to maximize yield from small biopsy samples. This is crucial as more emerging genes are being identified that affect NSCLC carcinogenesis, such as ROS (crizotinib sensitive), Met, PI3K, etc. (61,64). As minimally invasive diagnosis and staging, as well as therapeutic modalities with driver mutations now becoming available there is increasing need to maximize and refine the technology and processes for tissue sampling (i.e., multiplex sampling, to afford our patients the best treatment options).

Re-biopsy

The European Respiratory Society (ERS) has published a statement that at biopsy it is desirable to obtain as much useful tissue as possible to avoid time consuming delays (dead time) due to molecular analysis or having to re-biopsy (69) (*Figure 5*). In order to avoid a molecular analysis delay, many

facilities have introduced reflex testing, for example any biopsy sample identified as an adenocarcinoma and as primary lung origin is automatically sent for EGFR analysis (along with other chosen molecular markers) without requiring a release from the physician. Sampling techniques, such as ROSE ensure that there is sufficient sample for these various molecular techniques, which also translates to avoiding any associated procedural delays with re-biopsy.

This does not mean however, that patients should never be re-biopsied. There is a growing realization that in patients in whom a driver mutation has been discovered there is tumor heterogeneity and dynamism. An EGFR mutant does not remain static, especially under the selective forces of EGFR-Tyrosine kinase inhibition (TKI). Although initially demonstrating a dramatic response to TKIs most patients will eventually experience treatment failure usually through acquired resistance to EGFR TKI.

Arcila *et al.* re-biopsied 121 patients with known EGFR mutations and tumor progression and discovered the T790M mutation in 70% with persistence of the original EGFR mutation in all patients (70). Similar results have been found in studies by Sequist, Oxnam and Ohashi *et al.*, including other axonal additions, deletions, SCLC conversions, BRAF mutations and many more. It is clear that identification of the molecular mechanisms will be vital to overcoming EGFR TKI resistance. It is growing apparent that a static biopsy is inadequate to guide therapeutic decision-making during a patient's treatment course. Re-biopsy at the time of disease progression is becoming standard. Pulmonologists must be available to re-biopsy at progression to assess mutational status (71-76).

Therapeutic endobronchial tumor management

Navigational bronchoscopy/RP-EBUS in fiducial placement for SBRT

Navigational bronchoscopy has been utilized to assist the radiation oncologist for stereotactic body radiation therapy (SBRT). Small peripheral lung lesions that were previously inaccessible can be sampled and fiducial markers can be placed in the same procedure in anticipation for SBRT (77,78). Standard therapy for early stage (I and II) NSCLC is a lobectomy with ipsilateral hilar lymph node dissection. While parenchyma-preserving surgeries such as sublobar resection (wedge resection & segmentectomy) have advanced over the years with improved outcomes, there is still a significant subgroup of patients with poor lung function or other comorbidities that cannot tolerate surgery (79,80). Radiation therapy for this inoperable group of patients with potentially curable disease is an attractive option. SBRT has the ability to deliver high doses of radiation with fidelity to generate margins of 1 cm. SBRT trials consistently report loco-regional and 3-year overall survival rates of 78-87% and 55-88% respectively, both comparable to surgery (81,82). Continuous tracking of respiratory motion using fiducials improves the fidelity of SBRT even further, allowing it to deliver beams with tumor margins of 5 mm. To date, there are three ways to deliver fiducials to or near the target lesion: transthoracic, intravascular and bronchoscopically. Currently, CT-guided transthoracic placement has been generally used, but it has a high pneumothorax rate. CT guided lung biopsy has a pneumothorax rate of 15%, while in some studies this is as high as 38%. This is certainly deleterious in a population of patients selected by their marginal pulmonary health (25,83). Intravascular placement also has its problems too. Intravascular fiducial placement cause pleurisy (13-33%), pulmonary infarcts (5%) and groin hematomas (3%) (78,84,85). ENB placement of fiducials has the advantages of successful delivery of markers with great fidelity and with the low complication rates of bronchoscopy (*Figure 6*).

Several studies have looked at the use of radial EBUS and ENB use for fiducial placement, and have found a high success rate (Anatham found that 88% were able to be delivered to within the tumor itself) with very minimal migration of fiducials (78). Indeed, the 10% migration rate seen in studies using linear fiducials was greatly reduced if coil-spring fiducials were employed. Schroeder reported a pneumothorax complication rate of 5.3% (86). A meta-analysis by Wang *et al.* of over 3,000 bronchoscopies reports a much lower pneumothorax rate (25,87). Larger comparison studies need to confirm the role of ENB and fiducials for inoperable, early stage cancer patients. Currently, results are pending from an interventional trial-RTOG 0618-in comparing tumor control between SBRT and surgery among operable stage I/II patients.

Fiducial markers for localization for surgical biopsy

Navigational bronchoscopy can assist the thoracic surgeon with biopsy of small lesions that are difficult to palpate during video-assisted thoracoscopic surgery (VATS) or thoracotomy, particularly ground glass nodules that warrant

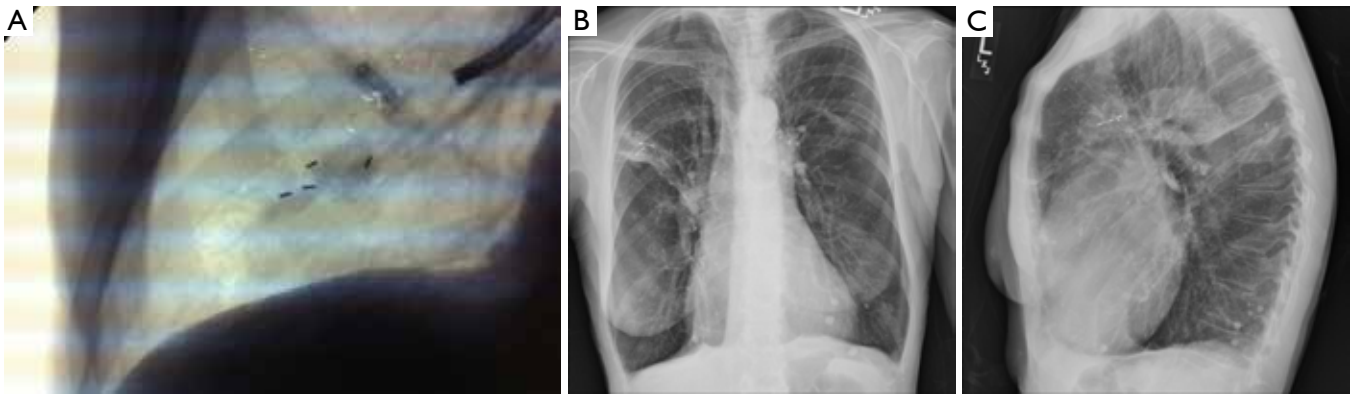


Figure 6 (A) Placement of fiducial markers by bronchoscopy under navigational guidance with real-time confirmation by fluoroscopy; (B) PA/LAT CXR after Fiducial Marker Placements (courtesy Joe Cicenia, M.D. Cleveland Clinic Foundation); (C) lateral.

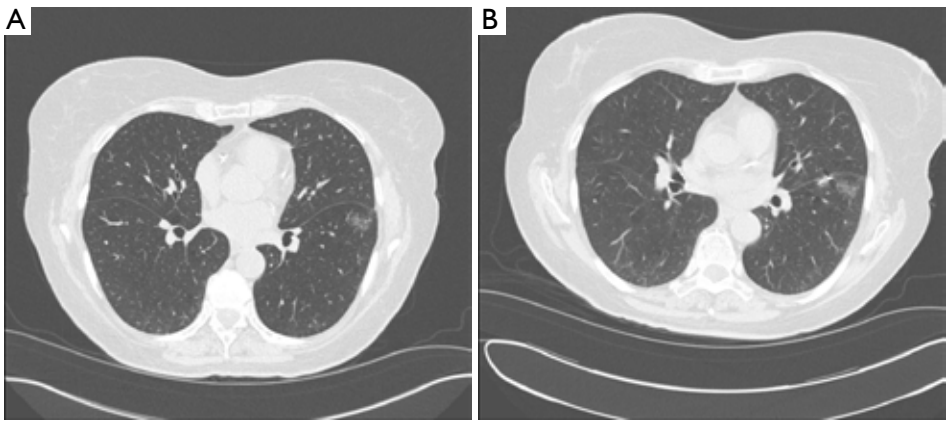


Figure 7 (A) Ground Glass Nodule of Left Lower lobe (courtesy Joe Cicenia, M.D, Cleveland Clinic Foundation); (B) post-fiducial placement for in preparation of surgical biopsy (white arrow) with subsequent resection.

histological confirmation of malignancy prior to anatomical resection or parenchymal sparing surgeries. CT-guidance can place fiducial markers with precision around the lesion prior to surgery with confirmation of position (*Figure* 7). Larger observational and interventional trials are needed to evaluate the efficacy of this complementary approach.

Palliative management in nonsurgical candidates

High dose rate brachytherapy

Henschke introduced the concept and technique of endobronchial brachytherapy in the 1960s as a method of introducing a radioactive source via a thin catheter (afterloader) intraluminally to targeted malignant tissue within the airways (16,88,89). A computerized, remote, 'afterloading' technique allows for safe delivery of radioactive material to endobronchial lesions at high doses in short periods of time while greatly minimizing radioactive exposure to staff. The most common radioisotope used is iridium-192 manufactured as a thin, flexible wire. The highly localized field of radiation around the flexible catheter allows for sparing of the surrounding tissue. High-dose rate endobronchial brachytherapy (HDREB) involves delivery of high-energy radiation over short periods (16). Although the available evidence for optimal radiation dosing is currently limited, the American Brachytherapy Society recommends 3 weekly fractions of 7.5 Gy each, 2 fractions of 10 Gy each, or 4 fractions of 6 Gy prescribed at 1 cm (16,90). These outpatient sessions are often well

tolerated and rapid with a response to therapy within 4-6 weeks (91). HDREB has shown to benefit patients with hemoptysis, dyspnea, post-obstructive pneumonia, and cough with centrally located lesions typically providing the best outcomes (16,90,91). Symptom control has been shown to be durable up to 6 months. Other potential indications for HDREB include patients who are poor surgical candidates; those who have maximized external beam radiation (EBR) doses; sole treatment for localized bronchial carcinomas; and carcinoma *in-situ* or pre-cancerous lesions (16,89,90,92). Although overall EBR alone has been more effective than HDREB in terms of durable palliation, combination of EBR with HDREB has also been shown to provide significant symptomatic control especially among patients with inoperable tumors or endobronchial obstruction causing atelectasis (89,91,93).

Photodynamic therapy

The targeted strategy of using photodynamic therapy (PDT) against malignant tissue has been in practice since the 1980s (16,94-96). It is an alternative treatment for cancer that involves administration of a systemic photosensitizing agent that preferentially accumulates in tumor cells. Palliative PDT for obstructive endobronchial tumors have been shown to be an effective strategy for patients experience persistent cough, progressive dyspnea, atelectasis and post-obstructive pneumonia (16,97-99). Results are optimal if the obstructive lesion is found in the segmental and subsegmental airways (100). PDT is generally well tolerated and can be administered regardless of prior chemotherapy, radiation or surgery. The most common photosensitizing agents used in lung cancer are hematoporphyrin derivatives, porfimer sodium (Photofrin®) and talaporfin sodium (Laserphyrin®). These agents are administered intravenously and peak extravascular concentration in tissues is achieved in 24 hours. While concentrations of the photosensitizing agent within peripheral organs decline over the next 2-3 days, tumors have been shown to selectively retain the chemical for much longer periods (16,101). For this reason the next stage of photoactivation typically does not occur until 24-72 hours when the tumor-normal tissue concentration ratio is optimal. During photosensitization a diode laser source emitting red or near-infrared light from a quartz catheter is delivered via flexible bronchoscopy to the endoluminal tumor cells. Currently the FDA recommended light dose is 200 J/cm with a total exposure time of 500 s (16,95,97,101). In addition PDT induces a thrombotic state within tumor microvessels leading to ischemic damage (16,96). As tumor cell death progresses necrotic tissue and debris accumulate in the airways in the next 48 hours after photoactivation. Repeat bronchoscopy is recommended at this point for debridement and prevention of obstruction (16,95,100). Further PDT sessions can be administered up to a maximum of 3 sessions within a 30-day period for residual tumor cells. Although PDT is generally well tolerated photosensitivity reaction in the form of sunburns can persist for up to 6 weeks after injection (16,96,101). The major disadvantage of PDT similar to brachytherapy is a delayed response after photoactivation. Thus this is not a feasible modality if rapid resolution of airway obstruction is needed (16,96,97,101).

Cryotherapy

Cryotherapy is an alternative method of controlling and debulking malignant endobronchial lesions by utilizing extreme cold energy to induce a cascade of events leading to tumor cell death (102,103). The principle of cryotherapy is the delivery of focused extreme cold energy via rapid expansion of compressed liquid nitrogen (the Joule-Thompson effect) at the tip of a cryoprobe. The high vascularity and water content of tumor tissue make it exceptionally vulnerable to extreme cold energy (16,102,104,105).

Endobronchial cryotherapy can be performed either via rigid or flexible bronchoscopy. It is considered a very safe procedure and is generally well tolerated. Patients who may be candidates for this procedure have advanced stage cancers and are poor surgical candidates. Similar to indications for photodynamic or brachytherapy, these patients require alleviation of symptoms attributed to endobronchial obstruction such as hemoptysis, atelectasis, intractable cough or post-obstructive pneumonia.

A recent systematic review noted mean response rates of 80% with minimal complications (0-11%) (106). In a series of 476 patients by Maiwand and colleagues, palliative cryotherapy has shown to provide significant alleviation of hemoptysis, cough, dyspnea and chest pain (76.4%, 69%, 59.2%, and 42.6%, respectively) in addition to improvements in Karnofsky performance scores (59.6 to 75.2) (103,106). Although the available data have shown variable survival rates, median survival time has not shown to be worse than other palliative-focuses endobronchial therapies (102,104-106). The major disadvantage of cryotherapy is its delayed response time and need for

repeat treatments relative to other palliative endobronchial procedures such as Nd:YAG laser, electrocautery and microdebridement. Tumor necrosis may continue for days after the initial treatment. Therefore cryotherapy is not optimal for patients experiencing massive hemoptysis or extensive endobronchial tumor involvement (16).

Rigid bronchoscopy and stenting

Nearly one-third of lung cancer patients experience some form of central airway obstruction (CAO) due to external compression, endoluminal disease or bulky lymphadenopathy (107-110). The quality of life and performance status of patients with CAO is significantly compromised due to dyspnea, stridor, hemorrhage and/or obstructive pneumonias. These airway-related symptoms may preclude the operability of a patient with early lung cancer. Endobronchial stenting has been shown to significantly relieve symptoms and improve quality of life among patients with malignant obstruction (107,111). While this technique has largely been used in advanced lung cancers it is, however, a palliative method that can be utilized in early lung cancer patients with poor functional status. Furthermore early stenting in this patient population may provide an additional survival advantage in addition to symptom relief (112). This will allow those who cannot tolerate surgery to undergo other definitive treatments such as brachytherapy or external radiation.

Conclusions

The last decade has seen many advances in lung cancer diagnosis and management options. Improvements in surgical techniques such as VATS, video mediastinoscopy and parenchymal sparing surgeries; discoveries of new targeted therapies for specific gene mutations in lung adenocarcinomas; and stereotactic radiotherapy for early stage lung cancers all contribute to improvement of quality of life and outcomes for patients with variable performance status. Furthermore, low-dose CT screening for early detection of lung cancers will inevitably revolutionize how lung cancer will be approached. It is paramount that the interventional pulmonologist integrate the armamentarium of minimally invasive approaches described in this review, coupled with sound clinical judgments to collaborate with all specialists of the lung cancer multidisciplinary team.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Smoking and tobacco use: fast facts. Atlanta: Centers for Disease Control and Prevention. Available online: http://www.cdc.gov/tobacco/data_statistics/fact_sheets/fast_facts
2. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
3. Wynder EL, Graham EA. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma; a study of 684 proved cases. J Am Med Assoc 1950;143:329-36.
4. Schmidt H, Voigt K, Emanuel EJ. The ethics of not hiring smokers. N Engl J Med 2013;368:1369-71.
5. Silvestri GA, Feller-Kopman D, Chen A, et al. Latest advances in advanced diagnostic and therapeutic pulmonary procedures. Chest 2012;142:1636-44.
6. Seijo LM, Sterman DH. Interventional pulmonology. N Engl J Med 2001;344:740-9.
7. Levine DJ, Angel LF. Role of the Interventional Pulmonologist. Clin Pulm Med 2006;13:128-41.
8. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395-409.
9. International Early Lung Cancer Action Program Investigators, Henschke CI, Yankelevitz DF, et al. Survival of patients with stage I lung cancer detected on CT screening. N Engl J Med 2006;355:1763-71.
10. Rivera MP. Preinvasive lesions of the bronchus. Clin Chest Med 2011;32:693-702.
11. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85.
12. Breuer RH, Pasic A, Smit EF, et al. The natural course of preneoplastic lesions in bronchial epithelium. Clin Cancer Res 2005;11:537-43.
13. Bota S, Auliac JB, Paris C, et al. Follow-up of bronchial precancerous lesions and carcinoma in situ using fluorescence endoscopy. Am J Respir Crit Care Med 2001;164:1688-93.
14. Kennedy TC, McWilliams A, Edell E, et al. Bronchial intraepithelial neoplasia/early central airways lung cancer: ACCP evidence-based clinical practice guidelines (2nd

edition). Chest 2007;132:221S-233S.
15. Ikeda N, Hayashi A, Iwasaki K, et al. Comprehensive diagnostic bronchoscopy of central type early stage lung cancer. Lung Cancer 2007;56:295-302.
16. Principles and Practice of Interventional Pulmonology. Springer, New York, 2013.
17. Zaric B, Perin B. Use of narrow-band imaging bronchoscopy in detection of lung cancer. Expert Rev Med Devices 2010;7:395-406.
18. Keith RL, Miller YE, Gemmill RM, et al. Angiogenic squamous dysplasia in bronchi of individuals at high risk for lung cancer. Clin Cancer Res 2000;6:1616-25.
19. Zaric B, Perin B, Becker HD, et al. Combination of narrow band imaging (NBI) and autofluorescence imaging (AFI) videobronchoscopy in endoscopic assessment of lung cancer extension. Med Oncol 2012;29:1638-42.
20. Vincent BD, Fraig M, Silvestri GA. A pilot study of narrow-band imaging compared to white light bronchoscopy for evaluation of normal airways and premalignant and malignant airways disease. Chest 2007;131:1794-9.
21. Herth FJ, Eberhardt R, Anantham D, et al. Narrow-band imaging bronchoscopy increases the specificity of bronchoscopic early lung cancer detection. J Thorac Oncol 2009;4:1060-5.
22. Zaric B, Becker HD, Perin B, et al. Narrow band imaging videobronchoscopy improves assessment of lung cancer extension and influences therapeutic strategy. Jpn J Clin Oncol 2009;39:657-63.
23. Detterbeck FC, Lewis SZ, Diekemper R, et al. Executive Summary: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:7S-37S.
24. Schreiber G, McCrory DC. Performance characteristics of different modalities for diagnosis of suspected lung cancer: summary of published evidence. Chest 2003;123:115S-128S.
25. Wang Memoli JS, Nietert PJ, et al. Meta-analysis of guided bronchoscopy for the evaluation of the pulmonary nodule. Chest 2012;142:385-93.
26. Baaklini WA, Reinoso MA, Gorin AB, et al. Diagnostic yield of fiberoptic bronchoscopy in evaluating solitary pulmonary nodules. Chest 2000;117:1049-54.
27. Kurimoto N, Murayama M, Yoshioka S, et al. Assessment of usefulness of endobronchial ultrasonography in determination of depth of tracheobronchial tumor invasion. Chest 1999;115:1500-6.
28. Baba M, Sekine Y, Suzuki M, et al. Correlation between endobronchial ultrasonography (EBUS) images and histologic findings in normal and tumor-invaded bronchial wall. Lung Cancer 2002;35:65-71.
29. Tanaka F, Muro K, Yamasaki S, et al. Evaluation of tracheo-bronchial wall invasion using transbronchial ultrasonography (TBUS). Eur J Cardiothorac Surg 2000;17:570-4.
30. Gildea TR, Mazzone PJ, Karnak D, et al. Electromagnetic navigation diagnostic bronchoscopy: a prospective study. Am J Respir Crit Care Med 2006;174:982-9.
31. Makris D, Scherpereel A, Leroy S, et al. Electromagnetic navigation diagnostic bronchoscopy for small peripheral lung lesions. Eur Respir J 2007;29:1187-92.
32. Herth F, Ernst A, Schulz M, et al. Endobronchial ultrasound reliably differentiates between airway infiltration and compression by tumor. Chest 2003;123:458-62.
33. Steinfort DP, Khor YH, Manser RL, et al. Radial probe endobronchial ultrasound for the diagnosis of peripheral lung cancer: systematic review and meta-analysis. Eur Respir J 2011;37:902-10.
34. Eberhardt R, Anantham D, Ernst A, et al. Multimodality bronchoscopic diagnosis of peripheral lung lesions: a randomized controlled trial. Am J Respir Crit Care Med 2007;176:36-41.
35. Ernst A, Anantham D. Update on interventional bronchoscopy for the thoracic radiologist. J Thorac Imaging 2011;26:263-77.
36. Eberhardt R, Anantham D, Herth F, et al. Electromagnetic navigation diagnostic bronchoscopy in peripheral lung lesions. Chest 2007;131:1800-5.
37. Eberhardt R, Anantham D, Ernst A, et al. Multimodality bronchoscopic diagnosis of peripheral lung lesions: a randomized controlled trial. Am J Respir Crit Care Med 2007;176:36-41.
38. Makris D, Scherpereel A, Leroy S, et al. Electromagnetic navigation diagnostic bronchoscopy for small peripheral lung lesions. Eur Respir J 2007;29:1187-92.
39. Seijo LM, de Torres JP, Lozano MD, et al. Diagnostic yield of electromagnetic navigation bronchoscopy is highly dependent on the presence of a Bronchus sign on CT imaging: results from a prospective study. Chest 2010;138:1316-21.
40. Lamprecht B, Porsch P, Wegleitner B, et al. Electromagnetic navigation bronchoscopy (ENB): Increasing diagnostic yield. Respir Med 2012;106:710-5.
41. Asano F, Shinagawa N, Ishida T, et al. Virtual bronchoscopic navigation combined with ultrathin

bronchoscopy. A randomized clinical trial. Am J Respir Crit Care Med 2013;188:327-33.
42. Eberhardt R, Kahn N, Gompelmann D, et al. LungPoint--a new approach to peripheral lesions. J Thorac Oncol 2010;5:1559-63.
43. Rusch VW, Asamura H, Watanabe H, et al. The IASLC lung cancer staging project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2009;4:568-77.
44. De Leyn, Lardinois D, Van Schil PE, et al. ESTS guidelines for preoperative lymph node staging for non-cmall cell lung cancer. Eur J Cardiothoracic Surg 2007;32:1-8.
45. Hwangbo B, Kim SK, Lee HS, et al. Application of endobronchial ultrasound-guided transbronchial needle aspiration following integrated PET/CT in mediastinal staging of potentially operable non-small cell lung cancer. Chest 2009;135:1280-7.
46. Lee PC, Port JL, Korst RJ, et al. Risk factors for occult mediastinal metastases in clinical stage I non-small cell lung cancer. Ann Thorac Surg 2007;84:177-81.
47. Herth F, Becker HD, Ernst A. Conventional vs endobronchial ultrasound-guided transbronchial needle aspiration: a randomized trial. Chest 2004;125:322-5.
48. Wallace MB, Pascual JM, Raimondo M, et al. Minimally invasive endoscopic staging of suspected lung cancer. JAMA 2008;299:540-6.
49. Yasufuku K, Chiyo M, Koh E, et al. Endobronchial ultrasound guided transbronchial needle aspiration for staging of lung cancer. Lung Cancer 2005;50:347-54.
50. Adams K, Shah PL, Edmonds L, et al. Test performance of endobronchial ultrasound and transbronchial needle aspiration biopsy for mediastinal staging in patients with lung cancer: systematic review and meta-analysis. Thorax 2009;64:757-62.
51. Gu P, Zhao YZ, Jiang LY, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for staging of lung cancer: a systematic review and meta-analysis. Eur J Cancer 2009;45:1389-96.
52. Varela-Lema L, Fernández-Villar A, Ruano-Ravina A. Effectiveness and safety of endobronchial ultrasound-transbronchial needle aspiration: a systematic review. Eur Respir J 2009;33:1156-64.
53. Yasufuku K, Pierre A, Darling G, et al. A prospective controlled trial of endobronchial ultrasound-guided transbronchial needle aspiration compared with mediastinoscopy for mediastinal lymph node staging of lung cancer. J Thorac Cardiovasc Surg 2011;142:1393-400.e1.
54. Vilmann P, Krasnik M, Larsen SS, et al. Transesophageal endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) biopsy: a combined approach in the evaluation of mediastinal lesions. Endoscopy 2005;37:833-9.
55. Annema JT, van Meerbeeck JP, Rintoul RC, et al. Mediastinoscopy vs endosonography for mediastinal nodal staging of lung cancer: a randomized trial. JAMA 2010;304:2245-52.
56. Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011;29:2866-74.
57. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
58. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306-11.
59. McDermott U, Iafrate AJ, Gray NS, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res 2008;68:3389-95.
60. Kwak EL, Camidge DR, Clark J, et al. Clinical activity observed in a phase I dose escalation trial of an oral c-MET and ALK inhibitor, PF-02341066. J Clin Oncol 2009;27:abstr 3509.
61. Nakajima T, Yasufuku K. How I do it--optimal methodology for multidirectional analysis of endobronchial ultrasound-guided transbronchial needle aspiration samples. J Thorac Oncol 2011;6:203-6.
62. Sakairi Y, Nakajima T, Yasufuku K, et al. EML4-ALK fusion gene assessment using metastatic lymph node samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration. Clin Cancer Res 2010;16:4938-45.
63. Navani N, Brown JM, Nankivell M, et al. Suitability of endobronchial ultrasound-guided transbronchial needle aspiration specimens for subtyping and genotyping of non-small cell lung cancer: a multicenter study of 774 patients. Am J Respir Crit Care Med 2012;185:1316-22.

64. Bulman W, Saqi A, Powell CA. Acquisition and processing of endobronchial ultrasound-guided transbronchial needle aspiration specimens in the era of targeted lung cancer chemotherapy. Am J Respir Crit Care Med 2012;185:606-11.
65. Loukeris K, Vazquez MF, Sica G, et al. Cytological cell blocks: Predictors of squamous cell carcinoma and adenocarcinoma subtypes. Diagn Cytopathol 2012;40:380-7.
66. Wallace WA, Rassl DM. Accuracy of cell typing in nonsmall cell lung cancer by EBUS/EUS-FNA cytological samples. Eur Respir J 2011;38:911-7.
67. Micames CG, McCrory DC, Pavey DA, et al. Endoscopic ultrasound-guided fine-needle aspiration for non-small cell lung cancer staging: A systematic review and metaanalysis. Chest 2007;131:539-48.
68. Nasuti JF, Gupta PK, Baloch ZW. Diagnostic value and cost-effectiveness of on-site evaluation of fine-needle aspiration specimens: review of 5,688 cases. Diagn Cytopathol 2002;27:1-4.
69. Pirker R, Herth FJ, Kerr KM, et al. Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. J Thorac Oncol 2010;5:1706-13.
70. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17:1169-80.
71. Majem M, Remon J. Tumor heterogeneity: evolution through space and time in EGFR mutant non small cell lung cancer patients. Transl Lung Cancer Res 2013;2:226-237.
72. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
73. Yano S, Nakataki E, Ohtsuka S, et al. Retreatment of lung adenocarcinoma patients with gefitinib who had experienced favorable results from their initial treatment with this selective epidermal growth factor receptor inhibitor: a report of three cases. Oncol Res 2005;15:107-11.
74. Yoshimoto A, Inuzuka K, Kita T, et al. Remarkable effect of gefitinib retreatment in a patient with nonsmall cell lung cancer who had a complete response to initial gefitinib. Am J Med Sci 2007;333:221-5.
75. Oxnard GR, Arcila ME, Sima CS, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res 2011;17:1616-22.
76. Ohashi K, Sequist LV, Arcila ME, et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. Proc Natl Acad Sci U S A 2012;109:E2127-33.
77. Eberhardt R, Kahn N, Herth FJ. 'Heat and destroy': bronchoscopic-guided therapy of peripheral lung lesions. Respiration 2010;79:265-73.
78. Anantham D, Feller-Kopman D, Shanmugham LN, et al. Electromagnetic navigation bronchoscopy-guided fiducial placement for robotic stereotactic radiosurgery of lung tumors: a feasibility study. Chest 2007;132:930-5.
79. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
80. Narsule CK, Ebright MI, Fernando HC. Sublobar versus lobar resection: current status. Cancer J 2011;17:23-7.
81. Timmerman R, McGarry R, Yiannoutsos C, et al. Excessive toxicity when treating central tumors in a phase II study of stereotactic body radiation therapy for medically inoperable early-stage lung cancer. J Clin Oncol 2006;24:4833-9.
82. Timmerman R, Papiez L, McGarry R, et al. Extracranial stereotactic radioablation: results of a phase I study in medically inoperable stage I non-small cell lung cancer. Chest 2003;124:1946-55.
83. Whyte RI, Crownover R, Murphy MJ, et al. Stereotactic radiosurgery for lung tumors: preliminary report of a phase I trial. Ann Thorac Surg 2003;75:1097-101.
84. Prasad V, Chan RP, Faughnan ME. Embolotherapy of pulmonary arteriovenous malformations: efficacy of platinum versus stainless steel coils. J Vasc Interv Radiol 2004;15:153-60.
85. Mager JJ, Overtoom TT, Blauw H, et al. Embolotherapy of pulmonary arteriovenous malformations: long-term results in 112 patients. J Vasc Interv Radiol 2004;15:451-6.
86. Schroeder C, Hejal R, Linden PA. Coil spring fiducial markers placed safely using navigation bronchoscopy in inoperable patients allows accurate delivery of CyberKnife stereotactic radiosurgery. J Thorac Cardiovasc Surg 2010;140:1137-42.
87. Harley DP, Krimsky WS, Sarkar S, et al. Fiducial marker placement using endobronchial ultrasound and

navigational bronchoscopy for stereotactic radiosurgery: an alternative strategy. Ann Thorac Surg 2010;89:368-73; discussion 373-4.
88. de Aquino Gorayeb MM, Gregório MG, de Oliveira EQ, et al. High-dose-rate brachytherapy in symptom palliation due to malignant endobronchial obstruction: A quantitative assessment. Brachytherapy 2013;12:471-8.
89. Nori D, Allison R, Kaplan B, et al. High dose-rate intraluminal irradiation in bronchogenic carcinoma. Technique and results. Chest 1993;104:1006-11.
90. Nag S, Cano ER, Demanes DJ, et al. The American Brachytherapy Society recommendations for high-dose-rate brachytherapy for head-and-neck carcinoma. Int J Radiat Oncol Biol Phys 2001;50:1190-8.
91. Guarnaschelli JN, Jose BO. Palliative high-dose-rate endobronchial brachytherapy for recurrent carcinoma: the University of Louisville experience. J Palliat Med 2010;13:981-9.
92. Ung YC, Yu E, Falkson C, et al. The role of high-dose-rate brachytherapy in the palliation of symptoms in patients with non-small-cell lung cancer: a systematic review. Brachytherapy 2006;5:189-202.
93. Kelly JF, Delclos ME, Morice RC, et al. High-dose-rate endobronchial brachytherapy effectively palliates symptoms due to airway tumors: the 10-year M. D. Anderson cancer center experience. Int J Radiat Oncol Biol Phys 2000;48:697-702.
94. Usuda J, Tsutsui H, Honda H, et al. Photodynamic therapy for lung cancers based on novel photodynamic diagnosis using talaporfin sodium (NPe6) and autofluorescence bronchoscopy. Lung Cancer 2007;58:317-23.
95. Sutedja TG. Photodynamic therapy in advanced tracheobronchial cancers. Diagn Ther Endosc 1999;5:245-51.
96. Owen J, Carroll C, Cooke J, et al. School-linked sexual health services for young people (SSHYP): a survey and systematic review concerning current models, effectiveness, cost-effectiveness and research opportunities. Health Technol Assess 2010;14:1-228, iii-iv.
97. Loewen GM, Pandey R, Bellnier D, et al. Endobronchial photodynamic therapy for lung cancer. Lasers Surg Med 2006;38:364-70.
98. Martin LK, Otterson GA, Bekaii-Saab T. Photodynamic therapy (PDT) may provide effective palliation in the treatment of primary tracheal carcinoma: a small case series. Photomed Laser Surg 2012;30:668-71.
99. Kato H. Our experience with photodynamic diagnosis and photodynamic therapy for lung cancer. J Natl Compr Canc Netw 2012;10 Suppl 2:S3-8.
100. Furukawa K, Okunaka T, Yamamoto H, et al. Effectiveness of Photodynamic Therapy and Nd-YAG Laser Treatment for Obstructed Tracheobronchial Malignancies. Diagn Ther Endosc 1999;5:161-6.
101. Usuda J, Kato H, Okunaka T, et al. Photodynamic therapy (PDT) for lung cancers. J Thorac Oncol 2006;1:489-93.
102. Maiwand MO. The role of cryosurgery in palliation of tracheo-bronchial carcinoma. Eur J Cardiothorac Surg 1999;15:764-8.
103. Maiwand MO, Asimakopoulos G. Cryosurgery for lung cancer: clinical results and technical aspects. Technol Cancer Res Treat 2004;3:143-50.
104. Homasson JP, Renault P, Angebault M, et al. Bronchoscopic cryotherapy for airway strictures caused by tumors. Chest 1986;90:159-64.
105. Asimakopoulos G, Beeson J, Evans J, et al. Cryosurgery for malignant endobronchial tumors: analysis of outcome. Chest 2005;127:2007-14.
106. Lee SH, Choi WJ, Sung SW, et al. Endoscopic cryotherapy of lung and bronchial tumors: a systematic review. Korean J Intern Med 2011;26:137-44.
107. Chin CS, Litle V, Yun J, et al. Airway stents. Ann Thorac Surg 2008;85:S792-6.
108. Theodore PR. Emergent management of malignancy-related acute airway obstruction. Emerg Med Clin North Am 2009;27:231-41.
109. Wood D. Airway stenting. Chest Surg Clin N Am 2003;13:211-29.
110. Lee P, Kupeli E, Mehta AC. Airway stents. Clin Chest Med 2010;31:141-50, Table of Contents.
111. Chhajed PN, Baty F, Pless M, et al. Outcome of treated advanced non-small cell lung cancer with and without central airway obstruction. Chest 2006;130:1803-7.
112. Razi SS, Lebovics RS, Schwartz G, et al. Timely airway stenting improves survival in patients with malignant central airway obstruction. Ann Thorac Surg 2010;90:1088-93.

Cite this article as: Tofts RP, Lee PM, Sung AW. Interventional pulmonology approaches in the diagnosis and treatment of early stage non small cell lung cancer. Transl Lung Cancer Res 2013;2(5):316-331. doi: 10.3978/j.issn.2218-6751.2013.10.11

Post-therapeutic positron emission tomography/computed tomography for early detection of non-small cell lung cancer recurrence

Sonja Sudarski, Thomas Henzler, Stefan O. Schoenberg

Institute of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Correspondence to: Thomas Henzler, MD. Institute of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68167 Mannheim, Germany.
Email: thomas.henzler@medma.uni-heidelberg.de.

Abstract: Patients after curative treatment of non-small cell lung cancer (NSCLC) have a high risk of loco-regional and/or distant tumor recurrence, especially within the first two years. Timely and accurate detection of recurrence is crucial in order to start salvage or palliative therapies with the overall goal of increasing patients' survival and quality of life. However, with the emerging use of non-surgical curative-intended therapies, follow-up of patients becomes even more challenging, as local recurrence has to be distinguished from various post-therapeutic changes at the site of the primary cancer. Integrated positron emission tomography/computed tomography (PET/CT), which is already an established imaging modality in the staging of NSCLC, is increasingly used in recurrence surveillance algorithms. By detailed morphological information being combined with additional information about the metabolic activity of suspicious sites, determination of suspicious lesions as benign or malignant can be improved. This article reviews the value of integrated PET/CT in assessing recurrence in NSCLC patients after potentially curative surgery and after curative-intended non-surgical therapies and raises as well the issue of cost-effectiveness of PET/CT for follow-up.

Keywords: Integrated positron emission tomography/computed tomography (PET/CT); non-small cell lung cancer (NSCLC); recurrence; follow-up

Submitted May 27, 2013. Accepted for publication May 30, 2013.
doi: 10.3978/j.issn.2218-6751.2013.05.02
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.05.02

Introduction

Lung cancer comprises almost 25% of the total cancer deaths worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers (2).

Although surgical resection remains the optimal treatment for early stage NSCLC, approximately 40% of patients with stage I and 60% of patients with stage II NSCLC relapse and die within 5 years after curative resection (3).

Timely and accurate detection of recurrence in patients with NSCLC plays a crucial role with regard to the initiation of salvage therapies with the overall goal of increasing survival.

Positron emission tomography (PET) has shown superior sensitivity and specificity in detecting NSCLC lymph node metastasis compared to standard CT alone (4). PET scans have widely replaced bone scintigraphy for detection of bone metastasis and PET is superior to all other clinically available imaging techniques for the detection of distant metastasis, except for cerebral metastasis (5).

The implementation of integrated positron emission tomography/computed tomography (PET/CT) systems, matching detailed morphological information of CT and

Figure 1 A 57-year-old asymptomatic patient that underwent annual PET/CT examinations for surveillance of recurrence. Between 2008 and 2009 PET/CT demonstrated stable disease with stable right hilar lymph node metastasis. One year later, PET/CT diagnosed loco-regional recurrence within the right lower lobe as well as distant lymph node metastasis.

metabolic information of structures provided by PET, has further improved accuracy compared to PET or CT alone and has therefore already become an integral imaging modality for diagnosis, staging and response assessment in NSCLC patients (6-9). PET/CT is now emerging as a follow-up imaging modality in these patients. In a study in 2004, reported overall sensitivities, specificities and positive and negative predictive values of integrated PET/CT for diagnosis of NSCLC recurrence were 96%, 82%, 89% and 93%, respectively, compared to 96%, 53%, 75% and 90%, respectively, for PET alone in patients with suspected recurrence who had previously undergone surgical therapy, surgery combined with chemo- or radiotherapy or combined chemo-radiotherapy alone (10).

This review focuses on the value of integrated PET/CT as a state-of-the-art technique in the detection of recurrence of NSCLC after curative surgery, (chemo-) radiotherapy as well as radiofrequency ablation and discusses the cost-effectiveness of PET/CT for recurrence detection.

NSCLC recurrence patterns

Recurrence of NSCLC may be classified as loco-regional recurrence or distant metastasis (*Figure 1*). Distant metastases are the most common form of NSCLC recurrence. Depending on the stage of disease at primary diagnosis and treatment administered, metastatic recurrence comprises 39% to 65.5% of all recurrences (11). About 30% of NSCLC recurrences are reported to be loco-regional. Loco-regional recurrence is located within the treated hemithorax and usually presents with nodules involving the resection staple line or the area that was treated with radiotherapy or RFA, as well as the bronchial stump, pleura, chest wall and lymph nodes (2).

In addition to recurrences, new primary lung cancer is also reported in 1% to 2% of NSCLC patients per year following initial radical therapy (12).

Technical aspects

Performing an integrated PET/CT scan, CT can either be run as low-dose CT, used predominantly for attenuation correction and solely approximate anatomical mapping, or CT is used for both attenuation correction and diagnostic purposes, being then performed with a standard radiation dose and *i.v.* and oral contrast material (13).

The two main advantages gained with the use of integrated PET/CT are on the one hand detection of lesions initially not seen on CT or PET alone, and on the other hand a more precise allocation of metabolic activity to an anatomic structure resulting in a better characterization of the lesion as benign or malignant (7,14). However, sensitivity of PET is decreased in tumors <1 cm, partly due to respiratory motion which can be reduced by respiratory triggered acquisitions at the expense of longer scan times and lower signal-to-noise-ratio (7). Furthermore, PET sensitivity is decreased in the brain. As the most common tracer used for PET scans is 2-deoxy-2-(18F)fluoro-D-glucose (FDG), a radioactively labeled glucose molecule, and the naturally high avidity of brain parenchyma for glucose leads to the problem that cerebral metastases can be obscured (5).

FDG uptake has been observed to vary between different NSCLC histologies, with adenocarcinomas generally being less FDG-avid than squamous cell carcinomas (15). Thus, detection of recurrence is extremely challenging for adenocarcinoma-in situ, minimally invasive adenocarcinoma and lepidic predominant adenocarcinoma since these tumors are often not FDG-avid and false-negative PET

findings have been reported for bronchioloalveolar carcinoma recurrence in 40% of cases (16).

Iatrogenic causes of focal or diffuse FDG parenchymal uptake include: talc deposits after pleurodesis, percutaneous needle biopsy, mediastinoscopy and FDG microembolism (17).

PET/CT in current follow-up guidelines and in clinical practice

Current recommendations for follow-up imaging after NSCLC treatment are based on the knowledge about the high incidence of recurrence during the first 2 years following therapy. The National Comprehensive Cancer Network (NCCN) guidelines from 2010 suggest for patients at all stages of NSCLC routine history and physical examinations every 4 to 6 months in the first 2 years and then annually (18). In patients treated with curative intent in good performance an additional contrast-enhanced chest CT scan is recommended every 4 to 6 months postoperatively for 2 years, followed by a non-contrast-enhanced chest CT annually thereafter. Routinely screening with chest CT alone should be omitted, because many recurrences are extrathoracic (11). PET or brain magnetic resonance imaging (MRI) is currently not recommended for routine follow-up (18).

Yumuk *et al.* performed a survey and interviewed physicians from 38 centres of 12 different countries on which tests they were performing on asymptomatic patients during their post-treatment follow-up. Contradictory to the guidelines, the most commonly used test was a chest CT scan as well as a CT scan of the abdomen at 3 months post treatment (19). PET/CT and contrast enhanced MRI of the brain were done solely in symptomatic patients. These results suggest that a CT scan at 3 months after the end of radical treatment has become a standard in clinical practice with little high quality evidence.

PET/CT for follow-up after surgery

Lung cancer recurs after surgery in 30% to 75% of patients (20). Differentiation of recurrence from post-surgical changes is challenging with CT alone since many benign conditions, including atelectasis, consolidations, and radiation induced fibrosis, are difficult to distinguish from loco-regional recurrence (2).

PET/CT on the other hand, can yield false-positive results from active inflammation, particularly in the acute post-operative phase (21).

False-positive PET/CT results can be explained by an increase in glycolysis due to macrophage infiltration where inflammation is present, and a subsequently higher glucose demand and FDG uptake. In 2008, a British study retrospectively assessed FDG uptake in post-thoracotomy scars of NSCLC patients (22). Increased uptake was seen in 100% of the cases at 1-3 months, in 92% at 3-12 months, and still in 40% of the studies more than one year after surgery all in patients with no evidence of disease on follow-up. FDG uptake was observed to be diffuse in 67% of cases. Tumor recurrence in the scar was found in three cases, with focally increased uptake at 3-8 months after thoracotomy. The authors concluded that increased FDG uptake in post-thoracotomy scars is mainly diffuse, and decreases in incidence and intensity with time, with 60% of studies showing no scar uptake more than one year after surgery. Focally intense scar uptake was suggested to prompt biopsy for suspected recurrence.

These results contradict the usefulness of early post-surgical follow-up with PET/CT within the first three months, whereas usefulness of PET/CT in follow-up as from three months on is supported by these data.

A large prospective study by Choi *et al.*, published in 2011, further evaluated the usefulness of PET/CT first performed one year after curative surgery (23). 358 patients having undergone complete resection of NSCLC were prospectively followed-up with PET/CT and conventional methods for recurrence of NSCLC at 3-month intervals for 2 years and after this at 6 month intervals for the next 3 years. Conventional methods comprised clinical, biochemical and radiographic assessment. Contrast-enhanced chest CT was done every 6 months whereas PET/CT was performed annually for 5 years after resection. Recurrence occurred in 31% of patients. In half of these patients, recurrence was detected with conventional methods. Concerning the other patients, recurrence was detected with both chest CT and PET/CT in 51% and solely with PET/CT in 37%. However, because PET/CT failed to detect 6 small or hypometabolic recurrent lesions, Choi *et al.* recommended as a screening algorithm annual PET/CT scans in combination with low-dose chest CT.

Besides the question of optimal timing of the first follow-up scan, the controversy whether to screen NSCLC patients after potentially curative treatment regardless of clinically suspected recurrence or whether to perform PET/CT only in symptomatic patients is still debated (*Figure 2*).

In this context, a Japanese study published in 2012 retrospectively evaluated the diagnostic accuracy of routinely

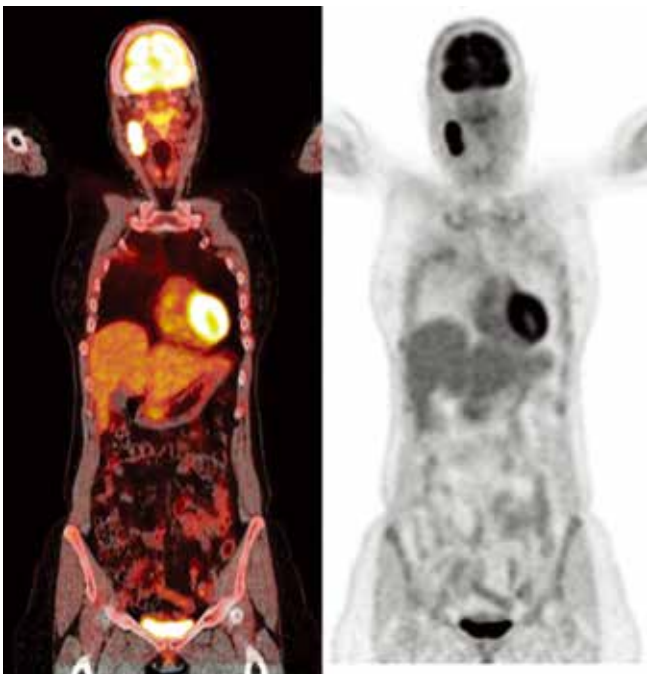


Figure 2 A 67-year-old asymptomatic patient with cervical metastatic disease that was detected during a follow-up PET/CT examination two years after curative intended chemo-radiation therapy.

performed PET/CT scans in post-operative asymptomatic NSCLC patients without suspicion of recurrence (24). A total of 101 NSCLC patients were followed-up for 5 years with a surveillance algorithm consisting of physical examination, chest radiograph, tumor marker, chest CT, PET/CT and brain MRI. Chest CT and PET/CT were performed in alternation every 6 months for the first 3 years. PET/CT was then performed every 12 months for the next 2 years. A total of 233 studies were acquired. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of PET/CT in recurrence evaluation were 94.4%, 97.6%, 89.5%, 98.8% and 97.0%, respectively. Recurrence rate in this asymptomatic patient cohort was 18%.

Another study with PET/CT in asymptomatic patients being performed at around 1 year after curative resection of NSCLC was conducted by the group of Cho *et al.* (25). The study enrolled 86 patients who had no suspicion of recurrence at the time of the PET/CT scan. 31.4% of the patients had recurrent disease in this cohort and 2 patients had extrathoracic double primary cancer. Six patients had extrathoracic recurrence without intrathoracic recurrence, contradicting the use of chest CT scans alone.

Jimenez-Bonilla *et al.* prospectively evaluated the contribution of PET/CT in patients with all stages of NSCLC with suspicion of recurrence in terms of sensitivity, specificity, impact on therapy and on survival (26). 59 suspicious lesions in 55 patients were investigated. PET/CT showed an overall sensitivity of 100% and 83% specificity. In 27 suspicious lesions where CT results were inconclusive, PET/CT showed 100% sensitivity and 78% specificity. PET/CT had an impact on patients' treatment in 42 of all 59 cases of suspected recurrence. Overall survival of PET/CT diagnosed recurrence at 20 months and 5 years was 44% and 11%, respectively.

In comparison, a large retrospective study from 2009 analyzed post-recurrence survival rates in 123 stage I NSCLC patients who had received curative surgery between 1980 and 2000 (27). Patients either had local recurrence only or both local recurrence and distant metastases. The overall 1 and 2 year post-recurrence survival rates were 48.0% and 18.7%, respectively (27).

Comparing the survival rate observed in the PET/CT study by Jimenez-Bonilla at 20 months (44%) to the survival rate of the Hung study after 2 years (18.7%) especially when further taking into consideration, that Jimenez-Bonilla's group also included patients at more advanced stages of NSCLC and not only stage I patients, these results are very encouraging: The outcome data of the study by Jimenez-Bonilla are suggesting a positive impact on survival using PET/CT for follow-up in the subgroup of symptomatic patients, with the limitation of the small number of patients enrolled.

Besides the high accuracy of PET/CT and its impact on treatment decisions and survival, another interesting issue—also with regard to cost-effectiveness—is the performance of PET/CT in detecting NSCLC recurrence compared to standard radiological examinations: two recent PET/CT studies prospectively enrolled patients that underwent NSCLC resection and assessed the accuracy of whole body PET/CT in recurrence detection in comparison to standard radiological examinations.

Takenaka *et al.* prospectively compared whole-body PET/CT and standard radiological follow-up examinations in the assessment of recurrence in post-operative NSCLC patients (28). A total of 92 consecutive patients with complete resection were enrolled. The standard radiological examination for distant metastasis assessment performed during the initial and the follow-up examinations and for local recurrence after surgery included contrast-enhanced MRI of the brain, contrast-enhanced whole-body CT

and bone scintigraphy. Final diagnosis of recurrence was based on the results of more than 1 year of follow-up and/or pathological examinations. ROC curves were used to compare the diagnostic capability of the two methods for assessment of post-operative recurrence on a per-patient basis. Sensitivity, specificity and accuracy were determined as well. There were no statistically significant differences in the area under the curve of sensitivity, specificity and accuracy between PET/CT and standard radiological examinations (P>0.05). Hence, the authors concluded that PET/CT can be used for assessment of post-operative recurrence in NSCLC patients with an accuracy as good as that of standard radiological examinations; yet, with the non-negligible advantage of only one examination for the patient instead of three. This factor might play a crucial role for an efficient workflow of large departments that follow-up large patient cohorts.

Onishi *et al.* investigated in a prospective study in 2011 the value of qualitative as well as of quantitative PET/CT for the assessment of post-operative intra- and extrathoracic recurrence in NSCLC patients compared to standard radiological examinations (29). 121 patients who had undergone complete resection were followed-up. Again, ROC analysis was used to compare the methods in their assessment of post-operative recurrence on a per-patient basis. Additionally, optimal cut-off values for FDG uptake measurement at a suspicious site detected on the basis of qualitative PET/CT were determined. Analogous to Takenaka's results, areas under the curve for accuracy of qualitative PET/CT and standard radiological examinations showed no significant differences (P>0.05). At an optimal cut-off value of 2.5, specificity and accuracy of combined quantitative and qualitative PET/CT were significantly higher than of qualitative PET/CT and standard radiological examinations alone (P<0.05). Accuracy in the evaluation of post-operative intra- and extrathoracic recurrence in NSCLC patients by qualitative and/or quantitative PET/CT was consequently rated equivalent to or higher than that of standard radiological examinations.

Kanzaki *et al.* retrospectively examined the clinical value of PET/CT in a large cohort of 241 patients with NSCLC after potentially curative surgery and even proposed that conventional imaging for the detection of extrathoracic metastases in patients who underwent potentially curative surgery for NSCLC can be completely omitted (with the exception of brain MRI) if PET/CT performed at least 6 months after surgery is negative, due to its high negative predictive value (30). 490 PET/CT studies were evaluated in this study. PET/CT correctly diagnosed recurrence in 34 of 35 patients and provided true negative findings in 198 of 206 patients who had no evidence of recurrence (sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of 97%, 96%, 96%, 81%, and 99%, respectively), indicating a high diagnostic performance in this patient group.

Follow-up of NSCLC after non-surgical treatment

The field of non-surgical therapies of primary lung cancer has grown rapidly in recent years. The use of external beam radiotherapy alone as a curative approach to therapy has been abandoned due to the high local recurrence rate of up to 70% (31). In contrary, minimally invasive image-guided therapies using thermal energies such as radiofrequency ablation, microwave ablation or cryoablation, and as the most common one stereotactic body radiation therapy (SBRT) have emerged as non-surgical treatment options (32). Yet, as the tumor is not resected, surveillance of recurrence and especially of tumor margins is crucial and challenging due to post-interventional parenchymal changes.

PET/CT in NSCLC follow-up after (chemo-) radiotherapy

SBRT has become the standard therapeutic approach for inoperable stage I NSCLC. SBRT induces parenchymal damage leading to fibrosis. It can be difficult to differentiate local recurrence from radiation-induced lung opacity. Radiation-induced fibrosis can appear more than 1 year after the end of therapy (33). Furthermore, secondary radiation-induced pneumonitis has been reported within 9 months after SBRT (32).

A small study by Hoopes *et al.* observed on PET scans in a patient cohort of inoperable stage I NSCLC after SBRT treatment a moderately hypermetabolic activity up to 2 years after SBRT (34). This persistent uptake is being attributed to a more persistent inflammation and fibrosis after SBRT compared to fractionated radiotherapy (7).

Takeda *et al.* retrospectively assessed the additional value of dual-time-point maximum standardized uptake values (SUV_{max}) in PET/CT for detection of local recurrence after SBRT of NSCLC in 214 scans of 154 patients (33). Tri-monthly follow-up CT scans were acquired and PET/CT scans were done one year after SBRT or when recurrence was clinically suspected. On early and late images, optimal SUV_{max} thresholds were identified as 3.2 and 4.2. Using these thresholds, sensitivity and specificity

were 100% and 96-98%, respectively. The authors therefore stated that SUV_{max} on PET/CT could predict local recurrence after SBRT for localized NSCLC. In a similar study, Zhang *et al.* also investigated whether the additional assessment of SUV_{max} on PET/CT after SBRT could help to predict local recurrence in 128 patients with stage I NSCLC or isolated recurrent/secondary parenchymal NSCLC patients (35). The authors found a SUV_{max} greater than 5, especially more than 6 months after SBRT to be associated with a higher local recurrence rate, whereas SUV_{max} from PET/CT scans performed within 6 months of treatment were not correlated with local recurrence. With the cutoff SUV_{max} of 5, sensitivity for correct prediction of local recurrence was calculated as 100%, specificity was 91%, positive predictive value was 50% and a negative predictive value of 100% was observed. The authors concluded that quantitative PET/CT was helpful for distinguishing SBRT-induced consolidation from local recurrence.

In contrast, van Loon *et al.* hypothesized that early PET/CT scans 3 months after curative-intended (chemo-) radiotherapy could lead to early detection of progressive disease (PD) amenable for radical treatment (36). Therefore, 100 patients with NSCLC were prospectively evaluated. All patients underwent a planned PET/CT scan 3 months after the start of radiotherapy. 24 patients had PD 3 months post-treatment of whom 16 patients were symptomatic. Yet, no curative treatment could be offered to any of these patients, which limits the impact of PET/CT on treatment decisions in the specific population of symptomatic patients. To 3/8 asymptomatic patients who were diagnosed PD, radical treatment could be offered. Progression—according to the EORTC criteria for PET and the RECIST criteria for CT—potentially amenable for radical therapy was in this study solely detected with PET/CT, but not with CT alone (37,38). Thus, van Loon suggested that asymptomatic patients would profit the most from an early PET/CT scan. However, it has still to be proven that the detection and therapy of early recurrence or PD leads to an overall higher survival in this patient cohort.

PET/CT in NSCLC follow-up after radiofrequency ablation (RFA)

Patients with stage I NSCLC who do not undergo surgical treatment are—besides SBRT—predominantly treated with RFA. The most common pattern of recurrence after RFA is loco-regional recurrence (39). As for SRBT, RFA causes focal changes in the lung parenchyma such as ground glass opacities around the treated tumor site (40). So far, there is no consensus existing on a standard protocol for post-RFA follow up. However, after RFA, continuous follow-up imaging seems to be beneficial to the patients because recurrence has been reported to occur throughout the first 2 years post-treatment (39).

Eradat *et al.* proposes an algorithm of CT follow-up 1-2 months after RFA followed by a PET/CT scan at 3 months thereafter alternated by contrast-enhanced CT every 3 months for 2 years (32). Similarly, the group by Beland is proposing contrast-enhanced CT at 3 weeks and 3 months followed by PET/CT at 6 months; alternating CT and PET/CT examinations then performed every 3 months (39).

Cost effectiveness

In spite of the experiences of PET/CT as a helpful staging imaging modality in the treatment of NSCLC and encouraging results concerning the accuracy of PET/CT in detecting recurrence reported in the few follow-up studies performed so far, and mostly enrolling patients with follow-up after surgical therapy, the 2nd edition of the American College of Chest Physicians (ACCP) evidence-based guidelines on the follow-up and surveillance of lung cancer patients did not recommend PET/CT for standard surveillance. The reason given for this decision was a lack of evidence that follow-up PET/CT improves either survival rates or quality of life of NSCLC patients (11).

In the only cost-effectiveness study of NSCLC follow-up so far with 100 patients, van Loon *et al.* prospectively compared long-term cost-effectiveness of 3 different follow-up strategies, all starting 3 months after therapy. The authors either performed a PET/CT scan, a chest CT scan or conventional follow-up with a chest radiograph (41). Cost-effectiveness was expressed in incremental cost-effectiveness ratios (ICERs), calculating the incremental costs per quality adjusted life year (QALY) gained. Both PET/CT- and CT-based follow-up were calculated to be more costly but at the same time also more effective than a chest radiograph follow-up. CT-based follow-up resulted in an incremental cost-effectiveness ratio (ICER) of euro 264.033 per QALY gained compared to a chest radiograph, whereas for PET/CT-based follow-up, the ICER was euro 69.086 per QALY gained. A subgroup analysis of asymptomatic patients undergoing PET/CT resulted in an ICER of euro 42.265 per QALY gained compared to

chest radiograph follow-up. Assuming a ceiling ratio of euro 80.000, PET/CT-based follow-up was calculated to have the highest probability of being cost-effective (73%). The authors therefore concluded that a PET/CT scan 3 months after curative-intended (chemo-) radiotherapy is a potentially cost-effective follow-up method, and is more cost-effective than CT alone. Performing PET/CT scans only in asymptomatic patients seems to be equally effective and even more cost-effective.

Conclusions

Current guidelines do not recommend the use of PET/CT for assessment of NSCLC recurrence. Recommendations of different authors concerning the initiation and frequency of follow-up with PET/CT scans are largely varying between post-surgical NSCLC follow-up and surveillance of patients treated with radiotherapy and radiofrequency ablation. Most studies on NSCLC follow-up were conducted in post-surgical stage I NSCLC patients and PET/CT was mostly performed annually, starting one year after surgical treatment.

Concerning follow-up after non-surgical potentially curative treatment of NSCLC patients, controversial results have been published on the optimal timing of the first PET/CT scans. Different algorithms from different working groups schedule the first PET/CT scan from 3 months on to one year in this patient cohort. Concerning follow-up after RFA, very few studies on follow-up of these patients have been published so far. In two existing follow-up algorithms, PET/CT is performed for the first time 3 months and 6 months after treatment, respectively.

The additional value of quantitative PET measurements in prediction of recurrence has been suggested in the evaluation of thoracotomy scars as well as in the surveillance of patients treated with SBRT.

Despite encouraging results of high accuracy of PET/CT for the assessment of NSCLC recurrence and reports of impact on changes in patient management, controversy exists about whether to follow-up symptom-based or whether to screen on a routinely basis independently of symptoms and clinical findings (10,36).

Currently, PET/CT is rather used in symptomatic patients with suspicion of recurrence. However, impact on therapeutic management was mainly reported for asymptomatic patients with regard to salvage therapies. Nevertheless, high quality evidence is still lacking that intensive surveillance programs and earlier detection of recurrence leads to a survival benefit and despite of one encouraging cost-effectiveness study, incremental costs of integrated PET/CT scanners might probably play a role in decisions for or against surveillance guidelines including PET/CT to come up (41-43).

In the future, large-scale randomized trials should predominantly focus on the impact of PET/CT on treatment outcome. Furthermore, optimal starting point and frequency of follow-up PET/CT scans should be determined, especially in patients treated with the emerging minimally-invasive image-guided therapies and lastly the utility of quantitative PET/CT measurements for recurrence detection has to be clarified.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Caulo A, Mirsadraee S, Maggi F, et al. Integrated imaging of non-small cell lung cancer recurrence: CT and PET-CT findings, possible pitfalls and risk of recurrence criteria. Eur Radiol 2012;22:588-606.
3. Hyun SH, Choi JY, Kim K, et al. Volume-based parameters of (18)F-fluorodeoxyglucose positron emission tomography/computed tomography improve outcome prediction in early-stage non-small cell lung cancer after surgical resection. Ann Surg 2013;257:364-70.
4. Pieterman RM, van Putten JW, Meuzelaar JJ, et al. Preoperative staging of non-small-cell lung cancer with positron-emission tomography. N Engl J Med 2000;343:254-61.
5. Marom EM, McAdams HP, Erasmus JJ, et al. Staging non-small cell lung cancer with whole-body PET. Radiology 1999;212:803-9.
6. Chao F, Zhang H. PET/CT in the staging of the non-small-cell lung cancer. J Biomed Biotechnol 2012;2012:783739.
7. Cuaron J, Dunphy M, Rimner A. Role of FDG-PET scans in staging, response assessment, and follow-up care for non-small cell lung cancer. Front Oncol 2012;2:208.
8. Kratochwil C, Haberkorn U, Giesel FL. PET/CT for diagnostics and therapy stratification of lung cancer. Radiologe 2010;50:684-91.
9. De Wever W, Stroobants S, Coolen J, et al. Integrated

PET/CT in the staging of nonsmall cell lung cancer: technical aspects and clinical integration. Eur Respir J 2009;33:201-12.
10. Keidar Z, Haim N, Guralnik L, et al. PET/CT using 18F-FDG in suspected lung cancer recurrence: diagnostic value and impact on patient management. J Nucl Med 2004;45:1640-6.
11. Rubins J, Unger M, Colice GL, et al. Follow-up and surveillance of the lung cancer patient following curative intent therapy: ACCP evidence-based clinical practice guideline (2nd edition). Chest 2007;132:355S-367S.
12. Ponn RB. Lightning can strike twice: second primary lung cancers. Chest 2000;118:1526-9.
13. Pfannenberg AC, Aschoff P, Brechtel K, et al. Low dose non-enhanced CT versus standard dose contrast-enhanced CT in combined PET/CT protocols for staging and therapy planning in non-small cell lung cancer. Eur J Nucl Med Mol Imaging 2007;34:36-44.
14. Bar-Shalom R, Yefremov N, Guralnik L, et al. Clinical performance of PET/CT in evaluation of cancer: additional value for diagnostic imaging and patient management. J Nucl Med 2003;44:1200-9.
15. Shim SS, Lee KS, Kim BT, et al. Focal parenchymal lung lesions showing a potential of false-positive and false-negative interpretations on integrated PET/CT. AJR Am J Roentgenol 2006;186:639-48.
16. Heyneman LE, Patz EF. PET imaging in patients with bronchioloalveolar cell carcinoma. Lung Cancer 2002;38:261-6.
17. Hany TF, Heuberger J, von Schulthess GK. Iatrogenic FDG foci in the lungs: a pitfall of PET image interpretation. Eur Radiol 2003;13:2122-7.
18. Ettinger DS, Akerley W, Bepler G, et al. Non-small cell lung cancer. J Natl Compr Canc Netw 2010;8:740-801.
19. Yumuk PF, Mohammed N, Maat AP, et al. How do lung cancer specialists follow their patients with stage III non-small cell lung cancer (NSCLC) after definitive treatment? A short report. Eur J Cancer 2012;48:2163-5.
20. Inoue T, Kim EE, Komaki R, et al. Detecting recurrent or residual lung cancer with FDG-PET. J Nucl Med 1995;36:788-93.
21. Opoka L, Szołkowska M, Podgajny Z, et al. Assessment of recurrence of non-small cell lung cancer after therapy using CT and Integrated PET/CT. Pneumonol Alergol Pol 2013;81:214-20.
22. Gorenberg M, Bar-Shalom R, Israel O. Patterns of FDG uptake in post-thoracotomy surgical scars in patients with lung cancer. Br J Radiol 2008;81:821-5.
23. Choi SH, Kim YT, Kim SK, et al. Positron emission tomography-computed tomography for postoperative surveillance in non-small cell lung cancer. Ann Thorac Surg 2011;92:1826-32; discussion 1832.
24. Toba H, Sakiyama S, Otsuka H, et al. 18F-fluorodeoxyglucose positron emission tomography/computed tomography is useful in postoperative follow-up of asymptomatic non-small-cell lung cancer patients. Interact Cardiovasc Thorac Surg 2012;15:859-64.
25. Cho S, Lee EB. A follow-up of integrated positron emission tomography/computed tomography after curative resection of non-small-cell lung cancer in asymptomatic patients. J Thorac Cardiovasc Surg 2010;139:1447-51.
26. Jiménez-Bonilla JF, Quirce R, Martínez-Rodríguez I, et al. Diagnosis of recurrence and assessment of post-recurrence survival in patients with extracranial non-small cell lung cancer evaluated by 18F-FDG PET/CT. Lung Cancer 2013;81:71-6.
27. Hung JJ, Hsu WH, Hsieh CC, et al. Post-recurrence survival in completely resected stage I non-small cell lung cancer with local recurrence. Thorax 2009;64:192-6.
28. Takenaka D, Ohno Y, Koyama H, et al. Integrated FDG-PET/CT vs. standard radiological examinations: comparison of capability for assessment of postoperative recurrence in non-small cell lung cancer patients. Eur J Radiol 2010;74:458-64.
29. Onishi Y, Ohno Y, Koyama H, et al. Non-small cell carcinoma: comparison of postoperative intra- and extrathoracic recurrence assessment capability of qualitatively and/or quantitatively assessed FDG-PET/CT and standard radiological examinations. Eur J Radiol 2011;79:473-9.
30. Kanzaki R, Higashiyama M, Maeda J, et al. Clinical value of F18-fluorodeoxyglucose positron emission tomography-computed tomography in patients with non-small cell lung cancer after potentially curative surgery: experience with 241 patients. Interact Cardiovasc Thorac Surg 2010;10:1009-14.
31. Qiao X, Tullgren O, Lax I, et al. The role of radiotherapy in treatment of stage I non-small cell lung cancer. Lung Cancer 2003;41:1-11.
32. Eradat J, Abtin F, Gutierrez A, et al. Evaluation of treatment response after nonoperative therapy for early-stage non-small cell lung carcinoma. Cancer J 2011;17:38-48.
33. Takeda A, Kunieda E, Fujii H, et al. Evaluation for local failure by 18F-FDG PET/CT in comparison with CT findings after stereotactic body radiotherapy (SBRT)

for localized non-small-cell lung cancer. Lung Cancer 2013;79:248-53.
34. Hoopes DJ, Tann M, Fletcher JW, et al. FDG-PET and stereotactic body radiotherapy (SBRT) for stage I non-small-cell lung cancer. Lung Cancer 2007;56:229-34.
35. Zhang X, Liu H, Balter P, et al. Positron emission tomography for assessing local failure after stereotactic body radiotherapy for non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2012;83:1558-65.
36. van Loon J, Grutters J, Wanders R, et al. Follow-up with 18FDG-PET-CT after radical radiotherapy with or without chemotherapy allows the detection of potentially curable progressive disease in non-small cell lung cancer patients: a prospective study. Eur J Cancer 2009;45:588-95.
37. Young H, Baum R, Cremerius U, et al. Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. Eur J Cancer 1999;35:1773-82.
38. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205-16.
39. Beland MD, Wasser EJ, Mayo-Smith WW, et al. Primary non-small cell lung cancer: review of frequency, location, and time of recurrence after radiofrequency ablation. Radiology 2010;254:301-7.
40. Bojarski JD, Dupuy DE, Mayo-Smith WW. CT imaging findings of pulmonary neoplasms after treatment with radiofrequency ablation: results in 32 tumors. AJR Am J Roentgenol 2005;185:466-71.
41. van Loon J, Grutters JP, Wanders R, et al. 18FDG-PET-CT in the follow-up of non-small cell lung cancer patients after radical radiotherapy with or without chemotherapy: an economic evaluation. Eur J Cancer 2010;46:110-9.
42. Crinò L, Weder W, van Meerbeeck J, et al. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v103-15.
43. Benamore R, Shepherd FA, Leighl N, et al. Does intensive follow-up alter outcome in patients with advanced lung cancer? J Thorac Oncol 2007;2:273-81.

Cite this article as: Sudarski S, Henzler T, Schoenberg SO. Post-therapeutic positron emission tomography/computed tomography for early detection of non-small cell lung cancer recurrence. Transl Lung Cancer Res 2013;2(4):295-303. doi: 10.3978/j.issn.2218-6751.2013.05.02

New TNM classification: achievements and hurdles

Peter Goldstraw[1,2]

[1]Thoracic Surgery, Royal Brompton Hospital, London, UK; [2]Thoracic Surgery, National Heart and Lung Institute, Imperial College, London, UK
Correspondence to: Peter Goldstraw, MB, FRCS, Honorary Consultant in Thoracic Surgery, Emeritus Professor of Thoracic Surgery, President of International Association for the Study of Lung Cancer (IASLC). Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK.
Email: p.goldstraw@rbht.nhs.uk.

Abstract: The 7th edition of TNM for Lung Cancer represented a major advance from previous editions, in the process of revision, the size and breadth of the data base used, its international character, the intensity of the analysis and the critical nature of the internal and external validation undertaken before its launch in January 2010. This all came about by the involvement of the International Association for the Study of Lung Cancer (IASLC), which assumed the role previously performed by Dr. Mountain, of developing data-driven revisions for the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC). In taking on this task the IASLC made the global lung cancer community aware of the limitations of previous revisions and now stand accountable and subject to the same scrutiny. In this article we describe the achievements of the IASLC TNM and Prognostic Factors Committee, but also the shortcomings of the 7th edition, as an essential step towards rectifying deficiencies and further improving the classification in future revisions.

Keywords: Lung cancer; prognostic factors; staging

Submitted Jul 22, 2013. Accepted for publication Jul 24, 2013.
doi: 10.3978/j.issn.2218-6751.2013.07.02
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.07.02

Introduction

To properly understand the achievements of the 7th edition of TNM for Lung Cancer it is necessary to give a brief history of the TNM Classification itself. A more detailed history of this topic is available elsewhere (1).

A system to describe the anatomical extent of a cancer using the "T", "N" and "M" descriptors was developed by Dr. Pierre Denoix, a surgical oncologist at the Institut Gustave-Roussy in Paris, and evolved over a series of articles in the 1940s and early 50s (2). The first international classification of malignant disease based on TNM was published in 1968 by the Union Internationale Contre le Cancer (UICC), which now prefers to be known by the English version of its title, Union for International Cancer Control (3), lung cancer being included under the section for "other sites". This initial attempt at classification was arrived at by discussion and consensus, there being no data available. The American Joint Committee for Cancer Staging and End Results Reporting, now the American Joint Committee on Cancer (AJCC), orchestrated the collection of data through its Task Force on Lung Cancer. The analysis of a data base of 2,155 lung cancer cases resulted in "A system for the clinical staging of lung cancer" reported by Drs. Mountain, Carr and Anderson in 1973 (4). This formed the basis of the 2nd edition of the UICC TNM Classification of Malignant Disease published in 1975 (5) and the 1st edition of the AJCC Manual for Cancer Staging published in 1977 (6). Thereafter Dr. Mountain developed his own data base which informed future revisions up to and including the 5th edition published in 1997 (7,8), by which time the data base had accumulated 5,319 cases. There were no changes in the lung cancer classification in the 6th edition (9,10).

At a workshop sponsored by the International Association for the Study of Lung Cancer (IASLC) and held at the Brompton Hospital in London in 1996 Dr. Mountain presented his revisions for the 5th edition of TNM which had been approved by the UICC and AJCC and were due to

come into force within a few weeks. The deficiencies of the underlying data were discussed: a relatively small number of cases, accumulated over 20 years, predominantly cases referred for surgical consideration and mostly derived from a single institution. The workshop attendees recommended "the establishment by the IASLC of a staging committee" to "represent the IASLC in negotiations with UICC and AJCC with regard to future revisions of classification" (11).

Achievements

With this introduction the achievements of the IASLC Staging and Prognostic Factors Committee can be put into perspective and enumerated.

In 1998, using the membership of the IASLC and pump-priming funding from the IASLC, a committee was established with members from all specialities involved in the treatment of lung cancer and from across the globe. The commitment of these early members was such that they largely self-funded their involvement for the first 2 years of the project (12).

High level support from the officers and head office of the IASLC secured long-term funding from philanthropic partners in the pharmaceutical industry. This provided administrative support for the committee and allowed us to contract with a not-for-profit data centre in Seattle, Cancer Research And Biostatistics (CRAB) with expertise in oncology and the collection and analysis of data from multi-centre, international studies.

Members of the lung cancer community supported this ambitious project by donating over 100,000 cases of lung cancer collected between 1990 and 2000. This data originated from 46 centres in over 20 countries around the globe and included cases treated by all modalities of care, including bi-modality and multi-modality regimens. Such a large data base allowed intensive internal and external validation, unprecedented in any previous revision (12).

As the proposals of each sub-committee were developed the data, analysis and proposals were published in the official journal of the IASLC, the Journal of Thoracic Oncology (JTO). These discussion articles were made available without subscription so that members and non-members were aware of the proposals and to enable an informed debate within national TNM committees. Once approved by the UICC and AJCC the IASLC produced site-specific educational materials (13) which were available at the 13th World Conference on Lung Cancer in September 2009. These contained precise figures illustrating each T, N and M descriptor. Never before had the global lung cancer community been so well informed of the pending changes for a new edition of TNM in lung cancer or been better prepared for its introduction.

The 7th edition of TNM for lung cancer was delivered, fully developed, on time and on budget to the UICC and AJCC. It complied with the requirements of both organisations with regard to process and timelines and was adopted in its entirety and without change. It came in to force on the 1st of January 2010 (14,15). The new edition retained the previous size cut-point of 3 cm separating T1 from T2 tumors. New size cut-points were introduced; 2 cm to separate T1a from T1b, 5 cm to separate T2a from T2b and 7 cm to separate T2b from T3 tumors. Size therefore became a T3 descriptor for the first time. There were changes to other T (16) and M categories (17). Cancers associated with additional tumor nodules (metastases) in the same lobe as the primary became T3, whilst those in other ipsilateral lobes became T4. Cancers associated with malignant pleural or pericardial effusions or nodules were moved from the T to an M category, linked with cases in which there were metastases in the opposite lung, as M1a. Distant haematogenous and nodal metastases became M1b. The N categories remained unchanged but for the first time these had been validated in an international data base of cases treated by all modalities of care (18). Some changes were also made in the resultant stage groupings (19). All of these changes were data driven and validated (20), aligning stage with prognosis more accurately than ever before. The use of TNM was shown to have prognostic value in the 13,000 cases of small-cell lung cancer (SCLC) within the database, in cases clinically staged (21) and the smaller number of surgically treated cases of SCLC in which pathological stage was available (22). Clinicians and pathologists were shown to have been correct in using the TNM classification for carcinoid tumors, although this was never previously sanctioned, and bronchopulmonary carcinoid tumors were included within the 7th edition of the TNM classification for the first time (23).

Additional issues, raised in discussions within the committee and in the literature review undertaken by the UICC, were addressed by consensus and, where available, by study of the available literature. The previous features used to distinguish pulmonary metastases from synchronous primary tumors were thought to have lagged behind developments in morphology, immunohistochemistry and molecular studies. The definition was therefore expanded and the role of the pathologist and these technological improvements

were emphasised. An internationally agreed definition of "visceral pleural invasion" (VPI) was developed (24) adapting a system long in use by the Japan Lung Cancer Society (25) and also espoused by Hammar (26). The inconsistencies between the nodal map developed by Naruke and the Japan Lung Cancer Society (27) and that of Mountain and Dresler (28) were reconciled by international agreement and defined by precise anatomical boundaries (29). This led to the UICC and AJCC recognising the IASLC nodal map and its accompanying table of definitions as the recommended means to describe regional lymph node spread for lung cancer. It then became possible to re-introduce minimum requirements for lymph node evaluation at surgery and subsequent pathological examination as part of the expanded definition of a complete, R0, resection (30). The concept of nodal "zones" was developed, covering larger anatomical areas than individual "stations" in the hope that this would assist oncologists, treating patients with bulky nodal disease which could encompass more than one station, and widen the relevance of nodal mapping beyond mere surgeons. A version of the IASLC nodal map is shown in *Figure 1* and the table of definitions in *Table 1*.

So much for the achievements. What "hurdles" were encountered and which are left for the next phase of the IASLC TNM and Prognostic Factors Committee?

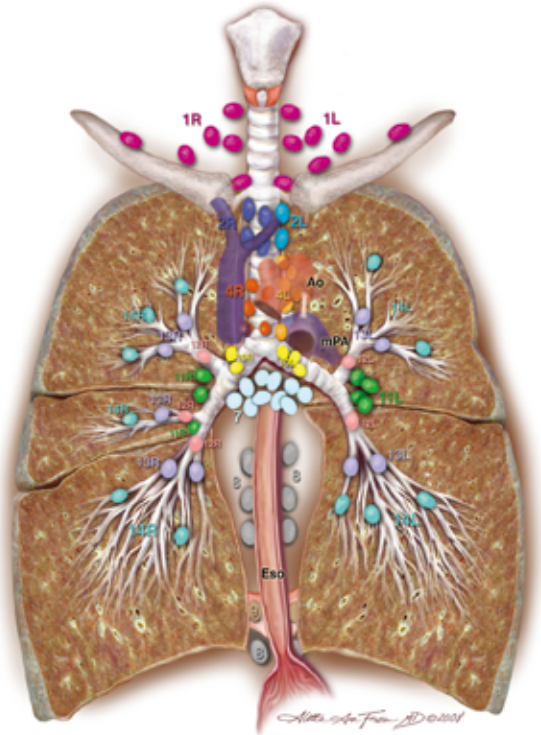
Hurdles

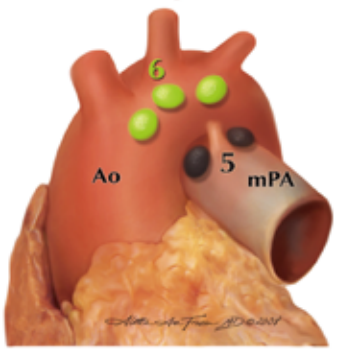
During the evolution of the IASLC proposals for the revision of the 6th edition we were aware of some limitations of our database and had to make difficult decisions. The solutions we settled upon and the retrospective nature of the data base have created some issues for those now using the 7th edition of the TNM Classification for Lung Cancer.

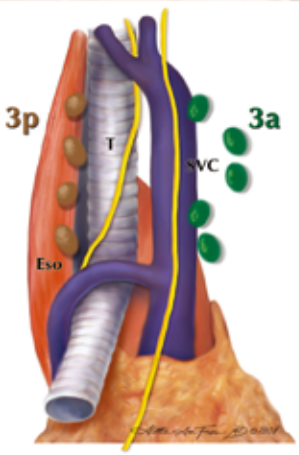
A dilemma was encountered as we sought to accommodate sub-groups of T or M cases that had been identified to have a prognosis that differed significantly from the rest of the cases within that category. If we kept the group within the original category and identified it by new alphabetical subscripts retrospective compatibility would be feasible and cases within existing data bases could be translated from the TNM version by which they were originally classified to the new edition of TNM. This had been managed with all previous revisions. Unfortunately it soon became apparent that the number of sub-categories required to accommodate all of the changes would exceed 20 and the number of resultant stage groupings would be in the region of 180 (19). This was clearly impractical with the technologies available globally at that time. For this reason it was reluctantly decided to move these sub-groups to other T and M categories which shared a similar prognosis, keeping the numbers of categories manageable but sacrificing backward compatibility for existing data bases, including our data donors!

A major limitation of our data was its retrospective nature. We chose to accept the short-coming of such data as the only way we could collect sufficient cases to inform revisions of the classification within the timelines dictated by the UICC and AJCC. However, the limitations of retrospective data collection brought with it several frustrations. Whilst in all cases we knew which category of T, N and M formed the basis of the clinical or pathological stage grouping, in only a minority did we know the descriptor which resulted in the case being assigned to that category and in few did we know that all other descriptors in that category were absent. For example, we would know that the case was assigned to T2, but only in a minority would we be told that this was because the tumor was 3 cm or larger. If this was so, we were rarely given information about the presence or absence of VPI or the proximity of the tumor to established anatomical levels on bronchoscopy. In addition we were not always told how was the size was measured, whether on chest radiography or CT, and in which dimension, a single reading or more, and in which axis? Similarly with no international guidance as to a definition of VPI we were unsure how this was assessed by individual pathologists at that time, and whether an elastic stain had been utilized to clarify this involvement? We had to accept that such cases were recorded in the data base as "VPI present". In accepting these limitations we should at least recognize that the same issues almost certainly applied to the data that informed all previous revisions. Although the 7th edition placed added emphasis on size, by including additional size cut-points and making size >7 cm a T3 descriptor, the problem of how best to measure size was just as pertinent for the 3 cm cut-point which had divided T1 and T2 tumors since the mid 1970s (4). In the prospective data set established by the IASLC Staging and Prognostic Factors Committee (31) to help inform future revisions of TNM we are collecting data on the largest dimension and the imaging modality used to measure size. In addition we ask for the status of all descriptors within each T and M category. Such data will allow us to investigate issues such as the interaction of VPI and size on prognosis.

A new category, T1a, has been created for very small







Supraclavicular zone
- 1 Low cervical, supraclavicular, and sternal notch nodes

SUPERIOR MEDIASTINAL NODES

Upper zone
- 2R Upper Paratracheal (right)
- 2L Upper Paratracheal (left)
- 3a Prevascular
- 3p Retrotracheal
- 4R Lower Paratracheal (right)
- 4L Lower Paratracheal (left)

AORTIC NODES

AP zone
- 5 Subaortic
- 6 Para-aortic (ascending aorta or phrenic)

INFERIOR MEDIASTINAL NODES

Subcarinal zone
- 7 Subcarinal

Lower zone
- 8 Paraesophageal (below carina)
- 9 Pulmonary ligament

N1 NODES

Hilar/Interlobar zone
- 10 Hilar
- 11 Interlobar

Peripheral zone
- 12 Lobar
- 13 Segmental
- 14 Subsegmental

Figure 1 The IASLC Nodal Map, reconciling the discrepancies between the Mountain/Dressler and Naruke maps. Each nodal station is colour-coded and listed. Those within a distinct nodal "zone" are grouped within the box. Reprinted courtesy of the International Association for the Study of Lung Cancer and with permission of Aletta Frazier, MD. Copyright ©2009, 2010 Aletta Ann Frazier, MD.

Table 1 The table of definitions for the nodal stations in the IASLC nodal map

Nodal station	Description	Definition
#1 (left/right)	Low cervical, supraclavicular and sternal notch nodes	Upper border: lower margin of cricoid cartilage Lower border: clavicles bilaterally and, in the midline, the upper border of the manubrium, 1R designates right-sided nodes, 1L, left-sided nodes in this region #L1 and #R1 limited by the midline of the trachea
#2 (left/right)	Upper paratracheal nodes	2R: Upper border, apex of the right lung and pleural space and, in the midline, the upper border of the manubrium; Lower border, intersection of caudal margin of innominate vein with the trachea 2L: Upper border, apex of the left lung and pleural space and, in the midline, the upper border of the manubrium; Lower border, superior border of the aortic arch As for #4, in #2 the oncologic midline is along the left lateral border of the trachea
#3	Pre-vascular and retrotracheal nodes	3a: prevascular On the right Upper border: apex of chest Lower border: level of carina Anterior border: posterior aspect of sternum Posterior border: anterior border of superior vena cava On the left Upper border: apex of chest Lower border: level of carina Anterior border: posterior aspect of sternum Posterior border: left carotid artery 3p: retrotracheal Upper border: apex of chest Lower border: carina
#4 (left/right)	Lower paratracheal nodes	4R: includes right paratracheal nodes, and pretracheal nodes extending to the left lateral border of trachea Upper border: intersection of caudal margin of innominate vein with the trachea Lower border: lower border of azygos vein 4L: includes nodes to the left of the left lateral border of the trachea, medial to the ligamentum arteriosum Upper border: upper margin of the aortic arch Lower border: upper rim of the left main pulmonary artery
#5	Subaortic (aorto-pulmonary window)	Subaortic lymph nodes lateral to the ligamentum arteriosum Upper border: the lower border of the aortic arch Lower border: upper rim of the left main pulmonary artery
#6	Para-aortic nodes (ascending aorta or phrenic)	Lymph nodes anterior and lateral to the ascending aorta and aortic arch Upper border: a line tangential to the upper border of the aortic arch Lower border: the lower border of the aortic arch
#7	Subcarinal nodes	Upper border: the carina of the trachea Lower border: the upper border of the lower lobe bronchus on the left; the lower border of the bronchus intermedius on the right

Table 1 (*continued*)

Table 1 (*continued*)

Nodal station	Description	Definition
#8 (left/right)	Para-esophageal nodes (below carina)	Nodes lying adjacent to the wall of the esophagus and to the right or left of the midline, excluding subcarinal nodes Upper border: the upper border of the lower lobe bronchus on the left; the lower border of the bronchus intermedius on the right Lower border: the diaphragm
#9 (left/right)	Pulmonary ligament nodes	Nodes lying within the pulmonary ligament Upper border: the inferior pulmonary vein Lower border: the diaphragm
#10 (left/right)	Hilar nodes	Includes nodes immediately adjacent to the mainstem bronchus and hilar vessels including the proximal portions of the pulmonary veins and main pulmonary artery Upper border: the lower rim of the azygos vein on the right; upper rim of the pulmonary artery on the left Lower border: interlobar region bilaterally
#11	Interlobar nodes	Between the origin of the lobar bronchi #11s: between the upper lobe bronchus and bronchus intermedius on the right #11i: between the middle and lower lobe bronchi on the right Optional sub-categories
#12	Lobar nodes	Adjacent to the lobar bronchi
#13	Segmental nodes	Adjacent to the segmental bronchi
#14	Sub-segmental nodes	Adjacent to the subsegmental bronchi

tumors, those no larger than 2 cm. The prognosis of these cases when associated with N0 disease is statistically more favorable than tumors greater than 2 cm but no larger than 3 cm, T1b. Does this have implications for structured observation in Low-Dose CT (LDCT) screening programmes? Does it suggest that sub-lobar resection may be sufficient for such cases? There are many shortcomings in such assumptions. The proportion of such cases in our data base which were screen-detected is unknown, but this will be clarified within the prospective data base. The issue regarding sub-lobar resection has become more topical with the increasing use of LDCT screening. Many of the cancers discovered with such screening are adenocarcinomas often with a proportion of ground-glass opacity (GGO). The classification of such lesions has been clarified in the new IASLC/ATS/ERS Classification for adenocarcinoma (32). The present role of sub-lobar resection has been summarized in a consensus report from the IASLC Strategic Screening Advisory Committee (33), which clearly favors anatomical segmentectomy over wedge resection and sets out the specific situation in which this is appropriate as an alternative to lobectomy, with carefully crafted caveats.

"It is recommended that anatomical segmentectomy be reserved for the CT screening detected pure GGO lesions or part-solid lesions below 2 cm located in the peripheral third of the lung, after frozen section of N1 and N2 lymph nodes have confirmed the T1aN0M0 status. In addition frozen section or cytological evaluation of resection margins is recommended."

There are 2 ongoing trials assessing the role of sub-lobar resection in small peripheral cancers, one in the United States, CALGB 140503, and another in Japan, JCOG 0802. These should provide definitive advice when the results become available (34).

In some situations we have moved descriptors between T and M categories which may result in a case being assigned to a different stage grouping from that of earlier editions of TNM. One such area concerned the classification of cancers associated with "additional tumor nodules" in the lobe of

the primary, moved from T4 to T3 in the 7th edition, and in other ipsilateral lobes, moved from M1 to T4. Could we be sure that such changes were appropriate for all such cases? The answer is almost certainly not, but does it only apply to cases with a single additional nodule, or those with several or many? Does it only apply to so called "satellite" nodules adjacent to the primary tumor? Can we be sure that none of these cases in our data base were actually synchronous primary tumors? Clarification of these questions will have to await an analysis of from prospective data bases such as the one the IASLC has established. However we have now clarified that "additional tumor nodules" are "pulmonary metastases", and improved the definition of "synchronous primary tumors". If the management of a cases hinges on the distinction between additional nodules being metastases or synchronous primary tumors then biopsy of more than just the main lesion may be necessary.

Stage does not dictate treatment, it is only one factor in this decision, acting to "aid the clinician in the planning of treatment" (14). Inevitably however when stage grouping change there is an understandable question as to whether this should influence treatment algorithms. Several such changes occurred in the 7th edition:

(I) Tumors larger than 5 cm have been re-classified as T2b, and those >7 cm as T3. These cases, when associated with the N0 category were previously stage IB but are now upstaged to IIA and IIB respectively. Clinical trials have now established that adjuvant chemotherapy is beneficial after complete resection of stage II cases (35). Should these "new" stage II cases, large tumors which are N0, be treated along these lines? We should remind ourselves that these trials were conducted on stage II cases associated with N1 disease and must await the results of appropriate trials addressing the issue of adjuvant chemotherapy in large, node negative tumors, stratifying by size using the 7th edition cut-points (36);

(II) The classification of T4 tumors associated with invasion of adjacent structures has not changed but the stage grouping assigned to such cases when T4 is associated with the N0 and N1 categories has been down-staged to stage IIIA. Should all such cases now be considered for multimodality regimens which include surgery? One has to be cautious about such sweeping statements. Most surgical series of "resectable" T4 cases have been small, with highly selected cases collected over decades. Many such cases did not go to theatre with a classification of T4 but were thought to be less extensive and only found to be "T4", "resectable" and node negative at surgery. The pre-operative assessment of "resectability" is always difficult, especially after induction chemotherapy and even more so after induction chemo-radiotherapy. It is also a very personal decision and one which cannot easily be conveyed by objective criteria. Until more data is accrued one can only advise that such advanced cases be assessed at specialist centres with experience in making these difficult decisions;

(III) The new descriptors appropriate for the classification of cases with additional tumor nodules in the lobe of the primary, and other ipsilateral lobes have already been alluded to and the reservations concerning this assignment mentioned. However, these changes have also resulted in down-staging in some circumstances. Those cases with additional tumor nodules in the lobe of the primary, classified as T3, when associated with the N0 category have been down-staged to stage IIB. One would expect that these cases would indeed be treated by surgery in patients who are sufficiently fit to withstand lobectomy as the additional lesions do not extend the extent of resection and subsequent pathological classification may show one (or more) to be synchronous primaries. The role of adjuvant chemotherapy will arise but at present there is no data to inform this decision. Such T3 cases associated with N1 and N2 categories, and the T4 lesions due to additional tumor nodules in other ipsilateral lobes associated with N0 and N1 categories have been down-staged to stage IIIA. Once again this stage has traditionally been the middle ground where most discussion at multi-disciplinary meetings is concentrated. One can only suggest that these cases now be subjected to the same deliberations and that treatment options include a discussion of surgery as part of the multi-modality care in appropriate cases. It is unlikely that trials will prove feasible in these cases and once again data from prospectively collected data with comprehensive data sets may help these decisions in future.

Conclusions

The 7th edition of TNM for lung cancer was an enormous improvement when compared to earlier editions. The process of revision has been dramatically altered and colleagues with data from around the globe have been able to influence the classification we all use in everyday practice. The new edition is based upon international data, on patients treated by all modalities of care and accrued over a relatively short period, during which time treatment and investigative algorithms were relatively stable. Stage has been aligned with prognosis more closely than ever

before. However, as outlined in this article, it is far from perfect. We responded to criticisms of earlier revisions and have taken a giant step forwards with the 7th edition. The IASLC is now committed, on behalf of its members and the global lung cancer community, to the long-term financial and scientific burden of improving future revisions and expanding our activities to cover other thoracic malignancies, including mesothelioma and thymic tumors. The 8th edition of the TNM Classification of Malignant Tumors is scheduled to be enacted in September 2015. The IASLC Staging and Prognostic Factors Committee is well advanced in its preparation and has accumulated an even larger data base than that previously used for the 7th edition. Once our proposals have been identified and validated they will again be released for scrutiny in discussion articles in JTO. Readers are encouraged to become members of the IASLC to assist in this endeavor, ensure they are kept abreast of impending changes and be in a position to obtain the educational materials the IASLC plans to launch at the 16th World Conference on Lung Cancer in Denver, September 2015.

Acknowledgements

Disclosure: The author declares no conflict of interest.

References

1. Goldstraw P. History of TNM in lung cancer. In: Goldstraw P. eds. IASLC staging manual in thoracic oncology (1st Edition). Florida: Editorial Rx, 2009:16-29.
2. Denoix PF. The TNM staging system. Bull Inst Nat Hyg 1952;7:743.
3. UICC. TNM classification of malignant tumours 1st, Geneva, 1968.
4. Mountain CF, Carr DT, Anderson WA. A system for the clinical staging of lung cancer. Am J Roentgenol Radium Ther Nucl Med 1974;120:130-8.
5. UICC. TNM classification of malignant tumours 2nd, Geneva, 1975.
6. American Joint Committee on cancer staging and end results reporting. AJCC cancer staging manual (1st Edition). Philadelphia: Lippincott-Raven, 1977.
7. UICC. eds. TNM classification of malignant tumours (5th Edition). Berlin: Springer Verlag, 1997.
8. American Joint Committee on Cancer. AJCC Cancer Staging Manual. In: Fleming ID, Cooper JS, Henson DE, et al. eds. 5th. Philadelphia: Lipincott Raven, 1997.
9. American Joint Committee on Cancer. eds. AJCC cancer staging manual (6th Edition). New York: Springer, 2002.
10. UICC International Union Against Cancer. eds. TNM classification of malignant tumours (6th Edition). New York: Wiley-Liss, 2002.
11. Goldstraw P. Report on the international workshop on intrathoracic staging. London, October 1996. Lung Cancer 1997;18:107-11.
12. Goldstraw P, Crowley JJ, IASLC International Staging Project. The international association for the study of lung cancer international staging project on lung cancer. J Thorac Oncol 2006;1:281-6.
13. Goldstraw P. eds. IASLC staging handbook in thoracic oncology (1st Edition). Florida: Editorial Rx Press, 2009.
14. Sobin LH, Gospodarowicz MK, Wittekind C. eds. UICC TNM classification of malignant tumours (7th Edition). New York: Wiley-Liss, 2009.
15. Edge SB, Byrd DR, Compton CC, et al. eds. AJCC cancer staging manual (7th Edition). New York: Springer, 2009.
16. Rami-Porta R, Ball D, Crowley J, et al. The IASLC lung cancer staging project: proposals for the revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. J Thorac Oncol 2007;2:593-602.
17. Postmus PE, Brambilla E, Chansky K, et al. The IASLC lung cancer staging project: proposals for revision of the M descriptors in the forthcoming (seventh) edition of the TNM classification of lung cancer. J Thorac Oncol 2007;2:686-93.
18. Rusch VW, Crowley J, Giroux DJ, et al. The IASLC lung cancer staging project: proposals for the revision of the N descriptors in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2007;2:603-12.
19. Goldstraw P, Crowley J, Chansky K, et al. The IASLC lung cancer staging project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
20. Groome PA, Bolejack V, Crowley JJ, et al. The IASLC lung cancer staging project: validation of the proposals for revision of the T, N, and M descriptors and consequent stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol 2007;2:694-705.
21. Shepherd FA, Crowley J, Van Houtte P, et al. The International Association for the Study of Lung Cancer lung cancer staging project: proposals regarding

the clinical staging of small cell lung cancer in the forthcoming (seventh) edition of the tumor, node, metastasis classification for lung cancer. J Thorac Oncol 2007;2:1067-77.
22. Vallières E, Shepherd FA, Crowley J, et al. The IASLC lung cancer staging project: proposals regarding the relevance of TNM in the pathologic staging of small cell lung cancer in the forthcoming (seventh) edition of the TNM classification for lung cancer. J Thorac Oncol 2009;4:1049-59.
23. Travis WD, Giroux DJ, Chansky K, et al. The IASLC lung cancer staging project: proposals for the inclusion of broncho-pulmonary carcinoid tumors in the forthcoming (seventh) edition of the TNM classification for lung cancer. J Thorac Oncol 2008;3:1213-23.
24. Travis WD, Brambilla E, Rami-Porta R, et al. Visceral pleural invasion: pathologic criteria and use of elastic stains: proposal for the 7th edition of the TNM classification for lung cancer. J Thorac Oncol 2008;3:1384-90.
25. The Japan Lung Cancer Society. eds. Classification of lung cancer: first English edition. Chiba: Kanehara and Co, 2000.
26. Hammar SP. Common tumors. In: Dail DH, Hammar SP. eds. Pulmonary pathology (2nd Edition). New York: Springer-Verlag, 1994:1138.
27. Naruke T, Suemasu K, Ishikawa S. Lymph node mapping and curability at various levels of metastasis in resected lung cancer. J Thorac Cardiovasc Surg 1978;76:832-9.
28. Mountain CF, Dresler CM. Regional lymph node classification for lung cancer staging. Chest 1997;111:1718-23.
29. Rusch VW, Asamura H, Watanabe H, et al. The IASLC lung cancer staging project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2009;4:568-77.
30. Union for International Cancer Control (UICC). eds. TNM supplement: a commentary on uniform use (4th Edition). Oxford: Wiley Blackwell, 2012.
31. Giroux DJ, Rami-Porta R, Chansky K, et al. The IASLC lung cancer staging project: data elements for the prospective project. J Thorac Oncol 2009;4:679-83.
32. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85.
33. Field JK, Smith RA, Aberle DR, et al. International association for the study of lung cancer computed tomography screening workshop 2011 report. J Thorac Oncol 2012;7:10-9.
34. Nakamura K, Saji H, Nakajima R, et al. A phase III randomized trial of lobectomy versus limited resection for small-sized peripheral non-small cell lung cancer (JCOG0802/WJOG4607L). Jpn J Clin Oncol 2010;40:271-4.
35. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
36. Cuffe S, Bourredjem A, Graziano S, et al. A pooled exploratory analysis of the effect of tumor size and KRAS mutations on survival benefit from adjuvant platinum-based chemotherapy in node-negative non-small cell lung cancer. J Thorac Oncol 2012;7:963-72.

Cite this article as: Goldstraw P. New TNM classification: achievements and hurdles. Transl Lung Cancer Res 2013;2(4):264-272. doi: 10.3978/j.issn.2218-6751.2013.07.02

A review of clinical practice guidelines for lung cancer

Jutta J. von Dincklage[1], David Ball[2], Gerard A. Silvestri[3]

[1]Cancer Council Australia, Sydney, Australia; [2]Peter MacCallum Cancer Centre, and Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Australia; [3]Division of Pulmonary and Critical Care Medicine Medical University of South Carolina, Charleston, South Carolina, USA

Correspondence to: Jutta J. von Dincklage, BA, MA. GPO Box 4708, Sydney, NSW 2001, Australia. Email: jutta.von@cancer.org.au.

Abstract: Clinical practice guidelines are important evidence-based resources to guide complex clinical decision making. However, it is challenging for health professionals to keep abreast available guidelines and to know how and where to access relevant guidelines. This review examines currently available guidelines for lung cancer published in the English language. Important key features are listed for each identified guideline. The methodology, approaches to dissemination and implementation, and associated resources are summarised. General challenges in the area of guideline development are highlighted. The potential to collaborate more widely across lung cancer guideline developers by sharing literature searches and assessments is discussed.

Keywords: Clinical practice guidelines; clinical practice guideline development; evidence-based medicine; lung neoplasms; non-small cell lung carcinoma; small cell lung carcinoma

Submitted Jul 15, 2013. Accepted for publication Jul 29, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.37
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.37

Introduction

A challenge for health professionals managing patients with lung cancer is to keep abreast with the rapidly growing evidence base in diagnosis, staging and treatment. Clinical practice guidelines for lung cancer provide a useful tool of synthesised evidence to guide complex clinical decision making. They have the potential to enhance the healthcare decisions of clinicians and patients, and to lead to better quality care and improved outcomes for patients when they are of high quality, accessible and successfully implemented (1).

Numerous guidelines have been developed for lung cancer across the world. With a vast number of lung cancer guidelines developed in different countries by different organisations and listed across numerous guideline databases, this review article aims to provide a comprehensive overview of available guidelines for lung cancer available in English language. Key features such as developing organisation(s), publication date, geographic context and access details are listed for each guideline. More detailed information in regards to the methodology, the dissemination and implementation approach, important background information and any associated resources are briefly summarised in the results section.

Methods

Clinical practice guidelines are defined as "statements that include recommendations intended to optimize patient care that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options" (1). This definition has been used to identify clinical practice guidelines for lung cancer to be included in this review article. A comprehensive literature search consisting of searching the Guidelines International Network (GIN) International Guideline Library, National Guideline Clearinghouse, Standards and Guidelines Evidence (SAGE) portal, Australia's Clinical Practice Guideline Portal, PubMed as well as Scottish International Guidelines Network's (SIGN) and National Institute for Health and Care Excellence's (NICE) databases was completed. In addition, snowballing was used to identify any further relevant guidelines that were missed in the database searches. The results were then screened and included if the following criteria were met (*Table 1*).

Guidelines addressing malignant pleural mesothelioma, thymoma, specific symptom management topics and other secondary topics were out of scope for this review article and therefore not considered. Clinical practice guidelines that met all criteria, but were based on a non-systematic literature review, were excluded from this review as the systematic review requirement according to the clinical practice guideline definition was not met (*Table 2*). Other forms of clinical guidance such as general consensus statements on clinical topics, expert advice, task force reports, health technology appraisal and appropriate use criteria were also excluded.

Table 1 Inclusion criteria

Inclusion criteria
Clinical practice guideline as per Institute of Medicine's definition (1)
Published in English language
Published between 2008 and 2013*
Addresses prevention, screening, diagnosis, staging, treatment and management of small cell and non-small cell lung cancer
*The date range is based on the maximum guideline validity period of five years according to National Health and Medical Research Council (2).

Results

In total 22 lung cancer guideline documents developed by 12 different organisations were identified as meeting the inclusion criteria (*Table 3*).

Table 3 shows that there is wide variation in nearly every aspect of guideline development between each of the guidelines. As *Table 3* indicates, the scope varied across the identified guidelines. Some guideline developers, such as NICE (37) or American College of Chest Physicians

Table 2 Excluded lung cancer guidelines

Guideline developer	Guideline title	Reason for exclusion
American Association for Thoracic Surgery (AATS)	The American Association for Thoracic Surgery guidelines for lung cancer screening using low-dose computed tomography scans for lung cancer survivors and other high-risk groups (3)	No explicit information in regards to literature search and review methods included (3)
Central European Cooperative Oncology Group (CECOG)	Third CECOG Consensus on the systemic treatment of non-small-cell lung cancer (4)	Not clear if systematic review was performed (4)
European Society for Medical Oncology (ESMO)	Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (5) Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (6) Small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (7)	Based on a narrative literature search (8)
European Respiratory Society (ERS), European Society of Thoracic Surgeons (ESTS)	ERS/ESTS clinical guidelines on fitness for radical therapy in lung cancer patients (surgery and chemo-radiotherapy) (9)	Based on a non-systematic literature review and expert panel consensus (9)
Italian Association of Thoracic Oncology (AIOT)	Treatment of advanced non-small-cell lung cancer: Italian Association of Thoracic Oncology (AIOT) clinical practice guidelines (10)	Based on a non-systematic literature review and expert panel consensus (10)
National Comprehensive Cancer Network (NCCN)	Lung cancer screening Version 1.2013 (11) Non-small cell lung cancer Version 2.2013 (12) Small cell lung cancer Version 2.2013 (13)	No information included in regards to literature search and review methods (11-13)
Spanish Society for Medical Oncology (SEOM)	SEOM clinical guidelines for the treatment of non-small cell lung cancer: an updated edition (14)	Methodology not included (14)

Table 3 Results of available lung cancer guidelines published between 2008-2013

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
Alberta Health Services	Non small cell lung cancer stage I (15)	2011	Canada	online, pdf format	Evidence-based clinical practice guidelines based on a systematic review and/ or systematic recommendation adoption from an existing guideline	Recommendations statements Example: "A lobectomy or greater lung resection is recommended over a sublobar resection for patients with stage I NSCLC who are medically fit for surgery." (15)	http://www.albertahealthservices.ca/1755.asp.
	Non small cell lung cancer stage II (16)	2011	Canada	online, pdf format			http://www.albertahealthservices.ca/1755.asp.
	Non small cell lung cancer stage III (17)	2011	Canada	online, pdf format			http://www.albertahealthservices.ca/1755.asp.
	Non small cell lung cancer stage IV (18)	2011	Canada	online, pdf format			http://www.albertahealthservices.ca/1755.asp.
	Small Cell Lung Cancer - Extensive Stage (19)	2011	Canada	online, pdf format			http://www.albertahealthservices.ca/1755.asp.
	Small Cell Lung Cancer - Limited Stage (20)	2011	Canada	online, pdf format			http://www.albertahealthservices.ca/1755.asp.
American College of Chest Physicians (ACCP)	Diagnosis and Management of Lung Cancer, 3rd ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (21)	2013	USA	Journal article	Evidence-based clinical practice guideline based on a systematic review	Graded recommendation statements according to ACCP's system (21) Example:" In patients with PET activity in a mediastinal lymph node and normal appearing nodes by CT (and no distant metastases), invasive staging of the mediastinum is recommended over staging by imaging alone (Grade 1C)." (21)	http://www.chestnet.org/Guidelines-and-Resources/Guidelines-and-Consensus-Statements/More-Guidelines/Lung-Cancer

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
American Society for Radiation Oncology (ASTRO)	Palliative thoracic radiotherapy in lung cancer: An American Society for Radiation Oncology evidence-based clinical practice guideline (22)	2011	USA	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Guideline statements Example: "At this time, there is no added benefit for the use of chemotherapy concurrently with radiation therapy (RT) in the palliation of thoracic symptoms in lung cancer patients. To date, there is 1 randomized phase III study directly addressing this issue." (22)	https://www.astro.org/Clinical-Practice/Guidelines/Palliative-thoracic.aspx
American Society of Clinical Oncology (ASCO)	2011 Focused Update of 2009 American Society of Clinical Oncology Clinical Practice Guideline Update on Chemotherapy for Stage IV Non–Small-Cell Lung Cancer (23)	2011	USA	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Narrative recommendation statements according to ASCO's system (24) Example: "Evidence supports the use of chemotherapy* in patients with stage IV non–small-cell lung cancer with Eastern Cooperative Oncology Group (ECOG)/Zubrod* PS 0, 1, and possibly 2." (23)	http://www.asco.org/institute-quality/asco-clinical-practice-guideline-update-chemotherapy-stage-iv-non-small-cell-lung
British Thoracic Society (BTS), Society for Cardiothoracic Surgery (SCTS)	Guidelines on the Radical Management of Patients with Lung Cancer (25)	2010	UK	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Graded recommendation statements according to Scottish Intercollegiate Guidelines Network (SIGN) system (25) For example: "View all available historical images at the onset of the diagnostic pathway and review them prior to treatment. [C]" (25)	http://www.brit-thoracic.org.uk/guidelines.aspx

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
Cancer Care Ontario	First-line systemic chemotherapy in the treatment of advanced non-small cell lung cancer (26)	2010	Canada	online, pdf format	Evidence-based clinical practice guidelines based on a systematic review	Recommendations, key evidence and qualifying statements Example: "Combination PET-CT imaging data may be used as part of research protocols in RT planning. Current evidence does not support the routine use of PET-CT imaging data in RT planning at this time outside of a research setting." (27)	https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Positron emission tomography in radiation treatment planning for lung cancer (27)	2010	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Postoperative adjuvant radiation therapy in stage II or IIIA completely resected non-small cell lung cancer (28)	2013	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Use of preoperative chemotherapy with or without postoperative radiotherapy in technically resectable stage IIIA non-small cell lung cancer (29)	2013	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Altered fractionation of radical radiation therapy in the management of unresectable non-small cell lung cancer (30)	2013	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Second-line or subsequent systemic therapy for recurrent or progressive non-small cell lung cancer (31)	2012	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
	The Role of Combination Chemotherapy in the Initial Management of Limited-Stage Small-Cell Lung Cancer (32)	2012	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Chemotherapy for relapsed small cell lung cancer (33)	2013	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
	Fluorodeoxyglucose positron emission tomography in the diagnosis and staging of lung cancer (34)	2012	Canada	online, pdf format			https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/lung-ebs/
Cancer Council Australia (CCA)	Clinical practice guidelines for the treatment of lung cancer (35)	2012	Australia	web-based	Evidence-based clinical practice guidelines based on a systematic review	Graded recommendations and levels of evidence according the NHMRC's system (35) Example:" Patients who have a good performance status (WHO 1, 2) and completely resected stage III non-small cell lung cancer should be offered adjuvant cisplatin-based chemotherapy." (35)	http://wiki.cancer.org.au/australia/Guidelines:Lung_cancer

Table 3 (*continued*)

Table 3 (*continued*)

Guideline Developer	Guideline Title	Year of publication	Country/ Region	Publication type	Guideline development approach	Recommendation format	Online access
College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), Association for Molecular Pathology (AMP)	Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors (36)	2013	USA	Journal article	Evidence-based clinical practice guidelines based on a systematic review	Graded recommendations including levels of evidence according to the NHMRC's system (36) Example: "ALK molecular testing should be used to select patients for ALK-targeted TKI therapy, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics. Evidence Grade: EGFR: A ALK: B…." (36)	http://www.archivesofpathology.org/doi/pdf/10.5858/arpa.2012-0720-OA
National Institute for Health and Care Excellence (NICE)	Lung cancer. The diagnosis and treatment of lung cancer (37)	2011	UK	web-based and pdf version	Evidence-based clinical practice guidelines based on a systematic review	Recommendation statements including qualifying statements (37) Example: "Ensure all patients potentially suitable for treatment with curative intent are offered PET-CT before treatment. [NEW 2011]" (37)	http://guidance.nice.org.uk/CG121

(ACCP) (21), published their lung cancer guideline as one large document covering all areas of lung cancer from epidemiology, screening, diagnosis, treatment, follow-up to end-of-life care. Others, such as Alberta Health Services (15-20) and College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC) and Association for Molecular Pathology (AMP) (36), developed more focused guidelines addressing specific area(s) of lung cancer. For example, Alberta Health Services released their lung cancer guidelines as separate discrete publications and published a guideline for each stage (15-20). Cancer Care Ontario's guidelines are even more specific and address only one or a few closely related clinical questions (26-34).

The guideline development approach also varied between organisations. All included clinical practice guidelines are based on formal systematic reviews to generate evidence-based recommendations. However, the evidence assessment tools, recommendation format and recommendation grading schemes vary (*Table 3*). A few developers even have a procedure in place to evaluate if recommendations from existing guidelines could be formally adopted (38) as opposed to developing de novo guidelines/recommendations.

Whereas all identified guidelines are disseminated and accessible online, the presentation varied from documents available for download, web-based clinical practice guidelines, guidelines available as published journal articles or a combination of approaches (*Table 3*). Many developers offer printed guideline copies or printed summaries of the recommendations available upon request.

The subsequent section summarises background information in regards to the relevant guideline(s), composition of guideline development group, conflict of interest (COI) management, guideline funding, the methodological as well as dissemination and implementation approach, planned update and any associated resources for the guideline(s) in narrative form under each guideline developer or collaboration of guideline developers. Together with *Table 3*, the summarised information covers the subject areas of the standards identified by the Institute of Medicine for developing trustworthy clinical practice guidelines (establishing transparency, management of COI, guideline development group composition, systematic review, evidence foundations and evidence level ratings, recommendation formulation, external review and updating) (1). Dissemination and implementation approach and any associated guideline resources were added for each guideline as these are key to achieve successful guideline uptake (39).

Alberta Health Services

Introduction

Alberta Health Services is a Canadian health authority that delivers health services in the Canadian province Alberta and develops clinical practice guidelines in oncology. For each stage in non-small cell and small cell lung cancer a separate lung cancer guideline document was produced. They are published as separate PDF publications on Alberta Health Services' website (*Table 3*) (15-20).

Guideline development methodology

Guideline development at Alberta Health Services follows a systematic guideline development approach as detailed in the Guideline Utilization Resource Unit Handbook (40). For each lung guideline, a multidisciplinary working group was recruited. The guideline scope was defined and clinical questions developed. The literature searches were carried out by an in-house knowledge management specialist. All retrieved literature results were screened, assessed and synthesised. Existing guidelines were also searched for in order to evaluate if an existing guideline could be formally adopted. Any retrieved existing guidelines were formally assessed with the AGREE II instrument to ensure minimum requirements for a good quality guideline were met, before considering the formal adoption or adaption of existing recommendation(s). Guideline recommendations were developed and formulated by the guideline working group members based on the evidence tables and expert clinical interpretation or, if applicable, an existing guideline. Recommendations were formulated in the form of action statements and the reasoning behind the recommendation, including the quality and level of evidence, was added in narrative form. Alberta Health Services did not use a formal grading scheme to assign specific grades to recommendations. The draft guidelines were then open for comment and reviewed by all members of the Provincial Tumour Team. Once the guideline documents were finalised, they were formally endorsed by Alberta Health Services.

COI management

COI statements are included in each lung cancer guideline as well as an overall statement from the developer that each guideline was satisfactorily developed in an unbiased manner (40).

Funding
Each lung cancer guideline document states that there was no direct industry involvement in the production or dissemination of the guideline (40).

Dissemination and implementation approach
The guidelines are published on the Alberta Health Services website. All members are notified when a guideline has been updated or added. Guidelines are presented at the local and provincial tumour team meeting as well as weekly hospital rounds to facilitate uptake (40).

Planned update
Alberta Health Services clinical practice guidelines are reviewed and updated every one to two years (40).

Associated resources
Treatment algorithms for each lung cancer guideline are available from http://www.albertahealthservices.ca/1755.asp.

American College of Chest Physicians

Introduction
The ACCP produces guidelines in chest medicine and has developed guidelines for lung cancer since 2003. The third edition of the ACCP lung cancer guidelines has been published in 2013 and is included in this review (*Table 3*) (21).

Guideline development methodology
ACCP used a formalised, systematic approach to develop the third edition of the lung cancer guidelines. A selected expert lung cancer guideline panel developed research questions in PICO [The acronym PICO refers to the 4 elements that should be included in a structured clinical question to govern systematic searches: patient, intervention, comparison and outcome. A framework commonly used in evidence-based medicine (41).] format and literature searches were designed and completed. The literature results were then screened against inclusion and exclusion criteria and formally assessed using standard quality assessment tools. If applicable, good quality meta-analysis (already published or performed by the authors specifically for the guideline) were used to inform the recommendations. Evidence summary tables and profiles were compiled for most PICO questions. Based on the evidence tables, recommendations were formulated and then graded according to the ACCP recommendation grading system. The whole guideline panel reviewed the guideline content, including formal anonymous voting to approve recommendations during face-to-face and virtual meetings. The draft guideline was then submitted through an internal and external review process before the guideline was finalised and published (42).

COI management
Each nominated guideline panel member had to submit a COI statement before the start of the guideline project. The COI statements were reviewed by the Guidelines Oversight Committee. All panellists were required to submit an updated COI statement before each meeting. COI management included strategies such as not drafting or voting on recommendations that were related to a particular conflict (42).

Funding
The majority of the guideline was funded by the ACCP. One private foundation and one pharmaceutical company financially supported the development and dissemination of the guideline. Those sponsoring companies were not allowed to participate in the guideline development process (42).

Dissemination and implementation approach
The ACCP lung guidelines are disseminated through the College's website (www.chestnet.org), the *CHEST journal* publication, National Guidelines Clearinghouse and GIN Library (42).

Planned update
The start of ongoing review is planned 1 year after publication unless the content experts, who continue to monitor the literature, suggest that recommendations need to be updated (42).

Associated resources
Additional clinical resources will be accessible in Chest Evidence. Associated patient guides will be available from www.onebreath.org (42).

American Society for Radiation Oncology (ASTRO)

Introduction
The Guidelines Subcommittee of the ASTRO identified a need for an evidence-based guideline on the use of palliative radiotherapy to lung cancer patients. The project proposal to develop this guideline was submitted and approved by the ASTRO Board of Directors in 2009 (22).

Guideline development methodology
A task force was established and assigned to review and synthesize the current available evidence to develop this guideline. The task force was divided into three topic groups and a systematic literature review was completed for each area. Evidence assessment, including the creation of evidence tables, and the formulation of the guideline content were completed and then revised by the complete expert group. The final draft was then circulated to three expert reviewers, the ASTRO legal counsel and also published on the ASTRO website for public comment. The feedback was reviewed and incorporated before the guideline was finally reviewed and approved by the ASTRO Board of Directors (22).

COI management
At the beginning of the guideline project, all members submitted COI declarations. The task group chairs reviewed all COI statements and determined that the disclosures would have no impact upon the content of the guideline manuscript (22).

Funding
Details in regards to the funding of the guidelines were not specified in the guideline document.

Dissemination and implementation approach
The guideline was formally published in the journal *Practical Radiation Oncology* (22) and the link to the article is listed on ASTRO's website.

Planned update
The ASTRO Guidelines Subcommittee will monitor this guideline and initiate an update when appropriate (22).

Associated resources
Not identified.

American Society for Clinical Oncology

Introduction
The American Society of Clinical Oncology (ASCO) has been developing clinical practice guidelines for lung cancer since 1997 and has published an update on chemotherapy for stage IV non-small lung cancer in 2011 that was eligible for inclusion in this review (23).

Guideline development methodology
The 2011 update on chemotherapy treatment for stage IV lung cancer is based on ASCO's 2009 lung cancer guideline update and addressed the clinical question, "What is the optimal duration of first-line chemotherapy for stage IV NSCLC?" from the previous guideline. The literature search for this guideline included an update of the original 2009 literature search and a systematic assessment of the updated evidence. ASCO's Guideline Procedures Manual provides details about ASCO's methodology for guideline development (24). The 2011 focused update was drafted by the co-chairs of the 2009 guideline as well as ASCO staff and was then circulated to the entire update committee for approval. The final document was reviewed and approved by ASCO's Clinical Practice Guideline Committee and Board of Directors Executive Committee. It was then submitted to *Journal of Clinical Oncology* for peer review before being finalized and published (23).

COI management
All members of the update committee completed the ASCO disclosure form prior to commencing the work on this guideline project. Further details about ASCO's COI management are published in ASCOS's COI management procedures summary (43).

Funding
Details in regards to guideline funding were not specified in the guideline publication (23).

Dissemination and implementation approach
The guideline was published in the *Journal of Clinical Oncology* (23) and is listed on ASCO's website in the clinical guideline section (*Table 3*).

Planned update
Not specified in guideline document.

Associated resources
Slide sets, patient guide and decision aids are available from http://www.asco.org/institute-quality/asco-clinical-practice-guideline-update-chemotherapy-stage-iv-non-small-cell-lung.

British Thoracic Society (BTS) and Society for Cardiothoracic Surgery (SCTS) in Great Britain and Ireland

Introduction
The BTS and the SCTS in Great Britain and Ireland had developed a guideline on the radical management of patients

with lung cancer in 2001 and decided to conduct an update of this guideline to provide comprehensive guidance on selection and risk assessment of suitable patients (*Table 3*) (25).

Guideline development methodology
The guideline development group determined the guideline scope based on the previous guideline and in consultation with members from both societies. A comprehensive literature search was performed and the evidence was assessed using the Scottish Intercollegiate Guidelines Network's (SIGN) methodology. Recommendations were developed based on the evidence tables and graded according to SIGN. Research recommendations were also incorporated. The draft document was distributed amongst BTS and SCTS members and presented at society meetings for consultation and review. All feedback was assessed and reviewed by the guideline committee before the guideline was finalised, approved and published (25).

COI management
COI statements are included in the guideline publication (25).

Funding
The BTS funded all committee meetings (25).

Dissemination and implementation approach
The guideline was published in the *Thorax journal* (25) and is also disseminated through a link on the BTS website (*Table 3*).

Planned update
2013 (44).

Associated resources
A quick reference guide is available from http://www.brit-thoracic.org.uk/Portals/0/Guidelines/Lung%20Cancer/Guidelines/LungCancerQRG.pdf.

Cancer Care Ontario

Introduction
Cancer Care Ontario, a Canadian health government agency, has published nine clinical practice guidelines for lung cancer between 2008 and 2013 (*Table 3*) covering specific clinical questions in the area of non-small cell and small cell lung cancer management (26-34).

Guideline development methodology
At Cancer Ontario, working groups consisting of two to six clinicians or content experts and one Research Coordinator were established to produce each lung cancer guideline. The working groups determined the overall guideline topic, the individual clinical questions for each topic as well as the overall scope of each lung cancer guideline. The literature review process, that formed the basis of each guideline document, consisted of two stages: first, existing lung cancer guidelines were identified to see if an existing guideline could be formally adapted. If not, a systematic review of the evidence considering the highest level evidence was conducted. After the evidence was assessed and synthesised, the working groups developed the initial recommendations. The reasoning behind each recommendation and the degree of how much it is evidence-based versus expert consensus is explicitly stated in the recommendations. All draft guideline documents went through an internal and external review process. The external review process consisted of targeted peer review and professional consultation. The draft guideline documents were then revised by the individual working groups to assess and incorporate the feedback. The process and results that arose from the consultation review are documented in the final guideline documents. Cancer Care Ontario's guideline development methodology is described in detail in the "Program in Evidence-Based Care Handbook" published by Cancer Care Ontario (45).

COI management
Working group authors had to declare COI as soon as they started on a guideline project and provide an update when the guideline was completed. The guideline chair and research coordinator were responsible to collate the declarations and updates and manage any conflicting interest according to Cancer Care Ontario's COI policy (46). Reviewers also had to declare any competing interests.

Funding
Guideline development is supported by the Ontario Ministry of Health and Long-term Care through Cancer Care Ontario and editorially independent from its funding source (26-34).

Dissemination and implementation approach
The guidelines are published on Cancer Care Ontario's website (*Table 3*) and indexed at National Guidelines Clearinghouse and CMA Infobase. In addition, the results of several systematic reviews are published in peer-reviewed journals (47-52).

Planned update
Each year the lung cancer guidelines are assessed with a document assessment tool developed by the Program in Evidence-Based Care at Cancer Ontario to determine if any guidelines are in need of an update (45).

Associated resources
Not identified.

Cancer Council Australia (CCA)

Introduction
CCA, a not for profit cancer charity, produces evidence-based clinical practice guidelines in oncology for the Australian health care context. In 2010, CCA was commissioned by Cancer Australia (an agency of the Australian Government) to update the lung guidelines originally published in 2004. The new web-based guideline covers treatment of non-small cell and small cell lung cancer, symptom management, supportive and palliative care (35).

Guideline development methodology
A multidisciplinary working group was established and the guideline objectives and scope were defined. Clinical questions according to PICO format were developed and systematic literature searches were carried out. The literature results were screened for relevance and formally assessed. The evidence was synthesised and analysed by the assigned working group members. Each question lead author developed the initial clinical question content, including formulation of evidence statements and draft recommendations and assigning the recommendation grades according to the NHMRC grading system (53). All draft content, including the recommendations and associated grades, was then internally reviewed and approved by all members of the working party before the draft guideline was released for public consultation. All externally received comments were considered by the working party and, where necessary, changes were made to the guideline. A formal response to each comment was documented. Once the guideline was finalized, it was published on CCA's Cancer Guidelines Wiki (35). CCA's Guideline Development Handbook provides a detailed description of the applied guideline development methodology (54).

COI management
COI statements were collected from each working group member at the start of the project. The management committee had the responsibility to collect and evaluate COI statements from all nominees. All working party members are responsible to provide updated COI statements if new interests arise (35).

Funding
Co-funding to develop these guidelines was received from Cancer Australia (35).

Dissemination and implementation approach
CCA's clinical practice guidelines are available online via the CCA Cancer Guidelines Wiki (35). The link to the guidelines was distributed directly to relevant professional and other interested groups via email, print and social media campaigns as well as through meetings, national conferences and other CME events. By allowing guideline stakeholders to comment on guidelines content and submit new evidence on an ongoing basis, CCA is encouraging its stakeholders to engage with the guideline content on a long-term basis (54).

CCA is developing online learning modules to reinforce content knowledge for participants and support guideline uptake. CCA is going to pilot the development of a lung cancer QStream module originally developed by Harvard Medical School (54).

Planned update
Ongoing (54).

Associated resources
Online QStream module is in development (54).

CAP, IASLC and AMP

Introduction
Three professional societies, CAP, IASLC, and AMP, systematically reviewed the literature to develop an evidence-based guideline for selection of lung cancer patients for EGFR mutation and ALK rearrangement testing. The guideline addresses which patients and samples should be tested and when and how testing should be performed (36).

Guideline development methodology
A systematic literature review, including blinded screening for relevant studies, was performed. A formal quality assessment and data extraction was completed for all selected studies. Evidence tables were created. Based on the evidence assessment, content and evidence-based

recommendations were formulated, evidence levels assigned and recommendation grades determined. In addition, recommendations based on formal expert consensus were added where appropriate and marked as such. The draft guideline then went through an extensive review process before it was finalised and published (36). The detailed methodological report is available from http://links.lww.com/JTO/A430 (55).

COI management

Before acceptance on the expert panel, all potential authors completed COI statements as per CAP' procedures and were required to disclose new conflicts at each conference call. They had to submit a general updated COI form on a yearly basis (55). The COI statements are published with the guidelines.

Funding

The guideline development was jointly funded by CAP, IASLC and AMP (36).

Dissemination and implementation approach

The guideline is disseminated through the organisations' websites and was released in *Archives of Pathology & Laboratory Medicine*, the *Journal of Thoracic Oncology*, and *the Journal of Molecular Diagnostics* (36).

Planned update

This guideline will be reviewed regularly, as mandated by publication of substantive and high-quality medical evidence that could potentially alter the original guideline recommendations (36).

Associated resources

A summary of recommendations is available from http://www.cap.org/apps/docs/membership/cap_iaslc_amp_summary_of_recommendations.pdf. A patient guide is available from http://www.cap.org/apps/docs/membership/lc_patient_guide.pdf. A frequently asked question sheet is available from http://www.cap.org/apps/docs/membership/lc_faqs.pdf.

National Institute for Health and Care Excellence

Introduction

NICE is a UK-based health authority that provides national guidance and advice to improve health and social care. In 2011, NICE published a revision of the clinical practice guideline for lung cancer titled "Lung cancer. The diagnosis and treatment of lung cancer" (37).

Guideline development methodology

The methods that were used to develop the lung cancer guideline are in accordance with those set out by NICE in *The guidelines manual* (56). After the decision was made to update the lung cancer guideline, the guideline scope was defined and a lung cancer guideline development group was established. The group formulated clinical questions using the PICO framework where applicable. Comprehensive, systematic literature searches were carried out for each question and the evidence critically appraised and assessed. Health economic evidence was also included, assessed and synthesized. Based on the evidence synthesis, recommendations were developed and agreed upon by the working group. Qualifying statements about the strength of evidence, about the benefits and harms for the intervention being considered, the degree of consensus within the GDG and the costs and cost-effectiveness of an intervention were added. The guideline draft went through a consultation process, which was documented and published as a separate report on the NICE website. Based on the stakeholder comments, the guideline content was revised and went through a pre-publication check process, before the final guideline version was published (37).

COI management

At the start of the guideline development process, all COI statements from each guideline development group member were recorded. At each subsequent meeting, members declared any new, arising interests. For group members, that declared any conflicting interests, an evaluation took place and a management plan was implemented (37). The code of practice for declaring and dealing with conflicts of interest outlines the COI management procedures in further detail (57).

Funding

NICE commissioned the National Collaborating Centre for Cancer to develop this guideline. The health economic analysis was conducted by the London School of Hygiene and Tropical Medicine and funded by the National Collaborating Centre for Cancer (37).

Dissemination and implementation approach

This guideline is disseminated as web-based and short and long PDF versions on the NICE website. Numerous implementation tools have been developed to facilitate

guideline update (see under associated resources). The NICE guidelines manual outlines the guideline dissemination and implementation approach for NICE guidelines in detail (56).

Planned update

After three years, the guideline will be formally evaluated to assess if an update is required (37).

Associated resources

A short version of this guideline, containing the key priorities, key research recommendations and all other recommendations, and a Quick Reference Guide (QRG) are available from http://www.nice.org.uk/guidance/index.jsp?action=byID&o=13465.

The following implementation tools are available from http://www.nice.org.uk/guidance/index.jsp?action=byID&o=13465: baseline assessment tool, clinical audit tool, costing report, costing template, multiple guidance audit tool, slide set, online educational tool about referral in case of suspected lung cancer.

Discussion

Considerable resources have been spent internationally on the development of lung cancer guidelines. This review article highlights that health professionals specialising in the treatment of lung cancer, patients and other stakeholders have access to numerous clinical practice guidelines developed for different local contexts. As the major concern around clinical practice guidelines is around quality, especially rigour of development, validity of recommendations and editorial independence, guideline users are encouraged to formally assess the quality of any identified lung cancer guideline (58). The guideline quality assessment instrument Agree II provides a validated tool to complete such quality assessments (59).

It was not part of this review to analyse and compare recommendations across guidelines addressing the same areas, nevertheless we are aware that variation does exist. For example, in patients with stage I non-small cell lung cancer who cannot tolerate surgery, the ACCP recommends stereotactic body radiotherapy (SBRT) (21), whereas NICE recommends patients should be offered continuous hyperfractionated accelerated radiotherapy (CHART) (37). ACCP does not mention CHART at all (21); NICE offers no guideline on SBRT, but recommends that further research should be undertaken (37). Undertaking a detailed content comparison across the identified lung cancer guidelines and investigating any variations, may be a worthwhile project to emerge from this initial review. It would be of interest to know if the reasons for any variations are resource related (for example Alberta Health Services does not recommend CHART because it is unavailable there), or a result of regional/cultural preferences in practice (for example the level of therapeutic aggression or nihilism). An example of the latter is the ACCP guideline for patients who have undergone resection of an isolated brain or adrenal metastasis, that adjuvant chemotherapy is suggested (21), whereas NICE only recommends adjuvant chemotherapy for patients without metastatic disease (37). The international variation in cultural attitudes to what are reasonable levels of medical intervention (as suggested by this example) could present an obstacle to the ultimate development of truly universal guidelines.

Compiling an overview of available lung cancer guidelines also pinpoints general challenges in the area of guideline development. Lung cancer guidelines, that follow an international standard, are presented in a validated, uniform format and are published together with the results of independently performed quality assessments, are still a vision of the future, even though significant efforts have been made to provide standards, methodologies and presentation guidelines (1,60-62).

Successful dissemination and implementation of lung cancer guidelines is another challenging area (39,63,64). Even if high quality evidence-based guidelines are available, it does not guarantee successful uptake by health professionals. Guideline developers, health care organisations, and governments need to put adequate resources into guideline dissemination and implementation and follow multiple implementation strategies to maximise uptake (39). Further there are many competing sources of information on lung cancer management besides guidelines which are readily available to health professionals and consumers. Although they may lack the endorsement of respected learned societies, these other sources, usually web-based, having avoided a lengthy development process, may provide more up-to-date information than traditional guidelines, and so become the first port of call for the information seeker. Conversely, without the rigour under which the guidelines are produced, use of that approach might lead to acceptance of faulty information.

It is therefore critical to keep the guidelines current if they are to be relevant and well used. Collaborating on lung cancer guidelines internationally by sharing literature

searches and assessments is considered an effective approach to reduce duplication of effort and help developers keep the existing guidelines current (65). We hope this review provides an information starting point to bring together potential future collaborators with a view to developing integrated, dynamic, so called "living guidelines", which can then be adapted to suit the different cultural and organisational contexts.

Summary

The aim of this review article was to provide a comprehensive overview of available clinical practice guidelines in the areas of small cell and non-small cell lung cancer. 22 clinical practice guidelines produced by 12 organisations with varying scopes and developed for different regions were identified and key features summarised. Health professionals in the area of lung cancer have no shortage of guidelines to assist the clinical decision making process. Future research needs to focus more on dissemination, implementation, guideline adherence and their effect on disease outcome. It is hoped this article will be a useful resource for clinicians and other stakeholders to easily access these different guidelines and assess relevance to their own practice. We also hope it may lead to organisations to pool their resources to develop consistent, internationally relevant guidelines for what is, after all, a global disease.

Acknowledgements

Jutta Johanna von Dincklage and David Ball are members of Cancer Council Australia's Lung Cancer Guidelines Working Party that developed Cancer Council Australia's Clinical practice guidelines for the treatment of lung cancer (35). Gerard Silvestri serves as American College of Chest Physicians' lung cancer panellist that developed the third edition of ACCP Lung Cancer Guidelines (21). In the last 12 months, David Ball has sat on advisory boards for Boehringer-Ingelheim and Lilly Oncology, and given a lecture at a Pfizer sponsored symposium. In all cases the honoraria for these services have been paid directly to his employing institution, Peter MacCallum Cancer Centre.
Disclosure: The authors declare no conflict of interest.

References

1. IOM (Institute of Medicine). Clinical Practice Guidelines We Can Trust, The National Academies Press, Washington DC, 2011.
2. National Health and Medical Research Council. Summary for developers, Procedures and requirements for meeting the 2011 NHMRC standard for clinical practice guidelines, Melbourne, 2011.
3. Jaklitsch MT, Jacobson FL, Austin JHM, et al. The American association for thoracic surgery guidelines for lung cancer screening using low-dose computed tomography scans for lung cancer survivors and other high-risk groups. J Thorac Cardiovasc Surg 2012;144:33-8.
4. Brodowicz T, Ciuleanu T, Crawford J, et al. Third CECOG consensus on the systemic treatment of non-small-cell lung cancer. Ann Oncol 2012;23:1223-9.
5. Crino L, Weder W, van MJ, et al. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v103-v115.
6. Peters S, Adjei AA, Gridelli C, et al. Metastatic non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23 Suppl 7:vii56-64.
7 Sørensen M, Pijls-Johannesma M, Felip E, et al. Small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v120-5.
8. Pavlidis N, Hansen H, Stahel R. ESMO clinical practice guidelines: development, implementation and dissemination. Ann Oncol 2010;21 Suppl 5:v7-8.
9. Brunelli A, Charloux A, Bolliger CT, et al. ERS/ESTS clinical guidelines on fitness for radical therapy in lung cancer patients (surgery and chemo-radiotherapy). Eur Respir J 2009;34:17-41.
10. De Marinis F, Rossi A, Di Maio M, et al. Treatment of advanced non-small-cell lung cancer: Italian Association of Thoracic Oncology (AIOT) clinical practice guidelines. Lung Cancer 2011;73:1-10.
11. National Comprehensive Cancer Network. eds. Lung cancer screening (Version 1). Washington: National Comprehensive Cancer Network, 2013.
12. National Comprehensive Cancer Network. eds. Non-small cell lung cancer (Version 2). Washington: National Comprehensive Cancer Network, 2013.
13. National Comprehensive Cancer Network. eds. Small cell lung cancer (Version 2). Washington: National Comprehensive Cancer Network, 2013.
14. Pérez J, López PG, Font EF, et al. SEOM clinical guidelines for the treatment of non-small cell lung cancer: an updated edition. Clin Transl Oncol 2010;12:735-41.

15. Alberta Provincial Thoracic Tumour team. eds. Non small cell lung cancer stage I. Alberta: Alberta Health Services, 2011.
16. Alberta Provincial Thoracic Tumour team. eds. Non small cell lung cancer stage II. Alberta: Alberta Health Services, 2011.
17. Alberta Provincial Thoracic Tumour team. eds. Non small cell lung cancer stage III. Alberta: Alberta Health Services, 2011.
18. Alberta Provincial Thoracic Tumour team. eds. Non small cell lung cancer stage IV. Alberta: Alberta Health Services, 2011.
19. Alberta Provincial Thoracic Tumour team. eds. Small cell lung cancer-extensive stage. Alberta: Alberta Health Services, 2011.
20. Alberta Provincial Thoracic Tumour team. eds. Small cell lung cancer-limited stage. Alberta: Alberta Health Services, 2011.
21. American College of Chest Physicians. Diagnosis and management of lung cancer, 3rd edition: American college of chest physicians evidence-based clinical practice guidelines. Chest 2013;143.
22. Rodrigues G, Videtic GMM, Sur R, et al. Palliative thoracic radiotherapy in lung cancer: an American society for radiation oncology evidence-based clinical practice guideline. Pract Radiat Oncol 2011;1:60-71.
23. Azzoli CG, Temin S, Aliff T, et al. 2011 Focused Update of 2009 American Society of Clinical Oncology Clinical Practice Guideline Update on Chemotherapy for Stage IV Non-Small-Cell Lung Cancer. J of Clin Oncol 2011;29:3825-31.
24. American Society of Clinical Oncology. eds. American Society of Clinical Oncology guideline procedures manual (Expert Panel Version 4.0). Alexandria: American Society of Clinical Oncology, 2011.
25. Lim E, Baldwin D, Beckles M, et al. Guidelines on the Radical Management of Patients with Lung Cancer. Thorax 2010;65 Suppl 3:iii1-27.
26. Goffin J, Coakley N, Lacchetti C, et al. First-line systemic chemotherapy in the treatment of advanced non-small cell lung cancer. Toronto (ON): Cancer Care Ontario; 2010 [In review 2012]. Program in Evidence-based CareEvidence-Based Series No.: 7-10 Version 2.2010.
27. Ung YC, Bezjak A, Coakley N, et al. Positron emission tomography in radiation treatment planning for lung cancer. Toronto (ON): Cancer Care Ontario, 2010. Program in Evidence-based Care Evidence-Based Series No.: 7-18.
28. Members of the Lung Cancer Disease Site Group. Postoperative adjuvant radiation therapy in stage II or IIIA completely resected non-small cell lung cancer Okawara G, Tey R, reviewers. Toronto, ON: Cancer Care Ontario, 2012. Program in Evidence-based Care Evidence-Based Series No.: 7-1-1 Version 2.
29. Members of the Lung Cancer Disease Site Group. Use of preoperative chemotherapy with or without postoperative radiotherapy in technically resectable stage IIIA non-small cell lung cancer. Toronto (ON): Cancer Care Ontario, 2013. Program in Evidence-based Care Practice Guideline Report No.: 7-4 Version 2.
30. Members of the Lung Cancer Disease Site Group. Altered fractionation of radical radiation therapy in the management of unresectable non-small cell lung cancer. Toronto (ON): Cancer Care Ontario, 2012. Program in Evidence-based Care Practice Guideline Report No.: 7-12 Version 2.
31. Noble J, Ellis P, Mackay JA, et al. Ellis P, Ismaila N, reviewers. Second-line or subsequent systemic therapy for recurrent or progressive non-small cell lung cancer. Toronto (ON): Cancer Care Ontario, 2012. Program in Evidence-based Care Evidence-based Series No.: 7-19.
32. Members of the Lung Cancer Disease Site Group. The role of combination chemotherapy in the initial management of limited-stage small-cell lung cancer. Laurie S, Souter L, reviewers. Toronto (ON): Cancer Care Ontario, 2012. Program in Evidence-based Care Evidence-based Series No.: 7-13-1 Version 2.
33. Members of the Lung Cancer Disease Site Group. Chemotherapy for relapsed small cell lung cancer. Toronto (ON): Cancer Care Ontario, 2013. Program in Evidence-based Care Evidence-based Series No.: 7-17 Version 2.
34. The Lung Cancer Disease Site Group. 18-Fluorodeoxyglucose positron emission tomography in the diagnosis and staging of lung cancer. Ung Y, Ismaili Nofisat, reviewers. Toronto (ON): Cancer Care Ontario, 2012. Program in Evidence-based Care Evidence-based Series No.: 7-20 Version 2.
35. Cancer Council Australia Lung Cancer Guidelines Working Party. Clinical practice guidelines for the treatment of lung cancer. Available online: http://wiki.cancer.org.au/australia/Guidelines:Lung_cancer
36. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for egfr and alk tyrosine kinase inhibitors. Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association

for Molecular Pathology. J Thorac Oncol 2013;8:823-59.
37. National Collaborating Centre for Cancer. eds. Lung cancer. The diagnosis and treatment of lung cancer. London: National Institute for Health and Clinical Excellence, 2011.
38. ADAPTE Collaboration. 2007. Manual for Guideline Adaptation Version 1.0. ADAPTE Collaboration.
39. Francke AL, Smit MC, de Veer AJE, et al. Factors influencing the implementation of clinical guidelines for health care professionals: a systematic meta-review. Available online: http://www.biomedcentral.com/1472-6947/8/38
40. Alberta Health Services. eds. Guideline utilization resource unit handbook (Version 2). Alberta: Alberta Health Services, 2013.
41. Akobeng AK. Principles of evidence based medicine. Arch Dis Child 2005;90:837-40.
42. Lewis SZ, Diekemper R, Addrizzo-Harris DJ, et al. Methodology for development of guidelines for lung cancer: diagnosis and management of lung cancer, 3rd ed: American college of chest physicians evidence-based clinical practice guidelines. Chest 2013;143:41S-50S.
43. American Society of Clinical Oncology. eds. Policy summary: conflict of interest management procedures for clinical practice guidelines. Alexandria: American Society of Clinical Oncology, 2008.
44. Guidelines. Available online: http://www.brit-thoracic.org.uk/guidelines.aspx
45. Cancer Care Ontario. eds. Program in evidence-based care handbook. Toronto: Cancer Care Ontario, 2011.
46. Cancer Care Ontario. eds. Program in evidence-based care (PEBC) conflict of interest policy. Toronto: Cancer Care Ontario, 2011.
47. Goffin J, Lacchetti C, Ellis PM, et al. First-Line systemic chemotherapy in the treatment of advanced non-small cell lung cancer: a systematic review. J Thorac Oncol 2010;5:260-74.
48. Ung YC, Bezjak A, Coakley N, et al. Positron emission tomography with 18fluorodeoxyglucose in radiation treatment planning for non-small cell lung cancer: a systematic review. J Thorac Oncol 2011;6:86-97.
49. Okawara G, Ung YC, Markman BR, et al. Postoperative radiotherapy in stage II or IIIA completely resected non-small cell lung cancer: a systematic review and practice guideline. Lung Cancer 2004;44:1-11.
50. Noble J, Ellis PM, Mackay JA, et al. Second-line or subsequent systemic therapy for recurrent or progressive non-small cell lung cancer: a systematic review and practice guideline. J Thorac Oncol 2006;1:1042-58.
51. Cheng S, Evans WK, Stys-Norman D, et al. Chemotherapy for relapsed small cell lung cancer: a systematic review and practice guideline. J Thorac Oncol 2007;2:348-54.
52. Ung YC, Maziak DE, Vanderveen JA, et al. 18Fluorodeoxyglucose positron emission tomography in the diagnosis and staging of lung cancer: a systematic review. J Natl Cancer Inst 2007; 99:1753-67.
53. NHMRC. eds. NHMRC additional levels of evidence and grades for recommendations for developers of guidelines. Canberra: NHMRC, 2009.
54. Cancer Council Australia. eds. Development of clinical practice guidelines using Cancer Council Australia's cancer guidelines wiki. Handbook 2012 for section authors and the guideline working party. Sydney: Cancer Council Australia, 2012.
55. Lindeman NI, Cagle PT, Beasley MB, et al. Supplemental digital content. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the college of American pathologists, international association for the study of lung cancer and the association for molecular pathology. Available online: http://links.lww.com/JTO/A430
56. National Institute for Health and Care Excellence. eds. The guidelines manual. London: National Institute for Health and Clinical Excellence (NICE), 2012.
57. National Institute for Health and Care Excellence. eds. A code of practice for declaring and dealing with conflicts of interest. London: National Institute for Health and Clinical Excellence (NICE), 2008.
58. Alonso-Coello P, Irfan A, Solà I, et al. The quality of clinical practice guidelines over the last two decades: a systematic review of guideline appraisal studies. Qual Saf Health Care 2010;19:e58.
59. Brouwers MC, Kho ME, Browman GP, et al. AGREE II: advancing guideline development, reporting and evaluation in health care. CMAJ 2010;182:E839-42.
60. Qaseem A, Forland F, Macbeth F, et al. Guidelines international network: toward international standards for clinical practice guidelines. Ann Intern Med 2012;156:525-31.
61. Jaeschke R, Guyatt GH, Dellinger P, et al. Use of GRADE grid to reach decisions on clinical practice guidelines when consensus is elusive. BMJ 2008;337:a744.
62. Treweek S, Oxman A, Alderson P, et al. Developing and evaluating communication strategies to support informed decisions and practice based on evidence (DECIDE):

protocol and preliminary results. Implement Sci 2013;8:6.
63. Grimshaw J, Eccles M, Thomas R, et al. Toward evidence-based quality improvement. Evidence (and its limitations) of the effectiveness of guideline dissemination and implementation strategies 1966–1998. J Gen Intern Med 2006;21:S14-20.
64. Grimshaw JM, Thomas RE, Maclennan G, et al. Effectiveness and efficiency of guideline dissemination and implementation strategies. Health Technol Assess 2004;8:iii-72.
65. Comprehensive Cancer Centre the Netherlands. eds. International collaboration. Sharing knowledge and experience: an international collaboration on guideline development. Utrecht: Comprehensive Cancer Centre the Netherlands, 2013.

Cite this article as: von Dincklage JJ, Ball D, Silvestri GA. A review of clinical practice guidelines for lung cancer. J Thorac Dis 2013;5(S5):S607-S622. doi: 10.3978/j.issn.2072-1439.2013.2013.07.37

Customized chemotherapy in metastatic non-small cell lung cancer (NSCLC)

Jia Wei[1], Teresa Moran[2], Zhengyun Zou[1], Xiaoping Qian[1], Lifeng Wang[1], Carlos Camps[3], Wenjing Hu[1], Imane Chaib[2], Belén Sanchez[2], Lixia Xu[1], Niki Karachaliou[4], María Sanchez-Ronco[5], Baorui Liu[1], Rafael Rosell[2,4]

[1]Comprehensive Cancer Centre of Drum Tower Hospital, Medical School of Nanjing University, Nanjing 210008, China; [2]Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Ctra Canyet s/n, 08916 Badalona, Spain; [3]Hospital General Universitario de Valencia, Av Tres Cruces, s/n, 46014 Valencia, Spain; [4]Breakthrough Cancer Research Unit, Pangaea Biotech S.L, Quiron Dexeus University Hospital, Sabino Arana 5-19, 08028 Barcelona; [5]Universidad Alcalá de Henares, Pza. San Diego s/n, 28801 Alcalá de Henares, Madrid, Spain

Correspondence to: Jia Wei. Comprehensive Cancer Centre of Drum Tower Hospital, Medical School of Nanjing University, Nanjing 210008, China. Email: weijia01627@hotmail.com.

Abstract: Metastatic non-small cell lung cancer (NSCLC) unfortunately remains a lethal disease, despite recent genetic characterization of subclasses of NSCLC, mainly adenocarcinoma, which has led to the development of targeted therapies that improve progression-free survival (PFS). Ultimately, however, patients fatally relapse. In this review we will focus on the search to improve survival for NSCLC patients deemed to be pan-negative for the common driver alterations susceptible to targeted therapy, above all those with EGFR mutations or ALK, ROS or RET translocations. Other uncommon driver mutations such as HER2 and BRAF mutations should be tested in order to rule out targeted treatment before assigning patients to chemotherapy. Chemotherapy yields short lived response with median survival still less than one year. Customized chemotherapy represents one way to attempt to prolong survival, although to date no prospective randomized customized studies have reported sufficient evidence to support this. In one attempt to demonstrate the role of tailoring chemotherapy, the Spanish Lung Cancer Group (SLCG) phase II customized chemotherapy trial (NCT00883480) showed that RAP80, a component of the BRCA1-A complex, influenced outcome in patients with low BRCA1 expression treated with cisplatin/gemcitabine, and in patients with intermediate/high BRCA1 levels receiving cisplatin/docetaxel or docetaxel alone. We are currently performing a prospective, randomized phase III trial comparing non-customized cisplatin/docetaxel with customized therapy in metastatic NSCLC patients (NCT00617656/GECP-BREC) and a parallel phase II study (ChiCTR-TRC-12001860) is being carried out in China (BREC-China) under the auspices of the SLCG.

Keywords: Non-small cell lung cancer; customized chemotherapy; BRCA1 and RAP80 expression customized (BREC); BRCA1; RAP80; RING finger protein 8 (RNF8)

Submitted Jan 18, 2013. Accepted for publication Feb 19, 2013.
doi: 10.3978/j.issn.2218-6751.2013.02.03
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.02.03

Introduction

Growing evidence points to the need for molecular characterization of non-small cell lung cancer (NSCLC), especially in adenocarcinomas and never smokers, for adequate identification of driver mutations or translocations that can be properly treated with targeted therapy. However, there is still a large proportion of NSCLCs for which genetic information to inform therapeutic intervention is still lacking. The benefit of chemotherapy is rather limited and almost all advanced NSCLC cases have poor prognosis with median survival of less than

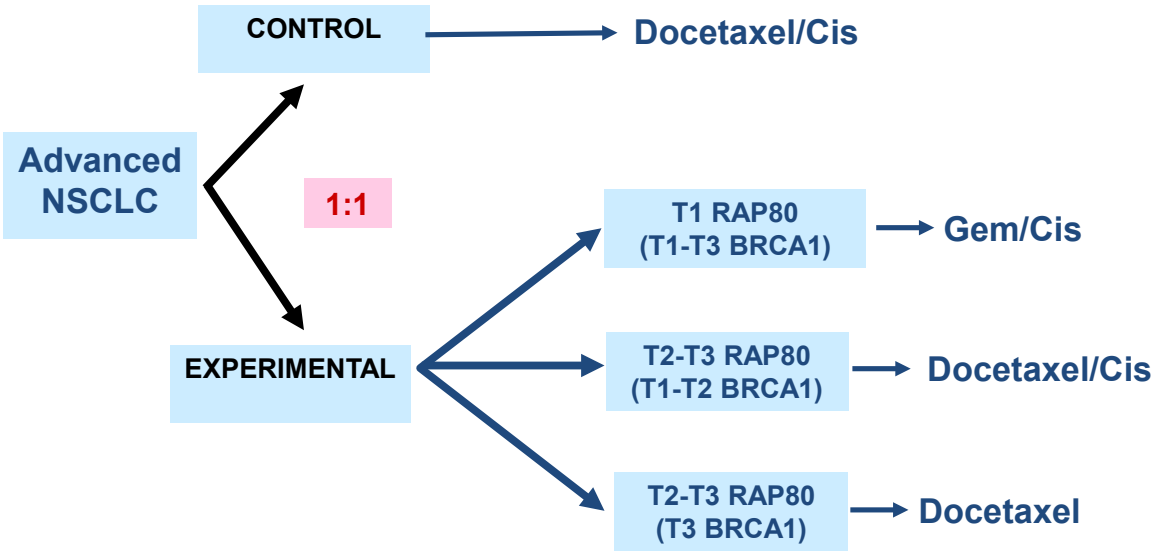


Figure 1 BREC trial design.

one year. Previous studies comparing chemotherapy with best supported care showed median survivals of between 8 and 4 months, respectively (1). Different studies of chemotherapy with cetuximab or pemetrexed as maintenance therapy have not significantly improved overall survival (2-4). In this review we will describe the significant components in DNA repair pathways that warrant investigation, with the aim of identifying a predictive model for optimal customization of chemotherapy which could translate to a meaningful improvement in survival. A BRCA1 and RAP80 Expression Customized (BREC) phase III trial (NCT00617656/GECP-BREC) and a parallel phase II study in China (BREC China, ChiCTR-TRC-12001860) are currently being performed based on the biological information available in 2007. Since then, great progress has been made in further defining DNA repair mechanisms. In this review we will summarize this important progress that has occurred whilst awaiting the results of the above mentioned trials. *Figure 1* shows the design of the randomized BREC trial.

RAP80 and BRCA1 mRNA levels in customizing chemotherapy in the BREC

The BREC studies were constructed based on a Spanish Lung Cancer Group (SLGC) phase II customized trial (NCT00883480) and information that was discovered in 2007 regarding the BRCA1-A complex (BRCA1, RAP80, ABRAXAS). As commented, information which has since been reported, during the accrual of the BREC, provides the rationale for exploring the mRNA levels of other genes in the BREC patients - above all, RNF8 could play a decisive role, since, when BRCA1 and RAP80 are low, if RNF8 is still expressed this will neutralize the predictive model. Other interesting genes and associations are explained below.

Double-strand breaks (DSBs) induced by chemotherapy lead to DNA damage response (DDR): ATM-related or tyrosine kinase-driven

DNA DSBs caused by chemotherapy are repaired by two major systems: non-homologous end joining (NHEJ) and homologous recombination (HR). Upon DNA DSB introduction, the following processes occur: the histone H2AX is phosphorylated by ataxia telangiectasia mutated (ATM); the mediator of DNA damage checkpoint 1 (MDC1) binds to the phosphorylated H2AX (H2AX); ATM phosphorylates MDC1 at the region surrounding the DSB. The E3 ubiquitin ligase RING finger protein 8 (RNF8) binds to phosphorylated MDC1 at DSB sites and promotes recruitment of another E3 ubiquitin ligase RNF168; RNF8 and RNF168 conjugate Lys 63-linked ubiquitin chains onto histone H2A with their cognate E2 ubiquitin-conjugating enzyme UBC13 and induce chromatin remodeling. UBC13-RNF8/RNF168-dependent ubiquitination promotes recruitment of BRCA1 and p53-binding protein 1 (53BP1) to DSBs (5) (*Figure 2*). Importantly, a large proportion

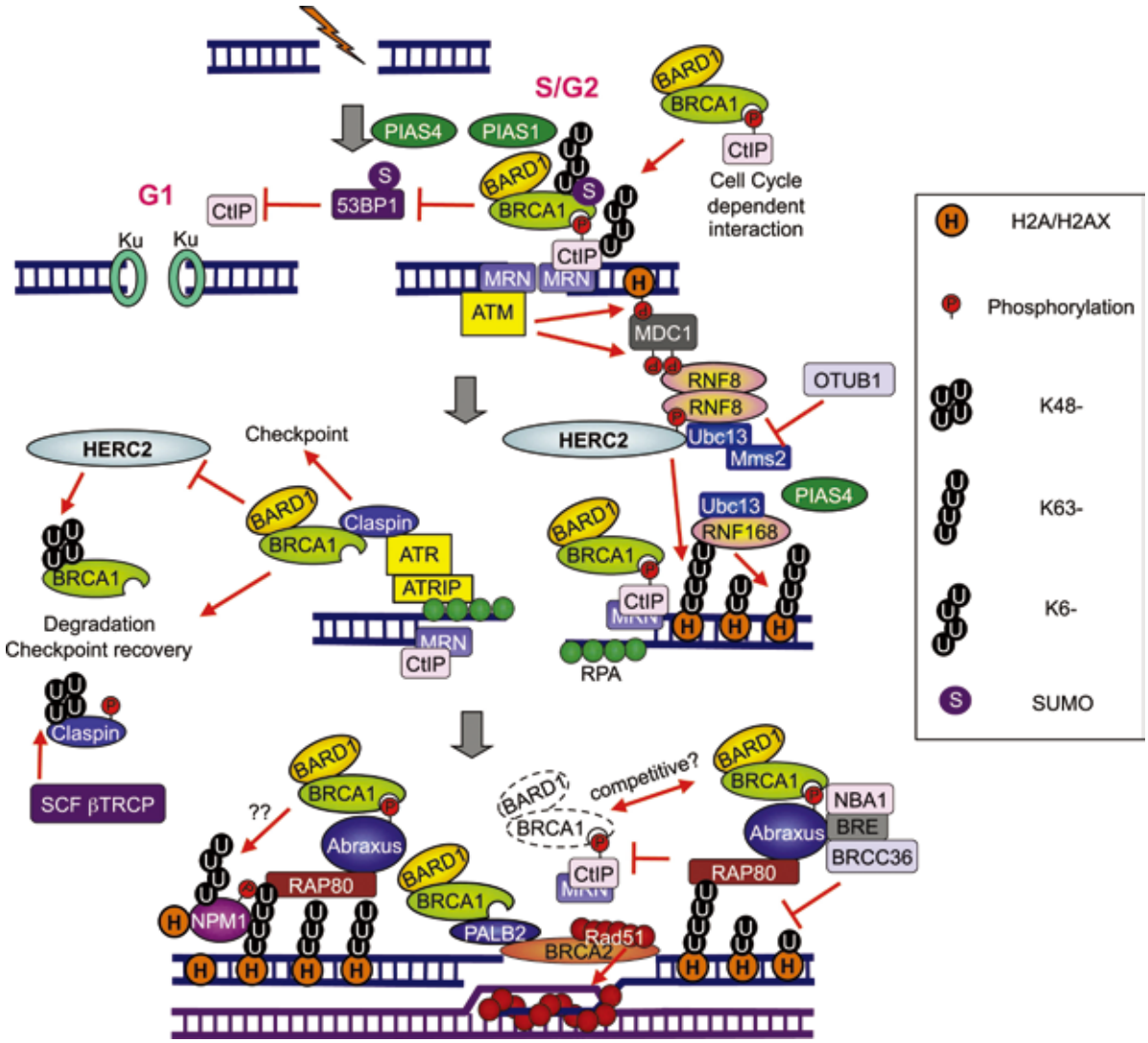


Figure 2 Ubiquitin modification activity of BRCA1 in homologous recombination repair. Reprinted from FEBS Letters 585, Ohta T, Sato K, Wu W. "The BRCA1 ubiquitin ligase and homologous recombination repair", pg 2836-44, Copyright 2011, with permission from Elsevier.

of BRCA1 that localizes to DSB sites is a component of the BRCA1-A complex, consisting of a BRCA1/BARD1 heterodimer, ubiquitin interacting motif (UIM)-containing protein RAP80, and adaptor protein ABRAXAS (6-9).

Based on this information, we performed an exploratory analysis of RAP80 and ABRAXAS mRNA levels in our previous customized phase II trial. Although the information provided by ABRAXAS was similar to that provided by RAP80, RAP80 was more significant (10). Mechanistically, loss of RAP80 suppresses recruitment of the BRCA1 complex to DNA damage sites and abrogates the DNA damage repair process at DSBs (11). It has since been discovered that the BRCA1-A complex also includes the deubiquitinating enzyme BRCC36, as well as BRCC45/BRE and MERIT40/NBA1 (5). Other groups have also demonstrated that, BRCA1 forms biochemically distinct complexes with certain other DNA damage response proteins [BRCA1-B and BRCA1-C complexes; *Figure 3* (6)] in response to DSBs. The simultaneous presence of multiple distinct BRCA1 complexes at DSBs suggests a crosstalk between complexes and increases the level of complexity; for example, the BRCA1/RAP80 complex can mitigate excessive resection by CtIP (12). Although a large proportion of BRCA1 fails to be retained at DSBs upon loss of RAP80, it is possible that relocation of a small amount of BRCA1 to the DSBs via the association with other protein

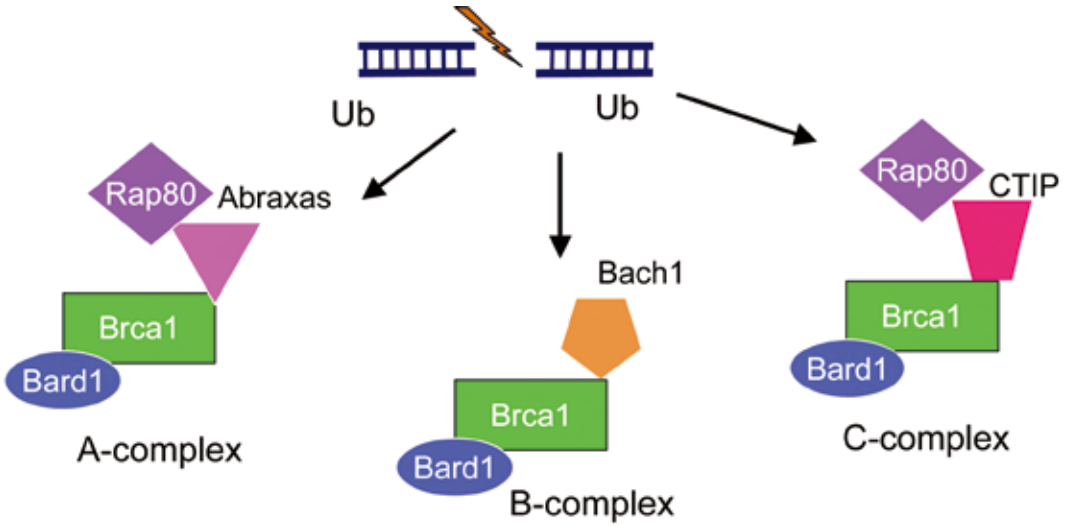


Figure 3 BRCA1-BRCT interacting complexes in DNA damage response. From Wang *et al.* "Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response". Science 2007;316:1194, Adapted with permission from AAAS.

complexes could occur.

In addition, BRCA1 can be recruited to DSBs through direct binding to phosphorylated CtIP, forming the BRCA1-C complex (6) [*Figure 3* (6)]. Importantly, CtIP is capable of generating limited DSB end resection without BRCA1 to promote altered NHEJ, an error-prone repair in G1 phase of the cell cycle [*Figure 2* (5)]. Interestingly, the DSB end resection promoted by CtIP is inhibited by 53BP1, and BRCA1 overwhelms 53BP1 to execute the resection (13,14). In addition, 53BP1 blocks HR and sustains the growth arrest induced by BRCA1 depletion. One major function of BRCA1 and BRCA1-C complex is the suppression of 53BP1 and prolongation of the CtIP activity for DSB end resection to generate ssDNA length long enough for HR [*Figure 2* (5)].

RAP80 interacts with Lys63-linked chain that is generated by UBC13-RNF8/RNF168 and brings BRCA1 to DSB sites. The overexpression of the deubiquitinating enzyme OUT domain, ubiquitin aldehyde binding 1 (OTUB1) suppresses DNA damage-dependent chromatin ubiquitination through inhibition of UBC13 activity, thus suppressing HR (15) [*Figure 2* (5)].

One of the major difficulties in the BREC study is that tumor cells have multiple DNA repair systems other than HR. These systems work redundantly, each operating to repair DNA in the event that other repair systems are ineffective. Recently, it has been demonstrated that inhibition of RNF8 or RNF168 activity can suppress BRCA1 independent of HR in tumor cells with low 53BP1. RNF8 is required for resistance to both irradiation and cytotoxic drugs (16). RNF8 can promote RAD51 assembly at DSB sites in BRCA1/53BP1-depleted cells (17). The model shows that in normal cells, an ubiquitin chain of RAP80, BRCA1, 53BP1 and RAD51 assembles at DSB sites. In BRCA1-depleted cells, RAP80 and 53BP1, but not RAD51, assemble at DSB sites. In RAP80-depleted cells, a small subset of BRCA1 protein, 53BP1 and RAD51 assemble at DSB sites. However, in RNF8/BRCA1-depleted cells or in RNF8/BRCA1/53BP1-depleted cells, RAD51 and RAP80 do not assemble at DSB sites (17) (*Figure 4*).

RNF8 displays dual non-catalytic and catalytic activities responsible for chromatin decondensation and histone ubiquitylation, respectively. An RNF8 dimer is recruited to a DSB by binding to phosphorylated MDC1. The recruited RNF8 dimer binds to the chromodomain helicase DNA-binding protein 4 (CHD4) in a phospho-independent manner, resulting in local chromatin decondensation, which permits enhanced ubiquitin conjugation at DSBs and association of RNF168 and BRCA1 (18). In addition, the ubiquitin-selective valosin-containing protein (VCP) is recruited by RNF8 and plays a critical role in mediating the recruitment of downstream repair factors. VCP stimulates 53BP1 recruitment (18).

The function of RNF8 could be vital to chemoresistance. The HECT type E3 ligase (HERC2), a large 4834-amino acid protein, interacts with the FHA domain of RNF8 in a phosphorylation-dependent manner, facilitating assembly of the RNF8/UBC13 complex (19) [*Figure 2* (5)].

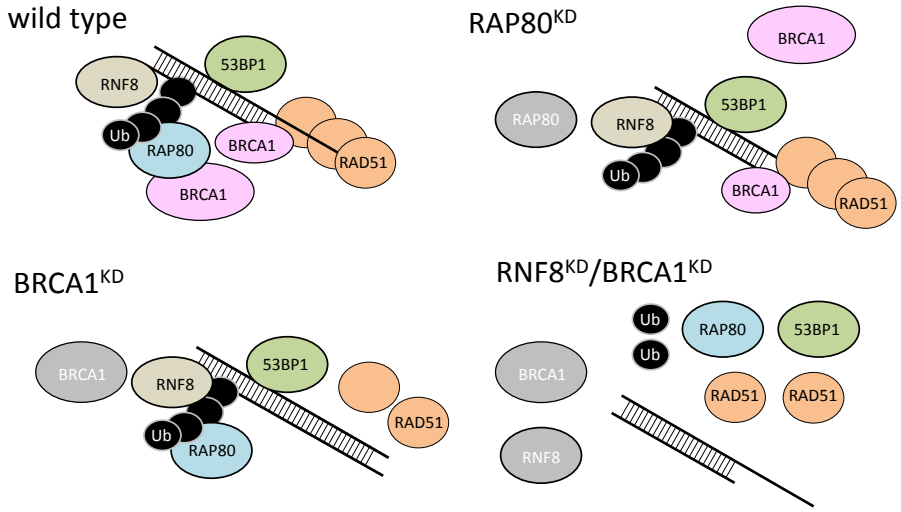


Figure 4 The ubiquitin chain, RAD51, BRCA1 and 53BP1 do not assemble at sites of double-strand breaks in RNF8/BRCA1-depleted cells or RNF8/BRCA1/53BP1-depleted cells. Adapted by permission from the American Association for Cancer Research: Nakada S. *et al.* "RNF8 regulates assembly of RAD51 at DNA double strand breaks in the absence of BRCA1 and 53BP1", Cancer Research 2012;72:4974-83.

Therefore, analysis of HERC2 and RNF8 could be of potential relevance in interpreting the results of the BREC. Interestingly, HERC2 can degrade BRCA1 (19). Also, nucleophosmin (NPM1) is recruited to DSBs in a manner dependent on the RNF8/RNF168-mediated ubiquitin conjugates (20).

PIAS1 and PIAS4 are recruited to DSBs. Depletion of PIAS1 or PIAS4 reduces the proportion of cells displaying BRCA1 accumulation and increase BRCA1 staining intensity at DSBs, increasing sensitivity to irradiation or cisplatin (21,22). Recruitment of RNF168 is impaired only in PIAS4- but not in PIAS1-depleted cells. 53BP1 recruitment does not require BRCA1 or PIAS1 but does require PIAS4 (21,22) [*Figure 2* (5)]. This important finding indicates the importance of examining BRCA1 levels together with those of PIAS1, as well as 53BP1 together with PIAS4 levels. High levels of PIAS4, PP2A/C and BRCA1mRNA were all independent markers of shorter PFS in EGFR-mutant non-small-cell lung cancer (NSCLC) patients treated with erlotinib (23). Along the same lines, low levels of BRCA1, PIAS1 and PIAS4 were independent markers of poor survival in gastric cancer patients receiving docetaxel as second-line treatment (24). BRCA1 was found to be a differential modulator of chemosensitivity, inducing a 10-1000-fold increase in resistance to several DNA-damaging agents, especially those that give rise to DSBs. In contrast, BRCA1 induced a more than 1000-fold increase in sensitivity to paclitaxel, docetaxel and vinorelbine (25,26).

RNF8 could establish a bridge between HR and the NHEJ repair. RNF8 regulates the abundance of the NHEJ repair protein KU80 at sites of DNA damage. RNF8 depletion results in prolonged retention of KU80 at damage sites and impaired NHEJ (27) [*Figure 2* (5)]. Therefore, we can assume that RNF8 depletion is important not only in enhancing the cytotoxic effect of chemotherapy, as described above, but also in impairing repair by NHEJ. On the other hand, NHEJ can function well in the presence of normal RNF8, which may contribute to the failure to predict outcome in the customized model of the BREC. Therefore, analysis of the BREC study can be re-interpreted according to expression of RNF8. In tumors with adequate RNF8 function, expression of Ligase IV could be a major determinant of shorter PFS. DNA Ligase IV is responsible for sealing of DSBs during NHEJ, which is one of the primary mechanisms of DSB repair and is active throughout the cell cycle. During NHEJ, KU70/KU80 heterodimer binds to the DNA ends and recruits proteins, such as DNA-PKcs, Artemis, and Pol, to the repair site, resulting in end-processing followed by Ligase IV, XRCC4 and XLF complex-mediated ligation (28). NHEJ plays a major role in resistance to chemotherapy and radiotherapy. DNA PKcs have been associated with radioresistance in lung cancer cell lines (29). Metnase is a recently described fusion protein composed of a SET

histone methylase domain and Transposase nuclease domain. Metnase enhances NHEJ. Both the SET histone methylase domain and the Transposase nuclease domain are essential for the enhancement of DSB repair (30). Metnase is overexpressed in acute leukemia (31), causing resistance to chemotherapy. Decreasing metnase enhanced cisplatin sensitivity in a lung cancer A549 xenograft (32).

PPP2R2A is also a critical effector of HR through modulation of ATM phosphorylation. PPP2R2A-depleted cells dramatically increase sensitivity to PARP inhibitors. Interestingly, PPP2R2A mRNA is commonly downregulated in NSCLC (33). We have previously observed that in EGFR-mutant NSCLC patients treated with erlotinib, high levels of PP2A/C mRNA significantly increased the hazard ratio for PFS in a multivariate model (23).

Modulator of apoptosis protein 1 (MOAP-1) is a Bax-interacting protein whose knockdown inhibits apoptosis. MOAP-1 association with Bax promotes Bax mitochondrial translocation and activation. The BH3-only proteins, like BIM or BID, serve as sentinels for the initiation of apoptosis by modulating the functions of multi-domain pro-survival (Bcl-2, Mcl-1, and others) or pro-apoptotic members like Bax, involved in regulating the mitochondrial outer membrane permeability (MOMP). MOAP-1 is highly enriched in mitochondria and is considered to act as an effector to facilitate apoptotic signaling of Bax in mitochondria. The intrinsic or mitochondrial programmed cell death pathway leads to the activation of caspase-9 and then caspase-3 (34). MOAP-1 degradation is inhibited by Trim39, a member of the tripartite motif (TRIM) family. Trim39 overexpression enhances etoposide-induced, Bax-mediated apoptosis through stabilization of MOAP-1 (35). Trim39 mRNA is highly expressed in the testis. The Trim 39 gene is located in the MHC class I region of genes within chromosome 6p21-23 (36). There is a mechanistic reason for this finding, since caspase-3 cleaves MDC1, separating the BRCT and FHA domains of MDC1, thus abrogating DNA damage repair (37). These observations prompt us to speculate that BIM mRNA levels could be crucial in inducing apoptosis and that downstream effectors, such as MOAP-1, Trim39 and caspase-3, could play an important role.

DNA damage checkpoint (DDC) signaling on DNA replication

In addition to homologous recombination and NHEJ, the genotoxic stress induced by chemotherapy also causes replication stress (38). This DDC pathway is less well known. The DNA repair scaffolding proteins Slx4 and Rtt107 prevent aberrant activation of DDC signaling by lesions generated during DNA replication. On replication stress, *Saccharomyces cerevisiae* cells lacking Slx4 and Rtt107 show hyperactivation of the downstream DDC kinase Rad53. The Slx4 or Rtt107 complex counteracts the checkpoint adaptor Rad9 by physically interacting with Dpb11 and phosphorylated histone H2A (39). It is hypothesized that modulation of Rad53 activation occurs by a DAMP (dampens checkpoint adaptor-mediated phosphor-signaling) (39).

It has recently been described that RNF126 is highly expressed in a subset of breast cancer cell lines and negatively correlates with p21 expression levels. RNF126 targets p21 for ubiquitin-mediated degradation (40).

DNA damage response (DDR) independent of ATM

Phosphoproteomic analysis have found that several kinases can be involved in DDR, with extensive crosstalk between them. One of the most important could be c-ABL. c-ABL is a non-receptor tyrosine kinase that is upregulated following irradiation, cisplatin and other drugs. c-ABL interacts with ATM and DNA-PK. c-ABL activated by irradiation mediates phosphorylation of PI3K and mTOR, leading to the inhibition of kinase activity (41).

c-ABL is a transducer in the process of apoptosis in response to DNA damage. It is a member of the Src family of non-receptor tyrosine kinases. Under normal conditions, c-ABL is inactive and sequestered into the cytoplasm by binding to the 14-3-3 protein. Upon DNA damage, c-Jun N-terminal kinase (JNK) is activated, phosphorylating 14-3-3 at the binding site to c-ABL, which releases c-ABL, which is localized in the nucleus and is activated by phosphorylation by ATM. Of great interest is that YAP1 is a direct substrate of c-ABL, and DNA damage stabilizes YAP1 in a c-ABL kinase-dependent manner. Then, the phosphorylated YAP1 binds to p73 and is selectively recruited onto the Bax promoter to induce apoptosis (42). The Hippo signaling pathway is a novel tumor suppressor pathway, and the downstream effect of the Hippo signaling cascade is to phosphorylate and inactivate YAP1 and its paralog TAZ. YAP1 and TAZ overexpression has been observed in NSCLC, conferring poor prognosis (43,44). It is interesting that YAP1 can induce apoptosis (Bax) via c-ABL.

Intriguingly, reinforcing the role of c-ABL, it has recently

been reported that overexpression of AXL causes resistance to cisplatin by inhibiting c-ABL/p73 signal (45). This allows us to reason that, since AXL is an effector of the YAP-TAZ pathway (when HIPPO is off) and can induce abrogation of c-ABL, disrupting the association with p73β (45). However, the previous work has demonstrated that c-ABL enhances apoptosis via activating YAP1 (42). This apparent contradiction can only be explained by requiring the Wnt pathway to be active since beta-catenin is then linked to YAP1 and may hamper YAP's transcriptional program, including activation of AXL. On these grounds, also recently, YAP1 and TAZ have been observed to be coupled with beta-catenin, and the degradation of YAP1 and TAZ is avoided when the Wnt pathway is active, which abrogates the beta-catenin destruction complex (AXIN1, GSK3, APC) (46,47). Binding of the Wnts to their receptors inactivates this complex, leading to accumulation and nuclear translocation of beta-catenin (48). Also, paradoxically, in melanomas with $BRAF^{V600E}$, the efficacy of the BRAF inhibitor PLX4720 is increased when beta-catenin is present, and this is achieved by eliminating AXIN1 (49).

Beta-catenin-independent signaling pathways

In addition to the FZD receptors, the Wnt receptors ROR1 and ROR2 also contribute to cancer proliferation (48). Wnt5A is the ligand for ROR1 (50). ROR1 repression inhibits lung adenocarcinoma regardless of EGFR status. ROR1 abrogates ASK1, which can lead to abrogation of BIM (51). In the EURTAC study, higher levels of ROR1 mRNA correlated significantly with poor survival.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Rapp E, Pater JL, Willan A, et al. Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer--report of a Canadian multicenter randomized trial. J Clin Oncol 1988;6:633-41.
2. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet 2009;373:1525-31.
3. Ciuleanu T, Brodowicz T, Zielinski C, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet 2009;374:1432-40.
4. Paz-Ares L, de Marinis F, Dediu M, et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): a double-blind, phase 3, randomised controlled trial. Lancet Oncol 2012;13:247-55.
5. Ohta T, Sato K, Wu W. The BRCA1 ubiquitin ligase and homologous recombination repair. FEBS Lett 2011;585:2836-44.
6. Wang B, Matsuoka S, Ballif BA, et al. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science 2007;316:1194-8.
7. Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science 2007;316:1202-5.
8. Sobhian B, Shao G, Lilli DR, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science 2007;316:1198-202.
9. Yan J, Kim YS, Yang XP, et al. The ubiquitin-interacting motif containing protein RAP80 interacts with BRCA1 and functions in DNA damage repair response. Cancer Res 2007;67:6647-56.
10. Rosell R, Perez-Roca L, Sanchez JJ, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. PLoS One 2009;4:e5133.
11. Wu J, Liu C, Chen J, et al. RAP80 protein is important for genomic stability and is required for stabilizing BRCA1-A complex at DNA damage sites in vivo. J Biol Chem 2012;287:22919-26.
12. Coleman KA, Greenberg RA. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. J Biol Chem 2011;286:13669-80.
13. Bunting SF, Callén E, Wong N, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 2010;141:243-54.
14. Bouwman P, Aly A, Escandell JM, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol 2010;17:688-95.
15. Nakada S, Tai I, Panier S, et al. Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. Nature 2010;466:941-6.
16. Sy SM, Jiang J, Dong SS, et al. Critical roles of ring finger

protein RNF8 in replication stress responses. J Biol Chem 2011;286:22355-61.
17. Nakada S, Yonamine RM, Matsuo K. RNF8 regulates assembly of RAD51 at DNA double-strand breaks in the absence of BRCA1 and 53BP1. Cancer Res 2012;72:4974-83.
18. Luijsterburg MS, Acs K, Ackermann L, et al. A new non-catalytic role for ubiquitin ligase RNF8 in unfolding higher-order chromatin structure. EMBO J 2012;31:2511-27.
19. Wu W, Sato K, Koike A, et al. HERC2 is an E3 ligase that targets BRCA1 for degradation. Cancer Res 2010;70:6384-92.
20. Koike A, Nishikawa H, Wu W, et al. Recruitment of phosphorylated NPM1 to sites of DNA damage through RNF8-dependent ubiquitin conjugates. Cancer Res 2010;70:6746-56.
21. Morris JR, Boutell C, Keppler M, et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 2009;462:886-90.
22. Galanty Y, Belotserkovskaya R, Coates J, et al. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 2009;462:935-9.
23. Rosell R, Molina MA, Costa C, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. Clin Cancer Res 2011;17:1160-8.
24. Wei J, Costa C, Ding Y, et al. mRNA expression of BRCA1, PIAS1, and PIAS4 and survival after second-line docetaxel in advanced gastric cancer. J Natl Cancer Inst 2011;103:1552-6.
25. Quinn JE, James CR, Stewart GE, et al. BRCA1 mRNA expression levels predict for overall survival in ovarian cancer after chemotherapy. Clin Cancer Res 2007;13:7413-20.
26. Quinn JE, Kennedy RD, Mullan PB, et al. BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. Cancer Res 2003;63:6221-8.
27. Feng L, Chen J. The E3 ligase RNF8 regulates KU80 removal and NHEJ repair. Nat Struct Mol Biol 2012;19:201-6.
28. Simsek D, Jasin M. Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4-ligase IV during chromosomal translocation formation. Nat Struct Mol Biol 2010;17:410-6.
29. Sirzén F, Nilsson A, Zhivotovsky B, et al. DNA-dependent protein kinase content and activity in lung carcinoma cell lines: correlation with intrinsic radiosensitivity. Eur J Cancer 1999;35:111-6.
30. Beck BD, Lee SS, Williamson E, et al. Biochemical characterization of metnase's endonuclease activity and its role in NHEJ repair. Biochemistry 2011;50:4360-70.
31. Wray J, Williamson EA, Sheema S, et al. Metnase mediates chromosome decatenation in acute leukemia cells. Blood 2009;114:1852-8.
32. Williamson EA, Damiani L, Leitao A, et al. Targeting the transposase domain of the DNA repair component Metnase to enhance chemotherapy. Cancer Res 2012;72:6200-8.
33. Kalev P, Simicek M, Vazquez I, et al. Loss of PPP2R2A inhibits homologous recombination DNA repair and predicts tumor sensitivity to PARP inhibition. Cancer Res 2012;72:6414-24.
34. Del Gaizo Moore V, Brown JR, Certo M, et al. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. J Clin Invest 2007;117:112-21.
35. Huang NJ, Zhang L, Tang W, et al. The Trim39 ubiquitin ligase inhibits APC/CCdh1-mediated degradation of the Bax activator MOAP-1. J Cell Biol 2012;197:361-7.
36. Lee SS, Fu NY, Sukumaran SK, et al. TRIM39 is a MOAP-1-binding protein that stabilizes MOAP-1 through inhibition of its poly-ubiquitination process. Exp Cell Res 2009;315:1313-25.
37. Solier S, Pommier Y. MDC1 cleavage by caspase-3: a novel mechanism for inactivating the DNA damage response during apoptosis. Cancer Res 2011;71:906-13.
38. Harper JW, Elledge SJ. The DNA damage response: ten years after. Mol Cell 2007;28:739-45.
39. Ohouo PY, Bastos de Oliveira FM, Liu Y, et al. DNA-repair scaffolds dampen checkpoint signalling by counteracting the adaptor Rad9. Nature 2013;493:120-4.
40. Zhi X, Zhao D, Wang Z, et al. E3 ubiquitin ligase RNF126 promotes cancer cell proliferation by targeting the tumor suppressor p21 for ubiquitin-mediated degradation. Cancer Res 2013;73:385-94.
41. Bensimon A, Aebersold R, Shiloh Y. Beyond ATM: the protein kinase landscape of the DNA damage response. FEBS Lett 2011;585:1625-39.
42. Levy D, Adamovich Y, Reuven N, et al. Yap1 phosphorylation by c-Abl is a critical step in selective activation of proapoptotic genes in response to DNA damage. Mol Cell 2008;29:350-61.
43. Wang Y, Dong Q, Zhang Q, et al. Overexpression of yes-associated protein contributes to progression and poor prognosis of non-small-cell lung cancer. Cancer Sci 2010;101:1279-85.
44. Zhou Z, Hao Y, Liu N, et al. TAZ is a novel oncogene in non-small cell lung cancer. Oncogene 2011;30:2181-6.

45. Hong J, Peng D, Chen Z, et al. ABL regulation by AXL promotes cisplatin resistance in esophageal cancer. Cancer Res 2013;73:331-40.
46. Azzolin L, Zanconato F, Bresolin S, et al. Role of TAZ as mediator of Wnt signaling. Cell 2012;151:1443-56.
47. Rosenbluh J, Nijhawan D, Cox AG, et al. β-Catenin-driven cancers require a YAP1 transcriptional complex for survival and tumorigenesis. Cell 2012;151:1457-73.
48. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer 2013;13:11-26.
49. Biechele TL, Kulikauskas RM, Toroni RA, et al. Wnt/β-catenin signaling and AXIN1 regulate apoptosis triggered by inhibition of the mutant kinase BRAFV600E in human melanoma. Sci Signal 2012;5:ra3.
50. Zhang S, Chen L, Cui B, et al. ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. PLoS One 2012;7:e31127.
51. Yamaguchi T, Yanagisawa K, Sugiyama R, et al. NKX2-1/TITF1/TTF-1-Induced ROR1 is required to sustain EGFR survival signaling in lung adenocarcinoma. Cancer Cell 2012;21:348-61.

Cite this article as: Wei J, Moran T, Zou Z, Qian X, Wang L, Camps C, Hu W, Chaib I, Sanchez B, Xu L, Karachaliou N, Sanchez-Ronco M, Liu B, Rosell R. Customized chemotherapy in metastatic non-small cell lung cancer (NSCLC). Transl Lung Cancer Res 2012;2(3):180-188. doi: 10.3978/j.issn.2218-6751.2013.02.03

Management of elderly patients

Alain Vergnenegre[1,2], Romain Corre[2,3], Hervé Lena[2,3], Hervé Le Caer[2,4]

[1]Service de Pathologie Respiratoire, CHU - Limoges, France; [2]Groupe Français de Pneumo - Cancerologie (GFPC), France; [3]Service de Pneumologie CHU - Rennes, France; [4]Service de Pneumologie CH - Draguignan, France

Correspondence to: Alain Vergnenegre. Service de l'Information Médicale et de l'Evaluation, Service de Pathologie Respiratoire et d'Allergologie, CHU Dupuytren, 87042 Limoges Cedex, France. Email: alain.vergnenegre@unilim.fr.

Background: Elderly patients are often excluded from clinical trials, yet more than two-thirds of patients diagnosed with lung cancer are over 65 years old. It is therefore important to develop specific tools and trials for this specific patient population.

Methods: This chapter first examines the management specificities of elderly patients. Randomized trials specifically involving elderly patients are then described, and likely future developments are considered.

Results: Older people have several specificities. In addition to traditional criteria such as age and performance status, other important factors include the number of comorbidities and age-related changes such as cognitive deficits and depression. Specific indices taking these factors into account have been published and validated. Single-agent therapy has been widely used to treat metastatic lung cancer in the elderly, following publication of negative results from randomized phase III trials of combination chemotherapy. Recently, however, a trial of doublet therapy gave positive results, in a subgroup of independent older patients. The benefit of patient selection based on a combination of these indices has been demonstrated in open-label and randomized trials. These results must now be confirmed in phase III trials including the use of tyrosine kinase inhibitors combined with chemotherapy.

Conclusions: Indices based on a combination of age-related factors, together with judicious use of biological markers, will further improve the prognosis of elderly lung cancer patients.

Keywords: Elderly; non-small cell lung cancer; geriatric assessment; management

Submitted Jan 18, 2013. Accepted for publication Feb 19, 2013.
doi: 10.3978/j.issn.2218-6751.2013.02.04
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.02.04

Introduction

Oncogeriatric medicine has now come of age. It involves a comprehensive, multidimensional and multidisciplinary approach to the elderly cancer patient (1). Life expectancy is increasing in all western countries, and projections show that, in France in 2020, more than 10% of inhabitants will be over 70 years old (2). However, elderly individuals are very heterogeneous, and their management must take into account both medical and social problems and specific cancer therapy (3). Elderly patients are generally excluded from clinical trials, however, representing only 8-13% of patients (4). Medical evaluation of elderly cancer patients is complicated not only by their age but also by comorbidities (5), which are independent prognostic factors.

In the United States, cancer registries show that patients over 65 years of age represent two-thirds of all lung cancer patients, and median age at diagnosis is around 70 years (6). A French observational study (7) showed that, in 2000, 32% of patients treated for lung cancer were over 70 years old, and that 18.1% were over 80.

Yet clinical trials specifically focusing on elderly patients are rare in the field of thoracic oncology, even though their value is now clear (8). Lung cancer management guidelines now include specific recommendations on the treatment of elderly patients (9,10). The international society of geriatric oncology has also issued similar guidelines (11).

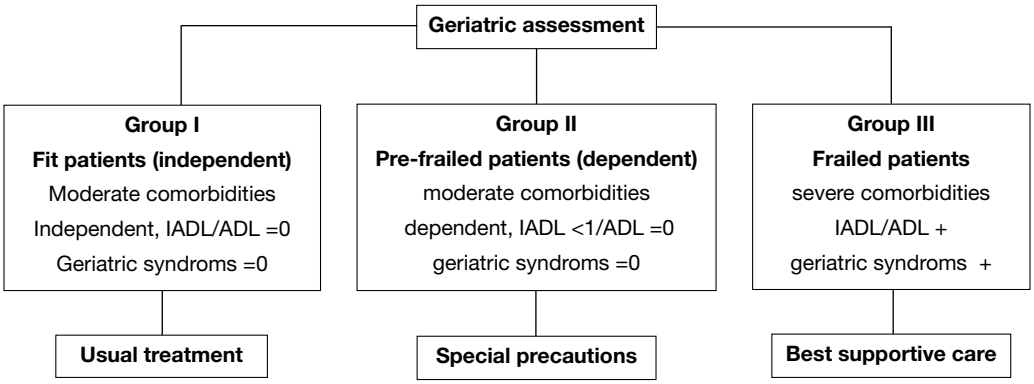


Figure 1 Subgroups of elderly patients identified using a geriatric assessment.

This article examines the specific assessment of elderly cancer patients, the use of certain tools for lung cancer treatment, and likely future developments.

Specificities of lung cancer management in seniors

The selection criteria are the same whether the elderly patient is a candidate for surgery or radiotherapy, and whether the lung cancer is locally advanced or metastatic.

Aging is accompanied by a number of physiological changes, including a decreased glomerular filtration rate, impaired hepatic metabolism, decreased serum albumin, and a decreased absorption-distribution ratio (3). Elderly patients often have comorbidities: Yancik's study (12) showed that 13% of patients aged between 55 and 65 years had more than 5 comorbidities, a figure rising to 24% between 66 and 74 years and 40% after 75 years. As stressed by Extermann (13), performance status, a prognostic factor in lung cancer, does not have the same impact on patient management as comorbidities, or on tolerance of either the disease or its treatment. Validated tools are available for assessing such comorbidities, such as the Charlson index (14) and the cumulative illness rating scale - geriatric (15). However, comorbidities, performance status are independent from age in the disease prognosis (14,15).

It is crucial to assess the impact of aging by using geriatric indexes (16,17). These multidimensional tools explore cognitive functions (18), depression (19), and other geriatric disorders (20) such as falls and incontinence, nutritional status, polypharmacy, mobility and environmental conditions. These disorders are combined in the standardized comprehensive geriatric assessment (CGA) proposed by Balducci (21-23). However, as the CGA was particularly time-consuming, a short questionnaire was developed and validated (24,25). This work allowed us to classify the elderly into three groups, as shown in *Figure 1*.

Recent studies have shown that the use of these indices influences the choice of initial care by multidisciplinary panels in about 1 in 5 cases (26-28).

Quality of life, which is widely assessed in lung cancer patients regardless of age, is particularly important in the elderly. Whatever the tool used, clinical trials must include QOL assessments to ensure that treatment does not have a major negative impact (29).

Management of early-stage lung cancer

Age itself does not contraindicate surgery (30), but elderly patients are less likely to be referred to a surgeon (31). There is a positive correlation between the survival rate and the use of limited surgery or video-assisted thoracoscopic surgery (32).

Management of patients with locally advanced lung cancer

There are currently no published trials of concurrent chemoradiotherapy in elderly patients, but trials not specifically devoted to seniors suggested that toxicity was greater in older patients (33). An ongoing French trial is studying the feasibility of using geriatric assessment for patient and treatment selection (34).

Management of patients with metastatic lung cancer

These are the patients who raise the most difficult

Table 1 Principal randomized trials in elderly subjects

Authors	Drugs	N° pts	Reponse rate (%)	Median survival	1-year survival (%)	P
Elvis 1999 (36)	VNR	76	19.7	6.5	32	0.03
	BSC	85	/	4.9	14	
Frasci 2000 (37)	Gem + VNR	60	15	7	30	<0.01
	VNR	60	20	4.5	13	
Gridelli 2003 (38)	VNR	233	21	8.5	42	ns
	Gem	233	16	6.5.	28	
	Gem + VNR	232	18.1	7.4	34	
Kudoh 2006 (39)	VNR	92	9.8	9.9	NR	ns
	Doc	90	22.7	14	NR	
Lilenbaum 2005 (40)	Carbo + Paclitaxel	561	28	9	38	ns
	Paclitaxel	155	36	8	33	
Comella 2004 (41)	Gem	68	18	5.1	29	ns
	Paclitaxel	63	13	6.4	25	
	Gem + Paclitaxel	65	32	9.2	44	
	Gem + VNR	68	23	9.7	32	
Quoix 2010 (42)	VNR or Gem	226	10	6.2	25.4	0.0004
	Carbo + weekly Paclitaxel	225	27	10.3	44.5	

issues. Standard treatment has consisted essentially of monotherapy, as trials conducted in the 2000s failed to show any improvement in survival with doublets. In 2010, however, Quoix *et al.* (35) showed the superiority of a weekly carboplatin-paclitaxel combination over gemcitabine or vinorelbine monotherapy, albeit at a cost of more severe hematological toxicity.

Table 1 summarizes the main phase III trials of single-agent and combination therapy in elderly patients.

It is important, in addition to traditional outcomes, to assess quality of life and particularly the impact of toxicities (29).

The choice between monotherapy and doublet therapy is still controversial, although the trial conducted by Quoix *et al.* (35) clearly marked a turning point. Des Guetz *et al.* (43) recently published a meta-analysis comparing the efficacy and safety of monotherapy versus doublet therapy in patients with metastatic lung cancer. This meta-analysis included 10 studies and 2,605 patients with an average age of 74 years. Overall survival at one year was not improved by the use of doublets versus monotherapy (HR 0.92, CI: 0.82-1.03, P=0.016). In contrast, the response rate was significantly better with doublet therapy (HR 1.51, 1.22-1.86, P>0.001). Gastrointestinal toxicity was similar in the two populations, but neutropenia, thrombocytopenia and anemia were more problematic with doublet therapy. Among grade III/IV adverse effects, thrombocytopenia and anemia were more frequent with doublet therapy. The authors concluded that there was little additional benefit to the use of doublets versus monotherapy in these patients. Further studies are required to confirm these results (35). In addition, as the authors pointed out, these findings are applicable to independent older patients and cannot be extrapolated to frailed patients, for whom the best treatment strategy remains to be defined.

In September 2012, ESMO (44) published its new guidelines favoring platinum-based doublets for elderly patients with PS =0-1 and for some selected patients with PS =2, while monotherapy should be offered to vulnerable patients and those with multiple comorbidities, owing to the higher risk of adverse effects. The "vulnerable" elderly patient was not defined.

While most of the studies presented in *Table 1* selected patients on the basis of standard criteria (age and performance status) (36-42), other teams attempted to define their geriatric patient population more precisely, based on a combination of age, performance status and a comorbidity index (Charlson score). Two open-label phase

II (45,46) trials involved two distinct populations: patients who were considered to be in good general condition with few comorbidities were treated with docetaxel and gemcitabine, while the most fragile patients were treated with docetaxel alone. Both trials were designed to assess the feasibility of the rating tools. Effectiveness was moderate in the monotherapy group, while patients treated with the combination had results similar to those observed in younger patients.

Two randomized phase II trials (47,48) were secondary published with the same selection and a targeted therapy with erlotinib into the treatment strategy. The docetaxel-gemcitabine combination followed by erlotinib gave the best results. Patients were selected on the basis of age, PS, the Charlson score, the number of comorbidities, and geriatric symptoms (falls, incontinence and dependency for ADL and IADL). The results were modest in the fragile patients treated with monotherapy (gemcitabine followed by erlotinib, or vice versa).

These latter two studies showed that geriatric assessment was feasible in clinical trials. Early use of geriatric criteria led to better-defined groups and favored the selection of patients for combination therapy or monotherapy.

Although quality of life was preserved in some clinical trials, such as that conducted by Quoix *et al.*, the risk-benefit assessment must take adverse effects into account (49).

Gradually, targeted therapies have started to be used in these patients. Numerous studies (50-53) have shown that, in Asian patients with activating EGFR mutations, EGFR-TKI significantly improved progression-free survival after frontline treatment, compared to platinum-based chemotherapy. These results were found with gefitinib in an Asian population [HR: 0.36 (0.25-0.51) (52); HR: 0.16 (0.10-0.26) (54)], and with erlotinib in a Caucasian population, HR: 0.37 (0.25 to 0.54) (53).

Following these results, gefitinib and erlotinib obtained marketing authorization for first-line treatment of advanced NSCLC in patients with activating EGFR mutations, even though these studies included very few elderly patients. The age limit for inclusion was 75 years in the studies by Maemondo *et al.* (52) and Zhou *et al.* (54), and median age was 65 years in the study by Rosell *et al.* (53). These activating mutations were a powerful predictor of intense and rapid responses [ORR 58% (53) to 73.7% (52)] to EGFR TKI, a drug with a favorable safety profile. Most elderly EGFR-mutated patients with symptoms or altered general condition (due mainly due to cancer extension) derive a major benefit. Inoue *et al.* (55) showed that some patients with activating EGFR mutations who were considered ineligible for chemotherapy because of poor PS (3 or 4) could regain a PS of 0 or 1, and that some even became eligible for second-line chemotherapy on disease progression.

There are no specific trials of angiogenesis inhibitors in elderly lung cancer patients.

In the ECOG 4599 trial (56), comparing carboplatin-paclitaxel to carboplatin-paclitaxel-bevacizumab. Bevacizumab did not improve survival in the subgroup of patients aged 70 years or more (median 74 years), although there was a trend towards a better response rate and longer progression-free survival in the bevacizumab group. Toxicity, and especially hematologic adverse effects, was higher in the bevacizumab arm. In the AVAIL study (57) of cisplatin-gemcitabine with or without bevacizumab, progression-free survival was significantly better with bevacizumab and was similar in the older and younger subgroups, without specific toxicity in the older group; however, the median age of patients over 65 was only 68 years. In the ARIES prospective cohort study (58) evaluating the use of bevacizumab in combination with first-line chemotherapy, PFS was respectively 6.6 and 6.7 months in patients <70 years (n=1,320) and ≥70 years (n=647), and overall survival was respectively 14.2 and 12.2 months, i.e. largely inferior in patients ≥70 years. There was no excess toxicity in these latter patients.

The role of bevacizumab in combination with platinum-based chemotherapy in patients ≥70 years of age needs to be determined in a phase III trial specifically dedicated to these patients.

Future developments

While clinical practice guidelines favored the use of monotherapy in elderly lung cancer patients, recent studies supported the use of doublets in selected patients.

A phase III trial is now needed to validate the use of a geriatric index as a criterion for patient selection. Enrolment in the Esogia trial (*Figure 2*) is now complete and the results should be available in 2013. If the results are positive, the short geriatric assessment could become a standard selection tool for the elderly population. The use of a complete or an abbreviated form might facilitate its application (59).

Elderly lung cancer patients cannot be selected on the basis of clinical criteria alone: biological factors must also be taken into account. Rosell *et al.* (60) have shown that the prevalence of EGFR mutations is higher (41%) among

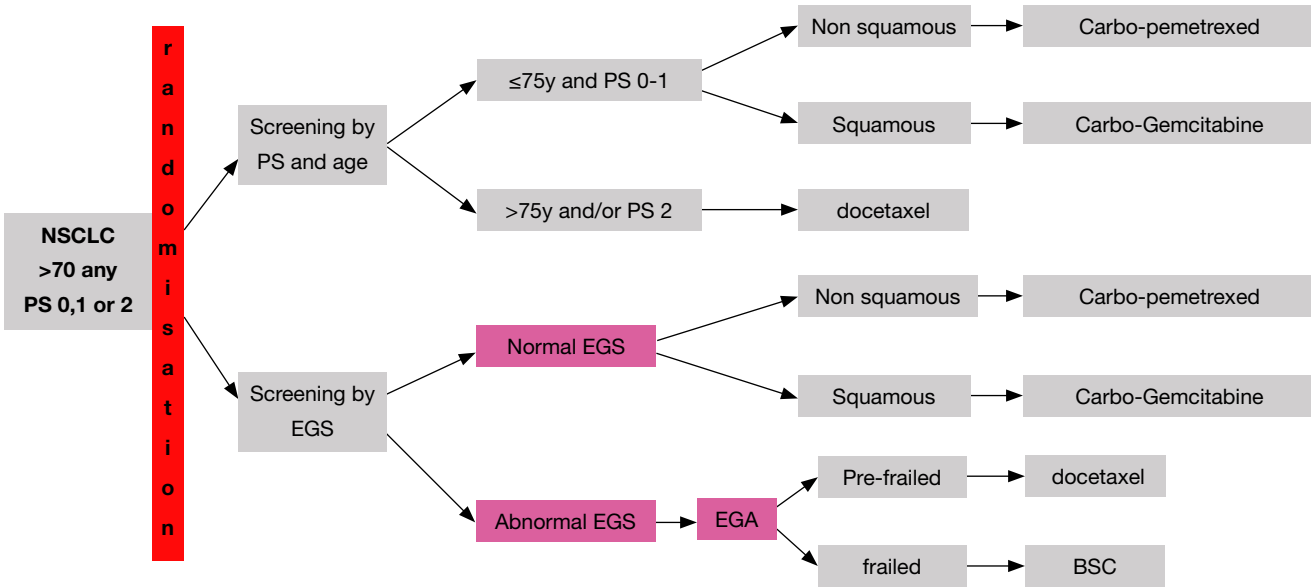


Figure 2 The ESOGIA trial.

patients over 70, supporting the use of EGFR inhibitors.

A recent report of the BATTLE trial (61) showed similar results in seniors and younger patients in an open trial in which treatment selection was based on a biomarker profile (EGFR, K-RAS, B-RAF, cyclin D1, VEGF receptor, and retinoid x receptor).

The future clearly lies in a combination of all these factors. Given the favorable harm-benefit ratio of targeted therapies (EGFR TKI and ALK inhibitors), these drugs might be used as first-line treatments for patients whose tumors bear the molecular target, including patients whose general condition is degraded by the disease. It is possible that, as new therapeutic targets and more effective and well-tolerated drugs are developed, the scope of geriatric assessment may change. Oncogeriatric tools will need to be adapted to these new treatments, including optimal use of biological markers and selection of eligible subpopulations on the basis of clinical criteria, including a geriatric assessment.

Acknowledgements

A. Vergnenegre has received honoraria from Roche, Amgen, Lilly and has received funding for clinical research from Astra-Zeneca, Chugaï, Lilly, Amgen, Roche and Boehringer-Ingelheim; R. Corre has funding for clinical research from Lilly, Roche, Chugai and Sanofi Aventis; H Léna has received honoraria from Lilly for board activity and from Astra Zeneca for speaker activity; H Le Caer has received honoraria from Roche and Lilly.

Disclosure: The authors declare no conflict of interest.

References

1. Zulian G, Terret C, Droz JP. Interdependance of oncologist and geriatrician. Médecine et hygiène 2003;61:1098-106.
2. Brutel C. Projections de population à l'horizon 2050 - un vieillissement inéluctable. Insee Première 2001:762:1 vol. Available online: http://www.insee.fr/fr/ffc/docs_ffc/ip762.pdf
3. Lichtman SM, Balducci L, Aapro M. Geriatric oncology: a field coming of age. J Clin Oncol 2007;25:1821-3.
4. Talarico L, Chen G, Pazdur R. Enrollment of elderly patients in clinical trials for cancer drug registration: a 7-year experience by the US Food and Drug Administration. J Clin Oncol 2004;22:4626-31.
5. Asmis TR, Ding K, Seymour L, et al. Age and comorbidity as independent prognostic factors in the treatment of non small-cell lung cancer: a review of National Cancer Institute of Canada Clinical Trials Group trials. J Clin Oncol 2008;26:54-9.
6. Owonikoko TK, Ragin CC, Belani CP, et al. Lung cancer in elderly patients: an analysis of the surveillance, epidemiology, and end results database. J Clin Oncol 2007;25:5570-7.

7. Piquet J, Blanchon F, Grivaux M, et al. Primary bronchial carcinoma in elderly subjects in France. Rev Mal Respir 2003;20:691-9.
8. Jatoi A, Hillman S, Stella P, et al. Should elderly non-small-cell lung cancer patients be offered elderly-specific trials? Results of a pooled analysis from the North Central Cancer Treatment Group. J Clin Oncol 2005;23:9113-9.
9. Azzoli CG, Baker S Jr, Temin S, et al. American Society of Clinical Oncology Clinical Practice Guideline update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol 2009;27:6251-66.
10. Felip E, Gridelli C, Baas P, et al. Metastatic non-small-cell lung cancer: consensus on pathology and molecular tests, first-line, second-line, and third-line therapy: 1st ESMO Consensus Conference in Lung Cancer; Lugano 2010. Ann Oncol 2011;22:1507-19.
11. Lichtman SM, Wildiers H, Chatelut E, et al. International Society of Geriatric Oncology Chemotherapy Taskforce: evaluation of chemotherapy in older patients--an analysis of the medical literature. J Clin Oncol 2007;25:1832-43.
12. Yancik R, Ganz PA, Varricchio CG, et al. Perspectives on comorbidity and cancer in older patients: approaches to expand the knowledge base. J Clin Oncol 2001;19:1147-51.
13. Extermann M, Overcash J, Lyman GH, et al. Comorbidity and functional status are independent in older cancer patients. J Clin Oncol 1998;16:1582-7.
14. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
15. Linn BS, Linn MW, Gurel L. Cumulative illness rating scale. J Am Geriatr Soc 1968;16:622-6.
16. Repetto L, Venturino A, Fratino L, et al. Geriatric oncology: a clinical approach to the older patient with cancer. Eur J Cancer 2003;39:870-80.
17. Ferrucci L, Guralnik JM, Cavazzini C, et al. The frailty syndrome: a critical issue in geriatric oncology. Crit Rev Oncol Hematol 2003;46:127-37.
18. Lichtman SM. Guidelines for the treatment of elderly cancer patients. Cancer Control 2003;10:445-53.
19. Roose SP, Katz IR, Pollock BG, et al. Contemporary issues in the diagnosis and treatment of late-life depression. J Am Med Dir Assoc 2002;3:H26-9.
20. Hardy C, Wallace C, Khansur T, et al. Nutrition, cancer, and aging: an annotated review. II. Cancer cachexia and aging. J Am Geriatr Soc 1986;34:219-28.
21. Balducci L, Extermann M. Management of cancer in the older person: a practical approach. Oncologist 2000;5:224-37.
22. Balducci L. Geriatric oncology. Crit Rev Oncol Hematol 2003;46:211-20.
23. Balducci L. Management of cancer in the elderly. Oncology (Williston Park) 2006;20:135-43; discussion 144, 146, 151-2.
24. Overcash JA, Beckstead J, Extermann M, et al. The abbreviated comprehensive geriatric assessment (aCGA): a retrospective analysis. Crit Rev Oncol Hematol 2005;54:129-36.
25. Extermann M, Hurria A. Comprehensive geriatric assessment for older patients with cancer. J Clin Oncol 2007;25:1824-31.
26. Caillet P, Canoui-Poitrine F, Vouriot J, et al. Comprehensive geriatric assessment in the decision-making process in elderly patients with cancer: ELCAPA study. J Clin Oncol 2011;29:3636-42.
27. Aliamus V, Adam C, Druet-Cabanac M, et al. Geriatric assessment contribution to treatment decision-making in thoracic oncology. Rev Mal Respir 2011;28:1124-30.
28. Yonnet S, Gazaille V, Grasset-Dupuy M, et al. Age and management decisions in patients with primary lung cancer. Rev Mal Respir 2008;25:295-302.
29. LeCaer H, Delhoume JY, Thomas PA, et al. Multicenter phase II trial of carboplatin/vinorelbine in elderly patients with advanced non-small-cell lung cancer efficacy and impact on quality of life: Groupe Francais de Pneumo-Cancerologie Study 9902. Clin Lung Cancer 2005;7:114-20.
30. Riquet M, Medioni J, Manac'h D, et al. Non-small cell lung cancer: surgical trends as a function of age. Rev Mal Respir 2001;18:173-84.
31. Riquet M, Le Pimpec Barthes F. Chirurgie thoracique du sujet âgé. ed. In: Morère JF, Rainfray M. eds. Cancer du sujet âgé. Paris: Springer Verlag France, 2002;1:33-45.
32. Jaklitsch MT, Mery CM, Audisio RA. The use of surgery to treat lung cancer in elderly patients. Lancet Oncol 2003;4:463-71.
33. Langer C, Hsu C, Curran W, et al. Elderly patients (pts) with locally advanced non-small cell lung cancer (LA-NSCLC) benefit from combined modality therapy: secondary analysis of Radiation Therapy Oncology Group (RTOG) 94-10. Proc Am Soc Clin Oncol 2002;21:abstr 1193.
34. Locher C, Pourel N, Marin B, et al. A phase II study of weekly cisplatine plus oral vinorelbine with concomittant radiotherapy in non-dependent elderly patients with localized inoperable non small cell lung carcinoma (Essai GFPC 08-06, Raccosa). Rev Mal Respir 2011;28:58-65.
35. Quoix E, Zalcman G, Oster JP, et al. Carboplatin and weekly paclitaxel doublet chemotherapy compared with monotherapy in elderly patients with advanced non-small-

cell lung cancer: IFCT-0501 randomised, phase 3 trial. Lancet 2011;378:1079-88.
36. Des Guetz G, Uzzan B, Nicolas P, et al. Comparison of the efficacy and safety of single-agent and doublet chemotherapy in advanced non-small cell lung cancer in the elderly: a meta-analysis. Crit Rev Oncol Hematol 2012;84:340-9.
37. Peters S, Adjei AA, Gridelli C, et al. Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23:vii56-64.
38. Effects of vinorelbine on quality of life and survival of elderly patients with advanced non-small-cell lung cancer. The Elderly Lung Cancer Vinorelbine Italian Study Group. J Natl Cancer Inst 1999;91:66-72.
39. Frasci G, Lorusso V, Panza N, et al. Gemcitabine plus vinorelbine versus vinorelbine alone in elderly patients with advanced non-small-cell lung cancer. J Clin Oncol 2000;18:2529-36.
40. Gridelli C, Perrone F, Gallo C, et al. Chemotherapy for elderly patients with advanced non-small-cell lung cancer: the Multicenter Italian Lung Cancer in the Elderly Study (MILES) phase III randomized trial. J Natl Cancer Inst 2003;95:362-72.
41. Kudoh S, Takeda K, Nakagawa K, et al. Phase III study of docetaxel compared with vinorelbine in elderly patients with advanced non-small-cell lung cancer: results of the West Japan Thoracic Oncology Group Trial (WJTOG 9904). J Clin Oncol 2006;24:3657-63.
42. Lilenbaum RC, Herndon JE 2nd, List MA, et al. Single-agent versus combination chemotherapy in advanced non-small-cell lung cancer: the cancer and leukemia group B (study 9730). J Clin Oncol 2005;23:190-6.
43. Comella P, Frasci G, Carnicelli P, et al. Gemcitabine with either paclitaxel or vinorelbine vs paclitaxel or gemcitabine alone for elderly or unfit advanced non-small-cell lung cancer patients. Br J Cancer 2004;91:489-97.
44. Quoix E, Monnet I, Scheid P, et al. Management and outcome of French elderly patients with lung cancer: an IFCT survey. Rev Mal Respir 2010;27:421-30.
45. LeCaer H, Barlesi F, Robinet G, et al. An open multicenter phase II trial of weekly docetaxel for advanced-stage non-small-cell lung cancer in elderly patients with significant comorbidity and/or poor performance status: The GFPC 02-02b study. Lung Cancer 2007;57:72-8.
46. LeCaer H, Fournel P, Jullian H, et al. An open multicenter phase II trial of docetaxel-gemcitabine in Charlson score and performance status (PS) selected elderly patients with stage IIIB pleura/IV non-small-cell lung cancer (NSCLC): the GFPC 02-02a study. Crit Rev Oncol Hematol 2007;64:73-81.
47. LeCaer H, Barlesi F, Corre R, et al. A multicentre phase II randomised trial of weekly docetaxel/gemcitabine followed by erlotinib on progression, vs the reverse sequence, in elderly patients with advanced non small-cell lung cancer selected with a comprehensive geriatric assessment (the GFPC 0504 study). Br J Cancer 2011;105:1123-30.
48. LeCaer H, Greillier L, Corre R, et al. A multicenter phase II randomized trial of gemcitabine followed by erlotinib at progression, versus the reverse sequence, in vulnerable elderly patients with advanced non small-cell lung cancer selected with a comprehensive geriatric assessment (the GFPC 0505 study). Lung Cancer 2012;77:97-103.
49. Janssen-Heijnen ML, Maas HA, Siesling S, et al. Treatment and survival of patients with small-cell lung cancer: small steps forward, but not for patients >80. Ann Oncol 2012;23:954-60.
50. Wheatley-Price P, Ding K, Seymour L, et al. Erlotinib for advanced non-small-cell lung cancer in the elderly: an analysis of the National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2008;26:2350-7.
51. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
52. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
53. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958-67.
54. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
55. Inoue A, Kobayashi K, Usui K, et al. First-line gefitinib for patients with advanced non-small-cell lung cancer harboring epidermal growth factor receptor mutations without indication for chemotherapy. J Clin Oncol 2009;27:1394-400.
56. Ramalingam SS, Dahlberg SE, Langer CJ, et al. Outcomes for elderly, advanced-stage non small-cell lung cancer patients treated with bevacizumab in combination with carboplatin and paclitaxel: analysis of Eastern Cooperative

Oncology Group Trial 4599. J Clin Oncol 2008;26:60-5.
57. Reck M, von Pawel J, Zatloukal P, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. J Clin Oncol 2009;27:1227-34.
58. Wozniak A, Garst J, Jahanzeb M, et al. Clinical outcomes (CO) for special populations of patients (pts) with advanced non-small cell lung cancer (NSCLC): Results from ARIES, a bevacizumab (BV) observational cohort study (OCS). J Clin Oncol 2012;28:abstr 7618.
59. Provencio M, Camps C, Alberola V, et al. Lung cancer and treatment in elderly patients: the Achilles Study. Lung Cancer 2009;66:103-6.
60. Rosell R, Santarpia M, Moran T, et al. Age-related genetic abnormalities: The Achilles Heel for customizing therapy in elderly lung cancer patients. Personal Med 2007;4:59-72.
61. Tsao AS, Liu S, Lee JJ, et al. Clinical outcomes and biomarker profiles of elderly pretreated NSCLC patients from the BATTLE trial. J Thorac Oncol 2012;7:1645-52.

Cite this article as: Vergnenegre A, Corre R, Lena H, Le Caer H. Management of elderly patients. Transl Lung Cancer Res 2013;2(3):200-207. doi: 10.3978/j.issn.2218-6751.2013.02.04

Alternative to surgery in early stage NSCLC—interventional radiologic approaches

Kyungmouk Steve Lee, Bradley B. Pua

Division of Interventional Radiology, Weill Cornell Medical College, New York-Presbyterian Hospital, New York, NY 10065, USA
Correspondence to: Bradley B. Pua, MD. Division of Interventional Radiology, Weill Cornell Medical College, New York-Presbyterian Hospital, 525 East 68th Street, New York, NY 10065, USA. Email: brp9018@med.cornell.edu.

Abstract: Interventional radiologists have a variety of techniques in their armamentarium to treat pulmonary tumors. While most therapies are targeted to metastasis or palliation, percutaneous thermal ablation represents a potential therapy for not only palliation, but to treat inoperable early stage disease. Although radiofrequency ablation (RFA) is the most studied of these ablative techniques, newer technologies of thermal ablation, such as microwave and cryoablation have emerged as additional options. In this article, we will review the three different thermal ablative modalities, including patient selection, technique, outcomes, complications, and imaging follow-up. A brief discussion of state of the art techniques such as irreversible electroporation (IRE) and catheter directed therapies will also be included.

Keywords: Non-small cell lung cancer (NSCLC); interventional radiologic; radiofrequency ablation (RFA); microwave ablation; cryoablation; irreversible electroporation (IRE); catheter directed therapies

Submitted Jul 24, 2013. Accepted for publication Sep 24, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.02
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.02

Introduction

The standard of care for operable patients with early stage non-small cell lung cancer (NSCLC) is lobectomy with lymph node evaluation (1,2). However, a significant number of patients with early stage NSCLC are not candidates for lobectomy due to diminished pulmonary function and other comorbidities (3-5). For non-surgical candidates, stereotactic body radiation therapy (SBRT) or image-guided percutaneous thermal ablation are attractive options.

For more than a decade, thermal ablation has been an effective, safe, repeatable, and relatively low-cost technique in the treatment of various solid tumors, including in the liver, kidney, adrenal gland, breast and bone (6). In 2000, Dupuy *et al.* first reported the use of radiofrequency ablation (RFA) in the treatment of lung tumors (7). Since then, RFA has been the most widely used form of thermal ablation in the lung, including in the treatment of medically inoperable or high-risk early stage NSCLC (8-14). In recent years, microwave ablation and cryoablation have also been applied with increasing frequency (15-19).

Percutaneous thermal ablation offers may advantages including its minimally invasive nature, its ability to preserve normal lung parenchyma with minimal effect on pulmonary function, and the ability to perform these procedure under moderate sedation or even local anesthesia (16,19). In fact, most procedures are performed in a single outpatient session and can even be done at the same time as a biopsy (20). Furthermore, percutaneous thermal ablation allows for repeated treatment sessions, which may improve survival in patients who have failed primary treatment (21). Repeated surgery, on the other hand, is often not feasible secondary to either technical difficulty or limited residual pulmonary reserves. SBRT is similarly limited in regards to retreatment of local tumor recurrence secondary to limitations in maximal tolerated radiation dosages to the lung for fear of radiation pneumonitis. Another limitation of some types of SBRT is the need for multiple gold fiducial markers, which are placed percutaneously or bronchoscopically. Percutaneous placement of fiducial markers is associated with higher complication rates than percutaneous thermal ablation (22).

In this article, we will review the three different thermal ablative modalities, including patient selection, technique, treatment outcomes, complications, and imaging follow-up. A brief discussion on state of the art techniques such as irreversible electroporation (IRE) and catheter directed therapies will also be included.

Patient selection and pre-procedural evaluation

In the treatment of early stage NSCLC, image-guided percutaneous thermal ablation is indicated for patients who are not surgical candidates due to cardiopulmonary comorbidities such as severe chronic obstructive pulmonary disease (7,12,18,19,23). Ablative techniques may be performed in patients who have limited pulmonary function. Pulmonary function is generally not significantly changed after thermal ablation. In fact, even in post-pneumonectomy patients with a single lung, RF ablation may be safely performed with preservation of pulmonary function (24,25).

Evaluation of patients prior to thermal ablation entails taking a history and physical examination with attention to bleeding diathesis and medications such as anticoagulants and antiplatelet agents. Medical comorbidities need to be assessed to determine the safety of administering moderate conscious sedation or general anesthesia. Patients should also be screened for the presence of cardiac devices because the energy from RFA may potentially interfere with pacemakers or defibrillators; these patients should have their treatment sessions coordinated with a cardiac electrophysiologist (26). However, this cardiac risk is obviated by bipolar microwave ablation systems as well as cryoablation devices.

Recent imaging, such as computed tomography and/or fluorodeoxyglucose positron emission tomography, are important for assessing tumor size and proximity to neurovascular structures as well as for selecting the type, number, and trajectory of ablation probes (27). An inherent limitation of non-surgical therapy is the inability to systematically assess for nodal disease. In one study, among patients with clinical stage I NSCLC, 13.8% of patients were upstaged to N1 disease on final surgical pathology and an additional 3.5% upstaged to N2 disease (28). The presence of nodal or extra-thoracic disease is generally a contraindication to thermal ablation unless the goal of the treatment is palliative.

Prior to the procedure, the potential side effects are explained to the patient including possible post-ablation syndrome, in which an inflammatory response may result in fever, malaise, and anorexia which may persist for several days (29). Patients may also experience post-procedural mild to moderate pain which is usually controlled with analgesics.

Techniques

RFA

RFA uses electromagnetic energy of a specific radiofrequency range, generally 375-500 kHz, to achieve controlled thermal destruction of cells and tissues (30). In RFA, an active electrode is placed into the tumor under image-guidance. A grounding electrode is placed on the opposite side of the chest or thigh. When the two electrodes are connected to an RF generator, a voltage gradient is produced. This voltage gradient results in an oscillating electric field that induces electrons to collide with the molecules closest to the applicator, which produces frictional heat (31). Tissue heating to a temperature greater than 60 degrees Celsius leads to immediate cell death secondary to coagulation necrosis (32).

In RFA of the lung, there are several obstacles that limit effective thermal ablation of tumor. First, pulmonary vessels and airways act as a "heat sink" to dissipate energy away from the adjacent normal lung parenchyma; this "heat sink" effect limits the size of the ablation margin surrounding the tumor (33,34). Second, there is inherent high-impedance in inflated lung due to its low water content, which limits the therapeutic ablative volume of ablation (35). Third, a fundamental limitation of RFA is its inability to heat charred or desiccated tissue (34,36).

Two popular RF devices for the treatment of pulmonary tumors are Starburst Radiofrequency Ablation System (Angiodynamics, Latham, NY) and Cool-tip (Covidien, Boulder, CO). The Starburst device uses a deployable array RF electrode via a 14- to 17-gauge needle. The Cool-tip device uses a cluster electrode that is perfused with cold saline or water pumped internally; this mechanism is designed to distribute tissue heating to reduce charring (37).

Microwave ablation

Microwave ablation uses electromagnetic energy at a much higher frequency range [generally 900-2,450 MHz (38)] compared to RFA and creates a larger zone of coagulation necrosis (18,39). Unlike RFA, microwave energy penetration does not occur by means of an electric current

Table 1 Comparison of image-guided percutaneous thermal ablation techniques (RFA, microwave ablation, and cryoablation)

	RFA	Microwave ablation	Cryoablation
Advantages	Experience regarding efficacy and safety (most widely studied and most outcome data available)	Compared to RF ablation: Larger tumor ablation volume. Faster ablation time. More effective ablation of cystic masses. Less "heat sink" effect. Less tissue charring. Less procedural pain. No grounding pad needed	Compared to RF ablation: Larger tumor ablation volume. Less procedural pain. No grounding pads needed
Disadvantages	Not suitable for tumors in mediastinum or lung apex due to non-target injury to neuro-vasculature structures and airways. Limited by "heat sink" effect from nearby vessels. Limited by tissue charring which may prevent tumor ablation at the periphery. Potential grounding pad injury	Limited safety and efficacy data available	Limited safety and efficacy data available. Longer procedural time due to freeze-thaw-freeze cycle. Higher hemorrhage risk secondary to lack of tissue cauterization

and therefore is thought not limited by the lower electrical conductivity of inflated lung, charred tissue, or desiccated tissue (18,38,40). Furthermore, no grounding pads are used in the microwave ablation. Microwave ablation utilizes rapidly alternating electric fields which cause polar water molecules to spin rapidly. These spinning water molecules then transfer their kinetic energy to the surrounding tissues resulting in hyperthermia (41).

Compared to RFA, microwave ablation can achieve larger ablative zones more quickly with less heat sink effect. In microwave ablation, a single probe may be used for tumors less than 3 cm. Two to three probes are generally used for tumors greater than 3 cm to produce a larger area of thermocoagulation. A thermocouple may be placed separately to measure the intratumoral temperature. Six microwave ablation devices are available for use. The 2,450-MHz generators are Amica (Hospital Service, Rome, Italy), Acculis MTA (Microsulis, Hamsphire, UK), Certus 140 (Neuwave, Madison, WI). The 915-MHz generators are Avecure (Medwaves, San Diego, CA), Evident (Covidien, Mansfield, MA), MicrothermX (BSD Medical, Salt Lake City, UT). The microwave antennae are straight applicators with active tips measuring 0.6 to 4.0 cm in length. The proximal portion of the antennae is cooled with room-temperature fluid or carbon dioxide to minimize damage of skin and tissues (30).

Cryoablation

Percutaneous cryoablation uses pressurized argon gas to achieve temperatures as low as –140 degrees Celsius based on the Joule-Thomson principle. At temperatures less than –40 degrees Celsius, cryogenic tissue destruction occurs due to protein denaturation, cell rupture from osmotic water shifts across cell membranes, as well as microvascular thrombosis-induced ischemia (42).

A freeze-thaw-freeze cycle is used for each cryoprobe to achieve thermal coagulation while minimizing air leak and bleeding (43). The thaw portion of the cycle is performed using helium and the cryoprobe is allowed to reach approximately 20 degrees Celsius. An example of a cryoablation protocol would consist of a 10-minute freeze of the tumor, followed by an 8-minute thaw, and then a 10-minute freeze, followed by an active or passive thaw.

Two cryoablation devices are available: Cryocare (Endocare, Irvine, CA) and Presice (Galil Medical, Arden Mills, MN). A cryoprobe measures 1.5 to 2.4 mm in diameter. One to 15 cryoprobes may be placed at a time with each probe achieving thermocoagulation after a single freeze-thaw-freeze cycle.

A comparison of the aforementioned thermal ablation modalities is summarized in *Table 1*.

Irreversible electroporation

IRE is the newest of the percutaneous ablation techniques with only a few reports of IRE use in human subjects. IRE uses very short high-voltage electrical pulses to create permanent nanopores in tumor cell membranes to induce apoptosis and cell death (44-46). IRE is largely non-thermal

Table 2 Summary of selected studies that evaluated thermal ablation treatment of inoperable patients with stage I NSCLC

	Study type	Tumor stage	Mean tumor size (cm)	Overall survival (%)				Cancer-specific survival (%)			
				1-yr	2-yr	3-yr	5-yr	1-yr	2-yr	3-yr	5-yr
RFA											
[Simon *et al.*, 2007] (14)	Retrospective	56 stage IA; 19 stage IB	3.0	78	57	36	27				
[Pennathur *et al.*, 2007] (13)	Retrospective	11 stage IA; 8 stage IB	2.6	95	68						
[Lencioni *et al.*, 2008] (12)	Prospective, intention-to-treat	10 stage IA; 3 stage IB	2.2		75				92		
[Lanuti *et al.*, 2009] (11)	Retrospective	29 stage IA; 5 stage IB	2.0	85	78	47		82	57	39	
[Hiraki *et al.*, 2011] (10)	Retrospective	38 stage IA; 12 stage IB	2.1	94	86	74		100	93	80	
[Ambrogi *et al.*, 2011] (8)	Prospective, intention-to-treat	44 stage IA; 15 stage IB	2.6					89		59	40
Microwave ablation											
[Liu and Steinke, 2013] (49)	Retrospective	15 stage I	2.5	N/A				N/A			
Cryoablation											
[Yamauchi *et al.*, 2012] (19)	Retrospective	34 stage IA (29 T1aN0, 5 T1bN0)	1.4		88	88					
RFA, Cryoablation											
[Zemlyak *et al.*, 2010] (50)	Retrospective RFA	12 stage I	Not reported			88				88	
	Cryo	27 stage I	Not reported			77				90	

and spares the surrounding extracellular matrix. Thus, the theoretical advantage of IRE is that tumor ablation occurs while sparing non-target injury to adjacent airways, blood vessels, and nerves (47). Therefore, IRE may potentially be used to treat pulmonary tumors near the hilum, mediastinum, and chest wall (48).

Preprocedural planning for IRE is essential for two reasons. Patients require general anesthesia and complete neuromuscular blockade to prevent generalized muscle contractions. Second, ECG-gated delivery of IRE is required in the chest to prevent cardiac arrhythmias. There is currently one IRE device approved by the FDA—Nanoknife (Angio Dynamics, Latham, NY). Nanoknife has already been used to ablate tumors in the lung, liver, kidney, prostate, and pancreas. Nanoknife electrodes are monopolar with a retractable sheath which allows for adjustable active length from 1 to 4 cm. Up to six electrodes may be used simultaneously for tumor ablation.

Outcomes in early stage disease

The literature on pulmonary thermal ablation in early stage disease is heterogeneous due to the diversity of study groups (mixture of primary and secondary lung tumors) and variations in follow-up lengths as well as reporting standards. Furthermore, the vast majority of the studies were performed retrospectively at single institutions (*Table 2*).

Ablation

Among the thermal ablation techniques, RFA has been the most widely used in the treatment of early stage NSCLC. The retrospective study by Simon *et al.* (14) is the largest to date and included 75 patients with stage I NSCLC who underwent percutaneous CT-guided RF ablation. There were 56 patients with stage IA disease and 19 patients with stage IB disease. The mean tumor diameter was 3.0 cm. The overall 1-, 2-, 3-, 4-, and 5-year survival rates, respectively, for stage I NSCLC were 78%, 57%, 36%, 27%, and 27%. The median survival was 29 months. Local tumor progression-free rates were as follows: 1 year, 83%; 2 years, 64%; 3 years, 57%; 4 years, 47%; and 5 years, 47% for tumors 3 cm or smaller. Tumor size was a statistically significant predictor of local tumor progression

in this study: median time to progression was 12 months for tumors >3 cm and 45 months for tumors <3 cm (14). It is worthwhile noting that the Charlson comorbidity index predicted patient outcome in cases of inoperable NSCLC (i.e., much better survival in patients with less comorbidities) (51).

The RAPTURE study by Lencioni *et al.* (12) was the first prospective, intention-to-treat clinical trial for RFA that reported the outcome of 106 patients with 183 lung tumors, among which only 13 patients had stage I NSCLC (stage IA, n=10; stage IB, n=3). The mean tumor size was 2.2 cm for all patients with NSCLC in this study. The patients with stage I disease had a 2-year overall survival of 75% and a 2-year cancer-specific survival of 92%.

The second prospective, intention-to-treat clinical trial by Ambriogi *et al.* (8) for RFA was of 57 inoperable patients with stage I NSCLC (stage IA tumor, n=44; stage IB tumor, n=15). The mean tumor size was 2.6 cm. Cancer-specific actuarial survivals were 89%, 59%, and 40% at 1, 3, and 5 years, respectively. The median overall survival was 33.4 months and the cancer-specific survival was 41.4 months.

In a retrospective study by Hiraki *et al.* (10) of 50 nonsurgical patients with stage I NSCLC (stage IA, n=38; stage IB, n=12) who were treated with RFA, the mean tumor size was 2.1 cm. After 37 months of follow-up, the local progression rate was 31%. The overall survival was 94%, 86%, and 74% at 1, 2, and 3 years, respectively. The cancer-specific survival was 100%, 93%, and 80% at 1, 2, and 3 years, respectively.

In a retrospective study by Lanuti *et al.* (11) of 31 patients with medically inoperable stage I NSCLC had 34 tumors treated with RFA (stage IA, n=29; stage IB, n=5), the mean size of the treated tumors was 2.0 cm. The overall survivals at 1, 2 and 3 years were 85%, 78% and 47%, respectively. Disease-free survivals at 1, 2 and 3 years were 82%, 57% and 39%, respectively. Local progression-free survivals at 1, 2, and 3 years were 71%, 58%, and 58%. The local failure rate was 32% after a median follow-up of 17 months.

Pennathur *et al.* (13) reported the outcome of 19 high-risk patients with stage I NSCLC (stage IA, n=11; stage IB, n=8) who underwent RFA. The mean tumor size was 2.6 cm. Overall survivals were 95% and 68% at 1 and 2 years, respectively.

Microwave ablation

The first study of microwave ablation in the lung was performed by Feng *et al.* (15) but no separate subset analysis of early stage NSCLC was performed. The largest study of microwave ablation was done by Wolf *et al.* (18) which retrospectively examined the recurrence and survival outcome of 50 patients, among which 27 patients had NSCLC and the remainder had small cell lung cancer or metastatic disease. While, the NSCLC stage was not specified, the overall mean tumor size was 3.5 cm. No subset analysis was done for patients with NSCLC versus other malignancies. These limitations limit this study's ability to assess the efficacy of microwave ablation in the early stage subgroup (18). While several other studies exist such as those by Belfiore *et al.* (52) and Lu *et al.* (53), these studies are similarly limited by either a lack of subgroup analysis or no reports of outcome data.

The only study focused on early stage NSCLC is a preliminary retrospective review by Liu *et al.* (49) of 15 patients with medically inoperable stage I NSCLC who were treated with CT-guided percutaneous microwave ablation. The mean tumor size was 2.5 cm (range, 0.8-4.0 cm). Local progression was 31% after 1-year follow-up; however, 80% of the local progression was observed in pleural-based tumors that were larger than 3.0 cm. No survival data was reported for this study.

Cryoablation

A study by Yamauchi *et al.* (19) retrospectively reviewed 20 patients with medically inoperable stage IA NSCLC who had 34 tumors treated with CT-guided cryoblation under local anesthesia. Twenty-nine tumors were T1aN0 and 5 tumors were T1bN0. The mean tumor size was 1.4 cm (range, 0.5-3.0 cm) and 12 tumors were subsolid on CT imaging. The 2- and 3-year overall survivals were 88% and 88%, respectively. One patient died of lung cancer progression at 68 months. Two patients died of acute exacerbations of idiopathic pulmonary fibrosis which were not considered to be directly related to the cryoablation, at 12 and 18 months, respectively. After a median follow-up of 23 months, the local control was 97%. Only one patient, who had a 1.6 cm squamous cell carcinoma, had local tumor progression at 8 months post-treatment; furthermore, in this patient, the local recurrence was re-treated with cryoablation and there was no evidence of further local recurrence. There was no significant change in the pulmonary function tests before and after cryoablation. The excellent survival data and local control for this study may partially be attributed to the small tumor size and the

Table 3 Complications following RFA

Complication	Incidence (%)
Pneumothorax	11-52
Pneumothorax requiring chest tube	6-29
Pleural effusion	6-19
Bronchopleural fistula	0.6
Hemoptysis	3-9
Pulmonary hemorrhage	6-18
Pulmonary artery pseudoaneurysm	0.2
Reactive pneumonitits	0.4
Needle tract tumor seeding	0.3-0.7
Death	0.6

significant number of subsolid lesions.

Wang *et al.* (17) also performed a retrospective study reviewing the outcome for cryoablation of 234 lung tumors but no specific data was reported for patients with early stage NSCLC.

The retrospective study by Zemlyak *et al.* (50) included 64 patients, among whom 25 underwent sublobar resection, 12 RFA, and 27 cryoablation. The mean tumor size was not reported in this study. However, the RFA group included patients with "larger lesions (≥3 cm)" while the cryoablation group was performed in patients with "lesions <3 cm". The 3-year overall survival was 87.1%, 87.5%, and 77% for sublobar resection, RFA, and cryoablation, respectively. The 3-year cancer-specific survival was 90.6%, 87.5%, and 90.2%. This study was limited by the small number of patients and selection bias.

Irreversible electroporation

There is currently very limited data of the use of IRE in human lung. The majority of the outcome data is derived from animal models (47,48). The theoretical advantage of IRE is preservation of underlying lung architecture and surrounding neurovascular structures, which may permit the use of IRE in ablating tumors in the mediastinum and lung apex. Another theoretical advantage is overcoming "heat sink" effect. Thomson *et al.* (54) performed IRE in 38 patients, among whom 3 patients had advanced lung cancer. All three patients had inadequate treatment response.

Complications

Image-guided percutaneous thermal ablation of lung cancer is generally safe and well-tolerated by most patients even with those with limited cardiopulmonary reserve. Most complications after percutaneous ablation are minor and treated conservatively or with minimal intervention (*Table 3*). There are, however, rare but serious complications such as massive pulmonary hemorrhage, bronchopleural fistula, and pulmonary artery pseudoaneurysm. Overall, procedural-related death is rare with the mortality rate reported to be 0.4% for RFA (55).

The most common complication after percutaneous lung ablation is pneumothorax. Pneumothorax occurs in 11% to 52% of cases, although only 6% to 29% of patients require chest tube placement (21,56-58). Rarely, there may be a bronchopleural fistula, which occurs in 0.6% of patients and is thought to be related to aggressive treatment (59). Pleural effusion is also a relatively common complication reported in 6% to 19% of cases and may be the result of non-target thermal injury to the pleura (21,57,60).

Hemoptysis and pulmonary hemorrhage are not uncommon after thermal ablation with reported incidences being 3-9% (57,61,62) and 6-18% (63,64), respectively, after RFA. Pulmonary hemorrhage and hemoptysis is more common after cryoablation than after RF or microwave ablation (16,43). Cryoablation has no cautery effect that is inherent in RFA or microwave ablation both of which use extreme heat; furthermore, cryoablation results in damage to microcirculation during the thaw cycle. Pulmonary hemorrhage is usually self-limiting and treated conservatively, although there are case reports of uncontrollable hemorrhage leading to death (23,63-65). Pulmonary artery pseudoaneurysm is a rare but life-threatening complication that occurred in 0.2% in a series of RF ablations (66). Two reports of a pulmonary artery pseudoaneurysm after RFA were both successfully treated using transcatheter coil embolization (67,68).

Non-target thermal damage to peripheral nerves may occur depending on the location of the tumor. If the tumor is in the lung apex, thermal ablation may cause injury to the caudal brachial plexus (69). Phrenic nerve injury is another potential complication if ablation is performed in close proximity (<1 cm) of the phrenic nerve (70). A thorough knowledge of the course of these nerves may reduce the rate of these non-target thermal injuries.

Pneumonitis after RFA is a rare but potentially lethal complication. In one series of RFA in the lung, there were two deaths attributed to interstitial pneumonia; both patients received radiotherapy prior to thermal ablation (60). In another series of patients, bronchiolitis obliterans

organizing pneumonia-like reactive pneumonitits occurred after RF ablation in 0.4% of patients (71).

A very rare but potentially life-threatening complication following any percutaneous needle-based procedure, including lung biopsy and thermal ablation, is systemic air embolism. There have been only two reported cases of systemic air embolism after RFA in the lung, both of which were nonfatal (72,73). The treatment for systemic air embolism is hyperbaric oxygen therapy.

Another rare complication is tumor seeding in the needle tract with reported incidence of 0.3% to 0.7% (74,75).

Follow-up imaging

Imaging is a critical component of thermal ablation because, unlike surgery, there is no tissue sample available for histopathologic evaluation of the ablative margin. Computed tomography (CT) and/or fluorodeoxyglucose positron emission tomography (FDG-PET) are used to ensure complete ablation and to evaluate for treatment response. There is no consensus on which imaging modality or imaging time interval that most accurately detects treatment success or failure or recurrent disease. However, follow-up imaging protocols after thermal ablation generally entail obtaining CT and/or FDG-PET one month post-ablation and then every three months thereafter (76,77).

After RFA, immediate post-procedural CT images demonstrate central consolidation (coagulation necrosis) surrounded by concentric rings of groundglass opacity (edema, inflammation, hemorrhage) (78,79). As the peripheral groundglass opacity overestimates the area of true coagulation necrosis by 4.1 mm (78), it is recommended that the ablation extend at least 5 mm (18,78,80) beyond the tumor. In one study (81), an ablation area at least four times larger than the pre-ablation tumor was predictive of complete ablation.

Within the first week of RFA, contrast-enhanced CT imaging may show a peripheral thin (<5 mm) rim of enhancement, which reflects benign reactive hyperemia (76). FDG-PET is not useful in the immediate post-ablation setting as there is non-specificity in its findings given the background of expected inflammatory reaction (82).

CT imaging 1-month post-RF ablation demonstrates a consolidated lesion that is larger than the pre-ablation tumor. Cavitation can develop in up to 25% of cases; cavitation is thought to occur as the sequelae of physiologic drainage of necrotic tissue (79,81,83). Two to six months post-RFA, CT imaging shows no change or increased size of the ablation cavity in comparison to the pre-ablation tumor. Because of this expected treatment response, Response Evaluation Criteria in Solid Tumors (RECIST) criteria is not ideal for evaluating changes after thermal ablation.

Beyond 6 months post-RFA, the ablation zone is expected to be smaller in size than the index tumor (84). Tumor recurrence or progression should be suspected if there is increased size of the ablation zone and/or central or nodular enhancement after 6 months (14,80). FDG uptake peaks at approximately 2 weeks post-RF ablation. Increased or new metabolic uptake on FDG-PET imaging at 2 months is suspicious for disease recurrence (85).

After microwave ablation, immediate post-procedural CT shows groundglass/consolidative opacity centrally (coagulation necrosis) with surrounding groundglass opacities (edema, hemorrhage, inflammation) penetrated by well-demarcated probe tracts (*Figure 1*) (18). The ablation zone increases in size for up to 6 months after treatment due to thermal changes in the adjacent lung parenchyma. After 6 months, the ablation zone should decrease in size and be replaced by consolidation. Cavitation within the ablation zone has been associated with decreased cancer-specific mortality (18), possibly due to cavitary changes occurring more often when there is more complete tumor destruction with thermocoagulation of local tissue blood supply (30). Similar to RFA, central or nodular enhancement on CT as well as increased or new metabolic uptake on FDG-PET imaging is suspicious for treatment failure.

Following the thaw cycle, the cryoablation zone is seen as a low attenuation central area (coagulation necrosis) surrounded by a concentric ring of groundglass opacity (hemorrhage and edema) (43,86). The “ice ball” seen on peri-procedural images overestimates the area of coagulation necrosis by 4 to 5 mm (87-89). After the first month post-ablation, the peripheral groundglass opacity resolves as the central zone of coagulation necrosis becomes well-marginated and consolidative. Cavitation may occur within this central area of necrosis (17). On immediate post-procedural contrast-enhanced CT, there may sometimes be peripheral or internal enhancement in the cryoablation zone that should resolve within 1 to 2 months (86). PET is not performed immediately post-cryoablation due to its nonspecificity in the background of reactive inflammation (*Figure 2*).

At 1 month post-cryoablation, the ablation zone should be decreasing in size. Growth in the ablation zone during the first two months post-ablation is suspicious for tumor recurrence or progression (86). In contrast to RFA or microwave ablative zones, the cryoablation zone generally

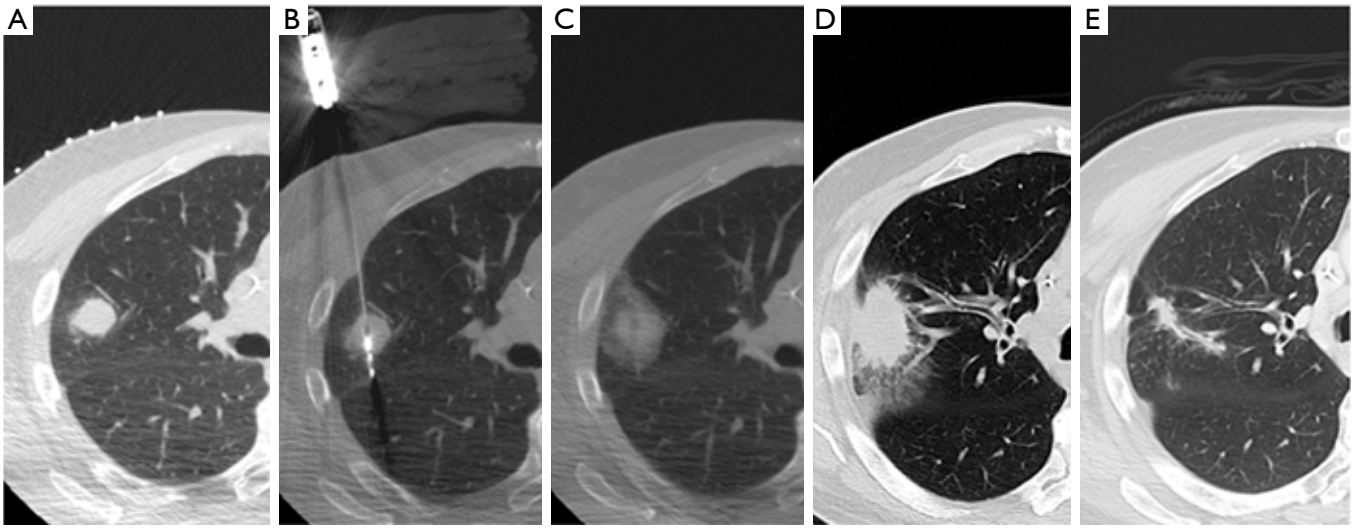


Figure 1 (A) Axial CT image shows a 2.2 cm right upper lobe lesion; (B) Image during microwave ablation shows a single microwave probe positioned within the tumor; (C) Immediate post-ablation image shows groundglass opacity surrounding the lesion, which is penetrated by probe tracts; (D) Follow-up CT image 3 months after microwave ablation shows a larger consolidation consistent with expected post-ablation change; (E) CT at 9-month follow-up shows near resolution of consolidation with residual parenchymal scar

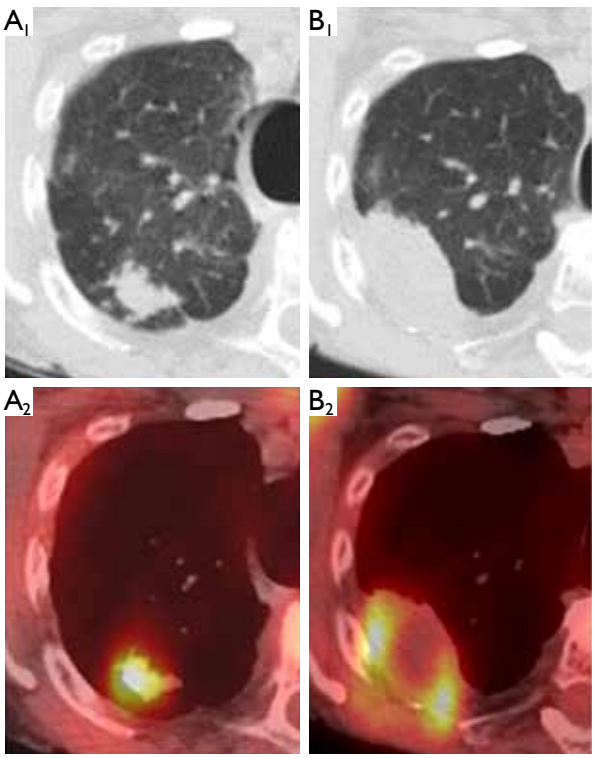


Figure 2 (A_1,A_2) Axial CT (A_1) and fused PET/CT (A_2) images show a right upper lobe nodule demonstrating intense metabolic activity; (B_1,B_2) Axial CT (B_1) and fused PET/CT (B_2) images 3 months after microwave ablation show a consolidation with rim FDG uptake, which are normal findings secondary to inflammation

involutes earlier and faster which would theoretically allow for earlier detection of treatment failure (17). FDG-PET imaging demonstrating new or increasing uptake after 2 months post-ablation is suggestive of tumor recurrence, whereas the absence of FDG uptake coupled with lesion resolution indicates adequate treatment response (*Figure 3*).

Palliative therapies

Image-guided thermal ablation is also useful in the palliative treatment of lung cancer through local control of advanced or metastatic disease. For example, painful bone metastases may be effectively palliated with RFA or cryoablation (90-93). Currently, there is a multicenter, prospective, single arm study (ECLIPSE trial) that is evaluating the safety and efficacy of cryoablation to patients with pulmonary metastatic disease.

Other palliative interventional radiologic procedures include endovascular stenting for superior vena cava syndrome and bronchial artery embolization for hemoptysis associated with lung cancer (94). Trans-arterial embolization may also be performed for preoperative purposes to decrease blood loss (95,96).

Emerging therapies

Less established in treatment of lung tumors but widely

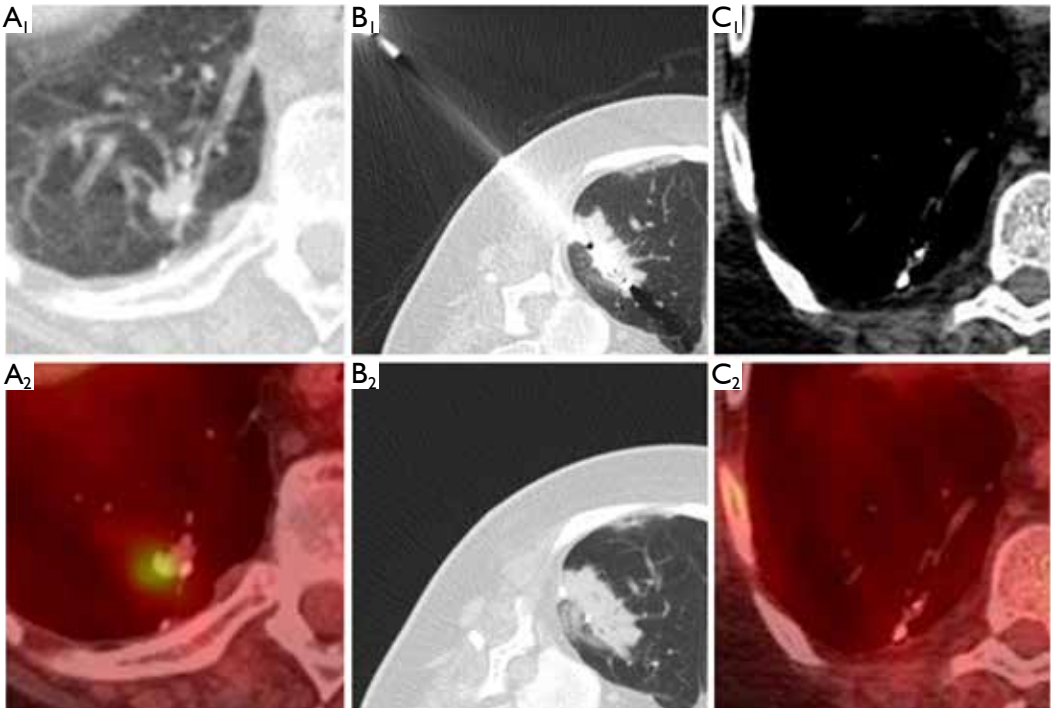


Figure 3 Right lower lobe nodule previously treated with surgery. (A_1) Axial CT image shows a small nodule next to a surgical staple line; (A_2) Correlating PET/CT image shows focal nodular hypermetabolic activity within the nodule, consistent with local tumor recurrence; (B_1) Axial CT image shows a cryoablation probe within this right lower lobe lesion; (B_2) Immediate post-cryoablation image shows expected groundglass opacity surrounding the lesion; (C_1) CT at 1-year follow-up shows resolution of previous nodule by staple line; (C_2) Correlating PET/CT image at 1-year follow-up shows no FDG uptake by staple line

used in treatment of tumors in solid organs such as the liver, transvascular therapy is being reexamined as a potential primary or adjuvant therapy. Several approaches are being investigated, including transpulmonary chemoembolization and image-guided percutaneous- or intra-arterial delivery of therapeutic nanoparticles.

In transpulmonary chemoembolization, the tumor-feeding pulmonary arteries are selectively catheterized after which a mixture of cytotoxic and embolic agents are administered locally. Vogl *et al.* (96-99) used a femoral vein puncture to access the tumor-supplying pulmonary artery and then infused a combination of lipiodol, mitomycin C, and microspheres to treat metastatic and primary lung tumors. In a study of 17 patients with unresectable primary lung tumors (97), transpulmonary chemoembolization was performed for symptomatic palliation. No major complications occurred with 35% of patients experiencing local progression after a mean follow-up of 11.3 months.

In the future, image-guided minimally invasive procedures will play a role in the delivery of a variety of nanotherapeutics via percutaneous or intravascular methods (100-102). The direct intra-tumoral delivery of therapeutic nanoparticles minimizes systemic toxicity while maximizing local efficacy of tumor destruction. For example, in a new investigational method called "magnetic" chemotherapy, chemotherapeutics are tagged with magnetic nanoparticles. After infusion of the magnetic nanotherapeutics into the vascular supply of the tumor, an external rare earth magnet is placed over the tumor. The resultant magnetic attraction directs the therapeutic particles out of the vessel and into the tumor.

Conclusions

Interventional radiologists play a key role in the image-guided thermal ablation of lung malignancies. RFA has been the most widely used form of thermal ablation in the lung. RFA for inoperable early stage NSCLC compares favorably to SBRT in regards to overall patient survival (8,10-14,19,20,49,50,103-108). However, RFA has worse

local control rates than SBRT although this does not appear to affect overall survival as many patients can easily undergo retreatment (20,109). As a more powerful form of thermal ablation, microwave ablation may potentially provide superior local control due to its larger zone of active heating and its ability to achieve higher intratumoral temperatures. There is still limited safety and efficacy data on the use of microwave ablation in early stage NSCLC although its use is growing. The preliminary data on cryoblation is promising, with one study of medically inoperable early stage NSCLC demonstrating a 3-year overall survival of 88% and a local control rate of 97% (19).

In a recent consensus statement by the American College of Chest Physicians and Society of Thoracic Surgeons regarding inoperable early stage NSCLC, image-guided thermal ablation was considered a treatment option only if the patient was not a candidate for SBRT (110). This statement is not surprising given that interventional radiology was not involved in the consensus and there is a paucity of multi-institutional clinical trials for thermal ablation. However, there is currently a multicenter pilot trial of inoperable patients with stage IA NSCLC treated with RFA, which is funded by the National Cancer Institute and performed through the American College of Surgeons Oncology Group (ACOSOG Z4033) (111). Although the results are not yet published, this RFA cohort was recently compared to sublobar resection and SBRT cohorts from other completed multi-center trials, and the survival was similar (despite the RFA cohort being older and sicker) (20).

Further studies need to done to determine which patients would benefit most from image-guided thermal ablation. Future research dictating the tumor location, size, and histology that is most suitable for ablation will help patients to achieve the best outcome.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
2. El-Sherif A, Gooding WE, Santos R, et al. Outcomes of sublobar resection versus lobectomy for stage I non-small cell lung cancer: a 13-year analysis. Ann Thorac Surg 2006;82:408-15; discussion 415-6.
3. Mery CM, Pappas AN, Bueno R, et al. Similar long-term survival of elderly patients with non-small cell lung cancer treated with lobectomy or wedge resection within the surveillance, epidemiology, and end results database. Chest 2005;128:237-45.
4. Raz DJ, Zell JA, Ou SH, et al. Natural history of stage I non-small cell lung cancer: implications for early detection. Chest 2007;132:193-9.
5. Bach PB, Cramer LD, Warren JL, et al. Racial differences in the treatment of early-stage lung cancer. N Engl J Med 1999;341:1198-205.
6. Dupuy DE, Goldberg SN. Image-guided radiofrequency tumor ablation: challenges and opportunities--part II. J Vasc Interv Radiol 2001;12:1135-48.
7. Dupuy DE, Zagoria RJ, Akerley W, et al. Percutaneous radiofrequency ablation of malignancies in the lung. AJR Am J Roentgenol 2000;174:57-9.
8. Ambrogi MC, Fanucchi O, Cioni R, et al. Long-term results of radiofrequency ablation treatment of stage I non-small cell lung cancer: a prospective intention-to-treat study. J Thorac Oncol 2011;6:2044-51.
9. Hiraki T, Gobara H, Iishi T, et al. Percutaneous radiofrequency ablation for clinical stage I non-small cell lung cancer: results in 20 nonsurgical candidates. J Thorac Cardiovasc Surg 2007;134:1306-12.
10. Hiraki T, Gobara H, Mimura H, et al. Percutaneous radiofrequency ablation of clinical stage I non-small cell lung cancer. J Thorac Cardiovasc Surg 2011;142:24-30.
11. Lanuti M, Sharma A, Digumarthy SR, et al. Radiofrequency ablation for treatment of medically inoperable stage I non-small cell lung cancer. J Thorac Cardiovasc Surg 2009;137:160-6.
12. Lencioni R, Crocetti L, Cioni R, et al. Response to radiofrequency ablation of pulmonary tumours: a prospective, intention-to-treat, multicentre clinical trial (the RAPTURE study). Lancet Oncol 2008;9:621-8.
13. Pennathur A, Luketich JD, Abbas G, et al. Radiofrequency ablation for the treatment of stage I non-small cell lung cancer in high-risk patients. J Thorac Cardiovasc Surg 2007;134:857-64.
14. Simon CJ, Dupuy DE, DiPetrillo TA, et al. Pulmonary radiofrequency ablation: long-term safety and efficacy in 153 patients. Radiology 2007;243:268-75.
15. Feng W, Liu W, Li C, et al. Percutaneous microwave coagulation therapy for lung cancer. Zhonghua Zhong Liu Za Zhi 2002;24:388-90.
16. Kawamura M, Izumi Y, Tsukada N, et al. Percutaneous

cryoablation of small pulmonary malignant tumors under computed tomographic guidance with local anesthesia for nonsurgical candidates. J Thorac Cardiovasc Surg 2006;131:1007-13.
17. Wang H, Littrup PJ, Duan Y, et al. Thoracic masses treated with percutaneous cryotherapy: initial experience with more than 200 procedures. Radiology 2005;235:289-98.
18. Wolf FJ, Grand DJ, Machan JT, et al. Microwave ablation of lung malignancies: effectiveness, CT findings, and safety in 50 patients. Radiology 2008;247:871-9.
19. Yamauchi Y, Izumi Y, Hashimoto K, et al. Percutaneous cryoablation for the treatment of medically inoperable stage I non-small cell lung cancer. PLoS One 2012;7:e33223.
20. Dupuy DE. Treatment of medically inoperable non-small-cell lung cancer with stereotactic body radiation therapy versus image-guided tumor ablation: can interventional radiology compete? J Vasc Interv Radiol 2013;24:1139-45.
21. Hiraki T, Tajiri N, Mimura H, et al. Pneumothorax, pleural effusion, and chest tube placement after radiofrequency ablation of lung tumors: incidence and risk factors. Radiology 2006;241:275-83.
22. Kothary N, Heit JJ, Louie JD, et al. Safety and efficacy of percutaneous fiducial marker implantation for image-guided radiation therapy. J Vasc Interv Radiol 2009;20:235-9.
23. Dupuy DE, Mayo-Smith WW, Abbott GF, et al. Clinical applications of radio-frequency tumor ablation in the thorax. Radiographics 2002;22 Spec No:S259-69.
24. Sofocleous CT, May B, Petre EN, et al. Pulmonary thermal ablation in patients with prior pneumonectomy. AJR Am J Roentgenol 2011;196:W606-12.
25. Ambrogi MC, Fanucchi O, Lencioni R, et al. Pulmonary radiofrequency ablation in a single lung patient. Thorax 2006;61:828-9.
26. Skonieczki BD, Wells C, Wasser EJ, et al. Radiofrequency and microwave tumor ablation in patients with implanted cardiac devices: is it safe? Eur J Radiol 2011;79:343-6.
27. Pua BB, Thornton RH, Solomon SB. Radiofrequency ablation: treatment of primary lung cancer. Semin Roentgenol 2011;46:224-9.
28. Crabtree TD, Denlinger CE, Meyers BF, et al. Stereotactic body radiation therapy versus surgical resection for stage I non-small cell lung cancer. J Thorac Cardiovasc Surg 2010;140:377-86.
29. Dupuy DE, Shulman M. Current status of thermal ablation treatments for lung malignancies. Semin Intervent Radiol 2010;27:268-75.
30. Dupuy DE. Image-guided thermal ablation of lung malignancies. Radiology 2011;260:633-55.
31. Organ LW. Electrophysiologic principles of radiofrequency lesion making. Appl Neurophysiol 1976-1977;39:69-76.
32. Nahum Goldberg S, Dupuy DE. Image-guided radiofrequency tumor ablation: challenges and opportunities--part I. J Vasc Interv Radiol 2001;12:1021-32.
33. Ahmed M, Liu Z, Afzal KS, et al. Radiofrequency ablation: effect of surrounding tissue composition on coagulation necrosis in a canine tumor model. Radiology 2004;230:761-7.
34. Oshima F, Yamakado K, Akeboshi M, et al. Lung radiofrequency ablation with and without bronchial occlusion: experimental study in porcine lungs. J Vasc Interv Radiol 2004;15:1451-6.
35. Morrison PR, vanSonnenberg E, Shankar S, et al. Radiofrequency ablation of thoracic lesions: part 1, experiments in the normal porcine thorax. AJR Am J Roentgenol 2005;184:375-80.
36. Lu DS, Raman SS, Limanond P, et al. Influence of large peritumoral vessels on outcome of radiofrequency ablation of liver tumors. J Vasc Interv Radiol 2003;14:1267-74.
37. Alexander ES, Dupuy DE. Lung Cancer Ablation: Technologies and Techniques. Semin intervent Radiol 2013;30:141-50
38. Simon CJ, Dupuy DE, Mayo-Smith WW. Microwave ablation: principles and applications. Radiographics 2005;25 Suppl 1:S69-83.
39. Skinner MG, Iizuka MN, Kolios MC, et al. A theoretical comparison of energy sources--microwave, ultrasound and laser--for interstitial thermal therapy. Phys Med Biol 1998;43:3535-47.
40. Brace CL, Hinshaw JL, Laeseke PF, et al. Pulmonary thermal ablation: comparison of radiofrequency and microwave devices by using gross pathologic and CT findings in a swine model. Radiology 2009;251:705-11.
41. Lubner MG, Brace CL, Hinshaw JL, et al. Microwave tumor ablation: mechanism of action, clinical results, and devices. J Vasc Interv Radiol 2010;21:S192-203.
42. Gage AA, Baust J. Mechanisms of tissue injury in cryosurgery. Cryobiology 1998;37:171-86.
43. Izumi Y, Oyama T, Ikeda E, et al. The acute effects of transthoracic cryoablation on normal lung evaluated in a porcine model. Ann Thorac Surg 2005;79:318-22; discussion 322.
44. Davalos RV, Mir IL, Rubinsky B. Tissue ablation

with irreversible electroporation. Ann Biomed Eng 2005;33:223-31.
45. Lee EW, Chen C, Prieto VE, et al. Advanced hepatic ablation technique for creating complete cell death: irreversible electroporation. Radiology 2010;255:426-33.
46. Rubinsky B, Onik G, Mikus P. Irreversible electroporation: a new ablation modality--clinical implications. Technol Cancer Res Treat 2007;6:37-48.
47. Dupuy DE, Aswad B, Ng T. Irreversible electroporation in a Swine lung model. Cardiovasc Intervent Radiol 2011;34:391-5.
48. Deodhar A, Monette S, Single GW Jr, et al. Percutaneous irreversible electroporation lung ablation: preliminary results in a porcine model. Cardiovasc Intervent Radiol 2011;34:1278-87.
49. Liu H, Steinke K. High-powered percutaneous microwave ablation of stage I medically inoperable non-small cell lung cancer: a preliminary study. J Med Imaging Radiat Oncol 2013;57:466-74.
50. Zemlyak A, Moore WH, Bilfinger TV. Comparison of survival after sublobar resections and ablative therapies for stage I non-small cell lung cancer. J Am Coll Surg 2010;211:68-72.
51. Simon TG, Beland MD, Machan JT, et al. Charlson Comorbidity Index predicts patient outcome, in cases of inoperable non-small cell lung cancer treated with radiofrequency ablation. Eur J Radiol 2012;81:4167-72.
52. Belfiore G, Ronza F, Belfiore MP, et al. Patients' survival in lung malignancies treated by microwave ablation: our experience on 56 patients. Eur J Radiol 2013;82:177-81.
53. Lu Q, Cao W, Huang L, et al. CT-guided percutaneous microwave ablation of pulmonary malignancies: results in 69 cases. World J Surg Oncol 2012;10:80.
54. Thomson KR, Cheung W, Ellis SJ, et al. Investigation of the safety of irreversible electroporation in humans. J Vasc Interv Radiol 2011;22:611-21.
55. Steinke K, Sewell PE, Dupuy D, et al. Pulmonary radiofrequency ablation--an international study survey. Anticancer Res 2004;24:339-43.
56. Nour-Eldin NE, Naguib NN, Saeed AS, et al. Risk factors involved in the development of pneumothorax during radiofrequency ablation of lung neoplasms. AJR Am J Roentgenol 2009;193:W43-8.
57. Okuma T, Matsuoka T, Yamamoto A, et al. Frequency and risk factors of various complications after computed tomography-guided radiofrequency ablation of lung tumors. Cardiovasc Intervent Radiol 2008;31:122-30.
58. Yamagami T, Kato T, Hirota T, et al. Pneumothorax as a complication of percutaneous radiofrequency ablation for lung neoplasms. J Vasc Interv Radiol 2006;17:1625-9.
59. Sakurai J, Hiraki T, Mukai T, et al. Intractable pneumothorax due to bronchopleural fistula after radiofrequency ablation of lung tumors. J Vasc Interv Radiol 2007;18:141-5.
60. Nomura M, Yamakado K, Nomoto Y, et al. Complications after lung radiofrequency ablation: risk factors for lung inflammation. Br J Radiol 2008;81:244-9.
61. Lee JM, Jin GY, Goldberg SN, et al. Percutaneous radiofrequency ablation for inoperable non-small cell lung cancer and metastases: preliminary report. Radiology 2004;230:125-34.
62. Sano Y, Kanazawa S, Gobara H, et al. Feasibility of percutaneous radiofrequency ablation for intrathoracic malignancies: a large single-center experience. Cancer 2007;109:1397-405.
63. Nour-Eldin NE, Naguib NN, Mack M, et al. Pulmonary hemorrhage complicating radiofrequency ablation, from mild hemoptysis to life-threatening pattern. Eur Radiol 2011;21:197-204.
64. Steinke K, King J, Glenn D, et al. Pulmonary hemorrhage during percutaneous radiofrequency ablation: a more frequent complication than assumed? Interact Cardiovasc Thorac Surg 2003;2:462-5.
65. Vaughn C, Mychaskiw G 2nd, Sewell P. Massive hemorrhage during radiofrequency ablation of a pulmonary neoplasm. Anesth Analg 2002;94:1149-51; table of contents.
66. Yamakado K, Takaki H, Takao M, et al. Massive hemoptysis from pulmonary artery pseudoaneurysm caused by lung radiofrequency ablation: successful treatment by coil embolization. Cardiovasc Intervent Radiol 2010;33:410-2.
67. Sakurai J, Mimura H, Gobara H, et al. Pulmonary artery pseudoaneurysm related to radiofrequency ablation of lung tumor. Cardiovasc Intervent Radiol 2010;33:413-6.
68. Soh J, Toyooka S, Gobara H, et al. A case of delayed massive hemothorax caused by the rupture of a pulmonary artery pseudoaneurysm after radiofrequency ablation of lung tumors. Jpn J Clin Oncol 2012;42:646-9.
69. Hiraki T, Gobara H, Mimura H, et al. Brachial nerve injury caused by percutaneous radiofrequency ablation of apical lung cancer: a report of four cases. J Vasc Interv Radiol 2010;21:1129-33.
70. Matsui Y, Hiraki T, Gobara H, et al. Phrenic nerve injury after radiofrequency ablation of lung tumors: retrospective evaluation of the incidence and risk factors. J Vasc Interv

Radiol 2012;23:780-5.
71. Hiraki T, Gobara H, Kato K, et al. Bronchiolitis obliterans organizing pneumonia after radiofrequency ablation of lung cancer: report of three cases. J Vasc Interv Radiol 2012;23:126-30.
72. Ghaye B, Bruyère PJ, Dondelinger RF. Nonfatal systemic air embolism during percutaneous radiofrequency ablation of a pulmonary metastasis. AJR Am J Roentgenol 2006;187:W327-8.
73. Okuma T, Matsuoka T, Tutumi S, et al. Air embolism during needle placement for CT-guided radiofrequency ablation of an unresectable metastatic lung lesion. J Vasc Interv Radiol 2007;18:1592-4.
74. Hiraki T, Mimura H, Gobara H, et al. Two cases of needle-tract seeding after percutaneous radiofrequency ablation for lung cancer. J Vasc Interv Radiol 2009;20:415-8.
75. Yamakado K, Akeboshi M, Nakatsuka A, et al. Tumor seeding following lung radiofrequency ablation: a case report. Cardiovasc Intervent Radiol 2005;28:530-2.
76. Abtin FG, Eradat J, Gutierrez AJ, et al. Radiofrequency ablation of lung tumors: imaging features of the postablation zone. Radiographics 2012;32:947-69.
77. Beland MD, Wasser EJ, Mayo-Smith WW, et al. Primary non-small cell lung cancer: review of frequency, location, and time of recurrence after radiofrequency ablation. Radiology 2010;254:301-7.
78. Yamamoto A, Nakamura K, Matsuoka T, et al. Radiofrequency ablation in a porcine lung model: correlation between CT and histopathologic findings. AJR Am J Roentgenol 2005;185:1299-306.
79. Gadaleta C, Mattioli V, Colucci G, et al. Radiofrequency ablation of 40 lung neoplasms: preliminary results. AJR Am J Roentgenol 2004;183:361-8.
80. Anderson EM, Lees WR, Gillams AR. Early indicators of treatment success after percutaneous radiofrequency of pulmonary tumors. Cardiovasc Intervent Radiol 2009;32:478-83.
81. de Baère T, Palussière J, Aupérin A, et al. Midterm local efficacy and survival after radiofrequency ablation of lung tumors with minimum follow-up of 1 year: prospective evaluation. Radiology 2006;240:587-96.
82. Yoo DC, Dupuy DE, Hillman SL, et al. Radiofrequency ablation of medically inoperable stage IA non-small cell lung cancer: are early posttreatment PET findings predictive of treatment outcome? AJR Am J Roentgenol 2011;197:334-40.
83. Steinke K, King J, Glenn D, et al. Radiologic appearance and complications of percutaneous computed tomography-guided radiofrequency-ablated pulmonary metastases from colorectal carcinoma. J Comput Assist Tomogr 2003;27:750-7.
84. Jin GY, Lee JM, Lee YC, et al. Primary and secondary lung malignancies treated with percutaneous radiofrequency ablation: evaluation with follow-up helical CT. AJR Am J Roentgenol 2004;183:1013-20.
85. Okuma T, Okamura T, Matsuoka T, et al. Fluorine-18-fluorodeoxyglucose positron emission tomography for assessment of patients with unresectable recurrent or metastatic lung cancers after CT-guided radiofrequency ablation: preliminary results. Ann Nucl Med 2006;20:115-21.
86. Ito N, Nakatsuka S, Inoue M, et al. Computed tomographic appearance of lung tumors treated with percutaneous cryoablation. J Vasc Interv Radiol 2012;23:1043-52.
87. Hinshaw JL, Lee FT Jr, Laeseke PF, et al. Temperature isotherms during pulmonary cryoablation and their correlation with the zone of ablation. J Vasc Interv Radiol 2010;21:1424-8.
88. Hinshaw JL, Littrup PJ, Durick N, et al. Optimizing the protocol for pulmonary cryoablation: a comparison of a dual- and triple-freeze protocol. Cardiovasc Intervent Radiol 2010;33:1180-5.
89. Permpongkosol S, Nicol TL, Link RE, et al. Differences in ablation size in porcine kidney, liver, and lung after cryoablation using the same ablation protocol. AJR Am J Roentgenol 2007;188:1028-32.
90. Callstrom MR, Charboneau JW, Goetz MP, et al. Image-guided ablation of painful metastatic bone tumors: a new and effective approach to a difficult problem. Skeletal Radiol 2006;35:1-15.
91. Callstrom MR, Dupuy DE, Solomon SB, et al. Percutaneous image-guided cryoablation of painful metastases involving bone: multicenter trial. Cancer 2013;119:1033-41.
92. Dupuy DE, Liu D, Hartfeil D, et al. Percutaneous radiofrequency ablation of painful osseous metastases: a multicenter American College of Radiology Imaging Network trial. Cancer 2010;116:989-97.
93. Kojima H, Tanigawa N, Kariya S, et al. Clinical assessment of percutaneous radiofrequency ablation for painful metastatic bone tumors. Cardiovasc Intervent Radiol 2006;29:1022-6.
94. Masuda E, Sista AK, Pua BB, et al. Palliative procedures in lung cancer. Semin intervent Radiol 2013;30:199-205

95. Gadaleta CD, Solbiati L, Mattioli V, et al. Unresectable lung malignancy: combination therapy with segmental pulmonary arterial chemoembolization with drug-eluting microspheres and radiofrequency ablation in 17 patients. Radiology 2013;267:627-37.
96. Lindemayr S, Lehnert T, Korkusuz H, et al. Transpulmonary chemoembolization: a novel approach for the treatment of unresectable lung tumors. Tech Vasc Interv Radiol 2007;10:114-9.
97. Vogl TJ, Herzog C, Zangos S, et al. Palliative treatment of primary lung tumors with transpulmonary chemoembolization (TPCE). Rofo 2007;179:300-7.
98. Vogl TJ, Lehnert T, Zangos S, et al. Transpulmonary chemoembolization (TPCE) as a treatment for unresectable lung metastases. Eur Radiol 2008;18:2449-55.
99. Vogl TJ, Wetter A, Lindemayr S, et al. Treatment of unresectable lung metastases with transpulmonary chemoembolization: preliminary experience. Radiology 2005;234:917-22.
100. Monsky WL, Vien DS, Link DP. Nanotechnology development and utilization: a primer for diagnostic and interventional radiologists. Radiographics 2011;31:1449-62.
101. Power S, Slattery MM, Lee MJ. Nanotechnology and its relationship to interventional radiology. Part II: Drug Delivery, Thermotherapy, and Vascular Intervention. Cardiovasc Intervent Radiol 2011;34:676-90.
102. Power S, Slattery MM, Lee MJ. Nanotechnology and its relationship to interventional radiology. Part I: imaging. Cardiovasc Intervent Radiol 2011;34:221-6.
103. Baumann P, Nyman J, Hoyer M, et al. Outcome in a prospective phase II trial of medically inoperable stage I non-small-cell lung cancer patients treated with stereotactic body radiotherapy. J Clin Oncol 2009;27:3290-6.
104. Fakiris AJ, McGarry RC, Yiannoutsos CT, et al. Stereotactic body radiation therapy for early-stage non-small-cell lung carcinoma: four-year results of a prospective phase II study. Int J Radiat Oncol Biol Phys 2009;75:677-82.
105. Lagerwaard FJ, Haasbeek CJ, Smit EF, et al. Outcomes of risk-adapted fractionated stereotactic radiotherapy for stage I non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2008;70:685-92.
106. McGarry RC, Papiez L, Williams M, et al. Stereotactic body radiation therapy of early-stage non-small-cell lung carcinoma: phase I study. Int J Radiat Oncol Biol Phys 2005;63:1010-5.
107. Onishi H, Araki T, Shirato H, et al. Stereotactic hypofractionated high-dose irradiation for stage I nonsmall cell lung carcinoma: clinical outcomes in 245 subjects in a Japanese multiinstitutional study. Cancer 2004;101:1623-31.
108. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. JAMA 2010;303:1070-6.
109. Lanuti M, Sharma A, Willers H, et al. Radiofrequency ablation for stage I non-small cell lung cancer: management of locoregional recurrence. Ann Thorac Surg 2012;93:921-7; discussion 927-88.
110. Donington J, Ferguson M, Mazzone P, et al. American College of Chest Physicians and Society of Thoracic Surgeons consensus statement for evaluation and management for high-risk patients with stage I non-small cell lung cancer. Chest 2012;142:1620-35.
111. Crabtree T, Puri V, Timmerman R, et al. Treatment of stage I lung cancer in high-risk and inoperable patients: comparison of prospective clinical trials using stereotactic body radiotherapy (RTOG 0236), sublobar resection (ACOSOG Z4032), and radiofrequency ablation (ACOSOG Z4033). J Thorac Cardiovasc Surg 2013;145:692-9.

Cite this article as: Lee KS, Pua BB. Alternative to surgery in early stage NSCLC—interventional radiologic approaches. Transl Lung Cancer Res 2013;2(5):340-353. doi: 10.3978/j.issn.2218-6751.2013.10.02

Supportive and palliative care for lung cancer patients

Patsy Yates[1], Penelope Schofield[2,3,4,5], Isabella Zhao[1], David Currow[6]

[1]Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia; [2]Department of Cancer Experiences Research, Peter MacCallum Cancer Centre, Melbourne, Australia; [3]Sir Peter MacCallum Department of Oncology, (Faculty of Medicine, Dentistry and Health Sciences), [4]School of Behavioural Science, [5]School of Nursing, The University of Melbourne, Parkville, Australia; [6]Discipline of Palliative and Supportive Services, Adelaide, Flinders University, Australia

Correspondence to: Patsy Yates. Institute of Health and Biomedical Innovation, 60 Musk Ave, Kelvin Grove, QLD 4059, Australia.
Email: p.yates@qut.edu.au.

Abstract: Lung cancer patients face poor survival and experience co-occurring chronic physical and psychological symptoms. These symptoms can result in significant burden, impaired physical and social function and poor quality of life. This paper provides a review of evidence based interventions that support best practice supportive and palliative care for patients with lung cancer. Specifically, interventions to manage dyspnoea, one of the most common symptoms experienced by this group, are discussed to illustrate the emerging evidence base in the field. The evidence base for the pharmacological management of dyspnoea report systemic opioids have the best available evidence to support their use. In particular, the evidence strongly supports systemic morphine preferably initiated and continued as a once daily sustained release preparation. Evidence supporting the use of a range of other adjunctive non-pharmacological interventions in managing the symptom is also emerging. Interventions to improve breathing efficiency that have been reported to be effective include pursed lip breathing, diaphragmatic breathing, positioning and pacing techniques. Psychosocial interventions seeking to reduce anxiety and distress can also improve the management of breathlessness although further studies are needed. In addition, evidence reviews have concluded that case management approaches and nurse led follow-up programs are effective in reducing breathlessness and psychological distress, providing a useful model for supporting implementation of evidence based symptom management strategies. Optimal outcomes from supportive and palliative care interventions thus require a multi-level approach, involving interventions at the patient, health professional and health service level.

Keywords: Lung cancer; palliative care; dyspnoea

Submitted Oct 07, 2013. Accepted for publication Oct 12, 2013.
doi: 10.3978/j.issn.2072-1439.2013.10.05
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.10.05

Introduction

Lung cancer patients experience multiple symptoms that often co-occur, the most common being dyspnoea, cough, fatigue, pain, anorexia, anxiety and depression. As survival rates for lung cancer are poor (five-year relative survival rates in Australia of 14.1%) (1), these effects often persist over time and intensify as the disease progresses. Studies report that more than 80% of lung cancer patients have multiple symptoms, often experiencing more symptoms and psychological distress than patients with other cancer types (2). It has been estimated that 43% of patients with lung cancer report psychological distress, compared to an overall prevalence rate of 35% across 14 cancer sites (3). Such symptoms can result in significant burden, impaired physical and social function and poor quality of life. Newly diagnosed lung cancer patients also report feeling shocked and frightened and display a high need for information (4). Given the burdensome nature of this disease, it is not surprising that studies have confirmed that patients with lung cancer report a higher burden of psychological and daily living unmet needs compared with patients who have other types of cancer (5-8).

This paper provides a review of evidence based interventions that support best practice supportive and palliative care for patients with lung cancer. Specifically, pharmacological and non-pharmacological interventions to manage dyspnoea, one of the most common symptoms experienced by this group, will be discussed to illustrate the emerging evidence base in the field. The evidence to support interventions that focus specifically on addressing psychological distress and unmet needs is also discussed. In addition, given the complex nature of the health and support needs experienced by patients with lung cancer, we consider recent evidence regarding health service level interventions designed to achieve optimal outcomes this population.

Interventions to manage dyspnoea in patients with lung cancer

Pharmacological management of dyspnoea in lung cancer

The evidence base for the pharmacological management of chronic refractory breathlessness is continuing to improve. In this context, chronic is defined as "daily for more than three of the last six months", and refractory refers to cases where all underlying causes contributing to breathlessness have been assessed as to whether they can be reversed and, if so, whether they should be reversed. Breathlessness in this case is defined as modified Medical Research Council (mMRC) scale 3 or 4--breathless at rest or on minimal exertion such as the basic activities of daily living (dressing, bathing or preparing food). It is likely, however, that people with mMRC scale 2 will also benefit from breathlessness interventions (9,10). The aim of a therapeutic intervention for this population is to reduce symptomatic breathlessness, as breathlessness will rarely be controlled at all times once chronic irreversible underlying causes of the symptom are established. Although this may translate for some people into improved or better maintained levels of function, ultimately the focus is on reducing the subjective experience that we call 'breathlessness'. It is important, therefore, that both the severity (intensity) of breathlessness and an affective component (the unpleasantness of breathlessness) should be assessed in this context.

Systemic opioids have the best available evidence to support their use in the clinical setting of people with chronic refractory breathlessness. A meta-analysis and an adequately powered, double blind, randomised controlled crossover trial both report the same order of magnitude of benefit (9,11). The major adverse effect in both of these studies was constipation, which should be treated expectantly, with no recorded episodes of respiratory depression. In prospectively done clinical trials, with carefully titrated opioids, patients have not been admitted to hospital with obtundation, respiratory depression nor confusion. Systemic opioids, where morphine has been the most frequently studied medication, are likely to offer the most benefit.

More recent work has followed patients who gained symptomatic benefit from opioids for chronic refractory breathlessness for up to 660 days to explore the long term efficacy of once daily sustained release morphine (12). In this case, between 10-30 mg of oral morphine per 24 hours was used and delivered a sustained benefit for two thirds of patients who were started on the medication. The majority of this sample derived benefit from just 10 mg per 24 hours.

Other opioids are starting to be studied, but the evidence base strongly supports systemic morphine preferably initiated and continued as a once daily sustained release preparation. In a sub study exploring response to the titration of sustained release morphine for chronic refractory breathlessness, when benefit was derived, there was not only a reduction in breathlessness in the first 24 hours, but continued improvement over the ensuing week (13). This suggests that sustained release morphine should be titrated to effect and, when benefit is gained, further titration delayed for at least one week.

However, the same systematic review did not demonstrate benefit from nebulised opioids, despite the wide-spread presence of opioids receptors in the bronchial tree. This potentially was a type II error and may relate to the way in which opioids were nebulised (14). However, more recent work suggests that opioids delivered at the alveolar level are likely to help reduce chronic refractory breathlessness. A recently reported randomised control trial demonstrated sustained reduction in breathlessness, improved sleep and decreased cough in a relatively small cohort of people who have long term respiratory damage from previous mustard gas exposure (15).

A number of other medications are being studied. A recent systematic review suggested that there may be benefits from nebulised frusemide unrelated to a diuretic effect (16). The first large study of this has recently been reported and suggests that there may be sustained symptomatic benefit by using nebulised frusemide at a dose of 40 mg/4 mls compared to 4 mls of normal saline. This therapy appeared to be well tolerated (17). Of note, the widespread use of benzodiazepines is not supported with current evidence (18). Although one randomised trial suggested symptomatic benefit was generated more quickly with benzodiazepines in

the setting of acute breathlessness where a diagnostic workup was required, the trade-off was increased somnolence.

Non-pharmacological management of dyspnoea in lung cancer

In addition to the growing evidence base supporting the role of various pharmacological agents in the management of breathlessness, evidence to support the use of a range of other adjunctive non-pharmacological interventions in managing the symptom is also emerging. Recently a landmark study has reported on the use of non-invasive ventilation in people with chronic refractory breathlessness and advanced disease without overt respiratory failure. Participants were randomised to oxygen or non-invasive ventilation set to support mode. Non-invasive ventilation was well tolerated in people with advanced disease many of whom derived symptomatic benefit at rates greater than those people treated only with oxygen (19). It is a therapy which will require careful ongoing evaluation in order to understand the net effect that such interventions will deliver to patients with chronic refractory breathlessness in the last days or weeks of life.

A number of recent systematic reviews have also reported benefits from use of behavioural, psychosocial and environmental modification interventions in the management of dyspnoea (20-22).

Most studies in this field involve testing of multicomponent interventions, where a range of strategies are combined into a bundled intervention, making it difficult to ascertain specific components that have most benefit. It is also difficult to conclude which groups of patients are most likely to benefit from these complex interventions, as there is significant variation in study samples (21). At least one review has concluded that patients who enroll and complete these types of interventions appear to be in the earlier stages of their disease or have better functional abilities than those who do not complete the study (21). The application of these approaches, and what modifications are required for patients as the disease progresses, has not been well established.

Notwithstanding these limitations, behavioural and psychosocial interventions for patients with lung cancer that have some supporting evidence can be categorised according to two main mechanisms of action (20). These categories include interventions to improve breathing efficiency and interventions targeting the affective component of breathlessness by seeking to reduce anxiety and distress. Interventions to improve breathing efficiency include a range of breathing retraining techniques, with systematic reviews concluding there is good evidence to support the effectiveness of these techniques, including pursed lip breathing, diaphragmatic breathing, 'blow-as-you-go', positioning and pacing techniques (20).

Another review has concluded that evidence supporting the benefits of exercise programs in controlling breathlessness is not conclusive (23). This review of 16 studies on 13 unique patient groups totaling 675 patients with NSCLC concluded that exercise interventions for patients with NSCLC is safe before and after cancer treatment. While not all studies in this review included breathlessness as an outcome, the authors did conclude there were some positive benefits on exercise capacity, symptoms and some domains of health-related quality of life. The majority of the studies reviewed were, however, small case series and focused mostly on patients immediately pre- and post-surgery. The authors concluded, therefore, that further research is required to establish the effect of exercise, especially in the advanced stage of disease, as well as to determine the optimum type and dose of exercise training.

With regard to interventions aiming to reduce distress associated with breathlessness, interventions including relaxation techniques, coping skills training, and general support for patients and their carers have been reported to achieve positive outcomes (20-22). Relaxation techniques in particular are reported to be beneficial, although the acceptability and sustainability of this approach for all patients has not been determined. As with other non-pharmacological interventions, the available Cochrane Reviews recommend further testing to determine the nature and scope of psychosocial interventions seeking to improve the management of breathlessness (20,22). Application of such techniques also requires careful assessment of a patient's preferences and capacity to implement them.

The use of a hand held fan has been considered in a limited number of studies. This intervention is thought to produce a flow of air which may alter ventilation when directed to the face, although the exact mechanism of this effect is unclear (24). One small randomised controlled trial (RCT) concluded that the effectiveness of the fan could not be proved, although a small group seemed to benefit, not necessarily related to a relief in breathlessness (24). Work is ongoing to evaluate the effectiveness of this approach.

Interventions to manage psychological distress and unmet needs in lung cancer patients

Despite high levels of distress and perceived unmet need experienced by this group, evidence to inform effective psycho-educational and supportive care interventions for

lung cancer patients is scarce (25). The probable reason is the considerable challenges faced in conducting trials of complex interventions with this population. Notably, high refusal rates and poor retention have been acknowledged as a significant difficulty in recruiting patients with lung cancer to these trials (26).

Given the high morbidity of people with lung cancer, a focus of work in this area has been on trialing multifaceted intervention targeting the psycho-social well-being of people with lung cancer. In one study, two sessions of nurse-led coaching in progressive muscle relaxation combined with education on self-management of symptoms at the beginning and middle of radiotherapy were compared against usual care in a RCT (n=140). The intervention was demonstrated to be more effective in terms of reducing breathlessness, fatigue and anxiety compared with usual care (27).

Another large trial (N=233) of education versus coping skills training for caregivers showed improvements in patient- and caregiver-reported outcomes, including depression and self-efficacy over time for both groups (28). Both interventions were telephone based and the education consisted of basic information on the illness and patient care and coping skills training incorporating relaxation practices, problem-solving and communication. Interpretation of benefits is complicated by the absence of a suitable 'no treatment' control. The most recent trial (n=108) tested whether a tailored, multidisciplinary supportive care program based on systematic needs assessment with two sessions at the commencement and end of treatment was effective in reducing unmet needs and psychological distress and improving quality of life (29). However, due to methodological limitations there were no differences between the two arms.

In summary, available studies of psychosocial and psychoeducational interventions have notable limitations in their design including selection, attrition and reporting bias, small samples, insufficient intervention dose and/or a lack of a suitable control group. Notwithstanding these limitations, it is highly plausible that psychosocial interventions can reduce distress associated with lung cancer. Such approaches are therefore an important part of a comprehensive management plan for this population, although further research is needed to define the precise nature and scope of these interventions and application in differing patient contexts.

Service delivery models to optimise outcomes for patients with lung cancer

The complex, multidimensional and chronic nature of lung cancer-related symptoms and associated psychological distress requires an approach to care that enables collaboration between a range of health care providers across inpatient and community settings to support consistent implementation of evidence based supportive care interventions. In recent years, a body of evidence has emerged regarding various health service level interventions that have been designed to achieve optimal outcomes for this group. For example, two studies have investigated post-treatment nurse follow-up versus standard physician follow-up. One three-arm study involved a sample size of 166 people with progressive lung cancer who were randomised to receive a specialised oncology home care program delivered by nurses, a standard home care program delivered by a multidisciplinary team or an office care program delivered by physicians (control group) (30). Participants who received one of the two home-based nurse groups had lower symptom distress, but self-perceived health was also poorer in comparison to the physician follow-up (30). Another study compared nurse follow up with physician follow up after the completion of initial treatment. In this study, patients randomised to nurse-led follow up had open access to nurse specialists Monday to Friday and contact through open access clinic, telephone, and message pager service, and telephone assessment or clinic appointment two weeks after baseline, then every four weeks while the patient was stable with no routine investigations. Emphasis was on rapid and comprehensive communication with general practitioners and the primary healthcare team with regular discussion and referral to a medical team on detection of any new symptom or rapid worsening of condition. Patients who received the nurse-led follow up intervention had less severe dyspnea at 3 months and had better scores for emotional functioning and less peripheral neuropathy at 12 months, although no other significant differences in quality of life domains were identified. Patients who received the nurse-led follow up also scored significantly higher compared to conventional follow up patients in satisfaction with the organisation of care, information and education and personal experience of care at 3, 6 and 12 months from baseline. Importantly, the authors also reported that the pattern of use of services differed between the two groups. Specifically, compared to conventional follow up patients, patients receiving nurse-led follow up had significantly fewer medical consultations with a hospital doctor at three months, had fewer radiographs taken (including chest radiographs) at 3 months and 6 months, and were more likely to have had radiotherapy treatment at 3 months. Additionally, when place of death was known, significantly more patients who received

nurse-led follow up than conventional follow up patients died at home rather than in a hospital or hospice. Comparison of the overall costs of care between groups showed no significant differences (31).

Given the poor prognosis associated with lung cancer, and the likely increasing burden of symptoms as the disease progresses, the potential benefits of referral to palliative care services has also been investigated in one recent study. This randomised trial compared the effect of early referral to palliative care for newly diagnosed metastatic non-small cell lung cancer patients alongside standard oncology care with standard oncology care alone. As hypothesised, patients who received early referral to palliative care had better quality of life and less depressive symptoms than those who received standard care alone. Additionally, and perhaps less expectedly, while patients in the early referral group had less aggressive care than those in the standard care alone group, median survival was longer for patients receiving palliative care compared to standard care (11.6 *vs.* 8.9 months) (32). While the study was conducted in one large cancer centre in the US with its unique health system and is yet to be tested in other health care contexts, the findings of the study raise important questions for clinicians and health service managers about the adequacy of existing linkages between specialist oncology and palliative care services.

Implications for practice and research

Patients with lung cancer experience significant symptom burden and will benefit from good supportive and palliative care. Over the past decade, there have been important advances in understanding of pharmacological and non-pharmacological approaches to managing some common symptoms experienced by this group. This is particularly the case for dyspnoea, although some gaps remain in how these interventions are implemented in practice. Other common symptoms are similarly gaining increased attention, although we have focused on dyspnoea in this review to illustrate advances in the field as the evidence base for this symptom has developed more rapidly than for other symptoms. In addition to the clinical approaches reviewed in this paper, research in this field needs to extend to identify service delivery models that enable implementation of best practice supportive and palliative care. For example, evidence reviews highlight that case management approaches and nurse-led follow-up programs are effective in reducing breathlessness (20,22) and may be useful in reducing symptom and psychological distress (27,30,31). Such models also have the potential to positively influence the way health services are used. Some evidence also exists to support early referral of patients with metastatic lung cancer to palliative care, alongside standard oncology care (32). While such service delivery models have not been tested across differing health care systems, the findings from these studies are noteworthy and their implications for health services are far reaching. To achieve optimal outcomes for patients with lung cancer requiring supportive and palliative interventions, it is important that these health system level reforms be considered.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Australian Institute of Health and Welfare 2012. Cancer survival and prevalence in Australia: period estimates from 1982 to 2010. Cancer series no. 69. Cat. no. CAN 65. Canberra: AIHW.
2. Tishelman C, Petersson LM, Degner LF, et al. Symptom prevalence, intensity, and distress in patients with inoperable lung cancer in relation to time of death. J Clin Oncol 2007;25:5381-9.
3. Zabora J, BrintzenhofeSzoc K, Curbow B, et al. The prevalence of psychological distress by cancer site. Psychooncology 2001;10:19-28.
4. Butow PN, Kazemi JN, Beeney LJ, et al. When the diagnosis is cancer: patient communication experiences and preferences. Cancer 1996;77:2630-7.
5. Edmonds P, Karlsen S, Khan S, et al. A comparison of the palliative care needs of patients dying from chronic respiratory diseases and lung cancer. Palliat Med 2001;15:287-95.
6. Murray SA, Boyd K, Kendall M, et al. Dying of lung cancer or cardiac failure: prospective qualitative interview study of patients and their carers in the community. BMJ 2002;325:929.
7. Hill KM, Amir Z, Muers MF, et al. Do newly diagnosed lung cancer patients feel their concerns are being met? Eur J Cancer Care (Engl) 2003;12:35-45.
8. Li J, Girgis A. Supportive care needs: are patients with lung cancer a neglected population? Psychooncology 2006;15:509-16.
9. Abernethy AP, Currow DC, Frith P, et al. Randomised, double blind, placebo controlled crossover trial of

sustained release morphine for the management of refractory dyspnoea. BMJ 2003;327:523.
10. Dyspnea. Mechanisms, assessment, and management: a consensus statement. American Thoracic Society. Am J Respir Crit Care Med 1999;159:321-40.
11. Jennings AL, Davies AN, Higgins JP, et al. A systematic review of the use of opioids in the management of dyspnoea. Thorax 2002;57:939-44.
12. Currow DC, McDonald C, Oaten S, et al. Once-daily opioids for chronic dyspnea: a dose increment and pharmacovigilance study. J Pain Symptom Manage 2011;42:388-99.
13. Currow DC, Quinn S, Greene A, et al. The longitudinal pattern of response when morphine is used to treat chronic refractory dyspnea. J Palliat Med 2013;16:881-6.
14. Krajnik M, Podolec Z, Siekierka M, et al. Morphine inhalation by cancer patients: a comparison of different nebulization techniques using pharmacokinetic, spirometric, and gasometric parameters. J Pain Symptom Manage 2009;38:747-57.
15. Krajnik M, Schäfer M, Sobański P, et al. Local pulmonary opioid network in patients with lung cancer: a putative modulator of respiratory function. Pharmacol Rep 2010;62:139-49.
16. Newton PJ, Davidson PM, Macdonald P, et al. Nebulized furosemide for the management of dyspnea: does the evidence support its use? J Pain Symptom Manage 2008;36:424-41.
17. Vahedi HS, Mahshidfar B, Rabiee H, et al. The adjunctive effect of nebulized furosemide in acute treatment of patients with chronic obstructive pulmonary disease exacerbation: a randomized controlled clinical trial. Respir Care 2013;58:1873-7.
18. Simon ST, Higginson IJ, Booth S, et al. Benzodiazepines for the relief of breathlessness in advanced malignant and non-malignant diseases in adults. Cochrane Database Syst Rev 2010;(1):CD007354.
19. Nava S, Ferrer M, Esquinas A, et al. Palliative use of non-invasive ventilation in end-of-life patients with solid tumours: a randomised feasibility trial. Lancet Oncol 2013;14:219-27.
20. Bausewein C, Booth S, Gysels M, et al. Non-pharmacological interventions for breathlessness in advanced stages of malignant and non-malignant diseases. Cochrane Database Syst Rev 2008;(2):CD005623.
21. Zhao I, Yates P. Non-pharmacological interventions for breathlessness management in patients with lung cancer: a systematic review. Palliat Med 2008;22:693-701.
22. Rueda JR, Solà I, Pascual A, et al. Non-invasive interventions for improving well-being and quality of life in patients with lung cancer. Cochrane Database Syst Rev 2011;(9):CD004282.
23. Granger CL, McDonald CF, Berney S, et al. Exercise intervention to improve exercise capacity and health related quality of life for patients with non-small cell lung cancer: a systematic review. Lung Cancer 2011;72:139-53.
24. Bausewein C, Booth S, Gysels M, et al. Effectiveness of a hand-held fan for breathlessness: a randomised phase II trial. BMC Palliat Care 2010;9:22.
25. Sanson-Fisher R, Bailey LJ, Aranda S, et al. Quality of life research: is there a difference in output between the major cancer types? Eur J Cancer Care (Engl) 2010;19:714-20.
26. Schofield P, Ugalde A, Carey M, et al. Lung cancer: challenges and solutions for supportive care intervention research. Palliat Support Care 2008;6:281-7.
27. McCorkle R, Benoliel JQ, Donaldson G, et al. A randomized clinical trial of home nursing care for lung cancer patients. Cancer 1989;64:1375-82.
28. Moore S, Corner J, Haviland J, et al. Nurse led follow up and conventional medical follow up in management of patients with lung cancer: randomised trial. BMJ 2002;325:1145.
29. Chan CW, Richardson A, Richardson J. Managing symptoms in patients with advanced lung cancer during radiotherapy: results of a psychoeducational randomized controlled trial. J Pain Symptom Manage 2011;41:347-57.
30. Porter LS, Keefe FJ, Garst J, et al. Caregiver-assisted coping skills training for lung cancer: results of a randomized clinical trial. J Pain Symptom Manage 2011;41:1-13.
31. Schofield P, Ugalde A, Gough K, et al. A tailored, supportive care intervention using systematic assessment designed for people with inoperable lung cancer: a randomised controlled trial. Psychooncology 2013;22:2445-53.
32. Temel JS, Greer JA, Muzikansky A, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. N Engl J Med 2010;363:733-42.

Cite this article as: Yates P, Schofield P, Zhao I, Currow D. Supportive and palliative care for lung cancer patients. J Thorac Dis 2013;5(S5):S625-S628. doi: 10.3978/j.issn.2072-1439.2013.10.15

Thoracoscopic sleeve resection—the better approach?

Calvin S. H. Ng

Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China
Correspondence to: Calvin S.H. Ng, BSc (Hons) MBBS (Hons) MD, FRCSEd (CTh). Associate Professor, Division of Cardiothoracic Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, N.T. Hong Kong SAR, China.
Email: calvinng@surgery.cuhk.edu.hk.

Submitted Jul 04, 2014. Accepted for publication Jul 06, 2014.
doi: 10.3978/j.issn.2072-1439.2014.07.28
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.07.28

In the past, thoracoscopic sleeve resection has been reserved for the most adventurous and capable minimal invasive thoracic surgeons. However, with improvements in thoracoscopic competency, greater exchange of knowledge and technical know-how, and advances in equipment, increasing number of centers are able to perform sleeve resections thoracoscopically. Jianxing He's team from China, a group known for their innovation and thoracoscopic excellence, has recently published their experience of bronchial sleeve resections (1). Among the 49 patients, 20 (41%) received the bronchial sleeve lobectomy thoracoscopically, with one patient requiring half-carinal reconstruction in combination with right upper sleeve lobectomy. A 3-port VATS technique was used, with the utility thoracotomy placed anteriorly, and the camera port inferiorly. In just under half of their initial cases, a modified interrupted suture anastomosis technique of closing the membranous posterior wall of the bronchus with continuous 4-O polypropylene followed by alternating figure-of-eight and mattress with 4-O single-strand absorbable suture for the cartilaginous anterior wall was used. For the subsequent remaining cases, a continuous suture technique was used for both the posterior and anterior bronchial walls. Neither covering nor buttressing techniques were needed for the anastomoses, and no postoperative anastomotic leakage was detected. With no perioperative mortality and excellent immediate results, this study seem to further support the relative safety and efficacy of thoracoscopic sleeve resection in experienced thoracoscopic surgery centers. In addition, the study has highlighted the evolution in thoracoscopic bronchial anastomotic technique from the traditional emphasis on the security of interrupted suturing (2), to the increasing use of the more convenient continuous suturing techniques over recent years (1,3,4). Evidently, continuous suturing techniques will result in less suture tangling and may be quicker, while proponents of interrupted suturing have emphasized the potential advantages of less anastomotic site ischemia and security of their technique. It seems impossible to have a meaningful comparison of clinical outcomes between the different anastomotic approaches for thoracoscopic sleeve lobectomy because of the relatively low case numbers, patient heterogeneity and the wide variations in technique within each anastomotic approach, for example, suture size and type used, or stitch spacing, just to mention a few. In thoracic surgery, perhaps more so in thoracoscopic surgery, it is often the technique which the surgeon has been trained and is most comfortable with which produces the best results. The bronchial anastomotic technique chosen should be the one most familiar to the surgeon.

Doing less for more

Although there are no randomized trials comparing outcomes following thoracoscopic sleeve resection lobectomy with thoracoscopic pneumonectomy in patients suitable for both procedures, it is well known that the latter is associated with a higher perioperative mortality rate and complications, including pleural space infection, bronchopleural fistula, atrial fibrillation and respiratory failure (5). Furthermore, less clinically apparent parameters such as right ventricular strain and pressure are likely to be higher following thoracoscopic pneumonectomy compared with thoracoscopic sleeve resection lobectomy. Therefore, despite the improving outcomes following thoracoscopic pneumonectomy over the years (6,7), few would argue

against sleeve resection lobectomy being the procedure of choice for those patients with suitable anatomy, to achieve better lung preservation, and lower morbidity and mortality.

There is currently no prospective study comparing outcomes between thoracoscopic and open sleeve lobectomy. However, we know that the thoracoscopic approach to major lung resection has been associated with attenuated inflammatory cytokine response (8), better preserved postoperative immune function (9,10), attenuated postoperative angiogenic environment (11), less impairment of lung function (12), reduced postoperative pain and less disturbed shoulder dysfunction (13) amongst other advantages, when compared with their open counterparts. Of greater importance is the positive effect of minimizing surgical access trauma through thoracoscopic lung cancer resection on patient survival. Several studies have shown a small 5-year survival advantage in those who underwent thoracoscopic lobectomy for early stage lung cancer when compared with open approach (14,15). Interestingly, a similar survival advantage can be detected in other cancers, such as colon cancer, when resections were performed laparoscopically rather than by open laparotomy (15). Another often forgotten advantage of a quicker postoperative recovery from the thoracoscopic approach is earlier commencement and higher tolerance to adjuvant therapy for advance lung cancer patients (16). Future studies may be needed to determine if similar advantages can be found following minimally invasive thoracoscopic sleeve lobectomy when compared with open approach.

The new horizon

Thoracoscopic sleeve lobectomy, and indeed the whole of minimal invasive thoracic surgery, is undergoing a major evolution (17), from hybrid mini thoracotomy procedures with video-assistance (18), to the 2-port thoracoscopic technique (19), and more recently the single port approach (20). The challenges of thoracoscopic sleeve lobectomy, particularly when the surgery is increasingly being performed through smaller and fewer incisions, are achieving good visualization, utilizing endoscopic instruments for tissue dissection and manipulation, and reducing the difficulty associated with thoracoscopic bronchial anastomosis. Specialized thoracoscopic instruments continue to undergo refinement by producing angulated double hinged and narrower shafted instruments which significantly improves ergonomics and minimize fencing when placed through small surgical incision(s) (21). Another recent advancement is the development of variable wide angled thoracoscopes that allow up to 120 degrees of vision by either flexible scope tip or rotating prism mechanism. These thoracoscopes improve the surgeon's visual field and flexibility, even when the scope movement and position is limited within the confines of a small single incision (22). The laborious task of intracorporeal knot tying for bronchial anastomosis can now be significantly simplified by using an endoscopic "knot tying" device, such as TK Ti-KNOT® (LSI Solutions, Rochester, USA), that conveniently tightens and then secures the suture using a titanium crimp (23). Also, rapid development in barbed suture technology may soon obviate the need for intracorporeal knot tying. On the horizon will be endoscopic robotic arm devices that open inside the thoracic cavity capable of tissue recognition and precision automated micro-suturing (24). Until that day, many of us flesh and bone mortals will need to continue to strive for technical excellence, and be acquainted with the latest and best equipment for our endeavours.

Acknowledgements

Disclosure: The author declares no conflict of interest.

References

1. Xu X, Chen H, Yin W, et al. Thoracoscopic Half Carina Resection and Bronchial Sleeve Resection for Central Lung Cancer. Surg Innov 2014;21:481-6.
2. Mahtabifard A, Fuller CB, McKenna RJ Jr. Video-assisted thoracic surgery sleeve lobectomy: a case series. Ann Thorac Surg 2008;85:S729-32.
3. Yu D, Han Y, Zhou S, et al. Video-assisted thoracic bronchial sleeve lobectomy with bronchoplasty for treatment of lung cancer confined to a single lung lobe: a case series of Chinese patients. J Cardiothorac Surg 2014;9:67.
4. Yang R, Shao F, Cao H, et al. Bronchial anastomosis using complete continuous suture in video-assisted thoracic surgery sleeve lobectomy. J Thorac Dis 2013;5:S321-2.
5. Ng CS, Wan S, Lee TW, et al. Post-pneumonectomy empyema: current management strategies. ANZ J Surg 2005;75:597-602.
6. Nwogu CE, Yendamuri S, Demmy TL. Does thoracoscopic pneumonectomy for lung cancer affect survival? Ann Thorac Surg 2010;89:S2102-6.
7. Lau KK, Ng CS, Wan IY, et al. Video-assisted

thoracoscopic pneumonectomy is safe and may have benefits over open pneumonectomy. European Society of Thoracic Surgeons (ESTS), 21st European Conference on General Thoracic Surgery, Birmingham, UK. 2013;17:abstract F108.
8. Yim AP, Wan S, Lee TW, et al. VATS lobectomy reduces cytokine responses compared with conventional surgery. Ann Thorac Surg 2000;70:243-7.
9. Ng CS, Lee TW, Wan S, et al. Thoracotomy is associated with significantly more profound suppression in lymphocytes and natural killer cells than video-assisted thoracic surgery following major lung resections for cancer. J Invest Surg 2005;18:81-8.
10. Ng CS, Wan S, Hui CW, et al. Video-assisted thoracic surgery lobectomy for lung cancer is associated with less immunochemokine disturbances than thoracotomy. Eur J Cardiothorac Surg 2007;31:83-7.
11. Ng CS, Wan S, Wong RH, et al. Angiogenic response to major lung resection for non-small cell lung cancer with video-assisted thoracic surgical and open access. ScientificWorldJournal 2012;2012:636754.
12. Garzon JC, Ng CS, Sihoe AD, et al. Video-assisted thoracic surgery pulmonary resection for lung cancer in patients with poor lung function. Ann Thorac Surg 2006;81:1996-2003.
13. Li WW, Lee RL, Lee TW, et al. The impact of thoracic surgical access on early shoulder function: video-assisted thoracic surgery versus posterolateral thoracotomy. Eur J Cardiothorac Surg 2003;23:390-6.
14. Ng CS, Wan S, Hui CW, et al. Video-assisted thoracic surgery for early stage lung cancer - can short-term immunological advantages improve long-term survival? Ann Thorac Cardiovasc Surg 2006;12:308-12.
15. Ng CS, Whelan RL, Lacy AM, et al. Is minimal access surgery for cancer associated with immunologic benefits? World J Surg 2005;29:975-81.
16. Petersen RP, Pham D, Burfeind WR, et al. Thoracoscopic lobectomy facilitates the delivery of chemotherapy after resection for lung cancer. Ann Thorac Surg 2007;83:1245-9; discussion 1250.
17. Ng CS, Lau KK, Gonzalez-Rivas D, et al. Evolution in surgical approach and techniques for lung cancer. Thorax 2013;68:681.
18. He J, Shao W, Cao C, et al. Long-term outcome of hybrid surgical approach of video-assisted minithoracotomy sleeve lobectomy for non-small-cell lung cancer. Surg Endosc 2011;25:2509-15.
19. Jiao W, Zhao Y, Huang T, et al. Two-port approach for fully thoracoscopic right upper lobe sleeve lobectomy. J Cardiothorac Surg 2013;8:99.
20. Gonzalez-Rivas D, Fernandez R, Fieira E, et al. Uniportal video-assisted thoracoscopic bronchial sleeve lobectomy: first report. J Thorac Cardiovasc Surg 2013;145:1676-7.
21. Ng CS, Wong RH, Lau RW, et al. Minimizing chest wall trauma in single-port video-assisted thoracic surgery. J Thorac Cardiovasc Surg 2014;147:1095-6.
22. Ng CS, Wong RH, Lau RW, et al. Single Port Video-Assisted Thoracic Surgery: Advancing Scope Technology. Eur J Cardiothorac Surg 2014. [Epub ahead of print].
23. Demmy TL. Thoracoscopic Left Upper Lobe Sleeve Lobectomy for Inflammatory Myofibroblastic Tumor. 94th Annual Meeting American Association for Thoracic Surgery (AATS), April 26-30, 2014, Toronto, Canada. [video presentation].
24. Ng CS, Rocco G, Wong RH, et al. Uniportal and single-incision video-assisted thoracic surgery: the state of the art. Interact Cardiovasc Thorac Surg 2014;19:661-6.

Cite this article as: Ng CS. Thoracoscopic sleeve resection—the better approach? J Thorac Dis 2014;6(9):1164-1166. doi: 10.3978/j.issn.2072-1439.2014.07.28

Surgery in 2013 and beyond

Rishendran Naidoo[1], Morgan N. Windsor[1], Peter Goldstraw[2]

[1]Department of Cardiothoracic Surgery, The Prince Charles Hospital, Brisbane, Australia; [2]Academic Department of Thoracic Surgery, Royal Brompton Hospital, London, UK

Correspondence to: Rishendran Naidoo, FRACS, Staff Specialist. Cardiac and Thoracic Surgery, Department of Cardiac Surgery, The Prince Charles Hospital, Brisbane, Queensland 4520, Australia. Email: naidoor21@yahoo.com.

Abstract: Lung cancer is a leading cause of cancer related mortality. The role of surgery continues to evolve and in the last ten years there have been a number of significant changes in the surgical management of lung cancer. These changes extend across the entire surgical spectrum of lung cancer management including diagnosis, staging, treatment and pathology. Positron Emission Tomography (PET) scanning and ultrasound (EBUS) have redefined traditional staging paradigms, and surgical techniques, including video-assisted thoracoscopy (VATS), robotic surgery and uniportal surgery, are now accepted as standard of care in many centers. The changing pathology of lung cancer, with more peripheral tumours and an increase in adenocarcinomas has important implications for the Thoracic surgeon. Screening, using Low-Dose CT scanning, is having an impact, with not only a higher percentage of lower stage cancers detected, but also redefining the role of sublobar resection. The incidence of pneumonectomy has reduced as have the rates of "exploratory thoracotomy". In general, lung resection is considered for stage I and II patients with a selected role in more advanced stage disease as part of a multimodality approach. This paper will look at these issues and how they impact on Thoracic Surgical practice in 2013 and beyond.

Keywords: Lung cancer; surgery

Submitted Jul 19, 2013. Accepted for publication Jul 29, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.39
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.39

Introduction

Epidemiology

Lung cancer remains a leading cause of cancer related mortality with the WHO reporting 1,380,000 deaths from it in 2008 (*Figure 1*) (1). It is the most common cancer in men worldwide, fourth in women and globally is responsible for more deaths that breast and prostate cancer combined. Tobacco consumption is incriminated in 85-90% of lung cancer cases.

In Australia, lung cancer is the 5th most commonly diagnosed cancer (2). It poses a significant health burden with an incidence rate of 43.2 cases per 100,000 people. Lung cancer is also the most common cause of cancer death accounting for 18.9% of all cancer deaths (2). Survival rates overall are poor, but the trend is improving with time, being 8.7% for 1982-1987 and increasing to 14.1% for 2006-2010 (3).

Worldwide the epidemiology varies due to socio-economic factors. In more developed countries the incidence is falling in men but is still rising in women (1) largely due to successful efforts at tobacco control and smoking cessation efforts (*Figures 2-4*). Peak incidence in more developed countries is now in the 8th decade. In less developed countries the lung cancer epidemic is in an earlier phase. Incidence is low but rising rapidly in men and women, and peak incidence occurs 2 decades earlier.

Tumour pathology—non-small cell lung cancer (NSCLC)

Recent developments in molecular profiling have accentuated

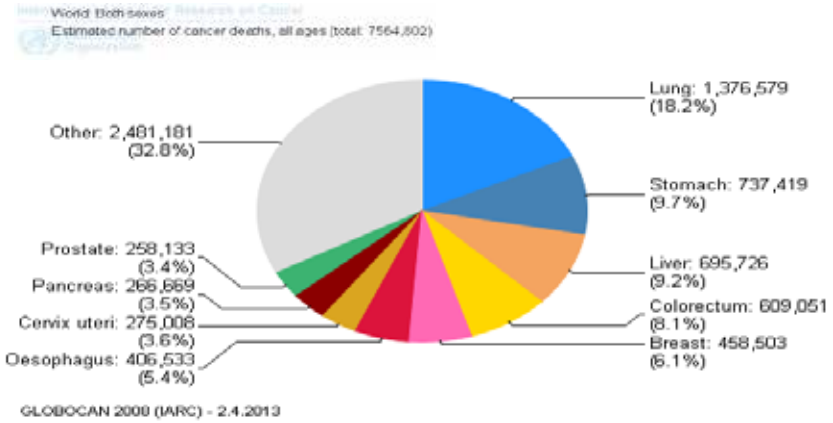


Figure 1 Cancer incidence and mortality worldwide. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 14/05/2013.

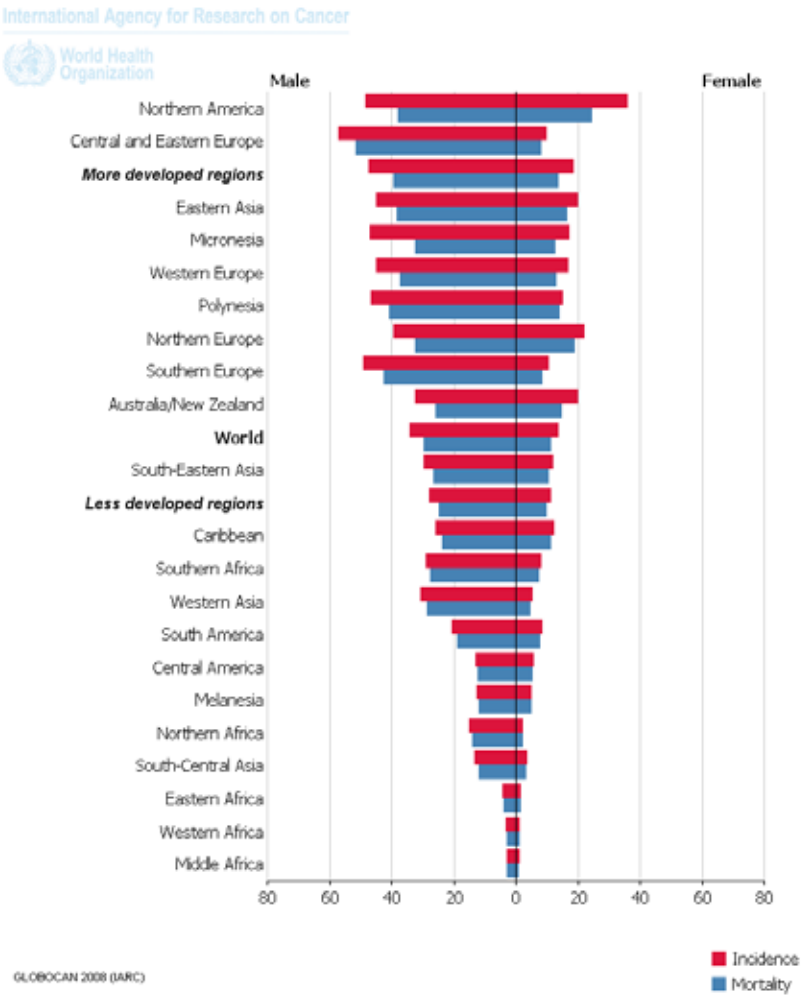


Figure 2 Incidence and mortality of lung cancer in men and women.

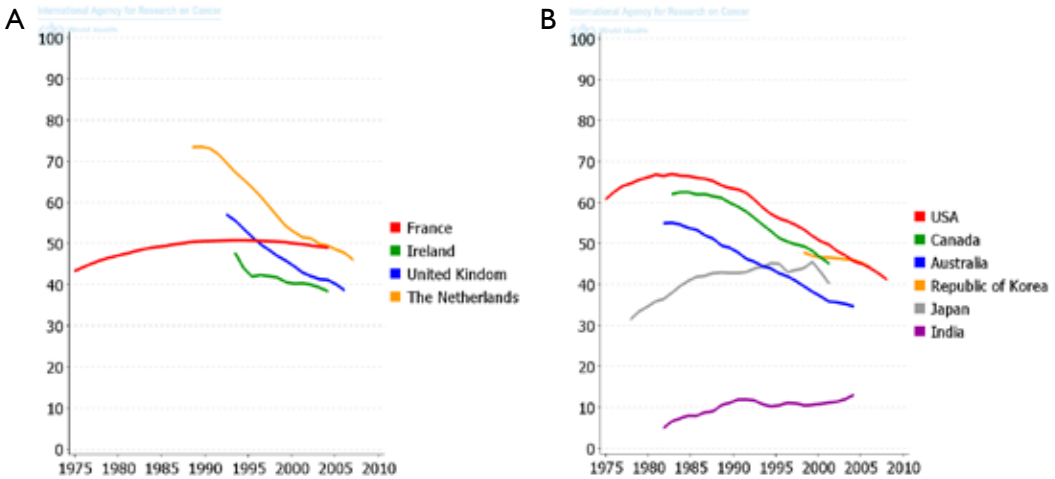


Figure 3 Trends in incidence of lung cancer in men.

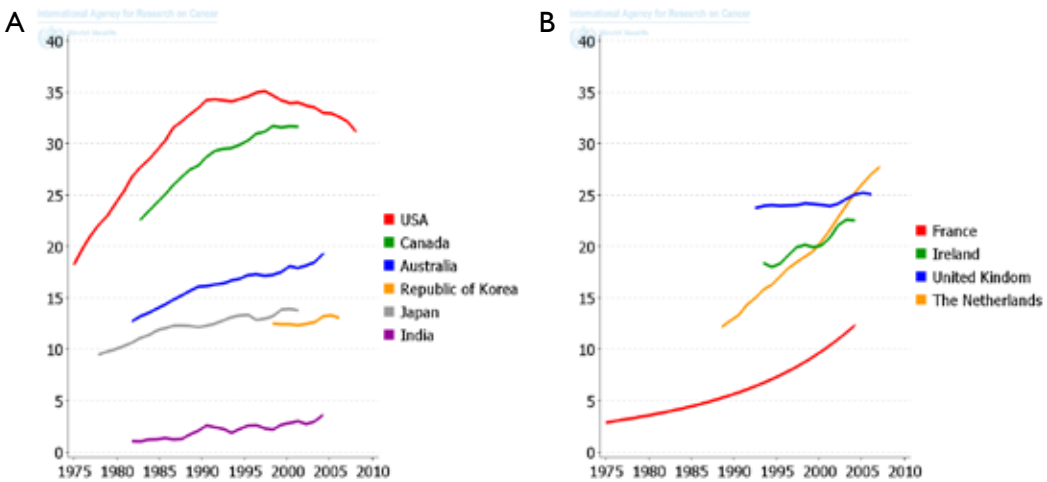


Figure 4 Trends in incidence of lung cancer in women.

the role of the pathologist within the multi-disciplinary team. No longer is it appropriate for the pathologist to determine simply whether a specimen is either small cell or NSCLC. In more developed countries there has been a marked change in the histopathologic profile of non-small cell carcinoma, squamous cell carcinoma no longer being the most common cell type. Recent trends show a significant increase in adenocarcinoma and a shift towards more peripheral squamous cell tumours (4).

Additionally the subclassification of adenocarcinoma into recognizable histologic groups has important prognostic and therapeutic implications. The pathologist will now routinely be required to perform an ever increasing array of tumour genomic assays as many unique tumour mutations and amplifications have been identified that allow targeted therapeutic options. The testing for these mutations is becoming cheaper and in many centres it is now routine to have results on the EGFR and ALK mutation status of all adenocarcinomas. These can be tested on fewer cells, in some cases only 100 cells may be required (5).

Koudelakova *et al*. recently reviewed the clinically relevant driver mutations (6). Epidermal growth factor receptor (EGFR) gene mutations occur in 10-30% of patients with non-small cell lung cancer (6,7). Tyrosine kinase inhibitors (TKI) have been demonstrated to show responses in 70-80% of patients with this mutation (6,7). Erlotinib and gefitinib have higher response rates and longer progression free survival compared to chemotherapy.

Response rates in EGFR negative patients are low. Adenocarcinomas, females and non-smokers have been shown to respond better. Current recommendations are that all newly diagnosed patients with advanced NSCLC be tested, and if positive, should be commenced on a TKI.

The anaplastic lymphoma kinase (ALK) oncogene has been found in 5% of patients, increasing to as high as 20% in light or non-smokers (8). Crizotinib, an ALK TKI, has been shown to be effective and phase III trials are ongoing. It is recommended that this mutation also be tested for.

The thoracic surgeon needs to be well aware of these developments not only to counsel the patient about the implications of such tests in resected specimens but to be fully involved within the multi-disciplinary team during discussions for "more tissue" (9). In patients with advanced metastatic disease, it is imperative that the surgeon brings to the table a realistic assessment of the risk/benefit of the proposed procedure, has knowledge of the chances of a positive result and is fully aware how much tissue is required before embarking on further invasive procedures.

Surgery—where are we now?

Surgical management is the standard of care for stage I and II in patients who are medically fit even though there are not randomised controlled trials of surgery versus other therapy in these patients (10,11). Expected 5-year survival figures are 60-80% for stage I and 40-60% for stage II. In a meta-analysis on the role of surgery, Wright *et al*. analysed trials of surgery against no treatment or non-surgical treatment, concluding that they could neither support nor discount the survival benefit of surgery but that "a little surgery was better than none" (12). There also is a role for surgery in selected stage IIIA cases, usually in a multi-modality setting, and even highly selected cases of stage IIIB and IV cases surgery may merit consideration.

Staging for lung cancer currently follows the TNM classification in its 7th edition and the reader is referred to the IALSC Staging Manual in Thoracic Oncology (13). There has been a logical evolution in trying to select those patients who will benefit from surgical resection and to exclude those in whom surgery will offer no assistance, the so called 'futile thoracotomy'. The dominant focus is the status of the mediastinal lymph nodes. After the introduction of invasive mediastinal assessment by Daniels [1949], Carlens [1959] and McNeill and Chamberlain [1966], these became the traditional preoperative modes of assessment for the next 40 years (14-16). Accuracy was quite high and these techniques became well established. Cervical mediastinoscopy however, is difficult to teach, and in inexperienced hands a procedure with morbidity and mortality rates. In general, there is strong evidence to suggest that it has been underutilized particularly in low volume centres as outlined in the review by Little *et al*. in 2005 (17). Video-assisted mediastinoscopy has been a considerable advance providing improved visualization especially for training purposes.

Over the last 30 years Computed Tomography (CT), has come to occupy a central role in assessing the intrathoracic extent of disease and occasionally detects occult distant disease. Assessment of the T component of stage is assisted by CT scan but all surgeons will be aware of the uncertainties in deciding resectability from the CT scan. MRI is usually reserved for apical sulcus lesions and sometimes T4 tumours in which the ability to reconstruct in oblique axes may be advantageous. Nodal assessment by CT scan has limited accuracy particularly with nodes <15 mm in short axis dimension. At least 20% of sub-centimetre nodes ultimately are confirmed to be malignant and around 40% of nodes 'enlarged' by CT criteria are benign (18).

Positron Emission Tomography (PET) combined with CT (PET-CT) scanning has revolutionized lung cancer staging and represents the biggest single advance in this field. When available, it should be a routine part of staging in all potentially resectable lung cancers, perhaps with the exception of sub-centimetre screen-detected lesions. PET scanning will often show unexpected uptake in nodal or distant sites. Whilst most of these lesions will be shown to be metastatic deposits, false positive uptake is known to occur, the incidence varying between geographical locations. It is thus important that each unit understands the incidence of false positive uptake in its own population and ensures that no one is denied curative surgery inappropriately. In doubtful cases biopsy of the area of uptake is recommended.

The introduction of endobronchial ultrasound (EBUS) has further revolutionised staging of the mediastinum and for that matter, assessment of all cases of mediastinal adenopathy. It is now possible to diagnose and stage the lung cancer patient in a single outpatient procedure, avoiding 'diagnostic' and then 'staging' bronchoscopies (19). Surgeons should be driving this process.

In experienced hands EBUS has been shown to be highly sensitive and accurate with a lower complication rate than mediastinoscopy. Yasafuku has demonstrated the equivalence of EBUS transbronchial needle aspiration (TBNA) *vs*. mediastinoscopy and this would now be the procedure

of choice for mediastinal staging (20). Endo-oesophageal ultrasound (EUS) has been used to stage the posterior mediastinum, evaluate the adrenals and even the left lobe of the liver. Whilst a meta-analysis has shown high sensitivity and specificity, the negative predictive value is limited (21). EBUS and EUS have a complementary role to play with reported accuracy of 95%, if available, they play an important role in minimally-invasive mediastinal staging (22).

In many units mediastinoscopy is reserved for the occasional patient where EBUS is negative but clinical suspicion of nodal disease is high, either as a primary staging or after induction therapy, or where mediastinal nodal involvement by sarcoid or lymphoma is suspected but the cores obtained at EBUS are non diagnostic. Surgeons should be performing these themselves and be *au fait* with on site pathologic assessment or have a close working relationship with physicians skilled in this technique. EBUS has an important role in preoperative determination of N1 disease. Far from irrelevant because it is still 'surgical', where resection is considered, N1 positivity may mean pneumonectomy and this has important implications for patient selection. At some centres, patients with N1 disease may undergo preoperative chemotherapy as it is better tolerated than in the adjuvant setting post pneumonectomy and downsizing bulky disease makes for a potentially more satisfactory surgical approach without an increase in morbidity.

The development of video-assisted mediastinal lymphadenectomy (VAMLA) and transcervical extended mediastinal lymphadenectomy (TEMLA) techniques have been described but as yet their role in primary evaluation of the mediastinum remains unclear (23).

VATS staging is occasionally necessary to evaluate a pleural effusion in which repeated aspirates have not confirmed a malignant cause, when nodal status remains unclear, especially in the aorto-pulmonary zone or if pathological confirmation of additional pulmonary nodules is needed to decide appropriate therapy.

In parallel with the assessment of disease extent it is important to assess patient fitness for surgery. Guidelines on pre-operative evaluation of patients outline the efficient way to stage patients to allow decision making on interventions (24).

The ageing population in the developed world has meant that decision making on suitability for surgery is imperative. Risk factors for surgical morbidity and mortality include patient age, sex, American Society of Anaesthesiologists (ASA) score, performance status, surgical priority, comorbidity, induction chemoradiation, forced expiratory volume in 1 second (FEV1), renal dysfunction and body mass index (24). Algorithms on the fitness for surgery have been described by the ACCP, ERS, ESTS and BTS (24-26). Functional assessment includes a walk test and cardiopulmonary exercise testing. Surgeons need to bear in mind though that these tests do have shortcomings and in recognition of this, Lim *et al*. have proposed greater involvement of the patient in the decision making process (13,27).

In evaluating the trends in surgical resection in England, Riaz *et al*. noted that the resection rates were increasing despite the patient population becoming older, and that more segmental resections were being performed (28). Increasing age was found to be associated with a decreased likelihood of undergoing pneumonectomy or sleeve resection.

Exploratory thoracotomy rates have also dropped, as have the number of pneumonectomies performed. Five year survival for lobectomy and patients with adenocarcinoma was increased and the overall prognosis over time was found to be improved on multivariate analysis, attributed to earlier diagnosis (28,29).

Surgical technique

Surgical access for lung cancer resection remains topical. Thoracotomy has been the traditional approach for resection of lung cancers. Video-assisted thoracoscopic (VAT) lobectomy generates controversy in the surgical field and has been slow to gain popularity. It is not new, celebrating its 20th anniversary this year. It has evolved and been assisted by improvements in hardware. Advocates argue that the advantages; reduced pain, shortened hospital duration, decreased air leak, pneumonia and atrial arrhythmias favour VATS over traditional thoractomy (30,31). In addition, it has been argued that the increase in inflammatory mediators is less exuberant than with open surgery (32). The counter argument relates to the learning curve safety, patient selection, long term survival and the ability to perform an oncologically complete operation with adequate nodal dissection. The latter has been given increased relevance as the 7th edition of TNM requires a minimum number of lymph nodes to be removed and examined pathologically, to allow a pN category to be assigned and a complete R0 resection to be confirmed (13).

The publication of the phase II CALGB 39802 study established the feasibility of this approach and sought to offer a precise definition (33). It was demonstrated that, with

a clear definition of the approach, complications were low and the survival compared favourably to open series. The VATS approach was less expensive, with lower morbidity than cases undergoing thoracotomy. In the absence of large scale randomised controlled trials, systematic reviews and meta-analyses of VATS lobectomy have demonstrated similar benefits (30,31,34). The use of propensity matching, despite some obvious limitations has also been utilised to demonstrate the superior benefits over open thoracotomy (31).

To address the concerns regarding the safety, specifically regarding the management of bleeding, Yamashita *et al*. published their results with management of intra-operative vessel injury. In a review of 557 patients, there were 26 (4.7%) vascular injuries, 17 of which involved pulmonary arterial branches. Fifty percent of these required conversion to thoracotomy and another 23% required mini-thoracotomy. They also noted no differences in hospital stay and overall morbidity but an increase in surgical time and blood loss (35). This led to the conclusion that safety concerns were not significant enough to preclude the VATS approach.

Hanna *et al*. compared cancer specific and overall survival in a propensity matched cohort of 190 VATS patient with open lobectomy (36). No statistically significant difference in cancer specific (76.7% *vs*. 82.9%, P=0.170) or overall survival (64% *vs*. 73%, P=0.170) was detected. Operative mortality and morbidity were similar in the 2 groups.

In a meta-analysis comparing the long term survival in patients undergoing VATS (n=2,106) and open surgery (n=2,661), Taioli *et al*. reviewed 20 observational studies. Long term survival was found to be increased in the VATS group with a 5% meta difference (95% CI, 3-6%) (37). Further evidence for at least an equivalent disease-free and overall survival was also provided by Kuritzky *et al*. (38).

Despite this, the uptake of this approach has been relatively slow. In a review of the STS General Thoracic Database, Paul *et al*. noted that in 2007, only 30% of all lobectomies were performed thoracoscopically (39).

Subsequent review of Nationwide Inpatient Sample Database by the same author encompassing 2007-2008, demonstrated that only 15% of lobectomies were thoracoscopic (40). Interestingly, the majority of these procedures (67%) were performed in teaching hospitals. Clear consensus on, and compliance with the definition of VATS lobectomy has hampered progress. Furthermore it is never clear what the comparator is for the open approach. Traditional posterolateral thoracotomy for lung cancer resection is clearly obsolete but this is often the "gold standard" against which VATS lobectomy is compared. The randomised trials do not address the observation that an experienced high volume surgeon, using a small 6-8 cm incision with limited rib spreading and standard techniques can achieve outcomes with open surgery equivalent or better than those published for VATS with less cost. Enthusiasm for 'minimally invasive' procedures needs to be tempered with tight cost evaluation and data that applies to the wider surgical community rather than specialist academic centres.

In an editorial commenting on VATS lobectomy, Wood noted that this procedure is still generally performed in high volume and academic centres. It is postulated that the improved outcomes noted in most studies relates to the surgeon rather than to the actual procedure (41). A similar point was raised by Farjah *et al*. noted that there was a higher hazard of death after VATS with low-volume surgeons (42). This is concerning and in our opinion likely to be under reported.

Many surgeons performing lung cancer surgery can do a safe operation with acceptable outcomes. The translation to the VATS approach however is not straightforward. Surgeons should look at their own results and outcomes. If they are equivalent (or better) to those published for VATS, they should not be under pressure to change technique. The vocal proponents of the VATS approach generally have set the baseline for the outcomes studies.

VATS lobectomy has clearly been demonstrated to be safe and oncologically effective, and more radical procedures are also being performed via this route. It is expected that uptake will increase with greater exposure during training of junior surgeons making it the standard of care in the future (43).

The extension of the multi-port approach is the single port technique pioneered initially by Rocco and now by Gonzales-Rivas (44,45).

The lobectomy performed through the uniport incision follows standard procedure with individual ligation of vascular structures and bronchus with mediastinal nodal dissection. Visualisation is entirely via the videoscope. The technique has been well described (45). Advantages include vision directed to the target tissue, similar to open surgery and reduction of post-operative pain. Uniportal VATS limited resections (wedge) can even be performed under locoregional anaesthesia in awake patients (46). The impending increase in referrals from the advent of screening programmes makes this approach important for the future. Clearly there is a learning curve and it has been suggested that surgeons already performing VATS lobectomy via the

anterior approach may adapt to this technique earlier. Once again, surgeons must evaluate their current practice and make an assessment whether such a technique would be useful to them.

Robotic surgery is an extension of the VATS minimally invasive approach. Proponents argue that there is improved operative field visualisation and better wrist-like motion of the instruments with tremor filtration (47,48). Disadvantages include the added cost associated with the robotic technology. Proponents of the VATS approach also believe that it does not add more to an already well established surgical approach. Evaluating the robotic approach in a systematic review, Cao *et al*. found that the robotic procedure was safe and effective in specialized centres. Long term efficacy data was limited however and warranted further study (49). Procedures are generally performed via 2-3 ports and a utility incision with no rib spreading, generally following the CALGB VATS technique. This approach still allows lymph node dissection. Studies have demonstrated similar operative times after the initial learning curve, reduced hospitalization and time to normal activity with some evidence for reduced post-operative pain. Equivalent oncological results to open thoracotomy have also been reported (50). The rate of conversion to open thoracotomy has been reported at 7-8% (47-52). In the largest reported series on robotic lobectomy, Park *et al*. reported an 80% overall 5-year survival with equivalent stage specific survival data compared to VATS approach. Whilst this was in a retrospective study that did not directly compare the 2 approaches, it still demonstrates the oncological efficacy of robotic lobectomy (53).

Whilst technically feasible, widespread uptake in an era of cost containment is highly unlikely. It is a nice marketing tool where competition for patients is high. The same concerns regarding learning curve, training etc. for VATS exist here but are even more pertinent (54).

How much lung is enough? The role of sub-lobar resection

Sub-lobar resections consist of anatomical segmental resection and wedge resections that are non-anatomical. Wedge resections generally have a poorer outcome compared to anatomical resection. Nakamura *et al*. reported a 55.4% 5-year survival after wedge excision, lower compared to lobectomy (82.1%) and segmental resection (87.2%) (55). It is with this in mind that the rest of this discussion will focus mainly on anatomical segmentectomy.

Segmental resections for early stage lung cancer have traditionally been reserved for patients with limited functional reserve, medical co-morbidity and for older patients. This is largely in view of the only randomized trial available comparing lobectomy to sub-lobar resection by Ginsberg *et al*. for the Lung Cancer Study Group (56). They demonstrated an inferior 5-year survival with limited resection as well as a threefold increase in local recurrence rates for tumours smaller than 3 cm confirmed to be N0 at thoracotomy. The locoregional recurrence rate per person/year was 0.044 for segmental resection and 0.086 for wedge resections. This study was limited by the low number included in the study and the unavailability of PET at this time.

Wolf *et al*. reported their experience with segmental resections compared to open lobectomy. They found a trend to increased local recurrence with shorter overall and recurrence free survival in segmental resections (57). This survival difference should be taken in context though; there were a larger number of older patients with poor lung function in the segmental group.

In a meta-analysis comparing survival to lobectomy for stage I disease, Nakamura *et al*. demonstrated better survival, albeit not statistically significant, following lobectomy. There was however considerable heterogeneity at time points 3 and 5 years after resection (58).

Interpretation of the data is difficult as there are differences in the application of segmental resection, as well as the extent of mediastinal nodal dissection at the time of resection. Tumour histology also plays a role in the outcomes with slow growing adenocarcinoma demonstrating better results.

In the absence of randomised controlled trials, Tsutani *et al*. published a propensity matched analysis limited to patients with stage IA lung adenocarcinoma (59). They excluded wedge resections and demonstrated no difference in survival and recurrence free survival in all cohorts before and after propensity matching. Of note was the fact that they included segmental resections for TIb tumours based on the standardized uptake value (SUVmax) and high resolution computerised tomography (HRCT) findings. Solid tumour size on HRCT and lower SUVmax were independent prognostic factors and tended to predict less invasive tumours that were managed by segmentectomy.

Reviewing the Surveillance, Epidemiology and End Result (SEER) database, Kates *et al*. examined the survival outcomes following lobectomy and segmentectomy for stage I tumours up to 1 cm. They noted equivalent survival and commented that segmentectomy may be preferable given the lower rate of complications. No

Table 1 Indications for sublobar resection.

Indications for sublobar resection in NSCLC
I. Peripheral tumour 2 cm or less
II. Predominant ground glass appearance on CT scan
III. Patients 75 years or older
IV. FEV1 less than 60% of predicted
V. Presence of synchronous tumours

survival difference could be demonstrated before and after propensity matching (60). Yang and D'Amico reviewed the results of thoracoscopic segmentectomy for lung cancer. The trends in the literature suggest that this approach, specifically for early stage tumours and the low-grade, ground glass opacity (GCO) cases, which if < than 3 cm are now classified as adenocarcinoma in situ (AIS), is safe and feasible (61). Zhong *et al*. demonstrated similar local recurrence and equivalent 5-year survival comparing thoracoscopic segmentectomy and lobectomy (62). The role of segmental resection is clearly being defined. Gorenstein *et al*. reviewed surgery for early stage cancer suggesting the following indications for sublobar resection (*Table 1*) (63). There are currently 2 randomised controlled trials that may clarify the role of limited resection, the CALGB 140503 (segmentectomy and wedge resection) and JCOG0802/WJOG4607L (segmentectomy). In these trials, selection for limited resection includes tumours 2 cm or less in size, peripheral tumours close to the outer third of the lung and good functional status. These results are awaited (64,65).

Lung preservation is also behind the push for sleeve resections, either bronchial, pulmonary arterial or the double sleeve resection. These are indicated for tumour involving either the origin of a lobar bronchus or lobar branch of the pulmonary artery that does not infiltrate as far as to require pneumonectomy. It allows patients who would not tolerate a pneumonectomy to undergo curative resection. D'Andrilli found that the oncological efficacy of sleeve resection is well established in stage I and II disease with some benefit in stage III over pneumonectomy. Quality of life, prognosis and morbidity were better in patients undergoing sleeve resection compared to pneumonectomy (66). Outcomes following resection and reconstruction of the pulmonary artery have been shown to be similar to standard lobectomy in selected patients (67).

Nodal dissection

There is ongoing controversy on the role of mediastinal node dissection during the resection. Nodal dissection allows accurate staging for prognostic purposes, thereby determining the need for adjuvant therapy and is necessary to ensure a complete R0 resection as defined by Rami-Porta *et al*. (68). There is good evidence of improved "stage specific" survival as the number of nodes removed increase. It also removes microscopic nodal disease that may result in local recurrence. The extent of this nodal dissection has long been the subject of discussion.

Wu *et al*. reported improved overall survival in patients undergoing systematic nodal dissection (SND) which is the only internationally standardized technique for intrathoracic nodal evaluation (69-71). The ACOSOG Z0030 trial reported no difference in survival between patients thought to have no nodal disease or non-hilar N1 disease randomised to nodal sampling or more extensive nodal dissection (72). Of note is the fact that the ACOSOG Z0030 utilised intra-operative frozen section analysis to ensure negative nodal status before randomisation. This is the practice of one of the authors (PG) as well.

In a retrospective review, Cerfolio *et al*. documented a higher rate of N2 pick-up with mediastinal nodal dissection with no impact on survival (73). This was in normal day to day surgical practice with no intra-operative frozen section.

Arguments against the routine mediastinal nodal dissection include the possibility of increased operative time or post-operative morbidity, a finding not supported by the ACOSOG Z0030. The 7th edition of TNM recommends that assessment of regional lymph node involvement be performed by the removal and subsequent pathological examination of a minimum of 6 nodes/stations, 3 from the mediastinum, including the subcarinal node (#7), and 3 from N1 zones.

Locally ablative therapy

Sub-lobar resection faces challenges from less invasive medical procedures. These include thermal ablation, either radio-frequency (RFA) or microwave, and stereotactic body radiotherapy (SBRT or stereotactic ablative radiotherapy—SABR) which has demonstrated excellent primary tumour control which some say approaches that of lobectomy (74,75).

RFA is currently utilised for medically inoperable patients with early stage tumours, either stage I or II. It has also been used to manage patients with pulmonary metastases if <5 cm. Reports on the long term benefits are limited though. In an editorial by Fernando, questions on

the role of RFA over SBRT are raised and highlight the possible deficiencies facing RFA (76).

Stereotactic body radiotherapy is mainly in patients deemed high risk for surgery. Senan *et al*. have demonstrated the efficacy of SABR for early NSCLC in medically inoperable patients (77). The data is based on progression-free survival which is a concern given the acknowledged difficulties in assessing progression after such treatment. In addition local progression may be underestimated if patients are not returned to the specialized centre for follow up. The diagnosis of malignancy was only confirmed in 31% of cases reported by Lagerwaard *et al*., a further area of concern (78). In a retrospective review of the SEER database, Fernandez *et al*. compared definitive radiation with sublobar resection in stage IA disease. The 3-year survival favoured sublobar resection in this cohort of high risk patients (79).

A phase III trial is currently underway comparing the sublobar resection with SBRT in high risk stage I disease (80). Patients will be randomised to either treatment arm but interestingly, no routine pre-operative mediastinal nodal staging will be performed which will result in some of the surgical arm being upstaged by nodal dissection. Despite this confounding factor the trial should help define the role of SBRT.

Lung cancer screening

The impact of Low-Dose CT (LDCT) screening for lung cancer will result in large numbers of patients being referred for the evaluation of nodules, many of which will not be malignant. Such evaluation requires a dedicated multidisciplinary approach if invasive investigations and resection for benign disease is to be kept at an acceptably low level. Such screening programmes will inevitably lead to an increased volume of patients with small lung cancers (1-2 cm) being presented for possible surgical resection.

The benefits of screening with low-dose CT scans are largely based on the results of The National Lung Screening Trial (NLST) published in 2011 (81). Comparing LDCT to chest radiographs, there was a 20.3% reduction in lung cancer related mortality and a 7% overall reduction in mortality. A caveat though was the false positivity rate of 95% in the NLST screening trial at prevalence screen. Surgical involvement in screening programmes is critical as it is anticipated that the number of referrals will increase.

Guidelines on intervention are currently available from the IALSC (82). Key recommendations include the use of a multidisciplinary team approach with surgery being performed in centres with minimally invasive programmes. Surgical resection, once diagnosis is confirmed, should also be anatomical by lobectomy with SND. Segmentectomy, and even wedge excision might be appropriate for (I) pure ground-glass opacities which if <3 cm with no invasive element and pure lepidic growth are now classified as "adenocarcinoma in-situ" and as such have almost 100% cure rate, and (II) screen-detected part-solid lesions <2 cm in the outer one third of the lung in whom frozen section has confirmed N0 disease and in which resection margins are checked by cytology of frozen section. The results of the JCOG and CALGB studies on segmentectomy may require us to re-evaluate these recommendations, especially as one becomes more concerned about second primaries in patients with high probability of cure from their first cancer.

Indeterminate lesions will require tissue for diagnosis, with CT-guided biopsy being encouraged. The decision to intervene will depend on the probability of lung cancer. It has been shown that lesions >20 mm have an 80% probability of being malignant. The risk of malignancy is reduced with numerous nodules (>6). Part solid (63%), non-solid (18%) and solid (7%) lesions all have varying degrees of malignancy associated with them (83).

We must await the results of ongoing trails, especially in Europe with the Dutch-Belgium randomised NELSON trial and the Danish Lung Cancer Screening Trial to see if the NLST translates across geographical regions and is cost-effective in varied health care systems (84,85).

Locally advanced disease: the role of surgery in a Multi-Modality setting

There remains significant controversy as to the role of surgery in locally advanced disease but for most surgeons resection performed as part of a multimodal therapy remains the cornerstone for any chance of cure for this group.

Stage III NSCLC represents a heterogeneous group and this is recognized by the recent American College of Chest Physicians (ACCP) clinical practice guidelines (86). Most surgeons would feel that isolated single N2 station nodal metastasis, assessed as 'resectable' should be considered for a treatment plan to include chemotherapy and surgical resection with or without thoracic radiotherapy which, whilst more contentious, is making a comeback. The order of the tri-modality therapy is variable. Neoadjuvant chemotherapy has been shown to improve survival

compared to surgery alone. Two landmark studies have compared the results of surgery alone in N2 disease versus the impact of neoadjuvant chemotherapy and surgery. Roth *et al*. showed a 5-year survival of 15% with surgery alone compared to 36% after pre-operative chemotherapy (87), and Rosell *et al*. obtained a significant overall survival advantage in the combined group (3-year survival of 15%) over surgery alone (3-year survival 0%) (88). Whilst the advantage to neoadjuvant chemotherapy in both arms is similar, an approximately 20% improvement in overall survival, the marked difference in the results of surgery alone (0% versus 15%) suggests the populations of N2 disease entered in to each trial differed significantly.

Recently, Ripley and Rusch have published an authoritative review of the role of induction therapy. After an extensive review of the current best available evidence they conclude that multimodality therapy should be standard of care for stage IIIA (N2) NSCLC, resection being offered to patients suitable for complete resection (89).

Randomised controlled trials of multimodality therapy in pre-operatively determined N2 disease, comparing regimens which included surgery with those using only chemotherapy and radiotherapy have shown the results to be similar. Arguments against the role of surgery in N2 disease cite Van Meerbeck *et al*., however, looking at the surgical group in this series, it was suggested that surgery less than pneumonectomy may provide a survival advantage (90).

Many surgical oncologists would agree however that the wide variety of findings mandates individualized assessment and treatment planning by a team experienced in lung cancer surgery. Similarly therefore, a smaller peripheral primary tumour and a single paratracheal or subcarinal metatstasis that would require a very large radiation field for radical treatment may be better treated with a multimodality approach including surgery. Finally, bulky central tumours with uncertain resectability (Likely T4) may be better treated with initial chemotherapy/chemoradiotherapy followed by surgical exploration once some cytoreduction has been achieved. However, there is concensus that bulky multi-station disease is better treated with definitive, concurrent chemoradiation. These special treatment issues are also well described in the ACCP guidelines (91).

There have been a significant number of clinical trials evaluating preoperative chemoradiation followed by surgery for locally advanced NSCLC. The most influential of these, including the SWOG 8805, German and Massachusetts General have shown increased resectablity rates, increased but acceptable perioperative morbidity and mortality with survival benefit (92-94). In the Prince Charles Hospital it is our preference to use induction chemotherapy alone and reserve surgery post chemoradiation as a salvage option only.

Oncologists in general, conclude from the EORTC 08941, that surgery does not improve survival in patients with N2 disease and therefore should not be used (95). Referral of patients with low volume N2 disease has been limited, which, in our opinion means denying these patients access to better treatment. Better local control occurred in the surgical arm and patients having an R0 resection had improved survival.

Further, the Intergroup trial 0139 showed no overall difference in survival in the surgical arm (96). There was a high mortality rate in the trial after pneumonectomy however and a clear survival advantage was present for patients having lobectomy after induction therapy, findings supporting the 'unplanned' analysis of the surgical group in the EORTC 08941. The differences in 5-year survival between the intergroup study and the EORTC are greater than can be explained by the difference between sequential and concurrent chemoradiation suggesting that the study populations were somewhat different. Weder *et al*. demonstrated that pneumonectomy can be performed with very acceptable morbidity and mortality after induction therapy (97). This further emphasizes the importance of such cases being dealt with by experienced multidisciplinary teams.

The T4 descriptor in the staging system has generally signified 'irresectable' disease. Whilst there are undoubtedly cases in which T4 cases, especially when associated with N0 of N1 disease (now stage IIIA in the 7th edition) can be resected and benefit from surgical treatment, it is difficult to support this in the literature since the case series are small and include an unspecified number of cases that were not characterized as T4 before surgery. In many cases in it difficult to be sure without exploration whether this is the case. Each of these patients needs to be assessed individually. Treatment options are between radical intent chemoradiation or surgery. Some centres may opt for surgical exploration and a 'trial dissection'. In our unit, in some cases, chemotherapy is given before exploration. Responders will undergo exploration with the aim of complete resection. In some units there is a move toward preoperative chemoradiation (98) with which there is little doubt that perioperative morbidity and mortality is higher, as is the pathologic complete response rate. Patient assessment should be in experienced units. Surgery for Pancoast tumour is well established as part of multimodality

therapy. Induction chemoradiation followed by complete surgical resection is the current standard of care.

Improvements in staging technologies have undoubtedly resulted in more patients being identified with unexpected and limited metastatic disease. The standard of care for stage IVB cases is definitive chemotherapy. Adjuvant surgery may be considered in occasional and highly selected patients with oligometastatic disease such as solitary brain and adrenal metastases. There are small series suggesting improved disease-free and overall survival in these circumstances (99,100). This radical approach would only be considered in the uncommon scenario of a resectable, node negative primary with an isolated metastatic deposit. Chemotherapy is an integral component in such cases and in our department would usually be administered after resection of the metastasis and before definitive pulmonary resection.

Adjuvant chemotherapy

Recurrence after complete resection for lung cancer is most commonly at distant sites.

There have been 4 positive adjuvant trials from 1994-2001 demonstrating a survival benefit with adjuvant chemotherapy (101-103). The greatest benefit was shown in the National Cancer Institute of Canada JBR.10, however, subset analysis showed no benefit for stage IB patients (104). These results were confirmed in the recent update of the trial results (105). The survival benefit reported in this trial was 15% at 5 years.

The Lung Adjuvant Cisplatin Evaluation (LACE) meta-analysis demonstrated a trend to benefit in stage IB and clear benefit in stage II N1 cases and IIIA mostly N2 cases disease (106).

Adjuvant chemotherapy has become the standard for resected stage II and IIIA disease, with level 1 evidence for cisplatin based chemotherapy in these patients. It has been suggested (level 2B) that high risk stage IB disease including; poorly differentiated carcinoma, vascular invasion, wedge resection and visceral pleural involvement, should also be offered treatment (101,102). In considering these results, it is worth remembering that in all these positive trials the 6th edition of the staging system was used. The benefit for Stage II was therefore in cases with N1 disease and in the stage IIIA cases with N2 disease. The CALGB 9633 trial for 6th edition stage IB was negative and only an unplanned post hoc analysis showed benefit for N0 cases 4 cm or larger. This finding in large N0 cases was not supported when Shepherd *et al.* pooled the data from the JBR 10 and CALGB 9633 trials (103). It appears clear that adjuvant chemotherapy offers benefit in node positive cases, the role in bulky but node negative cases is uncertain.

It is imperative that surgeons are familiar with this data as they are best placed to assess the suitability for adjuvant therapy. Whilst there is no conclusive data to suggest that neoadjuvant chemotherapy would be better than adjuvant chemotherapy there are reasons to think that it may be more effective (107). There is improved drug delivery to the tumour, particularly lymph nodes, better prospect of receiving the full dose of the planned regimen, earlier treatment of micrometastatic disease, and the possibility of improved resectability. For patients with bulky N1 disease requiring pneumonectomy in our institution neoadjuvant chemotherapy followed by resection is the preferred approach.

The CALGB 150803 trial is currently underway attempting to identify a subset of stage I patients that benefit from adjuvant chemotherapy using a 64-gene signature.

Small cell lung cancer

Small cell lung cancer represents 13% of newly diagnosed cases of lung cancer worldwide (108). It is common in heavy smokers, either current or previously, and is associated with early locoregional and distant spread. Treatment modalities are commonly limited to a combination of thoracic irradiation and multi-agent chemotherapy with surgery having a limited role.

Surgery was initially advocated based on the results of the Veterans Administration Surgical Oncology Group in 1982 with a 60% 5-year survival for T1N0 lesions with surgery and chemotherapy (109).

The Lung Cancer Study Group prospective trial of induction chemotherapy followed by either surgery or radiation demonstrated no survival benefit in either treatment arm, but excluded patients with stage I disease (110). It is one of the few randomised trials looking at the role of surgery.

Surgery is recommended for biopsy proven T1N0M0 disease, with adjuvant chemotherapy and prophylactic cranial irradiation (110). It may also be offered after neoadjuvant therapy (111,112). Surgery may also be offered as a salvage option to patients with relapse after remission or non-responders, Shepherd *et al.* reported a retrospective review with a 23% 5-year survival (113). There have also been selected reports of surgery for extensive disease with staging and selection being critical.

Conclusions

Surgical therapy for lung cancer has advanced since the first pneumonectomy by Evarts Graham (114). Advances in pre-operative, operative and post-operative care have revolutionized management and improved outcomes.

Multi-modality therapy, an expanding role for adjuvant therapy after complete resection and medical alternatives to surgery require that surgeons take an active role in the multidisciplinary discussions. They need to be fully conversant with the available literature and capable of strongly presenting the benefits of surgical options. The expanding use of LDCT screening will involve surgeons in the evaluation and treatment of smaller cancers which force us to re-evaluate our investigative algorithms and surgical options. Sub-lobar resections, minimally invasive strategies with earlier intervention for stage IA disease together with extending the role of surgery in advanced stages point the way forward for the thoracic surgical community.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Ferlay J, Shin HR, Bray F, et al. GLOBOCAN 2008 v2.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 10 (Internet). Lyon, France: International Agency for Research on Cancer; 2010. Available online: http://globocan.iarc.fr, accessed on 7/04/2013.
2. Australian Institute of Health and Welfare & Australasian Association of Cancer Registries 2012. Cancer in Australia: an overview, 2012. Cancer series no. 74. Cat. no. CAN 70. Canberra: AIHW.
3. Australian Institute of Health and Welfare 2012. Cancer survival and prevalence in Australia: period estimates from 1982 to 2010. Cancer series no. 69. Cat. No. CAN 65. Canberra: AIHW.
4. Drilon A, Rekhtman N, Ladanyi M, et al. Squamous-cell carcinomas of the lung: emerging biology, controversies, and the promise of targeted therapy. Lancet Oncol 2012;13:e418-26.
5. Rekhtman N, Brandt SM, Sigel CS, et al. Suitability of thoracic cytology for new therapeutic paradigms in non-small cell lung carcinoma: high accuracy of tumor subtyping and feasibility of EGFR and KRAS molecular testing. J Thorac Oncol 2011;6:451-8.
6. Koudelakova V, Kneblova M, Trojanec R, et al. Non-small cell lung cancer - genetic predictors. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2013;157:125-36.
7. Bunn PA Jr. Worldwide overview of the current status of lung cancer diagnosis and treatment. Arch Pathol Lab Med 2012;136:1478-81.
8. Ou SH, Bartlett CH, Mino-Kenudson M, et al. Crizotinib for the treatment of ALK-rearranged non-small cell lung cancer: a success story to usher in the second decade of molecular targeted therapy in oncology. Oncologist 2012;17:1351-75.
9. Travis WD, Rekhtman N. Pathological diagnosis and classification of lung cancer in small biopsies and cytology: strategic management of tissue for molecular testing. Semin Respir Crit Care Med 2011;32:22-31.
10. Mazzone P. Preoperative evaluation of the lung resection candidate. Cleve Clin J Med 2012;79 Electronic Suppl 1:eS17-22.
11. Gorenstein LA, Sonett JR. The surgical management of stage I and stage II lung cancer. Surg Oncol Clin N Am 2011;20:701-20.
12. Wright G, Manser RL, Byrnes G, et al. Surgery for non-small cell lung cancer: systematic review and meta-analysis of randomised controlled trials. Thorax 2006;61:597-603.
13. Gospodarowicz MK, O'Sullivan B, Koh ES. Prognostic factors: Principles and applications. In: Peter Goldstraw. eds. IASLC Manual - Staging Manual in Thoracic Oncology. Orange Park, FL, USA: Editorial Rx Press, 2009:111-28.
14. Daniels AC. A method of biopsy useful in diagnosing certain intrathoracic diseases. Dis Chest 1949;16:360-7.
15. Carlens E. Mediastinoscopy: a method for inspection and tissue biopsy in the superior mediastinum. Dis Chest 1959;36:343-52.
16. McNeill TM, Chamberlain JM. Diagnostic anterior mediastinotomy. Ann Thorac Surg 1966;2:532-9.
17. Little AG, Rusch VW, Bonner JA, et al. Patterns of surgical care of lung cancer patients. Ann Thorac Surg 2005;80:2051-6; discussion 2056.
18. Silvestri GA, Gould MK, Margolis ML, et al. Noninvasive staging of non-small cell lung cancer: ACCP evidenced-based clinical practice guidelines (2nd edition). Chest 2007;132:178S-201S.
19. Fielding D, Windsor M. Endobronchial ultrasound convex-probe transbronchial needle aspiration as the first diagnostic test in patients with pulmonary masses and associated hilar or mediastinal nodes. Intern Med J 2009;39:435-40.

20. Yasufuku K, Pierre A, Darling G, et al. A prospective controlled trial of endobronchial ultrasound-guided transbronchial needle aspiration compared with mediastinoscopy for mediastinal lymph node staging of lung cancer. J Thorac Cardiovasc Surg 2011;142:1393-400.e1.
21. Micames CG, McCrory DC, Pavey DA, et al. Endoscopic ultrasound-guided fine-needle aspiration for non-small cell lung cancer staging: A systematic review and metaanalysis. Chest 2007;131:539-48.
22. Herth FJ, Lunn W, Eberhardt R, et al. Transbronchial versus transesophageal ultrasound-guided aspiration of enlarged mediastinal lymph nodes. Am J Respir Crit Care Med 2005;171:1164-7.
23. Hürtgen M, Friedel G, Toomes H, et al. Radical video-assisted mediastinoscopic lymphadenectomy (VAMLA)--technique and first results. Eur J Cardiothorac Surg 2002;21:348-51.
24. Colice GL, Shafazand S, Griffin JP, et al. Physiologic evaluation of the patient with lung cancer being considered for resectional surgery: ACCP evidenced-based clinical practice guidelines (2nd edition). Chest 2007;132:161S-77S.
25. Brunelli A, Charloux A, Bolliger CT, et al. ERS/ESTS clinical guidelines on fitness for radical therapy in lung cancer patients (surgery and chemo-radiotherapy). Eur Respir J 2009;34:17-41.
26. Lim E, Baldwin D, Beckles M, et al. Guidelines on the radical management of patients with lung cancer. Thorax 2010;65 Suppl 3:iii1-27.
27. Lim E, Beckles M, Warburton C, et al. Cardiopulmonary exercise testing for the selection of patients undergoing surgery for lung cancer: friend or foe? Thorax 2010;65:847-9.
28. Riaz SP, Linklater KM, Page R, et al. Recent trends in resection rates among non-small cell lung cancer patients in England. Thorax 2012;67:811-4.
29. Riquet M, Berna P, Fabre E, et al. Evolving characteristics of lung cancer: a surgical appraisal. Eur J Cardiothorac Surg 2012;41:1019-24.
30. Yan TD, Black D, Bannon PG, et al. Systematic review and meta-analysis of randomized and nonrandomized trials on safety and efficacy of video-assisted thoracic surgery lobectomy for early-stage non-small-cell lung cancer. J Clin Oncol 2009;27:2553-62.
31. Cao C, Manganas C, Ang SC, et al. A meta-analysis of unmatched and matched patients comparing video-assisted thoracoscopic lobectomy and conventional open lobectomy. Ann Cardiothorac Surg 2012;1:16-23.
32. Yim AP, Wan S, Lee TW, et al. VATS lobectomy reduces cytokine responses compared with conventional surgery. Ann Thorac Surg 2000;70:243-7.
33. Swanson SJ, Herndon JE 2nd, D'Amico TA, et al. Video-assisted thoracic surgery lobectomy: report of CALGB 39802--a prospective, multi-institution feasibility study. J Clin Oncol 2007;25:4993-7.
34. Zhang Z, Zhang Y, Feng H, et al. Is video-assisted thoracic surgery lobectomy better than thoracotomy for early-stage non-small-cell lung cancer? A systematic review and meta-analysis. Eur J Cardiothorac Surg 2013;44:407-14.
35. Yamashita S, Tokuishi K, Moroga T, et al. Totally thoracoscopic surgery and troubleshooting for bleeding in non-small cell lung cancer. Ann Thorac Surg 2013;95:994-9.
36. Hanna WC, de Valence M, Atenafu EG, et al. Is video-assisted lobectomy for non-small-cell lung cancer oncologically equivalent to open lobectomy? Eur J Cardiothorac Surg 2013;43:1121-5.
37. Taioli E, Lee DS, Lesser M, et al. Long-term survival in video-assisted thoracoscopic lobectomy vs open lobectomy in lung-cancer patients: a meta-analysis. Eur J Cardiothorac Surg 2013;44:591-7.
38. Kuritzky AM, Ryder BA, Ng T. Long-term survival outcomes of Video-assisted Thoracic Surgery (VATS) lobectomy after transitioning from open lobectomy. Ann Surg Oncol 2013;20:2734-40.
39. Paul S, Altorki NK, Sheng S, et al. Thoracoscopic lobectomy is associated with lower morbidity than open lobectomy: a propensity-matched analysis from the STS database. J Thorac Cardiovasc Surg 2010;139:366-78.
40. Paul S, Sedrakyan A, Chiu YL, et al. Outcomes after lobectomy using thoracoscopy vs thoracotomy: a comparative effectiveness analysis utilizing the Nationwide Inpatient Sample database. Eur J Cardiothorac Surg 2013;43:813-7.
41. Wood DE. What is most important in improving outcomes after pulmonary lobectomy: the surgeon or the approach? Eur J Cardiothorac Surg 2013;43:817-9.
42. Farjah F, Flum DR, Varghese TK Jr, et al. Surgeon specialty and long-term survival after pulmonary resection for lung cancer. Ann Thorac Surg 2009;87:995-1004; discussion 1005-6.
43. Puri V, Meyers BF. Video-assisted thoracoscopic surgery lobectomy for lung cancer. Surg Oncol Clin N Am 2013;22:27-38, v.
44. Rocco G. One-port (uniportal) video-assisted thoracic surgical resections-- a clear advance. J Thorac Cardiovasc

Surg 2012;144:S27-31.
45. Gonzalez-Rivas D. VATS lobectomy: surgical evolution from conventional VATS to uniportal approach. ScientificWorldJournal 2012;2012:780842.
46. Rocco G, Romano V, Accardo R, et al. Awake single-access (uniportal) video-assisted thoracoscopic surgery for peripheral pulmonary nodules in a complete ambulatory setting. Ann Thorac Surg 2010;89:1625-7.
47. Park BJ, Flores RM, Rusch VW. Robotic assistance for video-assisted thoracic surgical lobectomy: technique and initial results. J Thorac Cardiovasc Surg 2006;131:54-9.
48. Veronesi G, Galetta D, Maisonneuve P, et al. Four-arm robotic lobectomy for the treatment of early-stage lung cancer. J Thorac Cardiovasc Surg 2010;140:19-25.
49. Cao C, Manganas C, Ang SC, et al. A systematic review and meta-analysis on pulmonary resections by robotic video-assisted thoracic surgery. Ann Cardiothorac Surg 2012;1:3-10.
50. Park BJ, Melfi F, Mussi A, et al. Robotic lobectomy for non-small cell lung cancer (NSCLC): long-term oncologic results. J Thorac Cardiovasc Surg 2012;143:383-9.
51. Louie BE, Farivar AS, Aye RW, et al. Early experience with robotic lung resection results in similar operative outcomes and morbidity when compared with matched video-assisted thoracoscopic surgery cases. Ann Thorac Surg 2012;93:1598-604; discussion 1604-5.
52. Cerfolio RJ, Bryant AS, Skylizard L, et al. Initial consecutive experience of completely portal robotic pulmonary resection with 4 arms. J Thorac Cardiovasc Surg 2011;142:740-6.
53. Park BJ. Robotic lobectomy for non-small cell lung cancer (NSCLC): Multi-center registry study of long-term oncologic results. Ann Cardiothorac Surg 2012;1:24-6.
54. Swanson SJ. Robotic pulmonary lobectomy--the future and probably should remain so. J Thorac Cardiovasc Surg 2010;140:954.
55. Nakamura H, Taniguchi Y, Miwa K, et al. Comparison of the surgical outcomes of thoracoscopic lobectomy, segmentectomy, and wedge resection for clinical stage I non-small cell lung cancer. Thorac Cardiovasc Surg 2011;59:137-41.
56. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
57. Wolf AS, Richards WG, Jaklitsch MT, et al. Lobectomy versus sublobar resection for small (2 cm or less) non-small cell lung cancers. Ann Thorac Surg 2011;92:1819-23; discussion 1824-5.
58. Nakamura H, Kawasaki N, Taguchi M, et al. Survival following lobectomy vs limited resection for stage I lung cancer: a meta-analysis. Br J Cancer 2005;92:1033-7.
59. Tsutani Y, Miyata Y, Nakayama H, et al. Oncologic outcomes of segmentectomy compared with lobectomy for clinical stage IA lung adenocarcinoma: propensity score-matched analysis in a multicenter study. J Thorac Cardiovasc Surg 2013;146:358-64.
60. Kates M, Swanson S, Wisnivesky JP. Survival following lobectomy and limited resection for the treatment of stage I non-small cell lung cancer<=1 cm in size: a review of SEER data. Chest 2011;139:491-6.
61. Yang CF, D'Amico TA. Thoracoscopic segmentectomy for lung cancer. Ann Thorac Surg 2012;94:668-81.
62. Zhong C, Fang W, Mao T, et al. Comparison of thoracoscopic segmentectomy and thoracoscopic lobectomy for small-sized stage IA lung cancer. Ann Thorac Surg 2012;94:362-7.
63. Gorenstein LA, Sonett JR. The surgical management of stage I and stage II lung cancer. Surg Oncol Clin N Am 2011;20:701-20.
64. CALGB 140503. A phase III randomised trial of lobectomy versus sublobar resection for small (≤2 cm) peripheral non-small cell lung cancer. Available online: http://www.calgb.org/public/Meetings/presentations/2007/cra_ws/03-140501-Altorki062007.pdf, accessed April 22,2013.
65. Nakamura K, Saji H, Nakajima R, et al. A phase III randomized trial of lobectomy versus limited resection for small-sized peripheral non-small cell lung cancer (JCOG0802/WJOG4607L). Jpn J Clin Oncol 2010;40:271-4.
66. D'Andrilli A, Venuta F, Menna C, et al. Extensive resections: pancoast tumors, chest wall resections, en bloc vascular resections. Surg Oncol Clin N Am 2011;20:733-56.
67. Rendina EA, Venuta F, De Giacomo T, et al. Sleeve resection and prosthetic reconstruction of the pulmonary artery for lung cancer. Ann Thorac Surg 1999;68:995-1001; discussion 1001-2.
68. Rami-Porta R, Wittekind C, Goldstraw P, et al. Complete resection in lung cancer surgery: proposed definition. Lung Cancer 2005;49:25-33.
69. Wu Yl, Huang ZF, Wang SY, et al. A randomized trial of systematic nodal dissection in resectable non-small cell lung cancer. Lung Cancer 2002;36:1-6.
70. Graham AN, Chan KJ, Pastorino U, et al. Systematic nodal dissection in the intrathoracic staging of patients with non-small cell lung cancer. J Thorac Cardiovasc Surg

1999;117:246-51.
71. Nicholson AG, Graham AN, Pezzella F, et al. Does the use of immunohistochemistry to identify micrometastases provide useful information in the staging of node-negative non-small cell lung carcinomas? Lung Cancer 1997;18:231-40.
72. Darling GE, Allen MS, Decker P, et al. Randomised trial of mediastinal lymph node sampling versus complete lymphadenectomy during pulmonary resection in patients with N0 or N1 (less than hilar) non-small cell carcinoma:results of the ACOSOG Z0030 trial. AATS Annual Meeting. Toronto: Canada, 2010.
73. Cerfolio RJ, Bryant AS, Minnich DJ. Complete thoracic mediastinal lymphadenectomy leads to a higher rate of pathologically proven N2 disease in patients with non-small cell lung cancer. Ann Thorac Surg 2012;94:902-6.
74. Onishi H, Shirato H, Nagata Y, et al. Hypofractionated stereotactic radiotherapy (HypoFXSRT) for stage I non-small cell lung cancer: updated results of 257 patients in a Japanese multi-institutional study. J Thorac Oncol 2007;2:S94-100.
75. Nagata Y, Takayama K, Matsuo Y, et al. Clinical outcomes of a phase I/II study of 48 Gy of stereotactic body radiotherapy in 4 fractions for primary lung cancer using a stereotactic body frame. Int J Radiat Oncol Biol Phys 2005;63:1427-31.
76. Fernando HC. The present and future of thermal ablation for lung cancer. Eur J Cardiothorac Surg 2013;43:687.
77. Senan S, Palma DA, Lagerwaard FJ. Stereotactic ablative radiotherapy for stage I NSCLC: Recent advances and controversies. J Thorac Dis 2011;3:189-96.
78. Lagerwaard FJ, Haasbeek CJ, Smit EF, et al. Outcomes of risk-adapted fractionated stereotactic radiotherapy for stage I non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2008;70:685-92.
79. Fernandez FG, Crabtree TD, Liu J, et al. Sublobar resection versus definitive radiation in patients with stage IA non-small cell lung cancer. Ann Thorac Surg 2012;94:354-60; discussion 360-1.
80. Fernando HC, Timmerman R. American College of Surgeons Oncology Group Z4099/Radiation Therapy Oncology Group 1021: a randomized study of sublobar resection compared with stereotactic body radiotherapy for high-risk stage I non-small cell lung cancer. J Thorac Cardiovasc Surg 2012;144:S35-8.
81. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395-409.
82. Field JK, Smith RA, Aberle DR, et al. International Association for the Study of Lung Cancer Computed Tomography Screening Workshop 2011 report. J Thorac Oncol 2012;7:10-9.
83. Stiles BM, Altorki NK. Screening for lung cancer: challenges for the thoracic surgeon. Surg Oncol Clin N Am 2011;20:619-35.
84. van Klaveren RJ, Oudkerk M, Prokop M, et al. Management of lung nodules detected by volume CT scanning. N Engl J Med 2009;361:2221-9.
85. Pedersen JH, Ashraf H, Dirksen A, et al. The Danish randomized lung cancer CT screening trial--overall design and results of the prevalence round. J Thorac Oncol 2009;4:608-14.
86. Ramnath N, Dilling TJ, Harris LJ, et al. Treatment of stage III non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e314S-40S.
87. Roth JA, Fossella F, Komaki R, et al. A randomized trial comparing perioperative chemotherapy and surgery with surgery alone in resectable stage IIIA non-small-cell lung cancer. J Natl Cancer Inst 1994;86:673-80.
88. Rosell R, Gómez-Codina J, Camps C, et al. A randomized trial comparing preoperative chemotherapy plus surgery with surgery alone in patients with non-small-cell lung cancer. N Engl J Med 1994;330:153-8.
89. Ripley RT, Rusch VW. Role of induction therapy: surgical resection of non-small cell lung cancer after induction therapy. Thorac Surg Clin 2013;23:273-85.
90. van Meerbeeck JP, Kramer GW, Van Schil PE, et al. Randomized controlled trial of resection versus radiotherapy after induction chemotherapy in stage IIIA-N2 non-small-cell lung cancer. J Natl Cancer Inst 2007;99:442-50.
91. Kozower BD, Larner JM, Detterbeck FC, et al. Special treatment issues in non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e369S-99S.
92. Albain KS, Rusch VW, Crowley JJ, et al. Concurrent cisplatin/etoposide plus chest radiotherapy followed by surgery for stages IIIA (N2) and IIIB non-small-cell lung cancer: mature results of Southwest Oncology Group phase II study 8805. J Clin Oncol 1995;13:1880-92.
93. Choi NC, Carey RW, Daly W, et al. Potential impact on survival of improved tumor downstaging and resection

rate by preoperative twice-daily radiation and concurrent chemotherapy in stage IIIA non-small-cell lung cancer. J Clin Oncol 1997;15:712-22.
94. Eberhardt WE, Albain KS, Pass H, et al. Induction treatment before surgery for non-small cell lung cancer. Lung Cancer 2003;42 Suppl 1:S9-14.
95. Splinter TA, van Schil PE, Kramer GW, et al. Randomized trial of surgery versus radiotherapy in patients with stage IIIA (N2) non small-cell lung cancer after a response to induction chemotherapy. EORTC 08941. Clin Lung Cancer 2000;2:69-72; discussion 73.
96. Uy KL, Darling G, Xu W, et al. Improved results of induction chemoradiation before surgical intervention for selected patients with stage IIIA-N2 non-small cell lung cancer. J Thorac Cardiovasc Surg 2007;134:188-93.
97. Weder W, Collaud S, Eberhardt WE, et al. Pneumonectomy is a valuable treatment option after neoadjuvant therapy for stage III non-small-cell lung cancer. J Thorac Cardiovasc Surg 2010;139:1424-30.
98. D'Angelillo RM, Trodella L, Ciresa M, et al. Multimodality treatment of stage III non-small cell lung cancer: analysis of a phase II trial using preoperative cisplatin and gemcitabine with concurrent radiotherapy. J Thorac Oncol 2009;4:1517-23.
99. Bindal AK, Bindal RK, Hess KR, et al. Surgery versus radiosurgery in the treatment of brain metastasis. J Neurosurg 1996;84:748-54.
100. Louie AV, Rodrigues G, Yaremko B, et al. Management and prognosis in synchronous solitary resected brain metastasis from non-small-cell lung cancer. Clin Lung Cancer 2009;10:174-9.
101. Coleman MH, Bueno R. Role of adjuvant chemotherapy in NSCLC (stages I to III). Surg Oncol Clin N Am 2011;20:757-67.
102. Reungwetwattana T, Eadens MJ, Molina JR. Chemotherapy for non-small-cell lung carcinoma: from a blanket approach to individual therapy. Semin Respir Crit Care Med 2011;32:78-93.
103. Cuffe S, Bourredjem A, Graziano S, et al. A pooled exploratory analysis of the effect of tumor size and KRAS mutations on survival benefit from adjuvant platinum-based chemotherapy in node-negative non-small cell lung cancer. J Thorac Oncol 2012;7:963-72.
104. Pepe C, Hasan B, Winton TL, et al. Adjuvant vinorelbine and cisplatin in elderly patients: National Cancer Institute of Canada and Intergroup Study JBR.10. J Clin Oncol 2007;25:1553-61.
105. Butts CA, Ding K, Seymour L, et al. Randomized phase III trial of vinorelbine plus cisplatin compared with observation in completely resected stage IB and II non-small-cell lung cancer: updated survival analysis of JBR-10. J Clin Oncol 2010;28:29-34.
106. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
107. Felip E, Rosell R, Maestre JA, et al. Preoperative chemotherapy plus surgery versus surgery plus adjuvant chemotherapy versus surgery alone in early-stage non-small-cell lung cancer. J Clin Oncol 2010;28:3138-45.
108. Shields TW, Higgins GA Jr, Matthews MJ, et al. Surgical resection in the management of small cell carcinoma of the lung. J Thorac Cardiovasc Surg 1982;84:481-8.
109. Lad T, Piantadosi S, Thomas P, et al. A prospective randomized trial to determine the benefit of surgical resection of residual disease following response of small cell lung cancer to combination chemotherapy. Chest 1994;106:320S-323S.
110. Goldstein SD, Yang SC. Role of surgery in small cell lung cancer. Surg Oncol Clin N Am 2011;20:769-77.
111. Badzio A, Kurowski K, Karnicka-Mlodkowska H, et al. A retrospective comparative study of surgery followed by chemotherapy vs. non-surgical management in limited-disease small cell lung cancer. Eur J Cardiothorac Surg 2004;26:183-8.
112. Brock MV, Hooker CM, Syphard JE, et al. Surgical resection of limited disease small cell lung cancer in the new era of platinum chemotherapy: Its time has come. J Thorac Cardiovasc Surg 2005;129:64-72.
113. Shepherd FA, Ginsberg R, Patterson GA, et al. Is there ever a role for salvage operations in limited small-cell lung cancer? J Thorac Cardiovasc Surg 1991;101:196-200.
114. Graham EA. The first total pneumonectomy. Tex Cancer Bull 1949;2:2-4.

Cite this article as: Naidoo R, Windsor MN, Goldstraw P. Surgery in 2013 and beyond. J Thorac Dis 2013;5(S5):S593-S606. doi: 10.3978/j.issn.2072-1439.2013.07.39

Management of ground-glass opacities: should all pulmonary lesions with ground-glass opacity be surgically resected?

Yoshihisa Kobayashi[1], Tetsuya Mitsudomi[2]

[1]Department of Thoracic Surgery, Aichi Cancer Center Hospital, Nagoya, Japan; [2]Department of Surgery, Division of Thoracic Surgery, Kinki University Faculty of Medicine, Osaka-Sayama, Japan

Correspondence to: Tetsuya Mitsudomi, MD. Department of Thoracic Surgery, Kinki University Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, 589-8511, Japan. Email: mitsudom@surg.med.kindai.ac.jp.

Abstract: Pulmonary nodules with ground-glass opacity (GGO) are frequently observed and will be increasingly detected. GGO can be observed in both benign and malignant conditions, including lung cancer and its preinvasive lesions. Atypical adenomatous hyperplasia and adenocarcinoma in situ are typically manifested as pure GGOs, whereas more advanced adenocarcinomas may include a larger solid component within the GGO region. The natural history of GGOs has been gradually clarified. Approximately 20% of pure GGOs and 40% of part-solid GGOs gradually grow or increase their solid component, whereas others remain unchanged for years. Therefore, it remains unclear whether all pulmonary lesions with GGO should be surgically resected or whether lesions without changes may not require resection. To distinguish GGOs with growth from those without growth, a 3-year follow-up observation period is a reasonable benchmark based on the data that the volume-doubling time (VDT) of pure GGOs ranges from approximately 600 to 900 days and that of part-solid GGOs ranges from 300 to 450 days. Future studies on the genetic differences between GGOs with growth and those without growth will help establish an appropriate management algorithm.

Keywords: Follow-up; ground-glass opacity (GGO); limited surgery; lung cancer; small lung lesion; volume doubling time (VDT)

Submitted Jul 30, 2013. Accepted for publication Sep 24, 2013.
doi: 10.3978/j.issn.2218-6751.2013.09.03
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.09.03

Introduction

Ground-glass opacity (GGO) is a radiological finding in computed tomography (CT) consisting of a hazy opacity that does not obscure the underlying bronchial structures or pulmonary vessels (1). Pure GGOs are those with no solid components, whereas part-solid GGOs contain both GGO and a solid component. Pulmonary nodules with GGO have been increasingly encountered in routine clinical practice with the increasingly widespread use of CT and the increased resolution of CT imaging. The recent positive results of the National Lung Screening Trial, which reported a 20% decrease in mortality from lung cancer as a result of low-dose CT screening for patients at high risk of developing lung cancer (2), are anticipated to support the use of CT examinations and to increase the detection of GGO lesions.

GGO can be a manifestation of a wide variety of clinical features, including malignancies and benign conditions, such as focal interstitial fibrosis, inflammation, and hemorrhage (3). However, lesions with GGO that do not disappear are often lung cancer or its precursor lesions (4). Favorable prognoses for the surgical resection of lesions with a considerable amount of GGO have been reported in several retrospective studies, in which the relapse rate was reported to be null (5-8).

Because some lesions with GGO remain unchanged for years, it is unclear whether all such lesions should be surgically resected, including those that microscopy shows

to contain cancer cells. It has also not yet been established which surgical procedures are well-balanced. In this article, we review the literature on GGO, with special emphasis on management of GGO-predominant pulmonary lesions.

Pathological features of lesions with GGO

Noguchi's classification

In 1995, Noguchi *et al.* reviewed 236 surgically resected small peripheral adenocarcinomas ≤2 cm in diameter and proposed a histologic classification of 6 types based on tumor growth patterns (9). Type A, localized bronchioloalveolar carcinoma (BAC), revealed the replacement of alveolar-lining epithelial cells with a relatively thin stroma. Type B was characterized by localized BAC with focal structural collapse of alveoli. Type C was characterized by localized BAC with foci of active fibroblastic proliferation. Type D (poorly differentiated adenocarcinoma), Type E (tubular adenocarcinoma) and Type F (papillary adenocarcinoma) showed compressive and expanding growth. Types A and B showed no lymph node metastasis and had a better 5-year survival rate (100%) than did Type C (75%) or Types D, E, and F (52%). According to Noguchi's classification, GGO can be found in Type A, B and C tumors that show a replacement growth pattern along the alveolar lining cells; for example, Yang *et al.* reported that the proportion of GGO in each of these tumor types was 92%, 52%, and 20%, respectively (10).

New international multidisciplinary classification of lung adenocarcinoma

In 2011, the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) proposed a new international multidisciplinary classification of lung adenocarcinoma (11). The terms BAC and mixed subtype adenocarcinoma are no longer used because these terms were applied to a broad spectrum of tumors. Adenocarcinomas are classified as preinvasive lesions [including atypical adenomatous hyperplasia (AAH) and adenocarcinoma in situ (AIS)], minimally invasive adenocarcinoma (MIA), and invasive adenocarcinoma. AAH is a localized small proliferation of atypical Type II pneumocytes and/or Clara cells lining the alveolar walls and respiratory bronchioles. AIS is a small (≤3 cm) solitary adenocarcinoma with pure lepidic growth, and the complete resection of AIS achieves 100% disease-specific survival. AIS corresponds to Types A and B in Noguchi's classification. MIA is a small (≤3 cm) solitary adenocarcinoma with a predominantly lepidic pattern and ≤5 mm invasion at the largest dimension. MIA does not invade lymphatics, blood vessels, or the pleura and contains no necrosis; therefore, complete resection achieves nearly 100% disease-specific survival. MIA roughly coincides with Type C in Noguchi's classification. In general, lung adenocarcinomas are thought to follow a linear multistep progression whereby AAH progresses to AIS, which is followed by invasive adenocarcinoma.

To discuss the association between the radiological findings of GGO and the pathological diagnosis based on the new IASLC/ATS/ERS classification, we present the updated data from our previous study on lesions with GGO. The inclusion criteria for the study were the following: (I) a lesion diameter ≤3 cm; (II) a GGO proportion >50%; and (III) observation without treatment in the prior 6 months (12). To date, 32 of the 120 lesions were surgically resected. The histological diagnoses were AAH in 3 lesions, AIS in 12, MIA in 11, and invasive adenocarcinoma in 6.

The correlation between the changes in size and the histological types is shown in *Figure 1*. None of the 3 AAHs increased in size, whereas some of the tumors belonging to the types other than AAH did so. From these observations, it is impossible to determine histopathologic types by changes in lesion size.

The association between the radiological findings at the time of the resection and the pathological types is shown in *Figure 2*. The solid component proportions were categorized as 0%, 1-25%, 26-50%, and 51-100%. Preinvasive lesions, including AAH and AIS, are typically manifested as pure GGOs, whereas more advanced adenocarcinomas may include a larger solid component within the GGO region.

Genetic features of lesions with GGO

Several reports have examined the relationship between pulmonary nodules with GGO and the relatively high frequency of epidermal growth factor receptor (EGFR) mutations. In a study of 38 patients with adenocarcinoma, the frequencies of GGO in patients with EGFR mutation and wild-types were 74% and 57%, respectively (13). In another study of 153 patients with adenocarcinoma, the GGO volume percentage in tumors with exon 21 mutation (61.7%±31.9%)

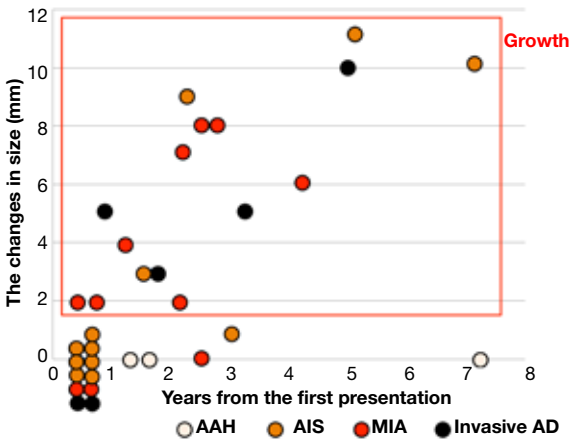


Figure 1 The correlation between the changes in size from the first presentation to the last CT examination and the histological types. AAH existed only in the no-growth group, whereas the remaining histological types existed in both groups.

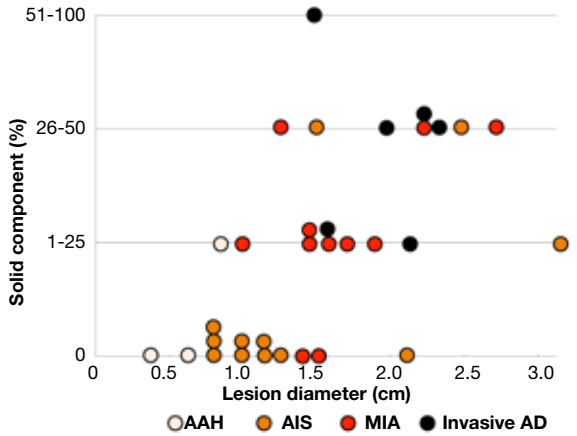


Figure 2 The association between the radiological findings at the time of resection and the pathological types. Solid component proportions are categorized as 0%, 1-25%, 26-50%, and 51-100%. The tendency toward pathological invasiveness is shown, along with the consistent increases in both the size and the solid component.

was significantly higher than that in EGFR wild-type tumors (30.0%±38.5%) (14). However, the frequencies of EGFR mutation did not significantly differ (25%, 36%, 86%, to 67% in AAH, AIS, MIA, and well-differentiated adenocarcinomas, respectively) (15). Both GGO and EGFR mutations are associated with adenocarcinoma histology, female gender, and nonsmoking status.

In comparison, the incidence of KRAS mutations was 33%, 12%, 8%, and 0% in AAH, AIS, MIA, and well-differentiated adenocarcinomas, respectively, in one report (15). The overall frequency of KRAS mutations in lung adenocarcinoma was limited to 13% (16). These findings cannot be explained without assuming that some tumors with KRAS mutations might undergo regression.

The association between radiological findings of GGO and pathological invasiveness

The accuracy rate of a CT-guided core needle biopsy for nodules with GGO depends on the lesion diameter and the proportion of the GGO component; it ranges from 64.6% to 93% (17-19). Recent CT fluoroscopy-guided biopsy has a higher accuracy rate ranging from 82% to 97% (20-22). Of course, we should interpret these results in light of a possible publication bias. The article on the new IASLC/ATS/ERS classification states that AIS and MIA should not be diagnosed in small biopsies or cytology specimens and that if a noninvasive pattern is present in a small biopsy, it should be referred to as a lepidic growth pattern (11). Therefore, diagnosis usually depends on radiographic findings, which correlate closely with the pathologic diagnosis in the determination of treatment options, including surgery.

A GGO proportion of 50% or more is suggested as a cutoff value for pathological noninvasiveness in each lesion size category (*Table 1*) (23-28). In lesions ≤3 cm with a GGO component <50%, the rate of lymph node metastasis ranges from 10% to 26% (23-28). Based on these data, in this article, we mainly address pulmonary nodules with GGO proportion >50%.

When pathological invasiveness is defined as the presence of vascular and lymphatic invasion and lymph node metastasis, the specificity of pathological invasiveness was 100% if the cut-off value was set as a consolidation/maximum tumor diameter (C/T) ratio of ≤0.5 for lesions ≤3 cm (29). There has only been one multi-institutional prospective study to predict pathological noninvasiveness. Based on the analysis of 545 patients, Suzuki *et al.* reported that the specificities for the diagnosis of pathological invasiveness were 96.4% for an adenocarcinoma ≤3 cm with a C/T ratio ≤0.5 and 98.7% for an adenocarcinoma ≤2 cm with a C/T ratio ≤0.25 (30). They concluded that radiological diagnosis of noninvasive lung cancer corresponded well with pathological invasiveness, and radiological noninvasive lung adenocarcinoma could be

Table 1 The association between GGO proportion and pathological invasiveness

First author [year] (references)	Lesion size (cm)	GGO proportion (%)	Total number	LN metastasis [%]	ly	v	pl
Asamua [2003] (23)	≤1	≥50	28	0	2*		–
		<50	20	3 [15]	7*		–
Ikeda [2004] (24)	≤2	≥50	44	0	–	–	–
		<50	115	12 [10]	–	–	–
Suzuki [2006] (25)	≤2	≥50	116	1 [0.9]	2	2	3
		<50	233	46 [20]	94	91	52
Aoki [2001] (26)	≤3	>50	24	1 [4]	–	3	–
		≤50	103	24 [23]	–	49	–
Matsuguma [2002] (27)	≤3	>50	26	0	0	1	–
		≤50	70	18 [26]	18	22	–
Nakata [2005] (28)	≤3	≥50	68	0	–	1	–
		<50	78	16 [21]	–	46	–

LN, lymph node; ly, lymphatic invasion; v, vascular invasion; pl, pleural invasion; *, lymphatic invasion or vascular invasion.

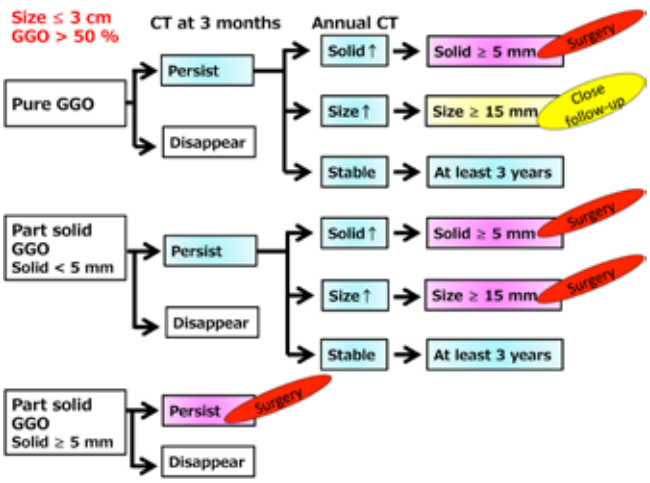


Figure 3 Conservative follow-up algorithm for pulmonary lesions ≤3 cm with a GGO component >50%. Currently, lesions a with solid component ≥5mm are recommended for resection. Pure GGOs ≥15 mm should be closely followed because of the tendency to grow. Part-solid GGOs ≥15 mm should be resected even if the solid component is <5 mm. All of the lesions without changes in the size and solid component should be followed for at least 3 years to accurately evaluate the tendency to grow.

defined as an adenocarcinoma ≤2 cm with a C/T ratio ≤0.25.

Appropriate timing for the decision to surgically resect

Because GGO-predominant lesions include malignancies, we must decide whether to resect at the first presentation. If the lesions were conservatively observed with CT examinations, we must decide when to resect them.

Recently, the Fleischner Society proposed recommendations for the management of GGOs (31). Briefly, they suggested that biopsy or surgical resection should be considered if the solid component becomes 5 mm or more.

The Japanese Society of CT Screening recommends that lesions with GGO ≥15 mm or a solid component ≥5 mm should be resected or biopsied (32).

Considering the Fleischner Society and the Japanese Society of CT Screening recommendations, we propose a conservative follow-up algorithm for pulmonary lesions ≤3 cm with a GGO component >50%, as illustrated in *Figure 3*.

Observation with CT examinations for lesions with GGO

Natural history of GGO

It is essential to understand the natural history of GGOs to discuss the conservative follow-up of GGO. Several reports have revealed that some lesions with GGO exhibit gradual growth, whereas others persist for years without changes (33-36). Representative CT images are presented in *Figure 4*. Recently, 5 reports analyzing more than 100 nodules with GGO have been published, and the results are summarized in *Table 2* (12,37-40). Our study is among these reports, and our results are further illustrated in *Figure 5* (12). Although the inclusion criteria and the definition of growth are

Table 2 Natural history of GGO based on more than 100 lesions

First author [year] (references)	Inclusion criteria			Patients	Lesions	Follow-up time (years)	With growth [n, %]
	Size	GGO proportion	Follow-up period				
Hiramatsu [2008] (37)	–	Any	≥3 months	125	125	2.9[a]	26[c] [21]
Matsuguma [2013] (38)	≤2 cm	>20%	–	171	174	2.4[a]	41[d] [24]
Chang [2013] (39)	–	100%	>2 years	89	122	4.9[b]	12[e] [10]
Lee [2013] (40)	–	Any	>2 years	114	175	3.8[b]	46[e] [26]
Kobayashi [2013] (12)	≤3 cm	≥50%	≥6 months	61	108	4.2[b]	29[e] [27]

[a], mean; [b], median; [c], growth was defined as ≥2 mm increase in whole GGO size, ≥2 mm increase in the solid component, or emerging new solid part of any size; [d], growth was defined as ≥2 mm increase in whole GGO size, ≥2 mm increase in the solid component, or emerging new solid part ≥2 mm; [e], growth was defined as ≥2 mm increase in whole GGO size.

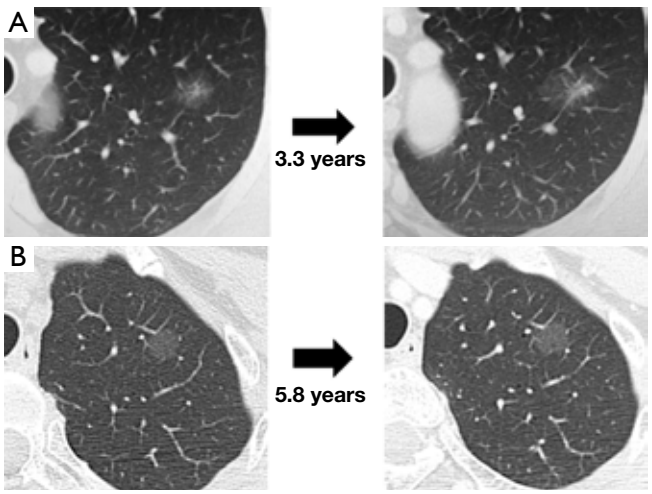


Figure 4 Computed tomography images of two representative pulmonary nodules with GGOs. A, A part-solid GGO lesion became larger, and its solid component increased after 3.3 years. B, A pure GGO lesion persisted without changing in size for 5.8 years.

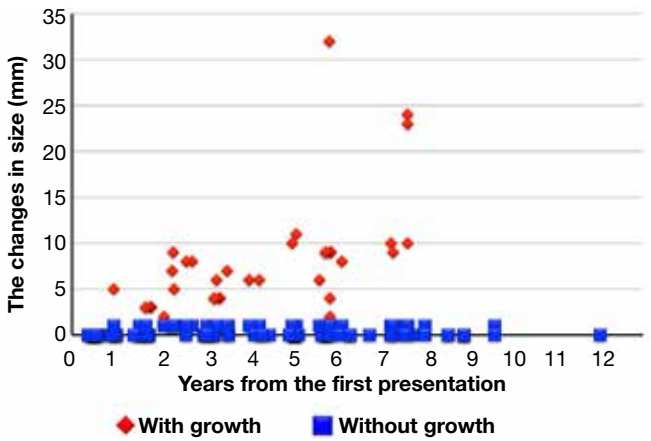


Figure 5 Changes in the sizes of the 108 evaluated lesions from the time of the first presentation to the last CT scan. Twenty-nine lesions (red) increased by 2 mm or more, whereas the remaining 79 lesions (blue) persisted without changing in size. Adapted with permission from Wolters Kluwer Health©. Kobayashi Y. *et al.* J Thorac Oncol 2013;8:309-14.

variable, 10% to 27% of GGOs gradually grow, whereas others persist without changes for years (12,37-40). It should be noted that according to the updated data from our study, even some part-solid GGOs remained unchanged for more than 3 years; these included 45 pure GGOs (size range, 4 to 16 mm) and 7 part-solid GGOs (size range, 7 to 12 mm). However, the solid component proportions of these 7 part-solid GGOs were only 1-25%.

To discuss the difference between the natural history of pure GGOs and that of part-solid GGOs, we summarized them separately. Among the 5 reports mentioned above, 4 included the natural histories of pure GGOs, and these are summarized in *Figure 6* (12,38-40). Approximately 80% of pure GGOs remained unchanged, while others grew in size or progressed to become part-solid GGOs. In comparison, the natural histories of part-solid GGOs were available in 3 reports; these histories are summarized in *Figure 7* (12,38,40). Approximately 60% of the part-solid GGOs remained unchanged. These findings indicate that part-solid GGOs seem more likely to grow than pure GGOs are.

Volume-doubling time (VDT) of nodules with GGO

The VDT is useful for objectively evaluating GGO-predominant lesions' tendency to grow. Based on the two-dimensional calculation method, the mean VDTs of 19 pure GGOs and 19 part-solid GGOs were 813 days (±375) and 457 days (±260), respectively (41). Other studies reported

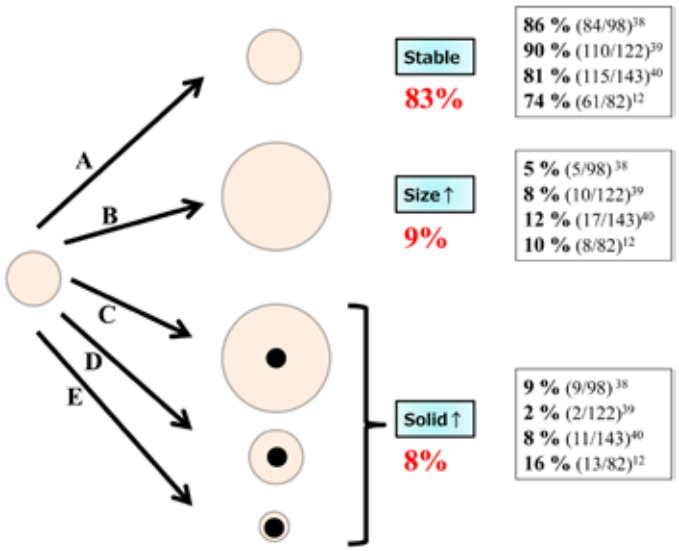


Figure 6 The natural history of pure GGOs. Five types of progression are suggested: (A) no change; (B) the size of the lesion increases, but there is no solid component; (C) the size of the lesion increases, and a solid component appears; (D) the solid component increases with no change in lesion size; and E. the size of the lesion decreases, and a solid component appears. The frequencies of each type are summarized. Approximately 80% of the pure GGOs remained unchanged.

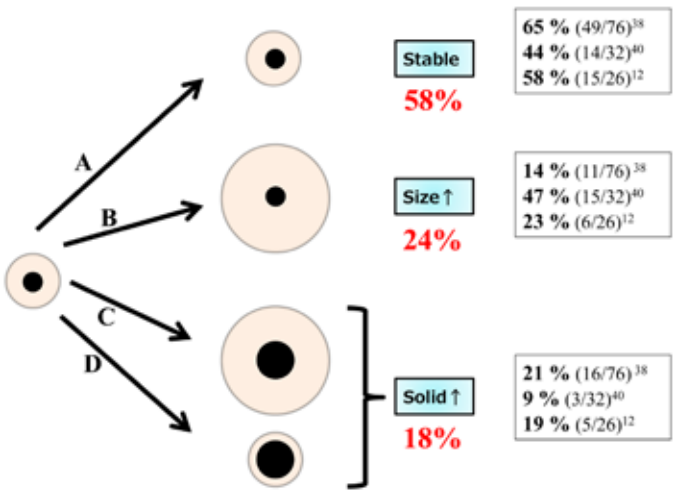


Figure 7 The natural history of part-solid GGOs. Four types of progression are suggested: (A) no change; (B) the size of the lesion increases, and the solid component remains unchanged; (C) the size of the lesion and the solid component increases; (D) the solid component increases, with no changes in the lesion size. The frequencies of each type are summarized. Approximately 60% of the part-solid GGOs remained unchanged.

similar results: the mean VDT of pure GGOs ranged from 769 to 880 days (39,40,42). In a recent study using computer-aided three-dimensional evaluation, the mean VDTs of 19 pure GGOs and 28 part-solid GGOs were 629 (±404) days and 277 (±156) days, respectively (43). Based on these data, the VDT of pure GGOs was consistently longer than that of part-solid GGOs.

How long should we follow up nodules with GGO?

It is unclear how long we should follow GGO-predominant lesions that do not meet the criteria for surgical intervention. We analyzed the time at which lesions with GGO began to grow. Among the 108 lesions that met the abovementioned criteria, 29 lesions grew at the median follow-up period of 4.2 years. All 29 of the lesions began to grow within 3 years from the time of the first observation; of these, 13 lesions grew within 1 year, 12 lesions grew within 1.1 to 2 years, and 4 lesions grew within 2.1 to 3 years (12). Therefore, we concluded that such lesions should be followed for at least 3 years to accurately evaluate the lesion growth.

We discuss the appropriate follow-up period based on the VDT of GGO-predominant lesions. We computationally simulated the size changes of pure GGO lesions using the VDT of 813 to 880 days. A 5-mm lesion would grow to 6.7 to 6.8 mm after 3 years of observation, whereas a 10-mm lesion would grow to 13.3 to 13.6 mm within the same period (12). Are these small changes in size (i.e., 1.7 to 1.8 mm and 3.3 to 3.6 mm) detectable on CT examinations? Measurement errors should be considered when we evaluate the increase in size. Kakinuma *et al.* reported that increase in diameter of >1.72 mm is necessary to identify true growth, considering interobserver measurement errors (44). Therefore, these calculated changes in size should be detectable with CT analysis, and the follow up period of 3 years seems to be reasonable.

It should be noted that the range of the VDTs stated above was wide in each study, and a few lesions actually began to grow after 3 years of observation (37-39). However, it is reasonable to regard the 3-year observation follow-up period as a benchmark for GGOs because the exceptional cases are in the minority.

Surgical procedure

When the GGO lesion in question is indicated for surgical resection, the extent of surgical resection presents another question. The standard treatment for operable non-small cell lung cancer is lobectomy with dissection of the ipsilateral hilar and mediastinal lymph nodes (45). Asamura *et al.* reported the prognosis of 545 patients who underwent lobectomy and lymph node dissection in the abovementioned multi-institutional prospective study (30) to predict pathological noninvasiveness. At the median follow-up period of 7.1 years, with the use of the cutoff value of an adenocarcinoma ≤3 cm with a C/T ratio ≤0.5, the 5-year overall survivals of radiologic noninvasive (121 patients) and invasive (424 patients) adenocarcinomas were 96.7% and 88.9%, respectively, and the difference was statistically significant (P<0.001). With the cutoff value of an adenocarcinoma ≤2 cm with a C/T ratio ≤0.25, the 5-year overall survivals of radiologic noninvasive (35 patients) and invasive (254 patients) adenocarcinomas were 97.1% and 92.4%, respectively, and the difference was not statistically significant (P=0.259) (46). These data showed that most of the patients with adenocarcinoma ≤3 cm with a GGO component >50% were cured by lobectomy.

Based on these favorable prognoses, limited surgical resection that preserves lung parenchyma might be indicated for patients with such GGO-predominant lesions. There have been many reports on recurrence-free survival after the limited resection of a GGO lesion. For example, 35 patients with pure GGOs ≤2 cm survived without recurrence after partial resection in 31 patients and segmentectomy in 4 patients (6). Similarly, 48 patients with lesions ≤2 cm with GGO proportions >50% survived without recurrence after partial resection in 33 patients and segmentectomy in 15 patients (47).

In contrast, local recurrence has also been reported. Nakao *et al.* reported that 4 out of 26 patients with GGO lesions ≤2 cm developed either cut-end recurrence or metachronous primary disease more than 5 years after the initial limited resection (48). In their study, a resection margin greater than 1 cm was ensured (48). Possible reasons for the cut-end recurrence are the difficulty of intraoperatively localizing the GGO and the vague GGO border. The preoperative CT-guided injection of agar near the target GGO lesion has been reported to be useful for making deeply located lesions palpable (49). Furthermore, intraoperative ultrasonography facilitated effective localization in a completely deflated lung and was useful for evaluating surgical margins (50). This method can be performed in complete video-assisted thoracic surgery.

Regardless of the favorable prognoses that were achieved by limited resection in the retrospective studies, prospective

clinical trials are necessary to establish the efficacy and safety of limited resection. There are two ongoing clinical studies in Japan to assess the efficacy of limited surgical resection for small lung cancer lesions. One study is a Phase III trial comparing lobectomy and segmentectomy for small radiologically invasive lung cancer, which is an adenocarcinoma ≤2 cm with a C/T ratio >0.25 (51). Another study is a Phase II trial of a wedge resection for small radiologically noninvasive lung cancer, which is an adenocarcinoma ≤2 cm with a C/T ratio ≤0.25 (52).

Conclusions

Surgery achieves favorable prognoses in patients with GGO-predominant lesions. However, the natural history of GGOs has been gradually clarified; some of them grow or increase their solid component, whereas others remain unchanged for years. Therefore, it remains unclear whether all GGO-predominant lesions should be surgically resected, and whether lesions without changes may not require resection. To distinguish GGOs with growth from those without growth, a 3-year observation period is a reasonable benchmark for follow-up. Future studies on the genetic differences between lesions with and without growth will help establish an appropriate management algorithm.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Hansell DM, Bankier AA, MacMahon H, et al. Fleischner Society: glossary of terms for thoracic imaging. Radiology 2008;246:697-722.
2. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395-409.
3. Park CM, Goo JM, Lee HJ, et al. Nodular ground-glass opacity at thin-section CT: histologic correlation and evaluation of change at follow-up. Radiographics 2007;27:391-408.
4. Takashima S, Maruyama Y, Hasegawa M, et al. CT findings and progression of small peripheral lung neoplasms having a replacement growth pattern. AJR Am J Roentgenol 2003;180:817-26.
5. Sawada S, Komori E, Nogami N, et al. Evaluation of lesions corresponding to ground-glass opacities that were resected after computed tomography follow-up examination. Lung Cancer 2009;65:176-9.
6. Yamada S, Kohno T. Video-assisted thoracic surgery for pure ground-glass opacities 2 cm or less in diameter. Ann Thorac Surg 2004;77:1911-5.
7. Mun M, Kohno T. Efficacy of thoracoscopic resection for multifocal bronchioloalveolar carcinoma showing pure ground-glass opacities of 20 mm or less in diameter. J Thorac Cardiovasc Surg 2007;134:877-82.
8. Yoshida J, Nagai K, Yokose T, et al. Limited resection trial for pulmonary ground-glass opacity nodules: fifty-case experience. J Thorac Cardiovasc Surg 2005;129:991-6.
9. Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Cancer 1995;75:2844-52.
10. Yang ZG, Sone S, Takashima S, et al. High-resolution CT analysis of small peripheral lung adenocarcinomas revealed on screening helical CT. AJR Am J Roentgenol 2001;176:1399-407.
11. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85.
12. Kobayashi Y, Fukui T, Ito S, et al. How long should small lung lesions of ground-glass opacity be followed? J Thorac Oncol 2013;8:309-14.
13. Yano M, Sasaki H, Kobayashi Y, et al. Epidermal growth factor receptor gene mutation and computed tomographic findings in peripheral pulmonary adenocarcinoma. J Thorac Oncol 2006;1:413-6.
14. Lee HJ, Kim YT, Kang CH, et al. Epidermal growth factor receptor mutation in lung adenocarcinomas: relationship with CT characteristics and histologic subtypes. Radiology 2013;268:254-64.
15. Sakamoto H, Shimizu J, Horio Y, et al. Disproportionate representation of KRAS gene mutation in atypical adenomatous hyperplasia, but even distribution of EGFR gene mutation from preinvasive to invasive adenocarcinomas. J Pathol 2007;212:287-94.
16. Kosaka T, Yatabe Y, Endoh H, et al. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919-23.
17. Shimizu K, Ikeda N, Tsuboi M, et al. Percutaneous CT-guided fine needle aspiration for lung cancer smaller than 2 cm and revealed by ground-glass opacity at CT. Lung

Cancer 2006;51:173-9.
18. Kim TJ, Lee JH, Lee CT, et al. Diagnostic accuracy of CT-guided core biopsy of ground-glass opacity pulmonary lesions. AJR Am J Roentgenol 2008;190:234-9.
19. Lu CH, Hsiao CH, Chang YC, et al. Percutaneous computed tomography-guided coaxial core biopsy for small pulmonary lesions with ground-glass attenuation. J Thorac Oncol 2012;7:143-50.
20. Hur J, Lee HJ, Nam JE, et al. Diagnostic accuracy of CT fluoroscopy-guided needle aspiration biopsy of ground-glass opacity pulmonary lesions. AJR Am J Roentgenol 2009;192:629-34.
21. Yamauchi Y, Izumi Y, Nakatsuka S, et al. Diagnostic performance of percutaneous core needle lung biopsy under multi-CT fluoroscopic guidance for ground-glass opacity pulmonary lesions. Eur J Radiol 2011;79:e85-9.
22. Inoue D, Gobara H, Hiraki T, et al. CT fluoroscopy-guided cutting needle biopsy of focal pure ground-glass opacity lung lesions: diagnostic yield in 83 lesions. Eur J Radiol 2012;81:354-9.
23. Asamura H, Suzuki K, Watanabe S, et al. A clinicopathological study of resected subcentimeter lung cancers: a favorable prognosis for ground glass opacity lesions. Ann Thorac Surg 2003;76:1016-22.
24. Ikeda N, Maeda J, Yashima K, et al. A clinicopathological study of resected adenocarcinoma 2 cm or less in diameter. Ann Thorac Surg 2004;78:1011-6.
25. Suzuki K, Kusumoto M, Watanabe S, et al. Radiologic classification of small adenocarcinoma of the lung: radiologic-pathologic correlation and its prognostic impact. Ann Thorac Surg 2006;81:413-9.
26. Aoki T, Tomoda Y, Watanabe H, et al. Peripheral lung adenocarcinoma: correlation of thin-section CT findings with histologic prognostic factors and survival. Radiology 2001;220:803-9.
27. Matsuguma H, Yokoi K, Anraku M, et al. Proportion of ground-glass opacity on high-resolution computed tomography in clinical T1 N0 M0 adenocarcinoma of the lung: A predictor of lymph node metastasis. J Thorac Cardiovasc Surg 2002;124:278-84.
28. Nakata M, Sawada S, Yamashita M, et al. Objective radiologic analysis of ground-glass opacity aimed at curative limited resection for small peripheral non-small cell lung cancer. J Thorac Cardiovasc Surg 2005;129:1226-31.
29. Ohde Y, Nagai K, Yoshida J, et al. The proportion of consolidation to ground-glass opacity on high resolution CT is a good predictor for distinguishing the population of non-invasive peripheral adenocarcinoma. Lung Cancer 2003;42:303-10.
30. Suzuki K, Koike T, Asakawa T, et al. A prospective radiological study of thin-section computed tomography to predict pathological noninvasiveness in peripheral clinical IA lung cancer (Japan Clinical Oncology Group 0201). J Thorac Oncol 2011;6:751-6.
31. Naidich DP, Bankier AA, MacMahon H, et al. Recommendations for the management of subsolid pulmonary nodules detected at CT: a statement from the Fleischner Society. Radiology 2013;266:304-17.
32. Guidelines for the Management of Pulmonary Nodules Detected by Low-dose CT Lung Cancer Screening. Available online: http://www.jscts.org/pdf/guideline/gls3rd_english120719.pdf
33. Kodama K, Higashiyama M, Yokouchi H, et al. Natural history of pure ground-glass opacity after long-term follow-up of more than 2 years. Ann Thorac Surg 2002;73:386-92; discussion 392-3.
34. Kim HK, Choi YS, Kim J, et al. Management of multiple pure ground-glass opacity lesions in patients with bronchioloalveolar carcinoma. J Thorac Oncol 2010;5:206-10.
35. Haro A, Yano T, Kohno M, et al. Ground-glass opacity lesions on computed tomography during postoperative surveillance for primary non-small cell lung cancer. Lung Cancer 2012;76:56-60.
36. Silva M, Sverzellati N, Manna C, et al. Long-term surveillance of ground-glass nodules: evidence from the MILD trial. J Thorac Oncol 2012;7:1541-6.
37. Hiramatsu M, Inagaki T, Inagaki T, et al. Pulmonary ground-glass opacity (GGO) lesions-large size and a history of lung cancer are risk factors for growth. J Thorac Oncol 2008;3:1245-50.
38. Matsuguma H, Mori K, Nakahara R, et al. Characteristics of subsolid pulmonary nodules showing growth during follow-up with CT scanning. Chest 2013;143:436-43.
39. Chang B, Hwang JH, Choi YH, et al. Natural history of pure ground-glass opacity lung nodules detected by low-dose CT scan. Chest 2013;143:172-8.
40. Lee SW, Leem CS, Kim TJ, et al. The long-term course of ground-glass opacities detected on thin-section computed tomography. Respir Med 2013;107:904-10.
41. Hasegawa M, Sone S, Takashima S, et al. Growth rate of small lung cancers detected on mass CT screening. Br J Radiol 2000;73:1252-9.
42. Aoki T, Nakata H, Watanabe H, et al. Evolution of peripheral lung adenocarcinomas: CT findings correlated

with histology and tumor doubling time. AJR Am J Roentgenol 2000;174:763-8.
43. Oda S, Awai K, Murao K, et al. Volume-doubling time of pulmonary nodules with ground glass opacity at multidetector CT: Assessment with computer-aided three-dimensional volumetry. Acad Radiol 2011;18:63-9.
44. Kakinuma R, Ashizawa K, Kuriyama K, et al. Measurement of focal ground-glass opacity diameters on CT images: interobserver agreement in regard to identifying increases in the size of ground-glass opacities. Acad Radiol 2012;19:389-94.
45. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
46. Asamura H, Hishida T, Suzuki K, et al. Radiographically determined noninvasive adenocarcinoma of the lung: survival outcomes of Japan Clinical Oncology Group 0201. J Thorac Cardiovasc Surg 2013;146:24-30.
47. Kodama K, Higashiyama M, Takami K, et al. Treatment strategy for patients with small peripheral lung lesion(s): intermediate-term results of prospective study. Eur J Cardiothorac Surg 2008;34:1068-74.
48. Nakao M, Yoshida J, Goto K, et al. Long-term outcomes of 50 cases of limited-resection trial for pulmonary ground-glass opacity nodules. J Thorac Oncol 2012;7:1563-6.
49. Tsuchida M, Yamato Y, Aoki T, et al. CT-guided agar marking for localization of nonpalpable peripheral pulmonary lesions. Chest 1999;116:139-43.
50. Kondo R, Yoshida K, Hamanaka K, et al. Intraoperative ultrasonographic localization of pulmonary ground-glass opacities. J Thorac Cardiovasc Surg 2009;138:837-42.
51. A phase III randomised trial of lobectomy versus limited resection (segmentectomy) for small (2 cm or less) peripheral non-small cell lung cancer (JCOG0802/WJOG4607L). Available online: https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&recptno=R000002300&type=summary&language=E, accessed July 30, 2013.
52. Phase II Study of Limited Surgical Resection for Peripheral Early Lung Cancer Defined with Thoracic Thin-section Computed Tomography (JCOG0804/WJOG4507L). Available online: https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&recptno=R000002262&type=summary&language=E, accessed July 30, 2013.

Cite this article as: Kobayashi Y, Mitsudomi T. Management of ground-glass opacities: should all pulmonary lesions with ground-glass opacity be surgically resected? Transl Lung Cancer Res 2013;2(5):354-363. doi: 10.3978/j.issn.2218-6751.2013.09.03

Video-assisted thoracoscopic lobectomy—from an experimental therapy to the standard of care

Tristan D. Yan[1,2]

[1]The Collaborative Research (CORE) group, Sydney, Australia; [2]The University of Sydney, Department of Cardiothoracic Surgery, Royal Prince Alfred Hospital, Sydney, Australia

Correspondence to: Associate Professor Tristan D. Yan. The Collaborative Research (CORE) Group, Sydney, Australia; Department of Cardiothoracic Surgery, Royal Prince Alfred Hospital, University of Sydney, Sydney, Australia. Email: tristanyan@annalscts.com.

Submitted Aug 10, 2013. Accepted for publication Aug 12, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.48
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.48

Without a doubt, video-assisted thoracoscopic surgery (VATS) has completely revolutionized modern thoracic surgery and significantly improved patient outcomes over the last two decades. Now is a crucial transition point—we are witnessing the VATS lobectomy technique transforming from an experimental procedure to the standard of care for patients with early-stage non-small cell lung cancer (NSCLC).

A recent meta-analysis of propensity score matched patients by Cao *et al*. demonstrated significantly lower incidences of overall complications, prolonged air leak, pneumonia, atrial arrhythmias and renal failure, as well as shorter hospitalization compared to open thoracotomy (1). This study further consolidated the benefits of VATS lobectomy for our patients and offered the highest clinical evidence on this topic. In 2012, the Cross-sectional Survey on Lobectomy Approach (X-SOLA) involving 850 general thoracic surgeons worldwide demonstrated that VATS lobectomy has been accepted as a standard surgical procedure (2). The debate regarding the safety of VATS lobectomy is clearly a flavor in the past (2). Not only is it safe to perform lobectomy and segmentectomy using a total VATS approach, it is also technically feasible for resection of locally advanced lung tumors (3,4). To the best of my knowledge, there has been no publication thus far demonstrating inferior outcomes of VATS lobectomy compared to conventional open thoracotomy. On the contrary, a meta-analysis published in the *Journal of Clinical Oncology* once again confirmed that VATS lobectomy is an appropriate procedure for early-stage NSCLC, in terms of its safety, local oncological control, and survival, when compared with open surgery (5).

The VATS Lobectomy Consensus Meeting was held in Edinburgh, UK in November 2012, which marked the 20th anniversary of this procedure. For the first time in history, 50 world-leading minimally invasive thoracic surgeons from 16 countries reached consensus agreements on several important issues on VATS lobectomy, including its definition, patient eligibility, surgical standard of care and future training (6). It is clear that the Cancer and Leukemia Group B (CALGB) definition represents the globally accepted state-of-the-art VATS lobectomy technique (7). Eligibility for VATS lobectomy should include tumor size ≤7 cm, N0 or N1 status and FEV1 or DLCO >30% (6). More interestingly, the great majority of the experts regarded a randomized-controlled trial (RCT) comparing VATS lobectomy with open thoracotomy for early-stage NSCLC not necessary. There are generally two groups of people who are still demanding a RCT to come forward. One group is the non-believers who use the lack of RCT as the argument for not doing VATS lobectomy at all and will likely carry on with the traditional open surgery, irrespective of a RCT. But there is little doubt that the trajectory of open lobectomy will eventually follow the course of open cholecystectomy. The other group includes the skeptics who are open-minded and waiting to be convinced. But as a RCT is not going to happen, a more pragmatic approach to evidence-based practice is required.

By now, we need to be realistic that a RCT is never going to happen. Although I agree that this research methodology may have scientific merit, the logistical problems with such a trial are probably insurmountable for several reasons. Few, (if any), patients would agree to the random assignment. I seriously doubt that any patients would subject themselves to open thoracotomy upfront, in a center where VATS lobectomy technique is proficient and the patient is

properly informed about both procedures. At the Royal Infirmary of Edinburgh, such an attempt of randomization was made with only two patients recruited during a 6-month period. As a result, this trial was terminated prematurely. Indeed, given the promising results of the VATS approach achieved today and the lack of any published evidence on the superiority of open lobectomy over VATS, one has to appreciate the real logistic difficulties of recruiting sufficient numbers of patients to identify small differences (if any) in long-term outcomes. In addition, the surgical quality control on such a multi-institutional, or even multinational study would be exceedingly difficult at best. Furthermore, there would be significant challenges to identify surgeons who are proficient in both VATS lobectomy and open thoracotomy and more importantly, willing to randomize their patients. Finally, we know of no available funding agency for such a trial, and the costs of involved would make participation costs prohibitive for what would be a low-accruing study at all but a handful of centers.

One needs to acknowledge that the acquisition of level I evidence by performing RCTs may not be necessary for experimental therapies to mature into the standard of care. For example, there was never a RCT demonstrating the superiority of metastasectomy for pulmonary metastases. It needs to be emphasized that a lack of RCT does not equate to a lack of evidence. Despite this, many of us continue to pursue high-level evidence for VATS lobectomy. The European and Asian collaborative groups are independently starting randomized studies comparing VATS segmentectomy versus VATS lobectomy for patients with small peripheral early-stage NSCLC. Our consensus project not only amalgamated the current expert recommendations, but also provided a pivotal role in setting the stage for further multi-institutional databases, the creation of mentoring workshops and standardized training programs to progressively develop this technique widely amongst thoracic surgical trainees and specialists (6).

The scientific question regarding the long-term oncologic efficacy of VATS lobectomy is an important one. However, the current data shows no long-term survival difference or even better survival outcomes with the VATS approach (5). Because of the marked perioperative benefits and equivalent long-term oncologic efficacy, VATS lobectomy must be considered as a standard surgical option for patients to choose. If the patient's informed decision is VATS lobectomy, the patient should be referred to specialist VATS center for assessment. Denying the patient a chance to choose VATS lobectomy due to the lack of surgical expertise is not justifiable.

In summary, both current evidence and expert consensus indicate that patients undergoing VATS lobectomy for early stage NSCLC, even with suboptimal pulmonary functions, will obtain better perioperative surgical outcomes and at least equivalent long-term efficacy when compared with the open thoracotomy approach. These patients should be considered for VATS lobectomy before embarking on an open thoracotomy, at least in a center with this surgical expertise. In other words, VATS lobectomy for NSCLC after 20 years of surgical refinement should be the current state-of-the-art treatment for early stage NSCLC, unless any future studies demonstrate superior results for open lobectomy.

Acknowledgements

Disclosure: The author declares no conflict of interest.

References

1. Cao C, Manganas C, Ang SC, et al. A meta-analysis of unmatched and matched patients comparing video-assisted thoracoscopic lobectomy and conventional open lobectomy. Ann Cardiothorac Surg 2012;1:16-23.
2. Cao CQ, Tian DH, Wolak K, et al. Cross-sectional survey on lobectomy approach (X-SOLA). Chest (submitted).
3. Hennon MW, Demmy TL. Video-assisted thoracoscopic surgery (VATS) for locally advanced lung cancer. Ann Cardiothorac Surg 2012;1:37-42.
4. Oparka J, Yan TD, Richards JMJ, et al. Video-assisted thoracoscopic pneumonectomy: The Edinburgh posterior approach. Ann Cardiothorac Surg 2012;1:105-8.
5. Yan TD, Black D, Bannon PG, et al. Systematic review and meta-analysis of randomized and nonrandomized trials on safety and efficacy of video-assisted thoracic surgery lobectomy for early-stage non-small-cell lung cancer. J Clin Oncol 2009;27:2553-62.
6. Yan TD, Cao C, D'Amico, et al. Video-assisted thoracoscopic surgery (VATS) lobectomy at 20 years: a consensus statement. Eur J Cardiothoracic Surg (In press).
7. Swanson SJ, Herndon JE 2nd, D'Amico TA, et al. Video-assisted thoracic surgery lobectomy: report of CALGB 39802--a prospective, multi-institution feasibility study. J Clin Oncol 2007;25:4993-7.

Cite this article as: Yan TD. Video-assisted thoracoscopic lobectomy—from an experimental therapy to the standard of care. J Thorac Dis 2013;5(S3):S175-S176. doi: 10.3978/j.issn.2072-1439.2013.08.48

Techniques of VATS lobectomy

John D. Mitchell

General Thoracic Surgery, Division of Cardiothoracic Surgery, University of Colorado School of Medicine, Aurora, Colorado, USA
Correspondence to: John D. Mitchell, M.D. Division of Cardiothoracic Surgery, University of Colorado School of Medicine, 12631 E. 17th Avenue, C-310, Aurora, Colorado 80045, USA. Email: John.Mitchell@ucdenver.edu.

Submitted Jul 10, 2013. Accepted for publication Jul 15, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.29
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.29

Introduction

Despite the objections of some zealots, there is clearly more than one way to successfully complete a video-assisted thoracoscopic or "VATS" lobectomy, and further refinements to the technique are added yearly. Most thoracic surgeons would define VATS lobectomy as one in which the dissection is completed with reliance on a video image, without the use of a retractor to spread the ribs and increase the width of the intercostal spaces. The actual number, location and aggregate length of the involved incisions are largely a matter of surgeon preference. Further, the actual methods used-fissure dissection compared with a "fissure-less" approach; Use of sharp, blunt or cautery techniques-is also at the discretion of the surgeon, as long as the basic tenet of individual dissection and ligation of the lobar structures is observed.

Operative technique

Preoperative assessment

Early stage (stages I and II) lung cancer is the most common indication for thoracoscopic lobectomy, although increasingly these techniques are applied in the setting of locally advanced disease following induction therapy. Benign tumors and focal areas of bronchiectasis are also usually amenable to a minimally invasive approach. The indications and contraindications to VATS lobectomy are covered in detail in another chapter in this monograph.

The preoperative assessment of patients considered for VATS lobectomy is routine, and is tailored to the indications for surgery. Preoperative imaging studies, including the use of computed tomography (CT) and positron emission tomography (PET), are helpful to confirm the planned extent of resection and the suitability of a VATS approach. Adequate pulmonary reserve is assessed through the use of pulmonary function testing, with occasional use of perfusion scanning and exercise testing when appropriate. Testing for occult cardiac disease is performed when indicated. In general, the preoperative assessment of a prospective patient is similar to any individual considered for pulmonary resection.

Anesthesia and preoperative bronchoscopy

The anesthetic technique for VATS lobectomy is similar to other cases of pulmonary resection. A means for lung isolation, either with the use of a double lumen endotracheal tube or bronchial blocker, is routine. Placement of a thoracic epidural catheter for postoperative pain control, while common in open thoracotomy cases, is usually not needed following thoracoscopic resection and is routinely omitted. It is often helpful to place intercostal blocks using 0.25% bupivacaine at the end of the procedure to aid immediate postoperative analgesia.

Surgeons are well advised to perform bronchoscopy prior to the procedure, to assess the targeted lobar orifice for abnormalities or any variations in anatomy which could have a significant impact on successful completion of the case. For example, encroachment of tumor on the planned line of bronchial resection could lead to abandonment of the minimally invasive approach.

Incisions and general dissection techniques

The vast majority of thoracoscopic lobectomy techniques employ either two, three, or four incisions, with three perhaps the most common. In all approaches, the camera

port (5 to 10 mm) is typically placed low in the chest—7th or 8th intercostal space—and either in the mid or anterior axillary line. A "utility" or "access" incision (3 to 6 cm) is usually placed in the anterior axillary line, over the anterior hilum (about 5th intercostal space) in the cases of upper lobectomy, and an interspace or two lower (adjacent to the major fissure) for middle and lower lobectomies. Third and fourth incisions, commonly 10 mm in size, are placed either through the auscultory triangle, high in the mid-axillary line, or low in the chest in the posterior axillary line. In all cases, no rib spreading is used at any of the incision sites. A soft tissue retractor, either a weitlaner or a commercially available device, is often used at the utility incision. Care must be used at all the incisions to avoid excessive "torqueing" of the rigid instruments on the adjacent ribs and intercostal bundles to avoid postoperative neuralgia.

The surgical procedure is facilitated by roughly aligning the view of the camera with the general direction of the dissection. This is most easily achieved with cameras designed to provide an angled view, either at 30 or 45 degrees from the long axis of the scope. This also allows the surgeon to "see around" the hilum with the camera in a trocar site low in the chest. It is important for the surgeon to remember that occasionally a better view may be available by placing the camera in the access or posterior incision; Flexibility with the operative technique in this fashion can often dramatically lessen the difficulty of the procedure.

Dissection of the hilar structures may be accomplished either using a largely blunt, sharp or cautery-based technique. A thorough knowledge of the hilar anatomy greatly enhances the safety of all of these techniques. Vital structures such as the phrenic nerve or recurrent laryngeal nerve should be identified early and preserved. While all of these techniques are useful, each has obvious drawbacks. It is likely that a combination of approaches probably produces the best results.

Pulmonary vessels and bronchi within the hilum are ligated with endoscopic staplers, although a "TA" type stapler may be used for the bronchus at the surgeon's discretion. It is important to introduce the stapler into the chest such that, once around the vessel or bronchus, it exits into "free space" and is not encumbered by other structures. This will avoid injury to other tissues, and assure a secure closure of the target. Bronchial arteries may be cauterized or clipped, or stapled in rare cases involving long standing pulmonary infection. Fissures are typically stapled unless complete, in which case cautery may be used. It is recommended that specimen removal is achieved with the use of a specimen bag, to minimize contact with the soft tissues at the access incision site. Use of this technique has reduced the incidence of "port-site" recurrence which plagued early attempts at thoracoscopic resection.

In cases of malignancy, nodal dissection may be performed either before or after completion of the pulmonary resection. Initial dissection often facilitates the subsequent lobectomy by increasing the mobility of the specimen at the hilar level. Further, identification of significant N2 disease, previously unrecognized, would allow for termination of the procedure prior to resection to allow for induction therapy. Alternatively, access to the various nodal stations is often improved after the pulmonary resection, thus enhancing the completeness of the dissection. Removal of the hilar and lobar nodes is performed during the ligation of the various hilar structures.

Recently, reports of minimally invasive lobectomies utilizing a single port, or "uniport" approach, have been published. This fascinating technique, still in evolution, is described in a separate submission to this monograph.

Right upper lobectomy (RUL)

The most common technique for "fissure-less" right upper lobectomy utilizes an "anterior to posterior" approach, wherein the dissection progresses from the anterior structures in the hilum to the more posterior structures, dividing the involved fissures last. This technique is felt to minimize complications of air leak which may be associated with significant dissection within an incomplete fissure.

The branches of the superior pulmonary vein pertaining to the RUL are dissected free, and divided with a vascular stapler. In most cases, the stapler is best introduced through the posterior trocar site, or through the camera port. The pleura is incised around the top of the hilum, extending posteriorly to the bronchus intermedius. This allows dissection of the truncus anterior branch of the pulmonary artery, which is divided in a similar fashion. Great care must be taken to avoid excessive retraction of the lobe posteriorly during this maneuver, which may result in arterial injury. It is a good practice to minimize traction on pulmonary vessels during staple ligation, leading to a more secure vascular closure.

Division of the truncus anterior branch will allow improved retraction of the lobe posteriorly, exposing the right upper lobe bronchus and the posterior ascending branch of the pulmonary artery. Either may ligated first,

allowing improved exposure for the second structure. Dissection along the ongoing pulmonary artery will allow identification of the middle lobe branches, as well as the branch to the superior segment of the lower lobe. Occasionally, a separate arterial branch may be identified to the anterior RUL segment. Access to the structures to be divided may be enhanced be initiating division of the minor fissure anteriorly; Alternatively, one may divide the RUL bronchus from a posterior approach.

Finally, one completes the major and minor fissures pertaining to the upper lobe with a stapler. As the RUL becomes more mobile, the surgeon must be careful not to prevent twisting or torsion of the lobe at this step, which may lead to inaccurate completion of the fissures.

If the major fissure is complete or nearly so, it is certainly permissible to dissect and expose the artery within the fissure. Doing so will likely aid in completion of the minor fissure, facilitate identification of the superior segmental pulmonary artery, and may improve exposure to the posterior ascending branch of the pulmonary artery for ligation. However, the surgeon should avoid routine dissection within the fissure for a RUL, as is commonly taught in open surgical techniques. Avoidance of air leak is important to maximize the benefits of a thoracoscopic approach, producing shorter chest tube duration and hospital stay.

Left upper lobectomy (LUL)

The location and number of incisions is analogous to those used in right upper lobectomy. An anterior to posterior, or fissure-less approach, is used. Retracting the lung posteriorly and caudally, the pleura overlying the anterior, superior, and posterior hilum is excised. The superior pulmonary vein is dissected free and ligated with a vascular endoscopic stapler. The surgeon must be assured that a separate inferior vein is present and not included in the stapler, as it is not uncommon on the left side for the two pulmonary veins to join prior to entry into the pericardium. The first branches of the pulmonary artery are then dissected free, a maneuver facilitated by removal of adjacent lymph nodes. Again, the surgeon must take care to avoid excessive traction on the LUL, which may lead to arterial injury as the surgeon attempts to expose these initial branches. Introduction of the vascular stapler for these branches is usually through the access incision or the camera port; the anterior location of these incisions allows the stapler anvil to slip around the branch into free space, with minimal torque on the vessel itself.

At this point, only the pulmonary artery branches to the posterior segment and the lingula remain. Exposing these branches is often helped by division of the LUL bronchus. After division of the superior vein, the surgeon has ready access to the crotch between the upper and lower lobe bronchi. Dissection in this area, along with separation of the pulmonary artery from the LUL bronchus as the former wraps around the bronchus superiorly, allows safe isolation of the LUL bronchus. Introduction of an appropriate endoscopic stapler from the anterior camera port will allow safe passage of the stapler between the bronchus and the pulmonary artery into the free space superior to the hilum. After bronchial division, it is fairly straightforward to identify and ligate the remaining pulmonary artery branches to the LUL. The fissure is then completed with a stapler. Occasionally, analogous to the RUL technique, it is advantageous to initiate fissure division prior to this point, to allow better exposure to the deeper hilar vessels.

Right middle lobectomy (RML)

A completely "fissure-less" technique for RML resection is not possible, due to the location of the lobe between the upper and middle lobes. However, as the dissection proceeds in a caudal to cranial direction, the minor fissure is divided last. Despite this, the RML is perhaps the easiest lobe to using thoracoscopic techniques. For this resection, it is helpful to employ an auscultory triangle port to allow passage of the endoscopic stapler, as noted below.

The RML vein is isolated and divided, with the vascular stapler introduced via the posterior (if present) or camera port. Minimal dissection within the major fissure usually yields the pulmonary artery, and the portion of the major fissure between the middle and lower lobes may be completed either with a stapler or the cautery if nearly complete. The surgeon must be careful to identify and preserve a small pulmonary artery branch, invariably present, arising in the medial major fissure to the medial basilar segments of the right lower lobe.

Completion of the fissure allows access to the RML bronchus. The bronchus is freed by developing the plane between the pulmonary artery in the fissure and the bronchus, following the artery more proximally as it wraps around the bronchus superiorly. More anteriorly, the bronchus is separated from the pulmonary venous branches to the RUL, and the bronchus is encircled and then ligated with an endoscopic stapler introduced via the posterior port.

With the bronchus divided, the lobe is retracted

cephalad, and one or two pulmonary artery branches are exposed to the RML. Just superior to this, the vein to the posterior segment of the RUL is seen. The arterial branches are isolated and divided either individually or occasionally with the same vascular stapler. If a posterior port is used at this point, it is important that it not be located too caudal, which will make the safe passage of the stapler more difficult. After arterial division, the minor fissure is completed, separating the middle from the upper lobe.

Lower lobectomy (RLL, LLL)

In the case of either right or left lower lobectomy, the operation starts with division of the inferior pulmonary ligament, followed by isolation and ligation of the inferior pulmonary vein. The surgeon should attempt to visualize and include the branch to the superior segment, which in some cases may arise low or even separate from the basilar vein branch. In addition, the left side identification of a separate superior vein is prudent, as mentioned previously. Pleural division posteriorly to the area of the upper lobe and anteriorly to the major fissure facilitates this portion of the case.

At this point, as the dissection proceeds cephalad into the subcarinal space, the surgeon makes a choice about the fissure. If complete or nearly so, the fissure may be completed first, allowing access to isolate and divide the pulmonary artery branches to the lower lobe. On the right, the posterior ascending branch to the RUL must be visualized and preserved, while on the left the lingular artery must be identified. After arterial division, only the bronchus remains, which is dissected free of adjacent nodal material for isolation and ligation using either an endoscopic or TA stapler. The bronchial stump should be short, but on the left care must be taken not to incorporate the bronchial side of a migrated double lumen endotracheal tube in the staple line.

If the fissure is incomplete, one may dissect down through the fissure, identify the pulmonary artery, and proceed as above. However, a better approach is to complete a "fissure-less" dissection in a caudal to cranial fashion, developing the fissure last. To do so, after vein ligation, the surgeon proceeds with the dissection into the lower subcarinal space. Anteriorly, the wall of the lower lobe bronchus is followed into the fissure. On the right, the RML bronchus is identified and kept cephalad to the line of dissection. On the left, a similar approach is used to the identified upper lobe bronchus. If the pulmonary artery is seen at this point, this greatly facilitates dissection between the two structures. A similar dissection technique is utilized posteriorly. On the left, the pulmonary artery is simple to identify posteriorly, enabling dissection between bronchus and artery. On the right, dissection posteriorly proceeds just cephalad to the identified superior segmental bronchus. Working from both anterior and posterior directions, some blunt dissection may be needed to complete bronchial isolation. Partial division of the fissure at this point of the case may greatly enhance visualization. When the lower lobe bronchus is encircled, it is divided with an endoscopic stapler. This then allows isolation and ligation of the pulmonary artery to the lower lobe. Again, care must be taken with respect to the lingular artery and the posterior ascending branch on the right. Finally, the remaining major fissure is completed.

Closure and perioperative management

Following placement of a single chest tube and assurance of hemostasis, chest closure is routine. Absorbable suture is used for the muscle layers and soft tissues external to the chest wall, with no intercostal sutures placed. The skin is closed with absorbable subcuticular suture.

Postoperative management is also routine, but should incorporate a paradigm shift from management strategies used for open lobectomy. As mentioned earlier, some of the advantages in minimally invasive surgery are lost if care plans based on a several day hospital stay after thoracotomy are used. Early mobilization and ambulation, combined with aggressive chest tube management, will result in earlier discharge from hospital, faster recovery and better patient satisfaction.

Outcomes and conclusions

The safety and efficacy of thoracoscopic lobectomy have been demonstrated in several large studies, comparable to open lobectomy (1-3). VATS lobectomy has been shown to be associated with less morbidity (4-7), at least equivalent mortality (4,8,9), shorter hospital stays (4-8), improved functional outcomes (10-12), and less costs (13-15) compared with an open approach. Perhaps most important, minimally invasive lobectomy is oncologically equivalent (1,4,8,9,16,17), at a minimum, to lobectomy through open thoracotomy. A direct comparison with open lobectomy remains lacking, though, and the concept of a prospective randomized trial comparing the open and VATS

approaches has been considered repeatedly. However, the recognized advantages of a thoracoscopic approach among dedicated thoracic surgeons have likely eroded any clinical equipoise needed for such a trial. Indeed, these advantages are not lost on practicing thoracic surgeons. Approximately 50% of lobectomies registered in the Society of Thoracic Surgeons General Thoracic Database are completed via a thoracoscopic approach (18), and the percentage continues to increase.

Current frontiers in thoracoscopic surgery now include chest wall resection and reconstruction, muscle flap transposition, sleeve resection, and the use of uniportal techniques. In the years ahead, we may expect advances in these areas, along with further refinement of established techniques in thoracoscopic surgery.

Acknowledgements

Disclosure: The author declares no conflict of interest.

References

1. McKenna RJ Jr, Houck W, Fuller CB. Video-assisted thoracic surgery lobectomy: experience with 1,100 cases. Ann Thorac Surg 2006;81:421-5; discussion 425-6.
2. Mitchell JD, Yu JA, Bishop A, et al. Thoracoscopic lobectomy and segmentectomy for infectious lung disease. Ann Thorac Surg 2012;93:1033-9; discussion 1039-40.
3. Onaitis MW, Petersen RP, Balderson SS, et al. Thoracoscopic lobectomy is a safe and versatile procedure: experience with 500 consecutive patients. Ann Surg 2006;244:420-5.
4. Flores RM, Park BJ, Dycoco J, et al. Lobectomy by video-assisted thoracic surgery (VATS) versus thoracotomy for lung cancer. J Thorac Cardiovasc Surg 2009;138:11-8.
5. Paul S, Altorki NK, Sheng S, et al. Thoracoscopic lobectomy is associated with lower morbidity than open lobectomy: a propensity-matched analysis from the STS database. J Thorac Cardiovasc Surg 2010;139:366-78.
6. Paul S, Sedrakyan A, Chiu YL, et al. Outcomes after lobectomy using thoracoscopy vs thoracotomy: a comparative effectiveness analysis utilizing the Nationwide Inpatient Sample database. Eur J Cardiothorac Surg 2013;43:813-7.
7. Villamizar NR, Darrabie MD, Burfeind WR, et al. Thoracoscopic lobectomy is associated with lower morbidity compared with thoracotomy. J Thorac Cardiovasc Surg 2009;138:419-25.
8. Whitson BA, Groth SS, Duval SJ, et al. Surgery for early-stage non-small cell lung cancer: a systematic review of the video-assisted thoracoscopic surgery versus thoracotomy approaches to lobectomy. Ann Thorac Surg 2008;86:2008-16; discussion 2016-8.
9. Yan TD, Black D, Bannon PG, et al. Systematic review and meta-analysis of randomized and nonrandomized trials on safety and efficacy of video-assisted thoracic surgery lobectomy for early-stage non-small-cell lung cancer. J Clin Oncol 2009;27:2553-62.
10. Demmy TL, Nwogu C. Is video-assisted thoracic surgery lobectomy better? Quality of life considerations. Ann Thorac Surg 2008;85:S719-28.
11. Handy JR Jr, Asaph JW, Douville EC, et al. Does video-assisted thoracoscopic lobectomy for lung cancer provide improved functional outcomes compared with open lobectomy? Eur J Cardiothorac Surg 2010;37:451-5.
12. Nakata M, Saeki H, Yokoyama N, et al. Pulmonary function after lobectomy: video-assisted thoracic surgery versus thoracotomy. Ann Thorac Surg 2000;70:938-41.
13. Burfeind WR Jr, Jaik NP, Villamizar N, et al. A cost-minimisation analysis of lobectomy: thoracoscopic versus posterolateral thoracotomy. Eur J Cardiothorac Surg 2010;37:827-32.
14. Casali G, Walker WS. Video-assisted thoracic surgery lobectomy: can we afford it? Eur J Cardiothorac Surg 2009;35:423-8.
15. Swanson SJ, Meyers BF, Gunnarsson CL, et al. Video-assisted thoracoscopic lobectomy is less costly and morbid than open lobectomy: a retrospective multiinstitutional database analysis. Ann Thorac Surg 2012;93:1027-32.
16. Farjah F, Wood DE, Mulligan MS, et al. Safety and efficacy of video-assisted versus conventional lung resection for lung cancer. J Thorac Cardiovasc Surg 2009;137:1415-21.
17. Sugi K, Kaneda Y, Esato K. Video-assisted thoracoscopic lobectomy achieves a satisfactory long-term prognosis in patients with clinical stage IA lung cancer. World J Surg 2000;24:27-30; discussion 30-1.
18. Boffa DJ, Kosinski AS, Paul S, et al. Lymph node evaluation by open or video-assisted approaches in 11,500 anatomic lung cancer resections. Ann Thorac Surg 2012;94:347-53; discussion 353.

Cite this article as: Mitchell JD. Techniques of VATS lobectomy. J Thorac Dis 2013;5(S3):S177-S181. doi: 10.3978/j.issn.2072-1439.2013.07.29

Contraindications of video-assisted thoracoscopic surgical lobectomy and determinants of conversion to open

Jennifer M. Hanna, Mark F. Berry, Thomas A. D'Amico

Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA

Correspondence to: Thomas A. D'Amico, MD, Professor and Vice Chair of Surgery. Duke University Medical Center, DUMC Box 3496, Duke South White Zone 3589, Durham, NC 27710, USA. Email: thomas.damico@dm.duke.edu.

Abstract: Since the introduction of anatomic lung resection by video-assisted thoracoscopic surgery (VATS) was introduced 20 years ago, VATS has experienced major advances in both equipment and technique, introducing a technical challenge in the surgical treatment of both benign and malignant lung disease. The demonstrated safety, decreased morbidity, and equivalent efficacy of this minimally invasive technique has led to the acceptance of VATS as a standard surgical modality for early-stage lung cancer and increasing application to more advanced disease. However, only a minority of lobectomies are performed using the VATS technique, likely owing to concern for intraoperative complications. Optimal operative planning, including obtaining baseline pulmonary function tests with diffusion measurements, positron emission tomography and/or computed tomography scans, bronchoscopy, and endobronchial ultrasound or mediastinoscopy, can be used to anticipate and potentially prevent the occurrence of complications. With increasing focus on operative planning, as well as comfort and experience with the VATS technique, the indications for which this technique is used has grown. As such, the absolute contraindications have narrowed to inability to tolerate single lung ventilation, inability to achieve complete resection with lobectomy, T3 or T4 tumors, and N2 or N3 disease. However, as VATS lobectomy has been applied to more advanced stage disease, the rate of conversion to open thoracotomy has increased, particularly early in the surgeon's learning curve. Causes of conversion are generally classified into four categories: intraoperative complications, technical problems, anatomical problems, and oncological conditions. Though it is difficult to anticipate which patients may require conversion, it appears that these patients do not suffer from increased morbidity or mortality as a result of conversion to open thoracotomy. Therefore, with a focus on a safe and complete resection, conversion should be regarded as a means of completing resections in a traditional manner rather than as a surgical failure.

Keywords: Video-assisted thoracoscopic surgery (VATS); lobectomy; contraindications; positron emission tomography (PET)

Submitted Jun 04, 2013. Accepted for publication Jun 15, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.08
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.08

Introduction

Since the introduction of anatomic lung resection or lobectomy for lung cancer by video-assisted thoracoscopic surgery (VATS) in the 1990s, VATS has experienced major advances in both equipment and technique and has subsequently been demonstrated to be safe and effective for the treatment of early-stage lung cancer (1-5). It is associated with decreased morbidity and length of stay and offers equivalence in terms of survival and recurrence rates (6,7). As such, VATS lobectomy is now accepted as a standard surgical modality for early-stage lung cancer and has been gradually applied to more advanced disease (8). However, only a minority of lobectomies are performed using the VATS technique, as only approximately 45% of lobectomies registered in the Society of Thoracic

Surgeons database are performed thoracoscopically (9). Its adoption has been variable, likely due to perceived technical challenges when compared to an open approach and the concern for intraoperative complications, especially during a surgeon's learning curve, discouraging smaller centers from adopting VATS lobectomy (10).

Operative planning

As with most surgical procedures, the optimal strategy for managing complications of VATS pulmonary resections is to prevent their occurrence. VATS represents a new approach and not a new procedure. Therefore, the preoperative evaluation and indications for VATS major resections remains the same as for conventional resection. Avoiding complications is dependent on appropriate preoperative workup and patient selection. Planning for as safe a VATS resection as possible involves consideration of patient characteristics, the radiographic appearance of the area of lung to be removed, and the anticipated technical aspects of the case.

All patients have a preoperative examination with a positron emission tomography (PET), computed tomography (CT) scan, bronchoscopy, and endobronchial ultrasound/mediastinoscopy for preoperative staging (unless it is benign lung disease or a peripherally-located T1 tumor on PET) (11). Additionally, preoperative evaluation and staging for thoracoscopic resection should include pulmonary function tests (PFTs) with diffusion measurements. The performance of thoracoscopic procedures is usually dependent on the ability to achieve and maintain single-lung ventilation, which involves careful consideration of the patient's contralateral lung status. Obtaining quantitative ventilation-perfusion scans can help in determining the ability of a patient with marginal functional status to tolerate pulmonary resection. The lowest limits in lung function parameters that would still be considered acceptable for VATS lobectomy have not been scientifically studied (12), but this would depend upon, among other factors, the surgeon's judgment, experience, and technique; the contribution of the excised lobe to overall lung function; and the exact location of the pathology. Additionally, VATS resections have been shown to be able to be accomplished in patients with lung function who have typically been thought to be too poor to undergo more conventional resection via thoracotomy (13,14). We have performed lobectomies on selected patients whose forced expiratory volume in 1 second (FEV_1) was less than 30% predicted with excellent outcomes (15). In fact, one major advantage of VATS resection is that it allows recruitment of older and sicker patients with multiple comorbidities who would otherwise not be suitable candidates for resection through a conventional thoracotomy approach (13,16). Moreover, aggressive preoperative pulmonary rehabilitation can be considered in patients initially considered not to be candidates for resection owing to poor PFTs (17). Finally, patients who are not candidates for an anatomical resection could still be considered for VATS wedge resection (18). In all such cases, it is imperative to consider that conversion to thoracotomy is possible for all patients for whom VATS resection is planned.

Contraindications to VATS lobectomy

Since major lung resection by VATS was first introduced in the early 1990s, the indications and contraindications of these procedures have changed over time. Thus, whereas initially a history of prior surgery, endobronchial lesion, or even the administration of induction chemotherapy were regarded as contraindications, the experience that has since been gained, together with improvements in instrumentation and thoracoscopic imaging, have now changed this situation in most hospitals with experience in VATS. As such, recent studies have shown that lobectomy by VATS in cases of bronchogenic carcinoma with prior chemotherapy can be carried out safely and effectively without an increase in the rate of complications (19). And although endobronchial lesions were previously considered a contraindication for VATS, some authors do not consider this issue a contraindication at present (20). Furthermore, there are publications reporting on thoracoscopic sleeve resections (21).

Nevertheless, in addition to the general contraindications, such as recent myocardial infarction and severe coagulopathy, there remain a few absolute contraindications that are specifically applicable to VATS major resections. Apart from the inability to tolerate single lung ventilation, which is relatively uncommon, absolute contraindications to thoracoscopic lobectomy include the inability to achieve complete resection with lobectomy, lobectomy, T4 tumors, and N3 disease (22). Absolute tumor size criteria that would preclude VATS resections have not been defined, though large specimens (tumors greater than 6 cm in diameter) may not be amenable to removal without rib spreading; this tends to negate the benefit of minimal access surgery. Despite these previously cited absolute contraindications, the ideal patient for thoracoscopic lobectomy, particularly

early in a surgeon's experience performing the operation, is one with a peripheral T1 or T2 lesion without nodal disease.

It remains controversial as to whether VATS lobectomy is justified for lung cancer patients with lymph node metastasis (23). It was generally considered that patients with lymph node metastasis were not suitable candidates for VATS lobectomy (8,24). Additionally, it has been suggested that if a suspicious looking mediastinal lymph node is detected, it should be biopsied and a frozen section examination performed; confirmation of N2 disease mandates conversion to open surgery for complete mediastinal lymphadenectomy or induction chemotherapy depending on the exact circumstances (25). These guidelines have stemmed from a concern over incomplete lymph node dissection during VATS lobectomy. However, Watanabe *et al.* reported that the outcomes of VATS lobectomy were comparable to those of thoracotomy in clinical N0 but postoperative pathological N2 patients (26). Additionally, previous studies have compared the efficacy of a lymph node dissection of a VATS lobectomy with standard thoracotomy and have demonstrated that the results are similar (23,27,28). Nevertheless, it remains that in some institutions, preoperative or intraoperative lymph node metastasis is a contraindication for a VATS lobectomy and mandates conversion if discovered intraoperatively (29).

True pleural symphysis that leads to abandonment of the VATS approach is uncommon in our experience, but it may represent a contraindication for surgeons without extensive experience. Once a space is created when the correct plane in the pleural space is entered, endoscopic adhesiolysis can proceed quickly and safely using a combination of sharp and blunt dissection under videoscopic vision. VATS has the advantage over conventional thoracotomy in visualizing, with high resolution for details, the apex and base of the hemithorax.

Relative contraindications include tumors that are visible at bronchoscopy and the presence of hilar lymphadenopathy that would complicate vascular dissection (benign or malignant). Tumors visible in the bronchus by bronchoscopy within 2 cm of the origin of the lobe to be resected and where a possible sleeve resection might be needed are likely not amenable to a VATS approach. Calcified hilar adenopathy, such as with histoplasmosis, can likewise complicate vascular dissection (30).

The use of prior thoracic irradiation and induction therapy have previously been considered relative contraindications, but thoracoscopic lobectomy has been shown to be both safe and effective for patients who received induction therapy for non-small cell lung cancer (NSCLC) (19,31). Prior thoracic surgery, incomplete or absent fissures, and benign mediastinal adenopathy should not be considered contraindications. Redo-VATS surgery has been reported, and prior surgery is no longer considered an absolute contraindication to VATS resection (32). Though fused fissures present a technical challenge to VATS lobectomy, with experience and proper operative planning, successful lobectomy can be accomplished—the fused fissure should be divided last following the pulmonary vasculature and the bronchus. Finally, though chest wall involvement requires thoracotomy for resection, VATS can be used to perform the lung portion of the surgery and allow placement of the incision better situated for the area of the chest wall to be removed.

It is important to note that with improving surgeon experience and comfort with VATS lobectomy, just as several indications have been modified and expanded, the number of contraindications has been reduced. However, there remains some institutional variability in contraindications for this same reason. In a high-volume tertiary care institution experienced in the technique of VATS lobectomy such as our own, contraindications evolved to include a narrow patient population. Other institutions cite chest wall invasion, tumor infiltration beyond the fissure, invasion of the pericardium or diaphragm, centrally placed tumors in the hilum and adherent to vessels, as well as induction radiotherapy or chemotherapy as contraindications (11,33). Nevertheless, we do not consider these absolute contraindications. Additionally, evidence from our institution has shown VATS lobectomy to be safe and technically viable in patients receiving induction chemotherapy (19,31). As such, these additional institutional contraindications likely represent surgeon comfort and experience with VATS techniques rather than those deemed necessary for patient safety, anatomical reasons, and complete oncological resection.

Conversion to open thoracotomy

Conversion rates for thoracoscopic lobectomy to open thoracotomy have been reported to range from 2% to as high as 23%, with these higher rates stemming from patients with more advanced NSCLC (34-40). Krasna *et al.* reported an 8% conversion rate in 321 patients undergoing VATS procedures for various indications

(41). Most commonly the conversion to thoracotomy was deemed necessary because of oncological reasons, such as centrally located tumors requiring vascular control or sleeve resection, or unexpected T3-T4 tumors that infiltrate to the chest wall, diaphragm, or superior vena cava. These authors concluded that abnormal hilar nodes with granulomatous or metastatic disease adherent to the superior pulmonary vein may be better evaluated and more safely resected with thoracotomy. However, about 30% of thoracotomy conversions in this series were for non-oncological reasons, such as pleural adhesions (41). In the series of the Memorial Sloan-Kettering Cancer Center Thoracic Service, conversion to open thoracotomy because VATS was not "technically adequate" occurred in 44/410 patients (11%) (42). In a recent institutional study, our conversion rate was 4% (36/916) when patients had an attempted VATS lobectomy for lung cancer, with patients with clinically node-positive disease (N1-N3) having statistically significantly higher conversion rates than clinical N0 patients (43).

Overall, causes of conversion can generally be classified into four categories: intraoperative complications (e.g., bleeding from vascular injury, usually to branches of the pulmonary artery and occasionally injury to the pulmonary vein; bronchus injury by the endotracheal tube), technical problems (e.g., equipment or stapler malfunction, failure to progress, poor visualization), anatomical problems (e.g., absent or thick fissure, calcified peri-arterial lymph nodes, diffuse pleural adhesions, chest wall invasion, tumor size precluding removal through the utility incision, need for sleeve resection), and oncological conditions (e.g., intraoperative discovery of N2 tumors, invasion of the artery, invasion of the parietal pleura, positive margins that need to be extended). However, the ability to predict which patients are more likely to require conversion to thoracotomy has not been thoroughly addressed to date. Given that studies have demonstrated that emergent conversion to open thoracotomy has been found to be significantly correlated with VATS-associated complications during the first 30 postoperative days (44), the ability to anticipate patients that may be high-risk for conversion may prevent this unexpected eventuality and its associated morbidity.

One of the most dreaded complications for surgeons is massive bleeding from pulmonary vessels. Dense adhesive disease often increases the risk of vascular injury, necessitating conversion to an open procedure. It is important to note that even in such cases, dissection of vessels can generally be difficult, and risk of vessel injury and bleeding can be high even by thoracotomy. Both Craig *et al.* and Yim *et al.* have reported mechanical failure of the staplers that resulted in massive bleeding (45,46). In these cases, bleeding was controlled by pressing on the bleeder with a sponge stick and conversion to thoracotomy. It should be pointed out that these are anecdotal cases, and the mechanical staplers available now are generally very reliable, and while stapler malfunction may occur, it is relatively rare. Certain avoidable conditions have been incorrectly associated with the stapler. For example, the use of metal clips in the hilar dissection is discouraged, as the stapler will not function if a clip is included in the stapler's jaw. Additionally, attention to the amount of tension when retracting during the stapling of pulmonary artery branches is essential. If excess retraction is applied during the stapling process, the arterial branch may tear before the completion of the stapling when the linear strength of the artery is reduced with the initiation of this process. Additionally, several technical developments have avoided the bleeding problems and consequent conversion to thoracotomy that are pitfalls of VATS techniques (46). These include us of visceral pleura to buttress staple lines, routine use of vertically apposed staplers, and expertise in extracorporeal and intracorporeal knot tying with fine suture.

Nevertheless, these results highlight the fact that even in the event of significant bleeding from a major pulmonary vein or artery branch injury that cannot be repaired thoracoscopically, the source of bleeding can usually be identified and controlled with a thoracoscopic instrument to allow controlled and stable conversion to thoracotomy. However, these injuries are usually managed successfully without conversion by the experienced thoracoscopic surgeon. With advanced skill and experience in endoscopic suturing, in the event of minor to moderate bleeding from the pulmonary vasculature, conversion can often be avoided.

Video equipment malfunctions are unique to VATS compared with open thoracotomy. The surgeon must be prepared when video equipment failures occur to prevent complications from taking place as a result. The operating room team must have someone familiar with the set-up of the camera, light source, and monitors present at all times as well as the ability to obtain back-up equipment or contact an expert in the event of equipment failure. Additionally, the surgeon and the entire operative team must always be prepared with the instruments needed to convert to thoracotomy in the event of patient instability or non-recoverable video equipment problems.

An additionally described cause of conversion to open lobectomy is particular to areas in which histoplasmosis is

endemic, specifically states bordering the Ohio River valley and the lower Mississippi River, making the hilar dissection challenging (30). In a recent study by Samson *et al.*, patients with evidence of calcifications specifically involving the hilum of resection had a 37% risk of conversion, and those with evidence of calcifications along the bronchial tree, but not along the hilum of resection had an intermediate rate of conversion at 25% (47). In fact, calcification score was the only predictor of conversion to open thoracotomy in multivariable modeling including lobe resection, race, gender, reoperation status, age, body mass index, tumor size, baseline PFTs, and time since first VATS lobectomy case to factor in the possible learning curve effect. In another study examining unplanned conversion for VATS lobectomy by Park and colleagues, 41% of conversions were due to hilar nodal anthracofibrosis and hilar adhesions, and were associated with increased operative time and length of stay (48). When the authors retrospectively reviewed the CT scans, hilar calcifications were seen in 71% of these patients. In these cases, careful review of the preoperative chest CT scan is essential, focusing on calcifications in the hilum, especially at the origin of the lobar bronchus that is to be divided. To date, however, there are few studies evaluating the role of imaging studies in selecting the surgical approach for lobectomy, and those that do are limited to the size and location of the tumor. Mason and colleagues evaluated the role of imaging studies in predicting complications associated with VATS and demonstrated that pleural thickening and calcifications on CT or chest X-ray predicted difficulties (49). However, this study included all VATS procedures with only a small number of lobectomies.

Samson and colleagues additionally demonstrated, not surprisingly, that when compared with completed VATS, converted VATS operations were significantly more likely to result in postoperative atrial fibrillation, increased length of stay, increased duration of chest tube drainage, longer surgery time, and increase in estimated blood loss (47). Interestingly, on comparison of converted VATS to planned open thoracotomy, VATS conversion was only an independent predictor of longer length of stay, and combined mortality and morbidity were similar. In fact, several studies have examined the implications of unplanned conversion from VATS to thoracotomy. One study evaluated the outcomes in 26 patients who underwent a converted VATS procedure and compared them with the outcomes of 52 patients who underwent a planned thoracotomy. There were no significant differences between the groups in perioperative (30-day) or long-term outcomes (50). Sawada and colleagues found that VATS conversion was associated with increased blood loss, perioperative complications, and length of surgery compared with completed VATS, similar to the recent data of Samson and colleagues (47,51). Nevertheless, these authors concluded that patients with evidence of calcifications involving the hilum of resection can undergo attempted VATS lobectomy, but perhaps this should not be attempted during the learning curve or by surgeons who are not as experienced with open pulmonary resection in these patients.

The number of patients undergoing VATS lobectomy as opposed to an open procedure has significantly increased over recent years but conversion rates have fallen (52). The anticipated learning curve for an advanced minimally invasive procedure can be clearly tracked. Cause of conversion initially was for a variety of reasons, but with experience and as confidence levels increased, reason for conversion for anatomical reasons has also increased, possibly reflecting bolder patient selection or discomfort with a perceived anatomical problem, such as chest wall adhesions. In addition, there are oncological reasons a decision to convert may be taken, with tumor size and location and extranodal invasion by a metastatic node being obvious markers. However, apart from the latter case, the decision of conversion depends solely on the surgeon's preference. Several reports have supported the use of VATS for complete lymph node dissection and showed no significant differences in survival or recurrence between VATS and thoracotomy (8,53-55). Thus, in cases of gross lymph node metastasis, the decision to convert must be carefully weighed.

But as programs developed, despite increasing numbers of VATS resections, conversions for anatomical reasons have tended to fall as have conversions for vascular injury (53). This is explained by the experience gained in vascular dissection and in the management of the fissure, particularly in complex cases, post-chemotherapy patients and even reoperations. The nature of the conversion and whether conversion is controlled is important both for the obvious safety aspects of the patient but also for how smoothly the minimally invasive approach is perceived amongst colleagues as well as the confidence of the surgeons performing the VATS lobectomy.

Generally, high conversion rates have declined as surgeons became more familiar with advanced thoracoscopic lobectomy, an operation with a challenging learning curve. This trend has been demonstrated previously, with a decreasing proportion of conversions as an increasing

number of thoracoscopic lobectomies were performed for advanced-stage disease (35). And although conversion to thoracotomy should always be considered as a tool available to manage any unexpected situation, conversion rates have been shown to be as low as 1.6% to 2.5% in large series by experienced thoracoscopic surgeons (35,56). Further, though it is clear that the accumulation of experience has improved the surgical team's skill, allowing them to avoid and/or manage problems, resulting in a reduced conversion rate, these results also suggest that there remains a patient population in which VATS lobectomy is difficult to perform. It is generally accepted that dense hilar lymphadenopathy, pleural symphysis and fused fissure make VATS lobectomy difficult, and increase the likelihood of conversion to an open procedure. Specifically, persistent air leak beyond seven days was the most common morbidity seen in earlier experience and almost certainly related to hilar dissection when the fissures were incomplete (57).

Ultimately, the decision for conversion is left to each surgeon's skills and patience. It is difficult to establish any guideline for the conversion; however, our approximate timing of the decision for conversion is as follows: in cases with bleeding, as previously described, a sponge stick is first applied in order to tamponade the bleeding. Once the bleeding is controlled, a decision about whether or not the repair can be performed under VATS is made. When the bleeding cannot be controlled or repair seems to be difficult under VATS, conversion to thoracotomy is considered. In cases with a fused fissure or dense hilar lymphadenopathy, if the pulmonary artery cannot be isolated, conversion is considered.

Finally, although it may ultimately be difficult to predict who will require conversion from VATS to open surgery, there are a few important considerations regarding this matter. First, one of the advantages of VATS lobectomy is the magnified visualization it affords, which is useful for dissecting vessels or identifying small bleeders and makes this technique useful even in cases where conversion to an open procedure may be considered likely preoperatively. Secondly, after the surgeon's learning curve with advanced VATS techniques is surpassed and the conversion rate presumable reaches its nadir, attempts at decreasing conversion rates may only serve to delay the timing of conversion and increase the risks. The first objective of the operation is to perform a safe and complete resection. Once problems arise, repair takes a longer time, and the risks are increased. It is important not only to plan safe maneuvers to avoid problems, but also to have the courage to convert if there is any sense of discomfort experienced by the surgeon with VATS. Finally, long-term outcome is an important parameter to evaluate the safety and feasibility of converted VATs lobectomy. Jones *et al.* reported that the long-term outcome of converted VATS lobectomy for lung cancer was equivalent to that of successful VATS lobectomy (50). Therefore, it is reasonable to conclude that VATSs lobectomy is feasible for lung cancer surgery even from the viewpoint of the safety rate of converted VATS.

Conclusions

VATS was introduced nearly 20 years ago. Since then, VATS has experienced major advances in both equipment and technique, especially for the treatment of benign lung disease (58). With the accumulation of experience for the treatment of benign diseases, VATS has gradually begun to be employed for radical resection of lung cancer (3,4). VATS lobectomy is now considered standard in thoracic surgery, with acceptable safety and efficacy for both lung cancer and benign lung diseases (59,60). Several investigators have reported that the outcomes of VATS lobectomy for lung cancer are comparable to those of thoracotomy (35,38,61,62). While no large, controlled studies have been conducted to compare VATS with thoracotomy, it is now generally accepted that the outcomes of VATS are not inferior to those of thoracotomy. However, another concern is the safety of VATS lobectomy. Subsequent to VATS lobectomy, perioperative complications and mortality have been reported to occur at rates of approximately 5-32% and 0-7%, respectively; these rates are also generally accepted to be comparable to those reported for thoracotomy (35,38,63,64).

However, VATS lobectomy sometimes requires, for a variety of reasons, emergency conversion to thoracotomy. There are difficulties with the procedure, including a narrow view angle, complicating conditions such as pleural adhesions and dense hilar lymphadenopathy, oncologic problems if the disease is lung cancer, and the surgeon's discomfort with VATS instruments. As such, even though the technical safety of VATS lobectomy is widely accepted, there remains a range of situations that can result in unplanned conversion to open thoracotomy during the procedure, especially during a surgeon's training period (30).

The most important concern with unplanned conversions is the possible increased risk of mortality, morbidity, and cancer recurrence. Patients who undergo unplanned conversion to open thoracotomy most likely experience a

longer operating time, extra lung manipulation, increased risk of injury to adjacent tissue, and increased blood loss, which may all adversely affect the outcome. And although the safety and efficacy of successful VATS lobectomy has been documented by many authors, there are fewer data regarding failed VATS lobectomy. The few studies regarding this problem report no significant increase in mortality or morbidity (50,51). Apart from vascular and bronchial injuries, which result from technical problems, the other causes of conversion may be predictable preoperatively. For example, in light of clear hilar calcifications on preoperative CT, conversions due to anthracofibrosis may be able to be anticipated. Certain vascular anomalies resulting in conversion are often visible on preoperative enhanced CT. Finally, preoperative PET scans can show a high probability of lymphatic metastasis in cases converted because of gross metastasis of these lymph nodes. Although unexpected conversion to thoracotomy during VATS does not appear to compromise prognosis, the decision to convert must be made promptly to reduce the operating time, blood loss, and possible complications. Accordingly, when attempting a VATS procedure, access ports must be placed to facilitate immediate conversion to open thoracotomy and to support instrument manipulation and anatomic accessibility of the stapler to close vessels and the bronchus. And in the context of narrowing contraindications for VATS lobectomy and surgeons overcoming the learning curve associated with increasingly complex resections, conversion should not be regarded as a surgical failure but rather as a way to safely complete resections in a traditional manner.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Daniels LJ, Balderson SS, Onaitis MW, et al. Thoracoscopic lobectomy: a safe and effective strategy for patients with stage I lung cancer. Ann Thorac Surg 2002;74:860-4.
2. Swanson SJ, Herndon JE 2nd, D'Amico TA, et al. Video-assisted thoracic surgery lobectomy: report of CALGB 39802--a prospective, multi-institution feasibility study. J Clin Oncol 2007;25:4993-7.
3. Roviaro G, Rebuffat C, Varoli F, et al. Videoendoscopic pulmonary lobectomy for cancer. Surg Laparosc Endosc 1992;2:244-7.
4. Landreneau RJ, Hazelrigg SR, Ferson PF, et al. Thoracoscopic resection of 85 pulmonary lesions. Ann Thorac Surg 1992;54:415-9; discussion 419-20.
5. Lewis RJ, Sisler GE, Caccavale RJ. Imaged thoracic lobectomy: should it be done? Ann Thorac Surg 1992;54:80-3.
6. Paul S, Altorki NK, Sheng S, et al. Thoracoscopic lobectomy is associated with lower morbidity than open lobectomy: a propensity-matched analysis from the STS database. J Thorac Cardiovasc Surg 2010;139:366-78.
7. Tomaszek SC, Cassivi SD, Shen KR, et al. Clinical outcomes of video-assisted thoracoscopic lobectomy. Mayo Clin Proc 2009;84:509-13.
8. Shiraishi T, Hiratsuka M, Yoshinaga Y, et al. Thoracoscopic lobectomy with systemic lymph node dissection for lymph node positive non-small cell lung cancer--is thoracoscopic lymph node dissection feasible? Thorac Cardiovasc Surg 2008;56:162-6.
9. Ceppa DP, Kosinski AS, Berry MF, et al. Thoracoscopic lobectomy has increasing benefit in patients with poor pulmonary function: a society of thoracic surgeons database analysis. Ann Surg 2012;256:487-93.
10. McKenna RJ Jr, Houck WV. New approaches to the minimally invasive treatment of lung cancer. Curr Opin Pulm Med 2005;11:282-6.
11. Hansen HJ, Petersen RH. Video-assisted thoracoscopic lobectomy using a standardized three-port anterior approach—the Copenhagen experience. Ann Cardiothorac Surg 2012;1:70-6.
12. Meyer DM. Preoperative assessment for video-assisted thoracic surgery. In: Yim APC, Hazelrigg SR, Izzat MB, et al. eds. Minimal access cardiothoracic surgery. Philadelphia: WB Saunders, 2000:52-67.
13. Demmy TL, Curtis JJ. Minimally invasive lobectomy directed toward frail and high-risk patients: a case-control study. Ann Thorac Surg 1999;68:194-200.
14. Garzon JC, Ng CS, Sihoe AD, et al. Video-assisted thoracic surgery pulmonary resection for lung cancer in patients with poor lung function. Ann Thorac Surg 2006;81:1996-2003.
15. Berry MF, Villamizar-Ortiz NR, Tong BC, et al. Pulmonary function tests do not predict pulmonary complications after thoracoscopic lobectomy. Ann Thorac Surg 2010;89:1044-51; discussion 1051-2.
16. Berry MF, Hanna J, Tong BC, et al. Risk factors for morbidity after lobectomy for lung cancer in elderly patients. Ann Thorac Surg 2009;88:1093-9.
17. Berry MF, D'Amico TA. Complications of thoracoscopic

pulmonary resection. Semin Thorac Cardiovasc Surg 2007;19:350-4.
18. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung cancer study group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
19. Petersen RP, Pham D, Toloza EM, et al. Thoracoscopic lobectomy: a safe and effective strategy for patients receiving induction therapy for non-small cell lung cancer. Ann Thorac Surg 2006;82:214-8; discussion 219.
20. Balsara KR, Balderson SS, D'Amico TA. Surgical techniques to avoid parenchymal injury during lung resection (fissureless lobectomy). Thorac Surg Clin 2010;20:365-9.
21. Mahtabifard A, Fuller CB, McKenna RJ Jr. Video-assisted thoracic surgery sleeve lobectomy: a case series. Ann Thorac Surg 2008;85:S729-32.
22. Burfiend WR, D'Amico TA. Thoracoscopic lobectomy. Operat Tech Thorac Cardiovasc Surg 2004;9:98-114.
23. Sagawa M, Sato M, Sakurada A, et al. A prospective trial of systematic nodal dissection for lung cancer by video-assisted thoracic surgery: can it be perfect? Ann Thorac Surg 2002;73:900-4.
24. Naruke T, Tsuchiya R, Kondo H, et al. Lymph node sampling in lung cancer: how should it be done? Eur J Cardiothorac Surg 1999;16:S17-24.
25. Yim AP. VATS major pulmonary resection revisited--controversies, techniques, and results. Ann Thorac Surg 2002;74:615-23.
26. Watanabe A, Mishina T, Ohori S, et al. Is video-assisted thoracoscopic surgery a feasible approach for clinical N0 and postoperatively pathological N2 non-small cell lung cancer? Eur J Cardiothorac Surg 2008;33:812-8.
27. Kaseda S, Hangai N, Yamamoto S, et al. Lobectomy with extended lymph node dissection by video-assisted thoracic surgery for lung cancer. Surg Endosc 1997;11:703-6.
28. Watanabe A, Koyanagi T, Ohsawa H, et al. Systematic node dissection by VATS is not inferior to that through an open thoracotomy: a comparative clinicopathologic retrospective study. Surgery 2005;138:510-7.
29. Roviaro G, Varoli F, Vergani C, et al. Long-term survival after videothoracoscopic lobectomy for stage I lung cancer. Chest 2004;126:725-32.
30. Reed MF, Lucia MW, Starnes SL, et al. Thoracoscopic lobectomy: introduction of a new technique into a thoracic surgery training program. J Thorac Cardiovasc Surg 2008;136:376-81.
31. Petersen RP, Pham D, Burfeind WR, et al. Thoracoscopic lobectomy facilitates the delivery of chemotherapy after resection for lung cancer. Ann Thorac Surg 2007;83:1245-9; discussion 1250.
32. Yim AP, Liu HP, Hazelrigg SR, et al. Thoracoscopic operations on reoperated chests. Ann Thorac Surg 1998;65:328-30.
33. Loscertales J, Quero Valen Zuela F, Congregado M, et al. Video-assisted thoracic surgery lobectomy: results in lung cancer. J Thorac Dis 2010;2:29-35.
34. Hennon M, Sahai RK, Yendamuri S, et al. Safety of thoracoscopic lobectomy in locally advanced lung cancer. Ann Surg Oncol 2011;18:3732-6.
35. McKenna RJ Jr, Houck W, Fuller CB. Video-assisted thoracic surgery lobectomy: experience with 1,100 cases. Ann Thorac Surg 2006;81:421-5; discussion 425-6.
36. Roviaro G, Varoli F, Vergani C, et al. Video-assisted thoracoscopic major pulmonary resections: technical aspects, personal series of 259 patients, and review of the literature. Surg Endosc 2004;18:1551-8.
37. Solaini L, Prusciano F, Bagioni P, et al. Video-assisted thoracic surgery (VATS) of the lung: analysis of intraoperative and postoperative complications over 15 years and review of the literature. Surg Endosc 2008;22:298-310.
38. Walker WS, Codispoti M, Soon SY, et al. Long-term outcomes following VATS lobectomy for non-small cell bronchogenic carcinoma. Eur J Cardiothorac Surg 2003;23:397-402.
39. Gharagozloo F, Tempesta B, Margolis M, et al. Video-assisted thoracic surgery lobectomy for stage I lung cancer. Ann Thorac Surg 2003;76:1009-14; discussion 1014-5.
40. Nomori H, Horio H, Naruke T, et al. What is the advantage of a thoracoscopic lobectomy over a limited thoracotomy procedure for lung cancer surgery? Ann Thorac Surg 2001;72:879-84.
41. Krasna MJ, Deshmukh S, McLaughlin JS. Complications of thoracoscopy. Ann Thorac Surg 1996;61:1066-9.
42. Yim AP. Port-site recurrence following video-assisted thoracoscopic surgery. Surg Endosc 1995;9:1133-5.
43. Villamizar NR, Darrabie M, Hanna J, et al. Impact of T status and N status on perioperative outcomes after thoracoscopic lobectomy for lung cancer. J Thorac Cardiovasc Surg 2013;145:514-20; discussion 520-1.
44. Winter H, Meimarakis G, Pirker M, et al. Predictors of general complications after video-assisted thoracoscopic surgical procedures. Surg Endosc 2008;22:640-5.
45. Yim AP, Ho JK. Malfunctioning of vascular staple cutter during thoracoscopic lobectomy. J Thorac Cardiovasc

Surg 1995;109:1252.
46. Craig SR, Walker WS. Potential complications of vascular stapling in thoracoscopic pulmonary resection. Ann Thorac Surg 1995;59:736-7; discussion 737-8.
47. Samson P, Guitron J, Reed MF, et al. Predictors of conversion to thoracotomy for video-assisted thoracoscopic lobectomy: a retrospective analysis and the influence of computed tomography-based calcification assessment. J Thorac Cardiovasc Surg 2013;145:1512-8.
48. Park JS, Kim HK, Choi YS, et al. Unplanned conversion to thoracotomy during video-assisted thoracic surgery lobectomy does not compromise the surgical outcome. World J Surg 2011;35:590-5.
49. Mason AC, Krasna MJ, White CS. The role of radiologic imaging in diagnosing complications of video-assisted thoracoscopic surgery. Chest 1998;113:820-5.
50. Jones RO, Casali G, Walker WS. Does failed video-assisted lobectomy for lung cancer prejudice immediate and long-term outcomes? Ann Thorac Surg 2008;86:235-9.
51. Sawada S, Komori E, Yamashita M. Evaluation of video-assisted thoracoscopic surgery lobectomy requiring emergency conversion to thoracotomy. Eur J Cardiothorac Surg 2009;36:487-90.
52. Gazala S, Hunt I, Valji A, et al. A method of assessing reasons for conversion during video-assisted thoracoscopic lobectomy. Interact Cardiovasc Thorac Surg 2011;12:962-4.
53. Kim K, Kim HK, Park JS, et al. Video-assisted thoracic surgery lobectomy: single institutional experience with 704 cases. Ann Thorac Surg 2010;89:S2118-22.
54. Shaw JP, Dembitzer FR, Wisnivesky JP, et al. Video-assisted thoracoscopic lobectomy: state of the art and future directions. Ann Thorac Surg 2008;85:S705-9.
55. Kim HK, Choi YS, Kim J, et al. Outcomes of unexpected pathologic N1 and N2 disease after video-assisted thoracic surgery lobectomy for clinical stage I non-small cell lung cancer. J Thorac Cardiovasc Surg 2010;140:1288-93.
56. Onaitis MW, Petersen RP, Balderson SS, et al. Thoracoscopic lobectomy is a safe and versatile procedure: experience with 500 consecutive patients. Ann Surg 2006;244:420-5.
57. Yim AP, Ko KM, Chau WS, et al. Video-assisted thoracoscopic anatomic lung resections. The initial Hong Kong experience. Chest 1996;109:13-7.
58. Levi JF, Kleinmann P, Riquet M, et al. Percutaneous parietal pleurectomy for recurrent spontaneous pneumothorax. Lancet 1990;336:1577-8.
59. Nicastri DG, Wisnivesky JP, Litle VR, et al. Thoracoscopic lobectomy: report on safety, discharge independence, pain, and chemotherapy tolerance. J Thorac Cardiovasc Surg 2008;135:642-7.
60. Yan TD, Black D, Bannon PG, et al. Systematic review and meta-analysis of randomized and nonrandomized trials on safety and efficacy of video-assisted thoracic surgery lobectomy for early-stage non-small-cell lung cancer. J Clin Oncol 2009;27:2553-62.
61. Shigemura N, Akashi A, Funaki S, et al. Long-term outcomes after a variety of video-assisted thoracoscopic lobectomy approaches for clinical stage IA lung cancer: a multi-institutional study. J Thorac Cardiovasc Surg 2006;132:507-12.
62. Sakuraba M, Miyamoto H, Oh S, et al. Video-assisted thoracoscopic lobectomy vs. conventional lobectomy via open thoracotomy in patients with clinical stage IA non-small cell lung carcinoma. Interact Cardiovasc Thorac Surg 2007;6:614-7.
63. Lewis RJ, Caccavale RJ, Bocage JP, et al. Video-assisted thoracic surgical non-rib spreading simultaneously stapled lobectomy: a more patient-friendly oncologic resection. Chest 1999;116:1119-24.
64. Whitson BA, Andrade RS, Boettcher A, et al. Video-assisted thoracoscopic surgery is more favorable than thoracotomy for resection of clinical stage I non-small cell lung cancer. Ann Thorac Surg 2007;83:1965-70.

Cite this article as: Hanna JM, Berry MF, D'Amico TA. Contraindications of video-assisted thoracoscopic surgical lobectomy and determinants of conversion to open. J Thorac Dis 2013;5(S3):S182-S189. doi: 10.3978/j.issn.2072-1439.2013.07.08

Current costs of video-assisted thoracic surgery (VATS) lobectomy

Tunc Lacin, Scott Swanson

Department of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Correspondence to: Tunc Lacin, MD. Department of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. Email: tlacin@partners.org or tunclacin@gmail.com.

Abstract: Video-assisted thoracoscopic lobectomy has many benefits over open surgery such as smaller incisions, less pain, less blood loss, faster postoperative recovery, shortened hospital stay, similar or superior survival rates. In contrast video-assisted thoracic surgery (VATS) has higher equipment costs, increased operating room times, at least initially, and a learning curve for the team. However when an experienced surgeon performs the surgery, significant hospital savings combined with better outcomes are achieved by video-assisted thoracoscopic lobectomy.

Keywords: Video-assisted thoracic surgery (VATS); lobectomy; costs; quality of life

Submitted Jul 05, 2013. Accepted for publication Jul 07, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.13
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.13

Background

Video-assisted thoracic surgery (VATS) has been used more and more in daily practice for diagnosis and treatment of lung diseases especially non-small cell lung carcinoma in the last decade (1,2). Despite the growing enthusiasm for VATS resections, this minimally invasive technique has had slow adoption due to concerns regarding oncologic principles, costs, possible complications, time spent on learning curve and lack of surgeon training (3). Potential benefits of VATS for lung resections are listed in the literature as smaller incisions, less pain, less blood loss, less respiratory compromise, shortened hospital lengths of stay and at least similar survival rates (3,4). VATS lobectomy is oncologically the same surgical procedure as a lobectomy through a thoracotomy; both use anatomic resection, individual hilar ligation, and lymph node sampling or dissection (4). Several reports indicate that the number of dissected lymph nodes is similar between VATS lobectomy and thoracotomy (5,6), although other reports question this assertion. Five year survival rates are comparable and in at least several meta-analyses better (7,8). The greatest advantage of a VATS lobectomy may be an improvement in perioperative quality of life (4). According to Demmy and colleagues' data, more patients who underwent thoracotomy required skilled nursing facilities after surgery (9) compared with a VATS approach. Several series have demonstrated that early postoperative pain is significantly less with VATS lobectomy (4,10). Patient who undergo VATS have a quicker recovery and have more strength to tolerate chemotherapy. As a result, theoretically, survival benefit will be higher if chemotherapy is started immediately after surgery (4). Postoperative pulmonary function also appears to be better after VATS than after a thoracotomy. In a nonrandomized comparison of patients who had a lobectomy by a thoracotomy or VATS, postoperative PaO_2, O_2 saturation, peak flow rates, forced expiratory volume in 1 second and forced vital capacity on both postoperative days 7 and 14 were better for the patients who had undergone the VATS procedure (11). The VATS patients have less impairment of pulmonary function and a better 6-min walk test than thoracotomy patients (12).

Recent data supporting advantages of VATS lobectomy

Several single institution series and a recent Society of Thoracic Surgeons (STS) database have demonstrated that compared with open thoracotomy, video-assisted thoracoscopic lobectomy may be associated with fewer postooperative complications (13). In the study of Paul

Table 1 The analysis of costs, surgery time and length of stay in open versus VATS lobectomy (3)

Procedure dependent variant	Lobectomy		P value
	Adjusted outcome	Standard deviation	
Hospital costs (dollars)			0.027
Open	$21,016.04	$5,645.14	
VATS	$20,316.19	$5,457.15	
Surgery time (hours)			0.000
Open	3.75	0.47	
VATS	4.09	0.52	
Length of stay (days)			0.000
Open	7.83	2.05	
VATS	6.15	1.61	

et al. 73.8 % of patients who underwent video-assisted thoracoscopic lobectomy had no complications, where as 65.3% of patients underwent lobectomy via thoracotomy had no complications. Compared with open lobectomy, video-assisted thoracoscopic lobectomy was associated with a lower incidence of arrhythmias, reintubation, blood transfusion as well as a shorter hospital stay and chest tube duration (13). In addition to these early functional advantages, video-assisted thoacoscopic lobectomy has been shown to have comparable long-term outcomes (14,15). The peri-operative advantages as well as the short and long-term outcomes reported have assuaged the concerns of the safety and efficacy aspects of video-assisted resections for the thoracic oncology patient population. However the drawbacks to VATS include higher equipment costs, longer operative room times and steeper learning curves for surgeons and operating room personnel (3).

Economic comparison of VATS versus open lobectomy

In a recent study our group compared hospital costs and perioperative outcomes for video-assisted thoracoscopic surgery and open lobectomy procedures in the United States using the Premier Prospective Database (Premier Inc, Charlotte, NC) (3). The study included the time period from the third quarter of 2007 through 2008. A total of 3,961 patients (open n=2,907, VATS n=1,054) were included in this evaluation. Length of stay was 7.83 days versus 6.15 days for open versus VATS. Surgery duration was shorter for open procedures at 3.75 versus 4.09 hours for VATS (*Table 1*) (3). The risk of adverse events was significantly lower in the VATS group (P=0.019) (3). Although statistically not significant, pneumonia occurred more frequently in the open group (9.1%) versus VATS (8.1%). Arrhythmias, other cardiac events and bleeding were found to be significantly more prevalent in the open group than in the VATS group. The frequency of patients with prolonged lengths of stay (>14 days) was higher in the open group than in the VATS group. Hospital costs were higher for open versus VATS; $21,016 versus $20,316 (P=0.027). Given that there is both a reduction in adverse events and a 1.68 day reduction in length of stay with VATS, one might expect the difference in cost between open and VATS to be greater than $700. Therefore, we looked at surgeon experience to determine if this played a role in cost. We examined surgeon experience with VATS over the 6 months prior to each operation and found a significant association between surgeon experience and cost. Average costs ranged from $22,050 for low volume surgeons to $18,133 for high volume surgeons. For open lobectomies, cost differences by surgeon experience were not significant and both levels were estimated at $21,000. These data suggest that economic impact is magnified as the surgeon's experience increases.

In another recent retrospective study the relationship between volume and outcome in VATS surgery was evaluated (16). This relationship was striking for cost and utilization outcomes and VATS lobectomy as compared to VATS wedge resection. Outcomes following VATS surgery seems to be strongly associated with experience (16). This report showed that the reduction in cost and resource utilization increases significantly with greater experience and is most marked for VATS lobectomy for lung cancer. Moreover, thoracic surgeons have better VATS outcomes than non-thoracic surgeons and greater experience with open procedures does not correlate with better VATS outcomes. These findings reinforce the need for surgeons to focus on their VATS technique to achieve the best outcomes.

Another report on cost of VATS lobectomies revealed that the total hospital costs in the VATS group were lower than for those in the open lobectomy group ($5,391 *vs.* $5,593) (17). The reasons for the higher total hospital costs for open lobectomy were explained as longer hospital stays, longer chest tube duration and the need for more medications to control pain. Pulmonary complications, including respiratory dysfunction, pneumonia, atelectasis, empyema and prolonged air leak were less common with VATS approach in this series. A subset of patients in this

group were compared according to the surgeon's experience (early learning period *vs.* experienced learning period). Because of the decreased operation duration during the experienced learning period, the cost of anesthesia was significantly lower for these patients compared with those during the early period (17).

As the cost of surgical disposables play an important role in the total cost of VATS lobectomy, differences in the cost of resection of different lobes are also recorded (17,18). Casali and Walker demonstrated that upper lobectomy is more expensive than other types of lobectomy and that the difference in cost is mainly due to different need for the number of stapler cartridges (18). Cho demonstrated that the cost of surgical materials for resection of a lower lobe was lower than that for resection of the an upper lobe. The cost was $1,630 *vs.* $1,981 for right side and $1,655 *vs.* $1,908 for left side. When the total hospital costs were evaluated between the VATS lobectomy and open lobectomy groups for the five different lobes, VATS lobectomy for the left lower lobe was much more cost-effective than open lobectomy, although the difference was not statistically significant (17).

Using robotic technology to perform pulmonary surgery is of great current interest to the thoracic surgical community (19). Robotic lobectomies have been performed on a limited basis, with the advocates suggesting that the visualization and dissection are superior compared with a VATS approach. Robotic technology does have a certain appeal. The arms have a wrist-like movement and the magnification and depth of field of the robotic camera are superior to the standard VATS camera. However, it is not clear that these are significant advantages compared with VATS in the realm of cancer surgery. Compared with a VATS approach, the robotic incisions are the same size, the stapling instruments are the same, and the removal of the specimen is the same. The safety of VATS dissection of the vascular structures is excellent, with minimal reported problems after more than 17 years of experience. The completeness of lymph node dissection is complete with VATS and is not better with the robot, at least to date. Also, the surgical time and cost are significantly less for VATS (20). Robotic lobectomy has higher associated costs than VATS, primarily attributed to increased costs of the first hospital day, but it is less costly than thoracotomy approach for lobectomy (21). The average cost of VATS is substantially less than thoracotomy primarily because of a decreased length of stay. The cost of robotic assistance for VATS is still less than thoracotomy, but greater than VATS alone (21).

Conclusions

Minimally invasive techniques, such as VATS and robotics, are becoming the preferred approach in many surgical disciplines. Lobectomy performed by the VATS approach as compared with an open technique results in shorter length of stay, fewer adverse events and less overall cost. Patients who undergo VATS are discharged without home assistance and have low opiate requirements. Where there may be concern over the cost of the thoracoscopic equipment required for VATS, the significant hospital savings combined with better outcomes, particularly when an experienced surgeon performs the surgery, clearly favor the VATS approach over a thoracotomy. As the demand for health care resources increases, we must pay more attention to cost. Data, to date, shows a significant cost savings when a VATS approach is used compared to a thoracotomy for resection of lung cancer while enhancing short term outcomes and likely comparable or improved long term survival.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. McKenna RJ Jr, Houck W, Fuller CB. Video-assisted thoracic surgery lobectomy: experience with 1,100 cases. Ann Thorac Surg 2006;81:421-5; discussion 425-6.
2. Swanson SJ, Herndon JE 2nd, D'Amico TA, et al. Video-assisted thoracic surgery lobectomy: report of CALGB 39802--a prospective, multi-institution feasibility study. J Clin Oncol 2007;25:4993-7.
3. Swanson SJ, Meyers BF, Gunnarsson CL, et al. Video-assisted thoracoscopic lobectomy is less costly and morbid than open lobectomy: a retrospective multiinstitutional database analysis. Ann Thorac Surg 2012;93:1027-32.
4. Nicastri DG, Wisnivesky JP, Litle VR, et al. Thoracoscopic lobectomy: report on safety, discharge independence, pain, and chemotherapy tolerance. J Thorac Cardiovasc Surg 2008;135:642-7.
5. Kondo T, Sagawa M, Tanita T, et al. Is complete systematic nodal dissection by thoracoscopic surgery possible? A prospective trial of video-assisted lobectomy for cancer of the right lung. J Thorac Cardiovasc Surg 1998;116:651-2.
6. Sagawa M, Sato M, Sakurada A, et al. A prospective trial of systematic nodal dissection for lung cancer by video-

assisted thoracic surgery: can it be perfect? Ann Thorac Surg 2002;73:900-4.
7. Walker WS, Codispoti M, Soon SY, et al. Long-term outcomes following VATS lobectomy for non-small cell bronchogenic carcinoma. Eur J Cardiothorac Surg 2003;23:397-402.
8. McKenna RJ Jr, Wolf RK, Brenner M, et al. Is lobectomy by video-assisted thoracic surgery an adequate cancer operation? Ann Thorac Surg 1998;66:1903-8.
9. Demmy TL, Plante AJ, Nwogu CE, et al. Discharge independence with minimally invasive lobectomy. Am J Surg 2004;188:698-702.
10. Nomori H, Horio H, Naruke T, et al. What is the advantage of a thoracoscopic lobectomy over a limited thoracotomy procedure for lung cancer surgery? Ann Thorac Surg 2001;72:879-84.
11. Nakata M, Saeki H, Yokoyama N, et al. Pulmonary function after lobectomy: video-assisted thoracic surgery versus thoracotomy. Ann Thorac Surg 2000;70:938-41.
12. Nomori H, Ohtsuka T, Horio H, et al. Difference in the impairment of vital capacity and 6-minute walking after a lobectomy performed by thoracoscopic surgery, an anterior limited thoracotomy, an anteroaxillary thoracotomy, and a posterolateral thoracotomy. Surg Today 2003;33:7-12.
13. Paul S, Altorki NK, Sheng S, et al. Thoracoscopic lobectomy is associated with lower morbidity than open lobectomy: a propensity-matched analysis from the STS database. J Thorac Cardiovasc Surg 2010;139:366-78.
14. D'Amico TA. Long-term outcomes of thoracoscopic lobectomy. Thorac Surg Clin 2008;18:259-62.
15. Yamamoto K, Ohsumi A, Kojima F, et al. Long-term survival after video-assisted thoracic surgery lobectomy for primary lung cancer. Ann Thorac Surg 2010;89:353-9.
16. David G, Gunnarsson CL, Moore M, et al. Surgeons' volume-outcome relationship for lobectomies and wedge resections for cancer using video-assisted thoracoscopic techniques. Minim Invasive Surg 2012;2012:760292.
17. Cho S, Do YW, Lee EB. Comparison of costs for video-assisted thoracic surgery lobectomy and open lobectomy for non-small cell lung cancer. Surg Endosc 2011;25:1054-61.
18. Casali G, Walker WS. Video-assisted thoracic surgery lobectomy: can we afford it? Eur J Cardiothorac Surg 2009;35:423-8.
19. Veronesi G, Galetta D, Maisonneuve P, et al. Four-arm robotic lobectomy for the treatment of early-stage lung cancer. J Thorac Cardiovasc Surg 2010;140:19-25.
20. Swanson SJ. Robotic pulmonary lobectomy--the future and probably should remain so. J Thorac Cardiovasc Surg 2010;140:954.
21. Park BJ, Flores RM. Cost comparison of robotic, video-assisted thoracic surgery and thoracotomy approaches to pulmonary lobectomy. Thorac Surg Clin 2008;18:297-300, vii.

Cite this article as: Lacin T, Swanson S. Current costs of video-assisted thoracic surgery (VATS) lobectomy. J Thorac Dis 2013;5(S3):S190-S193. doi: 10.3978/j.issn.2072-1439.2013.07.13

Indication for VATS sublobar resections in early lung cancer

Antonio E. Martin-Ucar[1], Maria Delgado Roel[2]

[1]Department of Thoracic Surgery, Nottingham University Hospitals NHS Trust, Nottingham, UK; [2]Complejo Hospitalario Universitario A Coruna, La Coruna, Spain

Correspondence to: Mr Antonio E. Martin-Ucar, Consultant, Thoracic Surgeon. Department of Thoracic Surgery, Nottingham University Hospitals NHS Trust, City Campus, Hucknall Road, Nottingham NG5 1PB, UK. Email: lungsout@yahoo.com.

Abstract: When dealing with early non-small cell lung cancer (NSCLC) sublobar resections still remain part of the surgical armamentarium. In selected patients with lung cancer, the combination of the potential benefits of parenchyma sparing procedures to the limited trauma provided by Video Assisted Thoracic Surgery (VATS) techniques can become very appealing. Two main groups are included: non-anatomical (wedges) and anatomical (segmentectomies) excisions. We describe the techniques, results and potential indications of both of these techniques.

Keywords: Minimally invasive surgery; segmentectomy; wedge

Submitted Jul 22, 2013. Accepted for publication Aug 10, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.41
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.41

Introduction

At present, surgery remains the most used radical treatment for early stage non-small cell lung cancer (NSCLC) (1). Lobectomy has been traditionally considered the gold standard procedure for early NSCLC following the Lung Cancer Study Group (LCSG) randomized controlled trial (2). However, the attempt to increase resection rates led to the need to offer surgery to patients with higher surgical risks: the elderly, the breathless and the ones with multiple co-morbidities (3-5). To manage these potential surgical risks and the possible long-term impairment in quality of life and respiratory function, surgeons have applied sublobar techniques to the management of lung cancer. These can be divided very clearly into two groups: non-anatomical resections (wedge) and anatomical resections (segmentectomies). The difference is the attempt during segmentectomies to follow the oncological principles of a lobectomy by achieving anatomical division of segmental veins, arteries and bronchi as well as good parenchymal clearance.

Video Assisted Thoracic Surgery (VATS) is on the increase in the management of benign and malignant processes. Large experiences have convinced the surgical community not only of the safety and possibilities of VATS surgery in early lung cancer, but of the benefits when compared to open surgery in terms of postoperative pain, length of recovery, return to activities, immune response to surgery and oncological results (6-9). As with open surgery where there is a variety of surgical approaches described (posterolateral, anterior, muscle-sparing, hybrid thoracotomies), VATS can also be performed with different surgical accesses: posterior approach, anterior approach, 2-port approach and single-port access (10-13).

We aimed to explore the potential possibilities and current experiences of the combination of sublobar resections and VATS techniques for early NSCLC.

Non-anatomical sublobar resections (wedge)

Wedge resections involve the excision of a pulmonary lesion with clear parenchymal margins with no attempt to deal with the hilar lobar structures (arteries, veins or bronchi). Although traditionally has been considered as a compromise operation due to the results of the LCSG trial that reported increase local recurrence compared to lobectomy, the indications for wedge excisions may be on the increase (2). Invariably, it is necessary that the lesion is peripheral so it can be identified and "wedged out" safely with sufficient margins. Despite the theoretical limitations as a sound

Video 1 Prompt identification and excision of a peripheral pulmonary nodule at the base of the left lower lobe.

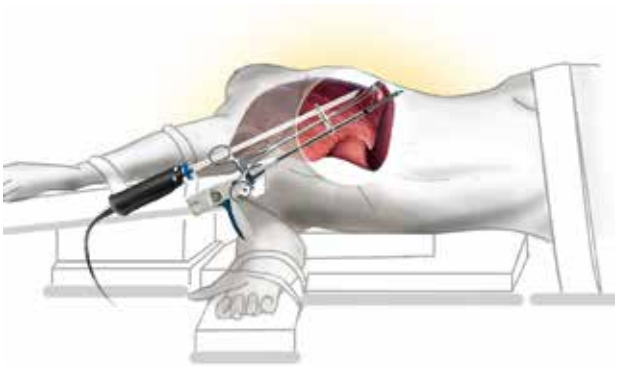


Figure 1 Diagram showing the position of the optics and instruments during VATS wedge excision of a pulmonary nodule.

oncologic procedure, wedge resection has continuously been used in certain circumstances for patients with lung cancer (14,15).

Technique

Wedge resections can be performed via VATS using a number of incisions including the single-port approach (16). Ideally the lung should be collapsed as it facilitates location of pulmonary nodules and instrumentation, but it can potentially be performed in a ventilated lung in patients that can't tolerate single lung ventilation. There are different ways to identify the lesions including palpation with instruments or the tip of the finger, but also more complex techniques using technology such as placement of metal wires/coils (17,18), instillation of different contrasts (19-21) or use of intraoperative ultrasound techniques (22).

Once the nodule has been identified, surgical staplers are applied to excise and seal the pulmonary parenchyma with clear margins. A brief example of a diagnostic excision of a nodule in the left lower lobe via a single port incision is demonstrated in *Video 1* with the position of the incision and instruments is illustrated in *Figure 1*.

Results

There is very limited evidence available to assess the role of wedge resections in lung cancer. One randomized controlled trial by the LCSG reported a similar survival, but increased recurrence of cancer in patients undergoing sublobar compared to lobar resections (2). The surgical community accepted the results and acknowledged the effort of the trialists and, even accepting the trial limitations, considered lobectomy as the procedure of choice for early lung cancer thus reserving sublobar resections for specific cohorts of patients who might benefit of the preservation of the parenchyma or a quicker procedure.

The experiences reported in the use of VATS wedge resections when compared to lobectomy are consistent with traditional reports in the thoracotomy approach. Wolf et al reported a retrospective comparative series of 154 sublobar resections (43% via VATS) and 84 lobectomies (10% via VATS) performed in patients with small early lung cancer. Patients who underwent lobectomy had a better survival and disease-free survival, but the sublobar group was significantly older and with worse respiratory reserve, highlighting the selection bias in this and every other study of its kind (23). Landreneau *et al.* reached similar conclusions in a multicenter study evaluating 102 wedge resections (60% by VATS) when compared to lobectomies (24).

One of the potential limitations of the use of VATS in deep-sited small lesions is the difficulty to locate them during surgery. The use of technologies has helped the identification of these nodules. Lee *et al.* were successful in 101 of 103 cases with small pulmonary nodules with the wire location techniques with an average operative time of 11 minutes (16). Molins *et al.* reported 50 out of 52 patients successfully underwent VATS excision of small nodules also identified by wires in the ambulatory setting (18). Similar success rates are reported by surgeons using different markers (methylene blue, radionuclides or contrast) (19-21). Finally, the use of intraoperative ultrasound has been reported by VATS, even in the single-port approach (25). Whatever the technology available, all these techniques seem to aid in identification of deep or small nodules during VATS surgery.

Video 2 Division of pulmonary artery, vein and segmental bronchus during anatomical left apical upper tri-segmentectomy.

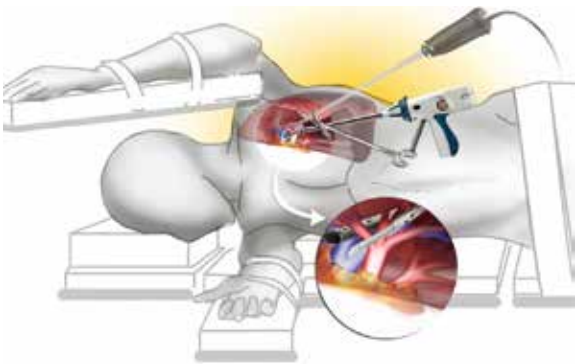


Figure 2 Diagram of a left apical upper tri-segmentectomy via single port VATS.

Indications

Based on the limited available evidence and the reported use of wedge resections in certain cohorts of patients with lung cancer we can identify possible indications for sublobar wedge resection in early NSCLC:

I. Cases in which preservation of parenchyma is mandatory. These include patients with very limited pulmonary reserve with COPD, significant pulmonary fibrosis that carry poor prognosis when lobectomy is performed, pulmonary hypertension and, more recently, in the management of metachronous or synchronous lung cancers;
II. Cases where preoperative histology could not be obtained or confirmed. Not only in very small pulmonary nodules unable to be biopsied percutaneously, but cases with history of distant malignancies where diagnosis metastasis/primary couldn't be made, or when radiological appearances are not very suggestive of cancer but patients request histological confirmation;
III. Diagnostic dilemmas in patients with underlying nodular lung disease (tuberculosis, sarcoid, rheumatoid) where one or more nodules are suspicious for malignancy during the course of their chronic disease in which a possible early NSCLC could be missed;
IV. Patients with severe comorbidities or very advanced age presenting with a peripheral nodule where a very short general anaesthesia period is preferred, where a wedge can be perform within few minutes, even with patients spontaneously ventilated.

Anatomical sublobar resections (segmentectomies)

Segmentectomies consist in the anatomical excision of one or more pulmonary segments. It is required to divide segmental branches of pulmonary artery, vein and bronchi related to the excised segments. The traditional technique of finding the segmental parenchymal plane by hand or electrocautery has now been substituted in many cases by the use of surgical staplers placed beyond the intersegmental plane with the potential benefit of reducing air leaks and parenchymal bleeding (26-28).

Segmentectomies for early lung cancer have been reported in the literature, and appear to be used more frequently (29,30). Surgeons have identified the potential role as an alternative to lobectomy in situations to increase operability (the elderly, patients with poor respiratory reserve, previous pulmonary resection) and resectability (multifocal ground-glass opacities, synchronous tumors, history of other solid malignancies where diagnosis of metastasis is a possibility), but also as the preferred option in small early stage NSCLC (31,32).

There is a limited but growing experience in the use of VATS segmentectomies, championed by experienced thoracoscopic surgeons but progressively being adopted by more units (33,34). The procedures can be performed via all the different VATS approaches including the Uniportal one (*Video 2*) and the instruments position is shown in *Figure 2*.

Technique

Segmentectomies can be divided into Typical (where parenchymal division involves 2 planes) or Atypical (more complex and technically demanding, when the segmental excision involves 3 planes). Examples of the former are

Table 1 Reports showing experiences in VATS segmentectomy for lung cancer

Author	Year	Operations	Number	Locoregional recurrence
Atkins	2007	Open segmentectomy; VATS segmentectomy	28; 48	8.3%; 7.7%
Saphiro	2009	VATS lobectomy; VATS segmentectomy	113; 31	3.6%; 3.5%
Yamashita	2011	VATS lobectomy; VATS segmentectomy	71; 38	5.6%; 7.1%
Soukiasian	2012	VATS lobectomy; VATS segmentectomy	266; 73	Ns (same survival)
Zhong	2012	VATS lobectomy; VATS segmentectomy	81; 39	4.9%; 5.1%
Zharo	2013	VATS lobectomy; VATS segmentectomy	138; 36	4.4%; 2.8%

excision of segments 6 on either side, lingulectomies, left apical upper tri-segmentectomies, left basal trisegmentectomies, right 7-10 segmentectomy. The rarer atypical segmentectomy examples are segmentectomy of 7-8 in the right, or 9-10 bilaterally.

With the patient on the lateral decubitus and forced hyperextension of the chest cavity to increase the intercostal space, a 4 cm incision is performed anterior to the latissimus dorsi edge at the level of 4^{th}-5^{th} intercostal space. The 30-degree thoracoscope is inserted to explore the pleural cavity. The thoracoscope is kept at the most posterior end of the wound allowing the insertion of 2, 3 or even more thoracoscopic instruments without interfering with them. Initially adhesions are divided with electrocautery and the left apical upper trisegmentectomy is performed. The Pulmonary Artery is identified and the initial branches are isolated and divided with an endo stapler. The segmental veins with preservation of the branches draining the lingula are then isolated and divided. Slightly more difficult is the identification of the segmental bronchus. Once this is isolated, we recommend that an inflation test is carried out prior to bronchial division as errors have been reported in VATS procedures. Once the bronchus has been divided, the parenchymal plane is identified by the inflation method prior to the excision. The specimen is removed with the help of a specimen bag in order to facilitate extraction and to minimize theoretical risk of wound seeding. A single intercostal drain is inserted after division of the inferior pulmonary ligament, lymph node excision and satisfactory lung re-expansion. Surgeons have employed other methods to identify the segmental plane: indocyanine green instillation or isolated inflation of the segments to be resected, all of them valid.

Results

The only randomized controlled trial including anatomical segmentectomies for lung cancer is the LCSG that, unfortunately, grouped segmentectomies together with wedge excisions. It concluded that survival after sublobar resections was equivalent to lobectomy but recurrence rates were much higher making a strong case for lobectomy to be considered the procedure of choice in early lung cancer. Unfortunately, the conclusions were impossible to extrapolate into a whole segmentectomy cohort due to the trial design (2).

Following this, few case-matched reports and several comparative series have indicated the value of anatomical segmentectomies to be similar to lobectomies in small size lung cancers, not only in the high-risk but also in the overall population (35-37). While survival or recurrence rates appear to be similar, there is evidence to demonstrate the lesser impact on pulmonary function after segmental resections.

If we apply the potential advantages seen in large experiences of surgeons performing VATS lobectomies compared with open lobectomies (less pain, early recovery, less complications and reduce immune response) the prospect of VATS anatomical segmentectomies might be very appealing (6-9). Several authors have described their experiences with a variety of VATS approaches from 4 to Single-port, and there are some comparative series between VATS and Open segmentectomy for lung cancer (38).

Overall, authors have not seen any significant differences in perioperative outcomes, survival or rates of recurrence between VATS segmentectomy and VATS lobectomy (*Table 1*) (39-43). The loco-regional recurrence rates vary between 2.8% and 7.7% in the different reports, similar to after VATS lobectomy by the same surgeons. One manuscript by Atkins et al compared the outcomes between open and VATS segmentectomies performed in an experienced thoracoscopic unit, with perioperative results indicating that VATS techniques do not compromise outcomes (38).

Authors have not seen a significant reduction in the patients' hospital stay after VATS segmentectomy compared to VATS lobectomy, maybe as a consequence of longer lasting air leaks after segmentectomy due to the more extensive parenchymal trauma than after a fisureless VATS lobectomy (39-43). In the VATS experience we are yet to confirm the benefits on pulmonary function that segmentectomy seems to have over lobectomy in thoracotomy cohorts (44).

Indications

Based on the limited available evidence, and pending the results of modern studies underway (CALBG-140503 trial of segmentectomy *vs.* lobectomy for early lung cancer), the possible indications for VATS sublobar resections in NSCLC include:

I. Nodules in patients with a previous history of solid malignancies in cases where intraoperative frozen sections can not differentiate a primary lung cancer from a distant metastasis;
II. Multicentric ground glass opacities previously described as bronchoalveolar carcinoma;
III. Second primary in cases who have undergone pulmonary resection in the past;
IV. Surgery in patients deemed to have a high-risk for a lobectomy including respiratory diseases, extreme age;
V. An increasing number of segmentectomies are being used as procedure of choice in patients with peripheral early lung cancer of less than 2 cm.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Lim E, Baldwin D, Beckles M, et al. Guidelines on the radical management of patients with lung cancer. Thorax 2010;65 Suppl 3:iii1-27.
2. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
3. Martin-Ucar AE, Waller DA, Atkins JL, et al. The beneficial effects of specialist thoracic surgery on the resection rate for non-small-cell lung cancer. Lung Cancer 2004;46:227-32.
4. Riaz SP, Linklater KM, Page R, et al. Recent trends in resection rates among non-small cell lung cancer patients in England. Thorax 2012;67:811-4.
5. Griffin JP, Eastridge CE, Tolley EA, et al. Wedge resection for non-small cell lung cancer in patients with pulmonary insufficiency: prospective ten-year survival. J Thorac Oncol 2006;1:960-4.
6. Yan TD, Black D, Bannon PG, et al. Systematic review and meta-analysis of randomized and nonrandomized trials on safety and efficacy of video-assisted thoracic surgery lobectomy for early-stage non-small-cell lung cancer. J Clin Oncol 2009;27:2553-62.
7. Paul S, Altorki NK, Sheng S, et al. Thoracoscopic lobectomy is associated with lower morbidity than open lobectomy: a propensity-matched analysis from the STS database. J Thorac Cardiovasc Surg 2010;139:366-78.
8. Petersen RP, Pham D, Burfeind WR, et al. Thoracoscopic lobectomy facilitates the delivery of chemotherapy after resection for lung cancer. Ann Thorac Surg 2007;83:1245-9; discussion 1250.
9. Yim AP, Wan S, Lee TW, et al. VATS lobectomy reduces cytokine responses compared with conventional surgery. Ann Thorac Surg 2000;70:243-7.
10. Walker WS, Carnochan FM, Pugh GC. Thoracoscopic pulmonary lobectomy. Early operative experience and preliminary clinical results. J Thorac Cardiovasc Surg 1993;106:1111-7.
11. Hansen HJ, Petersen RH, Christensen M. Video-assisted thoracoscopic surgery (VATS) lobectomy using a standardized anterior approach. Surg Endosc 2011;25:1263-9.
12. Onaitis MW, Petersen RP, Balderson SS, et al. Thoracoscopic lobectomy is a safe and versatile procedure: experience with 500 consecutive patients. Ann Surg 2006;244:420-5.
13. Gonzalez-Rivas D, Paradela M, Fernandez R, et al. Uniportal video-assisted thoracoscopic lobectomy: two years of experience. Ann Thorac Surg 2013;95:426-32.
14. Falcoz PE, Conti M, Brouchet L, et al. The Thoracic Surgery Scoring System (Thoracoscore): risk model for in-hospital death in 15,183 patients requiring thoracic surgery. J Thorac Cardiovasc Surg 2007;133:325-32.
15. Kozower BD, Sheng S, O'Brien SM, et al. STS database risk models: predictors of mortality and major morbidity for lung cancer resection. Ann Thorac Surg 2010;90:875-81; discussion 881-3.
16. Rocco G, Martin-Ucar A, Passera E. Uniportal VATS

wedge pulmonary resections. Ann Thorac Surg 2004;77:726-8.
17. Li W, Wang Y, He X, et al. Combination of CT-guided hookwire localization and video-assisted thoracoscopic surgery for pulmonary nodular lesions: analysis of 103 patients. Oncol Lett 2012;4:824-8.
18. Molins L, Mauri E, Sánchez M, et al. Locating pulmonary nodules with a computed axial tomography-guided harpoon prior to videothoracoscopic resection. Experience with 52 cases. Cir Esp 2013;91:184-8.
19. Vandoni RE, Cuttat JF, Wicky S, et al. CT-guided methylene-blue labeling before thoracoscopic resection of pulmonary nodules. Eur J Cardiothorac Surg 1998;14:265-70.
20. Choi BG, Kim HH, Kim BS, et al. Pulmonary nodules: CT-guided contrast material localization for thoracoscopic resection. Radiology 1998;208:399-401.
21. Stiles BM, Altes TA, Jones DR, et al. Clinical experience with radiotracer-guided thoracoscopic biopsy of small, indeterminate lung nodules. Ann Thorac Surg 2006;82:1191-6; discussion 1196-7.
22. Piolanti M, Coppola F, Papa S, et al. Ultrasonographic localization of occult pulmonary nodules during video-assisted thoracic surgery. Eur Radiol 2003;13:2358-64.
23. Wolf AS, Richards WG, Jaklitsch MT, et al. Lobectomy versus sublobar resection for small (2 cm or less) non-small cell lung cancers. Ann Thorac Surg 2011;92:1819-23; discussion 1824-5.
24. Landreneau RJ, Sugarbaker DJ, Mack MJ, et al. Wedge resection versus lobectomy for stage I (T1 N0 M0) non-small-cell lung cancer. J Thorac Cardiovasc Surg 1997;113:691-8; discussion 698-700.
25. Rocco G, Cicalese M, La Manna C, et al. Ultrasonographic identification of peripheral pulmonary nodules through uniportal video-assisted thoracic surgery. Ann Thorac Surg 2011;92:1099-101.
26. Jones DR, Stiles BM, Denlinger CE, et al. Pulmonary segmentectomy: results and complications. Ann Thorac Surg 2003;76:343-8; discussion 348-9.
27. Okada M, Mimura T, Ikegaki J, et al. A novel video-assisted anatomic segmentectomy technique: selective segmental inflation via bronchofiberoptic jet followed by cautery cutting. J Thorac Cardiovasc Surg 2007;133:753-8.
28. D'Amico TA. Thoracoscopic segmentectomy: technical considerations and outcomes. Ann Thorac Surg 2008;85:S716-8.
29. Jensik RJ, Faber LP, Milloy FJ, et al. Segmental resection for lung cancer. A fifteen-year experience. J Thorac Cardiovasc Surg 1973;66:563-72.
30. Yang CF, D'Amico TA. Thoracoscopic segmentectomy for lung cancer. Ann Thorac Surg 2012;94:668-81.
31. Lau KK, Martin-Ucar AE, Nakas A, et al. Lung cancer surgery in the breathless patient--the benefits of avoiding the gold standard. Eur J Cardiothorac Surg 2010;38:6-13.
32. Schuchert MJ, Pettiford BL, Pennathur A, et al. Anatomic segmentectomy for stage I non-small-cell lung cancer: comparison of video-assisted thoracic surgery versus open approach. J Thorac Cardiovasc Surg 2009;138:1318-25.e1.
33. Shiraishi T, Shirakusa T, Iwasaki A, et al. Video-assisted thoracoscopic surgery (VATS) segmentectomy for small peripheral lung cancer tumors: intermediate results. Surg Endosc 2004;18:1657-62.
34. Gonzalez-Rivas D, Fieira E, Mendez L, et al. Single-port video-assisted thoracoscopic anatomic segmentectomy and right upper lobectomy. Eur J Cardiothorac Surg 2012;42:e169-71.
35. Martin-Ucar AE, Nakas A, Pilling JE, et al. A case-matched study of anatomical segmentectomy versus lobectomy for stage I lung cancer in high-risk patients. Eur J Cardiothorac Surg 2005;27:675-9.
36. Date H, Andou A, Shimizu N. The value of limited resection for "clinical" stage I peripheral non-small cell lung cancer in poor-risk patients: comparison of limited resection and lobectomy by a computer-assisted matched study. Tumori 1994;80:422-6.
37. Okada M, Yoshikawa K, Hatta T, et al. Is segmentectomy with lymph node assessment an alternative to lobectomy for non-small cell lung cancer of 2 cm or smaller? Ann Thorac Surg 2001;71:956-60; discussion 961.
38. Atkins BZ, Harpole DH Jr, Mangum JH, et al. Pulmonary segmentectomy by thoracotomy or thoracoscopy: reduced hospital length of stay with a minimally-invasive approach. Ann Thorac Surg 2007;84:1107-12; discussion 1112-3.
39. Shapiro M, Weiser TS, Wisnivesky JP, et al. Thoracoscopic segmentectomy compares favorably with thoracoscopic lobectomy for patients with small stage I lung cancer. J Thorac Cardiovasc Surg 2009;137:1388-93.
40. Yamashita S, Chujo M, Kawano Y, et al. Clinical impact of segmentectomy compared with lobectomy under complete video-assisted thoracic surgery in the treatment of stage I non-small cell lung cancer. J Surg Res 2011;166:46-51.
41. Soukiasian HJ, Hong E, McKenna RJ Jr. Video-assisted thoracoscopic trisegmentectomy and left upper lobectomy provide equivalent survivals for stage IA and IB lung cancer. J Thorac Cardiovasc Surg 2012;144:S23-6.

42. Zhong C, Fang W, Mao T, et al. Comparison of thoracoscopic segmentectomy and thoracoscopic lobectomy for small-sized stage IA lung cancer. Ann Thorac Surg 2012;94:362-7.
43. Zhao X, Qian L, Luo Q, et al. Segmentectomy as a safe and equally effective surgical option under complete video-assisted thoracic surgery for patients of stage I non-small cell lung cancer. J Cardiothorac Surg 2013;8:116.
44. Keenan RJ, Landreneau RJ, Maley RH Jr, et al. Segmental resection spares pulmonary function in patients with stage I lung cancer. Ann Thorac Surg 2004;78:228-33; discussion 228-33.

Cite this article as: Martin-Ucar AE, Delgado Roel M. Indication for VATS sublobar resections in early lung cancer. J Thorac Dis 2013;5(S3):S194-S199. doi: 10.3978/j.issn.2072-1439.2013.08.41

Totally thoracoscopic pulmonary anatomic segmentectomies: technical considerations

Dominique Gossot, Rym Zaimi, Ludovic Fournel, Madalina Grigoroiu, Emmanuel Brian, Charles Neveu

Thoracic Department, Institut Mutualiste Montsouris, 42 Bd Jourdan, F-75014 Paris, France

Correspondence to: Dominique Gossot. Thoracic Department, IMM, 42 Bd Jourdan, F-75014 Paris, France. Email: dominique.gossot@imm.fr.

Background: While video-assisted thoracic surgery (VATS) lobectomies are being increasingly accepted, VATS segmentectomies are still considered as technically challenging. With the renewed interest for sublobar resection in the management of early stage lung carcinomas, the thoracoscopic approach may have a major role in a near future. We report our technique and results.

Patients and methods: Totally thoracoscopic anatomic segmentectomiy, i.e., using only endoscopic instrumentation and video-display without utility incision, was attempted on 117 patients (51 males and 66 females), aged 18 to 81 years (mean: 62 years). The indication was a clinical N0 non-small cell lung carcinoma in 69 cases, a solitary metastasis in 17 cases and a benign lesion in 31 cases. The following segmentectomies were performed: right apicosuperior [26] right superior [10], right basilar [18], lingula sparing left upper lobectomy [15], left apicosuperior [11], lingula [7], left superior [14], left basilar [13] and subsegmental resection [3]. Segmentectomy was associated with a radical lymphadenectomy in 69 cases.

Results: There were 5 conversions to thoracotomy. The mean operative time was 181±52 minutes, the mean intraoperative blood loss was 77±81 cc. There were 12 postoperative complications (11.7%). The median postoperative stay was 5.5±2.2 days. Out of the 69 patients operated on for a cN0 lung carcinoma, 6 were finally upstaged.

Conclusions: Totally thoracoscopic anatomic pulmonary segmentectomies are feasible and have a low complication rate.

Keywords: Segmentectomy; thoracoscopy; video-assisted thoracic surgery (VATS)

Submitted Apr 23, 2013. Accepted for publication Jun 20, 2013.

doi: 10.3978/j.issn.2072-1439.2013.06.25

View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.06.25

Video-assisted thoracic surgery (VATS) and thoracoscopic major pulmonary resections are accepted as a valid alternative to open surgery as it is now evident that minimally invasive surgery is beneficial in terms of reduced postoperative pain, shorter hospital stay, shorter recovery and better compliance to adjuvant chemotherapy, without compromising oncological principles (1). However few series of video-assisted pulmonary segmentectomies have been published and totally endoscopic-so-called complete VATS-segmentectomies series are even more infrequently reported (2,3). Many different techniques of thoracoscopic major pulmonary resections have been described, depending on the use of an accessory mini-thoracotomy, endoscopic instrumentation, and, video display. In the totally endoscopic approach only endoscopic instruments and monitor visualization are used. This is the technique that will be described in this article (4). By totally endoscopic we mean: (I) 100% video display; (II) no access incision and (III) only use of trocars and endoscopic instruments (5) (*Figures 1,2*). The aim of this article is not to discuss the oncologic validity of segmentectomies for early stage lung carcinomas but to describe and discuss some technical aspects and the results of totally thoracoscopic anatomic segmentectomies (TTAS).

Patients and methods

From January 2008 to January 2013, TTAS was attempted in 117 patients (51 males and 66 females) ranging in age

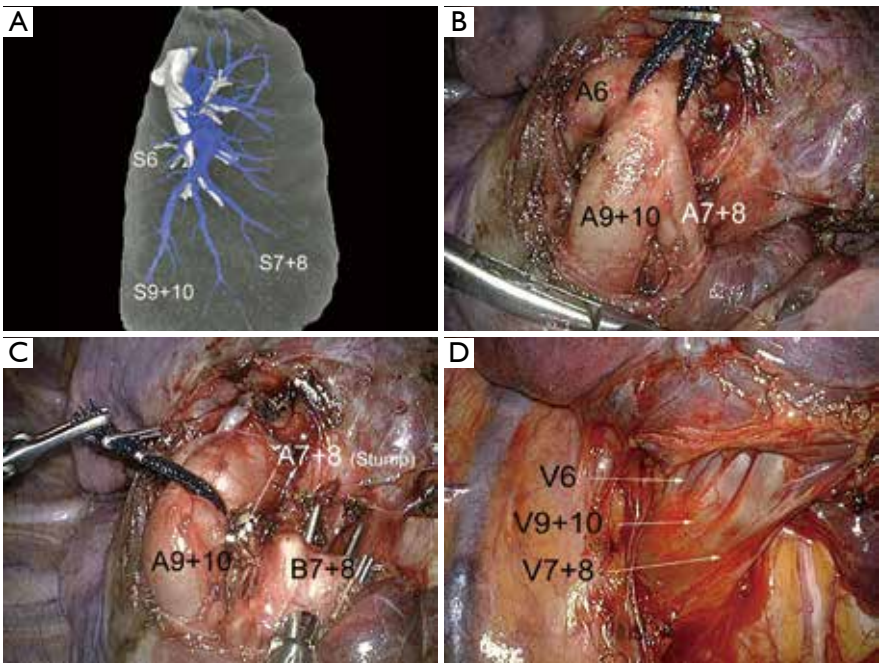


Figure 1 Main steps of a right anterior basilar subsegmenectomy of segments 7+8. (A) Three-dimensional reconstruction of arteries and bronchi; (B) a loop is passed around the main basilar arterial trunk and helps exposure of the arterial branches; (C) after division of the artery to the anterior segments, backward traction of the loop helps exposing the bronchus to segments 7+8; (D) segmental distribution of the branches of the right lower pulmonary vein. (A, artery; B, bronchus; V, vein; S, segment).

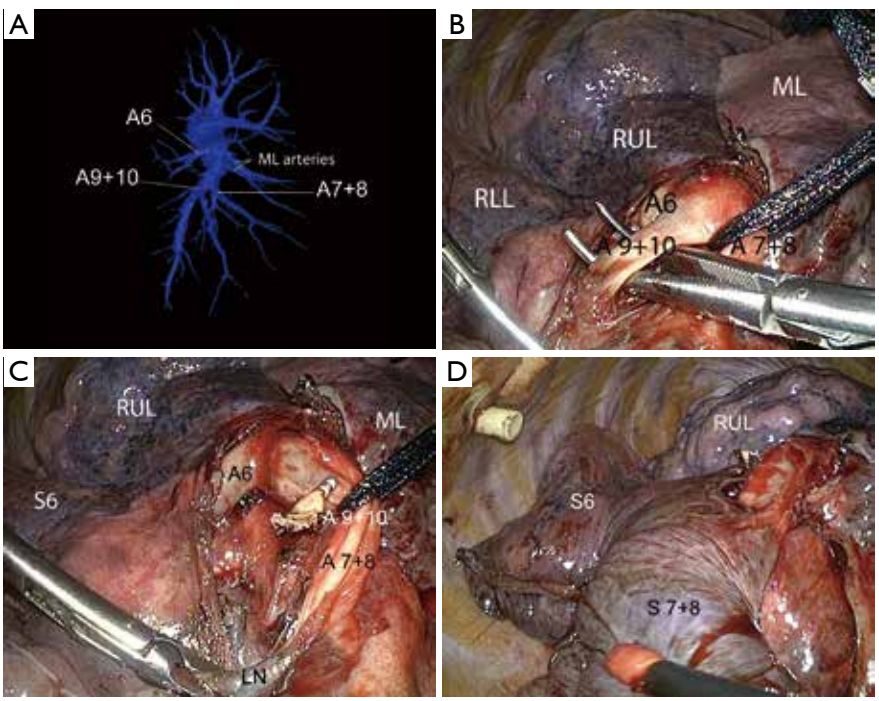


Figure 2 Main steps of a posterior subsegmenectomy of segments 9+10. (A) Three-dimensional reconstruction of arteries; (B) Dissection of the artery to the posterior segments; (C) after division of the artery to the posterior segments, forward traction of the loop helps exposing the bronchus to segments 9+10; (D) final aspect before reventilation after removal of the posterior segments. (RUL, Right upper lobe; ML, middle lobe; A, artery; B, bronchus; V, vein; S, segment).

Table 1 Resected segments (112 patients)

Right	N	Left	N
Apicoposterior (S1+2)	26	Upper division (S1+2+S3)	15
Superior (S6)	10	Apicoposterior (S1+2)	1
Basilar segments (S7-10)	18	Lingula (S4+5)	7
Posterior Basilar segments (S7-8)	1	Superior (S6)	14
Anterior Basilar segments (S9-10)	2	Basilar segments (S7-10)	13

from 18 to 81 years (mean: 62 years). The indication was either a benign lesion (31 patients), a solitary metastasis (17 patients), or a suspicion of clinical stage I non-small-cell lung carcinoma (NSCLC) (69 Patients). The reason for performing a segmentectomy for an NSCLC was an impaired lung function and/or a previous history of pulmonary resection, clinical stage IA in fragile patients or carcinoid tumor.

Patients' consent was routinely obtained. Intraoperative and postoperative data were recorded in a prospective manner into a database that was approved by our Institutional Review Board. The variables entered in the database were the following: need for conversion to thoracotomy, duration of the surgical procedure as noted on the operating room records, operative blood loss, intraoperative complications, number of collected lymph nodes and of dissected lymph node stations for patients operated on for NSCLC, duration of chest drainage, postoperative stay and postoperative complications. The types of segmentectomy are specified in *Table 1*.

Technical aspects

We have previously described our technique in detail (Gossot, 2010#53). In brief, the procedure was performed under general anesthesia with split ventilation using a double-lumen endotracheal tube. Patients were positioned in lateral decubitus as for a thoracotomy. The surgeon stood anterior or posterior to the patient, depending on the segments to be resected. He usually stood posterior to the patient for right sided resections and anteriorly for left sided ones. Two monitors were used and the thoracoscope was placed on a mechanical scope holder. In a fashion similar to our technique of totally endoscopic lobectomies, we used a deflectable thoracoscope housing a distal CCD (LTF, Olympus, Tokyo, Japan) (6) connected to a high definition camera system (HDTV) (Exera II, Olympus, Tokyo, Japan). Only specifically designed endoscopic instruments for VATS major resections were used. As a rule, trocars with a diameter ranging between 3 mm (micro-instruments) and 15 mm (endostapler and retrieval bag were utilized). For lung cancer patients, intersegmental lymph nodes, when present, were analyzed by frozen section to confirm the indication for segmentectomy. Larger vessels were divided with endostaplers while haemostasis of small caliber vessels was performed with clips, with a bipolar vessel sealing device (LigaSure™, Valleylab, Boulder, CO, USA) or with a combination of both methods. The root of the intersegmental veins was preserved and used as landmark for identification of the intersegmental plane. Demarcation between the resected and preserved segments was usually made possible by gentle reventilation and adequate application of a long 5-mm lung forceps whose position was adapted according to the inflation-deflation line. The intersegmental plane was divided by a combination of bipolar sealing device (for its peripheral and thin portion) and stapling (for its central and thick portion) using 4.8 mm staples (Endo-GIA II, Covidien Autosuture, Mansfield, MA). When the remaining segment was mobile and at risk of torsion, it was anchored to the adjacent lobe with a TA endostapler. An additional radical lymphadenectomy was performed for all patients operated on for a suspicion of lung carcinoma, according to a previously described technique (7). No utility incision was used. On completion of the pulmonary resection, the specimen was wrapped into an endobag and retrieved through one of the port sites that was enlarged to a length of 2 to 4 cm, depending on the specimen size. The use of a rib spreader was never required for specimen extraction. In most cases, only 1 chest tube was placed through one of the port site. Its removal was decided according to usual rules, i.e., no air leakage and output inferior to 200 cc per day.

Results

There were 5 conversions to thoracotomy (4.2%) for a

Table 2 Postoperative complications (112 patients)

None	100
Segmental ischemia requiring reoperation	2
Prolonged air leak (>5 days)	3
Pneumothorax requiring chest drainage	1
Sputum retention requiring bronchoscopy	2
Neurologic disorder	1
Pulmonary embolism	1
Pulmonary oedema	1
Arythmia	1

Table 3 Final pathological diagnosis (112 patients)

Primary malignant	69
Adenocarcinoma	33
Squamous cell carcinoma	3
Carcinoid tumor	9
Metastasis	17
Benign	31
Bronchectasia	3
Aspergillosis	2
Mucormycosis	1
Tuberculosis	1
Bronchial atresia	5
Bulla	1
Other benign conditions	6

fused fissure (2 cases) and for non-controllable hemorrhage (3 cases). In 1 of these hemorrhagic complications, the planned right apicoposterior segmentectomy was finally converted into an upper lobectomy. All 5 patients had a simple postoperative course. In the 112 other patients who had a totally thoracoscopic procedure, there were 3 intraoperative complications, i.e., a partial disruption of the staple line during division of the intersegmental plane requiring endoscopic suturing. The postoperative course of these 3 patients was simple and they were discharged between postoperative day 4 and 5. Operative time ranged from 87 to 315 minutes (mean, 181±52 minutes). The estimated blood loss ranged from 0 cc (non-measurable) to 450 cc (mean, 77±81 cc). No patient needed blood transfusion. All but 12 patients had an uneventful postoperative course (90%). Complications are listed in *Table 2*. Out of the 12 complications, 10 were minor whereas 2 were major, i.e., requiring a reoperation. These 2 patients had an ischemia of the remaining lingula after a lingula sparing left upper lobectomy. They underwent a lingulectomy by thoracoscopy (1 patient) or by thoracotomy (1 patient), with a simple postoperative course. The drainage duration ranged from 1 to 7 days (mean, 3.3±1.9 days) and the hospital stay from 2 to 22 days (mean, 5.5±2.2 days). The final pathological results are listed in *Table 3*. For the 69 patients who were operated on for a suspicion of primary lung carcinoma and who had an additional lymphadenectomy, the mean number of removed hilar lymph nodes (station 10) ranged from 0 to 6 (mean, 3±2) and from station 11-12 ranged from 1 to 9 (mean, 3±2) was. The mean number of collected mediastinal lymph nodes was 21±7 and the mean number of dissected lymph node stations was 3.5±1. For patients operated on for lung cancer, the tumors were staged pathological N0 in all but 2 cases which were upstaged N1 and 4 cases which were upstaged N2.

Discussion

Anatomical landmarks

Segmentectomy is considered a challenging procedure if done by thoracotomy and even more so if it is performed thoracoscopically (2). Not only the anatomical relationships are difficult to grasp, especially for the young and less experienced surgeons, but the identification and division of the intersegmental plane is a concern. The issue is more relevant for upper segmentectomies. Not only the number of arteries arising from the pulmonary artery is variable but their distribution is sometimes difficult to appreciate because the vessels can usually not been dissected to a sufficient length. This is especially true for the ascending arteries to the right upper lobe. These arteries can supply only the posterior segment of the upper lobe or both the posterior and anterior segments. The study of preoperative computed tomography three-dimensional reconstruction helps assessing the number, size and direction of these arteries without doubt (8). Having the vascular pattern in mind helps the surgeon performing a safer dissection of the branches of the pulmonary artery, especially when the fissure is fused and/or when lymph nodes are present. In a series of 49 patients selected for VATS lobectomy, Fukuhara *et al.* found that preoperative three-dimensional computed pulmonary angiography was identifying the PA branches in 95% of the cases (9). In their series, only some small

Table 4 Technical data available for published series of VATS or totally thoracoscopic segmentectomies

First author	N	VATS/TT	Number of trocars	Utility incision (cm)	Optics	Op. Time* [min]	Op.Blood loss* [mL]	Division of intersegmental plane
Shiraishi 2004 (13)	34	TT	6	None	Rigid 30°	240±72	169±68	Ultrasonic shears
Okada (14)	102	VATS	2	4-8	NS	129 [60-275]	50 [10-350]	Electrocautery + fibrin sealant
Atkins (15)	48	VATS	1	4	NS	136±45	250±200	Stapling
Oizumi (16)	29	TT	4	None	Rigid 30°	216 [146-425]	100 [3-305]	Stapling
Schuchert (17)	104	VATS	3	4	Rigid 0°	136 [120-152]	171 [133-209]	Stapling
Watanabe (11)	41	VATS	2	4 (3.5-6)	NS	220 [100-306]	183 [30-770]	Electrocautery + Stapling + fibrin sealant
Shapiro (18)	31	VATS	2	NS	NS	NS	NS	Stapling
Leshnower (19)	15	VATS	3	NS	Rigid 30°	145±55	NS	Stapling
Yamashita (20)	90	TT	4	None	Rigid 30°	257±91	132±181	Stapling
This series	117	TT	4-5	None	Deflectable			Stapling

N, number; VATS, video-assisted thoracic surgery; TT, totally thoracoscopic; cm, centimeter; min, minutes; mL, milliliter; NS, Not stated; *, expressed as mean and range or mean ± standard deviation.

branches (less than 2 mm in diameter) were missed. In the beginning of our experience, most patients candidate to an upper segmentectomy had a multidetector row preoperative computed tomography (CT) angiography with three-dimensional volume-rendering reconstruction of arterial and venous anatomy. Nevertheless, CT reconstruction was not done for the lower segments since anatomical variations of the vascular supply to the lower lobes has less impact on the surgical technique and can be easily managed (8-10). As we felt more confident with the technique and the thoracoscopic vision of anatomical landmarks, the resort to preoperative CT reconstruction was progressively abandoned.

Intersegmental plane

Another difficulty faced during thoracoscopic segmentectomy is the identification and division of the intersegmental plane. When performed through a thoracotomy, this step is facilitated by the use of manual palpation which is not possible via thoracoscopy. Several methods have been described. The most common is the creation of a ventilated-deflated line by reventilating the operated lung once the segmental bronchus has been stapled. This technique has drawbacks: (I) reventilation obscures the vision and this is a much more troublesome problem than during thoracotomy; (II) the segments to be resected can be partly reventilated through the collateral canals, leading to an unclear demarcation line. Therefore some authors have suggested acting reverse, i.e., reventilating the whole lung once the segmental bronchus has been divided and then collapsing it, so that only the diseased segments remain inflated (11). Others have suggested using selected jet ventilation in the segmental bronchi to be divided (12). In emphysematous patients we have used a similar method by injecting air through the channel of a bronchofiberscope, after selective endoscopy of the segmental bronchus.

Once the intersegmental plane has been determined, the last issue is the choice of the division method. Some authors have used a combination of blunt dissection, electrocautery and application of fibrin sealant (12). When air leaks were observed, some surgeons applied mattress suture with pledgets (12). These methods have the advantage of sparing parenchyma, but comprise a risk of postoperative air leak. Actually, most authors use staplers (*Table 4*). Stapling is however not that easy. First, it may require using many cartridges, up to 5 in the series of Watanabe (11). Second, the limited opening of the endostaplers and the thickness of the parenchyma expose to disruption of the staples line, an adverse event that occurred twice in our series. The consequences were not serious but leaded to troublesome blood loss and required hand suturing.

Segmental ischemia

In our series, 2 patients had to be reoperated for an ischemia

Table 5 Results for published series of VATS or totally thoracoscopic segmentectomies

First author	N	VATS/TT	Conversion rate	Morbidity	Chest tube duration* [days]	Postoperative. stay* [days]
Shiraishi (13)	34	TT	0%	11.7%	4.5±3.2	12.7±3.6
Okada (14)	102	VATS	NS	9.8%	1	NS
Atkins (15)	48	VATS	0%	31.3%	3.5±4	4.3±3
Oizumi (16)	29	TT	0%	10%	1 [1-7]	NS
Schuchert (17)	104	VATS	NS	26%	NS	5
Watanabe (11)	41	VATS	0	10%	3 [1-9]	NS
Shapiro (18)	31	VATS	13%	26%	2 [1-33]	4 [1-98]
Leshnower (19)	15	VATS	0%	0%	2.8±1.3	3.5±1.4
Yamashita (20)	90	TT	4.8%	19%	4.8.±3.4	12.2±8.2
This series	117	TT	4.3%	11.7%	3.3±1.9	5.6±2.4

N, number; VATS, video-assisted thoracic surgery; TT, totally thoracoscopic; NS, Not stated; *, expressed as mean and range or mean ± standard deviation.

of the lingula after an upper division of the left upper lobe. In one case, it was unclear whether ischemia was related to the torsion of the remaining segment or to an injury of the lingular vein, while torsion was obvious in the second case. This complication has been reported by others (21).

Although the thoracoscopic approach offers a clear and magnified view, one of its limitations is the difficulty in obtaining a global vision of the operative field, especially as the lung is reinflated. Therefore, a wrong positioning of the remaining segment can be overlooked. In addition, securing the segment to the adjacent lobe by thoracoscopy is not that easy. When performed by thoracotomy, it is usually done by applying anchoring stiches on a partially reventilated parenchyma. This is almost impossible to perform by thoracoscopy due to the lack of space caused by reinflation of the lung. We have overcome this difficulty by applying 1 or 2 cartridges of staples, using an endostapler with no knife (Endo-TA, Covidien). Thorough examination of the remaining segment is required to avoid mispositioning. Should a reoperation be necessary, it can be performed by re-thoracoscopy (22), as occurred in one of our patient.

Lymph node dissection

Several works dealing with the issue of the validity of lymph node dissection during VATS lobectomy and segmentectomy have been recently published. Basing on a cohort of 14,473 patients, Whitson *et al.* have shown that survival was less after segmentectomy than after lobectomy, even for T1a tumors (23). This was confirmed by the work of Wolf *et al.* (23), but these authors demonstrated that survival was not statistically different between lobectomy and segmentectomy if a lymph node dissection was performed (24).Therefore, the quality of lymph node dissection during segmentectomy for lung cancer is most likely a crucial part of the procedure. Recently, Hattori *et al.* showed that the rate of positive lymph nodes was high for solid T1A tumors especially in case of high standardized uptake value (SUV_{max}). They advocate for a thorough intraoperative evaluation of lymph nodes to prevent locoregional recurrence (25). However, it seems that lobar and segmental lymph node clearance is a weak point of the thoracoscopic approach for sublobar resection. Boffa *et al.* have demonstrated that nodal upstaging form cN0 to pN2 was no statistically different between the open and thoracoscopic approach but that upstaging form cN0 to pN1 was significantly higher when the patient was operated on via thoracotomy (9.3% versus 6.7%) (26). This difference tended to be minimized with experience of the surgeon (26). A satisfactory clearance of stations 11 and 12 can be achieved with the use of patience, appropriate dissection and hemostatic tools and frozen section if any suspicion of nodal metastasis (24).

Tumor-free margins

In case of lung cancer, frozen section must also be used for examination of the margins after completion of segmentectomy. Indeed, local recurrence after limited resection is related not only to nodal involvement but also to the size of the lesion and to the width of the surgical margins (19).The majority of recurrences are seen when the

ratio between the margin and the tumor size is less than (27). Accordingly, frozen section should be used if any doubt exists as to completeness of resection.

Conclusions

Although a totally endoscopic approach to anatomic segmentectomies can seem challenging and difficult, the operation time in our series was acceptable and the morbidity rate was low (*Table 5*). Combining the advantages of an endoscopic approach and an anatomic limited resection could be highly beneficial for those of the patients who fulfill the criteria of a sublobar resection. With the renewed interest for sublobar resection in the management of early stage lung carcinomas, the thoracoscopic approach may have a major role in a near future (28,29), provided the following criteria are fulfilled: (I) true anatomic resection with hilar division of bronchovascular elements; (II) adequate clearance of intersegmental lymph nodes and (III) tumor- free margins.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Yan TD, Black D, Bannon PG, et al. Systematic review and meta-analysis of randomized and nonrandomized trials on safety and efficacy of video-assisted thoracic surgery lobectomy for early-stage non-small-cell lung cancer. J Clin Oncol 2009;27:2553-62.
2. Oizumi H, Kanauchi N, Kato H, et al. Anatomic thoracoscopic pulmonary segmentectomy under 3-dimensional multidetector computed tomography simulation: a report of 52 consecutive cases. J Thorac Cardiovasc Surg 2011;141:678-82.
3. Witte B, Wolf M, Hillebrand H, et al. Complete video-assisted thoracoscopic surgery anatomic segmentectomy for clinical stage I lung carcinoma - technique and feasibility. Interact Cardiovasc Thorac Surg 2011;13:148-52.
4. Gossot D. eds. Atlas of endoscopic major pulmonary resections. Paris: Springer Editions, 2010.
5. Shigemura N, Akashi A, Nakagiri T, et al. Complete versus assisted thoracoscopic approach: a prospective randomized trial comparing a variety of video-assisted thoracoscopic lobectomy techniques. Surg Endosc 2004;18:1492-7.
6. Gossot D. Technical tricks to facilitate totally endoscopic major pulmonary resections. Ann Thorac Surg 2008;86:323-6.
7. Ramos R, Girard P, Masuet C, et al. Mediastinal lymph node dissection in early-stage non-small cell lung cancer: totally thoracoscopic vs thoracotomy. Eur J Cardiothorac Surg 2012;41:1342-8; discussion 1348.
8. Yamada S, Suga A, Inoue Y, et al. Use of multi-detector row angiography for the arrangement of video-assisted modified segmental resection. Eur J Cardiothorac Surg 2009;36:727-30.
9. Fukuhara K, Akashi A, Nakane S, et al. Preoperative assessment of the pulmonary artery by three-dimensional computed tomography before video-assisted thoracic surgery lobectomy. Eur J Cardiothorac Surg 2008;34:875-7.
10. Kanzaki M, Kikkawa T, Shimizu T, et al. Presurgical planning using a three-dimensional pulmonary model of the actual anatomy of patient with primary lung cancer. Thorac Cardiovasc Surg 2013;61:144-50.
11. Watanabe A, Ohori S, Nakashima S, et al. Feasibility of video-assisted thoracoscopic surgery segmentectomy for selected peripheral lung carcinomas. Eur J Cardiothorac Surg 2009;35:775-80; discussion 780.
12. Okada M, Mimura T, Ikegaki J, et al. A novel video-assisted anatomic segmentectomy technique: selective segmental inflation via bronchofiberoptic jet followed by cautery cutting. J Thorac Cardiovasc Surg 2007;133:753-8.
13. Shiraishi T, Shirakusa T, Iwasaki A, et al. Video-assisted thoracoscopic surgery (VATS) segmentectomy for small peripheral lung cancer tumors: intermediate results. Surg Endosc 2004;18:1657-62.
14. Okada M, Tsutani Y, Ikeda T, et al. Radical hybrid video-assisted thoracic segmentectomy: long-term results of minimally invasive anatomical sublobar resection for treating lung cancer. Interact Cardiovasc Thorac Surg 2012;14:5-11.
15. Atkins BZ, Harpole DH Jr, Mangum JH, et al. Pulmonary segmentectomy by thoracotomy or thoracoscopy: reduced hospital length of stay with a minimally-invasive approach. Ann Thorac Surg 2007;84:1107-12; discussion 1112-3.
16. Oizumi H, Kanauchi N, Kato H, et al. Total thoracoscopic pulmonary segmentectomy. Eur J Cardiothorac Surg 2009;36:374-7; discussion 377.
17. Schuchert MJ, Pettiford BL, Pennathur A, et al. Anatomic segmentectomy for stage I non-small-cell lung cancer: comparison of video-assisted thoracic surgery versus open approach. J Thorac Cardiovasc Surg 2009;138:1318-25.e1.
18. Shapiro M, Weiser TS, Wisnivesky JP, et al. Thoracoscopic segmentectomy compares favorably with thoracoscopic

lobectomy for patients with small stage I lung cancer. J Thorac Cardiovasc Surg 2009;137:1388-93.
19. Leshnower BG, Miller DL, Fernandez FG, et al. Video-assisted thoracoscopic surgery segmentectomy: a safe and effective procedure. Ann Thorac Surg 2010;89:1571-6.
20. Yamashita S, Tokuishi K, Anami K, et al. Thoracoscopic segmentectomy for T1 classification of non-small cell lung cancer: a single center experience. Eur J Cardiothorac Surg 2012;42:83-8.
21. Eguchi T, Kato K, Shiina T, et al. Pulmonary torsion of the lingula following a segmentectomy of the left upper division. Gen Thorac Cardiovasc Surg 2008;56:505-8.
22. Sung HK, Kim HK, Choi YH. Re-thoracoscopic surgery for middle lobe torsion after right upper lobectomy. Eur J Cardiothorac Surg 2012;42:582-3.
23. Whitson BA, Groth SS, Andrade RS, et al. Survival after lobectomy versus segmentectomy for stage I non-small cell lung cancer: a population-based analysis. Ann Thorac Surg 2011;92:1943-50.
24. Wolf AS, Richards WG, Jaklitsch MT, et al. Lobectomy versus sublobar resection for small (2 cm or less) non-small cell lung cancers. Ann Thorac Surg 2011;92:1819-23; discussion 1824-5.
25. Hattori A, Suzuki K, Matsunaga T, et al. Is limited resection appropriate for radiologically "solid" tumors in small lung cancers? Ann Thorac Surg 2012;94:212-5.
26. Boffa DJ, Kosinski AS, Paul S, et al. Lymph node evaluation by open or video-assisted approaches in 11,500 anatomic lung cancer resections. Ann Thorac Surg 2012;94:347-53; discussion 353.
27. Schuchert MJ, Pettiford BL, Keeley S, et al. Anatomic segmentectomy in the treatment of stage I non-small cell lung cancer. Ann Thorac Surg 2007;84:926-32; discussion 932-3.
28. Yang CF, D'Amico TA. Thoracoscopic segmentectomy for lung cancer. Ann Thorac Surg 2012;94:668-81.
29. Swanson SJ. Video-assisted thoracic surgery segmentectomy: the future of surgery for lung cancer? Ann Thorac Surg 2010;89:S2096-7.

Cite this article as: Gossot D, Zaimi R, Fournel L, Grigoroiu M, Brian E, Neveu C. Totally thoracoscopic pulmonary anatomic segmentectomies: technical considerations. J Thorac Dis 2013;5(S3):S200-S206. doi: 10.3978/j.issn.2072-1439.2013.06.25

Teaching video-assisted thoracic surgery (VATS) lobectomy

Philip W. Carrott Jr[1], David R. Jones[2]

[1]Department of Surgery, University of Michigan, Ann Arbor MI, USA; [2]Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Correspondence to: David R. Jones, MD, Professor of Surgery, Chief, Thoracic Surgery. Department of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 49, New York, NY 10065, USA. Email: jonesd2@mskcc.org.

Abstract: Video-assisted thoracic surgery (VATS) lobectomy has become the standard of care for early stage lung cancer throughout the world. Teaching this complex procedure requires adequate case volume, adequate instrumentation, a committed operating room team and baseline experience with open lobectomy. We outline what key maneuvers and steps are required to teach and learn VATS lobectomy. This is most easily performed as part of a thoracic surgery training program, but with adequate commitment and proctoring, there is no reason experienced open surgeons cannot become proficient VATS surgeons. We provide videos showing the key portions of a subcarinal lymph node dissection, posterior hilar dissection of the right upper lobe, fissureless right middle lobectomy, and fissureless left lower lobectomy. These videos highlight what we feel are important principals in VATS lobectomy, i.e., N2 and N1 lymph node dissection, fissureless techniques, and progressive responsibility of the learner. Current literature in simulation of VATS lobectomy is also outlined as this will be the future of teaching in VATS lobectomy.

Keywords: video-assisted thoracic surgery (VATS) lobectomy; teaching; simulation

Submitted Jul 10, 2013. Accepted for publication Jul 22, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.31
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.31

Introduction

Video-assisted thoracic surgery (VATS) lobectomy has rapidly become the standard of care for early-stage lung cancer treatment throughout North America and increasingly in the world. A VATS lobectomy is defined as the use of a 3-6 cm access incision without rib-spreading, one to three additional 1 cm ports, and the use of a thoracoscope to visualize the dissection and subsequent lobectomy. Compared to an open thoracotomy and lobectomy, a VATS lobectomy has equivalent oncologic results, less post-operative pain, shorter hospitalization, earlier return to activities of daily living, earlier administration of adjuvant therapies, and is less expensive (1,2). Despite these advantages there are several barriers to the adoption of more advanced VATS procedures including lobectomy. These include a lack of formal education and training, cost, lack of access to technology (particularly in non-North American or Western European countries), and a continued lack of education about the oncologic merits of the procedure relative to an open thoracotomy.

A recent survey of thoracic surgery residents reveals that 58% believe they are proficient in performing a VATS lobectomy at the completion of their residency program. Those individuals who were dedicated thoracic surgeons were much more likely (86%) to be comfortable performing a VATS lobectomy relative to those individuals with a mixed practice (28%) (3). Collectively, this suggests that there needs to more emphasis on introducing, teaching, and monitoring progression of the VATS lobectomy procedure to our trainees as well as those surgeons who are interested in incorporating the procedure into their existing practice.

There is an increasing literature on how advanced technologic procedures should be introduced into surgical practice (4-6). It is now well established that there is distinct learning curve for learning how to safely and proficiently perform a VATS lobectomy (4-9). The actual technical aspects of the procedure including number of incisions and methodologies to dissect and divide bronchovascular

structures will vary amongst surgeons and are also dependent on the tumor stage and biology. For these reasons, the purpose of this review is to highlight important aspects of teaching and learning VATS lobectomy with an emphasis on programmatic requirements, patient selection, and strategies to facilitate the learning process, including simulation. We will also discuss some basic technical considerations that apply to all VATS lobectomy procedures.

Programmatic and individual requirements

McKenna describes several important pre-requisites relative to beginning a VATS lobectomy program (9). One the most important points is that the entire operating room team (nurses, scrub technicians, first assistants) need to be familiar with *open* procedures before attempting VATS lobectomies. In addition, there should be an adequate volume of lobectomies (>25/year) in the practice. The surgeon who is performing VATS lobectomy procedures should have done a relative large number of smaller VATS procedures (i.e., wedge resection, lymph node biopsies, etc.). In addition, the surgeon should have observed several "live" VATS lobectomies and, if at all possible, assisted in the operations. There is no substitute (i.e., simulation, workshop, or video) for actual experience when one is adopting a new surgical technique. Frequently, this requires more than one observation or active participation. In addition, the best approach is for the scrub and circulating nurses to have also observed a live case or two so they can also become familiar with the basics of the procedure. These individual and programmatic pre-requisites apply to both new thoracic surgery residents and more experienced surgeons who are adopting this technology to their practices.

An additional pre-requisite that is rarely mentioned is the need for the appropriate VATS instrumentation, endostaplers, and the necessary instruments should conversion to an open procedure be indicated. Failure to have the appropriate VATS instruments, thoracoscopes and monitors can result in inadvertent intraoperative injuries, prolong the case, increase conversion rates, and demoralize surgeon and team morale and interest in the procedure. We routinely use a 45° thoracoscope while others prefer a 30° or flexible tipped camera (10). These angled scopes offer the most versatility in providing alternate angles to view the anterior and posterior hilum without switching camera port access sites. Use of dissecting two-point scissors, needle holders, long Harken or Semb clamps, DeBakey clamps and axial handle forceps are all basic and required instruments to facilitate performing a VATS lobectomy.

The last pre-requisite is for the surgeon and the other team members to understand their responsibilities should the case require conversion from VATS to open procedure. It is extraordinarily rare to require conversion emergently as most complications, including major bleeding, can be managed with elective or urgent conversion maneuvers.

Intraoperative teaching

Incisions and surgeon positions

Once the patient is positioned, attention is given to selection of the appropriate locations of the incisions. We use a 5 mm thoracoscope and therefore place a small trocar in the 7th or 8th intercostal space (ICS) in the middle to posterior axillary line to guide subsequent incision placement. A 4 cm access incision is then made anteriorly in the 4th ICS for upper and middle lobectomies and in the 5th ICS for lower lobectomies. This incision needs to be quite anterior. A third 1 cm incision is then made depending on surgeon preference.

If the teaching surgeon is going to stand posteriorly at the patient's back, then it is easier to teach, guide, and first assist if the third incision is placed posterior to the camera port. If the teaching surgeon is going to stand anteriorly on the same side as the learner, then the third 1 cm incision is best placed anterior to the camera port. We prefer to have the teaching surgeon stand posteriorly and the learner stands anteriorly. We typically do not place trocars in these third incisions and thus only need a 5 mm trocar for the entire procedure. Additional ports are placed at the discretion of the surgeon. All ports should be separated by 6-8 cm in order to avoid unnecessary fencing of intrathoracic instruments. In teaching VATS lobectomy, as with other cases, there is a progression of responsibility for the case.

It is important to remember that an open lobectomy is typically performed via a posterior approach while a VATS lobectomy is almost always an anterior approach. Thus, a VATS lobectomy offers a "different view" for many surgeons. A final caveat is that if a two- or three-incision VATS lobe strategy is used, then the operating surgeon will need to operate more exclusively through the anterior access incision and therefore will most certainly need the full armamentarium of VATS instrumentation.

The correct placement of the access incision and ancillary

Video 1 Station 7 Lymph node dissection. We prefer to start our procedure with this posterior hilar dissection and removing N2 nodes during the initial dissection. The sub-carinal lymph nodes are removed as a packet whenever possible.

Video 2 Posterior dissection RUL Bronch and PA. The bronchus and first pulmonary artery branch are dissected and divided from the posterior approach. This may be necessary for large anterior tumors that prevent anterior visualization.

ports is one of the most critical aspects of performing a VATS lobectomy proficiently. One also needs to consider the patient's body habitus, a history of prior intra-thoracic procedures, and other considerations (i.e., breast implants, pacemakers, etc.).

Lymph node dissection

We perform the mediastinal nodal dissection first when performing a VATS lobectomy. Routine nodal dissection for right-sided tumors includes stations 2R, 4R, 7, and 10R. For left sided tumors we dissect stations 5, 7, and 10 L and station 6 if we observe a node in that region. Teaching the learner to dissect all the nodal tissue while avoiding bronchopulmonary structures as well as the superior vena cava (SVC), esophagus, and vagus, phrenic, and recurrent laryngeal nerves, is terrific first step in the learning process. This exposes the learner to much of the anatomy from an anterior approach as well as the various lung positioning and retraction maneuvers to facilitate the nodal dissection. While not routine, there are maneuvers that can be done to facilitate the N2 nodal dissection for the novice VATS lobe surgeon. For instance, division of the azygous vein at the junction of the SVC facilitates the dissection of 2R and 4R nodal stations.

We routinely begin our nodal dissection by retracting the lung anteriorly to completely dissect of station 7 (*Video 1*) and the posterior station 10L nodes. When indicated for more anteriorly located right upper lobe tumors or tumors near the minor fissure, it is also possible to begin to isolate and divide lobar bronchovascular structures from this posterior approach, as outlined in *Video 2*. In these cases the right upper lobe bronchus is isolated and divided first followed by the truncus arterial branch next, with the remainder of the segmental pulmonary arterial vessels and lobar veins taken from a continued posterior approach or from an anterior approach.

Teaching the N1 nodal dissection can be challenging for both the instructor and the learner. Notwithstanding the oncologic benefit, it is imperative that all N1 nodes be removed in order to facilitate the accurate identification of the lobar bronchi and perhaps more importantly the segmental branches of the pulmonary artery. We prefer a combination of blunt (metal suction device) and sharp dissection with either scissors or low-dose cautery to remove these nodes. The primary difficulty the learner has when performing a N1 dissection is the loss of haptic perception. Intraoperative teaching of this aspect of the procedure is best done by (I) having the correct VATS instrumentation; (II) explaining normal and common variant anatomy; and (III) moving anterior to posterior in the nodal dissection. Analysis of the STS database for upstaging of pulmonary malignancies following either VATS or open lobectomy found that significantly fewer N1 nodes were obtained following VATS lobectomy, indicating that VATS surgeons need to be more complete in sending N1 nodal tissue (11). The routine dissection and removal of the N1 lymph nodes makes subsequent isolation and division of the segmental pulmonary arteries with the endostapler much more expeditious and safer.

Fissureless VATS lobectomy

The majority of VATS lobectomies do not require identification of the pulmonary artery in the fissure and

Video 3 Anterior approach to the RML. Right middle lobectomy is performed in a "Fissureless" technique, taking the hilar vessels and bronchus first, then the fissures to perform the lobectomy.

Video 4 Fissureless LLL. The key steps of a fissureless left lower lobectomy are shown. Smaller portions of the dissection are shown to keep the video short.

thus division of the parenchyma is commonly the last step of the procedure. This fissureless approach is best taught during open thoracotomies for lobectomies. We perform a fissureless VATS lobectomy in the majority of cases. As shown in *Video 3* (a VATS middle lobectomy) and *Video 4* (a VATS left lower lobectomy) a fissureless approach is simple, straightforward and in my opinion is less likely to result in injury to segmental pulmonary arterial branches. On occasion partial division of a fissure may facilitate the dissection and when appropriate should be performed. In addition, an experienced VATS surgeon will occasionally need to dissect vascular structures in the fissure to safely remove centrally-located or large tumors. These types of operations are not, however, appropriate beginning cases for the novice VATS lobectomy surgeon.

In general, when teaching a fissureless approach the pulmonary vein is isolated and divided first. This is a relatively simple maneuver most of the time and one that an intermediate learner can do within 10-15 minutes. Once complete it offers exposure to the lobar bronchus (lower and middle lobectomies) and pulmonary arterial segmental vessels. Each successive division opens up the dissection of the next structure, until the fissures are the only remaining attachments. We find that dissection and confirmation with various instruments that mimic the angles of the stapling devices are helpful in orienting subsequent endostapler application. We utilize the VATS curved and straight DeBakey clamps to approximate the angles one must have for the endostaplers.

Case progression

When teaching any new surgical technique there needs to be a progression toward independence for all steps of the procedure. In general, one can divide the steps in a VATS lobectomy into discrete, defined maneuvers (see *Table 1* example). The learner and the instructor can both track progress, operative times per maneuver, and technical results and then make necessary adjustments on this data.

Simulation and VATS lobectomy

Advanced minimally-invasive procedures such as a VATS lobectomy require a specialized surgical skill set. Surgical simulation may be able to facilitate a more rapid and safe introduction into surgical practice without exposing the patient to unnecessary risk. There are a number of relevant issues regarding simulation in thoracic surgery including identification of an appropriate and realistic model (computer-based, animal, or tissue block) and validation of the model (12-15). As outlined by Tong *et al.* the utility of a task-based simulator depends on its fidelity and validity. Fidelity, also known as face validity, refers to how real the simulator experience feels to the student. Content validity evaluates whether the steps performed in the simulator are accurate to what is done in the actual procedure. Construct validity evaluates the ability of the simulator to discriminate between learners at different levels of experience (14).

Groups at the University of North Carolina at Chapel Hill and New York University have developed a porcine-block and a virtual reality trainer VATS lobectomy model, respectively (13,15). The porcine lung block model has been shown to have a high fidelity and is perhaps the best studied and most validated model for teaching VATS lobectomy (14,15). The porcine block left lung model is not anatomically identical

Table 1 Incremental steps of a VATS right upper lobectomy (RUL)	
(I)	Correct placement of the access incision, thoracoscope and additional ports;
(I)	Inspection and retraction of lung;
(I)	Dissection of 7, 4R, 2R and 10R nodal stations;
(II)	Incision of posterior mediastinal pleura to expose the right mainstem and upper lobe bronchi;
(III)	Dissection of the sump node at the junction of the RUL bronchus and proximal bronchus intermedius;
(IV)	Incision of the anterior mediastinal pleura and isolation and division of the superior pulmonary vein;
(V)	Isolation and division of the truncus anterior and posterior ascending pulmonary arteries;
(VI)	Removal of peribronchial nodal tissue followed by isolation and division of the RUL bronchus;
(VII)	Division of the lung parenchyma;
(VIII)	Placement of the RUL specimen into an endocatch bag followed by removal from the pleural cavity;
(IX)	Apposition of the RML to the RLL to prevent a RML torsion syndrome (if indicated).

to human anatomy, but the tissue and advanced dissection techniques are reproducible and are detailed in an evaluation by senior surgeons (15). Additional groups have developed simulators for open surgery as well, which could be easily transitioned to VATS (16).

The virtual trainer has advantages in ease of set-up and fidelity to human anatomic variants as well as the ability to improve the model as technology improves. The upfront costs are estimated to be $25,000-35,000 for required infrastructure and will need further development. The virtual reality trainer can score the movements of the surgeon, allowing users to track their progress and set benchmarks for resident progress (13). Validation studies of the porcine block model were performed by Tong *et al.* and showed that in 31 residents with varying experience with VATS lobectomy that this model discriminated well between novice, intermediate, and experienced VATS lobectomy surgeons (14).

In all likelihood, the use of both platforms will be advantageous at different points in thoracic surgery training and in learning the VAS lobectomy procedure. The virtual reality platform can be used as often as one likes, and would be a good starting point for novice VATS lobectomy surgeons. The porcine model can then be used once surgeons gain some operative experience and will facilitate the development of fine dissection skills and gain a "feel" for tissue strength with sharp and blunt dissection of hilar vessels. In the United States, thoracic surgery education and training is transitioning to shorter, integrated programs which will certainly need simulation to adequately prepare surgeons with a reduced time in training. Unfortunately, there is still no universally identified simulation model and exposure opportunities are varied and limited to individual institutions. A more uniform and accessible simulation strategy for teaching and learning the skills required to perform a VATS lobectomy is needed.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Grogan EL, Jones DR. VATS lobectomy is better than open thoracotomy: what is the evidence for short-term outcomes? Thorac Surg Clin 2008;18:249-58.
2. Cajipe MD, Chu D, Bakaeen FG, et al. Video-assisted thoracoscopic lobectomy is associated with better perioperative outcomes than open lobectomy in a veteran population. Am J Surg 2012;204:607-12.
3. Boffa DJ, Gangadharan S, Kent M, et al. Self-perceived video-assisted thoracic surgery lobectomy proficiency by recent graduates of North American thoracic residencies. Interact Cardiovasc Thorac Surg 2012;14:797-800.
4. Reed MF, Lucia MW, Starnes SL, et al. Thoracoscopic lobectomy: introduction of a new technique into a thoracic surgery training program. J Thorac Cardiovasc Surg 2008;136:376-81.
5. Ng T, Ryder BA. Evolution to video-assisted thoracic surgery lobectomy after training: initial results of the first 30 patients. J Am Coll Surg 2006;203:551-7.
6. Rocco G, Internullo E, Cassivi SD, et al. The variability of practice in minimally invasive thoracic surgery for pulmonary resections. Thorac Surg Clin 2008;18:235-47.
7. Petersen RH, Hansen HJ. Learning thoracoscopic lobectomy. Eur J Cardiothorac Surg 2010;37:516-20.

8. Belgers EH, Siebenga J, Bosch AM, et al. Complete video-assisted thoracoscopic surgery lobectomy and its learning curve. A single center study introducing the technique in The Netherlands. Interact Cardiovasc Thorac Surg 2010;10:176-80.
9. McKenna RJ Jr. Complications and learning curves for video-assisted thoracic surgery lobectomy. Thorac Surg Clin 2008;18:275-80.
10. Demmy TL, James TA, Swanson SJ, et al. Troubleshooting video-assisted thoracic surgery lobectomy. Ann Thorac Surg 2005;79:1744-52; discussion 1753.
11. Boffa DJ, Kosinski AS, Paul S, et al. Lymph node evaluation by open or video-assisted approaches in 11,500 anatomic lung cancer resections. Ann Thorac Surg 2012;94:347-53; discussion 353.
12. Meyerson SL, LoCascio F, Balderson SS, et al. An inexpensive, reproducible tissue simulator for teaching thoracoscopic lobectomy. Ann Thorac Surg 2010;89:594-7.
13. Solomon B, Bizekis C, Dellis SL, et al. Simulating video-assisted thoracoscopic lobectomy: a virtual reality cognitive task simulation. J Thorac Cardiovasc Surg 2011;141:249-55.
14. Tong BC, Gustafson MR, Balderson SS, et al. Validation of a thoracoscopic lobectomy simulator. Eur J Cardiothorac Surg 2012;42:364-9; discussion 369.
15. Fann JI, Feins RH, Hicks GL Jr, et al. Evaluation of simulation training in cardiothoracic surgery: the senior tour perspective. J Thorac Cardiovasc Surg 2012;143:264-72.
16. Carter YM, Marshall MB. Open lobectomy simulator is an effective tool for teaching thoracic surgical skills. Ann Thorac Surg 2009;87:1546-50; discussion 1551.

Cite this article as: Carrott PW Jr, Jones DR. Teaching video-assisted thoracic surgery (VATS) lobectomy. J Thorac Dis 2013;5(S3):S207-S211. doi: 10.3978/j.issn.2072-1439.2013.07.31

Right, middle, and lower bronchial sleeve lobectomy by video-assisted thoracic surgery

Xin Zhang, Gui-Lin Peng, Li-Xia Liang, Jian-Xing He

Department of Throcic Surgery, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China

Correspondence to: Jian-Xing He. Department of Throcic Surgery, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China. Email: drjianxing.he@gmail.com.

Submitted Aug 06, 2013. Accepted for publication Aug 21, 2013.

doi: 10.3978/j.issn.2072-1439.2013.08.61

View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.61

Introduction

Surgical treatment remains the most effective approach to extending the long-term survival of patients with lung cancer (1).

However, for tumors that have already invaded the orifice of upper lobe bronchus and/or main bronchus, lobectomy alone can not completely remove the tumors, whereas pneumonectomy will severely damage the lung functions. On the contrary, bronchial sleeve lobectomy is featured by not only the maximal resection of tumors but also the maximal reservation of the normal lung tissues and lung functions and the remarkably decreased complications. Thus, it has shown good effectiveness in treating central-type lung cancer (2). The bronchial sleeve lobectomy extends the indications of lung cancer surgeries (3); When applied under thoracoscope, it can reduce the damage to the chest organs/tissues and the post-operative pain and therefore is particularly superior (4).

Clinical data

An 18 years old female patient was admitted on May 18, 2013 due to "heart palpitations on exertion with shortness of breath, occasionally accompanied with dry cough". Chest computed tomography (CT) at admission showed right middle lobe atelectasis (*Figure 1*). Bronchoscopy displayed a mass at the orifice of right middle-lower bronchus. Pathology indicated the presence of "bronchial mucoepidermoid carcinoma" (*Figure 2A*). After the pre-operative preparation was well performed, he received right, middle, and lower bronchial sleeve lobectomy and lymph node dissection by video-assisted thoracic surgery (VATS) under general anesthesia (*Video 1*). During the surgery, obstructive atelectasis was seen in the right, middle and lower lung and excessive expansion seen in the right upper lung. Also, some paratracheal and subcarinal lymph nodes were found to be swollen. After the surgery, the tracheal stump was sent for pathologic examination, which showed no residual cancer. The post-operative pathology showed bronchial mucoepidermoid carcinoma (*Figure 2B*), whereas no metastasis was seen in lymph node stations 2, 4, 7, 8, 11, and 12. Anti-inflammatory and symptomatic treatment was provided after the surgery. The patient recovered well from the surgery and was discharged on the tenth post-operative day. One month later, the Chest CT scan showed right upper lobar inflation (*Figure 3*).

Pre-operative preparation

The patient underwent blood tests, urine analysis, ECG, and pulmonary function tests before the surgery to comprehensively evaluate the general conditions and his tolerance to the surgery. Informed consent was obtained before the surgery.

Surgical procedures

The patient was under general anesthesia with double-lumen endotracheal intubation. One lung ventilation of the healthy side was done when the patient was asked to take a supine position on the healthy side. A 1-cm incision was made at the sixth intercostal space on the right anterior axillary line as the observation port; A 5-cm incision was made at the fourth intercostal space on the

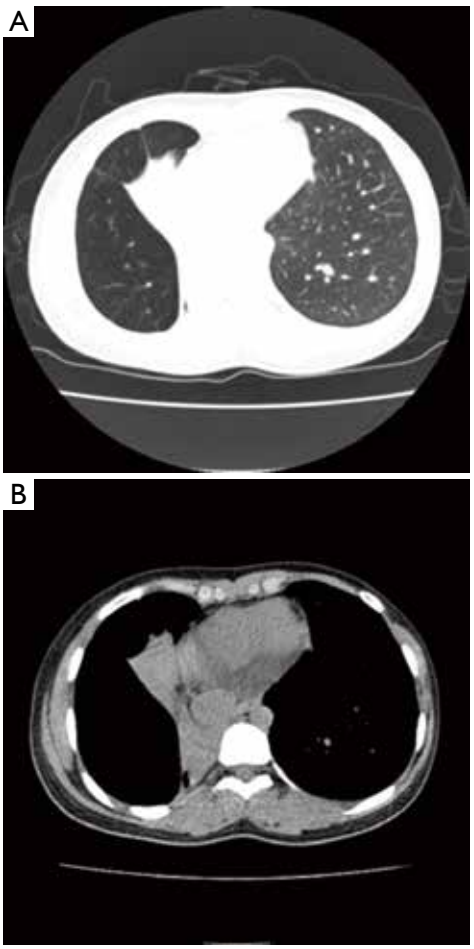


Figure 1 Chest CT shows right middle lobe atelectasis. (A) Lung window; (B) Mediastinal window.

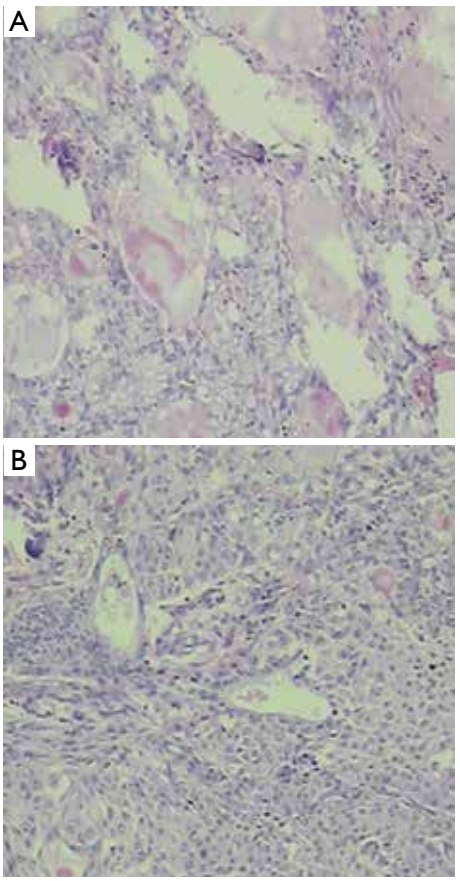


Figure 2 Both the bronchoscopic biopsy and post-operative pathology indicate the presence of bronchial mucoepidermoid carcinoma. (A) Bronchoscopic biopsy; (B) post-operative pathology.

Video 1 VATS right middle-low bronchial sleeve lobectomy for lung cancer.

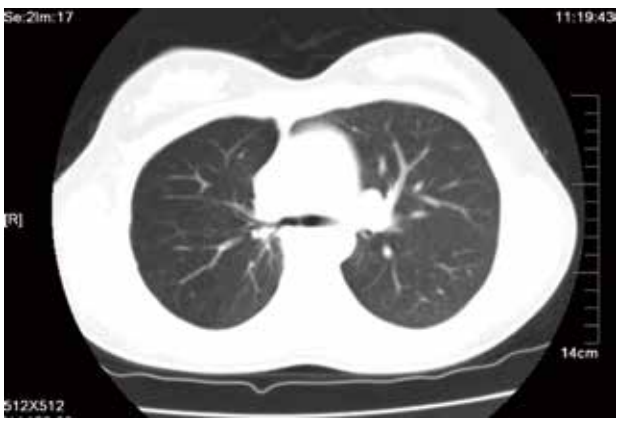


Figure 3 Chest CT scan showed right upper lobar inflation.

anterior axillary line as the main operation port; And a 1-cm incision was made near the sixth intercostal space on the posterior axillary line as the auxiliary operation port. After the insertion of instruments, the mediastinal pleura of portopulmonary was dissected and the right inferior pulmonary ligament was disconnected. However, the hyperinflation of the lower right lung hampered the operation. Thus, the right, middle, and lower bronchi were dissected firstly and then transected, so as to relieve the hyperinflation of the lower right lung. The arteries and veins in the right, middle, and lower lungs were divided and then transected. The swollen lymph nodes in the hilum of lung were removed. End-to-end anastomosis of the right upper lobe bronchus to the main bronchus was performed using the prolene 3-0 suture. After the anastomosis, sterile water was injected into the thoracic cavity to find out if there was any gas leakage. A right lower chest tube was placed. Bronchoscopic sputum suction was performed. The condition of the anastomotic stoma was checked; Since it was patent, the incisions were then sutured.

Post-operative management

The post-operative management of patients who have undergone full thoracoscopic bronchial sleeve lobectomy is basically the same as those who have received conventional thoracoscopic lobectomy. It mainly includes adequate postoperative analgesia, prophylactic use of antibiotics, and resolving sputum. Effective expectoration and early ambulation should also be encouraged. A second chest X-ray showed good recruitment of the residual lung. The drain was electively removed when drainage was less than 100 mL/day.

Discussion

Currently the bronchial sleeve lobectomy is still based on the traditional open surgery. However, the open surgery-related trauma can severely affect the post-operative quality of life. Even worse, its damage to the respiratory muscles on the chest wall can increase the perioperative mortality. However, in some patients with central lung cancer, bronchial sleeve lobectomy can achieve an effectiveness similar as the pneumonectomy, and meanwhile can achieve the maximal reservation of the post-operative lung functions, improve the quality of life, and extend the survival (5). The min-invasive bronchial or vascular sleeve lobectomy by VATS can minimize the above problems. However, the full thoracoscopic surgery can be challenging for the operators. The bronchial sleeve lobectomy should only be performed by thoracic surgeons who have been well trained in laparoscopic techniques in carefully selected patients to ensure the safety.

Cite this article as: Zhang X, Peng GL, Liang LX, He JX. Right, middle, and lower bronchial sleeve lobectomy by video-assisted thoracic surgery. J Thorac Dis 2013;5(S3):S277-S279. doi: 10.3978/j.issn.2072-1439.2013.08.61

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Ohta M, Sawabata N, Maeda H, et al. Efficacy and safety of tracheobronchoplasty after induction therapy for locally advanced lung cancer. J Thorac Cardiovasc Surg 2003;125:96-100.
2. Maurizi G, D'Andrilli A, Anile M, et al. Sleeve lobectomy compared with pneumonectomy after induction therapy for non-small-cell lung cancer. J Thorac Oncol 2013;8:637-43.
3. Okada M, Yamagishi H, Satake S, et al. Survival related to lymph node involvement in lung cancer after sleeve lobectomy compared with pneumonectomy. J Thorac Cardiovasc Surg 2000;119:814-9.
4. Mahtabifard A, Fuller CB, McKenna RJ Jr. Video-assisted thoracic surgery sleeve lobectomy: a case series. Ann Thorac Surg 2008;85:S729-32.
5. Ma Z, Dong A, Fan J, et al. Does sleeve lobectomy concomitant with or without pulmonary artery reconstruction (double sleeve) have favorable results for non-small cell lung cancer compared with pneumonectomy? A meta-analysis. Eur J Cardiothorac Surg 2007;32:20-8.

Non-intubated complete thoracoscopic bronchial sleeve resection for central lung cancer

Wenlong Shao[1,2,3], Kevin Phan[4], Xiaotong Guo[5], Jun Liu[1,2,3], Qinglong Dong[6], Jianxing He[1,2,3]

[1]Department of Thoracic Surgery, First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; [2]Department of Surgery, Guangzhou Institute of Respiratory Diseases, Guangzhou 510120, China; [3]National Respiratory Disease Clinical Research Center, Guangzhou 510000, China; [4]The Collaborative Research (CORE) Group, Macquarie University Hospital, New South Wales, Australia; [5]Department of Thoracic Surgery, Second Affiliated Hospital of Harbin Medical University, Harbin 15000, China; [6]Department of Anesthesiology, First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China

Correspondence to: Jianxing He, MD, PhD, FACS. Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, No. 151, Yanjiang Rd, Guangzhou 510120, China. Email: drjianxing.he@gmail.com.

Abstract: Bronchial sleeve resection has emerged as an effective thoracoscopic approach for central lung cancer with reduced operation mortality rates, optimal lung function and long-term survival. Endobronchial intubation is a commonly used method of anesthesia for such thoracoscopic procedures, but is associated with increased intubation-related and lung complications. Non-intubated epidural anesthesia represents an alternative approach which may avoid such difficulties, particularly in complicated sleeve resection situations. Here we have described a case of complete endoscopic bronchial sleeve resection of right lower lung cancer under non-intubated epidural anesthesia.

Keywords: Lung cancer; bronchial sleeve resection; non-intubated epidural anesthesia

Submitted Aug 09, 2014. Accepted for publication Aug 11, 2014.
doi: 10.3978/j.issn.2072-1439.2014.08.10
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.08.10

Introduction

Bronchial sleeve resection of lung tumors has emerged as an effective approach which not only removes the lesion but also avoids pneumonectomy, thereby reducing surgical mortality and maximizing lung function and long-term survival (1). Chen *et al*. reported a video-assisted thoracic surgery (VATS) lobectomy for lung cancer under non-intubated epidural anesthesia, which demonstrated acceptable safety and feasibility (2). However, there are no reports describing bronchial sleeve resection under non-intubated anesthesia. Here, we describe a case of complete endoscopic bronchial sleeve resection of right lower lung cancer under non-intubated epidural anesthesia.

Case report

A 70-year-old man presented with a mass in the right lower lung during physical examination. Lung function tests showed forced vital capacity (FVC) of 73.1% and forced expiratory volume in 1 s (FEV_1) of 71.5%. Computed tomography (CT) showed a mass at the dorsal segment of the lower right pulmonary lobe (*Figure 1*), measuring approximately 3×4 cm^2, as a thick-walled eccentric cavity. On November 11, 2013, the patient underwent complete thoracoscopic resection under non-intubated epidural anesthesia. Intramuscular midazolam (0.07 mg/kg) and atropine (0.01 mg/kg) were administered at 30 min before anesthesia. Epidural puncture was performed at the T7-8 intervertebral space, with the epidural catheter tip pointed towards the head and fixed after confirming successful placement. Following epidural injection of 0.375% ropivacaine and a test dose of 2 mL ropivacaine, the patient was observed for 5 min for signs of total spinal anesthesia. If total spinal anesthesia was not achieved, two more injections of 0.375% ropivacaine were administered, totaling 8 mL.

With a mask to supply oxygen and remove nitrogen, 2 μg/mL of intravenous propofol was given via target-

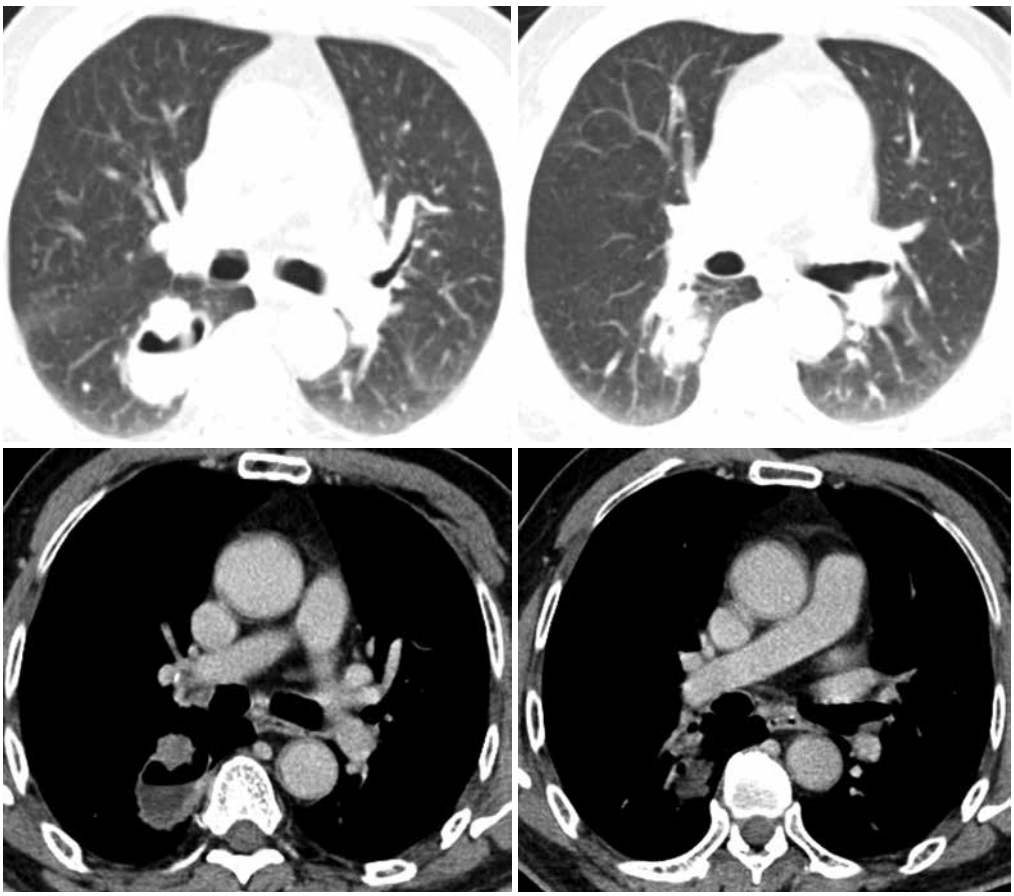


Figure 1 Mass at the dorsal segment of the right lung on computed tomography (CT).

controlled infusion (TCI) in combination with 0.2 μg/kg intravenous infusion of sufentanil. When adequate sedation was achieved, a laryngeal mask airway (LMA) was inserted and the anesthesia machine was connected to provide simultaneous intermittent mandatory ventilation (SIMV). Arterial catheterization was performed at the right internal jugular vein and the radial artery on the non-operative side.

Epidural injection of 4 mL 0.375% ropivacaine was administered at an interval of 60 min. Continuous intravenous infusion of 1.0-1.5 μg/mL propofol was performed via TCI. Continuous infusion of remifentanil 0.03 μg/kg·min and dexmedetomidine hydrochloride 0.5-1.0 μg/kg·h was administered to maintain sedation. An intraoperative spectrum analyzer was used to monitor the sedative effect, with the bispectral index (BIS) maintained at 40-60. The sedation depth was adjusted according to the monitored parameters. Spontaneous breathing was maintained, with a respiratory rate of 12-20 beats/min.

To suppress the cough reflex caused by lung tissue stretch during the thoracoscopic operation, the intrathoracic vagus nerve was blocked. Under direct vision in thoracoscopy, 3-5 mL of 0.375% ropivacaine was injected near the vagus nerve inferior to the mediastinal pleura above the azygos arch adjacent to the trachea.

The approach for non-intubated epidural thoracoscopic surgery was the 3-port method. With the patient in a left lateral position, the endoscopic observation port was made in the 7^{th} intercostal space at the anterior axillary line, the working port in the 5^{th} intercostal space at the anterior axillary line, and the auxiliary port in the 7^{th} intercostal space at the posterior axillary line. Using a 30° endoscope, the observation field covered the entire chest cavity. Using the connection between the operated side and the outer atmosphere and a gentle push on the lesion side, an iatrogenic pneumothorax was formed to collapse the right lung. After vagus nerve blockade, exploration of the dorsal side of the right lower lung was performed, where a mass measuring 4×5×5 cm^3 was found, with evident pleural

surface indentation. Johnson's endoscopic automatic stapler was initially used to isolate the incomplete fissure, and the right lower pulmonary artery and vein were incised. The right lower lobular bronchus was then similarly transected, sent for frozen biopsy and shown to be "bronchial margin residual cancer". While waiting for the pathological result, systematic lymphadenectomy was performed. To preserve the right and middle lung, bronchial sleeve resection was planned, and the surgery was continued without switching to intubation. The right middle lobe and the bronchi in the middle segment were transected at the root. The frozen pathology showed no residual lesions in the margin of the intermediate segment and the proximal middle bronchus. The right middle lobular bronchus was then joined with the right intermediate bronchus, and was continuously sutured with single 3-0 Prolene suture silk. After anastomosis, a pressurized balloon was applied in conjunction with laryngeal mask ventilation to expand the lungs, and no leakage was observed at the bronchial anastomosis. Upon confirmation of hemostasis, the operation was completed.

Results

The operation time was 165 min, involving 25 min of bronchial anastomosis and 120 mL blood loss. Five groups of a total of 18 lymph nodes were dissected during surgery. Histopathology results were as follows: moderately differentiated squamous cell carcinoma of the right lower lung, stump carcinoma in situ of the lower lobe bronchus, no tumor in the proximal margins of the right middle lobe and intermediate bronchi, and no lymph node metastasis in any dissected group (0/18). The patient did not require assisted breathing postoperatively. He was able to drink and eat at 4 h postoperatively and was mobile at postoperative day 1. At postoperative day 3, the drainage was removed, and no leaks, pulmonary infection, atelectasis, bronchial fistula, or other complications were observed. He was discharged on postoperative day 6. Pulmonary CT at 1 month postoperatively showed no anastomotic strictures (*Figure 2*).

Comment

In the present case, since there was a stump residual tumor in the bronchus after lobular resection, we switched to the sleeve resection technique. Given that the patient was stable and the advantage of reduced operative time required for bronchial anastomosis, the non-intubated anesthetic approach was undertaken with the hope of avoiding further injury. Therefore, we performed bronchial sleeve resection under non-intubated anesthesia with satisfactory results, demonstrating that non-intubated anesthesia could be successfully used in not only conventional VATS lobectomy but also for complicated bronchial anastomosis.

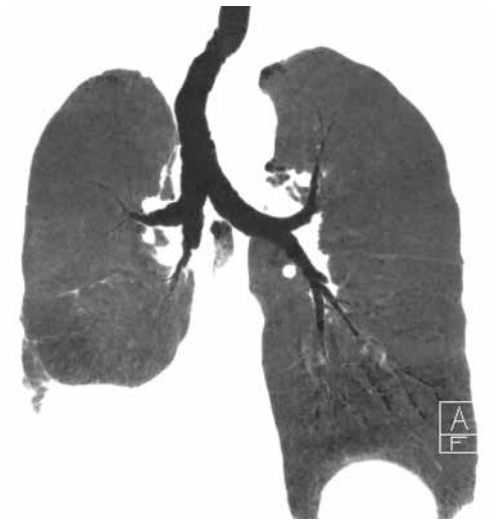


Figure 2 Postoperative pulmonary computed tomography (CT) revealed no anastomotic stricture at 1 month after surgery.

To avoid perioperative respiratory failure, non-intubated epidural anesthesia is usually performed only in a select group of patients, with estimated operation time within 3 h and have ASA grade I-II, body mass index <25, and good lung function reserves. In such patients, SPO_2 ≥90% can be maintained (2-4). In this patient, since we needed to open the airway for bronchial sleeve resection, which stopped the inhalation of oxygen from the nostrils, the inhaled oxygen concentration was reduced and the SPO_2 briefly decreased to 80%. We therefore provided assisted ventilation with a laryngeal mask and balloon to increase the oxygen flow and ventilation, rapidly improving the SPO_2 to a safe range of 90-95%, reversing the hypoxemia while reducing CO_2 reabsorption. After completing bronchial anastomosis, the patient's ventilation recovered immediately, and hypoxemia and hypercapnia improved significantly.

In conclusion, thoracoscopic bronchial resection under non-intubated epidural anesthesia can be performed, allowing successful removal of the tumor while retaining adequate functionality of the lung tissue. The patient recovered rapidly, awakened quickly postoperatively, began to eat and drink and was mobile soon after surgery, with a short hospital stay.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Schmid T, Augustin F, Kainz G, et al. Hybrid video-assisted thoracic surgery-robotic minimally invasive right upper lobe sleeve lobectomy. Ann Thorac Surg 2011;91:1961-5.
2. Chen JS, Cheng YJ, Hung MH, et al. Nonintubated thoracoscopic lobectomy for lung cancer. Ann Surg 2011;254:1038-43.
3. Dong Q, Liang L, Li Y, et al. Anesthesia with nontracheal intubation in thoracic surgery. J Thorac Dis 2012;4:126-30.
4. Chen KC, Cheng YJ, Hung MH, et al. Nonintubated thoracoscopic lung resection: a 3-year experience with 285 cases in a single institution. J Thorac Dis 2012;4:347-51.

Cite this article as: Shao W, Phan K, Guo X, Liu J, Dong Q, He J. Non-intubated complete thoracoscopic bronchial sleeve resection for central lung cancer. J Thorac Dis 2014;6(10):1485-1488. doi: 10.3978/j.issn.2072-1439.2014.08.10

Anesthesia with nontracheal intubation in thoracic surgery

Qinglong Dong[1], Lixia Liang[1], Yingfen Li[1], Jun Liu[2,3], Weiqiang Yin[2,3], Hanzhang Chen[2,3], Xin Xu[2,3], Wenlong Shao[2,3], Jianxing He[2,3]

[1]Department of anesthesia; [2]Department of Cardiothoracic Surgery, The First Hospital Affiliated to Guangzhou Medical College; [3]Guangzhou Institute of Respiratory Disease, State Key Laboratary of Respiratory Disease, Guangzhou 510120, China

Correspondence to: Jianxing He, MD, PhD, FACS. Department of Cardiothoracic Surgery, The First Hospital Affiliated to Guangzhou Medical College, Guangzhou Institute of Respiratory Disease, State Key Laboratary of Respiratory Disease, No. 151 Yanjiang Rd, Guangzhou 510120, China. Email: drjianxing.he@gmail.com. Lixia Liang, MD. Department of anesthesia, The First Hospital Affiliated to Guangzhou Medical College, No. 151 Yanjiang Rd, Guangzhou 510120, China. Email: drllx@hotmail.com.

Objective: To study one-lung respiration during VATS wedge resection of bullae and pulmonary nodules with nontracheal intubation, and to explore the changes of vital signs when patients return to two-lung ventilation.

Methods: Twenty-two patients with normal cardiopulmonary function and absence of contraindications to epidural anesthesia were included in this study. VATS wedge resection of bullae or pulmonary nodules was performed. 0.5% Ropivacain was administrated for epidural anesthesia (T8-9), and 2 mL of 2% lidocaine was used for local anesthetic block of the intrathoracic vagus nerves. The BIS value was maintained between 50 and 70 by target-controlled infusion of propofol and remifentanil. Electrocardiogram (ECG), heart rate (HR), blood pressure (Bp), pulse oxygen saturation (SpO_2), respiratory rate (RR), bispectral index (BIS) and urine volume were monitored.

Results: None patients were converted to endotracheal intubation during anesthesia. MAP and SpO_2 after wound disclosure were stable ($P>0.05$), level of CVP significantly elevated, HR and RR increased ($P<0.05$), $PaCO_2$ increased gradually while PaO_2 remained stable. Fifteen minutes after wound closure, MAP, RR and SpO_2 returned to their pre-anesthesia levels, PH value gradually recovered, $PaCO_2$ tended to decrease and returned to normal one hour after wound closure. Physical agitation occurred in one case due to inadequate epidural anesthesia during skin incision. Cough before intrathoracic vagal blockade was noted in two cases (9.1%) because of lobe traction.

Conclusions: Nontracheal intubation is feasible in VATS wedge resection of bullae and pulmonary nodules. The patients are with stable intraoperative vital signs and none experiences hypoxemia; intraoperative hypercapnia is tolerable and transient, which can be improved quickly when bilateral lungs resume spontaneous respiration.

Keywords: Anesthesia; nontracheal intubation; thoracic surgery

Submitted Mar 06, 2012. Accepted for publication Mar 13, 2012.
doi: 10.3978/j.issn.2072-1439.2012.03.10
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2012.03.10

Introduction

As lung separation techniques and anesthesia management advances in thoracic surgery, video-assisted thoracic surgery rapidly develops as well. As a result, the operating time and surgical trauma in wedge resections of bullae and pulmonary nodules have been significantly reduced. Since Pompe reported awake VATS wedge resection of solitary pulmonary nodules under thoracic epidural anesthesia in 2004, thoracic sympathectomy, lung metastases resection, pulmonary nodule resection, pulmonary bulla resection, biopsy of lung and pleura, resection of mediastinum nodules and pulmonary lobectomy in a similar anesthetic manner have continually been reported (1-7).

Although VATS wedge resection of bullae and pulmonary nodules with nontracheal intubation has been proven to be feasible, various factors, such as spontaneous respiration with one-lung ventilation during operation, intercostal muscle damage induced by thoracic epidural anesthesia intravenous administration of sedatives and analgesics, and operative position, may aggravate respiratory impairment, causing hypoxemia and hypercapnia, or even serious complication as well.

In addition, as for VATS operations under anesthesia with nontracheal intubation, it is not clear how the vital signs, such as respiration and circulation, will change during one-lung ventilation. Moreover, the trend and time of vital signs recovery after conversion to two-lung ventilation have not been reported. This clinical observation of 22 cases of VATS wedge resection of bullae or pulmonary nodules under anesthesia with nontracheal intubation explored changes of the vital signs during one-lung ventilation and subsequent two-lung ventilation.

Patients and methods

Research design

Anesthesia protocols were audited and approved by the Hospital Ethical Committee. The inclusion criteria for subjects were ASA I-II, age between 18 and 65, BMI <25, Mallampati grade I-II, little airway secretion and absence of epidural puncture contraindication. VATS wedge resections of bullae and pulmonary nodules were performed. Anesthetic protocols were explained to the participants before the informed consent was obtained.

Anesthesia

Patients received intramuscular Midazolam 0.06 mg/kg and Atropine 0.01 mg/kg 30 minutes before anesthesia. Electrocardiogram (ECG), heart rate (HR), blood pressure (Bp), pulse oxygen saturation (SpO_2), respiratory rate (RR) and bispectral index (BIS) and urine volume were continuously monitored after the patients entered the operation room. The thoracic epidural catheter was inserted at the T8-9 interspace, 3 cm towards the head, after intravenous infusion had been established. 2 mL of 2% Lidocaine was injected with the patients in supine position. Five minutes after the injection when no abnormal reaction to the anesthesia was observed, 3 mL of 0.5% Ropivacain was administrated followed by re-injection of another 3 mL 5 minutes later to reach a level of anesthesia between T2 and T10. Target-controlled infusion of Propofol and Remifentanil was started, and the BIS value was maintained between 50 and 70 by adjusting target concentration. During the whole research process, nasopharyngeal airway and face mask were used for oxygen inhalation, with an oxygen flow of 3-5 L/min.

Catheters were inserted via the right internal jugular vein or the right subclavian vein to continuously monitor the central venous pressure (CVP). And catheterization via the radial artery was performed to continuously monitor the invasive blood pressure (IBP). An incision into the chest wall on the operated side caused pulmonary collapse, leading to iatrogenic pneumothorax. Patients received local administration of 2 mL of 2% Lidocain injected under thoracoscopic guidance to achieve local anesthetic block of the intrathoracic vagus nerves. After the pleural cavity was closed and the wound was sutured, a face mask was used to assist the patients in ventilation to inflate the lung tissue. After the target controlled infusion was stopped and the epidural catheter was removed, the patients were transferred to a post anesthesia care unit (PACU).

If SpO_2 gradually decreased below 90% during anesthesia, a face mask was needed to assist ventilation in order to improve systematic oxygenation; if $PaCO_2$ ≥80 mmHg, operation had to be suspended and mechanical ventilation was delivered via a face mask to assist gas exchange. If ventilation could not be improved by the face mask, endotracheal intubation would be resorted.

Vital signs were monitored at pre-anesthesia, before and 15, 30, 45 minutes after wound disclosure as well as 15, 30, 45 minutes after wound closure. At the above time points, arterial blood was simultaneously extracted for blood gas analysis to detect values of pH, PaO_2, $PaCO_2$ and Lac. Operating time, arrhythmia, physical agitation, coughs before and after local anesthetic block of the intrathoracic vagus nerves and the cases transferred to endotracheal intubation were all recorded.

Statistical analysis

Primary outcome measures included values of HR, SpO_2, RR, Bp, CVP and arterial blood gas analysis. Secondary outcome measures included BIS, operating time, physical agitation and coughs. Age, height, weight and BMI were expressed by average value ± standard deviation. Two-sample t-test was used for statistical analyses. All data were analyzed with SPSS 13.0. A P value of <0.05 was considered statistically significant.

Results

From July to December of 2011, 9 VATS resections of bullae and 13 wedge resections of lung nodules were performed under combined anesthesia with epidural block, local block of the thoracic vagus nerve and analgesic sedation.

Table 1 General information of the patients

Items	Results
Male/Female (n)	14/8
Age (y)	39.18±18.52
Height (m)	1.66±0.09
Weight (kg)	56. 36±7.30
BMI	20.57±2.35
Arrhythmia (n)	0
Conversion to intubation (n)	0
Agitation (n)	1
Cough before intrathoracic vagal blockade (n)	2
Cough after intrathoracic vagal blockade (n)	0
Mean anesthetic duration (min)	143.9±24.5
Mean operative duration (min)	57.5±14.2
Bullectomy	9
Wedge resection of pulmonary lump	13
Location: right upper lobe	8
Right lower lobe	3
Left upper lobe	8
Left lower lobe	3

The general clinical data of the patients were detailed in *Table 1*. Their average age was 39.18±18.52 years and their average BMI was 20.57±2.35. No arrhythmia was found by ECG monitoring. No patients needed conversion to endotracheal intubation during anesthesia. Physical agitation caused by inadequate epidural anesthesia was noted in one case (4.5%) during skin incision. Cough occurred before local anesthetic block of the vagus nerves in two cases (9.1%), which was caused by stretching of pulmonary lobes when exploring and exposing the vagus nerves, but no cough occurred after completion of the vagus nerve blockade.

The HR and SpO_2 values before wound disclosure were almost the same as those before anesthesia; the BIS value obviously declined by 28.8% ($P<0.01$); the mean arterial pressure (MAP) slightly declined by 15.4%; the respiration rate decreased by 30.3% (*Table 2*). The changes of operative indexes after wound disclosure were detailed in *Table 3*. The

Table 2 Vital signs before anesthesia and before wound disclosure ($\bar{x}\pm s$)

	Before anesthesia	Before wound disclosure
BIS	92.5±5.6	66.2±8.5*
MAP (mmHg)	84.9±9.5	71.7±15.1*
HR (bpm)	78.9±13.9	75.1±17.7
RR (bpm)	15.8±1.4	11.0±3.3*
SpO_2 (%)	99.2±1.1	99.8±0.5

Compared with those before anesthesia. *, $P<0.01$.

Table 3 Vital signs and blood gas analyses before and after wound disclosure ($\bar{x}\pm s$)

	Before wound disclosure	15 min after wound disclosure	30 min after wound disclosure
HR (bpm)	75.1±17.7	85.9±16.3*	89.2±14.8**
MAP (mmHg)	71.7±15.1	71.1±12.2	75.5±7.7
CVP (cmH_2O)	8.2±4.1	11.4±5.0	11.0±4.6
SpO_2 (%)	99.8±0.5	99.1±2.7	99.7±0.7
RR (bpm)	11.0±3.3	14.7±4.4*	14.5±5.6*
BIS	66.2±8.5	62.5±13.3**	62.6±9.9**
pH	7.30±0.06	7.23±0.06**	7.25±0.05**
$PaCO_2$ (mmHg)	57.6±10.6	68.1±12	65.7±8.6
PaO_2 (mmHg)	260.7±119.2	241.0±122.6	248.3±121.8
Oxygenation index	411.1±149.9	358.1±172.8	365.9±179.3
HCO_3^- (mmol/L)	27.7±2.1	28.1±1.9	28.4±1.5
BE (mmol/L)	1.10±1.76	0.56±1.83	1.12±1.69
Lac (mmol/L)	0.65±0.44	0.65±0.51	0.52±0.36

Compared with those before wound disclosure, *, $P<0.05$; **, $P<0.01$.

MAP and SpO_2 changed slightly (P>0.05) while the CVP rose significantly; the sedation level deepened with gradually decreased BIS value; HR and respiratory rate (P<0.05) gradually increased; acidemia was gradually aggravated with increasing $PaCO_2$ but no hypoxemia occurred after PaO_2 was maintained stable.

Compared with those before anesthesia, the MAP, RR and SpO_2 values 15 minutes after wound closure returned to their pre-anesthesia levels. Patients were still in light sedation, with slightly increased HR and BIS value of 73.4±13.6 (*Table 4*). Under spontaneous respiration with oxygen inhalation via a nasal tube (2-3 L/min), arterial blood gas analysis showed that PH value gradually recovered and $PaCO_2$ tended to decrease but returned to normal one hour after wound disclosure. The oxygenation index significantly declined 15 minutes after thoracotomy but recovered to that before thoracotomy 30 minutes later. Although the value of Lac after thoracotomy was higher than that before wound disclosure, both Lac values were within the normal range (*Table 5*).

Discussion

The present study enrolled subjects to undergo VATS bullectomy or lumpectomy which can be accomplished simply and in a short time. In our series, the operation duration was (57.5±14.2) min, and the duration from wound disclosure to closure when the negative pressure restored in the thoracic cavity in all cases did not exceeded 45 minutes. As a result, we did not have a long time to observe the pathophysiologic changes after pneumothorax.

Incisions through the chest wall for VATS are generally made between the 4th and 7th costal interspace, so we chose the T8/9 thoracic interspace as the puncture site to perform thoracic epidural blockade because it could maintain effective analgesia in the operative field. In this study, limb agitation occurred during skin incision due to insufficient epidural anesthesia in one patient, whose operation was then completed after further TCI anesthesia.

Cough reflex is a complicated process of neuro-physiological reflex. The cough center is located in the solitary nucleus over the medulla oblongata area of the brain, associated with respiratory neurons. Cough receptors are located mainly on the posterior wall of trachea, pharynx, and mucosa of bronchus. Receptors above secondary bronchi are sensitive to mechanical stimuli while those below are sensitive to chemical stimuli. Impulses caused by stimuli travel via the vagus nerve to the medulla of the brain and trigger a cough. Two cases in our study coughed during operative exploration and lobe traction before intrathoracic vagal blockade, but none had operation-irritated cough during the whole procedure after local anesthesia with lidocaine over the intrathoracic vagus nerve. This indicates intrathoracic vagal blockade may effectively prevent cough reflex, which is in consistency with another relevant study (7).

While patients maintained spontaneous breathing during anesthesia, the operated lung collapsed after iatrogenic pneumothorax. Moreover, factors related to operation and anesthesia aggravated the impaired respiratory function,

Table 4 Vital signs before anesthesia and 15 min after wound closure ($\bar{x}$±s)

	Before anesthesia	15 min after wound closure
BIS	92.5±5.6	73.4±13.6**
MAP (mmHg)	84.9±9.5	84.1±11.4
HR (bpm)	78.9±13.9	90.3±12.9*
RR (bpm)	15.8±1.4	15.0±4.3
SpO_2 (%)	99.2±1.1	99.0±2.6

Compared with those before anesthesia, *, P<0.05; **, P<0.01.

Table 5 Blood gas analyses before wound disclosure, 15, 30 and 60 min after wound closure ($\bar{x}$±s)

	Before wound disclosure	15 min after wound closure	30 min after wound closure	60 min after wound closure
pH	7.30±0.06	7.29±0.06	7.32±0.05	7.34±0.03
$PaCO_2$ (mmHg)	57.6±10.6	54.1±11.3	48.9±7.30*	47.7±4.45**
PaO_2 (mmHg)	260.7±119.2	144.1±98.8*	144.1±69.3**	133.8±42.5**
Oxygenation index	411.1±149.9	289.5±141.7**	410.5±117.6	420.7±146.7
HCO_3^- (mmol/L)	27.7±2.1	25.5±2.5**	25.0±2.6**	25.6±2.2**
BE (mmol/L)	1.10±1.76	–1.00±2.41*	–1.19±2.80*	–0.39±2.43
Lac (mmol/L)	0.65±0.44	1.08±1.33	1.13±0.98	0.97±0.63

Compared with those before wound disclosure. *, P<0.05; **, P<0.01.

mainly as follows: (I) decreased activity of the non-operated thoracic cage due to operative posture related compression; (II) further decreased activity of the thoracic cage caused by impaired intercostal muscle function following thoracic epidural anesthesia; (III) inhibition of the respiratory center caused by any anesthetic, sedative and analgesic agent; (IV) paradoxical breathing due to the collapse and insufficiency of the operated lung; (V) muscle flaccidity over the laryngopharynx in sedation which may produce and accelerate glossoptosis, leading to upper respiratory obstruction and aggravating paradoxical respiration and mediastinal flutter.

We strictly selected subjects with good cardiorespiratory functions and without difficult airway. The patients breathed oxygen through a ventimask during the procedure to maintain a good oxygenation index, with SpO_2 above 95%. When epidural anesthesia worked and TCI analgesia was administered, the breathing slowed down and hypercapnia was observed. When iatrogenic pneumothorax occurred on the operated side, the respiratory rate grew compensatingly and $PaCO_2$ increased continuously, which reached to the peak 15 minutes after pneumothorax but began to relieve slightly 30 minutes later. We consider that the hypercapnia occurring in this procedure is tolerable and has little effect on the hemodynamics. It is believed that the increase of $PaCO_2$ along with the decrease of pH mainly depends on the increasing speed of $PaCO_2$ and functional compensation of the kidney. The side effects and tolerance of hypercarbia are mainly related to the cardiovascular and cerebrovascular status of the patients. Studies have indicated that permissive hypercarbia relieves as well as deteriorates cerebral-ischemia-reperfusion injury in rats. Which role it will play is closely correlated with its severity. In a range of 60-100 mmHg, $PaCO_2$ relieves cerebral-ischemia-reperfusion injury in rats by inhibiting neuron apoptosis while it aggravates cerebral edema induced by cerebral-ischemia-reperfusion injury in a range of 101-120 mmHg (8). Propofol may significantly decrease the intracranial pressure and maintain the balance of cerebral oxygen supply and demand in patients with permissive hypercapnia (9).

Patients breathed with bilateral lungs after wound closure, lung dilatation and thoracic negative-pressure drainage. The arterial blood gas analysis 15 minutes later showed all values returned almost to their levels before wound disclosure. Our results also showed that the hypercapnia during pneumothorax was quickly and effectively improved after operation, particularly one hour later.

The blood pressure before operation was lower than that before anesthesia, which was induced by epidural anesthesia and TCI sedation. Thoracic epidural blockade may significantly influence the thoracic sympathetic nerve system, inducing vasodilatation and decreased blood pressure. The arterial blood pressure maintained basically normal after iatrogenic pneumothorax, with no arrhythmia noted by continuous ECG monitoring, which indicates that the mediastinal flutter has no significant influence on circulation. The increases of heart rate and CVP may be compensations for the slowed down venous return following the disappearance of negative pressure in unilateral thoracic cavity.

In the present study, we demonstrated that VATS wedge resection of bullae and pulmonary nodules with nontracheal intubation are feasible in operations that can be accomplished in a short time. Patients can maintain stable intraoperative physical signs without severe hypoxemia. The intraoperative hypercapnia is tolerable and transient and can be improved quickly when the bilateral lungs resume spontaneous respiration. Further research, however, is still to be further studied to characterize the hypercarbia 30 minutes after pneumothorax and to explore its systematic impacts.

References

1. Pompeo E, Mineo D, Rogliani P, Sabato AF, Mineo TC. Feasibility and results of awake thoracoscopic resection of solitary pulmonary nodules. Ann Thorac Surg 2004;78:1761-8.
2. Sugimoto S, Date H, Sugimoto R, Okazaki M, Aoe M, Sano Y, et al. Thoracoscopic operation with local and epidural anesthesia in the treatment of pneumothorax after lung transplantation. J Thorac Cardiovasc Surg 2005;130:1219-20.
3. Mineo TC, Pompeo E, Mineo D, Tacconi F, Marino M, Sabato AF. Awake nonresectional lung volume reduction surgery. Ann Surg 2006;243:131-6.
4. Mineo TC. Epidural anesthesia in awake thoracic surgery. Eur J Cardiothorac Surg 2007;32:13-9.
5. Pompeo E, Tacconi F, Mineo D, Mineo TC. The role of awake video-assisted thoracoscopic surgery in spontaneous pneumothorax. J Thorac Cardiovasc Surg 2007;133:786-90.
6. Pompeo E, Mineo TC. Awake pulmonary metastasectomy. J Thorac Cardiovasc Surg 2007;133:960-6.
7. Chen JS, Cheng YJ, Hung MH, Tseng YD, Chen KC, Lee YC. Nonintubated thoracoscopic lobectomy for lung

cancer. Ann Surg 2011;254:1038-43.
8. Zhou Q, Cao B, Niu L, Cui X, Yu H, Liu J, et al. Effects of permissive hypercapnia on transient global cerebral ischemia-reperfusion injury in rats. Anesthesiology 2010;112:288-97.
9. Wang LP, Chen GZ. Effects of sedation induced with dexmedetomidine versus propofol on intracranial pressure and cerebral oxygen metabolism in patients with permissive hypercapnia. Chinese Journal of Anesthesiology 2011;31:397-400.

Cite this article as: Dong Q, Liang L, Li Y, Liu J, Yin W, Chen H, Xu X, Shao W, He J. Anesthesia with nontracheal intubation in thoracic surgery. J Thorac Dis 2012;4(2):126-130. doi: 10.3978/j.issn.2072-1439.2012.03.10

Video-assisted thoracoscopic lobectomy for non-small cell lung cancer in patients with severe chronic obstructive pulmonary disease

Wei Wang[1,2*], Zhiqiang Xu[1,2*], Xinguo Xiong[1,2*], Weiqiang Yin[1,2], Xin Xu[1,2], Wenlong Shao[1,2], Hanzhang Chen[1,2], Jianxing He[1,2]

[1]Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; [2]Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

Correspondence to: Jianxing He, MD, PhD, FACS. Department of Cardiothoracic Surgery, the First Affiliated Hospital of Guangzhou Medical University, No. 151, Yanjiang Rd, Guangzhou 510120, China. Email: drjianxing.he@gmail.com.

Objective: To assess the feasibility, safety and long-term outcomes of video-assisted thoracic surgery (VATS) lobectomy for the treatment of non-small cell lung cancer (NSCLC) in patients with severe chronic obstructive pulmonary disease (COPD).

Methods: The clinical data of patients with NSCLC and severe COPD (preoperative FEV1% <50%) who underwent VATS lobectomy from January 2000 to January 2011 were retrospectively analyzed to identify their demographic parameters, postoperative complications and outcomes.

Results: The preoperative FEV1/FVC was <70% and FEV1% <50% in all 61 patients in this study, with a mean preoperative FEV1 of 0.99 L (0.54-1.58 L) and mean FEV1% of 38.4% (22-49.82%). All of the 61 patients underwent the VATS lobectomy or sleeve resection plus systemic lymph node dissection. The mean operative time was 218 minutes (120-355 minutes), with a mean intraoperative blood loss of 342 mL (50-1,600 mL). None of the patients converted to thoracotomy. Multivariate statistical analysis revealed that age and TNM staging after tumor resection were independent predictive factors for the 5-year survival in those patients (P=0.014 and 0.013).

Conclusions: With preoperative imaging studies, pulmonary function assessment and target positioning, VATS lobectomy can be safely and effectively performed for patients with NSCLC and severe COPD to achieve a satisfying long-term survival outcome.

Keywords: Non-small cell lung cancer (NSCLC); video-assisted thoracic surgery (VATS); lobectomy; chronic obstructive pulmonary disease (COPD)

Submitted Jul 08, 2013. Accepted for publication Jul 30, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.23
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.23

Introduction

A disease of serious harm to human health and life, lung cancer has shown evidently increasing morbidity and mortality worldwide in recent years, and ranked first in both figures in developed and developing countries (1). Although surgery has been recognized as the most effective method of treatment for early-stage non-small cell lung cancer (NSCLC), most patients with lung dysfunction, due to chronic obstructive pulmonary disease (COPD) after a history of smoking, are at a higher risk of complications after lung surgery. Therefore, a history of lung cancer with severe COPD is a contraindication to lobectomy. With the ongoing application of lung volume reduction surgery both at home and abroad, it has been shown that, after the removal of part of the lesions in lung tissue, lung function can be improved to varying extents for some patients with severe emphysema (2). An increasing number of studies have confirmed improvement in the lung function of patients with

lung cancer and severe COPD following lobectomy (3-5). Those findings have shed new light on the indications for lobectomy in patients with lung cancer and COPD.

Video-assisted thoracoscopic lobectomy was first applied for the treatment of lung cancer in 1992. Its greatest advantage included the minimal invasiveness, reduced postoperative pain and less damage to the respiratory muscle and pulmonary function (6). The video-assisted thoracic surgery (VATS) has been reported (7) to allow significantly faster recovery of pulmonary functionality for in the early stages after lobectomy, compared with open-chest surgery, which further suggests that VATS protects lung function more efficiently as it causes less damage to respiratory muscles. With the wide application of VATS and continuous advancement in the technology of anesthesia, intensive care and preoperative respiratory function management, the indications for pulmonary resection are also expanding to include more and more elderly patients or long-term smokers whose lung function is already impaired. At present, favorable short- and long-term outcomes have been reported in a few studies using VATS lung resection to treat patients with lung cancer and severe COPD (8). So far, however, only a small number of such cases undergoing VATS lobectomy have been reported, and the findings are not sufficient to provide a comprehensive evaluation of the safety and effectiveness of this approach in this regard. Hence, this study is conducted to assess the safety and effectiveness of VATS lobectomy based on the findings of 61 patients with lung cancer and severe COPD who underwent this treatment in our department.

Materials and methods

Clinical data

The clinical data of patients undergoing VATS lobectomy in First Affiliated Hospital of Guangzhou Medical College from January 2000 to January 2011 were retrospectively analyzed. Sixty-one patients complicated with COPD were identified and enrolled in this study based on the GOLD classification standard for COPD (9). Upon enrollment, all participants were engaged in a series of preparation before surgery, including quitting smoking, respiratory function exercise, administration of phlegm drugs and chest physiotherapy.

Preoperative examination and surgical methods

Before surgery, all participants received physical examination, routine blood tests, ECG, cardiac color Doppler ultrasound and lower extremity deep venous color Doppler ultrasound. Respiratory function tests include pulmonary ventilation-dispersion function tests and ventilation-perfusion radionuclide scans. Coronary CT or treadmill activity tests were performed in patients with suspected coronary heart disease over the age of 60, as well as coronary interventional examination, if necessary.

Preoperative tumor staging was based mainly on the chest X-ray examination, chest CT, head and abdominal MRI, whole body bone scan, and bronchoscopy. PET/CT scans were recommended for patients considered to be stage II or above. All participants underwent VATS lobectomy and hilar and mediastinal lymph node dissection, of which the specific surgical techniques were already reported in our previous study (10).

Data collection and follow-up

The demographic data, smoking status, lung function test results, operative time, blood loss, postoperative hospital stay, postoperative chest tube residence time, postoperative tumor stage, postoperative complications, and pre- and post-operative ECOG performance status of all enrolled patients were collected. The following postoperative complications were recorded: perioperative mortality (in-hospital mortality or death of any cause in 30 days after surgery), severe complications (surgery-related: second thoracotomy due to postoperative bleeding; Respiratory: ARDS and bronchopleural fistula, pneumonia, pulmonary embolism, empyema, pulmonary edema, tracheostomy or second endotracheal intubation; Cardiac: myocardial infarction, myocardial ischemia or angina pectoris, cerebrovascular event, deep vein thrombosis; Others: acute renal failure, acute gastrointestinal bleeding, etc); and mild complications (atelectasis, postoperative air leakage for more than seven days, pleural effusion, atrial fibrillation or other arrhythmias, wound infection, etc). Long-term follow-up was conducted to identify the breathing status, tumor recurrence and survival of all patients, for a period of 1-60 months.

Statistical analysis

Measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$). The chi-square test was used in the correlation analysis of changes in the ECOG performance status of the participants, and Kaplan-Meier survival analysis was conducted to identify the correlation with postoperative

Table 1 Demographics and clinical data

Characteristics	No (%)
Age, years	64 (range, 46-83)
Male:female	53:8
Smoking	
Yes	51
No	10
Preoperative lung function	
FEV1 (L)	0.99 (0.54-1.58)
FEV1%	38.40 (22-49.82)
FEV1/FVC%	47.88 (25.79-69)
VATS operations	
Lobectomy	57 (93.4%)
Sleeve resection	4 (6.6%)
Right upper lobe	23 (37.7%)
Right middle lobe	3 (5.0%)
Right lower lobe	11 (18.0%)
Left upper lobe	13 (21.3%)
Left lower lobe	11 (18.0%)
Mean operative time (mins)	218 (range, 120-355)
Bleeding (L)	342 (range, 50-1,600)
Hospital stay (days)	16 (5-54)
Histology	
Adenocarcinoma	34 (55.7%)
Squamous cell carcinoma	20 (32.8%)
Others	7 (11.5%)
Staging	
IA	9 (14.8%)
IB	19 (31.1%)
IIA	14 (23.0%)
IIB	6 (9.8%)
IIIA	13 (21.3%)

survival. The Cox regression model test was performed for each variable with a P value of ≤0.20 in the univariate analysis. The statistical analysis was completed in SPSS 13, with P<0.05 indicating a statistically significant difference.

Results

Clinical data

Sixty-one cases were finally included in the retrospective study, including 53 men (86.9%) and eight women (*Table 1*). The average age was 64 years (46-83 years). Fifty-one patients were long-term smokers. The preoperative FEV1/FVC was <70% and FEV1% <50% in all patients, with a mean preoperative FEV1 of 0.99 L (0.54-1.58 L) and mean FEV1% of 38.4% (22-49.82%).

All of the 61 patients underwent the VATS lobectomy or sleeve resection plus systemic lymph node dissection [right upper lobe in 23 cases (37.7%), right middle lobe in three (5.0%), right lower lobe in eleven (18.0%), left upper lob in thirteen (21.3%) and left lower lobe in eleven (18.0%)]. The mean operative time was 218 minutes (120-355 minutes), with a mean intraoperative blood loss of 342 mL (50-1,600 mL). None of the patients converted to thoracotomy. Postoperative pathology reported 34 cases of adenocarcinoma (55.7%),

20 cases of squamous cell carcinoma (32.8%) and seven of other tumors (11.5%). All participants were subject to pathological and clinical staging according to the TNM Classification of the UICC, 7th edition (11). As a result, there were nine patients of IA (14.8%), nineteen of IB (31.1%), fourteen of IIA (23.0%), six of IIB (9.8%), and thirteen of IIIA (21.3%).

Table 2 Complications

Complication	Patients, No[a]
Mortality	2
Air leak	16
Atrial fibrillation	3
Pneumonia	6
Respiratory failure	3
Atelectasis	2
Empyema	0
Pulmonary embolism	2
Wound infection	0
Bleeding	0

[a], Some patients had more than one complication.

Complications after surgery

Two patients died of ARDS during the perioperative period, and 24 patients (39.3%) presented postoperative complications (*Table 2*). Twenty-two patients (36.1%) had respiratory complications postoperatively, including air leakage in 16 cases (25.8%), pulmonary infection in six, respiratory failure in three, atelectasis in two and pulmonary embolism in two. The average hospital stay was 16±1.1 days (5-54 days).

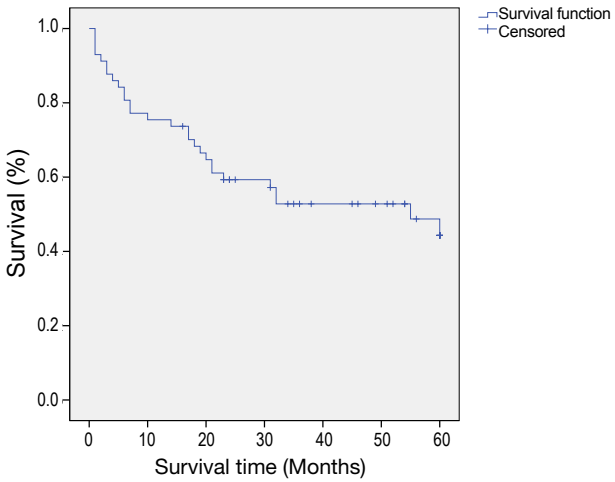


Figure 1 Overall survival (n=56).

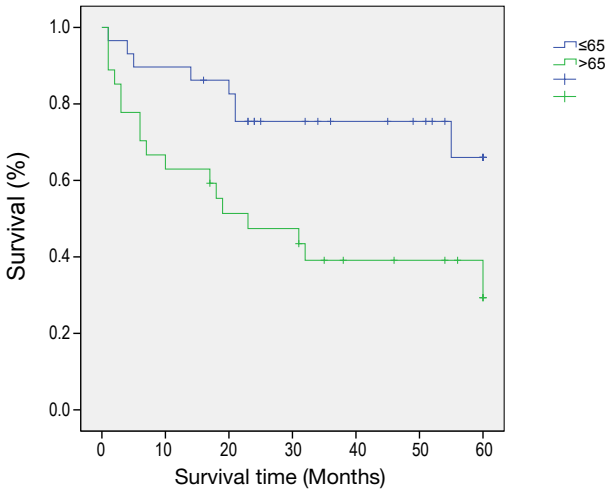


Figure 2 Survival according to age: ≤65 years old (n=27) versus >65 years old (n=29) (P=0.007).

Table 3 Univariate analysis of factors associated with overall survival

	No.	5-y OS rate % (95% CI)	P Log-rank test
Gender			
Male	50	40.2 (33.3-47.1)	0.434
Female	6	28.7 (12.0-45.3)	
Age			
≤65	29	47.8 (40.2-55.4)	0.007
>65	27	30.6 (20.7-40.4)	
Smoking status			
Nonsmoker	48	40.6 (33.6-47.6)	0.335
Smoker	8	28.0 (13.2-42.8)	
ECOG performance status			
0-1	27	42.0 (32.9-51.0)	0.787
2	29	37.0 (27.5-46.4)	
Histology			
Squamous cell carcinoma	19	47.2 (35.9-58.6)	0.216
Non-Squamous cell carcinoma	37	35.5 (27.6-43.4)	
Lobe location			
Upper lobe	33	40.8 (32.6-49.0)	0.557
Middle-lower lobe	23	37.4 (26.4-48.5)	
pTNM stage			
I	26	49.4 (41.1-57.6)	0.006
II/III	30	30.7 (21.7-39.8)	

Overall survival

During a median follow-up time of 39 months (1-60 months), five patients were lost to follow-up and 27 died. The survival rate was 75.4% in the first year, and 50.9% in five years (*Figure 1*). In the univariate analysis using the Log-rank test, the outcomes were correlated with age and postoperative TNM staging (P=0.007 and 0.006, *Table 3*). The median survival of patients not older than 65 years was 48 months, and reduced to 31 months in those older than 65 (P=0.007, *Figure 2*). Patients with stage I tumors had a median survival of 49 months, while those had stage II/III tumors had only 28 months. The difference was significant between them (P=0.006, *Figure 3*). In the Cox regression model, when taking into account those factors showing significant effect on survival in the univariate analysis, age and TNM staging after tumor resection were independent predictive factors for the 5-year survival in those patients (P=0.014 and 0.013, *Table 4*).

The ECOG scores were recorded three months before and after surgery to evaluate the changes of lung function and quality of life for the patients (12). The results showed that mean ECOG scores of 1.51 and 1.31 before and after surgery, respectively, among the 59 patients, excluding two who died during the perioperative period. The difference between those scores was significant (P<0.05).

Discussion

Lung cancer and COPD are two common diseases of human beings. The presence of both conditions in a patient

can increase the risk of complications after lung surgery due to underlying lung function damage. Since lung cancer patients with severe COPD are at a higher risk of postoperative complications, most of they have to receive non-radical partial lung resection (wedge or segmental resection) instead of lobectomy, which is currently recognized as the most effective means of treatment for early stage lung cancer. For patients with lung cancer, however, both pulmonary wedge resection and segmental resection are associated with a significantly increased recurrence rate and lower postoperative survival compared with standard lobectomy (13,14).

With the ongoing application of lung volume reduction surgery, it has been found that partial lung resection can achieve the similar result to volume reduction for patients with lung cancer and emphysema (3), which can minimize or even improve postoperative pulmonary function loss. Those findings have shed new light on the surgical options for patients with lung cancer and severe COPD. With the development of surgical techniques, anesthesia and intensive medical technology, an increasing number of studies have reported that lung resection can be tolerated by patients with lung cancer and severe pulmonary insufficiency, and can lead to satisfying outcomes (3,8,15-18).

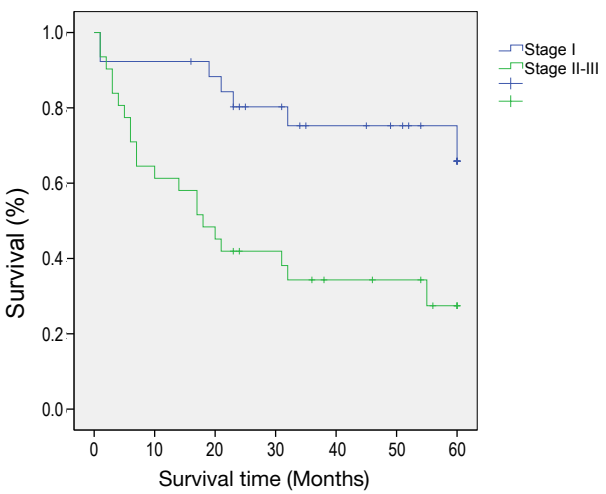


Figure 3 Survival according to stage: stage I disease (n=26) versus stages II/III (n=30) (P=0.006).

Since the early 1990s, VATS has been rapidly developed and widely applied in the world, involving almost all areas of general thoracic surgery. Compared with thoracotomy, VATS enables a smaller incision without removing or stretching the ribs open, sparing respiratory muscles from injures and thus minimizing the loss of lung function. Moreover, with a smaller incision, patients will suffer less pain postoperatively and expectorate more easily, reducing the incidence of postoperative pulmonary infection and complications as well. In view of those advantages, VATS procedures have been used in a growing number of studies to treat patients with lung cancer and severe pulmonary dysfunction (8,19).

Previous studies have shown that, however, patients with lung cancer and COPD have an increased risk of cardiopulmonary complications compared to patients with lung cancer alone (20). In the present study, two patients died of respiratory failure in the perioperative period and 24 patients (39.3%) had postoperative complications, of which 22 (36.1%) had respiratory complications with an average hospital stay of 16 days after surgery. It can be seen that the incidence of postoperative respiratory complications in this study is not unacceptable compared with the previous reports (*Table 5*). According to the existing studies, open chest surgery is associated with a longer postoperative hospital stay and higher incidence of respiratory complications in patients with lung cancer and severe pulmonary dysfunction compared with the VATS procedures, which further demonstrates that the VATS technique is an ideal option for such patients. A possible explanation for the lower risk of postoperative pulmonary complications is that reduced injury to respiratory muscles, smaller chest wall incision and consequently less pain allows patients to cough and expectorate more easily and get out of bed sooner after VATS, and this in turn reduces the likelihood of other complications of the respiratory system. In the present study, pulmonary complications were observed in 36.1% of the patients, which is lower than the report of most studies with open chest surgery (3,16,17) but higher than those with VATS surgery (8). Martin *et al.* (8)

Table 4 Multivariate analysis of overall survival.

Factors	Characteristics		Hazard ratio	95% CI	P value
	Unfavorable	Favorable			
Age	>65	≤65	2.899	1.239-6.787	0.014
pTNM stage	II/III	I	3.113	1.273-7.609	0.013

Table 5 Lung resection in poor pulmonary function

References	No. of Patients	Pulmonary function tests	Approach	Hospital stay (days)	Mortality 30 days %	Morbidity 30 days %	Respiratory complications (pulmonary and pleural) and remarks
Nakajima T *et al*. 2009 (17)	36	Pred FEV% <50%	Open: wedge + segment 4, lobectomy 26, pneumonectomy 6		0	11 (30.6)	Pneumonia 5 Bronchopleural fistula 3 Empyema 3
Garzon *et al*. 2006 (10)	25	Pred FEV1 <0.8 or FEV1% <50%	VATS: lobectomy 13, wedge resection 12	7.4 [2-26]	0	5 (20.0)	Air leak 2 Atelectasis 2 Pneumonia 1
Linden PA *et al*. 2005 (18)	100	Pred FEV1% <35%	Open: wedge resection 65, lobectomy 10, wedge resection 5, segmentectomy 4, lung volume reduction/wedge 8. VATS: lobectomy 4, segmentectomy 4	8.37	1 (1)	41 (41.0)	Prolonged air leak 22 New oxygen requirement 11 Respiratory failure 4 Pneumonia 4
Magdeleinat *et al*. 2005 (19)	106	Pred FEV% ≤50%	Open: pneumonectomy 16, lobectomy 73, z segmentectomy 7, wedge resection 10	20	5 (8.5)	74 (69.8)	Pneumonia 27 Atelectasis 16 Bronchitis 1
Martin U *et al*. 2005 (8)	34	ppoFEV1% <40%	VATS: lobectomy 17, segmentectomy 17	7 [3-31]	2 (5.8)	10 (29.4)	Pneumonia 3 Air leak 3 Empyema 1
Choong *et al*. 2004 (3)	21	Mean FEV1 =0.7 L mean Pred FEV1% =29%	Open: lobectomy 18, wedge resection 3	9 [5-24]	0	19 (90.5)	Respiratory failure 2 Air leak 11 Mini-tracheostomy 7
Solli *et al*. 2003 (20)	31	Pred FEV% <50% or DLCO% <50%	Open: pneumonectomy 10, lobectomy 11, z sublobar resection 10, segmentectomy 7, wedge 2	7 [4-21]	0	16 (31.6)	Respiratory failure 1 Atelectasis 3 Pneumonia 1 Air leak 3
Present study	62	Pred FEV% <50%	VATS: lobectomy 62	16±1 [5-54]	2 (3.2)	22 (36.1)	Air-leak 16 Pneumonia 6 Respiratory fail 3 Atelectasis 2 Pulmonary embolism 2

FEV, forced expiratory volume; ppoFEV1, predicted postoperative percentage of FEV1; Pred, predicted; VATS, video-assisted thoracic surgery.

carried out VATS lung resection for 34 patients with lung cancer and a FEV1% <40%; Although there were two dead cases, respiratory complications were observed in only ten patients (29.4%). In the present study, although the incidence of postoperative respiratory complications was higher than the above findings (8), systemic radical surgery was administered to all of the patients with lung cancer and severe COPD in the former, while VATS lobectomy accounted for up to 50.2% and 50% (8) in the other two studies. Lobectomy is associated with much greater surgical injury and loss of functional alveolar areas than either wedge resection or segmentectomy, and there were seven patients with extremely severe COPD and a preoperative FEV1% of only 27.8% (22-29.9 %) in this study.

Patients in this study had a relatively long hospital stay, averaging 16 days. Although it is slightly shorter than 20 days as reported by Magdeleinat *et al*. (17), it is longer than all of the other studies, which may be largely due to the surgical approaches. In this study, all 61 patients received either lobectomy or sleeve resection, whereas lobectomy accounts for a relatively small part in all of the remaining studies.

In the present study, both short- and long-term survival rates are observed in patients with moderate COPD who received lobectomy or sleeve resection after a 5-year follow-up. The survival analysis showed a 1-year survival rate of 75.4%, which was basically consistent with the findings of Magdeleinat (17), and a 5-year survival rate of 50.9%, which was higher than the report of Magdeleinat. Further analysis showed significantly better outcomes in patients with stage I lung cancer than in those with stages II or III, with the 5-year survival rates being 73.1% and 32.3%, respectively ($P<0.05$), which were generally consistent with other reports (8,15,17). According to the report by Martin *et al*. (8), the analysis of 34 patients with stage I lung cancer and severe pulmonary dysfunction who underwent VATS lobectomy or segmental resection revealed a 5-year survival up to 69.7%, without significant difference between the two groups. Nakajima *et al*. (15) found a 5-year survival of 57.9% in the stage I group as a part of 36 patients with lung cancer and severe lung dysfunction, but the 5-year survival was merely 11.9% in the more advanced groups.

Lung cancer and COPD are mostly found in elderly people, while patients over the age of 65 years account for about 50% and those over the age of 70 years account for 30-40% of all cases (21). COPD and cardiovascular diseases are the common concomitant diseases in elderly smokers with lung cancer, and the presence of such conditions may directly or indirectly affect their therapy and outcomes. In the study of Janssen-Heijnen *et al*. (22), age was regarded as an independent factor for the survival outcomes of patients with stages I and II NSCLC, though it had no significant impact on the survival outcomes of patients at more advanced stages. Li *et al*. (23) also found that the 5-year survival rate was significantly higher in patients with stage I lung cancer who were not older than 65 years, compared with those older. In our previous study, we also found that age could be a critical factor in predicting the outcomes of those patients (24). A number of studies (15,17,25) have shown that, for patients complicated with severe pulmonary dysfunction, those with stage I lung cancer would have a better outcome than patients with the condition at stages II and III ($P<0.05$). In the present study, multivariate statistical analysis also suggested that age could be an independent prognostic factor for patients with lung cancer and severe COPD, which was consistent with previous reports.

However, there are several limitations in this study due to its retrospective nature. Although it has included the largest number of patients with lung cancer and severe COPD undergoing VATS lobectomy so far, the absolute number is not significantly large. Secondly, the present analysis included only the 5-year survival but not the time to progression, and did not take into account the subsequent treatment patients received after the surgery when calculating the 5-year survival rates. Finally, an objective comparison between the lung function data before and after the surgery is unavailable because some of patients did not receive postoperative pulmonary function tests. Hence, the changes in the quality of life can merely be analyzed based on some relatively objective indicators in the present study. A more comprehensive prospective study will be needed to further determine the safety and effectiveness of VATS lobectomy as the treatment for patients with lung cancer and severe COPD.

In conclusion, VATS lobectomy can be safely and effectively performed for patients with NSCLC and severe COPD to achieve a satisfying long-term survival outcome as good as the routine VATS procedure, with an acceptable incidence of postoperative complications. Therefore, our preliminary conclusion is that for younger patients at an earlier stage (stage I), VATS lobectomy can be used as a more effective treatment option.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Zeng Q, Jiang S. Update in diagnosis and therapy of coexistent chronic obstructive pulmonary disease and chronic heart failure. J Thorac Dis 2012;4:310-5.
3. Choong CK, Meyers BF, Battafarano RJ, et al. Lung cancer resection combined with lung volume reduction in patients with severe emphysema. J Thorac Cardiovasc Surg 2004;127:1323-31.
4. Zhong N. Nipping it in the bud: An inspiring mission for prevention and management of COPD. J Thorac Dis 2012;4:102-5.
5. Korst RJ, Ginsberg RJ, Ailawadi M, et al. Lobectomy improves ventilatory function in selected patients with severe COPD. Ann Thorac Surg 1998;66:898-902.
6. Richards JMJ, Dunning J, Oparka J, et al. Video-assisted thoracoscopic lobectomy: the Edinburgh posterior approach. Ann Cardiothorac Surg 2012;1:61-9.
7. Hennon MW, Demmy TL. Video-assisted thoracoscopic surgery (VATS) for locally advanced lung cancer. Ann Cardiothorac Surg 2012;1:37-42.
8. Martin-Ucar AE, Nakas A, Pilling JE, et al. A case-matched study of anatomical segmentectomy versus lobectomy for stage I lung cancer in high-risk patients. Eur J Cardiothorac Surg 2005;27:675-9.
9. Pauwels RA, Buist AS, Calverley PM, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med 2001;163:1256-76.
10. He J, Shao W, Cao C, et al. Long-term outcome and cost-effectiveness of complete versus assisted video-assisted thoracic surgery for non-small cell lung cancer. J Surg Oncol 2011;104:162-8.
11. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
12. Berrisford R, Brunelli A, Rocco G, et al. The European Thoracic Surgery Database project: modelling the risk of in-hospital death following lung resection. Eur J Cardiothorac Surg 2005;28:306-11.
13. Warren WH, Faber LP. Segmentectomy versus lobectomy in patients with stage I pulmonary carcinoma. Five-year survival and patterns of intrathoracic recurrence. J Thorac Cardiovasc Surg 1994;107:1087-93; discussion 1093-4.
14. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
15. Nakajima T, Sekine Y, Yamada Y, et al. Long-term surgical outcome in patients with lung cancer and coexisting severe COPD. Thorac Cardiovasc Surg 2009;57:339-42.
16. Linden PA, Bueno R, Colson YL, et al. Lung resection in patients with preoperative FEV1 <35% predicted. Chest 2005;127:1984-90.
17. Magdeleinat P, Seguin A, Alifano M, et al. Early and long-term results of lung resection for non-small-cell lung cancer in patients with severe ventilatory impairment. Eur J Cardiothorac Surg 2005;27:1099-105.
18. Solli P, Leo F, Veronesi G, et al. Impact of limited pulmonary function on the management of resectable lung cancer. Lung Cancer 2003;41:71-9.
19. Demmy TL, Curtis JJ. Minimally invasive lobectomy directed toward frail and high-risk patients: a case-control study. Ann Thorac Surg 1999;68:194-200.
20. Pompili C, Brunelli A, Refai M, et al. Does chronic obstructive pulmonary disease affect postoperative quality of life in patients undergoing lobectomy for lung cancer? A case-matched study. Eur J Cardiothorac Surg 2010;37:525-30.
21. Yancik R. Cancer burden in the aged: an epidemiologic and demographic overview. Cancer 1997;80:1273-83.
22. Janssen-Heijnen ML, Smulders S, Lemmens VE, et al. Effect of comorbidity on the treatment and prognosis of elderly patients with non-small cell lung cancer. Thorax 2004;59:602-7.
23. Li Z, Yu Y, Lu J, et al. Analysis of the T descriptors and other prognosis factors in pathologic stage I non-small cell lung cancer in China. J Thorac Oncol 2009;4:702-9.
24. Wang W, Xu X, Wang W, et al. The expression and clinical significance of CLIC1 and HSP27 in lung adenocarcinoma. Tumour Biol 2011;32:1199-208.
25. Lau KK, Martin-Ucar AE, Nakas A, et al. Lung cancer surgery in the breathless patient--the benefits of avoiding the gold standard. Eur J Cardiothorac Surg 2010;38:6-13.

Cite this article as: Wang W, Xu Z, Xiong X, Yin W, Xu X, Shao W, Chen H, He J. Video-assisted thoracoscopic lobectomy for non-small cell lung cancer in patients with severe chronic obstructive pulmonary disease. J Thorac Dis 2013;5(S3):S253-S259. doi: 10.3978/j.issn.2072-1439.2013.08.23

Analysis of feasibility and safety of complete video-assisted thoracoscopic resection of anatomic pulmonary segments under non-intubated anesthesia

Zhihua Guo[1,2*], Wenlong Shao[1,2*], Weiqiang Yin[1,2*], Hanzhang Chen[1,2], Xin Zhang[1,2], Qinglong Dong[3], Lixia Liang[3], Wei Wang[1,2], Guilin Peng[1,2], Jianxing He[1,2]

[1]Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; [2]Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China; [3]Department of Anesthesiology, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China

*These authors contributed equally in this study.

Correspondence to: Jianxing He, MD, PhD, FACS. Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, No. 151, Yanjiang Rd, Guangzhou 510120, China. Email: drjianxing.he@gmail.com.

Objective: To explore the feasibility and safety of complete video-assisted thoracoscopic surgery (C-VATS) under non-intubated anesthesia for the resection of anatomic pulmonary segments in the treatment of early lung cancer (T1N0M0), benign lung diseases and lung metastases.

Methods: The clinical data of patients undergoing resection of anatomic pulmonary segments using C-VATS under non-intubated anesthesia in the First Affiliated Hospital of Guangzhou Medical University from July 2011 to November 2013 were retrospectively analyzed to evaluate the feasibility and safety of this technique.

Results: The procedures were successfully completed in 15 patients, including four men and eleven women. The average age was 47 [21-74] years. There were ten patients with adenocarcinoma, one with pulmonary metastases, and four with benign lung lesions. The resected sites included: right upper apical segment, two; right lower dorsal segment, one; right lower basal segment, two; left upper lingular segment, three; left upper apical segment, one; left upper anterior apical segment, two; left upper posterior segment, one; left lower basal segment, one; left upper posterior and apical segments, one; and left upper anterior and apical segments plus wedge resection of the posterior segment, one. One case had intraoperative bleeding, which was controlled with thoracoscopic operation and no blood transfusion was required. No thoracotomy or perioperative death was noted. Two patients had postoperative bleeding without the need for blood transfusions, and were cured and discharged. The pathologic stage for all patients with primary lung cancer was IA. After 4-19 months of follow-up, no tumor recurrence and metastasis was found. The overall mean operative length was 166 minutes (range 65-285 minutes), mean blood loss 75 mL (range 5-1,450 mL), mean postoperative chest drainage 294 mL (range 0-1,165 mL), mean chest drainage time 2 days (range 0-5 days), and mean postoperative hospital stay 5 days (range 3-8 days).

Conclusions: Complete video-assisted throacoscopic segmentectomy under anesthesia without endotracheal intubation is a safe and feasible technique that can be used to treat a selected group of IA patients with primary lung cancer, lung metastases and benign diseases.

Keywords: Video-assisted thoracoscopic surgery (VATS); segmentectomy; lung cancer

Submitted Jan 01, 2014. Accepted for publication Jan 06, 2014.
doi: 10.3978/j.issn.2072-1439.2014.01.06
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.01.06

Introduction

Lung cancer is the most common cancer worldwide, accounting for about 15% of cancer cases around the world, and 28% of cancer deaths (1). Lung cancer is also associated with the highest morbidity and mortality among all malignant conditions in China (2). Surgical resection by thoracotomy or thoracoscopy is the preferred treatment for early-stage non-small cell lung cancer (3). Since the early 1990s, video-assisted thoracoscopic surgery (VATS) has been rapidly developed and widely applied in the world, involving almost all areas of general thoracic surgery. Compared with thoracotomy, VATS enables a smaller incision without removing or stretching the ribs open, sparing respiratory muscles from injures and thus minimizing the loss of lung function. Moreover, with a smaller incision, patients will suffer less pain postoperatively and expectorate more easily, reducing the incidence of postoperative pulmonary infection and complications as well (4). Thoracoscopic lobectomy is a representative application of thoracoscopic surgical techniques in thoracic surgery.

With the development and extensive application of imaging techniques such as high-resolution computed tomography (HRCT) and low-dose spiral computed tomography (CT), the detection rate of small lung nodule of unknown nature has been increasing. Lung resection is considered to be applicable for early lung cancer (T1N0M0), small metastases and localized benign lesions (such as bronchiectasis and tuberculosis) (5-8). Compared with lobectomy, segment resection better preserves lung functions while removing small nodules (9). With the intensified aging population, some patients are often complicated with cardiovascular diseases that make them unable to tolerate lobectomy, and therefore segmental resection has also been considered for the treatment of patients with primary lung cancer and poor cardiopulmonary function (3).

For now, general anesthesia with one-lung intubated ventilation is the standard anesthesia in thoracic surgery. Intubated anesthesia is, however, often associated with postoperative throat discomfort, including primarily irritating cough, and throat pain in some patients. On the other hand, non-intubated anesthesia can reduce general anesthesia-related complications, and many investigators have therefore begun to explore its application in general thoracic surgery. Dong *et al.* reported that thoracoscopic wedge resection under non-intubated anesthesia was feasible and safe (10). Chen *et al.* reported the safety and feasibility of thoracoscopic resection under non-intubated anesthesia (lobectomy, lung resection and wedge resection) in 285 patients (11). Hung *et al.* reported segmental resection under non-intubated anesthesia in 21 patients, finding that the technique preserved maximum normal lung tissue while reducing the loss of lung functions, and general anesthesia-related adverse reactions (12). This study summarizes 15 patients undergoing C-VATS resection of anatomic pulmonary segments under non-intubated anesthesia in our department.

Subjects and methods

Clinical data

Patients undergoing C-VATS resection of anatomic pulmonary segments from July 2011 to November 2013 were enrolled. All patients received pre-operative chest high-resolution thin-slice enhanced CT scans and pulmonary function tests. For those suspected of lung cancer, additional upper abdomen CT, head MRI, whole body bone scintigraphy or whole body PCT examination was needed to exclude distant metastases. Patients were eligible when they had an ASA grade of I-II, BMI <25 and no evident airway secretions or contraindications for epidural puncture in preoperative anesthesia assessment (11). All operations were performed by the same group of thoracic surgeons and anesthesiologist team. The primary outcome measures included the operative time, intraoperative blood loss, hospital stay, chest drainage, chest tube duration, and type of lung resection.

Indications for segmental resection

The indications for segmental resection included: (I) a lung mass close to the hilum in which wedge resection is not possible; (II) history of lung lobe resection, leading to the consideration of an additional primary lesion; (III) past history of other malignancies and lung solitary tumors, for which differentiation with primary lung cancer is not possible via intraoperative frozen sections; (IV) multiple pulmonary ground-glass shadows, for which atypical adenomatous hyperplasia (AAH), adenocarcinoma *in situ* (AIS) or minimally invasive adenocarcinoma (MIA) may be suspected; (V) a complication with any cardiopulmonary disease that makes lobectomy intolerable; and (VI) peripheral early lung cancer ≤2 cm in diameter.

Surgical methods

Administration of anesthesia: with established intravenous

rehydration, an epidural catheter is inserted in the thoracic T6-7 space. In the supine position, 2 mL of 2% lidocaine is injected through the epidural catheter. If signs of spinal anesthesia are not present in five minutes, fractionated injection of 12 mL 0.375% ropivacaine is administered. Before surgery, the anesthesia level should reach between T2 and T10. Propofol and remifentanil are infused for sedation and analgesia during surgery, with the BIS values maintained between 40 and 60. During surgery, masked and nasopharyngeal airway assisted ventilation is given with an inhaled oxygen concentration FiO_2 of 0.33. Monitors are mounted on both sides along the patient's head, which generally lies on the opposite side to the operating site, with the hilum and waist padded to further widen the intercostal space. The operator stands in front of the patient, the first assistant on the patient's back side, and the second assistant handles the thoracoscope. The first port is generally made in the 7th or 8th intercostal space at the anterior axillary as the observation port. It should be noted that, in case that the diaphragm is too high or unclear on the X-ray images, this port should be positioned at a higher intercostal space to avoid injuring the abdominal organs. The second port is usually in the 7th intercostal space at the posterior axillary line and the third port close to the lesion, which form a triangle on the chest wall. All of them are treated with soft incision protectors to serve as the surgical operation channels. All video-assisted thoracic operations are performed using Stryker 1288 HD 3-Chip Camera/1288 with a three-chip HD camera system and specially designed endoscopic instruments in our department. After insertion of the thoracoscope from the first port, full chest exploration is conducted to determine whether there is evidence that the lesion is unresectable, such as pleural metastasis or other sign of metastases. Local vagus nerve block is achieved with 2 mL of 2% lidocaine under thoracoscopic guidance in the chest cavity, followed by spray of appropriate amount of the same concentration on the surface to reduce coughing that may induced by pulling of the lung tissue, ensuring a steady operation environment.

The thoracoscopic lung resection is done following the basic principle for lobectomy, in the order of arteries, bronchi, veins, and lung parenchyma in general. For resection of upper segments in the left upper lung, the veins are treated first because the superior branch of the superior pulmonary vein is anterior to, and blocks part of, its anterior branch, and thereby it should be first transected. The use of staplers and vascular clips is at the discretion of the operator depending on the vessel sizes during the surgery. According to the experience of the surgeons in our department, the use of hemolok and titanium clips should be avoided when clamping blood vessels. That is mainly because their application may affect the appropriate operation of other equipment such as stapler. (For example, a clip being caught in the stapler may prevent it from being successfully triggered.). Although in the event that vessels are well exposed, a stapler can be used to directly close or ligate and cut them off, there are still many factors that may affect those operations to such an extent that vessels are excessively pulled and injured when the stapler passes through them. In such cases, the tip of a linear stapler can be guided through the stapler guiding catheter to safely pass the posterior part of a vessel to successfully cut it off. The same method can be used to cut off bronchi, with satisfactory results. After the vessels and bronchi at the lesion segment are resected, the lung segment is in an atelectasis state. The anesthesiologist is instructed to maintain low volume low pressure ventilation to help determine the intersegmental plane. In addition, when the veins around the segment and in the surrounding segments to be preserved are well exposed, they can also be used to help identify the intersegmental plane. Mediastinal lymph node assessment is an essential component in thoracoscopic segmental resection for non-small cell lung cancer. Systemic lymph node dissection is performed following the segmental resection. Frozen sections of the segmental bronchus stumps and lymph nodes are sent for pathological tests. When positive intersegmental or interlobular metastases are present, switch to lobular resection is always preferred as long as the patient's physical conditions allow. If there is so little residual tissue following the resection that the high mobility makes lung torsion likely, Gossot *et al.* suggests connecting with the adjacent lobes via TA to reduce the postoperative complication (10). During surgery, if SpO_2 drops to below 90%, mask assisted ventilation is needed to improve oxygenation. If blood gas analysis shows an arterial carbon dioxide partial pressure of ≥80 mmHg, the operation needs to be suspended followed by mask-assisted gas exchange. If the ventilation does not improve in this way, endotracheal intubation is required (9). Chest tube drainage is routinely used after the surgery. When there is no leakage and thoracic fluid volume is less than 200 mL per day, removal of the drainage can be considered.

Specific methods of segmental resection

(I) Resection of right upper posterior apical segments: the

Table 1 Basic characteristics of patients

Characteristics	Number of patients (n=15)	Percentage
Median age (years)	47 [21-74]	
Gender		
Male	4	27
Female	11	73
Smoking history		
No smoking history	15	100

Table 2 Postoperative pathology

Pathological type	Number of patients (n=15)	Percentage
Primary bronchogenic carcinoma		
Adenocarcinoma	10	66.7
Metastasis		
Lung metastasis of breast cancer	1	6.7
Benign disease		
Pulmonary sclerosing hemangioma	1	6.7
Bulla	1	6.7
Proliferation of fibrous connective tissue	1	6.7
Arteriovenous fistula	1	6.7

apical and posterior segments can be treated separately, but they are usually removed at the same time. The posterior ascending aorta anterior to the upper lobular bronchus is treated before the bronchi. The upper lobe is pulled forward to expose the posterior mediastinum. The pleura of the upper lobe bronchus close to the mediastinum are opened using coagulation hook, "peanut" gauze or a combination of both. A 45-mm endoscopic stapler is used to open the posterior part of the oblique fissure to help expose the ascending aorta, and the artery is transected. With combined use of the cautery hook, right-angle clamp and ultrasonic scalpel, the surrounding soft tissue is separated until the apical segmental bronchus is fully exposed. The apical artery is located posterior to it. A cutting stapler is used to close the bronchus while the posterior arteries are properly protected. After transection of the segmental bronchus, the apical artery is revealed. The upper lung lobe is pulled backwards to expose the apical vein anterior to the hilum, which is then closed and cut. When eventually cutting the lung parenchyma, the anesthetist is instructed to maintain low-pressure ventilation so that the boundary line between ventilated and non-ventilated areas can be followed as the cutting line.

(II) Resection of the upper segment in the right lower lung: with combined use of the coagulation hook and ultrasonic scalpel, the pleura around the hilum in the right lower lung are divided and the oblique fissure opened using a stapler. The pulmonary arteries are gradually exposed. After the upper segmental artery is divided and cut, the posterior bronchus is revealed, separated, stapled and cut. The inferior pulmonary ligament is transected through to the inferior pulmonary vein. Gauze is used to expose the superior segmental vein upwards from the inferior pulmonary vein, and the former is then cut with a vascular clamp or stapler.

(III) Resection of the basal segment in the right lower lung: the anterior part of the oblique fissure is opened to expose the basal segment artery, which is transected and closed. The segmental bronchus is separated from the deep structure of the artery. The anesthesiologist is instructed to help identify if the basal segment bronchus is closed off by ventilation. The inferior pulmonary ligament is transected through to the inferior pulmonary vein. With the inferior lobe is pulled up, the surrounding tissue of the inferior pulmonary vein is divided using the cautery hook and peanut gauze. The basal segment vein is exposed and transected.

(IV) Lingular segment of the left upper lung: the lingular artery is separated and transected to reveal the upper lobular bronchus and lingular segmental bronchus. The latter is clamped, and low ventilation is used to identify its closure before transaction. The superior pulmonary vein is separated until its lowermost branch is exposed. If the lingular segmental vein can be located, it is transected before the intersegmental pulmonary tissue is handled. Otherwise, the lingular segmental vein can be treated until the lingular segmental tissue is fully separated.

Results

The procedures were successfully completed in 15 patients, including four men and eleven women. The average age was 47 [21-74] years. The patient characteristics are listed in *Table 1*. Pathological examination showed ten patients with adenocarcinoma, one with pulmonary metastases, and four with benign lung lesions (*Table 2*).

Segmental resections were successful in all patients without switching to thoracotomy or lobectomy. The

Table 3 Thoracoscopic resection of lung segments

Sites	Number
Left	
S4 + S5	3
S1 + S3 + PS2	1
S1	1
S2	1
S7 + S8 + S9 + S10	1
S1 + S3	2
S1 + S2	1
Total	10
Right	
S1	2
S6	1
S7 + S8 + S9 + S10	2
Total	5

Note: S1, apical; S2, posterior; S3, anterior; S4 + S5, lingular; S6, superior; S7, medial basal; S8, anterior basal; S9, external basal; S10, posterior basal.

resected sites included: right upper apical segment, two; right lower dorsal segment, one; right lower basal segment, two; left upper lingular segment, three; left upper apical segment, one; left upper anterior apical segment, two; left upper posterior segment, one; left lower basal segment, one; left upper posterior and apical segments, one; and left upper anterior and apical segments plus wedge resection of the posterior segment, one. Resected lung segments are shown in *Table 3*.

One case had intraoperative bleeding of 1,450 mL, which was controlled with thoracoscopic operation and no blood transfusion was required. There were no perioperative deaths. Two patients of postoperative bleeding were controlled with hemostatic medicine without the need for blood transfusions, and no other serious complications occurred. All patients were cured and discharged. The overall mean operative length was 166 minutes (range 65-285 minutes), mean blood loss 75 mL (range 5-1,450 mL), mean postoperative chest drainage 294 mL (range 0-1,165 mL), mean chest drainage time 2 days (range 0-5 days), and mean postoperative hospital stay 5 days (range 3-8 days) (*Table 4*).

Of the ten patients with primary lung cancer, nine received mediastinal lymph node dissection or systemic lymph node sampling, and the pathological staging showed stage IA for them; one patient who did not receive the above procedure had micro invasive adenocarcinoma in the left lung. After 4-19 months of follow-up for the patients, no tumor recurrence and metastasis was found.

Table 4 Intra- and post-operative conditions of lung resection surgery

Characteristics	Value/number of patients
Mean operation length (min)	166 [65-285]
Mean intraoperative blood loss (mL)	75 [5-1,450]
Mean drainage volume, mL	294 [0-1,165]
Mean drainage days	2 [0-5]
Mean postoperative stay (days)	5 [3-8]
Perioperative complications	
Postoperative bleeding, n (%)	2 (13.4)

Discussion

Whether segmental resection can achieve comparable effects to lobectomy for the treatment of early stage lung cancer is still controversial. Previous studies have shown that for early lung cancer, particularly when the tumor diameter is ≤2 cm, segmental resection can yield comparable long-term survival as with lobectomy (13,14). However, evidence in this regard comes mainly from retrospective case comparisons and meta-analyses, and the role of segmental resection in NSCLC needs to be further confirmed by large international multi-center randomized controlled clinical studies (CALGB 140503 in the United States and JCOG0802/WJOG4607L in Japan).

Complete thoracoscopic segmental resectionis a complex and technically demanding procedure, requiring the surgeon to be extremely familiar with the anatomic structures of every segmental vessel and bronchus. One of the major technical difficulties is confirmation of the plane between segments. Most investigators traditionally suggest low-pressure ventilation after occlusion or transection of segmental bronchi, so that the plane can be determined by differentiating between the collapsed and expanded interface. The purpose of the ventilation is to avoid the influence on endoscopic vision and operation by excessive expansion of lung tissue. According to our experience, a long-handled tong may be used to clamp the plane after low-pressure ventilation, as it provides two main advantages: (I) in view of the traffic between the lung segments, adjacent lung segments can be expanded with ventilation, blurring the lung segment boundary;

(II) a stapler only provides a limited opening angle that is likely to injury the lung parenchyma when coming across the thicker portion of it, leading to the need of manual stitches and bleeding control after the resection, which will increase the length of operation. The use of this recommended instrument can provide local compression, making it easier for a stapler to pass the lung segment boundary. Some investigators on the other hand suggest the use of selective lung ventilation in patients with COPD, in which the target segment is expanded through bronchoscopy and separated from other collapsed lung segments, reducing the impact of endoscopic vision by lung expansion (15). Segmental veins can also be helpful in identifying the intersegmental plane, and separation along pulmonary veins and loose connective tissue in the lung segments usually does not damage large bronchi and pulmonary arterial branches. Some lesions are located between segments, and when reliable surgical margins are not secured, resection of the adjacent segments can be considered.

Compared with traditional surgery under general anesthesia, epidural analgesia reduces intubation-related complications and facilitates early mobility of patients (10,11,16). It also reduces the dose of intraoperative anesthesia drugs, which will help restore the breathing and digestive functions. Four to six hours after non-intubation segmental resection, the patients could start eating, drinking, and get out of bed. Chest X-ray scans could be performed on the same the day after surgery. If imaging tests suggest good lung recruitment and no air leaks, and 24-h chest drainage is less than 200 mL, the drainage can be removed. With non-intubated anesthesia, coughing induced by postoperative throat discomfort is significantly reduced. Coughing may worsen wound pain, which in turn suppresses the cough reflex, making pulmonary secretions difficult to discharge after surgery, and indirectly leading to alveolar hypoventilation due to rapid and shallow breathing; some patients may even experience atelectasis or lung infection after surgery. Therefore, non-intubation endoscopic resection of lung segments may reduce the incidence of pulmonary complications, maximize protection of lung function and reduce postoperative pain, shorten chest tube duration, shorten the length of hospital stay, and allow faster recovery to preoperative mobility.

Non-intubated anesthesia combined with C-VATS lung resection surgery should be one of the most minimally invasive lung cancer surgery at present. With non-intubation anesthesia, the biggest challenge for surgeons is the remarkable mediastinal motion, which requires full cooperation among the surgeon, anesthetists and assistants. Mediastinal movement occurs when the ipsilateral intrathoracic pressure was significantly higher than that of the contralateral side in open pneumothorax, resulting in mediastinal shift to the contralateral area that further limits expansion of the contralateral lung. During inhalation and exhalation, the unbalanced pleural pressure on both sides experiences cyclical changes so that the contralateral mediastinum moves toward the contralateral side during inhalation and the opposite side during exhalation. In non-intubation segmental resection, the patient's spontaneous breathing has to be retained in order to achieve atelectasis of the operative side and good ventilation of the contralateral lung, so that both the oxygen supply and a favorable operating field can be secured. With collapsed ipsilateral lung after thoracotomy, some patients will have obvious mediastinal swing, which will affect the surgeon's surgical operation, particularly when dealing with blood vessels in which excessive traction may lead to bleeding. To mitigate the impact of the mediastinal swing during surgery, anesthesiologists can increase the amount of opioids based on the operation, reduce the breathing frequency or the respiratory tidal volume, thereby reducing the amplitude of the swing. At the same time, appropriate ventilation can be given based on the results of blood gas analysis to avoid serious hypercapnia, so as to maintain the body's acid-base balance.

Based on the fifteen patients undergoing non-intubated anesthesia combined with C-VATS lung resection in our department, the technique is feasible and safe with the help of skilled anesthetists with experience in thoracoscopic lobectomy and non-intubated anesthesia. So far, there has been no shift to thoracotomy and lobectomy. Although there was one case of bleeding, it was well controlled endoscopically without the need of blood transfusion. As for the two cases of postoperative bleeding, no blood transfusions were needed and no other complications were observed. The incidence of perioperative complication was 13.4%. The mean operative time was 166 minutes, mean intraoperative blood loss 75 mL, mean postoperative chest drainage two days, and mean postoperative hospital stay five days. The operative time and the number of days in hospital are comparable to those reported with VATS under general anesthesia, while intraoperative blood loss, chest drainage time and perioperative complications were better than the latter (*Table 5*).

In summary, complete video-assisted thoracoscopic surgery (C-VATS) under non-intubated anesthesia for

Table 5 Thoracoscopic segmental resection (17-29)

Lead author	Year of publication	Number of cases	Operation time (min)	Intraoperative blood loss (mL)	Chest tube drainage (days)	Postoperative hospital stay (days)	Perioperative complications (%)
Tracheal intubation, VATS segmentectomy							
Shiraishi	2004	34	240±72	169±168	4.5±3.2	–	11.8
Atkins	2007	48	136±45	250±200	3.5±4.0	–	25.8
Watanabe	2009	41	220±56	183±195	3.0±2.0	–	31.3
Shapiro	2009	31	–	–	2 [1-33]	4 [1-98]	26.0
Schuchert	2009	104	136 [120-152]	171 [133-209]	–	5	6.9
Oizumi	2009	30	216 [146-425]	100 [3-305]	1 [1-7]	–	0
Leshnower	2010	15	145±55	–	2.8±1.3	–	11.6
Gossot	2011	50	188±54	91±82	3.3±1.0	–	19.0
Moroga	2011	20 a	303±103	182±291	4.6±3.4	–	20.0
Moroga	2011	63 b	241±82	118±127	5.1±3.8	–	34.5
Dylewski	2012	35	146 [82-229]	50 [20-100]	–	2 [1-15]	33.9
Yamashita	2012	90	257±91	132±181	4.8±3.4	–	34.6
Pu	2012	20	155 [120-235]	50 [10-600]	3 [1-6]	6 [3-9]	25.0
Lin	2012	20	133 [90-240]	85 [50-200]	3.2 [2-7]	6.7 [4-11]	0
Nonintubated VATS segmentectomy							
Present study		15	166 [65-285]	75 [5-1,450]	2 [0-5]	5 [3-8]	13.4

a, with SNB; b, without SNB; SNB, sentinel node biopsy.

the resection of anatomic pulmonary segments in the treatment of early lung cancer (T1N0M0), benign lung diseases and lung metastases is safe and feasible, and can reduce postoperative pain, improve the appearance with small incisions, shorten chest drainage duration and postoperative hospital stay, provide maximum protection of lung functions, and reduce complications after general anesthesia. However, it requires that the surgeon has extensive experience in thoracoscopic lung resection in good cooperation with anesthesia doctors. Due to the short follow-up period, the long-term efficacy needs to be further confirmed. The long-term effect of non-intubated thoracoscopic anatomic segmental resection needs to be further studied and identified in a larger-scale study.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. American cancer Society, Lung Cancer Statistics, 2010.
2. Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Available online: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx
3. Ettinger DS, Akerley W, Borghaei H, et al. Non-small cell lung cancer, version 2.2013. J Natl Compr Canc Netw 2013;11:645-53; quiz 653.
4. He J, Xu X. Thoracoscopic anatomic pulmonary resection. J Thorac Dis 2012;4:520-47.
5. Shapiro M, Weiser TS, Wisnivesky JP, et al. Thoracoscopic segmentectomy compares favorably with thoracoscopic lobectomy for patients with small stage I lung cancer. J Thorac Cardiovasc Surg 2009;137:1388-93.
6. Zhong C, Fang W, Mao T, et al. Comparison of thoracoscopic segmentectomy and thoracoscopic lobectomy for small-sized stage IA lung cancer. Ann Thorac Surg 2012;94:362-7.
7. Donahue JM, Morse CR, Wigle DA, et al. Oncologic efficacy of anatomic segmentectomy in stage IA lung cancer patients with T1a tumors. Ann Thorac Surg 2012;93:381-7; discussion 387-8.
8. Xiong X, Shao W, Yin W, et al. Video-assisted thoracoscopic surgery for stage I non-small cell lung cancer: long-term survival and prognostic factors. Tumour Biol 2013;34:3389-96.

9. Yoshimoto K, Nomori H, Mori T, et a1. Quantification of the impact of segmentectomy on pulmonary function by perfusion single-photon-emission computed tomography and multidetector computed tomography. J Thorac Cardiovasc Surg 2009;137:1200-5.
10. Dong Q, Liang L, Li, Y, et al. Anesthesia with nontracheal intubation in thoracic surgery. J Thorac Dis 2012;4:126-30.
11. Chen KC, Cheng YJ, Hung MH, et al. Nonintubated thoracoscopic lung resection: a 3-year experience with 285 cases in a single institution. J Thorac Dis 2012;4:347-51.
12. Hung MH, Hsu HH, Chen KC, et al. Nonintubated thoracoscopic anatomical segmentectomy for lung tumors. Ann Thorac Surg 2013;96:1209-15.
13. Read RC, Yoder G, Schaeffer RC. Survival after conservative resection for T1 N0 M0 non-small cell lung cancer. Ann Thorac Surg 1990;49:391-8; discussion 399-400.
14. Koike T, Togashi K, Shirato T, et a1. Limited resection for noninvasive bronchioloalveolar carcinoma diagnosed by intraoperative pathologic examination. Ann Thorac Surg 2009;88:1106-11.
15. Okada M, Mimura T, Ikegaki J, et al. A novel video-assisted anatomic segmentectomy technique:Selective segmental inflation via bronchofiberoptic jet followed by cautery cutting . J Thorac Cardiovasc Surg 2007;133:753-8.
16. Shao W, Wang W, Yin W, et al. Nonintubated thoracoscopic lobectomy plus lymph node dissection following segmentectomy for central type pulmonary masses. Chin J Cancer Res 2013;25:124-7.
17. Shiraishi T, Shirakusa T, Iwasaki A, et al. Video-assisted thoracoscopic (VATS) segmentectomy for small peripheral lung cancer tumors. Surg Endosc 2004;18:1657-62.
18. Atkins BZ, Harpole DH Jr, Mangum JH, et al. Pulmonary segmentectomy by thoracotomy or thoracoscopy: reduced hospital length of stay with a minimally-invasive approach. Ann Thorac Surg 2007;84:1107-12; discussion 1112-3.
19. Watanabe A, Ohori S, Nakashima S, et al. Feasibility of video-assisted thoracoscopic surgery segmentectomy for selected peripheral lung carcinomas. Eur J Cardiothorac Surg 2009;35:775-80; discussion 780.
20. Shapiro M, Weiser TS, Wisnivesky JP, et al. Thoracoscopic segmentectomy compares favorably with thoracoscopic lobectomy for small stage I lung cancers. J Thorac Cardiovasc Surg 2009;137:1388-93.
21. Schuchert MJ, Pettiford BL, Pennathur A, et al. Anatomic segmentectomy for stage I non-small-cell lung cancer: comparison of video-assisted thoracic surgery versus open approach. J Thorac Cardiovasc Surg 2009;138:1318-25.e1.
22. Oizumi H, Kanauchi N, Kato H, et al. Total thoracoscopic pulmonary segmentectomy. Eur J Cardiothorac Surg 2009;36:374-7; discussion 377.
23. Leshnower BG, Miller DL, Fernandez FG, et al. Video-assisted thoracoscopic surgery segmentectomy: a safe and effective procedure. Ann Thorac Surg 2010;89:1571-6.
24. Gossot D, Ramosa R, Brian E, et al. A totally thoracoscopic approach for pulmonary anatomic segmentectomies. Interact Cardiovasc Thorac Surg 2011;12:529-32.
25. Moroga T, Yamashita S, Tokuishi K, et al. Thoracoscopic segmentectomy with intraoperative evaluation of sentinel nodes for stage I non–small cell lung cancer. Ann Thorac Cardiovasc Surg 2012;18:89-94.
26. Dylewski MR, Ohaeto AC, Pereira JF. Pulmonary resection using a total endoscopic robotic video-assisted approach. Semin Thorac Cardiovasc Surg 2011;23:36-42.
27. Yamashita S, Tokuishi K, Anami K, et al. Thoracoscopic segmentectomy for T1 classification of non-small cell lung cancer: a single center experience. Eur J Cardiothorac Surg 2012;42:83-8.
28. Pu Q, Mei JD, Liao H, et al. Complete video-assisted thoracoscopic anatomic segmentectomy for pulmonary diseases: the early experiences. Zhonghua Wai Ke Za Zhi 2012;50:823-6.
29. Lin ZW, Jiang W, Wang Q, et al. Total thoracosenpic anatomic pulmonary segmentectomy for 20 patients. Chin J Clin Thorac Cardiovasc Surg 2012;19:270-3.

Cite this article as: Guo Z, Shao W, Yin W, Chen H, Zhang X, Dong Q, Liang L, Wang W, Peng G, He J. Analysis of feasibility and safety of complete video-assisted thoracoscopic resection of anatomic pulmonary segments under non-intubated anesthesia. J Thorac Dis 2014;6(1):37-44. doi: 10.3978/j.issn.2072-1439.2014.01.06

Comparative study of systematic thoracoscopic lymphadenectomy and conventional thoracotomy in resectable non-small cell lung cancer

Wei Wang[1,2]*, Weiqiang Yin[1,2]*, Wenlong Shao[1,2]*, Gening Jiang[3], Qun Wang[4], Lunxu Liu[5], Deruo Liu[6], Zheng Wang[7], Zhihua Zhu[8], Hanzhang Chen[1,2], Jianxing He[1,2]

[1]Department of Cardiothoracic Surgery, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; [2]Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China; [3]Department of Thoracic Surgery, Shanghai Pulmonary Hospital of Tongji University, Shanghai 200433, China; [4]Department of Thoracic Surgery, Shanghai Zhongshan Hospital of Fudan University, Shanghai 200032, China; [5]Department of Cardiovascular and Thoracic Surgery, West China Hospital, Sichuan University, Chengdu 610041, China; [6]Department of Thoracic Surgery, China and Japan Friendship Hospital, Beijing 100029, China; [7]Department of Thoracic Surgery, Shenzhen People's Hospital, Shenzhen 518020, China; [8]Department of Thoracic Oncology, Cancer Center of Sun Yat-Sen University, Guangzhou 510060, China

*These authors contributed equally in this work.

Correspondence to: Jianxing He, MD, PhD, FACS. Department of Cardiothoracic Surgery, the First Affiliated Hospital of Guangzhou Medical University. No. 151, Yanjiang Rd, Guangzhou 510120, PR China. Email: drjianxing.he@gmail.com.

Objective: To assess the feasibility and safety of the video-assisted thoracoscopy surgery (VATS) systematic lymph node dissection in resectable non-small cell lung cancer (NSCLC).

Methods: The clinical data of patients with NSCLC who underwent VATS or thoracotomy combined with lobectomy and systematic lymphadenectomy from January 2001 to January 2008 were retrospectively analyzed to identify their demographic parameters, number of dissected lymph nodes and postoperative complications.

Results: A total of 5,620 patients were enrolled in this study, with 2,703 in the VATS group, including 1,742 men (64.4%), and 961 women (35.6%), aged 59.5±10.9 years; and 2,917 in the thoracotomy group, including 2,163 men (74.2%), and 754 women (25.8%), aged 58.5±10.4 years. Comparing the VATS with the thoracotomy groups, the mean operative time was 146 *vs.* 157 min, with a significant difference (P<0.001); and the average blood loss was 162 *vs.* 267 mL, with a significant difference (P<0.001). Comparing the two groups of patients data, the number of lymph node dissection: 18.03 in the VATS group and 15.07 in the thoracotomy group on average, with a significant difference (P<0.001); postoperative drainage time: 4.5 days in the VATS group and 6.37 days in the thoracotomy group on average, with a significant difference (P<0.001); postoperative hospital stay: 6.5 days in the VATS group and 8.37 days in the thoracotomy group on average, with a significant difference (P<0.001); proportion of postoperative chylothorax: 0.2% (4/2,579) in the VATS group and 0.4% (10/2,799) in the thoracotomy group, without significant difference (P>0.05).

Conclusions: For patients with resectable NSCLC, VATS systematic lymph node dissection is safe and effective with fewer postoperative complications, and significantly faster postoperative recovery compared with traditional open chest surgery.

Keywords: Non-small cell lung cancer (NSCLC); video-assisted thoracoscopy surgery (VATS); systematic lymph node dissection

Submitted Jan 03, 2014. Accepted for publication Jan 06, 2014.
doi: 10.3978/j.issn.2072-1439.2014.01.07
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.01.07

Introduction

Lung cancer is a serious hazard to human health and life, with a significant rising trend in terms of morbidity and mortality around the world in recent years. This condition has become the leading cause of morbidity and mortality worldwide, both for developed and developing countries (1). Although there are many methods for treating lung cancer at present, the recognized option of choice for the treatment of early- and mid-stage non-small cell lung cancer (NSCLC) is surgical excision, and the standard surgical method is lobectomy combined with systematic lymph node dissection. As early as in 1983, Martini *et al*. (2) first reported the use of lobectomy and mediastinal lymph node dissection for the treatment of primary lung cancer.

With the wide application of minimally invasive techniques in the surgical field, the use of video-assisted thoracoscopy surgery (VATS) in the treatment of NSCLC has been increasingly valued by thoracic surgeons. With the greatest advantage of minimal invasiveness, reduced postoperative pain and less damage to the respiratory muscle and pulmonary function, the VATS technique has been applied in the lobectomy of lung cancer as early as in 1992 (3). In 1995, McKenna *et al*. (4) first reported the use of VATS lobectomy combined with mediastinal lymph node dissection in the treatment of primary lung cancer.

Thorough lymph node dissection is one of the keys for successful comprehensive treatment of lung cancer, as it provides definite staging and guidance for the prognosis and the next treatment, and can improve the local remission rate and prolong disease-free survival time. According to the guidelines issued by the European-Society of Thoracic Surgeons (ESTS), systematic lymph node dissection is required for resectable NSCLC regardless of VATS or thoracotomy (5). Whether VATS allows thorough mediastinal lymph node dissection and can achieve comparable effects to thoracotomy has been controversial. At present, the reported results varied in different studies on the use of VATS for lobectomy combined with lymphadenectomy of resectable NSCLC compared with thoracotomy (6-14). So far, however, the number of studies comparing the two techniques is not large enough for a comprehensive assessment of the effectiveness and safety of systematic lymphadenectomy using VATS versus thoracotomy. This study aims to determine the effectiveness and safety of VATS-based systematic lymphadenectomy by retrospectively analyzing the related multi-center, large-scale clinical data.

Materials and methods

Clinical data

The clinical data of patients with NSCLC who underwent VATS or thoracotomy combined with lobectomy and systematic lymphadenectomy in eight hospitals in China from January 2001 to January 2008 were retrospectively analyzed, and 5,620 patients were included in this study. Upon enrollment, all participants were engaged in a series of preparation before surgery, including quitting smoking, respiratory function exercise, administration of phlegm drugs and chest physiotherapy.

Preoperative examination and surgical methods

Before surgery, all participants received physical examination, routine blood tests, ECG, cardiac color Doppler ultrasound and lower extremity deep venous color Doppler ultrasound. Respiratory function tests included pulmonary ventilation-dispersion function tests. Coronary artery CT or treadmill activity tests were performed in patients with suspected coronary heart disease over the age of 60, as well as coronary interventional examination, if necessary.

Preoperative tumor staging was based mainly on chest CT, head and abdominal MRI, whole body bone scan, and bronchoscopy. PET/CT scans were recommended for patients considered to be stage II or above.

All participants underwent VATS or open chest lobectomy and hilar and mediastinal lymph node dissection, of which the specific surgical techniques were already reported in our previous study (15).

Thoracotomy group: a standard posterolateral incision of about 10-20 cm was made for placement of intercostal distraction to carry out the thoracotomy under direct vision. The operation included anatomic lobectomy plus systematic mediastinal lymph node dissection.

Systematic mediastinal lymph node dissection was common in both procedures, instead of lymph node sampling, involving at least three groups of mediastinal and intrapulmonary lymph nodes (including subcarinal lymph nodes). The surrounding fat tissue was be resected together with the lymph nodes en bloc. The resected lymph node specimens were independently examined and interpreted by two or more senior pathologists.

Data collection and follow-up

The demographic data, operative time, blood loss, number

Table 1 Characteristics of included patients

	VATS (%)	Open (%)	P
Numbers	2,703	2,917	
Sex			<0.001
Male	1,742 (64.4)	2,163 (74.2)	
Female	961 (35.6)	754 (25.8)	
Age (mean ± SD), years	59.5±10.9	58.5±10.4	0.002
Histology			<0.001
Squamous carcinoma	675 (25.0)	1,081 (37.1)	
Adenocarcinoma	1,663 (61.5)	1,326 (45.5)	
Adenosquamous carcinoma	126 (4.7)	168 (5.8)	
Large cell carcinoma	62 (2.3)		
BAC	75 (2.8)	198 (6.8)	
Others	102 (3.8)	101 (3.5)	
TNM stage			<0.001
Stage I	1,415 (52.3)	1,246 (42.7)	
Stage II	657 (24.3)	794 (27.2)	
Stage III (A)	631 (23.3)	877 (30.1)	

Abbreviations: VATS, video-assisted thoracoscopy surgery; BAC, bronchioloalveolar carcinoma.

of dissected lymph nodes, postoperative hospital stay, postoperative chest tube duration, postoperative tumor type, stage, and occurrence of postoperative chylothorax were collected for all patients.

Statistical analysis

Measurement data were expressed as mean ± standard deviation ($x \pm s$). The statistical analysis was completed in SPSS 13, with $P<0.05$ indicating a statistically significant difference.

Results

Clinical data

A total of 5,620 patients were finally included in the retrospective study, with 2,703 in the VATS group, including 1,742 men (64.4%) and 961 women (35.6%), aged 59.5±10.9 years; and 2,917 in the thoracotomy group, including 2,163 men (74.2%), and 754 women (25.8%), aged 58.5±10.4 years (*Table 1*).

All patients underwent VATS or open chest lobectomy plus systematic lymphadenectomy. Comparing the VATS with the thoracotomy groups, the mean operative time was 146 *vs.* 157 min, with a significant difference ($P<0.001$); and the average blood loss was 162 *vs.* 267 mL, with a significant difference ($P<0.001$) (*Table 2*). The postoperative pathological test showed 1,663 patients with adenocarcinoma (61.5%), 675 patients with squamous cell carcinoma (25.0%), 126 patients with adenosquamous carcinoma (4.7%), and 239 patients with other types of tumors (8.9%) in the VATS group; and 1,326 patients with adenocarcinoma (45.5%), 1,081 patients with squamous cell carcinoma (37.1%), 168 patients with adenosquamous carcinoma (5.8%), and 342 patients with other types of tumors (11.8%) in the thoracotomy group (*Table 1*). According to the 2009 International Association for the Study of Lung Cancer (IASLC) staging criteria (16), all patients were subject to clinical pathological staging classification. There were 1,415 patients at stage I (52.3%), 657 patients at stage II (24.3%), and 631 patients at stage IIIA (23.3%) in the VATS group; and 1,246 patients at stage I (42.7%), 794 patients at stage II (27.2%), and 877 patients at stage IIIA (30.1%) in the thoracotomy group (*Table 2*).

Postoperative conditions (Table 2)

Comparing the two groups of patients data, the number of lymph node dissection (*Figure 1*): 18.03 in the VATS

Table 2 Comparisons of numbers of sampled lymphnodes and operation duration between VATS and open surgery for resectable stage NSCLC

Mean (SD)	VATS (N=2,703)	Open (N=2,917)	P
No. of sampled LNs			
Total	18.03 (10.14)	15.07 (8.55)	<0.001
Stage I	17.26 (9.29)	14.32 (7.98)	<0.001
Stage II	18.53 (11.20)	15.38 (8.91)	<0.001
Stage IIIA	19.27 (10.68)	15.86 (8.90)	<0.001
Operation length/minutes			
Total	145.71 (13.03)	156.72 (17.03)	<0.001
Stage I	145.75 (12.95)	156.09 (17.06)	<0.001
Stage II	145.40 (12.51)	157.63 (16.95)	<0.001
Stage IIIA	145.96 (13.71)	156.80 (17.04)	<0.001
Blood loss/mL	162.20 (142.56)	267.34 (220.31)	<0.001
Drainage days	4.50 (1.84)	6.37 (3.45)	<0.001
Length of hospitalization/days	6.50 (1.84)	8.37 (3.45)	<0.001
Chylothorax	4/2,579 (0.2%)	10/2,799 (0.4%)	0.117

Abbreviations: NSCLC, Non-small cell lung cancer; VATS, video-assisted thoracoscopy surgery; LNs, lymph nodes; Total, all stages (stage I-III).

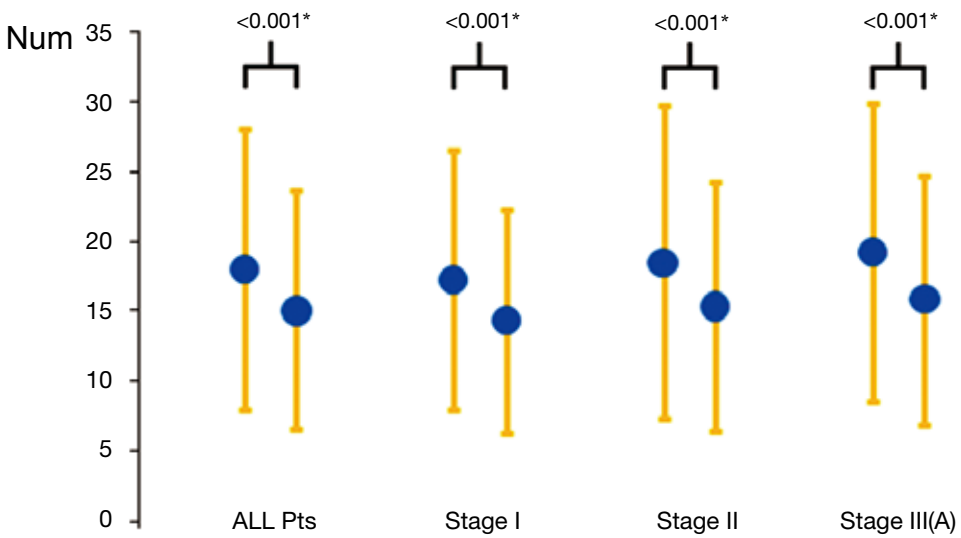


Figure 1 Comparisons of numbers of sampled lymph nodes between VATS and open surgery for resectable stage NSCLC. Abbreviations: NSCLC, Non-small cell lung cancer; VATS, video-assisted thoracoscopy surgery. *, With a significant difference.

group and 15.07 in the thoracotomy group on average, with a significant difference (P<0.001); blood loss: 162.2 mL in the VATS group and 267.34 mL in the thoracotomy group on average, with a significant difference (P<0.001); postoperative drainage time: 4.5 days in the VATS group and 6.37 days in the thoracotomy group on average, with a significant difference (P<0.001); postoperative hospital stay: 6.5 days in the VATS group and 8.37 days in the thoracotomy group on average, with a significant difference (P<0.001); proportion of postoperative chylothorax: 0.2% (4/2,579) in the VATS group and 0.4% (10/2,799) in the thoracotomy group, without significant difference (P>0.05).

Discussion

Lymph node metastasis is an important way of local and distant metastases in malignant cancer, as well as in NSCLC. It has a very important role in the prognostic determination and development of therapeutic strategies. Thus, for resectable NSCLC, the standard surgical method is lobectomy in combination with systematic lymph node dissection, which can improve the local control rate and prolong disease-free survival time.

Although it remains unconfirmed whether systematic lymphadenectomy can benefit patients with NSCLC oncologically, accurate lymph node staging still plays an important role in determining the need of postoperative adjuvant therapy and prognosis. Studies have shown that systematic lymphadenectomy is significantly superior to lymph node sampling in accurate staging. Investigators have found 4% patients at N2 stage with systematic lymph node dissection from 524 stage I patients who were identified with negative lymph nodes based on the sampling (17).

In the past, standard posterior lateral open chest lobectomy and lymph node dissection was mostly used for early and mid-stage resectable NSCLC. However, it is associated with a surgical incision often larger than 10 cm, extensive injury, slower postoperative recovery and higher incidence of postoperative complications. Since the early 1990s, VATS has been rapidly developed and widely applied in the world, involving almost all areas of general thoracic surgery. Compared with thoracotomy, VATS enables a smaller incision without removing or stretching the ribs open, sparing respiratory muscles from injures and thus minimizing the loss of lung function. Moreover, with a smaller incision, patients will suffer less pain postoperatively and expectorate more easily, reducing the incidence of postoperative pulmonary infection and complications as well.

The safety and effectiveness of VATS lobectomy combined with lymph node dissection for the treatment of early NSCLC has been confirmed, more and more studies have shown that this technique has comparable long-term oncological outcomes as a radical option to traditional open thoracic surgery (18,19). Moreover, National Comprehensive Cancer Network (NCCN) treatment guidelines for NSCLC has also clarified that VATS is a viable option for treating resectable lung cancer, particularly for those who can not tolerate standard thoracotomy due to physical conditions. This means that VATS treatment of NSCLC has covered most internationally recognized indications for surgical treatment of lung cancer.

As we all know, a thorough lymph node dissection is essential for the prognosis of patients with NSCLC, but it remains controversial whether this can be achieved with thoracoscopic systematic lymphadenectomy for NSCLC. In contrast to the thoracic surgery, many surgeons suspect the feasibility and thoroughness of thoracoscopic lymph node dissection. The primary concern is residual lymph nodes. In this regard, many studies have confirmed that after VATS lymph node dissection, the residual lymph node rate is very low. Hoksch *et al.* (20) did VATS lymphadenectomy in corpses followed by standard lateral open chest exploration, and the results showed no significant residual hilar and mediastinal lymph nodes. Sagawa *et al.* (21) performed VATS lymph node dissection in 29 NSCLC stage I patients followed by open chest exploration, and confirmed that there were only 2-3% of residues.

Since it has been applied in lymph node dissection, VATS has witnessed numerous controversies about whether it is superior or inferior to thoracotomy in this regard. Retrospective or prospective clinical studies yielded varying results as well (6-14,22). Ramos *et al.* (11) conducted a retrospective study to compare the number of dissected lymph nodes and stations with the two approaches by collecting the clinical and pathological data from patients with stage I non-small cell lung cancer patients. The results showed that an average dissection number of 5.1 stations in the VATS group, which was more than 4.5 stations in the open chest group, with a significant difference. However, the average number of 22.6 dissected nodes in the VATS group was far fewer than 25.4 nodes in the open chest group, with a significant difference. Lee *et al.* (23) analyzed 141 VATS patients and 115 cases of thoracic surgery for resectable NSCLC, finding that VATS yielded fewer dissected nodes compared with the open chest group (11.3±6.4 *vs.* 14.3±8.8, P=0.001), and the total number of dissected stations (3.1±1.1 *vs.* 3.8±1.2, P<0.001). Further analysis revealed that both differences came mainly from the dissection of mediastinal lymph nodes. On the other hand, some studies have confirmed that there is no difference in the number of either dissected nodes or dissected stations between the two approaches. Yang *et al.* (22) compared 62 patients with resectable NSCLC, which 31 cases in each of the VATS and thoracotomy groups, and found no significant difference in the number of either node or station dissected. In the present study, we found through statistical analysis that there was a mean number of dissected nodes of 18.03 in the VATS group and 15.07 in the thoracotomy group, with a significant difference (P<0.001)

Table 3 Comparisons between VATS and open surgery for resectable stage NSCLC

References	No. of patients		No. of sampled LNs		Hospital stay/days		Drainage days		Chylothorax	
	VATS	Open	VATS	Open	VATS	Open	VATS	Open	VATS	Open
Merritt RE, *et al*. 2013 (12)	60	69	9.9	14.7	4.5	5.1				
Ramos R, *et al*. 2012 (11)	96	200	22.6	25.4	7	10.3	4	5.7	2	3
Denlinger CE, *et al*. 2010 (13)	79	464	7.4	8.9	5.1	7.3				
Scott WJ, *et al*. 2010 (10)	66	686	15	19	5	7			0	7
Yang H, *et al*. 2013 (22)	31	31	28.2	29.8	10.6	12.4	6.3	8.3	0	1
Our study	2,703	2,917	18.03	15.07	6.5	8.37	4.5	6.37	4	10

Abbreviations: NSCLC, Non-small cell lung cancer; VATS, video-assisted thoracoscopy surgery; LNs, lymph nodes.

between the two groups, which is inconsistent with previous reports. We believe that the thoracoscopic vision has almost zero dead angles during intrathoracic operations. It can provide a good surgical field and has a visual zoom effect to magnify the surgical field, with which the hilar structures and mediastinal lymph node stations can be more clearly identified and exposed. In this way, we are able to clean out more mediastinal lymph node, reducing the incidence of residual lymph nodes.

The safety of VATS lobectomy in combination with systematic lymphadenectomy for resectable NSCLC is another concern. We have found through literature review and comparison (*Table 3*) that the majority of studies suggest that VATS has great advantages in terms of postoperative complications, postoperative chest tube drainage duration and postoperative hospital stay compared with thoracotomy. This study also confirms this conclusion. We believe that the smaller surgical wound and more clearly exposed blood vessels, lymph nodes and lymph vessels during VATS have made it possible to accurately dissect target tissue during dissection without damaging small blood vessels and lymph nodes, thus reducing lymphatic drainage and the occurrence of postoperative chylothorax, allowing earlier postoperative extubation and reduced postoperative hospital stay.

However, there are several limitations in this study due to its retrospective nature. Although this study has involved the most cases in comparison of VATS and open chest lymph node dissection, the origination of data from several studies with surgeons of varying thoracoscopic technical levels may have contributed to certain data deviation. Secondly, this study only analyzes two surgical procedures only in terms of the number of lymph node dissection and related postoperative complications, without comparing the differences in the prognosis. Therefore, a more comprehensive prospective study will be needed to further determine the safety and effectiveness of VATS lymph node dissection.

In conclusion, for patients with resectable NSCLC, VATS systematic lymph node dissection is safe and effective with acceptably low incidences of postoperative complications, and significantly faster postoperative recovery compared with traditional open chest surgery.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Martini N, Flehinger BJ, Nagasaki F, et al. Prognostic significance of N1 disease in carcinoma of the lung. J Thorac Cardiovasc Surg 1983;86:646-53.
3. Roviaro G, Rebuffat C, Varoli F, et al. Videoendoscopic pulmonary lobectomy for cancer. Surg Laparosc Endosc 1992;2:244-7.
4. McKenna R Jr. Vats lobectomy with mediastinal lymph node sampling or dissection. Chest Surg Clin N Am 1995;5:223-32.
5. Lardinois D, De Leyn P, Van Schil P, et al. ESTS guidelines for intraoperative lymph node staging in non-small cell lung cancer. Eur J Cardiothorac Surg 2006;30:787-92.
6. Yan TD, Black D, Bannon PG, et al. Systematic review and meta-analysis of randomized and nonrandomized trials on safety and efficacy of video-assisted thoracic surgery lobectomy for early-stage non-small-cell lung cancer. J Clin Oncol 2009;27:2553-62.
7. Rueth NM, Andrade RS. Is VATS lobectomy better:

perioperatively, biologically and oncologically? Ann Thorac Surg 2010;89:S2107-11.
8. Watanabe A, Mishina T, Ohori S, et al. Is video-assisted thoracoscopic surgery a feasible approach for clinical N0 and postoperatively pathological N2 non-small cell lung cancer? Eur J Cardiothorac Surg 2008;33:812-8.
9. Kim HK, Choi YS, Kim J, et al. Outcomes of unexpected pathologic N1 and N2 disease after video-assisted thoracic surgery lobectomy for clinical stage I non-small cell lung cancer. J Thorac Cardiovasc Surg 2010;140:1288-93.
10. Scott WJ, Allen MS, Darling G, et al. Video-assisted thoracic surgery versus open lobectomy for lung cancer: a secondary analysis of data from the American College of Surgeons Oncology Group Z0030 randomized clinical trial. J Thorac Cardiovasc Surg 2010;139:976-81; discussion 981-3.
11. Ramos R, Girard P, Masuet C, et al. Mediastinal lymph node dissection in early-stage non-small cell lung cancer: totally thoracoscopic vs thoracotomy. Eur J Cardiothorac Surg 2012;41:1342-8; discussion 1348.
12. Merritt RE, Hoang CD, Shrager JB. Lymph node evaluation achieved by open lobectomy compared with thoracoscopic lobectomy for N0 lung cancer. Ann Thorac Surg 2013;96:1171-7.
13. Denlinger CE, Fernandez F, Meyers BF, et al. Lymph node evaluation in video-assisted thoracoscopic lobectomy versus lobectomy by thoracotomy. Ann Thorac Surg 2010;89:1730-5; discussion 1736.
14. Flores RM, Park BJ, Dycoco J, et al. Lobectomy by video-assisted thoracic surgery (VATS) versus thoracotomy for lung cancer. J Thorac Cardiovasc Surg 2009;138:11-8.
15. He J, Shao W, Cao C, et al. Long-term outcome and cost-effectiveness of complete versus assisted video-assisted thoracic surgery for non-small cell lung cancer. J Surg Oncol 2011;104:162-8.
16. Rusch VW, Asamura H, Watanabe H, et al. The IASLC lung cancer staging project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2009;4:568-77.
17. Darling GE, Allen MS, Decker PA, et al. Number of lymph nodes harvested from a mediastinal lymphadenectomy: results of the randomized, prospective American College of Surgeons Oncology Group Z0030 trial. Chest 2011;139:1124-9.
18. Cao C, Zhu ZH, Yan TD, et al. Video-assisted thoracic surgery versus open thoracotomy for non-small-cell lung cancer: a propensity score analysis based on a multi-institutional registry. Eur J Cardiothorac Surg 2013;44:849-54.
19. Flores RM, Park BJ, Dycoco J, et al. Lobectomy by video-assisted thoracic surgery (VATS) versus thoracotomy for lung cancer. J Thorac Cardiovasc Surg 2009;138:11-8.
20. Hoksch B, Ablassmaler B, Walter M, et al. Radical thoracoscopic lobectomy with lymphadenectomy in a cadaver model. Can J Surg 2002;45:376-80.
21. Sagawa M, Sato M, Sakurada A, et al. A prospective trial of systematic nodal dissection for lung cancer by video-assisted thoracic surgery: can it be perfect? Ann Thorac Surg 2002;73:900-4.
22. Yang H, Li XD, Lai RC, et al. Complete mediastinal lymph node dissection in video-assisted thoracoscopic lobectomy versus lobectomy by thoracotomy. Thorac Cardiovasc Surg 2013;61:116-23.
23. Lee PC, Nasar A, Port JL, et al. Long-term survival after lobectomy for non-small cell lung cancer by video-assisted thoracic surgery versus thoracotomy. Ann Thorac Surg 2013;96:951-60; discussion 960-1.

Cite this article as: Wang W, Yin W, Shao W, Jiang G, Wang Q, Liu L, Liu D, Wang Z, Zhu Z, Chen H, He J. Comparative study of systematic thoracoscopic lymphadenectomy and conventional thoracotomy in resectable non-small cell lung cancer. J Thorac Dis 2014;6(1):45-51. doi: 10.3978/j.issn.2072-1439.2014.01.07

Medical Oncology

Neoadjuvant chemotherapy in early-stage non-small cell lung cancer

Francesc Salvà, Enriqueta Felip

Oncology Department, Valld'Hebron University Hospital, Barcelona, Spain

Correspondence to: Enriqueta Felip. Oncology Department, Valld'Hebron University Hospital, Barcelona, Spain. Email: efelip@vhebron.net.

Abstract: Surgical resection followed by adjuvant chemotherapy is the standard of care for completely resected stages II and III non-small cell lung cancer (NSCLC) patients. In order to improve survival in patients with early-stage NSCLC, efforts have been focused on the use of chemotherapy and radiotherapy before surgery with the aim of reducing the risk of relapse. Neoadjuvant chemotherapy is an attractive treatment option which is employed in different tumors and may well be associated with certain advantages in NSCLC patients such as being effective in treating occult microscopic systemic disease, downstaging mediastinal lymph node and improving the success of surgery by tumor reduction. Furthermore, chemotherapy compliance prior to surgery is generally better than after surgery. The potential disadvantages are treatment-related toxicities and the delay of surgery. At present, neoadjuvant chemotherapy is still considered an experimental treatment modality in early-stage disease and its role should be more clearly defined.

Keywords: Neoadjuvant; early-stage; non-small cell lung cancer (NSCLC)

Submitted Jul 08, 2013. Accepted for publication Oct 09, 2013.

doi: 10.3978/j.issn.2218-6751.2013.10.07

View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.07

Introduction

Current standard care for treating early stage non-small cell lung cancer (NSCLC) is surgical resection, when feasible, followed by adjuvant chemotherapy in stages II and III. However, chemotherapy compliance in the post-surgery setting is relatively poor and other strategies, such as neoadjuvant chemotherapy, have been addressed in clinical trials.

The role of neoadjuvant or induction chemotherapy in non-metastatic NSCLC has been evaluated in non-randomized and randomized clinical trials since neoadjuvant or induction therapy in resectable patients carries several theoretical advantages including locoregional cytoreduction, control of distant micrometastases, and a higher preoperative chemotherapy compliance compared with chemotherapy compliance after surgery. When this neoadjuvant approach was first discussed, the main potential disadvantages were treatment-associated toxicities and a delay in the surgical procedure, although at present, these drawbacks are considered barely relevant.

Studies analyzing neoadjuvant chemotherapy, and neoadjuvant chemoradiotherapy

In the 1990s, two small randomized trials comparing neoadjuvant platinum-based chemotherapy followed by surgery *versus* surgery alone in stage IIIA NSCLC had a profound impact because they demonstrated a survival benefit in patients receiving preoperative chemotherapy. Rosell *et al*. (1) compared resection and post-operative radiation (50 Gy) *versus* induction chemotherapy with three courses of cisplatin, mitomycin C and ifosfamide followed by resection and post-operative radiation in 60 patients with stage IIIA NSCLC. A three-fold survival advantage was seen in those patients who received induction chemotherapy (26 *versus* 8 months, P<0.001). Roth *et al*. (2) reported the results of a similar clinical trial in which 60 patients with stage IIIAN2 disease were randomly assigned to receive induction chemotherapy with three cycles of cyclophosphamide, etoposide and cisplatin followed by resection *versus* surgical resection alone. Radiation was

administered to more than half the patients in both groups. Induction chemotherapy was associated with a six-fold increase in median survival (64 *versus* 11 months, P<0.008).

Updated analyses of both studies continue to favor survival in the neoadjuvant chemotherapy arms. Long-term results of Rosell *et al*. study (3) confirmed a statistically significant survival difference (22 *versus* 10 months, P=0.005). In the long-term report of the Roth *et al*. (4) study with a follow-up of 82 months, 32% of patients who underwent neoadjuvant chemotherapy remained alive *versus* 16% of those who had undergone surgery alone (P=0.056).

The results of these two studies were discussed extensively as the survival advantages for the neoadjuvant chemotherapy groups were far greater than could be reasonably expected (5) and because both studies have a number of weaknesses in their design. These include variable prescription of adjuvant radiotherapy, the use of older drugs, and the application of the 1986 staging classification, in which stage III is even more heterogeneous than in the present one. Furthermore, in the Rosell *et al*. study there was a poor outcome in the surgery-alone group, which may be attributable to an imbalance of biological prognostic factors.

Since these early studies, several groups have evaluated neoadjuvant chemotherapy combinations prior to surgery in patients with early-stage disease.

In 2001, the French Thoracic Cooperative Group reported the results of a phase III study including 355 patients with stage IB, II and IIIA disease randomized to receive neoadjuvant chemotherapy (two courses of mitomycin C, ifosfamide and cisplatin) followed by surgery *versus* surgery alone (6). In both arms, patients with pT3 or pN2 disease received adjuvant thoracic radiotherapy, and responding patients received two additional cycles of adjuvant chemotherapy. Overall response to induction chemotherapy was 64%. The median survival was 37 months for the combined arm *versus* 26 months for the surgical arm (P=0.15). Interestingly, a survival benefit was observed in patients with stage I or II (P=0.027), but not in patients with stage IIIA (P=0.85). A major limitation of this study was the chemotherapy regimen employed, which resulted in poor compliance and an excess of toxicity in the initial phases of the trial.

The role of induction chemotherapy in stages IB to T3N1 NSCLC has also been evaluated by the Biomodality Lung Oncology Team (BLOT) trial in a phase II study in order to assess the feasibility of this approach. A total of 94 patients with early-stage NSCLC were scheduled to receive two courses of paclitaxel and carboplatin administered every three weeks followed by surgery and then, 3 cycles of adjuvant chemotherapy with the same agents for patients undergoing complete resection (7). Ninety-two patients completed preoperative chemotherapy, 59% of major responses were observed, and 82% underwent complete resection. However, only 45% of the patients received the planned adjuvant chemotherapy. In this trial, the 5-year survival rate was 42%. Based on this study, the Southwest Oncology Group (SWOG) 9900 trial randomly assigned 354 patients with stages IB, II or IIIA (excluding superior sulcus tumors and N2 disease) NSCLC to either three cycles of induction chemotherapy with paclitaxel and carboplatin followed by surgery *versus* surgery alone (8). This trial was closed to accrual early, owing to the data available in 2004 showing that adjuvant therapy improved survival after surgery. In the study, a response rate of 41% was seen in the neoadjuvant chemotherapy arm. In both arms of the trial, 84% of the patients underwent complete resection. The median overall survival was 62 months for neoadjuvant chemotherapy arm *versus* 41 months for the surgery alone arm, with a 19% reduction in the risk of death in favor of induction chemotherapy. However, this difference did not achieve statistical significance (HR 0.80, P=0.10).

The Medical Research Council LU22/NVALT 2/EORTC 08012 trial evaluated the role of induction chemotherapy with one of six platinum-based combinations followed by surgery *versus* surgery alone in 519 patients with stages IA to III NSCLC (9). The study was negative with regard to overall survival (HR 1.02, P=0.86). Subgroup analyses were not reported.

The Spanish Lung Cancer Group led the NATCH (Neo-adjuvant *Versus* Adjuvant Taxol/Carbo Hope) trial which included 624 patients with stages IA (size >2 cm), IB, II, T3N1 NSCLC (10). It was a three-arm study in which participants were randomly assigned to receive induction chemotherapy followed by surgery, surgery followed by adjuvant chemotherapy or surgery alone. The chemotherapy regimen was paclitaxel and carboplatin. Although a trend for improved 5-year disease-free survival rates with neoadjuvant therapy was observed (38.3% with neoadjuvant chemotherapy, 36.6% with adjuvant chemotherapy, and 34.1% with surgery alone), there were no statistical differences (P=0.71) among the three arms; it is noteworthy that the majority of patients had stage I disease. In this trial, in the subgroup of patients with stage II-T3N1, the 5-year disease-free survival rates favored the neoadjuvant arm (36.6% in the neoadjuvant group, 31%

in the adjuvant arm, and 25% in the surgery group). A greater proportion (90%) of patients in the neoadjuvant group received the planned three cycles of neoadjuvant chemotherapy compared with the adjuvant group in which only 66% of the patients started adjuvant treatment.

Recently, the CHEST (Chemotherapy for Early Stages Trial) has reported surprisingly different results (11). This study randomly assigned 270 patients with stages IB, II and IIIA NSCLC to three cycles of induction chemotherapy with cisplatin and gemcitabine followed by surgery *versus* surgery alone. Overall, a significant advantage for induction chemotherapy was found with regard to progression-free survival (HR 0.70, P=0.003) and overall survival (HR 0.63, P=0.02), the study being positive in its primary end point (progression-free survival). However, the benefit of induction chemotherapy in progression-free survival was limited only to the subgroup of patients with stages IIB or IIIA disease (92% were IIB); progression-free survival at 3 years was 23% better in the chemotherapy group (P=0.002). The risk of death was reduced by almost 60% among patients with stage IIB/IIIA disease who were randomly assigned to receive induction chemotherapy (HR 0.42, P<0.001). In contrast, in the stage IB/IIA subgroup (93% were IB) there were no differences in progression-free survival (HR 1.06, P=0.83) or overall survival (HR 1.02, P=0.94). Interestingly, in this study, slightly more patients in the surgery alone arm (25%) required pneumonectomy compared with 17% of patients in the chemotherapy arm.

Meta-analyses from data of randomized trials addressing the role of neoadjuvant chemotherapy in early-stage NSCLC are of interest. Berghmans *et al*. reported data from six randomized trials, published between 1990 and 2003, including 590 patients (12). The addition of neoadjuvant chemotherapy to surgery was associated with a non-significant improvement in overall survival (HR 0.65, 95% CI, 0.41-1.04).

Burdett *et al*. examined data from seven randomized trials including 988 patients, published between 1990 and 2005. Neoadjuvant chemotherapy improved survival (HR 0.82, 95% CI, 0.69-0.97), with an absolute benefit of 6% at 5 years (13).

In the CHEST trial results, Scagliotti *et al*. reported the results of a meta-analysis including 10 randomized clinical trials with a total of 2,188 patients comparing neoadjuvant chemotherapy followed by surgery *versus* surgery alone (including NATCH trial and CHEST data). This meta-analysis did show a significant survival advantage for those patients randomly assigned to receive induction chemotherapy (HR 0.89, P=0.02) (11).

Finally, preliminary results from a systematic review and meta-analysis of individual patient data from 13 randomized trials reported that neoadjuvant chemotherapy was associated with an improvement in survival in operable patients with 5% absolute benefit at 5 years (HR 0.88, P=0.025) (14).

Another strategy is the addition of thoracic radiotherapy to chemotherapy in the preoperative setting, which may improve local control and help sterilize mediastinal disease. The principal drawback of preoperative chemoradiotherapy is that it can lead to an increase in surgical complications, principally bronchopleural fistula and post-pneumectomy mortality. Neoadjuvant chemoradiotherapy has been analyzed mainly in stage III disease. The phase III randomized North American Intergroup Trial (Intergroup 0139 trial) addresses the role of surgery after neoadjuvant chemoradiation in resectable stage III NSCLC; 429 potentially resectable patients with biopsy-proven stage IIIA N2 NSCLC were randomly assigned to concurrent chemoradiotherapy (two cycles of cisplatin and etoposide plus radiotherapy up to 45 Gy) followed by surgical resection or further radiation to a definitive dose of 61 Gy (15). Consolidation chemotherapy with cisplatin/etoposide was given to patients in both arms (15). The 5-year disease-free survival rate was 22% for the surgical group and 11.1% for the definitive radiation group. However, the two groups did not differ in their median overall survival (23.6 *versus* 22.2 months, respectively, HR 0.87, P=0.24). The mortality rate observed in the surgical arm was 7.9%, compared with 2.1% in the definitive radiation arm. After neoadjuvant chemoradiotherapy, postoperative mortality was 26% for those patients who underwent pneumonectomy compared with only 1% in patients who had a lobectomy. In an exploratory unplanned, matched subgroup analysis, patients treated with a lobectomy after induction concurrent chemoradiotherapy had a significantly better survival than those who underwent a pneumonectomy or were treated non-surgically.

Two randomized studies address the potentially favorable contribution of adding thoracic radiotherapy to chemotherapy before surgery in patients with stage III disease. The German Lung Cancer Cooperative Group conducted a clinical trial including 558 stage III NSCLC patients, all of whom received three cycles of cisplatin and etoposide; the control group then underwent surgery followed by post-operative radiotherapy while

the investigational arm received further chemotherapy with hyperfractionated radiotherapy (1,5 Gy twice daily to 45 Gy) followed by surgery (16). Although the addition of radiotherapy in the preoperative setting increased the rate of mediastinal clearance (46% *versus* 29%) and decreased the rate of positive surgical margins (8% *versus* 14%), no differences were observed in progression-free survival or overall survival between the two groups. The risk of bronchopleural fistula (5% *versus* 1%) and post-pneumectomy mortality (14% *versus* 6%) was higher in patients receiving preoperative radiotherapy.

At the ASCO-2013 meeting the results of a Swiss trial analyzing neoadjuvant chemotherapy with or without preoperative irradiation in stage IIIAN2 disease (SAKK trial 16/00) were presented (17). Patients with resectable stage IIIAN2 were randomized to receive 3 cycles of neoadjuvant cisplatin and docetaxel followed by accelerated boost radiotherapy or neoadjuvant chemotherapy alone with subsequent surgery for all patients. They reported the results of a planned interim analysis on data of the first 219 patients. In this study, preoperative radiotherapy did not improve median event-free survival (12.8 months for the preoperative chemotherapy followed by radiotherapy arm *versus* 11.8 months for the preoperative chemotherapy alone arm) or survival (27.1 months for the preoperative chemotherapy followed by radiotherapy arm *versus* 26.2 months for the preoperative chemotherapy alone arm).

Overall, these two randomized studies (16,17) suggest that the addition of preoperative radiotherapy seems not to improve overall survival.

Summary and conclusions

In the light of available data, there is, at present, clearer evidence favoring adjuvant strategies when compared with neoadjuvant strategies in early-stage NSCLC. Overall, neoadjuvant approaches are less well studied than adjuvant strategies and the majority of neoadjuvant trials have closed early and have been small in size. Some advantages are associated with neoadjuvant chemotherapy. The compliance with neoadjuvant chemotherapy is better; in the NATCH trial, in which patients were randomized before surgery, a considerable number of patients were unable to receive adjuvant chemotherapy due to slow recovery from surgery. There are subgroups of NSCLC patients who clearly benefit from neoadjuvant strategies, such as those with pathologic complete response at surgery (18), but there are no markers to identify those patients at diagnosis. In our opinion, in the light of the NATCH and CHEST trial results, neoadjuvant strategies may be considered for patients with more advanced disease (T3N1, and patients with multiple N1 regions involved in the preoperative staging) and for those in whom we believe adjuvant chemotherapy could be difficult to administer.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Rosell R, Gómez-Codina J, Camps C, et al. A randomized trial comparing preoperative chemotherapy plus surgery with surgery alone in patients with non-small-cell lung cancer. N Engl J Med 1994;330:153-8.
2. Roth JA, Fossella F, Komaki R, et al. A randomized trial comparing perioperative chemotherapy and surgery with surgery alone in resectable stage IIIA non-small-cell lung cancer. J Natl Cancer Inst 1994;86:673-80.
3. Schirren J, Eberlein M, Fischer A, et al. The role of sleeve resections in advanced nodal disease. Eur J Cardiothorac Surg 2011;40:1157-63.
4. Roth JA, Atkinson EN, Fossella F, et al. Long-term follow-up of patients enrolled in a randomized trial comparing perioperative chemotherapy and surgery with surgery alone in resectable stage IIIA non-small-cell lung cancer. Lung Cancer 1998;21:1-6.
5. Johnson DH, Piantadosi S. Chemotherapy for resectable stage III non-small-cell lung cancer--can that dog hunt? J Natl Cancer Inst 1994;86:650-1.
6. Depierre A, Milleron B, Moro-Sibilot D, et al. Preoperative chemotherapy followed by surgery compared with primary surgery in resectable stage I (except T1N0), II, and IIIa non-small-cell lung cancer. J Clin Oncol 2002;20:247-53.
7. Pisters KM, Ginsberg RJ, Giroux DJ, et al. Induction chemotherapy before surgery for early-stage lung cancer: A novel approach. Bimodality Lung Oncology Team. J Thorac Cardiovasc Surg 2000;119:429-39.
8. Pisters KM, Vallières E, Crowley JJ, et al. Surgery with or without preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer: Southwest Oncology Group Trial S9900, an intergroup, randomized, phase III trial. J Clin Oncol 2010;28:1843-9.
9. Gilligan D, Nicolson M, Smith I, et al. Preoperative chemotherapy in patients with resectable non-small

cell lung cancer: results of the MRC LU22/NVALT 2/EORTC 08012 multicentre randomised trial and update of systematic review. Lancet 2007;369:1929-37.
10. Felip E, Rosell R, Maestre JA, et al. Preoperative chemotherapy plus surgery versus surgery plus adjuvant chemotherapy versus surgery alone in early-stage non-small-cell lung cancer. J Clin Oncol 2010;28:3138-45.
11. Scagliotti GV, Pastorino U, Vansteenkiste JF, et al. Randomized phase III study of surgery alone or surgery plus preoperative cisplatin and gemcitabine in stages IB to IIIA non-small-cell lung cancer. J Clin Oncol 2012;30:172-8.
12. Berghmans T, Paesmans M, Meert AP, et al. Survival improvement in resectable non-small cell lung cancer with (neo)adjuvant chemotherapy: results of a meta-analysis of the literature. Lung Cancer 2005;49:13-23.
13. Burdett SS, Stewart LA, Rydzewska L. Chemotherapy and surgery versus surgery alone in non-small cell lung cancer. Cochrane Database Syst Rev 2007;(3):CD006157.
14. Burdett S, Rydzewska LH, Tierney JF on behalf of the NSCLC Meta-analyses Collaborative Group. Pre-operative chemotherapy improves survival and reduces recurrence in operable non-small cell lung cancer: preliminary results of a systematic review and meta-analysis of individual patient data from 13 randomised trials. 14th World Conference on Lung Cancer, July 3-7, 2011 Amsterdam, The Netherlands (abstract O23.01).
15. Albain KS, Swann RS, Rusch VW, et al. Radiotherapy plus chemotherapy with or without surgical resection for stage III non-small-cell lung cancer: a phase III randomised controlled trial. Lancet 2009;374:379-86.
16. Thomas M, Rübe C, Hoffknecht P, et al. Effect of preoperative chemoradiation in addition to preoperative chemotherapy: a randomised trial in stage III non-small-cell lung cancer. Lancet Oncol 2008;9:636-48.
17. Pless M, Stupp R, Ris HB, et al. Neoadjuvant chemotherapy with or without preoperative irradiation in stage IIIA/N2 non-small cell lung cancer (NSCLC): A randomized phase III trial by the Swiss Group for Clinical Cancer Research (SAKK trial 16/00). J Clin Oncol 2013;31:abstr 7503.
18. Mouillet G, Monnet E, Milleron B, et al. Pathologic complete response to preoperative chemotherapy predicts cure in early-stage non-small-cell lung cancer: combined analysis of two IFCT randomized trials. J Thorac Oncol 2012;7:841-9.

Cite this article as: Salvà F, Felip E. Neoadjuvant chemotherapy in early-stage non-small cell lung cancer. Transl Lung Cancer Res 2013;2(5):398-402. doi: 10.3978/j.issn.2218-6751.2013.10.07

Adjuvant chemotherapy of completely resected early stage non-small cell lung cancer (NSCLC)

Ying Liang[1], Heather A. Wakelee[2]

[1]Department of Medical Oncology, Cancer Center, Sun Yat-sen University, Guangzhou 510060, China; [2]Department of Medicine, Division of Oncology, Stanford University School of Medicine, Stanford, CA, USA

Correspondence to: Heather A. Wakelee. Department of Medicine, Division of Oncology, Stanford Cancer Institute, Stanford University, 875 Blake Wilbur Drive, Stanford, CA 94305-5826, USA. Email: hwakelee@stanford.edu.

Abstract: Surgery is regarded as the primary treatment modality for early stage non-small cell lung cancer (NSCLC), but even after complete resection, a substantial percentage of these patients eventually develop local recurrence or distant metastases. Therefore more effective treatment strategies to reduce lung cancer mortality and recurrence rate are needed. Only recently has the use of adjuvant chemotherapy become standard in early stage NSCLC, at least for stage II and resected IIIA NSCLC. Controversies remain about the benefit for stage I patients. Five-year survival improvements of 5% to 10% have been reported with cisplatin-based adjuvant chemotherapy from multiple large randomized phase III clinical trials and meta-analyses. Questions remain as to which patients benefit and which regimens are best. In this paper, important clinical research in the field of adjuvant chemotherapy of NSCLC is reviewed.

Keywords: Non-small cell lung cancer (NSCLC); adjuvant chemotherapy; elderly patient

Submitted Jul 09, 2013. Accepted for publication Jul 15, 2013.
doi: 10.3978/j.issn.2218-6751.2013.07.01
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.07.01

Although surgery is regarded as the primary treatment modality for non-small cell lung cancer (NSCLC), only 20-25% of tumors are suitable for potentially curative resection (1) and even after resection, a substantial percentage of these patients eventually develop local recurrences or distant metastasis. Consequently, 5-year survival rates after surgery are disappointingly low, ranging from 58% to 73% in stage I, 36% to 46% in stage II, and only 19% to 24% in patients with stage IIIA tumors (2).

Therefore more effective treatment strategies to reduce lung cancer mortality and recurrence rate are needed. High expectations for post-operative (adjuvant) chemotherapy were based on the fact that this is a standard treatment after complete resection in malignancies such as breast (3) and colon (4). But only recently has adjuvant chemotherapy become standard in early stage NSCLC. Questions remain as to which patients benefit and which regimens are best. In this paper, important clinical research in the field of adjuvant chemotherapy of NSCLC is reviewed, in order to provide a reference for further clinical practice. Data with pre-operative (neo-adjuvant) therapy, alternatives to chemotherapy, and prognostic or predictive biomarkers are discussed elsewhere.

Early trials of adjuvant chemotherapy with regimens consisting of alkylating agents and older chemotherapy did not show any clear impact on survival. In 1995 an individual patient data-based meta-analysis (5) from 52 randomized trials and 9,387 patients was initiated by the British Medical Research Council's Cancer Trials Office, Cambridge; the Institut Gustave Roussy, Villejuif, France; and the Istituto Mario Negri, Milan, Italy, and was carried out on behalf of the Non-small Cell Lung Cancer Collaborative Group (NSCLCCG). Data were available from 14 trials (4,357 patients and 2,574 deaths) for evaluating surgery versus surgery plus chemotherapy. The trials included platinum-based and non-platinum based regimens. The overall hazard ratio of 0.87; 95% CI, 0.74 to 1.02; (P=0.08), or 13% reduction in the risk of death, suggested an absolute benefit from

chemotherapy, but it was not statistically significant. This ignited the interest of study groups for NSCLC adjuvant chemotherapy after surgery and led to multiple large randomized trials, which have subsequently proven the benefit suggested by the original 1995 meta-analysis. The standard of care has now become adjuvant chemotherapy for resected non-small cell lung cancer.

The Adjuvant Lung Project Italy (ALPI) trial (6) was the first large, prospective adjuvant study designed to detect small differences in survival that were in the range detected by the NSCLCCG meta-analysis. This trial was a randomized controlled study to evaluate the Mitomycin C, Vindesine, Cisplatin (MVP) regimen in patients with radically resected stages I-IIIA non small-cell NSCLC. A total of 1,209 patients (1,086 from the Italian centers and 123 from EORTC-LCCG centers) were enrolled in this study. Patients were randomly assigned to the MVP arm or the control arm and received adjuvant radiotherapy according to the policy of the individual participating center. In total, 69% of patients on the experimental arm completed the planned 3 cycles of MVP treatment. Sixty-five percent of patients received radiotherapy in the MVP arm, and 82% in the control arm. Treatment-related deaths were documented in 10 patients (three patients in the MVP arm and seven patients in the control arm), respectively. After median duration of follow-up for 64.5 months (52.1-79.6 months), no significant difference in overall survival (OS) was seen with an OS HR of 0.96 (95% CI, 0.81-1.13; P=0.589), nor in progression-free survival (HR=0.89, 95% CI, 0.76-1.03; P=0.128). Median overall survival was 55 months in the MVP arm and 48 months in the control arm. One possible reason for this result may be low compliance with chemotherapy, or the regimen utilized. In the multivariable analysis, only disease stage and sex were associated with survival (P<0.001 for stage II or III versus stage I and P=0.034 for male versus female, respectively). Another theory about the lack of benefit seen with adjuvant chemotherapy in this NSCLC population is that the health of patients who have undergone a major thoracic surgical procedure is very often compromised by the procedure itself, and these patients usually require a long time to fully recover.

Investigators in the United Kingdom (UK) reported a randomized trial (Big Lung Trial-BLT) (7) evaluating cisplatin-based adjuvant chemotherapy in patients with completely resected stage I-IIIA NSCLC. Between November 1995 and November 2001 a total of 381 patients were enrolled into the trial from 52 UK and 4 non-UK centers. One hundred ninety-two patients were randomized to receive chemotherapy(C), and 189 to no chemotherapy (NoC). Twenty-seven percent of patients had stage I disease, 38% stage II, and 26% stage IIIA, respectively. Fifty-two (14%) patients received radiotherapy as part of their planned primary treatment. In the chemotherapy arm the patients were prescribed three cycles of 3-weekly cisplatin-based chemotherapy, primarily doublet regimens. Only 64% of patients finished all three cycles of the chemotherapy as planned, with the rest requiring dose reductions or delays. There were 6 treatment related deaths and 30% of patients experienced grade 3/4 toxicity. With a median-follow time of 34.6 months, the median survival was 33.9 months for Chemotherapy patients, and 32.6 months for No-chemotherapy patients. The overall survival hazard ratio was 1.02 (95% CI, 0.77-1.35, P=0.90) and PFS hazard ratio was 0.97 (95% CI, 0.74-1.26, P=0.81). The results of ALPI and this trial taken together cast doubt on the utility of adjuvant chemotherapy.

More recent trials, however, have been positive and have led to a change in the standard of care. The International Adjuvant Lung Cancer Trial (IALT) (8) was the largest prospective, randomized trial to test the hypothesis from the NSCLCCG Meta-analysis (5). From February 1995 to December 31, 2000, 1,867 completely resected stage I-III NSCLC patients were recruited by 148 centers in 33 countries. They were randomized to adjuvant chemotherapy or best supportive care. Each participating center could determine the pathological stages of disease to include, the dose of cisplatin given per cycle, the drug that was combined with cisplatin, and the postoperative radiotherapy policy. Adjuvant chemotherapy regimens included cisplatin combined with etoposide, vindesine or vinblastine. In the chemotherapy group, 73.8 percent of patients received at least 240 mg/m^2 of cisplatin. Twenty-seven percent of patients received postoperative radiotherapy. The median follow-up was 56 months. A total of 22.6% of the patients had at least one episode of grade 4 toxic effect and seven patients (0.8%) died of toxic effects of chemotherapy. The disease-free survival rate was significantly higher in the chemotherapy group (HR=0.83, 95% CI, 0.74-0.94, P<0.003). The overall survival rate was also significantly higher in the chemotherapy group (HR=0.86, 95% CI, 0.76-0.98, P<0.03); the five-year survival rates were 44.5% *vs.* 40.4%. The absolute five-year benefit in overall survival was 4.1 percent, a value that is concordant with the estimation from the chemotherapy meta-analysis (5). However, in 2009, the long-term follow-up results with a median follow-

up 7.5 years was reported (9), and while the results still showed potential benefit, the significance was lost with an overall survival HR=0.91 (95% CI, 0.81-1.02; P=0.10), but a persistently significant benefit on disease-free survival (HR, 0.88; 95% CI, 0.78-0.98; P=0.02). The results of overall survival were significantly different before and after 5 years of follow-up (HR, 0.86; 95% CI, 0.76-0.97; P=0 .01 *vs.* HR, 1.45; 95% CI, 1.02-2.07; P=0 .04). The reasons behind this are not clear. The non-lung cancer deaths analysis showed a HR of 1.34 (95% CI, 0.99-1.81; P=0.06). The second malignancies were not significantly different (8-year rate of approximately 10%) between the arms. Although absolute 5-year survival benefits of 4% are fairly modest, and the long-term benefit is not as robust, on a global scale the use of cisplatin based adjuvant chemotherapy could potentially help keep approximately 10,000 more NSCLC patients alive at 5 years. The positive results of this study and others that matured around the same time, laid the foundation for the routine use of adjuvant chemotherapy and future trials.

Most of the earlier trials have used a variety of chemotherapy combination regimens, including toxic triplet regimens, but the National Cancer Institute of Canada Clinical Trials Group JBR.10 trial in patients with completely resected stage IB or stage II non-small-cell lung cancer utilized a regimen of vinorelbine (VNR) plus cisplatin (DDP) as adjuvant chemotherapy (10). This study was a North American intergroup, phase III, randomized trial and between 1994-2001 it enrolled 482 patients who were randomly assigned to observation or vinorelbine plus cisplatin chemotherapy. No patients received adjuvant radiotherapy. Fifty-eight percent of the patients received three or more cycles of cisplatin, 77% had at least one dose reduction or omission, and 55% required one dose delay or more, most related to neutropenia at the expected time of vinorelbine administration on day 15 (cycle week 3). Seventy-three percent of patients had grade 3 or 4 neutropenia. With median follow-up of just over 5 years, five-year survival rate was 69% in the vinorelbine-cisplatin group and 54% in the observation alone (P=0.03). In subgroup analysis there was no statistically significant improvement in overall survival among patients with stage IB disease. In the quality-of-life (QOL) analyses (11), despite toxicity, the decline in function and symptom-related domains during chemotherapy in this trial was limited and resolved rapidly (within three months after completion of therapy). In 2010, an updated survival analysis (12) with a median follow-up of 9.3 years was published. Patients in the adjuvant chemotherapy arm continued to show a significant survival advantage compared with observation (HR, 0.78; 95% CI, 0.61-0.99; P=0.04). The absolute benefit for 5-year survival was 11% (67% chemotherapy *vs.* 56% observation). For patients without lymph node involvement, patients with tumors 4 cm or larger in size derived clinically meaningful benefit from chemotherapy (HR, 0.66; 95% CI, 0.39-1.14; P=0.13), while those with tumors smaller than 4 cm did not (HR, 1.73; 95% CI, 0.98-3.04; P=0.06). Seventy-three percent of patients died of disease or complications of treatment of their NSCLC and 10.6% patients developed second malignancies. This was the first clinical trial in which all patients on chemotherapy received third-generation chemotherapy drugs in an adjuvant setting for completely resected NSCLC, and has the best reported outcomes.

The Adjuvant Navelbine International Trialist Association (ANITA) reported positive results from their phase III randomized trial in patients with completely resected stage IB, II, and IIIA NSCLC (13). From December 1994, to December 2000, 840 patients were enrolled and randomly assigned to vinorelbine plus cisplatin or to observation (control). Postoperative radiotherapy was optional, decided by every participating center, and was to be decided before patients were included into the trial. Among the patients, 37% underwent pneumonectomy and 39% had stage IIIA disease. Fifty percent of patients completed the planned four cycles. Grade 3-4 neutropenia was seen in 85% patients in the chemotherapy arm and there were seven (2%) treatment-related deaths in the chemotherapy group. Median survival was 65.7 months (95% CI, 47.9-88.5) for the chemotherapy group and 43.7 months (35.7-52.3 months) for controls [hazard ratio 0.80 (0.66-0.96), P=0.017]. The absolute overall survival benefit for patients receiving chemotherapy compared with controls was 8.6% at 5 years. Relapse was lower in the chemotherapy group than in the observation group (local relapse, 12% of patients *vs.* 18% of patients, P=0.025). Subgroup analysis indicated that the benefit is seen in patients with stage II and IIIA disease. Postoperative radiotherapy was delivered to 232 (28%) patients (> N0) with improved 5-year survival in patients with N2 disease who received PORT from both groups. This trial showed survival benefits of the vinorelbine-cisplatin combination in the adjuvant setting and confirmed the JBR.10 (10) findings in patients with stage II disease; and also provided new data for patients with stage IIIA NSCLC.

Paclitaxel/carboplatin remains one of the most widely used regimens in the United States for advanced stage

NSCLC, partly due to a favorable toxicity profile. There has only been one large randomized trial to explore this regimen in the adjuvant setting, CALGB9633, which randomly assigned completely resected stage IB (T2N0) patients to four postoperative cycles of paclitaxel (200 mg/m^2) and carboplatin (AUC =6) chemotherapy versus surgery alone. The trial started in September 1996 and was closed in November 2003 by the Data and Safety Monitoring Board after a planned interim analysis following accrual of 344 patients. Chemotherapy was well tolerated, and there were no treatment-related toxic deaths. The predominant toxicity was grade 3 to 4 neutropenia, which was observed in 35%. Fifty-seven percent (77 of 136) of patients received four cycles of chemotherapy at full dose. This trial was initially presented at ASCO 2004 (14) as positive (HR: 0.62; 95% CI, 0.44-0.89; P=0.014) with median follow-up of 34 months. However in the final publication of the mature results with median follow-up of 74 months, the survival gain lost statistical significance in OS (HR, 0.83; 90% CI, 0.64-1.08; P=0.125), and DFS (HR, 0.80; 90% CI, 0.62-1.02; P=0.065); respectively. In the subgroup of tumor size ≥4.0 cm in diameter though, there were significant advantages in OS (HR, 0.69; 90% CI, 0.48 to 0.99; P=0.043) and DFS (HR, 0.69; 90% CI, 0.49 to 0.97; P=0.035) for patients who received chemotherapy. Results of CALGB 9633 (and confirmatory findings from NCIC-CTG-JBR-10) support consideration for adjuvant chemotherapy in stage IB patients who have tumors ≥4.0 cm in diameter. However, the routine use of carboplatin/paclitaxel as an adjuvant regimen is discouraged based on the results of this trial.

The Lung Adjuvant Cisplatin Evaluation (LACE) analysis (15) collected and pooled data on 4,584 patients from the 5 randomized adjuvant cisplatin-based chemotherapy trials which were conducted after the NSCLCCG 1995 meta-analysis (5) and whose cohorts were larger than 300 patients: ALPI (6), IALT (8), ANITA (13), BR.10 (10), BLT (7). With a median follow-up of 5.1 years (3.1-5.9 years) the result showed there was a statistically significant benefit (HR: 0.89; 95% CI, 0.82-0.96; P=0.005) in OS for the chemotherapy group compared with the control group corresponding to an 11% reduction in the risk of death and absolute survival benefits of 3.9% and 5.4% at 3 and 5 years, respectively. The benefit varied with stage (test for trend, P=0.046) with an HR of 1.41 (0.96-2.09) for stage IA, 0.93 (0.78-1.10) for stage IB, 0.83 (0.73-0.95) for stage II, and 0.83 (0.73-0.95) for stage III. The rate of overall grade 3 to 4 toxicity was 66% and the most common toxicity was neutropenia (9% grade 3 and 28% grade 4). This analysis clearly confirmed that cisplatin-based adjuvant chemotherapy is of benefit for completely resected NSCLC and further supports its use in routine clinical practice. Of note, 59% of patients received at least 240 mg/m^2 of cisplatin.

Results from LACE were very closely mirrored by the results of the updated NSCLC Meta-analyses Collaborative Group (16) which conducted a meta-analysis to assess the effectiveness of adjuvant chemotherapy in patients with NSCLC from randomized trials starting from Jan 1, 1965. The individual data was from 11,107 patients from 47 comparisons in 33 trials, which is more than three times that available in 1995. The comparison of surgery plus chemotherapy versus surgery alone was based on 34 trial comparisons and 8,447 patients (3,323 deaths) and showed a benefit of adding chemotherapy after surgery [hazard ratio (HR) 0.86, 95% CI, 0.81-0.92, P<0.0001], with an absolute increase in survival of 4% (95% CI, 3-6%) at 5 years (from 60% to 64%). Results for recurrence-free survival (HR 0.83, 95% CI, 0.77-0.90, P<0.0001), time to locoregional recurrence (0.75, 0.66-0.85, P<0.0001), and time to distant recurrence (0.80, 0.72-0.89, P=0.0007) all significantly favored chemotherapy. Another comparison of surgery plus radiotherapy and chemotherapy versus surgery plus radiotherapy was based on 13 trial comparisons and 2,660 patients (1,909 deaths) and showed a benefit of adding chemotherapy to surgery plus radiotherapy (HR 0.88, 95% CI, 0.81-0.97, P=0.009), representing an absolute improvement in survival of 4% (95% CI, 1-8%) at 5 years (from 29% to 33%).

The largest proportion of patients randomized to date to adjuvant chemotherapy have received cisplatin/vinorelbine (CVb, 41%) (17), which also was the most homogeneous subgroup in terms of drug doses and eligibility. As shown in a LACE meta-analysis subgroup analysis (17), the cisplatin/vinorelbine combination was associated with a substantially superior survival benefit compared with other cisplatin-based regimens. However, toxicity has been a critical issue in platinum-based adjuvant protocols with neutropenia in up to 85% and febrile neutropenia in up to 9% reported. Further points of concern are incomplete treatment delivery in up to 50% of the patients, mainly due to toxicity and patient refusal (18). In a phase III trial (19) in advanced stage NSCLC, the combination of cisplatin and pemetrexed (CPx), a multi-target folate antimetabolite, showed a good safety profile and convenient administration schedule and also OS superiority in adenocarcinoma (HR=0.84, P=0.03) and large cell (HR=0.67, P=0.03), both separately and

grouped together as "nonsquamous" (HR=0.81, P=0.005) when compared with gemcitabine combined with cisplatin. The question of whether this regimen could be used in the adjuvant setting was addressed in the TREAT study (20), a prospective, open-label, randomized phase 2 trial conducted in 16 centers in Germany and Belgium. A total of 132 patients with completely resected IB, IIA, IIB orT3N1 NSCLC were randomly assigned to cisplatin/pemetrexed (CPx) or cisplatin/vinorelbine (CVb). Postoperative radiotherapy was not allowed. The primary objective was the clinical feasibility rate. The results showed that the feasibility rate differed significantly and was higher in CPx than CVb [95.5% (95% CI, 87.5-99.1%) *vs.* 75.4% (95% CI, 63.1-85.2%), P=0.0010]. However, for efficacy data, the limitations of the size of a phase II trial, and of a large proportion of patients with stage IB or with squamous cell carcinoma, have to be taken into account. Further results of adjuvant cisplatin combined with pemetrexed are expected from the ITACA (EudraCT #: 2008-001764-36) and the ECOG E1505 (NCT00324805) trials. Although there is a lack of level 1 data regarding the utility of cisplatin/pemetrexed in an adjuvant setting, the NCCN guidelines (21) still recommend it as an option for non-squamous histology adjuvant chemotherapy. In addition to cisplatin/vinorelbine and cisplatin/pemetrexed, other regimens included on the E1505 trial, which closed to accrual in September 2013, include cisplatin/gemcitabine and cisplatin/docetaxed, which are also included as options per the NCCN. In interim data presented on E1505 (22), all 4 options have been selected on a fairly equal basis for patient enrolled onto the trial.

The treatment of lung cancer in the elderly bears special consideration. Between 2003 and 2007, 68% of cases of lung cancer were diagnosed in patients more than 65 years old and approximately 37% in patients over age 75 in the United States (23). However, although the incidence of NSCLC in elderly patients is high, they are underrepresented in clinical trials frequently (24). In a retrospective subgroup analysis of the JBR.10 trial (25), there were in total 155 patients (nearly 1/3 of the total) aged 65 years or older and the eldest patient was 82 years old, and in this subgroup adjuvant chemotherapy significantly prolonged overall survival (HR 0.61; 95% CI, 0.38-0.98; P=0.04). In a pooled analysis of the effect of age on adjuvant cisplatin-based chemotherapy in the LACE meta-analysis (26), patient and treatment characteristics, overall and event-free survival, delivery, chemotherapy toxicity and cause-specific mortality were compared among different age groups. There are 414 patients (9%) age 70 years or older. A trend toward survival benefit with adjuvant chemotherapy in elderly patients was shown (HR 0.90; 95% CI, 0.70-1.16; P=0.29), and no differences in severe toxicity rates were observed. This pooled analysis concluded that adjuvant cisplatin-based chemotherapy should not be withheld from elderly patients with NSCLC purely on the basis of age. These findings improved the probability of chemotherapy administration in the elderly in North America. A population based study based on the Ontario Cancer Registry (27) demonstrated that the percentage of patients aged at least 70 years of age who received adjuvant chemotherapy increased from 3.3% (2001 to 2003) to 16.2% (2004 to 2006). Twenty-eight percent of patients received carboplatin-based and 70% of patients received cisplatin-based regimens. In the Ontario analysis, the four-year survival of elderly patients increased significantly (47.1% for patients diagnosed from 2001 to 2003; 49.9% for patients diagnosed from 2004 to 2006; P=0.01). Survival improved in all subgroups except patients age ≥80 years. In US 16,420 patients >65 years with resected stage IB-IIIA NSCLC diagnosed between 1992 and 2007 were identified from the SEER-Medicare database (28). Among these patients, 1,803 (11%) received platinum-base adjuvant chemotherapy and this was associated with improved OS. However, 83% of the treated patients received carboplatin-based adjuvant chemotherapy and had a comparable OS advantage, and more favorable toxicity profile when compared with cisplatin-based adjuvant chemotherapy. In clinical practice, biologic age instead of chronologic age should be considered.

The combination of uracil and tegafur (also referred to as UFT, a pro-drug of 5FU) is an oral anticancer agent with good absorption in the small intestine (29). The West Japan Study Group for Lung Cancer Surgery reported that survival was significantly longer in patients assigned to adjuvant treatment with uracil-tegafur than in patients assigned to observation alone after complete resection of stage I, II, or III non–small-cell lung cancer. The five-year survival rate was 64 percent in the uracil-tegafur group and 49 percent in the control group (P=0.02). In a subgroup analysis, there was no significant difference in overall survival between the UFT group and the control group among patients with squamous-cell carcinoma (P=0.24) (30). Another Japanese Phase III randomized trial enrolled 979 patients with stage I (T1N0M0 or T2N0M0) adenocarcinoma of the lung and randomized them to receive either UFT 250 mg/m^2 for 2 years or observation. With median follow-up for 73 months, the difference in overall

survival between the two groups was statistically significant in favor of the UFT group (P=0.04). In subgroup analysis, the survival rate among patients with T2 disease in the UFT group was significantly higher than that in the control group, whereas among patients with T1 disease, there was no significant difference in survival between the UFT and control groups (31). A meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small-cell lung cancer (32) showed that postoperative adjuvant chemotherapy with UFT improved 5- and 7-year survival in a Japanese patient population composed primarily of stage I adenocarcinoma patients. Now in Japan, UFT is used as the standard postoperative adjuvant chemotherapy for stage I NSCLC patients with a tumor larger than 2 cm (33). S-1 is an orally active combination of tegafur and gimeracil [an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil and oteracil (which inhibits the phosphorylation of fluorouracil in the gastrointestinal tract, thereby reducing the gastrointestinal toxic effects of fluorouracil)] (34). Tsuchiya *et al.* (35) reported a phase II trial that included 51 curatively resected pathologic stage IB-IIIA non-small-cell lung cancer patients who received 8 courses (4-week administration, 2-week withdrawal) of S-1 at 80-120 mg per day. Postoperative 1-year administration of S-1 seems feasible as an oral adjuvant chemotherapy for lung cancer and further trials are ongoing. The Japanese WJOG4107 trial enrolled 200 patients with completely resected stage II and IIIA (excluded multi-station N2 cases) NSCLC who were randomized to receive either oral S-1 (40 mg/m^2 twice per day) for consecutive 2 weeks repeated every 3 weeks for 1 year or cisplatin (60 mg/m^2 day1) plus oral S-1 (40 mg/m^2 twice per day) for consecutive 2 weeks repeated every 3 weeks for 4 cycles. Relapse free survival rate on 2 years was 65.6% (95% confidence interval, 55.3-74.0%) in the S-1 arm and 58.1% (95% confidence interval, 47.7-67.2%) in the cisplatin plus S-1 arm. OS data was not mature.

In summary, adjuvant therapy for NSCLC has reached a new era, but continued progress must be made. At this time, the role of adjuvant cisplatin-based chemotherapy has been established by multiple large randomized phase III trials for resected stage II and IIIA NSCLC, but it is controversial in high risk stage IB patients and is not recommended for those with resected stage IA disease. The majority of the adjuvant chemotherapy data is with cisplatin/vinorelbine but other cisplatin/based regimens are commonly utilized and are included in ongoing clinical trials. The carboplatin/paclitaxel regimen is only recommended if patients have comorbidities and are not able to tolerate cisplatin.

Acknowledgements

Disclosure: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Datta D, Lahiri B. Preoperative evaluation of patients undergoing lung resection surgery. Chest 2003;123:2096-103.
2. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
3. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;365:1687-717.
4. André T, Boni C, Mounedji-Boudiaf L, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 2004;350:2343-51.
5. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899-909.
6. Scagliotti GV, Fossati R, Torri V, et al. Randomized study of adjuvant chemotherapy for completely resected stage I, II, or IIIA non-small-cell Lung cancer. J Natl Cancer Inst 2003;95:1453-61.
7. Waller D, Peake MD, Stephens RJ, et al. Chemotherapy for patients with non-small cell lung cancer: the surgical setting of the Big Lung Trial. Eur J Cardiothorac Surg 2004;26:173-82.
8. Arriagada R, Bergman B, Dunant A, et al. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351-60.
9. Arriagada R, Dunant A, Pignon JP, et al. Long-term results of the international adjuvant lung cancer trial evaluating adjuvant Cisplatin-based chemotherapy in resected lung cancer. J Clin Oncol 2010;28:35-42.
10. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell

lung cancer. N Engl J Med 2005;352:2589-97.
11. Bezjak A, Lee CW, Ding K, et al. Quality-of-life outcomes for adjuvant chemotherapy in early-stage non-small-cell lung cancer: results from a randomized trial, JBR.10. J Clin Oncol 2008;26:5052-9.
12. Butts CA, Ding K, Seymour L, et al. Randomized phase III trial of vinorelbine plus cisplatin compared with observation in completely resected stage IB and II non-small-cell lung cancer: updated survival analysis of JBR-10. J Clin Oncol 2010;28:29-34.
13. Douillard JY, Rosell R, Delena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719-27.
14. Strauss GM, Herndon JE 2nd, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. J Clin Oncol 2008;26:5043-51.
15. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
16. NSCLC Meta-analyses Collaborative Group, Arriagada R, Auperin A, et al. Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. Lancet 2010;375:1267-77.
17. Douillard JY, Tribodet H, Aubert D, et al. Adjuvant cisplatin and vinorelbine for completely resected non-small cell lung cancer: subgroup analysis of the Lung Adjuvant Cisplatin Evaluation. J Thorac Oncol 2010;5:220-8.
18. Alam N, Shepherd FA, Winton T, et al. Compliance with post-operative adjuvant chemotherapy in non-small cell lung cancer. An analysis of National Cancer Institute of Canada and intergroup trial JBR.10 and a review of the literature. Lung Cancer 2005;47:385-94.
19. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26:3543-51.
20. Kreuter M, Vansteenkiste J, Fischer JR, et al. Randomized phase 2 trial on refinement of early-stage NSCLC adjuvant chemotherapy with cisplatin and pemetrexed versus cisplatin and vinorelbine: the TREAT study. Ann Oncol 2013;24:986-92.
21. NCCN Guildlines: Non-small-cell Lung Cancer. Available online: http://www.nccn.org/ professionals/ physician_gls/ f_guidelines.asp#site
22. Wakelee HA, Dahlberg SE, Keller SM, et al. Interim report of on-study demographics and toxicity from E1505, a phase III randomized trial of adjuvant (adj) chemotherapy (chemo) with or without bevacizumab (B) for completely resected early-stage non-small cell lung cancer (NSCLC). J Clin Oncol 2011;29:abstr 7013.
23. Altekruse SF, Kosary CL, Krapcho M, et al. eds. SEER Cancer Statistics Review, 1975-2007. Available online: http://seer.cancer.gov/csr/1975_2007, Accessed October 25, 2010.
24. Hutchins LF, Unger JM, Crowley JJ, et al. Underrepresentation of patients 65 years of age or older in cancer-treatment trials. N Engl J Med 1999;341:2061-7.
25. Pepe C, Hasan B, Winton T, et al. Adjuvant chemotherapy in elderly patients: An analysis of National Cancer Institute of Canada Clinical Trials Group and Intergroup BR.10. J Clin Oncol 2006;24:366s.
26. Früh M, Rolland E, Pignon JP, et al. Pooled analysis of the effect of age on adjuvant cisplatin-based chemotherapy for completely resected non-small-cell lung cancer. J Clin Oncol 2008;26:3573-81.
27. Cuffe S, Booth CM, Peng Y, et al. Adjuvant chemotherapy for non-small-cell lung cancer in the elderly: a population-based study in Ontario, Canada. J Clin Oncol 2012;30:1813-21.
28. Gu F, Wisnivesky JP, Mhango G, et al. Carboplatin versus cisplatin-based adjuvant chemotherapy in elderly patients with stages IB, II, and IIIA non-small cell lung cancer in the community setting. Proc Am Soc Clin Oncol 2013:31;abstr 7533.
29. Fujii S, Kitano S, Ikenaka K, et al. Effect of coadministration of uracil or cytosine on the anti-tumor activity of clinical doses of 1-(2-tetrahydrofuryl)-5-fluorouracil and level of 5-fluorouracil in rodents. Gann 1979;70:209-14.
30. Wada H, Hitomi S, Teramatsu T. Adjuvant chemotherapy after complete resection in non-small-cell lung cancer. West Japan Study Group for Lung Cancer Surgery. J Clin Oncol 1996;14:1048-54.
31. Kato H, Ichinose Y, Ohta M, et al. A randomized trial of adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. N Engl J Med 2004;350:1713-21.

32. Hamada C, Tanaka F, Ohta M, et al. Meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small-cell lung cancer. J Clin Oncol 2005;23:4999-5006.
33. Nakagawa K, Kondo H. Lung cancer. Gan To Kagaku Ryoho 2011;38:1412-5.
34. Shirasaka T, Shimamato Y, Ohshimo H, et al. Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 1996;7:548-57.
35. Tsuchiya T, Nagayasu T, Yamasaki N, et al. A multicenter phase II study of adjuvant chemotherapy with oral fluoropyrimidine S-1 for non-small-cell lung cancer: high completion and survival rates. Clin Lung Cancer 2012;13:464-9.

Cite this article as: Liang Y, Wakelee HA. Adjuvant chemotherapy of completely resected early stage non-small cell lung cancer (NSCLC). Transl Lung Cancer 2013;2(5):403-410. doi: 10.3978/j.issn.2218-6751.2013.07.01

VATS lobectomy facilitates the delivery of adjuvant docetaxel-carboplatin chemotherapy in patients with non-small cell lung cancer

Xiuyi Zhi[1*], Wen Gao[2*], Baohui Han[3*], Yue Yang[4*], Hui Li[1*], Deruo Liu[5*], Changli Wang[6*], Gong Min[1*], Hao Long[7*], James R. Rigas[8*], Mark Carey[8*], Thierry Jahan[9*], Amanda Sammann[9], Joseph Reza[9], Daoyuan Wang[10], Michael J. Mann[9*], David M. Jablons[9*], Jianxing He[10*], for the China Clinical Trials Consortium

[1]Beijing Lung Cancer Center, Capital Medical University, Beijing, China; [2]Shanghai Pulmonary Hospital, Tongji University, Shanghai, China; [3]Shanghai Chest Hospital, Jiaotong University, Shanghai, China; [4]Beijing Cancer Hospital, Peking University, Beijing, China; [5]Beijing Sino-Japan Hospital, Beijing, China; [6]Tianjin Cancer Hospital, Tianjin Medical University, Tianjin, China; [7]Cancer Center of Sun Yat-sen University, Guangzhou, China; [8]Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA; [9]University of California, San Francisco, CA, USA; [10]First Affiliated Hospital of Guangzhou Medical College, Guangzhou, China

Correspondence to: Jianxing He, MD, PhD, FACS. Department of Cardiothoracic Surgery, the First Affiliated Hospital of Guangzhou Medical College, Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, No. 151, Yanjiang Rd, Guangzhou 510120, Guangdong Province, PR China. Email: drjianxing.he@gmail.com; David M. Jablons, MD. University of California, San Francisco, CA, USA. Email: David.Jablons@ucsfmedctr.org.

Background: To evaluate the safety and tolerability of docetaxel/carboplatin regimen in the post-operative setting of patients with non-small cell lung cancer (NSCLC).

Methods: Enrolment of 133 patients with stage Ib - IIIa NSCLC was undertaken in an open-label, single arm study to assess the safety and tolerability of docetaxel (75 mg/kg) and carboplatin (AUC 5.5) administered for 3 cycles after resection for curative intent. The primary endpoint of the study was safety, as reflected by a febrile neutropenia rate of <10%. Other endpoints assessed protocol compliance and the impact of minimally invasive surgical technique.

Results: Patient accrual was completed at 1 center in the US and 10 centers in China in <6 months. Febrile neutropenia complicated treatment in 12 patients (9.0%), below the predetermined safety threshold of 14 patients. Four VATS and 8 open thoracotomy patients experienced febrile neutropenia (P=0.26). Completion of the three-cycle adjuvant regimen was achieved in 86% (95% CI, 77-95%) of patients. Sixty-two of 66 VATS patients compared to 53 of 67 open thoracotomy patients received all three doses according to protocol (P<0.01). Thirteen serious adverse events (9.8%) and no deaths were attributed to the study regimen.

Conclusions: In this rapidly accrued study, docetaxel and carboplatin were well-tolerated in the adjuvant treatment of NSCLC. Adjuvant treatment compliance was higher among patients undergoing a minimally invasive surgical approach. (ClinicalTrials.gov number NCT00883675).

Keywords: Non-small cell lung cancer; adjuvant chemotherapy; docetaxel; carboplatin

Submitted Jan 01, 2012. Accepted for publication Feb 04, 2012.
doi: 10.3978/j.issn.2072-1439.2012.02.05
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2012.02.05

Introduction

With an ever-increasing array of novel therapeutics competing for clinical resources, the rate at which new cancer treatments can be evaluated and made available to under-served populations depends heavily on the recruitment of patients into mid-stage and late-stage clinical trials. For many cancers, including non-small cell lung cancer (NSCLC), this rate of development traditionally has been slow; many trials require years to complete patient accrual, while others close enrollment prior to attaining target sample sizes and compromise the studies' power to fully evaluate their therapeutic strategies (1). This problem has been particularly pronounced in the area of post-operative adjuvant therapies. The China Clinical Trials Consortium (CCTC), a cooperative group formed to facilitate the development of advanced clinical research in China, has been designed to access in a rapid, efficient and rigorous manner extremely large pools of cancer patients previously unavailable to participate in the development of new cancer therapies.

Compared to many other cancers, overall survival among patients with NSCLC remains poor, even when the disease is discovered in its earliest stages (2). Attempt at curative resection even for stage IA patients, for example, is associated with only 70-75% survival (3,4); survival after resection for stages II-IIIA ranges from 30-50% (4). Early, undetected metastasis is responsible for the vast majority of the failures of these local treatments, and adjuvant systemic therapies for NSCLC have therefore been investigated extensively. Whereas alkylating agents do not seem to add any survival benefit in this patient population, platinum-based combination (doublet) therapies have consistently been shown to confer an absolute 5-year survival benefit ranging between 4% and 15% (5-9).

Positive randomized studies of adjuvant chemotherapy for NSCLC have studied combinations of cisplatin with either etoposide or a vinca alkaloid (e.g., vinorelbine), or of carboplatin and paclitaxel (6-9). The combination of carboplatin and docetaxel has been studied in patients with advanced-stage NSCLC in patients who had not received any prior therapy (10). In that study, the efficacy of this combination was comparable to that of a cisplatin-vinorelbine doublet. Grade 3 or 4 toxicity in the form of nausea, vomiting or anemia, however, was higher among patients receiving vinorelbine than among patients receiving docetaxel combined either with cisplatin or carboplatin. A number of quality of life parameters were also improved in the docetaxel arms compared to the patients who had received vinorelbine.

Benefit from chemotherapy, whether administered as a first line agent for late-stage patients or in the adjuvant setting after an attempt at curative resection, depends on successful completion of a prescribed course of therapy (11). In this regard, successful completion of adjuvant regimens has been somewhat disappointing in previously reported studies (7,8), likely related to the reduced functional status of patients still recovering from anatomic lung resection. In two studies of adjuvant cisplatin-vinorelbine, for example, only 58-61% of eligible patients completed at least 3 cycles of post-operative chemotherapy; in one study 77% of patients required at least one dose reduction or omission (7), while in the other 62% of patients received <66% of the planned dose of vinorelbine and 37% tolerated only <66% of the total dose of cisplatin (8). The combination of docetaxel and carboplatin, on the other hand, has been well-tolerated as a first line therapy for late stage patients (10). Only one study, however, has evaluated the safety and tolerability of this regimen in the post-operative setting, and its small sample size and long accrual period likely compromised its ability to establish its pre-defined safety target (12). We therefore assessed these parameters in a cohort of 133 patients in an open-label, single arm study. The CCTC was enlisted to accelerate completion of the study.

Methods

Study design and patients

For this open label study, the primary endpoint of safety was defined as a febrile neutropenia rate of 10% or less. A Simon's two-stage sequential design (13) indicated that a sample size of 133 would yield a power of 90% with the alpha set 5% to test the null hypothesis that $P<0.850$ versus the alternative hypothesis that $P\geq0.930$ where P was the absence of febrile neutropenia. Accordingly, the combination of docetaxel and carboplatin would be rejected if 14 or more cases of febrile neutropenia were observed in conjunction with this adjuvant therapy.

Enrollment of patients took place at a single center in the United States (Dartmouth-Hitchcock Norris Cotton Cancer Center) and at ten sites in China that were early members of the CCTC. Five patients were enrolled during a pilot phase at CCTC sites between July and September 2009. The remaining 128 patients were subsequently enrolled within a six-month period between October 2009 and April 2010. Two patients were enrolled during this period in the US; 39, 45 and 47 patients were enrolled at centers in Beijing-Tianjin, Guangzhou and Shanghai, respectively.

The protocol was approved by an ethics committee at each center, and was conducted according to the Declaration of Helsinki and the International Committee on Harmonization guidelines for Good Clinical Practice (ICH-GCP, E6). Every patient provided written informed consent according to GCP guidelines. An independent data and safety monitoring board was also appointed.

Eligibility criteria included: 2-8 weeks within complete resection (R0) of pathologically confirmed NSCLC, stage IB-IIIA according to IASLC 7th TNM staging system (14), via lobectomy, bilobectomy or pneumonectomy plus formal lymph node dissection; age >17 and ECOG status 0-1 with normal organ function based on blood counts and chemistries. Patients with concurrent malignancies or who received any prior therapy for NSCLC were excluded, as were HIV-positive patients and patients with grade 2 or higher neuropathy, treatment within 30 days with any other investigational anti-cancer agent, previous treatment with docetaxel or carboplatin, or hypersensitivity to platinum.

Procedures

Patients were registered in the study after undergoing resection with curative intent and after confirmation of eligibility criteria. After providing written informed consent, patients were prescribed a total of three courses of combination therapy with docetaxel (75 mg/m2 IV) and carboplatin [AUC 5.5 × (estimated creatinine +25)]. Docetaxel was administered before carboplatin on the first day of each three-week cycle. Complete blood counts were measured each week until the completion of all three cycles, and blood chemistries were measured prior to initiation of each cycle. An absolute neutrophil count of at least 1,200/mm3 was required before each cycle, and specified dose modifications were instituted for various grades of neutropenia and/or thrombocytopenia. Patients requiring more than 2 dose reductions were removed from the study; colony-stimulating factors were used at the discretion of physicians according to published American Society of Clinical Oncology (ASCO) guidelines. Patient follow up visits were scheduled at 3, 6, 12, 18 and 24 months after the last dose of chemotherapy, and every 12 months thereafter until the 60-month follow up is reached.

Statistical analysis

The primary endpoint was defined as the rate of febrile neutropenia associated with the adjuvant administration of docetaxel/carboplatin. Secondary endpoints such as rates of adverse events (AEs) and serious adverse events (SAEs), specific toxicities (based on highest grade according to Common Terminology Criteria for Adverse Events, version 4.0), dose modifications, removal from the study, and completion of the three prescribed cycles of chemotherapy were assessed as indicators of the regimen's tolerability. Post-hoc comparison of chemotherapy utilization was made between patients undergoing a minimally invasive video-assisted thoracoscopic surgery (VATS) approach versus traditional open thoracotomy. Dichotomous variables were compared using a Chi-squared test, while continuous variables were compared using Student's *t*-test. A P-value of <0.05 was considered statistically significant.

Results

After a preliminary three-month period of protocol initiation, 96% of patient enrollment was completed between October 2009 and April 2010. A total of 89 men (67%) and 44 women (33%) were enrolled; patient characteristics are summarized in *Table 1*. Lobectomy was performed in 118 patients (89%, including 3 sleeve lobectomies), while the remaining patients received either bilobectomy (7.5%) or pneumonectomy (8.6%). Sixty-six procedures (50%) were performed using a VATS approach, including 62 lobectomies (53% of lobectomies), 1 bilobectomy (14% of bilobectomies), and 3 pneumonectomies (38% of pneumonectomies).

A total of 115 patients (86%) completed three cycles of docetaxel-carboplatin therapy according to protocol (*Table 2*). Discontinuation of therapy resulted from adverse reactions in 6 cases (4.5%) and patient withdrawal in 12 (9%). Among patients receiving all three cycles, only 2 (1.5%) received less than 66% of their target doses of carboplatin, and 3 (2.3%) received between 67 and 75% of their carboplatin doses, all 5 tolerating their full docetaxel doses (*Table 3*). Docetaxel dose reduction was required in only 4 patients (3.5%) receiving all three cycles. Among patients receiving all three cycles, 74 (64%) received full dose chemotherapy; 56% of patients in the study therefore received all three cycles of full-dose chemotherapy. Utilization of a VATS approach for resection was associated with a lower rate of therapy discontinuation; 62 of 66 VATS patients compared to 53 of 67 open thoracotomy patients received all three doses according to protocol ($P<0.01$). In addition, only 1 VATS patient receiving all three doses required a dose reduction of >10% of the target doses,

Table 1 Patient characteristics

	Open thoracotomy	VATS	Total
Age (years)			
Median [Range]	60 [32-80]	63 [40-80]	61 [32-80]
Sex			
Male	46	43	89 (67%)
Female	21	23	44 (33%)
Days till First Chemotherapy (+/– SD)	34 (+/– 9)	32 (+/– 10)	33 (+/– 10)
Type of surgery			
Pneumonectomy	5	3	8 (6%)
Bilobectomy	6	1	7 (5%)
Lobectomy	56	62	118 (89%)
Stage			
IB	14	6	20 (15%)
IIA	22	30	52 (39%)
IIB	11	11	22 (17%)
IIIA	20	19	39 (29%)
Histology			
Adenocarcinoma	37	42	79 (59%)
Squamous cell	23	17	40 (30%)
Large cell	4	1	5 (4%)
Other	3	6	9 (7%)
LN Status			
N0	32	35	67 (50%)
N1	17	14	31 (23%)
N2	18	17	35 (27%)
ECOG			
0	9	16	25 (19%)
1	58	50	108 (81%)

compared to 16 patients undergoing open thoracotomy (P<0.001). Interestingly, there was no difference in the time from surgery to initiation of chemotherapy between the VATS patients and the open thoracotomy patients (32±10 and 34±9 days, respectively, P=0.4).

Febrile neutropenia was encountered in a total of 12 patients (9.0%), below the pre-defined safety threshold rate of 14 cases. Five cases occurred after the second cycle of adjuvant chemotherapy, with the remainder occurring after the third cycle. Hospitalization for febrile neutropenia was required only in 1 case; each case resolved without

Table 2 Protocol compliance

Patients who completed	Open thoracotomy	VATS
Cycle I	67 (100%)	66 (100%)
Cycle II	58 (87%)	62 (94%)
Cycle III	53 (79%)	62 (94%)*

*P<0.01 *vs*. Open thoracotomy.

Table 3 Percent of target carboplatin dose in patients completing 3 cycles

Percent of target carboplatin dose in patients completing 3 cycles	Open thoracotomy (Percent of 53 patients who received 3 doses)	VATS (Percent of 62 patients who completed 3 doses)
<66%	2 (4%)	0 (0%)
67-75%	3 (6%)	0 (0%)
76-90%	11 (21%)	1 (2%)
91-99%	15 (28%)	9 (14%)
100%	22 (41%)	52 (84%)**

**P<0.001 *vs*. Open thoracotomy.

serious complication (*Table 4*). Four VATS patients and 8 open thoracotomy patients experienced febrile neutropenia (P=0.26). There were no treatment related deaths, and grade 3 or 4 toxicities are reported in *Table 5*. Some degree of neutropenia was observed in 74 patients (56%); grade 3 or 4 neutropenia was observed in 55 patients (41%). Grade 3 or 4 nausea, vomiting or diarrhea was encountered in only 3 patients (2%) during the course of therapy.

Eighteen serious adverse events (SAEs) were encountered in 14 (10.5%) patients, including 4 allergic reactions to docetaxel that required discontinuation of therapy, two of which required hospitalization for hypotension (*Table 6*). Nine of the other SAEs involved hospitalization, either for febrile neutropenia (1 patient), infection (3 patients), severe neutropenia (3 patients) or severe vomiting/diarrhea (2 patients); the last SAE involved a pelvic infection requiring intravenous antibiotics.

Discussion

According to the pre-specified criteria, the combination of docetaxel and carboplatin was determined in this study to be safe in the adjuvant treatment of NSCLC. There were no treatment-related deaths. Febrile neutropenia was

Table 4 Regimen toxicity of febrile neutropenia

Febrile neutropenia	
Total	12 (9%)
Requiring hospitalization	1 (1%)

Table 5 Regimen safety and toxicity

Toxicity	Grade 3	Grade 4
Death	0	0
Neutropenia	26 (19%)	29 (22%)
Anemia	1 (1%)	0
Thrombocytopenia	1 (1%)	1 (1%)
Fatigue	1 (1%)	0
Nausea	1 (1%)	0
Vomiting	1 (1%)	0
Diarrhea	2 (2%)	0
Infection	2 (2%)	0
Pleural effusion	1 (1%)	0
Hyperbilirubinemia	1 (1%)	0

Table 6 Serious adverse events

Serious adverse events	Patients (events)
Febrile neutropenia requiring hospitalization	1 (1)
Allergic reaction not requiring hospitalization	2 (2)
Allergic reaction requiring hospitalization	2 (2)
Infection not requiring hospitalization	1 (1)
Infection requiring hospitalization	3 (4)
Neutropenia requiring hospitalization	3 (4)
Vomiting/Diarrhea requiring hospitalization	2 (4)
Requiring Hospitalization	1 (1%)

encountered in 9.0% of patients, which is comparable to febrile neutropenia rates of 5-14% reported with other common regimens for NSCLC, either in the first line or adjuvant setting (5-10,15). The rate of treatment-related hospitalization (11/133, 8.3%) was similarly low, with the only other SAEs involving allergic reactions or infection that did not require hospitalization. Other Grade 3 or 4 toxicities were also quite comparable in incidence to the reported literature for other first line and adjuvant therapies for NSCLC (5-10,15).

Accrual of patients in this study occurred at an unusually rapid pace compared to other recent trials involving adjuvant therapy after resection of NSCLC with curative intent (6-10). In fact, several important adjuvant trials have been halted due to poor patient accrual (6,16). This efficient enrollment was accomplished via the participation of the CCTC, an organization recently formed to advance fully ICH-GCP compliant clinical research at leading cancer centers in China. Although concern was raised prior to study initiation about the use of 'Western' doses of chemotherapy in an Asian population that often receives reduced doses (17), the full dose of docetaxel and carboplatin was well-tolerated in this study. In fact, as has been seen in a previous study of carboplatin-based adjuvant chemotherapy for NSCLC (9), compliance with the treatment protocol was high, with 86% of patients receiving all three cycles, compared to approximately 60% of patients in adjuvant studies of vinorelbine and cisplatin (7,8). Enforcement of strict protocol adherence among CCTC investigators and the tolerability of the regimen under study were likely responsible for the high compliance rate observed.

A similar study of the feasibility of docetaxel and carboplatin in the adjuvant treatment of NSCLC was recently conducted at seven centers in the United States (12). Although that study failed to meet its predetermined safety and feasibility target by a very small margin, the overall results were very similar to the current study. Those investigators reported completion of the study regimen in 79% of patients, and a febrile neutropenia rate of 11%. Interestingly, however, nearly two and a half years were required to complete enrollment of 72 patients in that phase II trial. Had those investigators attempted to accrue the sample size of 133 patients included in this study, enrollment at the same pace would have required four and a half years, compared to the enrollment period of approximately 6 months in this CCTC clinical trial.

In this initial study, the CCTC was therefore able to accelerate enrollment by nearly an order of magnitude over accrual in a comparable American trial. Importantly, this accrual speed did not result in a compromise of protocol or GCP compliance. Similar acceleration of other important, large-scale clinical projects may transform current capabilities for the development of novel cancer therapies. An unprecedented ability to rapidly conduct several phase II studies of a single agent in parallel, for example, may allow a much broader range of indications for a novel therapeutic to receive reasonable consideration, and may prevent the oversight of an important new indication for drug usage that might have resulted from a previous limitation of clinical resources.

This study was not designed prospectively to compare a

traditional open thoracotomy to a more minimally invasive VATS approach to anatomic resection, and patients were therefore not randomized between these two forms of surgery. A relatively comparable of patients in our cohort, however, received each form of treatment, which allowed some post hoc comparisons to be made. In another retrospective, non-randomized study that compared 43 patients who underwent complete resection by thoracotomy to 57 patients treated via thoracoscopy (18), VATS lobectomy patients had statistically significantly fewer delayed doses and fewer dose reductions than thoracotomy patients. In addition, 61% of VATS patients versus 40% of thoracotomy patients in that study received 75% or more of their planned adjuvant regimen (P=0.03). In comparison, only 57% of the thoracotomy patients in the Cancer and Leukemia Group B trial 9633 received full-dose chemotherapy (19), and the Intergroup JBR.10 trial reported that 55% of their thoracotomy patients had at least 1 dose delay (7). Similarly, only 34% of patients in the chemotherapy arm of the Adjuvant Lung Project Italy series received all scheduled doses without adjustment or delay; only 69% completed their treatments with or without adjustments or delay (20). The potential benefit of a minimally invasive surgical approach, with a likely reduction in post-operative pain and an easier post-operative recovery period prior to initiation of chemotherapy (21), was supported by the observation that treatment protocol compliance and delivery of target doses of adjuvant chemotherapy were higher among patients undergoing VATS lobectomy in the current study.

Taken together, the results of this study suggest that the combination of docetaxel and carboplatin is both safe and well-tolerated in the adjuvant treatment of NSCLC, and that adjuvant treatment compliance was highest among patients undergoing a minimally invasive surgical approach. Ongoing follow-up of the cohort of patients enrolled in this study will provide some insight into the relative efficacy of this regimen compared to the reported experience in other adjuvant studies. Perhaps more important, this study also demonstrated successful implementation of the founding premise of the CCTC: that substantial improvement in the current landscape for the development of novel cancer therapies can be achieved through rigorous organization and management of dedicated clinical study groups involving emerging centers of excellence in cancer care and research.

Acknowledgement

This study was supported by a grant from Sanofi-Aventis.

Disclosure: No potential conflict of interest. Presented at the 14th World Conference on Lung Cancer, Amsterdam, July 6, 2011.

References

1. Zhao Y, Kosorok MR, Zeng D. Reinforcement learning design for cancer clinical trials. Stat Med 2009;28:3294-315.
2. Shao W, Wang D, He J. The role of gene expression profiling in early-stage non-small cell lung cancer. J Thorac Dis 2010;2:89-99.
3. Martini N, Bains MS, Burt ME, et al. Incidence of local recurrence and second primary tumors in resected stage I lung cancer. J Thorac Cardiovasc Surg 1995;109:120-9.
4. Voon PJ, Chul Cho B, Yeo WL, et al. The role of epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of advanced stage non-small cell lung cancer. J Thorac Dis 2010;2:144-53.
5. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899-909.
6. Arriagada R, Bergman B, Dunant A, et al. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351-60.
7. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005;352:2589-97.
8. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719-27.
9. Strauss GM, Herndon JE 2nd, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. J Clin Oncol 2008;26:5043-51.
10. Fossella F, Pereira JR, von Pawel J, et al. Randomized, multinational, phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small-cell lung cancer: the TAX 326 study group. J Clin Oncol 2003;21:3016-24.

11. Fidias P, Novello S. Strategies for prolonged therapy in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:5116-23.
12. Stinchcombe TE, Harper HD, Hensing TA, et al. The feasibility of adjuvant carboplatin and docetaxel in patients with curatively resected non-small cell lung cancer. J Thorac Oncol 2008;3:145-51.
13. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials 1989;10:1-10.
14. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
15. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92-8.
16. Chhatwani L, Cabebe E, Wakelee HA. Adjuvant treatment of resected lung cancer. Proc Am Thorac Soc 2009;6:194-200.
17. Millward MJ, Boyer MJ, Lehnert M, et al. Docetaxel and carboplatin is an active regimen in advanced non-small-cell lung cancer: a phase II study in Caucasian and Asian patients. Ann Oncol 2003;14:449-54.
18. Petersen RP, Pham D, Burfeind WR, et al. Thoracoscopic lobectomy facilitates the delivery of chemotherapy after resection for lung cancer. Ann Thorac Surg 2007;83:1245-9; discussion 1250.
19. Strauss GM, Herndon JE 2nd, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. J Clin Oncol 2008;26:5043-51.
20. Scagliotti GV, Fossati R, Torri V, et al. Randomized study of adjuvant chemotherapy for completely resected stage I, II, or IIIA non-small-cell Lung cancer. J Natl Cancer Inst 2003;95:1453-61.
21. He J, Shao W, Li S, et al. Feasibility Of Administering Adjuvant Chemotherapy Of Pemetrexed Followed By Pemetrexed/oxaliplatin Immediately Post-VATS In Patients With Completely Resected NSCLC. J Thorac Dis 2009;1:55-62.

Cite this article as: Zhi X, Gao W, Han B, Yang Y, Li H, Liu D, Wang C, Min G, Long H, Rigas JR, Carey M, Jahan T, Sammann A, Reza J, Wang D, Mann MJ, Jablons DM, He J, for the China Clinical Trials Consortium. VATS lobectomy facilitates the delivery of adjuvant docetaxel-carboplatin chemotherapy in patients with non-small cell lung cancer. J Thorac Dis 2013;5(5):578-584. doi: 10.3978/j.issn.2072-1439.2012.02.05

Medical Oncology

Anti-tumor immune response in early stage non small cell lung cancer (NSCLC): implications for adjuvant therapy

Charles A. Butts

Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada

Correspondence to: Charles A. Butts, MD, FRCP(C), Associate Professor of Oncology. Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada. Email: Charles.Butts@albertahealthservices.ca.

Abstract: The demonstration that systemic chemotherapy improves survival in patients who have had resection of early stage non-small cell lung cancer (NSCLC) represents a significant advance. The absolute benefit of adjuvant chemotherapy in this setting is small with an overall survival improvement of 5%. In addition, there are many patients who have contraindications to cisplatin-based adjuvant therapy. Adjuvant chemotherapy is intended to target systemic micrometastases that remain after primary resection. The observation that cancers can relapse months or years after initial surgery implies that the residual micrometastases exist in a latent or dormant state. The concept of tumor dormancy offers therapeutic potential through induction or maintenance of the dormant state in disseminated tumor cells or through eradication of these dormant cells. Cancer dormancy is a complex process with multiple potential mechanisms. This review will focus on some of the evidence for immune related tumor dormancy and the potential for immune therapies to improve outcomes in the adjuvant setting in NSCLC.

Keywords: Non-small cell lung cancer (NSCLC); anti-tumor immune response; immunotherapy; checkpoint inhibitors; gene signature (GS); immune dormancy

Submitted Sep 28, 2013. Accepted for publication Oct 12, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.09
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.09

Introduction

The demonstration that systemic chemotherapy improves survival in patients who have had resection of early stage non-small cell lung cancer (NSCLC) represents a significant advance in the treatment of this disease (1). The absolute benefit of adjuvant chemotherapy in this setting is small with an overall survival improvement of 5%. In addition, there are many patients who have contraindications to cisplatin-based adjuvant therapy. Hence, a non-cytotoxic therapy that could be combined with chemotherapy to improve survival or could be applied in patients not eligible for adjuvant chemotherapy would be in a welcomed advance.

Adjuvant chemotherapy is intended to target systemic micrometastases that remain after primary resection. The observation that cancers can relapse months or years after initial surgery implies that the residual micrometastases exist in a latent or dormant state. In addition, experimental models of dormancy have been developed that allow for investigation into different mechanisms of tumor dormancy (2). The concept of tumor dormancy offers therapeutic potential through induction or maintenance of the dormant state in disseminated tumor cells or through eradication of these dormant cells.

Cancer dormancy is a complex process with multiple potential mechanisms. More broadly, these can be categorized into cellular dormancy, resulting from tumor growth arrest of disseminated tumor cells, or tumor mass dormancy related to limitations in vascular supply or to an active host immune response (3).

This review will focus on the evidence for immune related tumor dormancy and the potential for immune

therapies to improve outcomes in the adjuvant setting in NSCLC.

Immune dormancy

It has been more than half a century since Burnet and Thomas proposed a formal hypothesis of cancer immune-surveillance wherein one of the primary functions of the immune system is to protect against cancers. Dunn *et al.* (4) expanded this into a broader proposal of immunoediting, which envisages not only elimination of cancer cells (immune-surveillance), but also a dynamic interaction between cancer cells and immune system, which leads to an equilibrium phase. This process selects for less immunogenic cancer cells until the selection eventually leads to cancer cell escape from immune control. The equilibrium phase could account for the period of immune dormancy while escape would signal clinical relapse. The extensive experimental data from those models and correlated clinical studies in human patients that support the concept of immune dormancy have been reviewed elsewhere (5-9).

One line of clinical evidence supporting an interaction between host immune system and human cancer cells are the numerous studies documenting infiltration of human malignancies by immune cells. While many reports focus on tumor infiltrating lymphocytes (TILs), effector cells of the innate immune system (macrophages, mast cells, dendritic cells and natural killer cells) have also been shown to be major constituents of tumor infiltrates (10).

Colorectal cancer is one of the most studied human cancers linking TILs and prognosis. Initial reports linking TILs to better prognosis date back more than thirty years (11). More recently, Galon *et al.* (12) found that, in colorectal cancer, the type, density, and location of TILs within the tumor were strongly linked to survival independent of tumor stage.

Tumor infiltrating immune cells and prognosis in NSCLC

A number of studies have investigated the link between tumor-associated immune response in the primary tumor and patient outcome after surgical resection in early stage NSCLC. While these studies have a number of limitations including small patient numbers, specificity of the markers of immune cells used, lack of correlation with micro-anatomical location and failure to assess functional status of immune cells, they do suggest a potential prognostic role for assessing tumor infiltrating immune cells after resection of early stage non small cell lung cancer.

In an early report of over 700 resected NSCLC specimens, Johnston *et al.* (13) found that non-specific evaluation of tumor infiltrating immune cells was not correlated with prognosis. However, the presence of T cells infiltrating among the cancer cells was associated with favorable prognosis. Wakabayashi (14) also pointed out the importance of assessing immune cell infiltration in various tumor compartments: within the tumor cell nests (epithelial), within the central stroma (stromal), and along the invasive margins.

Tumor associated macrophages (TAMs) constitute a major component of tumor infiltrating immune cells (10) and have the potential to promote tumor progression or support an immune response (15). A number of groups have reported that the number of tumor infiltrating macrophages could be associated with favorable survival in early stage NSCLC (16,17). In contrast, the largest reported trial (18) found no association between TAMs in either stromal or tumor epithelial compartments and survival in 335 resected NSCLCs. These contradictory results might possibly be due to failure to distinguish M2 macrophages, which are tumor angiogenesis promoting, and M1 macrophages which may exert a cytotoxic effect on cancer cells (15). Studies by Ohri (19) and Ma (20) found that macrophages expressing markers for M1 phenotype were associated with better prognosis in early stage NSCLC.

Dendritic cells are the most efficient antigen presenting cells for inducing an immune response to cancer. Al-Shibli *et al.* (18) measured immature dendritic cells in the tumor epithelial and stromal compartments from resected NCLC. Higher stromal DCs were associated with absence of nodal involvement and significantly better disease specific survival. Similar results were reported by Inoshima (21), who assessed 132 resected NSCLC specimens for dendritic cell infiltration in tumor nests. Higher DC counts were more common in stage I patients and an independent prognostic factor for overall survival. This study also looked at the correlation between VEGF expression and DC infiltration. In addition to its role in angiogenesis, VEGF has been shown to inhibit both the maturation and function of DCs (22). Tumors expressing high levels of VEGF had less DC infiltration and the combination of high VEGF expression and low DC infiltration resulted in a significantly lower 5-year survival than patients with low VEGF and high DC infiltration (14.5% versus 43.4%). These results

suggest a potential clinically important interaction between VEGF and an anti-tumor immune response and a role for anti-VEGF therapy in restoring DC function.

A favorable effect on prognosis in early stage NSCLC was seen in studies investigating infiltration of primary NSCLC by $CD8^+$ cytotoxic T lymphocytes (16,23,24). In a report of 335 consecutive stage I-IIIA NSCLC specimens, Al-Shibli *et al*. evaluated tissue microarrays for epithelial and stromal $CD4^+$, $CD8^+$ and $CD20^+$ lymphocytes. High density of both $CD4^+$ and $CD8^+$ lymphocytes in the stromal but not the epithelial compartment were associated independent predictors of disease specific survival.

Malignant tumors are composed not solely of the malignant cells but also stromal, endothelial, and immune/inflammatory cells that interact in complex ways. These interactions may lead to presence of immune cells that are immature or anergic. As a marker of a functional anti-tumor immune response Gottlin (25) looked at the relationship between presence of organized lymphoid structures, germinal centers (GC) and survival in early stage NSCLC. The GCs are organized loci containing mature dendritic cells and T cells adjacent to B cells and are an adaptive immune response. They assessed 91 early stage NSCLC specimens for the presence of GCs and found 32 (35%) to have GCs at tumor margins or tumor center. The presence of intratumoral but not marginal GCs was associated with earlier stage (Stage I) and in stage I patients, presence of intratumoral GCs was associated with better survival than no GCs. A separate study from France (26), looked at the presence of tertiary lymphoid structures, which they designated tumor-induced bronchus associated lymphoid tissues (Ti-BALT) in 74 patients with resected early stage NSCLC. The Ti-BALT is composed of mature dendritic cell/T-cell clusters adjacent to B-cell follicles. The used the density of mature DCs as a marker of Ti-BALT. The density of mature Dcs was significant predictor of overall, disease specific and disease free survival. They concluded that their data suggested that infiltration of tumor cells by mature DCs resulted in organization and proliferation of T and B-cells in Ti-BALT. They proposed a potential role of mature DC density as a prognostic factor for relapse in early stage NSCLC.

The studies cited provide substantial evidence for the existence an antitumor response in NSCLC. These studies suggest a potential role for correlating host immune response with survival in early stage NSCLC and a role in potentially selecting patients for adjuvant therapy. However, there is no convincing evidence that assessment of any component of the innate or adaptive immune response can reliably predict outcomes after surgical resection of NSCLC. The large tumor banks from randomized trials of adjuvant chemotherapy provide the potential to assess the prognostic ability of host immune response in early NSCLC in a large sample size taken in a multi-center setting and whether or not a local immune response to the primary tumor might predict for benefit of adjuvant chemotherapy.

Gene signature (GS) predicting response to immunotherapy

It is believed that a cancer phenotype associated with immune response does occur and may identify patients more likely to respond to immunotherapy. Ulloa-Montoya *et al*. (27) used tissue microarrays from patients with advanced melanoma treated with MAGE-A3 antigen specific cancer immunotherapy (ASCI) to identify a pre-treatment gene expression signature associated with clinical benefit. Clinical benefit was defined as objective response, stable disease for more than 4 months or mixed response with unequivocal tumor shrinkage. Genes selected from the microarray data were corroborated using quantitative polymerase chain reaction (qRT-PCR) and these. The GS identified not only identified patients with clinical benefit but also was predictive of better overall survival (29 versus 16.2 months) with MAGE-A3 ASCI treatment in patients whose tumors were GS positive versus those who were GS negative.

This GS was applied to resection specimens from patients enrolled in an independent randomized phase II trial of MAGE-A3 ASCI as adjuvant therapy in stage IB or II NSCLC. The GS was assessed from 157 of the 162 randomized patients. There were 61 patients who were GS positive. A positive GS was associated with a better disease free interval (DFI), the primary end-point of the trial (HR 0.42; 95% CI: 0.17-1.03; P=0.06). Although no benefit in terms of overall survival was seen for MAGE-A3 ASCI in the overall study population, in patients with GS positive tumors a strong trend in favor of benefit from MAGE-A3 ASCI was seen (HR 0.63; 95% CI: 0.22-1.78; P=0.38).

Analysis of the genes included in the GS positive tumors showed an over-representation of immune related genes. Genes involved included MHC class I and II, T cell markers regulated by interferon gamma (IFN-G), genes involved in antigen presentation and chemokines. The authors concluded that a specific tumor microenvironment favors the presence of immune effector cells in responding

patients. Of interest, the GS did not appear to be prognostic in placebo arm. This might reflect the small sample size or the selection of patients for the trial based on MAGE-A3 expression.

Prospective evaluation of this GS in randomized phase III trials in melanoma and NSCLC are planned. Confirmation of the predictive value of a GS for immune response would be valuable in patient selection for on-going trials and potential could be valuable in selecting patients likely to benefit from MAGE-A3 immunotherapy.

Checkpoint inhibitors

The demonstration of agents targeting immune check-points can result in tumor response in human solid tumors (28) and improve survival (29) has renewed interest in cancer immunotherapy. The fact that these agents have activity when used alone is support for an endogenous host immune response to cancer cells.

Under normal circumstances, immune checkpoint inhibitors are integral to maintaining cell tolerance and protecting normal tissue from damage during immune response (30). Counter-balancing stimulatory and inhibitory signals regulate T cell activation. The two most relevant immune checkpoint inhibitors are cytotoxic T-lymphocyte associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD1).

CTLA4 is expressed exclusively on T cells and primarily acts to regulate the amplitude of early T cell activation (30). It is not expressed on the naive or memory T cells and expression is triggered only after antigen binds to T cell receptor (TCR). Hence, the clinical activity of the CTLA blocking antibodies implies that a host T cell response to tumor antigens exists but is suppressed by factors in the tumor microenvironment.

The major role for PD1 is to limit activity of T cells in peripheral tissue and to limit autoimmunity (30). The expression of PD1 is induced after T cell activation. PD1 must bind to one of its ligands, PD1 Ligand1 (PD L1 or B7-H1) or PD1 Ligand2 (PD L2 or B7-DC) in order to inhibit T cell activation signals. Unlike CTLA4, the expression of PDL1 is not limited to T cells. It is also expressed on B cells and natural killer cells.

The ligands for PD1 are commonly upregulated on tumor cells including NSCLC (31) and they have potential as biomarkers for response to PD1 ligand blockade. The expression of PD L1 on tumors may be a form of adoptive immune resistance and is further evidence of an endogenous host immune response and a potential mechanism of immune escape. Zhang *et al*. (32) compared PD1 expression on peripheral blood CTLs from healthy controls to those obtained from peripheral blood of 21 NSCLC patients undergoing surgical resection and to PD1 expression on TILs from resected specimens from 16 of these patients. The expression of PD1 was higher in peripheral blood CTLs from NSCLC patients than healthy controls and highest in the TILs from the surgical resection specimens. The PD1 expressing TILs showed less differentiated phenotype and were less capable of production of IFN G and IL-2 and of proliferation. Blocking antibodies to PDL1 but not PDL2 lead to increased cytokine production and T cell proliferation.

Immunosuppressive regulatory T cells (Treg) also highly express PD1 and early expression of PD1 can shift T cells from an activated state to one of anergy. Assessing TILs for expression of PD1 or its ligands may be important in studies evaluating TILs and the association with prognosis in NSCLC. Schneider *et al*. (33) assessed tumors from 12 patients undergoing potentially curative resection for early stage NSCLC for the expression of B7-H3, a member of the PDL1 ligand family, on DCs from tumor and normal lung distant from tumor. Expression of B7-H3 was significantly upregulated on DCs from tumor compared to healthy lung and these DCs were inferior at stimulating T cell proliferation. The ability to stimulate T cell proliferation could be restored by blocking antibodies for B7-H3.

The above evidence, although taken from small, single institution studies, suggests that the PD1/PDL1 pathway may play a role in immune escape in human NSCLC, even at an early stage. The potential to reverse the immunosuppression with blockade of PD1/PDL1 pathway provides rationale for studies of PD1/PDL1 blocking agents in the adjuvant setting.

Immunotherapy as adjuvant therapy in NSCLC

The concept of immunotherapy as an adjuvant treatment after resection of early NSCLC is not a new one. The Ludwig Lung Cancer Study Group investigated immune-stimulation with intrapleural Bacillus Calmette-Guérin (BCG) versus placebo as adjuvant therapy in early stage NSCLC more than 30 years ago (34). This non-specific immunotherapy which was administered once in early post-operative setting, resulted in an increased complications, mainly empyema, and inferior disease-free survival in the BCG group. Since that time, our understanding of cancer

immunology has increased tremendously (35) and has led to the development of more specific immunotherapies. Recent successes with specific immunotherapy strategies in castrate resistant prostate cancer (36) and melanoma (29) have renewed excitement in the potential immunotherapy to modify the clinical course of solid malignancies.

There are few cancer immunotherapies that have been assessed in the adjuvant setting in NSCLC. The two agents that have completed phase III clinical trials are both antigen specific (vaccine) strategies: tecemotide, previously known as L-BLP25, and MAGE-A3 ASCI.

Tecemotide is an antigen specific immunotherapy targeting the MUC1 glyco-peptide. A randomized phase IIB (37) trial with tecemotide versus observation showed a potential survival advantage in patients with stage III NSCLC. This led to the design of a global randomized placebo controlled phase III trial of tecemotide versus placebo in patients with stage III NSCLC after primary therapy with chemo-radiation (START). Hence, the tecemotide was given as adjuvant to the primary chemo-radiation. Patients with stage III NSCLC who achieved partial response or stable disease to the primary chemo-radiation were randomized to tecemotide or placebo until disease progression. Patients were stratified based on stage at initial presentation, response to primary chemo-radiation (stable disease versus partial response), region of the world, and mode of delivery of chemo-radiation (sequential versus concurrent). Despite challenges related to a clinical hold imposed on this START trial, more than 1,500 patients were randomized and 1,239 patients were included in the primary analysis (38). Tecemotide was well tolerated even when administered for prolonged periods. However, the primary end point of improvement in overall survival in the primary analysis population was not met. A preplanned subgroup analysis based on stratification variables did show, in the largest sub-group of patients treated with concurrent chemo-radiation (n=806), tecemotide adjuvant therapy resulted in a 10.2 months improvement in median survival (HR, 0.78; 95% CI: 0.64-0.95; P=0.016). Although this could not be considered a statistically significant result, the clinically significant difference in survival seen with tecemotide in a large sub-group of patients with stage III disease suggests a strong signal of efficacy.

MAGE-A3 gene is expressed in a number of cancers, including melanoma and NCLC. It is not expressed on normal tissues with the exception of testis and placenta and is considered a tumor specific antigen and ideal candidate for active immunotherapy. MAGE-A3 ASCI targets the MAGE-A3 tumor specific antigen. In a randomized phase II trial (39) in 182 patients with resected stage IB or II NSCLC, MAGE-A3 ASCI showed a strong trend for improved disease-free interval compared to observation. This trial was conducted prior to wide-spread of the use of adjuvant chemotherapy. An updated survival analysis of this phase II trial was recently published (40). Further follow up to 70 months continues to show a strong trend in favor of MAGE A3 ASCI in terms of DFI (HR 0.78; 95% CI: 0.49-1.24; P=0.248) although no difference was seen in overall survival (HR 0.99). A very large global double-blind placebo controlled phase III trial was initiated to test MAGE-A3 ASCI as adjuvant therapy in NSCLC. The MAGE-A3 as Adjuvant Non-Small Cell LunG CanceR Immuno Therapy (MAGRIT) trial is perhaps the largest adjuvant trial in NSCLC. More than 9,300 patients with stage I-IIIA NSCLC who had undergone surgical resection were screened for MAGE-A3 expression. Patients with tumors expressing MAGE-A3 were stratified based on whether they received adjuvant chemotherapy or not and then randomized to receive MAGE-A3 ASCI or placebo. The MAGRIT trial completed its target accrual of 2,270 patients in late 2011. The results of this trial are eagerly awaited.

Future directions

The limited success of adjuvant chemotherapy in early stage NSCLC is not surprising given the multiple mechanisms involved in tumor dormancy. Cytotoxic chemotherapy is likely to target those micro-metastases that are actively proliferating during the relatively brief time adjuvant therapy is administered. Although elimination of micro-metastases may be the optimal goal, maintaining them in a dormant state may prove an equally valuable strategy. The recent demonstration that very prolonged (10 years) adjuvant anti-estrogen therapy with tamoxifen is superior to shorter durations (5 years) would suggest that maintaining dormancy could be an effective adjuvant strategy (41). Future improvements in adjuvant therapy for NSLC will likely involve combination strategies that target different aspects of dormancy and can build on the gains made with adjuvant chemotherapy or be effective in patients for whom cytotoxic therapy is not an option.

The wide range of immunotherapies currently under investigation makes it difficult to generalize, but there are a number of features that that make immunotherapy particularly attractive in the adjuvant setting. Vaccine or

active specific immunotherapies are easy to administer, have a favorable toxicity profile and can thus be administered for prolonged periods. Immunotherapy has the potential to induce T cell memory and hence, the effect may persist long after the treatment is completed. Finally, contrary to what is popularly believed, immunotherapy may be synergistic when combined with chemotherapy (42) and targeted therapies (43).

The evidence currently available on the prognostic implications of tumor infiltrating immune cells is insufficient to support routine use. The tumor banks available for the large randomized adjuvant trials present an ideal opportunity to explore not only their prognostic value but also to help better understand the complex interactions between cancer and the immune system in early stage NSCLC. Such studies should focus not only on TILs and their location, but also the potential role of PD1/PDL1 in early stage NSCLC. This could help define whether a population suitable for a potential adjuvant trial with agents targeting this pathway might be identified. This is particularly relevant given the demonstration of clinical activity of checkpoint inhibitors such as anti-CTLA4 and anti-PD1/PDL1 anti-bodies. However, these are but a fraction of the receptors and ligands the have been identified as modulators of an anti-cancer immune response (30). It will be important to define which, if any, of these are relevant in a particular cancer.

The ultimate value of immunotherapies in the adjuvant setting will await demonstration of improved clinical outcomes in randomized trials. The recently completed START trial, while not meeting its primary end-point, shows a strong signal for improved survival with tecemotide as adjuvant therapy after concurrent chemo-radiation in stage III NSCLC. A confirmatory trial, focusing on patients completing concurrent chemo-radiation is being planned. The MAGRIT trial of MAGE-A3 ASCI in the adjuvant setting will define whether this immunotherapy can improve outcomes when given after adjuvant cytotoxic chemotherapy and/or in patients not suitable for adjuvant chemotherapy. This trial will also help define whether a GS might be used to predict patients likely to benefit from the MAGE-A3 ASCI.

Conclusions

The multitude of strategies used by tumors to circumvent immune recognition means that immunotherapy strategies aimed at enhancing one aspect of the immune response or overcoming one aspect of immune resistance are likely to meet with limited success. The availability of clinically active immunotherapies targeting different aspects of the immune response allow for the exploration of combinations of immune therapies. As an example, the activity seen with the checkpoint inhibitors is likely to be limited to patients in which a pre-existing anti-tumor immune response has occurred. By combining checkpoint inhibitors with other strategies such as ASCI, cytotoxic chemotherapy or certain targeted therapies that stimulate an anti-tumor immune response, their clinical activity might be enhanced. Although developing combination therapies presents many challenges, the opportunity to improve clinical outcomes is greatest with such strategies.

Acknowledgements

Disclosure: The author declares no conflict of interest.

References

1. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
2. Akslen LA, Naumov GN. Tumor dormancy--from basic mechanisms to clinical practice. APMIS 2008;116:545-7.
3. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer 2007;7:834-46.
4. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991-8.
5. Teng MW, Swann JB, Koebel CM, et al. Immune-mediated dormancy: an equilibrium with cancer. J Leukoc Biol 2008;84:988-93.
6. Zitvogel L, Apetoh L, Ghiringhelli F, et al. The anticancer immune response: indispensable for therapeutic success? J Clin Invest 2008;118:1991-2001.
7. Bhardwaj N. Harnessing the immune system to treat cancer. J Clin Invest 2007;117:1130-6.
8. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest 2007;117:1137-46.
9. Quesnel B. Tumor dormancy and immunoescape. APMIS 2008;116:685-94.
10. Talmadge JE, Donkor M, Scholar E. Inflammatory cell infiltration of tumors: Jekyll or Hyde. Cancer Metastasis Rev 2007;26:373-400.
11. House AK, Watt AG. Survival and the immune response

in patients with carcinoma of the colorectum. Gut 1979;20:868-74.
12. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960-4.
13. Johnson SK, Kerr KM, Chapman AD, et al. Immune cell infiltrates and prognosis in primary carcinoma of the lung. Lung Cancer 2000;27:27-35.
14. Wakabayashi O, Yamazaki K, Oizumi S, et al. CD4+ T cells in cancer stroma, not CD8+ T cells in cancer cell nests, are associated with favorable prognosis in human non-small cell lung cancers. Cancer Sci 2003;94:1003-9.
15. Bremnes RM, Al-Shibli K, Donnem T, et al. The role of tumor-infiltrating immune cells and chronic inflammation at the tumor site on cancer development, progression, and prognosis: emphasis on non-small cell lung cancer. J Thorac Oncol 2011;6:824-33.
16. Dai F, Liu L, Che G, et al. The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. BMC Cancer 2010;10:220.
17. Welsh TJ, Green RH, Richardson D, et al. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. J Clin Oncol 2005;23:8959-67.
18. Al-Shibli K, Al-Saad S, Donnem T, et al. The prognostic value of intraepithelial and stromal innate immune system cells in non-small cell lung carcinoma. Histopathology 2009;55:301-12.
19. Ohri CM, Shikotra A, Green RH, et al. Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. Eur Respir J 2009;33:118-26.
20. Ma J, Liu L, Che G, et al. The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time. BMC Cancer 2010;10:112.
21. Inoshima N, Nakanishi Y, Minami T, et al. The influence of dendritic cell infiltration and vascular endothelial growth factor expression on the prognosis of non-small cell lung cancer. Clin Cancer Res 2002;8:3480-6.
22. Gabrilovich DI, Chen HL, Girgis KR, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996;2:1096-103.
23. Al-Shibli KI, Donnem T, Al-Saad S, et al. Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. Clin Cancer Res 2008;14:5220-7.
24. Kawai O, Ishii G, Kubota K, et al. Predominant infiltration of macrophages and CD8(+) T Cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. Cancer 2008;113:1387-95.
25. Gottlin EB, Bentley RC, Campa MJ, et al. The Association of Intratumoral Germinal Centers with early-stage non-small cell lung cancer. J Thorac Oncol 2011;6:1687-90.
26. Dieu-Nosjean MC, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 2008;26:4410-7.
27. Ulloa-Montoya F, Louahed J, Dizier B, et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. J Clin Oncol 2013;31:2388-95.
28. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65.
29. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
30. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.
31. Konishi J, Yamazaki K, Azuma M, et al. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res 2004;10:5094-100.
32. Zhang Y, Huang S, Gong D, et al. Programmed death-1 upregulation is correlated with dysfunction of tumor-infiltrating CD8+ T lymphocytes in human non-small cell lung cancer. Cell Mol Immunol 2010;7:389-95.
33. Schneider T, Hoffmann H, Dienemann H, et al. Non-small cell lung cancer induces an immunosuppressive phenotype of dendritic cells in tumor microenvironment by upregulating B7-H3. J Thorac Oncol 2011;6:1162-8.
34. Immunostimulation with intrapleural BCG as adjuvant therapy in resected non-small cell lung cancer. The Ludwig Lung Cancer Study Group (LLCSG). Cancer 1986;58:2411-6.
35. Finn OJ. Cancer immunology. N Engl J Med 2008;358:2704-15.
36. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-22.
37. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol 2005;23:6674-81.

38. Butts CA, Socinski M, Mitchell P, et al. START: A phase III study of L-BLP25 cancer immunotherapy for unresectable stage III non-small cell lung cancer. J Clin Oncol 2013;31:abstr 7500.
39. Vansteenkiste J, Zielinski M, Linder A, et al. Final results of a multicenter, double-blind, placebo-controlled randomized phase II study to assess MAGE-A3 immunotherapeutic as adjuvant therapy in stage IB/II non-small cell lung cancer (NSCLC). J Clin Oncol 2007:25:abstr 7554.
40. Vansteenkiste J, Zielinski M, Linder A, et al. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. J Clin Oncol 2013;31:2396-403.
41. Gray RG, Rea D, Handley K, et al. aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. J Clin Oncol 2013;31:abstr 5.
42. Lake RA, Robinson BW. Immunotherapy and chemotherapy--a practical partnership. Nat Rev Cancer 2005;5:397-405.
43. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012;12:237-51.

Cite this article as: Butts CA. Anti-tumor immune response in early stage non small cell lung cancer (NSCLC): implications for adjuvant therapy. Transl Lung Cancer 2013;2(5):415-422. doi: 10.3978/j.issn.2218-6751.2013.10.09

Adjuvant molecularly targeted therapy—epidermal growth factor tyrosine kinase inhibition and beyond

Joel W. Neal[1], Lecia V. Sequist[2]

[1]Stanford Cancer Institute, Stanford University, USA; [2]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA
Correspondence to: Joel W. Neal, MD, PhD, Assistant Professor of Medicine (Oncology). Stanford Cancer Center, 875 Blake Wilbur Drive, CC 2220, Stanford, CA 94305-5826, USA. Email: jwneal@stanford.edu.

Submitted Jul 02, 2013. Accepted for publication Oct 12, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.06
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.06

Introduction

In stage IV non-small cell lung cancer (NSCLC), DNA molecular testing for mutations in epidermal growth factor receptor (*EGFR*) and gene rearrangements of anaplastic lymphoma kinase (*ALK*) has become the new standard of care. This is based on the unprecedented efficacy of small molecule EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib against *EGFR* mutant NSCLC (1,2), and the ALK TKI crizotinib against ALK positive NSCLC (3). While these highly active drugs should conceptually be effective as a component of the curative treatment of earlier stage NSCLC, the presently available evidence is minimal as pivotal studies are either underway or still in development. This article reviews current evidence about the use of adjuvant therapy for molecular targets in NSCLC, in particular regarding the use of EGFR TKIs in the treatment of early stage NSCLC harboring EGFR mutations (*Video 1*).

Video 1 Adjuvant molecularly targeted therapy—epidermal growth factor tyrosine kinase inhibition and beyond.

Retrospective studies regarding adjuvant gefitinib and erlotinib

In 2004, activating *EGFR* mutations were identified as a key biomarker of sensitivity to the EGFR-TKIs gefitinib and erlotinib (4-6). Based on the observed cytotoxicity of these agents upon cell lines, and the availability of EGFR TKIs by prescription for the treatment of stage IV NSCLC, some physicians began to use these drugs in patients with early stage disease. Memorial Sloan Kettering performed a retrospective analysis of 167 patients with stage I-III *EGFR* mutant NSCLC and compared a cohort of 56 patients who received neoadjuvant or adjuvant EGFR TKI to a separate cohort of 111 patients who did not receive TKI (7). In a multivariate analysis that adjusted for stage and treatment with adjuvant chemotherapy, patients who received an EGFR-TKI had a 2-year disease free survival (DFS) rate of 89%, as compared with 72% for patients not treated with TKI (P=0.06), suggesting possible benefit and supporting the need for prospective research. The 2-year overall survival was ≥90% in both groups, and was not statistically different.

In another retrospective study from Memorial, 22 patients who recurred after adjuvant EGFR TKI treatment were identified, of whom 11 were retreated with TKI and 8 responded for a median duration of 10 months (8). In this study, the resistance mutation T790M was only identified in tumors from patients who were either in the midst of adjuvant EGFR TKI therapy or less than 6 months from completion. This suggests that, similar to estrogen-receptor positive breast cancer treated with adjuvant tamoxifen (9),

longer duration of an active adjuvant therapy may potentially be beneficial, and that adjuvant TKI therapy may not be increasing cure rates, but may simply be delaying recurrences.

Adjuvant gefitinib

Over 10 years ago, two large randomized trials were designed to test EGFR TKIs in early stage NSCLC (not molecularly-selected patients)—one involving chemoradiation followed by gefitinib in stage III NSCLC (SWOG S0023), and the other with adjuvant gefitinib in stage I-III NSCLC. Unfortunately, in 2005, the large ISEL trial of second line gefitinib in unselected stage IV NSCLC failed to meet its overall survival endpoint, which prematurely disrupted enrollment in both early stage trials (10).

The phase III S0023 study enrolled a total of 243 patients with stage III NSCLC expected to receive concurrent chemotherapy and radiation and randomized them to outback gefitinib for up to 5 years or placebo (11). An unplanned interim analysis in 2005 at the time of the ISEL read-out demonstrated a signal of harm for gefitinib, with a median survival time of 23 months for patients receiving gefitinib, and 35 months for patients who received placebo (P=0.013). A subset analysis to look for potential benefit, or at least lack of harm, in patients with *EGFR* mutation-positive disease could not be retrospectively performed. Based on this study, EGFR-TKI therapy after combined chemoradiation is not recommended outside of a clinical trial.

In the phase III BR.19 study, patients with stage IB-IIIA NSCLC were randomized, following surgical resection and optional adjuvant chemotherapy, to 2 years of adjuvant gefitinib or equivalent placebo. Of a planned 1,160 patients, enrollment stopped at 503 in 2005 based on the negative ISEL trial and S0023 interim report. All patients were taken off of their assigned therapy. The analysis reported in 2010 demonstrated no difference between the groups, but a trend toward harm with gefitinib was observed for both disease free and overall survival (12). In the subgroup analysis of patients with *EGFR* mutant NSCLC, 40 patients treated with placebo had a numerically, but not significantly, improved overall survival compared with 36 patients who received adjuvant gefitinib. However, given the small numbers of patients and the shorter-than-planned 5 months median duration of adjuvant TKI, firm conclusions regarding the efficacy of adjuvant TKIs for *EGFR* mutant NSCLC should not be based on this trial.

At ASCO 2013, a relatively small Chinese trial was presented in which 60 patients with primarily resected stage IIIA-N2 NSCLC were treated with either 4 cycles of adjuvant carboplatin and pemetrexed, or the same chemotherapy followed by 6 months of gefitinib (13). Unlike the S0023 and BR.19 trials, no patients received radiation, and all patients had tumors with sensitizing *EGFR* mutations. An improvement was observed for the gefitinib arm versus the control arm for median DFS (39.8 *vs.* 27.0 mo, P=0.014, HR 0.37) and a trend toward improved median overall survival was noted (41.6 *vs.* 32.6 mo, P=0.066, HR 0.37). While this study is small, it does suggest benefit for an *EGFR* mutant population with adjuvant gefitinib treatment.

Adjuvant erlotinib—SELECT and RADIANT

Following FDA approval for erlotinib in the second line treatment of stage IV NSCLC based on the BR.21 trial (14), the potential efficacy of erlotinib in the adjuvant setting became an important question. The RADIANT trial is an ongoing phase III trial which targeted 945 patients with stage I-IIIA NSCLC whose tumors have EGFR protein expression by immunohistochemistry (IHC), or increased *EGFR* gene copy number by fluorescence in situ hybridization (FISH) (15). Following surgical resection and optional adjuvant chemotherapy, patients were randomized 2:1 to erlotinib for 2 years or placebo. The biomarker analysis presented early demonstrated an *EGFR* mutation positive rate of about 17%, suggesting that approximately 40 patients will be on the control arm and 80 on the erlotinib arm (16). The initial results of this trial, including a biomarker based outcome analysis, are expected soon.

During the time the large RADIANT trial became underway, increasing evidence suggested that *EGFR* mutation status was a bigger determinant of response to EGFR TKI than either EGFR protein expression or *EGFR* gene amplification. Therefore, we initiated a single arm phase II trial with 2 years of adjuvant erlotinib following surgery for stage I-IIIA surgically resected *EGFR* mutant NSCLC patients, dubbed the SELECT (Surgically resected *EGFR* mutant Lung cancer with adjuvant Erlotinib Cancer Treatment) trial (17). Following primary surgical resection, patients received standard-of-care adjuvant chemotherapy and/or radiation at the discretion of their treating physician, followed by adjuvant erlotinib at 150 mg po daily. A report at ASCO 2012 of the first 36 enrolled patients demonstrated that the majority were able to tolerate 2 years of adjuvant treatment, though some required dose reductions. Only two patients recurred before two years (one during adjuvant

erlotinib, and one following early discontinuation), for an observed 2-year DFS of 94%. An additional 10 patients recurred after 2 years and most still were responsive to subsequent EGFR TKI therapy. This trial was subsequently expanded to 100 patients total and has been completely enrolled, with results for the entire study population expected in another 1-2 years.

Future directions/conclusions

Based on the mixed results of existing trials, there are plans for larger phase III trial to further establish the magnitude of benefit, and potential risk, of adjuvant TKI in molecularly selected subgroups of patients. In the United States, the NCI and cooperative oncology groups are designing randomized placebo-controlled phase III trials that add adjuvant TKI to standard therapy regimens: one with adjuvant EGFR TKI in surgically resected *EGFR* mutant NSCLC (n=410), and another with adjuvant crizotinib in resected ALK-positive NSCLC (n=336) (18). Randomized trials that test substitution of EGFR-TKI with adjuvant chemotherapy are also ongoing in Asia, including the WJOG6410L trial in Japan (19) and the ADJUVANT trial in China (20), each of which are randomizing more than 200 patients between adjuvant cisplatin/vinorelbine and adjuvant gefitinib for stage II-III patients after resection. If these phase III trials show promise of efficacy, the next generation of TKIs, with their expanded spectrum of activity, may present additional opportunity for improvement. Furthermore, some fundamental biological questions, such as whether these agents are cytotoxic against micrometastatic disease or simply cytostatic, will require ongoing long-term follow up, and potentially investigating extended durations of treatment.

Acknowledgements

Neal—Support for clinical research (Genentech/OSI/Astellas, Boehringer Ingelheim). Sequist—Unpaid consulting for Boehringer-Ingelheim, Clovis Oncology, Merrimack Pharmaceuticals, AstraZeneca. Paid consulting for GSK. Support for clinical research (Genentech/OSI, Boehringer-Ingelheim).
Disclosure: The authors declare no conflict of interest.

References

1. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
2. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
3. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
4. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
5. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
6. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306-11.
7. Janjigian YY, Park BJ, Zakowski MF, et al. Impact on disease-free survival of adjuvant erlotinib or gefitinib in patients with resected lung adenocarcinomas that harbor EGFR mutations. J Thorac Oncol 2011;6:569-75.
8. Oxnard GR, Janjigian YY, Arcila ME, et al. Maintained sensitivity to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer recurring after adjuvant erlotinib or gefitinib. Clin Cancer Res 2011;17:6322-8.
9. Gray RG, Rea D, Handley K, et al. aTTom: long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. J Clin Oncol 2013;31:abstr 5.
10. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527-37.
11. Kelly K, Chansky K, Gaspar LE, et al. Phase III trial of maintenance gefitinib or placebo after concurrent chemoradiotherapy and docetaxel consolidation in inoperable stage III non-small-cell lung cancer: SWOG S0023. J Clin Oncol 2008;26:2450-6.
12. Goss GD, O'Callaghan C, Lorimer I, et al. Gefitinib Versus Placebo in Completely Resected Non-Small-Cell

Lung Cancer: Results of the NCIC CTG BR19 Study. J Clin Oncol 2013;31:3320-6.
13. Wang SY, Ou W, Li N, et al. Pemetrexed-carboplatin adjuvant chemotherapy with or without gefitinib in resected stage IIIA-N2 non-small cell lung cancer harbouring EGFR mutations: a randomized phase II study. J Clin Oncol 2013;31:abstr 7519.
14. Shepherd FA, Rodrigues Pereira J, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123-32.
15. A Study of Tarceva After Surgery With or Without Adjuvant Chemotherapy in Non-Small Cell Lung Carcinoma (NSCLC) Patients Who Have Epidermal Growth Factor Receptor (EGFR) Positive Tumors (RADIANT). NCT00373425.
16. Richardson F, Richardson K, Sennello G, et al. Biomarker analysis from completely resected NSCLC patients enrolled in an adjuvant erlotinib clinical trial (RADIANT). J Clin Oncol 2009;27:abstr 7520.
17. Neal JW, Pennell NA, Govindan R, et al. The SELECT study: a multicenter phase II trial of adjuvant erlotinib in resected epidermal growth factor receptor (EGFR) mutation-positive non-small cell lung cancer (NSCLC). J Clin Oncol 2012;30:abstr 7010.
18. Govindan R. Lung Cancer: Molecular Profiling and Genomics (Eductation Session Presentation). ASCO2013.
19. Tada H, Takeda K, Nakagawa K, et al. Vinorelbine plus cisplatin versus gefitinib in resected non-small cell lung cancer haboring activating EGFR mutation (WJOG6410L). J Clin Oncol 2012;30:TPS7110.
20. Gefitinib Versus Vinorelbine/Platinum as Adjuvant Treatment in Stage II-IIIA(N1-N2) NSCLC With EGFR Mutation (ADJUVANT). NCT01405079.

Cite this article as: Neal JW, Sequist LV. Adjuvant molecularly targeted therapy—epidermal growth factor tyrosine kinase inhibition and beyond. Transl Lung Cancer 2013;2(5):411-414. doi: 10.3978/j.issn.2218-6751.2013.10.06

Medical Oncology

The continuing role of chemotherapy for advanced non-small cell lung cancer in the targeted therapy era

Zarnie Lwin[1], Jonathan W. Riess[2], David Gandara[2]

[1]Royal Brisbane and Women's Hospital, Brisbane, Australia; [2]University of California Davis Cancer Center, Sacramento, California, USA
Correspondence to: Dr. Zarnie Lwin. Department of Medical Oncology, Royal Brisbane and Women's Hospital, Butterfield Street, Herston, Brisbane 4006, Australia. Email: Zarnie_Lwin@health.qld.gov.au.

Abstract: There have been remarkable advances in the targeted treatment of advanced non-small cell lung cancer (NSCLC) over the past several years. Survival outcomes are steadily improving as management paradigms shift in the diagnosis and treatment of advanced NSCLC. Customizing treatment based on histology and molecular typing has become a standard of care in this era of targeted therapy. While new chemotherapeutic agents have proven effective, the pivotal role of platinum-based chemotherapy doublets has been confirmed. Maintenance chemotherapy has become an option, but who to employ it in remains unclear in the real-world setting. Efforts to overcome resistance to targeted agents are ongoing utilizing combination regimens of chemotherapy plus targeted agents, but optimizing combination strategies needs further exploration. This review highlights recent developments in novel chemotherapeutics and in chemotherapy strategies over the past two years. Despite advances in molecular medicine, there remains an essential role for chemotherapy in advanced NSCLC, even in the recent targeted therapy era.

Keywords: Advanced non-small cell lung cancer (NSCLC); recent developments; chemotherapy strategies; targeted therapy

Submitted Aug 05, 2013. Accepted for publication Aug 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.47
View this article at: http://dx.doi.org/doi: 10.3978/j.issn.2072-1439.2013.08.47

The continuing role of chemotherapy for advanced non-small cell lung cancer in the recent targeted therapy era

Despite remarkable advances in the targeted treatment of non-small cell lung cancer (NSCLC) over the past several years, chemotherapy remains of paramount importance in the treatment of advanced NSCLC. Even in patients whose tumors contain *EGFR* activating mutations or *ALK* gene rearrangements and are treated with first line tyrosine kinase inhibitors, resistance invariably develops, with chemotherapy remaining the cornerstone of subsequent therapy. In profiling mutations of 1,000 metastatic lung adenocarcinoma patients, although the Lung Cancer Mutation Consortium was able to identify actionable mutations, including molecular aberrations linked to approved drugs and clinical trials in 54% of cases (1), in only a small minority, about 14-18% in Western populations, are there approved targeted drugs (EGFR and ALK TKIs) with which to treat them. As of yet, no drugs targeting oncogenic-driver pathways have been approved in squamous cell lung cancers, though clinical trials are ongoing. With the majority of advanced lung cancer patients not harboring actionable driver mutations with paired targeted agents that effectively improve outcomes, advancing chemotherapy regimens through rational drug combinations and discovery of new potent chemotherapeutics remains critical. This review highlights advances in chemotherapy of advanced NSCLC over the past two years.

Continuing central role of platinum compounds in first line chemotherapy of advanced stage NSCLC

Although recently implemented treatment guidelines recommend that patients with advanced stage NSCLC

whose tumors harbor *EGFR* activating mutations or *ALK* gene rearrangements be treated first line with erlotinib or crizotinib, respectively, it is with the realization that there is no overall survival benefit to patients with *EGFR* mutated cancers whether they receive an EGFR TKI first line or second line. This TKI-first recommendation is true even in patients with tumor-related poor performance status (2). For 'fit' patients who do not have an oncogene-driven cancer, platinum doublet chemotherapy (with consideration of bevacizumab in non-squamous histology patients) remains the cornerstone of treatment. In an attempt to preserve efficacy and minimize toxicity, platinum-free combinations of newer agents have been tested against conventional platinum-based combinations. Although a recent meta-analysis of 16 randomized trials found that the efficacy was comparable between non-platinum doublets of third-generation agents and platinum-based doublets for pooled overall survival (HR =1.03, 95% CI: 0.98-1.08, P=0.290) (3), all evidence based guidelines support platinum-based therapy as standard of care. Subgroup analyses by different non-platinum doublet protocols revealed that none of the four non-platinum doublets achieved a different survival when compared with platinum-based doublets. The pooled progression-free survival showed that platinum-based doublets may have an advantage over non-platinum doublets (HR =1.06, 95% CI: 1.01-1.12, P=0.03). In this study, a meta-analysis of toxicity could not be performed.

In an attempt to show that platinum compounds were non-essential, a recent Phase III trial in advanced stage NSCLC with performance status 2 randomized patients to receive pemetrexed with or without carboplatin. All efficacy parameters favored the carboplatin-pemetrexed combination over pemetrexed alone: response rate 23.8% *vs.* 10.3%, PFS 5.8 *vs.* 2.8 months, and OS 9.3 *vs.* 5.3 months (4). Clearly, the weight of evidence in all categories of advanced NSCLC without *EGFR* mutation or *ALK* fusion favors platinum-based doublet therapy.

Biomarkers to select platinum and non-platinum chemotherapy

Utilizing DNA repair enzymes as biomarkers for better selecting front-line chemotherapy is an area of active investigation. Low ERCC1 expression by either IHC or RT-PCR has been shown in preliminary studies to be a potential biomarker of benefit to platinum compounds and low RRM1 a potential biomarker of benefit to gemcitabine. The ERCC1 enzyme removes platinum-induced DNA adducts, and thus low ERCC1 levels are associated with platinum sensitivity (5). RRM1 is a subunit of ribonucleotide reductase which is the main target of gemcitabine; thus, low RRM1 levels are associated with gemcitabine sensitivity (6). In the recently published phase III TASTE trial in metastatic NSCLC, patients were randomly assigned 2:1 to the experimental arms: (I) gemcitabine/carboplatin if RRM1 and ERCC1 were low; (II) docetaxel/carboplatin if RRM1 was high and ERCC1 was low; (III) gemcitabine/docetaxel if RRM1 was low and ERCC1 was high; and (IV) docetaxel/vinorelbine if both were high (7). Control arm patients received gemcitabine/carboplatin. There were no statistical differences for progression-free survival or overall survival. The authors note they required real-time processing of tumor specimens for ERCC1, RRM1 and *in situ* protein levels. Therefore day-to-day variations in the reagent assay reliability and processing procedures may have affected the reliability and reproducibility of these assays. A recent attempt to validate ERCC1 by IHC as a prognostic marker to platinum based chemotherapy in the adjuvant setting failed as the same antibody to ERCC1 (but a different batch) could not detect the functional ERCC1 isoform (8).

Thymidylate synthase (TS), the *de novo* source of thymidylate synthesis, is an essential enzyme for DNA replication and cell growth and one of the primary targets of pemetrexed. Pemetrexed has a potential histology-specific benefit which may be related to higher levels of TS expression in squamous histology of the lung compared to adenocarcinoma with overexpression of TS is related to a reduced sensitivity to pemetrexed (9). *In vitro* studies have correlated differential expression of TS and pemetrexed sensitivity (10). In an analysis of the largest data set for gene expression of biomarkers reported to date, significant histology-related associations for ERCC1, RRM1, and TS were seen, warranting randomized phase III trials assessing the predictive value of these chemotherapy-related biomarkers (11).

Another biomarker that may assist in chemotherapy selection is SPARC (secreted protein acidic and rich in cysteine), a matricellular glycoprotein that is produced by tumor and/or neighboring stroma. SPARC expression is thought to facilitate the intracellular accumulation of nanoparticle albumin-bound paclitaxel (nab-paclitaxel) (12). Multiple issues in assay development, standardization, tissue processing and antibody reliability have affected the potential utility of these biomarkers to better select rationale chemotherapy combinations in advanced NSCLC. Further development of these predictive biomarkers is of interest to

convert chemotherapy into targeted chemotherapy.

Pemetrexed first line therapy for non-squamous histology

Pemetrexed is a multi-targeted anti-folate employed: with platinum derivates for first-line treatment, as single agent for subsequent lines of treatment, and as maintenance therapy. In the landmark JMDB trial, Scagliotti *et al*. demonstrated no difference in overall survival between cisplatin/gemcitabine and cisplatin/pemetrexed as first-line treatment of patients with metastatic NSCLC. However, in a preplanned subset analysis the cisplatin-pemetrexed combination was superior in non-squamous histology with a median overall survival of 12.6 months in the cisplatin-pemetrexed arm and 10.9 months in the cisplatin-gemcitabine arm (HR =0.84; 95% CI: 0.71-0.99; P=0.03) (13). By contrast, patients with squamous carcinoma had a worse median overall survival in the cisplatin-pemetrexed arm than in the cisplatin-gemcitabine arm (9.4 *vs*. 10.8 months; HR =1.23; 95% CI: 1.0-1.5; P=0.05).

In a more recent study the Norwegian Lung Cancer Study Group enrolled 436 patients to compare health-related quality of life (HRQoL) between carboplatin-pemetrexed and carboplatin-gemcitabine as first-line treatments for advanced NSCLC. The two regimens achieved similar results in terms of HRQoL and overall survival (7.3 months for carboplatin-pemetrexed *vs*. 7.0 months for carboplatin-gemcitabine; P=0.63) (14). Multivariate analyses and interaction tests did not reveal any significant associations between specific histology and survival. Carboplatin-pemetrexed combination was not superior in non-squamous histology, in contrast to the JMDB trial. In another randomized phase III trial carboplatin-pemetrexed achieved a longer median survival without toxicity when compared to carboplatin-docetaxel in advanced non-squamous NSCLC (3.2 *vs*. 0.7 months; HR =0.45; 95% CI: 0.34-0.61). The primary end-point of survival without toxicity was defined as the interval from randomization to the first treatment-induced grade 3-4 adverse event (15). In a meta-analysis published in 2012, Li and colleagues evaluated a selection of clinical trials in which platinum-based combinations including pemetrexed were compared with platinum-based combinations including other third- generation agents for first-line treatment. A consistent survival advantage with pemetrexed was observed especially in non-squamous NSCLC (which represented the majority of the patients) (16). A meta-analysis of five trials (three first-line trials, one second-line trial, one maintenance trial) confirmed that pemetrexed, when compared with alternative treatments or placebo, is consistently associated with a significant overall survival improvement in non-squamous histology (HR =0.82) but not in squamous histology (HR =1.19) (17).

Combining chemotherapy with targeted agents

The diagnosis and management paradigm of metastatic NSCLC has transitioned into an algorithm of presence or absence of oncogene addiction as a key branch point to selecting appropriate treatment. As described above, with the identification of driver mutations such as *EGFR* and *ALK*, EGFR-TKIs and crizotinib are supplanting traditional chemotherapy for upfront treatment of these patients (18). However, initial TKI responders inevitably relapse due to acquired resistance. More recently, an added layer of complexity related to intrapatient tumor heterogeneity has been observed, particularly relevant to the clonal evolution of somatic mutations from the primary tumor to metastatic lesions and the mixed response to treatment in different tumor sites (2). At the same time, chemotherapy combinations have reached a therapeutic plateau for metastatic disease (19). Therefore, an area of focus has therefore been on interrogating the combination of novel targeted agents together with chemotherapy to optimize efficacy, survival and overcome acquired resistance. Early studies done combining EGFR-inhibitors with concurrent chemotherapy in unselected populations did not confer a survival advantage (20).

Given the lack of benefit seen in combining concurrent chemotherapy and EGFR TKIs in an unselected patient population, efforts to best integrate chemotherapy and TKI regimens are ongoing. One such approach is intercalating a TKI with chemotherapy based on the preclinical rationale that EGFR TKIs cause G1 cell-cycle arrest thus inhibiting cell-cycle dependent cytotoxic effects of chemotherapy (21). Because the mechanism of action of EGFR-TKIs has the theoretical potential to interfere with or even negate the effects of chemotherapy, it has been hypothesized that sequential or intermittent schedules to confer pharmacodynamic separation may confer better benefit (18).

Table 1 lists recent phase III trial results combining chemotherapy with a targeted agent or novel small molecule inhibitors for within the past two years. The treatment algorithms include single-target agents, multi-target agents, concurrently, intercalated with chemotherapy and as maintenance.

Table 1 Recent Phase III Trials combining chemotherapy and targeted agents in the past two years.

Targeted agent	Trial design and chemo partner	Sequencing of targeted agent	N	Outcome
EGFR inhibitors Erlotinib FAST-ACT2 (22)	First line, unselected platinum/ gemcitabine + erlotinib or placebo on days 15-28 f/by erlotinib or placebo	Intercalated + maintenance	451	PFS =7.6 *vs*. 6.0 months, HR =0.57 (0.47-0.69) P<0.0001; OS =18.3 *vs*. 15.2 months, HR =0.79 (0.64-0.99) P=0.0420; intercalated erlotinib *vs*. placebo
Cetuximab SELECT (23)	Second line, squamous Pem/cetux *vs*. Pem *vs*. docetaxel/cetux *vs*. docetaxel f/by cetux in cetux arms	Concurrent + maintenance	Pem =605; Docetaxel =333	PFS =2.70 *vs*. 2.27 months, HR =0.93 (0.81-1.08) P=0.305; OS =6.74 *vs*. 7.85 months HR 1.05, (0.91-1.2) P=0.47; cetux + chemo *vs*. chemo
VEGF Bevacizumab PRONOUNCE (24)	First line, non squamous PemC *vs*. PCB f/by Pem (PemC Arm) or Bev (PCBArm)	Concurrent + maintenance	361	PFS =4.4 *vs*. 5.5months, HR =1.06 (0.84-1.35) P=0.610; OS =10.5 *vs*. 11.7 months HR =1.07 (0.83-1.36) P=0.616; PemC *vs*. PCB
Bevacizumab PointBreak (25)	First line, non squamous PemCB f/by Pem+Bev *vs*. PCB f/by Bev	Concurrent + maintenance	939	PFS =6 *vs*. 5.6 months, HR =0.83 (0.71-0.9) P=0.012; OS =12.6 *vs*. 13.4 months HR =1.00 (0.86-1.16) P=0.943; PemCB *vs*. PCB
Cediranib NCIC BR29 (26)	First line, unselected Carbo/taxol + CED or placebo f/by CED or placebo	Concurrent + maintenance	306	PFS= 5.5 *vs*. 5.5 months, HR =0.91 (0.71-1.18); OS =12.2 *vs*. 12.1 months, HR =0.95 (0.69-1.30) P=0.74; CED *vs*. placebo
Sorafenib NExUS (27)	First line, Non squamous cis/gem plus sorafenib or placebo f/by sorafenib or placebo	Concurrent + maintenance	772	PFS = 6.0 *vs*. 5.5 months, HR =0.83 (0.71-0.97) P=0.008; OS = 12.4 *vs*. 12.5 months, HR =0.98 (0.83-1.16) P=0.40; sorafenib *vs*. placebo
Aflibercept VITAL (28)	Second line, non-squamous Docetaxel + Aflibercept or placebo	Concurrent	913	PFS =5.2 *vs*. 4.1 months, HR =0.82 (0.72-0.94) P=0.820; OS =10.1 *vs*. 10.4 months, HR =1.01 (0.87-1.17) P=0.9; aflibercept *vs*. placebo
Multitargeted agents Nintedanib (VEGFR, FGFR,PDGFR inhibitor) LUME-lung2 (29)	Second line, non-squamous PEM + Nintedanib or Placebo	Concurrent	713	PFS =4.4 *vs*. 3.6 months, HR =0.83 (0.7-0.99) P=0.04; OS = No difference in OS, HR =1.03; nintedanib *vs*. placebo
Nintedanib LUME-lung1 (30)	Second line Docetaxel + Nintedanib or Placebo	Concurrent	1,314	PFS =3.4 *vs*. 2.7 months, HR =0.79 (0.68-0.92) P=0.0019; OS in all pts 10.1 *vs*. 9.1 months HR =0.94 P=0.272; nintedanib *vs*. placebo
Vandetanib (31) (VEGF, EGFR inhibitor)	Second line PEM + Vandetanib or Placebo	Concurrent	534	PFS =17.6 *vs*. 11.9 weeks, HR =0.86 (0.69 to 1.06) P=0.108; OS =10.5 *vs*. 9.2 months, HR, 0.86 (0.65-1.13) P=0.219; vandetanib *vs*. placebo

Pem/cetux, Pemetrexed/cetuximab; Pem, Pemetrexed; docetaxel/cetux, docetaxel/cetuximab; PemCB, Pem/Carbo/Bev; CED, Cedirinib; PCB, Paclitaxel/ Carboplatin/Bevacizumab; PemC, Pemetrexed/Carboplatin; Carbo/taxol, Carboplatin/paclitaxel.

The recently published FASTACT-2 study shows that intercalating erlotinib and chemotherapy yields improved progression-free survival and overall survival in East Asian patients enriched for EGFR-activating mutations. However, progression-free survival and overall survival were not significantly different in EGFR wild-types groups (22). Treatment benefit was noted only in patients whose tumors harbored an EGFR activating mutation (median progression-free survival 16.8 *vs*. 6.9 months, HR =0.25; P<0.0001; median overall survival 31.4 *vs*. 20.6 months, HR =0.48; P=0.0092).

The anti-VEGF monoclonal antibody Bevacizumab has a demonstrated overall survival benefit in combination with carboplatin and paclitaxel in a phase III trial and this combination can be considered an option in treating nonsquamous NSCLC. However, since the year 2000, over 11 other phase III trials have been negative to date for an overall survival benefit when combining bevacizumab or other anti-angiogenic agents to platinum based chemotherapies. One important issue in employing anti-angiogenesis therapy is absence of a predictive marker for therapeutic benefit. Differences in progression-free survival *vs*. overall survival benefits may also be confounded by effect of further therapies, given the existence of a variety of moderately active agents now available for second and third line treatments.

In the recent PRONOUCE study the primary objective was to compare progression-free survival without Grade 4 toxicity (G4PFS) between a two drug regimen (Pem/Carbo) *vs*. three (Pac/Carbo + Bev) in a phase III superiority trial (24). The rationale for this trial design can be questioned. Nevertheless, study outcomes were negative. In the PointBreak trial patients were randomized to carboplatin/paclitaxel/bevacizumab followed by bevacizumab maintenance and compared to carboplatin/pemetrexed/bevacizumab followed by pemetrexed/bevacizumab maintenance (25). There was no overall survival advantage inclusive of all age subgroups. In fact, OS was numerically in favor of paclitaxel.

Nintedanib is a novel multitargeted oral inhibitor of VEGFR, FGFR, and PDGFR, which showed improved progression-free survival when combined with chemotherapy (29,30). Other exploratory avenues showing early signals in combination with chemotherapy include combining immunotherapies such as Ipilumumab or PD-L1 or chaperone proteins such as Hsp90 inhibitor Ganetespib (32-34).

In summary, optimal methods for combing chemotherapy and targeted therapies remain unclear. In addition, these trials emphasize that patient selection factors may dictate outcomes independent of the therapies being evaluated.

Maintenance therapy in advanced NSCLC

Maintenance therapy strategies that improve patient outcomes are an area of active investigation in NSCLC. Both continuation and switch maintenance approaches have been actively studied. Continuation maintenance strategies hope to suppress tumor growth beyond the time of 4 cycles of standard front-line chemotherapy. Alternatively, switch maintenance strategies hope to delay resistance to treatment by incorporating a new chemotherapeutic agent with a different mechanism of action. Ultimately, the goal of maintenance therapy is not just enhance progression-free survival, but to prolong overall survival without decreasing QoL.

The most prominent recently published study of maintenance chemotherapy is PARAMOUNT. In this large, phase III trial patients with non-squamous NSCLC were randomized to pemetrexed or placebo plus best supportive care after induction with 4 cycles of cisplatin/pemetrexed. Both progression-free (HR =0.62, P<0.0001) and overall survival (HR =0.78, P=0.019) were significantly prolonged with continuation maintenance pemetrexed (35,36). Discontinuation of maintenance pemetrexed due to toxicity was low (5%). A comparable number of patients in both treatment arms received post-discontinuation therapy (64% of patients treated with placebo and 58% of patients treated with pemetrexed maintenance). However, maintenance therapy is expensive. A recent Chinese cost-effectiveness analysis estimated cost per quality adjusted life year of maintenance pemetrexed in the Chinese health care system to be between $125,000 and $180,000 (37). Furthermore, it remains unclear in non-squamous patients whether close follow up with timely second line therapy or re-initiation of pemetrexed upon progression would have comparable efficacy to pemetrexed maintenance, particularly in patients who initially benefit from a first-line platinum/pemetrexed doublet, and then are observed without maintenance. Lastly, there is considerable debate as to whether 4 cycles of induction chemotherapy is an adequate point for consideration of maintenance, or whether the 2 months increase in median PFS could be achieved with further induction therapy.

In another pemetrexed maintenance trial (JMEN) that used a switch maintenance strategy, overall survival was improved and patients' QoL was similar compared to placebo except for a slight decrease in appetite and delayed

worsening of hemoptysis and pain (38). In particular, the results of this trial are confounded by a very low rate of second line crossover to pemetrexed in the placebo arm, making real world interpretation difficult. Other trials employing maintenance with gemcitabine and docetaxel after frontline chemotherapy did not show any overall survival benefit when compared to initiating treatment after progression of disease (39,40). A criticism of many maintenance trials is the high percentage of patients randomized to the best-supportive care only arm failing to receive second-line therapy upon progression. Subset analyses of some maintenance treatment trials suggest that patients with stable disease may benefit more from a maintenance strategy, rather than those who respond. Though hypothesis generating, the rationale is sound: patients who do not have a response may progress quicker and would typically receive early second line agents. Thus, regardless of terminology, a switch to docetaxel or gemcitabine could be considered second line therapy instead of maintenance therapy, particularly in squamous histology patients with good functional status who do not have a response to frontline therapy.

New chemotherapeutics

Albumin-bound paclitaxel

Taxanes have been a backbone of NSCLC therapy for well over a decade. 130-nm albumin bound paclitaxel (nab-paclitaxel) differs from standard bound paclitaxel (sb-paclitaxel) by being preferentially taken up into cancer cells via caveolae mediated transcytosis. The proposed mechanism involves enhanced drug delivery to tumor by albumin binding to SPARC (secreted protein, acidic and rich in cysteine), which is preferentially expressed on tumor cells compared to normal tissue (41). It also lacks the cremophor vehicle present in standard bound paclitaxel that can trigger allergic reactions. Nab-paclitaxel was studied in combination with carboplatin and compared to sb-paclitaxel plus carboplatin as first-line therapy of metastatic NSCLC in a large, randomized phase III trial. This trial met its primary endpoint of increased response rate for the carboplatin and nab-paclitaxel combination (33% *vs.* 25%, P=0.005) (42). The largest gains in response rates were noted in squamous cell histology patients (41% *vs.* 24%) and no increase in ORR was seen in non-squamous histology. There also was less grade ≥3 neuropathy compared to the sb-paclitaxel combination. However, no significant improvement in overall or progression free survival was noted. In a subset analysis, patients from North America and age ≥70 had significantly improved overall survival with nab-paclitaxel, however this subset analysis should be considered hypothesis generating only. Nab-paclitaxel is clearly a suitable substitute for sb-paclitaxel when allergy to the cremophor vehicle is present or in patients with baseline neuropathy. In addition, nab-paclitaxel could be considered preferential in those with squamous histology when a response is needed, where a subset analysis showed a higher difference in response rates. This rationale is also supported by the realization that new treatment options for NSCLC patients with squamous histology lag far behind those for lung adenocarcinoma.

Cabazitaxel

Cabazitaxel is another taxane currently being studied in a phase II trial in advanced NSCLC (NCT01438307). Recent data in metastatic prostate cancer that showed a significant overall survival benefit underlies the merit of its evaluation in NSCLC (43). Trial results with cabazitaxel in metastatic NSCLC are not yet mature.

Vintafolide (EC145): a folate-vinca alkaloid conjugate

Vinca alkaloids have documented activity in NSCLC, but have largely been supplanted by taxanes and pemetrexed for first or second line systemic treatment of NSCLC. Vintafolide is a conjugate folate molecule linked to vinblastine. Over 75% of NSCLC is folate receptor positive (by immunohistochemistry), offering the potential of folate receptor-targeted therapy. In a recent phase II trial, companion imaging of the folate receptor via ^{99}Tc-EC20 CT scans was used to select patients with folate receptor expressing tumors for treatment with vintafolide. Thus, EC20 uptake is under development as a potential predictive biomarker to vintafolide. In a phase II trial of heavily pretreated relapsed/refractory NSCLC patients with positive EC20 scans, clinical benefit (stable disease + overall response rate) was seen in 26% of patients (44). Currently vintafolide is being studied in combination with docetaxel in a randomized phase II trial of relapsed/refractory NSCLC patients (NCT01577654).

Eribulin mesylate

Eribulin mesylate is a synthetic analogue of halichondron B

Table 2 Newly studied chemotherapeutics in metastatic NSCLC

Drug	Trial design	Clinical setting	Outcome
Nab-paclitaxel	Randomized, phase III (with carboplatin) compared to carboplatin sb-paclitaxel	1st line	ORR 33% *vs*. 25% (P=0.005); median PFS 6.3 *vs*. 5.8 mo. (P=0.214); median OS 12.1 *vs*. 11.2 (P=0.27)
Vintafolide	Phase II, relapsed/refractory NSCLC with companion EC20 Scans	Beyond 2nd line	Clinical benefit (CB) =31%; CB in patients with EC20+ imaging 50%
Eribulin	Single agent phase II	2nd and 3rd line	ORR =5%; SD =24%
Ixabepilone	Randomized, phase II (with carboplatin) vs. carboplatin/ixabepilone	1st Line	PFS; HR 1.04 (0.78-1.41)
Pralatrexate	Randomized, phase II (compared to erlotinib)	2nd and 3rd line	OS HR 0.84 (95% CI: 0.61-1.14); Non-sq NSCLC; OS HR 0.65 (0.42-1.0)

isolated from a rare marine sponge. It inhibits microtubule dynamics using a distinct mechanism from taxanes or vinca alkaloids. It was recently approved for breast cancer based on a trial showing improved overall survival in heavily pre-treated metastatic breast cancer patients who previously received an anthracycline and a taxane (45). In a phase II trial in NSCLC patients who had previously received a taxane, response rates were low (5%), but 50% of patients achieved stable disease (46). Eribulin is currently being studied in combination with erlotinib (NCT01104155), pemetrexed (NCT01126736) or physicians choice of control drug (NCT01454934) in 3 separate clinical trials.

Ixabepilone

Ixabepilone is an epithilone (a novel anti-microtubule class of agent) that similar to taxanes binds and stabilizes microtubules, eventually resulting in G2/M cell-cycle arrest. Some preclinical studies show it is active in taxane-resistant models and ixabepilone is approved for treatment of metastatic breast cancer. In a randomized phase II trial in NSCLC, it did not improve overall survival or achieve any other clinically meaningful endpoint (47). The investigators stratified patients based on beta-3 tubulin immunohistochemistry and showed it to be a negative prognostic indicator, but not a predictive marker of benefit to ixabepilone. As there is no clear signal of superiority compared to paclitaxel, the future development of ixabepilone in advanced NSCLC treatment is unclear.

Pralatrexate

Pralatrexate, a folate analogue targeting dihydrofolate reductase, was recently studied in a randomized phase II trial compared to erlotinib in metastatic NSCLC patients who progressed on first-line therapy. A trend towards increased overall survival was observed and an increase in progression free survival was noted (48). In this study 18 of 100 patients treated with pralatrexate had prior pemetrexed. There was a high rate of mucositis with pralatrexate despite B12 and folic acid supplementation. As pemetrexed is increasingly being incorporated into upfront treatment regimens of non-squamous NSCLC and the toxicity of pralatrexate appears higher, the role of additional anti-folate therapies is unclear.

Summary of new chemotherapeutic agents

Multiple new chemotherapeutic agents are currently in clinical development or have been recently evaluated in NSCLC (*Table 2*).

Several of these drugs are from similar drug classes to those already shown to be active in NSCLC (cabazitaxel, pralatrexate) while others have been reformulated to preferentially target tumor cells (albumin-bound paclitaxel, vintafolide). Ixabepilone and eribulin affect microtubule dynamics through distinct mechanisms of action compared to taxanes. None of the clinical trials to date with these drugs suggest dramatic benefits in advanced NSCLC patients, but some of these new agents may have a role in specific treatment settings, as per nab-paclitaxel discussed above.

Discussion

Chemotherapy remains the indispensible choice for the vast majority of patients with advanced NSCLC, given the relative rarity of currently defined and treatable oncogene-

driven patient subsets. Several new chemotherapeutic agents for NSCLC are in clinical development, though their actual role in the current treatment paradigm is yet to be determined. As we seek to rank, order and rationally combine existing chemotherapies to achieve optimal patient outcomes, some promising results have emerged. Switch or continuation maintenance strategies are of benefit, but defining exactly who to treat remains problematic, as the trial designs may not have always reflected real-world considerations. Several aspects of maintenance therapy need further examination including the optimal number of induction chemotherapy cycles, the role of treatment-free intervals, QoL, economic considerations, and whether progression-free survival is a worthy therapeutic goal in this disease setting (49). Platinum based cytotoxic chemotherapy has been the backbone of treatment for metastatic NSCLC for decades and non-platinum combinations have not shown superiority. Attempts to employ biomarkers of DNA repair or other biomarkers for chemotherapy have been hindered by methodological issues to date. Optimal strategies for integrating chemotherapy and targeted therapeutics are an area of active investigation with promising results.

Despite the remarkable advances in the targeted treatment of NSCLC in the past several years, chemotherapy remains of paramount importance in the treatment of advanced NSCLC.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Kris MG, Johnson BE, Kwiatkowski DJ, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI's Lung Cancer Mutation Consortium (LCMC). J Clin Oncol 2011;29:abstr CRA7506.
2. Li T, Kung HJ, Mack PC, et al. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. J Clin Oncol 2013;31:1039-49.
3. Jiang J, Liang X, Zhou X, et al. Non-platinum doublets were as effective as platinum-based doublets for chemotherapy-naïve advanced non-small-cell lung cancer in the era of third-generation agents. J Cancer Res Clin Oncol 2013;139:25-38.
4. Zukin M, Barrios CH, Rodrigues Pereira J, et al. Randomized Phase III Trial of Single-Agent Pemetrexed Versus Carboplatin and Pemetrexed in Patients With Advanced Non-Small-Cell Lung Cancer and Eastern Cooperative Oncology Group Performance Status of 2. J Clin Oncol 2013;31:2849-53.
5. Altaha R, Liang X, Yu JJ, et al. Excision repair cross complementing-group 1: gene expression and platinum resistance. Int J Mol Med 2004;14:959-70.
6. Bepler G, Kusmartseva I, Sharma S, et al. RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. J Clin Oncol 2006;24:4731-7.
7. Bepler G, Williams C, Schell MJ, et al. Randomized International Phase III Trial of ERCC1 and RRM1 Expression-Based Chemotherapy Versus Gemcitabine/Carboplatin in Advanced Non-Small-Cell Lung Cancer. J Clin Oncol 2013;31:2404-12.
8. Friboulet L, Olaussen KA, Pignon JP, et al. ERCC1 isoform expression and DNA repair in non-small-cell lung cancer. N Engl J Med 2013;368:1101-10.
9. Sigmond J, Backus HH, Wouters D, et al. Induction of resistance to the multitargeted antifolate Pemetrexed (ALIMTA) in WiDr human colon cancer cells is associated with thymidylate synthase overexpression. Biochem Pharmacol 2003;66:431-8.
10. Giovannetti E, Mey V, Nannizzi S, et al. Cellular and pharmacogenetics foundation of synergistic interaction of pemetrexed and gemcitabine in human non-small-cell lung cancer cells. Mol Pharmacol 2005;68:110-8.
11. Maus MK, Mack PC, Astrow SH, et al. Histology-related associations of ERCC1, RRM1, and TS biomarkers in patients with non-small-cell lung cancer: implications for therapy. J Thorac Oncol 2013;8:582-6.
12. Shao H, Tang H, Salavaggione OE, et al. Improved response to nab-paclitaxel compared with cremophor-solubilized paclitaxel is independent of secreted protein acidic and rich in cysteine expression in non-small cell lung cancer. J Thorac Oncol 2011;6:998-1005.
13. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26:3543-51.
14. Grønberg BH, Bremnes RM, Fløtten O, et al. Phase III study by the Norwegian lung cancer study group: pemetrexed plus carboplatin compared with gemcitabine plus carboplatin as first-line chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol 2009;27:3217-24.
15. Rodrigues-Pereira J, Kim JH, Magallanes M, et al.

A randomized phase 3 trial comparing pemetrexed/carboplatin and docetaxel/carboplatin as first-line treatment for advanced, nonsquamous non-small cell lung cancer. J Thorac Oncol 2011;6:1907-14.
16. Li M, Zhang Q, Fu P, et al. Pemetrexed plus platinum as the first-line treatment option for advanced non-small cell lung cancer: a meta-analysis of randomized controlled trials. PLoS One 2012;7:e37229.
17. Al-Saleh K, Quinton C, Ellis PM. Role of pemetrexed in advanced non-small-cell lung cancer: meta-analysis of randomized controlled trials, with histology subgroup analysis. Curr Oncol 2012;19:e9-e15.
18. Pennell NA. Integration of EGFR inhibitors and conventional chemotherapy in the treatment of non-small-cell lung cancer. Clin Lung Cancer 2011;12:350-9.
19. Belani CP, Goss G, Blumenschein G Jr. Recent clinical developments and rationale for combining targeted agents in non-small cell lung cancer (NSCLC). Cancer Treat Rev 2012;38:173-84.
20. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol 2005;23:5892-9.
21. Gandara DR, Gumerlock PH. Epidermal growth factor receptor tyrosine kinase inhibitors plus chemotherapy: case closed or is the jury still out? J Clin Oncol 2005;23:5856-8.
22. Wu YL, Lee JS, Thongprasert S, et al. Intercalated combination of chemotherapy and erlotinib for patients with advanced stage non-small-cell lung cancer (FASTACT-2): a randomised, double-blind trial. Lancet Oncol 2013;14:777-86.
23. Kim ES, Neubauer MA, Cohn AL, et al. SELECT: Randomized phase III study of docetaxel (D) or pemetrexed (P) with or without cetuximab (C) in recurrent or progressive non-small cell lung cancer (NSCLC) after platinum-based therapy. J Clin Oncol 2012;30:abstr 7502.
24. Zinner R, Ross HJ, Weaver R, et al. Randomized, open-label, phase III study of pemetrexed plus carboplatin (PemC) followed by maintenance pemetrexed versus paclitaxel/carboplatin/bevacizumab (PCB) followed by maintenance bevacizumab in patients with advanced nonsquamous (NS) non-small cell lung cancer (NSCLC). J Clin Oncol 2013;31:abstr LBA8003.
25. Socinski MA, Patel JD, Garon EB, et al. A phase III study of pemetrexed (Pem) plus carboplatin (Cb) plus bevacizumab (Bev) followed by maintenance pem plus bev versus paclitaxel (Pac) plus cb plus bev followed by maintenance bev in stage IIIb or IV nonsquamous non-small cell lung cancer (NS-NSCLC): overall and age group results. J Clin Oncol 2013;31:abstr 8004.
26. Laurie SA, Solomon BJ, Seymour L, et al. A randomized double-blind trial of carboplatin plus paclitaxel (CP) with daily oral cediranib (CED), an inhibitor of vascular endothelial growth factor receptors, or placebo (PLA) in patients (pts) with previously untreated advanced non-small cell lung cancer (NSCLC): NCIC Clinical Trials Group study BR29. J Clin Oncol 2012;30:abstr 7511.
27. Paz-Ares LG, Biesma B, Heigener D, et al. Phase III, randomized, double-blind, placebo-controlled trial of gemcitabine/cisplatin alone or with sorafenib for the first-line treatment of advanced, nonsquamous non-small-cell lung cancer. J Clin Oncol 2012;30:3084-92.
28. Ramlau R, Gorbunova V, Ciuleanu TE, et al. Aflibercept and Docetaxel versus Docetaxel alone after platinum failure in patients with advanced or metastatic non-small-cell lung cancer: a randomized, controlled phase III trial. J Clin Oncol 2012;30:3640-7.
29. Hanna NH, Kaiser R, Sullivan RN, et al. Lume-lung 2: a multicenter, randomized, double-blind, phase III study of nintedanib plus pemetrexed versus placebo plus pemetrexed in patients with advanced nonsquamous non-small cell lung cancer (NSCLC) after failure of first-line chemotherapy. J Clin Oncol 2013;31:abstr 8034.
30. Reck M, Kaiser R, Mellemgaard A, et al. Nintedanib (BIBF 1120) plus docetaxel in NSCLC patients progressing after first-line chemotherapy: LUME Lung 1, a randomized, double-blind phase III tria. J Clin Oncol 2013;31:abstr LBA8011.
31. de Boer RH, Arrieta Ó, Yang CH, et al. Vandetanib plus pemetrexed for the second-line treatment of advanced non-small-cell lung cancer: a randomized, double-blind phase III trial. J Clin Oncol 2011;29:1067-74.
32. Tomasini P, Khobta N, Greillier L, et al. Ipilimumab: its potential in non-small cell lung cancer. Ther Adv Med Oncol 2012;4:43-50.
33. Fennell DA, Goss DG, Socinski MA, et al. GALAXY-2 trial: a randomized phase III study of ganetespib in combination with docetaxel versus docetaxel alone in patients with advanced non-small cell lung adenocarcinoma. J Clin Oncol 2013;31:abstr TPS8126.
34. Spigel DR, S NG, Horn L, et al. Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). J Clin Oncol 2013;31:abstr 8008.

35. Paz-Ares L, de Marinis F, Dediu M, et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): a double-blind, phase 3, randomised controlled trial. Lancet Oncol 2012;13:247-55.
36. Paz-Ares L, F DM, Dediu M, et al. PARAMOUNT: Final overall survival (OS) results of the phase III study of maintenance pemetrexed (pem) plus best supportive care (BSC) versus placebo (plb) plus BSC immediately following induction treatment with pem plus cisplatin (cis) for advanced nonsquamous (NS) non-small cell lung cancer (NSCLC). J Clin Oncol 2012;30:abstr LBA7507.
37. Zeng X, Peng L, Li J, et al. Cost-effectiveness of continuation maintenance pemetrexed after cisplatin and pemetrexed chemotherapy for advanced nonsquamous non-small-cell lung cancer: estimates from the perspective of the Chinese health care system. Clin Ther 2013;35:54-65.
38. Belani CP, Brodowicz T, Ciuleanu TE, et al. Quality of life in patients with advanced non-small-cell lung cancer given maintenance treatment with pemetrexed versus placebo (H3E-MC-JMEN): results from a randomised, double-blind, phase 3 study. Lancet Oncol 2012;13:292-9.
39. Fidias PM, Dakhil SR, Lyss AP, et al. Phase III study of immediate compared with delayed docetaxel after front-line therapy with gemcitabine plus carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 2009;27:591-8.
40. Belani CP, Waterhouse DM, Ghazal H, et al. Phase III study of maintenance gemcitabine (G) and best supportive care (BSC) versus BSC, following standard combination therapy with gemcitabine-carboplatin (G-Cb) for patients with advanced non-small cell lung cancer (NSCLC). J Clin Oncol 2010;28:15:abstr 7506
41. Gradishar WJ. Albumin-bound paclitaxel: a next-generation taxane. Expert Opin Pharmacother 2006;7:1041-53.
42. Socinski MA, Bondarenko I, Karaseva NA, et al. Weekly nab-paclitaxel in combination with carboplatin versus solvent-based paclitaxel plus carboplatin as first-line therapy in patients with advanced non-small-cell lung cancer: final results of a phase III trial. J Clin Oncol 2012;30:2055-62.
43. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet 2010;376:1147-54.
44. Edelman MJ, Harb WA, Pal SE, et al. Multicenter trial of EC145 in advanced, folate-receptor positive adenocarcinoma of the lung. J Thorac Oncol 2012;7:1618-21.
45. Cortes J, O'Shaughnessy J, Loesch D, et al. Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study. Lancet 2011;377:914-23.
46. Gitlitz BJ, Tsao-Wei DD, Groshen S, et al. A phase II study of halichondrin B analog eribulin mesylate (E7389) in patients with advanced non-small cell lung cancer previously treated with a taxane: a California cancer consortium trial. J Thorac Oncol 2012;7:574-8.
47. Edelman MJ, Schneider CP, Tsai CM, et al. Randomized phase II study of ixabepilone or paclitaxel plus carboplatin in patients with non-small-cell lung cancer prospectively stratified by beta-3 tubulin status. J Clin Oncol 2013;31:1990-6.
48. Kelly K, Azzoli CG, Zatloukal P, et al. Randomized phase 2b study of pralatrexate versus erlotinib in patients with stage IIIB/IV non-small-cell lung cancer (NSCLC) after failure of prior platinum-based therapy. J Thorac Oncol 2012;7:1041-8.
49. Gerber DE, Schiller JH. Maintenance chemotherapy for advanced non-small-cell lung cancer: new life for an old idea. J Clin Oncol 2013;31:1009-20.

Cite this article as: Lwin Z, Riess JW, Gandara D. The continuing role of chemotherapy for advanced non-small cell lung cancer in the targeted therapy era. J Thorac Dis 2013;5(S5):S556-S564. doi: 10.3978/j.issn.2072-1439.2013.08.47

Medical Oncology

Chemotherapy advances in small-cell lung cancer

Bryan A. Chan[1,2], Jermaine I. G. Coward[1,2,3]

[1]Mater Adult Hospital, Department of Medical Oncology, Raymond Terrace, Brisbane, QLD 4101, Australia; [2]School of Medicine, University of Queensland, St Lucia, Brisbane, QLD 4072, Australia; [3]Inflammation & Cancer Therapeutics Group, Mater Research, Level 4, Translational Research Institute, Woolloongabba, Brisbane, QLD 4102, Australia

Correspondence to: Dr. Jermaine I. G. Coward, MRCP (UK), FRACP, PhD. Inflammation & Cancer Therapeutics Group, Mater Research, Level 4, Translational Research Institute, 37 Kent Street, Woolloongabba, Brisbane, QLD 4102, Australia. Email: jcoward@mmri.mater.org.au.

Abstract: Although chemotherapeutic advances have recently been heralded in lung adenocarcinomas, such success with small-cell lung cancer (SCLC) has been ominously absent. Indeed, the dismal outlook of this disease is exemplified by the failure of any significant advances in first line therapy since the introduction of the current standard platinum-etoposide doublet over 30 years ago. Moreover, such sluggish progress is compounded by the dearth of FDA-approved agents for patients with relapsed disease. However, over the past decade, novel formulations of drug classes commonly used in SCLC (e.g., topoisomerase inhibitors, anthracyclines, alkylating and platinum agents) are emerging as potential alternatives that could effectively add to the armamentarium of agents currently at our disposal. This review is introduced with an overview on the historical development of chemotherapeutic regimens used in this disease and followed by the recent encouraging advances witnessed in clinical trials with drugs such as amrubicin and belotecan which are forging new horizons for future treatment algorithms.

Keywords: Small cell lung cancer (SCLC); amrubicin; belotecan; picoplatin; relapsed SCLC

Submitted Jul 18, 2013. Accepted for publication Jul 30, 2013.
doi: 10.3978/j.issn.2072-1439.2013.07.43
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.07.43

Introduction

For several decades, lung cancer has been and remains by far the most common malignancy in the world with an estimated 1.6 million new cases per annum (12.7% of total) (1). It is also the leading cause of cancer-related mortality with an estimated 1.38 million deaths per annum (1). Small-cell lung cancer (SCLC) accounts for between 10% to 15% of all lung cancer cases and is closely linked to the intensity and duration of tobacco smoking (2). As such, typical SCLC patients are elderly, current or past heavy smokers with multiple cardiovascular and pulmonary comorbidities that may impede optimal management. SCLC is characterised by its aggressive nature with rapid growth, paraneoplastic endocrinopathies and early metastasis (3). In developed countries, the incidence of SCLC peaked in the 1980s corresponding to peak rates of cigarette smoking 20 years prior, but is now slowly decreasing due to changing smoking patterns (2).

Untreated SCLC is rapidly fatal within two to four months (3,4). Initial management strategies for SCLC included surgery or radiotherapy alone if deemed unresectable (3,5). Ultimately, both modalities proved to be suboptimal with very low long-term survival rates and early relapses, usually with distant metastatic disease. In 1969, chemotherapy with single agent cyclophosphamide doubled survival when compared to best supportive care alone (6). Following that, combination chemotherapy was trialled and shown to be superior to single agents (7,8). Dramatic response rates, including complete responses (CR), brought forward the tantalising promise of a cure in the 1980s. However, whilst SCLC is initially sensitive to chemotherapy and radiotherapy, relapse is almost inevitable and the efficacy of treatment beyond first line dwindles as it becomes increasingly resistant to treatment (9,10).

For many other solid-tumour malignancies, advances in diagnosis and treatment have resulted in improved survival. However for SCLC, the 5-year survival rates have not improved significantly over the last 40 years and

Table 1 History of treatments for SCLC

1940s	Surgery
	Radiotherapy
	Nitrogen mustard
1960s	Recognition that SCLC was a different entity compared to other bronchogenic carcinomas (non-small cell lung carcinoma)
	2 tier clinical staging system (limited and extensive) introduced by the Veteran's Administration Lung Cancer Study Group for SCLC
	Single agent chemotherapy trials—cyclophosphamide
1970s	Combination chemotherapy superior to single agents
	Combination anthracycline-based chemotherapy (CAV or CEV)
1980s	Combination platinum-based chemotherapy (EP)
	Chemotherapy combined with thoracic radiation for LD-SCLC
1990s	Early concurrent thoracic radiation with chemotherapy for LD-SCLC
	Chemotherapy for relapsed disease
	Prophylactic cranial irradiation (PCI) for those with good performance status and complete response following combined chemoradiation for LD-SCLC
2000s	Hyperfractionated thoracic radiation
	Irinotecan plus cisplatin (IP) for ED-SCLC (Japan)
	PCI also offered to those with ED-SCLC with good performance status and good response following initial treatment
	Novel regimens (incorporating taxanes, gemcitabine)
	Trials of sequencing, cycling and maintenance chemotherapy
2010s	IASLC introduce TNM staging for SCLC
	Novel agents (amrubicin, belotecan, bendamustine, picoplatin, palifosfamide)

CAV, cyclophosphamide, doxorubicin, vincristine; CEV, cyclophosphamide, epirubicin, vincristine; EP, etoposide, cisplatin; IP, irinotecan, cisplatin.

have currently plateaued (2,11,12). In Australia, the 5-year survival rate improved only marginally between the years of 1982-1987 and 2000-2007 with males improving from 3% to 5% and females 5% to 8% (12).

Over the last 30 years, phase III trials of chemotherapy for SCLC have yielded only a two month improvement in median survival time (10). Radiotherapy in the form of prophylactic cranial irradiation (PCI) has provided incremental improvements in those achieving a complete or near-complete response with initial chemotherapy (5.4% improvement in 3-year survival rate from 15.3% to 20.7%) (13).

In contrast to non-small cell lung cancer, the advances in tumour genomics, chemotherapy and targeted therapy have been relatively sluggish for SCLC. There has been a distinct paucity of change to chemotherapy regimens beyond those first used in the 1970s and 1980s and currently platinum-etoposide remains the backbone of therapy (14,15). Recent advances in understanding molecular pathways and genomic aberrations involved in SCLC pathogenesis will hopefully translate into novel therapeutic targets to improve outcomes (16,17).

This review commences with a synopsis of the history and evolution of SCLC and its treatment (*Table 1*), with a focus on chemotherapy. This is followed by a comprehensive overview of the current systemic options for de novo and relapsed disease as well as novel chemotherapeutic agents and regimens on the horizon.

SCLC: histology and staging

SCLC was initially believed to be caused by arsenic exposure in miners and was previously labelled as 'lymphosarcoma of the mediastinum' (18). In 1926, Barnard discovered that the 'oat cell sarcoma tumour' in fact had an epithelial origin arising from the lung (19). In 1967, the World Health Organisation (WHO) first categorised SCLC into four histological subtypes based on Barnard's observations including: (I) lymphocyte-like; (II) polygonal; (III) fusiform

and (IV) other (3,9). Numerous revisions were made by the WHO before the International Association for the Study of Lung Cancer (IASLC) modified it further in 1988, replacing the term 'oat-cell' with 'small cell carcinoma'.

The original staging system for SCLC was introduced in 1968 by the Veterans Administration Lung Cancer Study Group and consisted of two clinical subgroups namely 'limited disease' (LD-SCLC) and 'extensive disease' (ED-SCLC) (20). LD-SCLC was defined as tumour and nodes confined to one hemithorax and able to be encompassed within a single radiotherapy port, whilst all else was ED-SCLC (11,20).

Approximately 30-40% of patients present with LD-SCLC and are optimally treated with combination chemotherapy with thoracic radiation. Median survival is between 15 to 20 months with 2- and 5- year survival rates of 20-40% and 10-20% respectively (21). Unfortunately, most patients (60-70%) will present with ED-SCLC and are treated with combination chemotherapy resulting in a median survival between 8 to 13 months. Moreover, both 2- and 5-year survival rates remain poor at approximately 5% and 1-2% respectively (21).

As most of SCLC literature utilises the two-subgroup clinical staging system, it remains relevant for clinical decision-making regarding therapy. However there are significant differences between survival outcomes within the 'limited disease' subgroup. When LD-SCLC is further stratified according to the IASLC's Tumour, Node, Metastasis (TNM) classification (7th edition 2010), 5-year survival rates range from 38% for stage IA to 9% for stage IIIB (11). This highlights the need for more precise stratification and as such the TNM staging is now recommended at least in clinical trials for non-metastatic disease (11,15).

Evolution of combination chemotherapy

Although combination chemotherapy is now widely accepted to be integral in the treatment of all stages of SCLC, this contrasts with historical systemic strategies (15,22,23). In the 1940s, initial efforts to treat SCLC involved surgery until radiotherapy was shown to be superior, even for operable cases in 1969 (5,14,18). Alkylating agents such as nitrogen mustard were used as early as 1942, but at the time, the true nature of SCLC was yet to be discovered and all bronchogenic carcinomas were treated similarly (18,23-26). Nitrogen mustard did improve inoperable bronchogenic carcinoma's median survival time from 93 to 121 days (notably only 81 of 468 had oat cell carcinoma) (25,26). In 1962, Watson and Berg argued that 'oat cell carcinoma' with its distinctly aggressive nature and propensity for early metastasis might be better treated with combination intensive chemotherapy and radiation rather than local treatments such as surgery or radiation alone (23).

Cyclophosphamide was the first cytotoxic chemotherapy agent to demonstrate a statistically significant survival advantage over placebo [1969] for bronchogenic carcinoma including SCLC (4.0 *vs*. 1.5 months) (6). Furthermore, in 1979, the combination of cyclophosphamide-based chemotherapy plus thoracic radiation was shown to be superior compared to radiotherapy alone (7,27).

Following these promising results with cyclophosphamide, further single agent cytotoxics were studied with objective overall response rates (ORR) of up to 62% including; anthracyclines, etoposide, tenoposide, ifosfamide, hexamethylmelamine, cisplatin, carboplatin, vindesine, vincristine and nimustine (28). From this, it was recognised that the epipodophyllotoxins (etoposide and tenoposide) were some of the most active single agents in SCLC (29-32). Indeed, a randomised trial using three different schedules of etoposide showed response rates between 20-62% (33). Alkylating agents including ifosfamide showed response rates of up to 46% (28) and other alkylators including cisplatin and carboplatin were less active but animal studies suggested synergism with etoposide (28-33). As single agents in heavily pre-treated SCLC, cisplatin and carboplatin had ORRs of 15% and 24% respectively (28).

Following this, the combination of cyclophosphamide with an anthracycline (doxorubicin or epirubicin) and vincristine (CAV or CEV) was investigated. In extensive disease, CAV showed 14% CR rate, 57% ORR and median survival of 26 weeks. In limited disease, CAV had a 41% CR rate, 75% ORR and median survival of 52 weeks (8). The addition of etoposide to the CAV regimen (CAVE) did not reproducibly improve survival but came at the cost of increased haematological toxicity (34). Thus until the mid-1980s, CAV was the standard regimen for first line induction chemotherapy (34,35).

In cases where anthracyclines were contraindicated due to severe cardiac or hepatic dysfunction, an alternative regimen was suggested using a combination of the most active and synergistic drugs in pre-clinical models. VP-16 or etoposide was combined with cisplatin (EP) and the combination yielded an impressive ORR of 86-89% (29,30). ORR approximated 55% in those refractory to previous anthracycline-based chemotherapy. Median survival times

were 70 and 43 weeks for limited and extensive stage disease respectively (30,31). In the realms of SCLC management, this study proved to be ground-breaking as it yielded responses comparable to anthracycline-based chemotherapy in patients with poorer performance status, serious cardiac disease or extensive liver and brain metastases (30,31).

Following this, direct comparisons between CAV and EP showed equivalent response rates (61% for CAV versus 51% for EP) (36). CR rates and median survival rates were 10% versus 7% and 8.6 versus 8.1 months for CAV and EP respectively (36). Alternating CAV and EP was also investigated and was no different except for a trend towards longer median time to progression (4 months with EP versus 5.2 months with EP/CAV alternating) (36). However, Fukuoka *et al*. conducted a similar trial in Japan showing that EP or CAV alternating with EP (CAV/EP) had significantly higher response rates compared to CAV (78%, 76% and 55% respectively) (37). Survival times favoured the alternating regimen CAV/EP (11.8 months) compared to EP (9.9 months) (P=0.056) or CAV (9.9 months) (P=0.027) (37).

These results favouring platinum-containing regimens have been confirmed by a subsequent randomised phase III trial with 5 years of follow up (38). In LD-SCLC, EP was superior to CEV with 2- and 5-year survival rates of 25% and 10% respectively in the EP arm compared to 8% and 3% in the CEV arm (P=0.0001) (38). For ED-SCLC, there was a trend towards survival benefit with EP over CEV but these were not statistically significant with median survival 8.4 versus 6.5 months respectively (38). When combined with concurrent thoracic radiation, EP is also better tolerated than anthracycline-based regimens (e.g., less oesophagitis and pneumonitis) and so became the most frequently used chemotherapy regimen for SCLC (10,22,30,31,37-40).

The increasing use of platinum in a host of solid tumours has stimulated a plethora of studies comparing its efficacy with non-platinum regimens along with head to head comparisons between cisplatin and carboplatin. With respect to SCLC, a meta-analysis by Pujol *et al*. found that cisplatin-based regimens had an increased probability of response over those without cisplatin (OR 1.35, 95% confidence interval of 1.18-1.55) (41). Cisplatin is associated with significant nephrotoxicity, neurotoxicity and gastrointestinal adverse effects whereas carboplatin is associated with more myelosuppression (42). The COCIS meta-analysis by Rossi focused on whether or not cisplatin was required or if carboplatin could be substituted (42). It suggested that carboplatin-based regimens were equivalent in terms of ORR, progression-free survival (PFS) and overall survival (OS) compared to cisplatin-based regimens (42). Thus it seems reasonable to substitute carboplatin for cisplatin to avoid non-haematological toxicities.

First-line chemotherapy

Current combination chemotherapy with either EP or CAV achieves partial or complete responses rates between 50% to 85% alongside median survival times ranging from 9 to 12 months (4,10). In the hope of improving the outlook for SCLC, several novel agents have been investigated upfront in view of encouraging preliminary results witnessed with these drugs in relapsed disease. Much of the progress seems to have been focussed around the DNA topoisomerase enzymes that are critical for DNA replication and ultimately cell survival (*Table 2*). Dual inhibition of both topoisomerase I and II can produce significant cytotoxic effects by arresting both DNA and RNA replication by maintaining torsional stresses that ultimately impede tumour cell division (53).

Irinotecan

Irinotecan, a topoisomerase I inhibitor, has shown much promise in numerous phase II trials. The Japanese Clinical Oncology Group (JCOG) conducted a phase III trial combining cisplatin with irinotecan (IP) and compared it to EP in treatment naïve ED-SCLC (43). The trial was terminated early due to an interim analysis showing a significant benefit in median survival with IP compared to EP (12.8 versus 9.4 months respectively, P=0.002) (43). OS rates at 2 years were 19.5% and 5.2% respectively suggesting new hope in ED-SCLC (43). Myelosuppression was more common with EP whilst diarrhoea was more common in the IP arm (43).

Whilst this regimen was adopted as first-line therapy for SCLC in Japan, confirmatory studies were required prior to changing standard practice in other countries. Two large North American studies looked at the IP combination but found conflicting results to the JCOG study (44,45). The first used a slightly modified protocol (cisplatin 30 mg/m^2 i.v.i. plus irinotecan 65 mg/m^2 i.v.i. on days 1 and 8 every 21 days) compared to the JCOG (cisplatin 60 mg/m^2 i.v.i. day 1 and irinotecan 60 mg/m^2 i.v.i. on days 1, 8, and 15 q28 days) and found no differences in survival (44). The follow up SWOG S0124 trial used an IP protocol identical to that used in the JCOG trial but found that IP was equivalent to, but not superior to EP, both in terms of ORR and OS (45).

Table 2 Trials of first-line chemotherapy in small-cell lung cancer

Author [Year]	Phase	Disease stage	Regimen	Number	ORR (%)	Median TTP or PFS (wks/mo.)	Median survival time (wks/mo.)	1 yr OS (%)	2 yr OS (%)
Evans *et al*. [1985] (30)		ED & LD	EP	31 (ED: 20/31; LD: 11/31)	86	LD (39 wks) ED (26 wks)	LD (70 wks) ED (43 wks)	NR	NR
Noda *et al*. [2002] (43)	III	ED	IP EP	77 77	84 68 (P=0.02)	– –	12.8 mo. 9.4 mo. (P=0.002)	58.4 37.7	19.5 5.2
Hanna *et al*. [2006] (44)	III	ED	IP EP	221 110	48 43.6 P value NR	4.1 mo. (TTP) 4.6 mo. (TTP) (P=0.37)	9.3 mo. 10.2 mo. (P=0.74)	34.95 35.19	8 7.9
Lara *et al*. [2009] (45)	III	ED	IP EP	324 327	60 57 (P=0.56)	5.8 mo. (PFS) 5.2 mo. (PFS) (P=0.07)	9.9 mo. 9.1 mo. (P=0.71)	41 34	NR NR
Kim *et al*. [2010] (46)	II	ED	B	62	53.2	4.6 mo. (TTP)	10.4 mo.	49.9	NR
Hong *et al*. [2012] (47)	II	ED	BP	35	71.4	5.7 mo. (PFS)	10.2 mo.	NR	NR
Lim *et al*. [2013] (48)	II	ED	BP	42	73.8	6.9 mo. (PFS)	11.2 mo.	NR	NR
Ohe *et al*. [2005] (49)	I-II	ED	AP	44	87.8	NR	13.6 mo.	56.1	NR
Yana *et al*. [2007] (50)	II	ED	A	33	75.8	NR	11.7 mo.	48.5	20.2
Kobayashi *et al*. [2010] (51)	II	ED	IP-A	45	79	6.5 mo. (PFS)	15.4 mo.	61	NR
O'Brien *et al*. [2011] (52)	II	ED	A AP EP	28 30 30	61 67 67	5.2 mo. (PFS) 6.9 mo. (PFS) 5.8 mo. (PFS)	11.1 mo. 11.1 mo. 10 mo.	NR NR NR	NR NR NR

ED, extensive disease; LD, limited disease; NR, not recorded; TTP, time to Progression; PFS, progression free survival; OS, overall survival; BSC, best supportive care; EP, etoposide/cisplatin; IP, irinotecan/cisplatin; B, belotecan; BP, belotecan/cisplatin; A, amrubicin; AP, amrubicin/cisplatin; IP-A, irinotecan/cisplatin followed by amrubicin.

It is postulated that pharmacogenomic variability amongst different ethnic populations could be a potential reason for the differing results; a concept covered further in this review.

Belotecan

Belotecan is a novel camptothecin derivative that inhibits topoisomerase I and positive results from single agent therapy in previously untreated ED-SCLC were seen in a phase II trial (46). It had an impressive ORR of 53.2%, time to progression (TTP) of 4.6- and 10.4-month median OS (46). The most common toxicity was haematological with up to 71% grade 3/4 neutropenia (46). Subsequently, belotecan was combined with cisplatin in two phase II studies which both showed an ORR ≥70% and median survival time of ≥10 months (47,48). The results of an ongoing phase III trial (COMBAT) are eagerly anticipated as it compares belotecan-cisplatin with the gold standard EP in chemotherapy naïve SCLC (54).

Amrubicin

Amrubicin is a synthetic anthracycline derivative which shares structural features with doxorubicin and also stabilises the topoisomerase II-DNA complex (55). Its active metabolite amrubicinol is believed to preferentially accumulate in tumour cells and is thus associated with reduced toxicity including anthracycline-cardiotoxicity (53,56,57). A phase II study in previously untreated ED-SCLC patients found that single agent amrubicin had an ORR of 75.8%, median survival time (MST) of 11.7 months and 2-year survival rate of 20.2% (50).

Consequently, the introduction of amrubicin in first

line platinum doublet therapy has been investigated with response and survival rates comparable to those documented with platinum-etoposide regimens. Ohe *et al.* conducted a phase I-II study of amrubicin combined with cisplatin in first line ED-SCLC to determine the maximum tolerated and recommended dose of the novel combination consisting of amrubicin 40 mg/m^2/day and cisplatin 60 mg/m^2/day (49). They reported an impressive ORR of 87.8% (36 of 41 patients) at the recommended dose schedule. The MST was 13.6 months and 1-year survival rate 56.1%, however these outcomes were counteracted by significant grade 3/4 neutropenia (95.1%) (49).

The West Japan Thoracic Oncology Group 0301 trial was a phase II study investigating sequential triplet chemotherapy with IP followed by amrubicin in previously treated ED-SCLC (51). They reported an ORR of 79% with median PFS 6.5 months. Median OS was 15.4 months but this came at the cost of significant myelosuppression with 91% grade 3/4 neutropenia and 15% febrile neutropenia associated with amrubicin (51).

The EORTC 08062 randomised phase II trial compared amrubicin monotherapy (A) or in combination with cisplatin (AP) versus the standard EP regimen in a non-Asian population (52). Independent reviewer ORR was reported as 61%, 67% and 67% for A, AP and EP respectively (52,58). Although amrubicin is associated with significantly more grade ≥3 haematological toxicities, its impressive response rates are generating interest to further investigate its potential use for SCLC (52).

More recently, Noro *et al.* conducted a phase II study of non-cross resistant chemotherapy by alternating AP with weekly IP for treatment naïve ED-SCLC (59). Whilst this showed an impressive ORR of 85% including 20% CR, significant myelosuppression was evident with 83.3% grade ≥3 neutropenia. However, weekly IP was associated with significantly more diarrhoea. The MST was 359 days (12 months), median PFS 227 days (7.5 months) and one-year OS rate of 40% (59). Hence, the combination of amrubicin-cisplatin (AP) or alternating AP with IP seems to be a very active regimen for SCLC and AP is now being compared to EP in a phase III trial (60).

Maintenance and consolidation therapy

Due to the propensity for SCLC to promptly relapse, maintenance therapy has been a strategy employed to prolong time to recurrence or progression. The Eastern Cooperative Oncology Group (ECOG) conducted a phase III trial of maintenance topotecan (topoisomerase I inhibitor) for patients with stable or responding disease following four cycles of induction cisplatin-etoposide (61). Although PFS was significantly improved, there was no difference in patient-related quality of life or OS between observation and topotecan arms (8.9 versus 9.3 months; P=0.43) (61). Subsequently, a systematic review and meta-analysis by Rossi *et al.* found that the addition of maintenance chemotherapy, interferons or biological agents only produced a very small and clinically insignificant survival benefit (62).

Second-line chemotherapy

Although initial objective chemotherapeutic responses to first line treatment are generally observed, this is seldom witnessed beyond this setting with a median OS often <6 months from the point of relapse (63). In line with other diseases where platinum agents represent the core of primary gold standard therapy (e.g. gynaecological cancers), the extent of initial response is a reasonably robust predictor of future outcome in the event of tumour progression. However, as the usual definition of true platinum sensitivity (i.e. platinum free interval of ≥12 months) used in such diseases is rarely applicable in SCLC, historical classifications have adopted a relatively sombre tone reflecting the unrelenting course of this disease.

Initial reports in the 1980s defined chemoresistant patients with disease that had either progressed during first-line therapy or within 90 days of its completion (64). In turn, PFS extensions beyond this time period are generally categorised as having 'sensitive' disease. Moreover, in particular cases with both high responses from initial induction chemotherapy and prolonged treatment free intervals (TFI) of >6 months, rechallenging with the same drugs used in primary therapy can achieve response rates of 50% (65,66). These early studies helped define the current nomenclature of 'sensitive relapsed' (PFS >3 months), 'resistant' (PFS <3 months) and 'refractory' (progression through first line treatment) SCLC (67). However, amongst the literature, the 'refractory' and 'resistant' definitions increasingly appear to be used interchangeably.

With respect to the second-line cytotoxic strategies employed, there is no general consensus on the most effective regimen. However, there is a leaning towards standard therapy with the camptothecin; topotecan, which to date represents the sole agent with FDA approval specifically for this setting. In comparison with commonly used combinatorial approaches such as CAV, topotecan appears to have

equivalent response (24.3% *vs.* 18.3%; P=0.285) and median survival rates (TTP: 13.3 *vs.* 12.3 weeks; P=0.552; OS: 25 *vs.* 24.7 weeks; P=0.795) but superior palliation of symptoms such as dyspnoea, anorexia, hoarseness, and fatigue (68). Furthermore, the addition of oral topotecan to best supportive care (BSC) resulted in improved symptom control and significant OS advantages over BSC alone (25.9 versus 13.9 weeks; P=0.01) (69). Of interest, the direct comparisons of oral and intravenous administration have revealed equivalence in terms of response rates (18.3% *vs.* 21.9%), median OS (33 *vs.* 35 weeks) and quality of life (70).

Patients with relapsed SCLC will exhibit reasonable responses to other single agents including paclitaxel (71), irinotecan (72), gemcitabine (73) and vinorelbine (74). Nevertheless, although the response rates with such monotherapies are often inferior to combinations of these drugs with platinum agents (75-77), the benefits of combinatorial approaches are often offset by increased toxicity. However, for patients deemed to have sensitive relapse with a PFS of greater >3 months, rechallenging with platinum-based doublets presents a possible option. This approach has been confirmed in a recent meta-analysis conducted by Garassino *et al.* amongst 161 patients with SCLC undergoing second line therapy having failed EP (78). In this study, subjects were treated independent of their platinum sensitivity and only 30 (18.6%) were rechallenged with platinum. Notably, patients from this particular cohort with platinum sensitive disease showed a trend towards superior ORR (34.5% *vs.* 17.5%, P=0.06) and OS (9.2 *vs.* 5.8 months, P=0.08) in comparison with those treated with non-platinum agents (78). Interestingly, clinical benefit (i.e. SD + PR) was obtained in 30% of patients with refractory/resistant disease who underwent platinum rechallenge (78). Despite these results, rechallenging with platinum is mainly reserved for patients with both sensitive relapsed disease and a TFI >6 months.

Despite the modicum of success with such regimens, a clear therapeutic ceiling has been reached with the current armament of cytotoxic agents available for second line treatment and beyond. For this reason, research has focused on developing novel formulations of drug classes such as platinum salts, anthracyclines, camptothecins and alkylating agents; all of which have been the cornerstone of progressive SCLC treatment for several decades (*Table 3*).

Amrubicin

The encouraging results emanating from the aforementioned first-line phase II/III studies with amrubicin containing regimens have stimulated significant interest in relapsed SCLC. Within this sphere, several small Phase II trials have been conducted for both sensitive and refractory SCLC (53) (*Table 3*) which could potentially help establish an alternative 2nd line regimen to topetecan.

Table 3 Trials of second-line chemotherapy in small-cell lung cancer

Author [Year]	Phase	Treatment free interval	Regimen	Number	ORR (%)	Median TTP or PFS (wks/mo.)	Median survival time (weeks/mo.)	Survival rates (%)
Von Pawel *et al*. [1999] (68)	III	>6 mo.	T *vs*. CAV	107	T: 24.3 CAV: 18.3 P=0.285	TTP T: 13.3 wks CAV: 12.3 wks P=0.552	T: 25 wks CAV: 24.7 wks P=0.795	NR
O'Brien *et al*. [2006] (69)	III	All relapsed SCLC	T (oral) *vs*. BSC	141	T: 7; (44 SD)	TTP T: 16.3 wks	T: 25.9 wks BSC: 13.9 wks (P=0.01)	6 month survival, T: 49 BSC, 26
Eckhardt *et al*. [2007] (70)	III	≥90 days	T (oral) *vs*. T (i.v.i.)	309	T (oral): 18.3 T (i.v.i.): 21.9 P value NR	NR	T (oral): 33 wks T (i.v.i.): 35 wks	1 yr survival, T (oral): 32.6, T (i.v.i.) : 29.2 2 yr survival, T (oral): 12.4, T (i.v.i.) : 7.1
Onada *et al*. [2006] (79)	II	</>60 days	A	60 (16 refractory, 44 sensitive)	Refractory: 50 sensitive: 52	PFS refractory: 2.6 mo. sensitive: 4.2 mo.	Refractory: 10.3 mo. sensitive: 11.6 mo.	1 yr survival, refractory: 40, sensitive: 46

Table 3 (*continued*)

Table 3 (*continued*)

Author [Year]	Phase	Treatment free interval	Regimen	Number	ORR (%)	Median TTP or PFS (wks/mo.)	Median survival time (weeks/mo.)	Survival rates (%)
Inoue *et al*. [2008] (80)	II	</>90 days	A *vs*. T	59 evaluable (A=29, T=30) 23 refractory, 36 sensitive)	A: 38 (refractory 17, sensitive 53) T: 13 (refractory 0, sensitive 21)	PFS A: 3.5 mo. (refractory 2.6 mo., sensitive 3.9 mo.) T: 2.2 mo. (refractory 1.5 mo., sensitive 3.0mo)	A: 8.1 mo. (refractory 5.3 mo., sensitive 9.9 mo.) T: 8.4 mo. (refractory 5.4 mo., sensitive: 11.7 mo.)	NR
Ettinger *et al*. [2010] (81)	II	<90 days	A	75	21.3 (1.3 CR, 20 PR)	PFS: 3.2 mo.	6.0 mo.	6 month survival, 48; 1 yr survival, 15.7
Jotte *et al*. [2011] (82)	II	≥90 days	A *vs*. T	76 (A=50, T=26)	A: 44 T: 15 P=0.021	PFS A: 4.5 mo. T: 3.3 mo.	A: 9.2 mo. T: 7.6 mo.	6 month survival, A: 60, T: 54 1 yr survival, A: 36, T: 33
Jotte *et al*. [2011] (83) (ACT-1 study)	III	</>90 days	A *vs*. T	637 (A=424, T=213)	A: 31 T: 17 P=0.0002	PFS A: 4.1 mo. T: 4.0 mo. P=0.98	A: 7.8 mo. T: 7.5 mo. refractory; A:6.2 mo., T: 5.7 mo., P=0.049)	1 yr survival, A: 17, T: 8 (P=0.019) 18-month survival, A: 12, T: 0 (P=0.0006)
Treat *et al*. [2002] (84)	II	</>8 wks	PIC	37 (13 resistant, 24 sensitive)	Resistant: 15.4 sensitive: 8.3	NR	Resistant: 27.3 wks sensitive: 35.7 wks	NR
Eckhardt *et al*. [2009] (85)	II	Refractory: PD through 1st line therapy Resistant: <90 days Sensitive: ≥91 days <180 days	PIC	77 (44 refractory, 27 resistant, 6 sensitive)	4	PFS: 9.1 wks	26.9 wks	6 month survival, 50.6 1 yr survival, 16.9
Ciuleanu *et al*. [2010] (86) (SPEAR study)	III	<6 mo.	PIC + BSC *vs*. BSC	401 (268 PIC + BSC, 133 BSC)	NR	PFS refractory with no post study treatment) PIC + BSC: 9 wks BSC: 7 wks P=0.03	PIC + BSC: 21 wks BSC: 20 wks NS	NR
Rhee *et al*. [2011] (87)	II	All relapsed SCLC	B	25	24	PFS: 2.2 mo.	9.9 mo.	1yr survival, 38.3
Jeong *et al*. [2010] (88)	II	≥3 mo.	B	27	22	PFS: 4.7 mo.	13.1 mo.	NR
Kim *et al*. [2012] (89)	II	All relapsed SCLC (Platinum sensitivity not defined)	B	50 (30 refractory, 20 sensitive)	14 refractory: 10 sensitive: 20	PFS: 1.6 mo. refractory: 1.5 mo. sensitive: 2.8 mo.	4.5 mo. refractory: 4.0 mo. sensitive: 6.5 mo.	NR
Schmittel *et al*. [2007] (90)	II	≥60 days	BEN	21	29	PFS 4.0 mo.	7.0 mo.	1 yr survival, 16 2 yr survival, 8

T, topetcan; CAV, cyclophosphamide/doxorubicin/vincristine; A, amrubicin; PIC, picoplatin; B, belotecan; BEN, bendamustine; BSC, best supportive care; NR, not reported.

The first of these studies highlighting the salvage potential of amrubicin was published by Onoda *et al*. in 2006 (79). This multicentre phase II study enrolled 60 patients with relapsed SCLC; 16 refractory (i.e. TTP <60 days from treatment discontinuation) and 44 with sensitive disease (i.e. demonstrable 1st line treatment response and PD >60 days post treatment discontinuation). In line with the current recommended dosing schedule, single agent amrubicin was administered at 40 mg/m^2 d1-3 every 3 weeks. The median number of treatment cycles was 4 cycles(range, 1-8 cycles). Interestingly, the ORR for refractory and sensitive patients were almost equivalent at 50% (95% CI, 25% to 75%) and 52% (95% CI, 37% to 68%) respectively. However, superior PFS (2.6 *vs*. 4.2 months), OS (10.3 *vs*. 11.6 months) and 1-year survival (40% *vs*. 46%) favoured patients with sensitive disease (79). With respect to toxicity, grade 3/4 myelosuppression was most commonplace with high rates of neutropenia (83%), followed by anaemia (33%) and thrombocytopenia (20%). Importantly, only 3 patients (5%) experienced febrile neutropenia and no treatment-related deaths were documented (79).

Naturally, these findings fuelled the development of a subsequent study directly comparing the efficacy of amrubicin (40 mg/m^2 d1-3 q3 weeks) and topetecan (1 mg/m^2 d1-5 q3 weeks) within the second line setting. Another phase II Japanese study conducted by Inoue *et al*. enrolled 60 SCLC patients pre-treated with platinum-based chemotherapy (80). Of the 59 evaluable, 23 had refractory (defined as no response to 1st line therapy or relapse <90 days of discontinuation) and 36 had sensitive disease. Clear benefits of amrubicin (n=29) over topotecan (n=30) were evident with ORR of 38% (95% CI, 20% to 56%) and 13% (95% CI, 1% to 25%) respectively. In addition, these advantages were highlighted further with patients stratified according to sensitive (53% *vs*. 21%) or refractory (17% *vs*. 0%) disease (80). Although the superiority of amrubicin over topotecan reflected in the PFS (3.5 *vs*. 2.2 months), this did not extend to the MST (8.1 *vs*. 8.4 months). Rates of neutropenia (79% *vs*. 43%), febrile neutropenia (14% *vs*. 3%) and non-haematological toxicities grade >3 were higher in the amrubicin arm and unfortunately, one treatment related death secondary to neutropenia was observed in this group of patients (80).

Analogous to other success stories with novel therapeutics initially trialled in Asian populations [e.g. IPASS in non-small cell lung cancer (NSCLC) (91)], these results were greeted with initial caution as certain pharmacogenomic profiles exclusive to such cohorts could possibly preclude the same responses in Caucasian patients. Specifically, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is an enzyme critically involved in the metabolism of amrubicin and the polymorphisms of this enzyme which are recognised in Asian populations could potentially influence response (92). Consequently, two studies focusing on 2nd line amrubicin treatment in refractory and sensitive SCLC have been conducted in patients from Western populations. With respect to platinum-refractory disease, Ettinger *et al*. conducted a phase II study with single agent amrubicin in 75 patients who achieved a median PFS of 38 days following 1st line chemotherapy (81). Of these, 69 patients received a median of 4 cycles (range, 1-12 cycles), with a modest ORR of 21.3% (95% CI, 12.7% to 32.3%). In addition 1 CR (1.3%) and 15 PR (20%) were witnessed alongside a PFS and OS of 3.2 months (95% CI, 2.4 to 4.0 months) and 6.0 months (95% CI, 4.8 to 7.1 months), respectively (81). Interestingly, amongst the 43 (57%) patients who failed to respond to initial platinum-based therapy, a 16.3% ORR (95% CI, 6.8% to 30.7%) was observed (81).

The subsequent Jotte *et al*. study with amrubicin in platinum-sensitive SCLC (i.e. TFI ≥90 days) bore similarities to the Inoue trial by employing a topotecan-containing comparator arm (82). Patients (n=76) were randomised 2:1 to amrubicin (n=50; 40 mg/m^2 i.v.i. d1-3, q21 days) or topotecan (n=26; 1.5 mg/m^2 i.v.i. d1-5, q21 days). Again, significantly higher ORR was witnessed with amrubicin compared with topotecan (44% *vs*. 15%; P=0.021) and this also translated into superior median PFS (4.5 *vs*. 3.3 months) and OS (9.2 *vs*. 7.6 months). In contrast to the Inoue study, there was a trend towards more myelosuppression (≥ grade 3) with topotecan as opposed to amrubicin (82). In conclusion, the favourable results witnessed with amrubicin in ORR, PFS, OS in sensitive/refractory SCLC and the superiority over topotecan in Asian cohorts are also apparent in patients from the Western world and has consequently stimulated the development of a further larger scale study. Namely, the randomised phase III ACT-1 study aimed to compare the efficacy of 2nd line amrubicin with topotecan in patients with relapsed SCLC (83). In this trial 637 patients were randomized 2:1 to amrubicin (n=424) 40 mg/m^2 i.v.i. d1-3 or topotecan (n=213) 1.5 mg/m^2 i.v.i. d1-5. In line with similar aforementioned studies with these regimens, the results presented at the 2011 American Society of Clinical Oncology (ASCO) Annual Meeting confirmed that amrubicin had significantly improved ORR compared to topotecan (31% *vs*. 17%; P=0.0002) (83). Furthermore, despite no differences in PFS, OS trends favoured amrubicin (HR 0.88; 95% CI, 0.73-1.06; P=0.17), with a particular leaning towards patients with

refractory disease (HR 0.77; 95% CI, 0.59-1.00; P=0.049) (83).

In addition, small Phase I/II studies have explored the efficacy of combining amrubicin and topotecan as a potential 2nd line regimen (93,94). However despite the 60-70% ORR achieved, any optimism generated from these trials is tempered by unacceptable toxicities including grade 4 myelosuppression, fatal diarrhoea and pneumonitis (94). Nevertheless, the results from the larger amrubicin monotherapy studies have certainly shed significant light on a plausible alternative therapeutic agent that could salvage patients with relapsed SCLC.

Picoplatin

Picoplatin (ZD0473) is a novel organic platinum analogue developed specifically to circumvent the development of platinum resistance mediated by sulphur-containing compounds such as glutathione and metallothionein (95,96); thiol agents that detoxify through avid platinum binding (97). This property extends its anti-neoplastic activity beyond the standard functionality of platinum revolving around DNA alkylation, inter- and intra-strand cross-linking which all facilitate apoptosis. More specifically, an *in vitro* study has confirmed the reversal of resistance to both cisplatin and carboplatin with picoplatin in platinum resistant H69 and SBC-3 SCLC lines (98). Moreover, it appears that the mechanism of action underlying this phenomenon relates to a decrease in platinum accumulation (98). The first clinical reports confirming single agent activity of picoplatin in relapsed SCLC were published by Treat *et al.* with a phase II study in SCLC patients with platinum resistant (defined as PD <8 weeks from 1st line platinum based treatment, n=13) or sensitive disease (n=24) (84). The ORR was modest at 15.4% and 8.3% and median OS was 27.3 and 35.7 weeks for the resistant and sensitive groups respectively (84,96). A subsequent larger study with picoplatin monotherapy (150 mg/m^2 i.v.i. q3 wks consisted of 77 patients with relapsed SCLC); 57% (n=44) with platinum refractory disease (no response to platinum based therapy), 35% (n=27) with platinum resistance (i.e. relapse <90 days from completing 1st line platinum based therapy) and 8% (n=6) with platinum sensitive disease (i.e. relapse ≥91 days <180 days following completion of 1st line platinum-based therapy) (85). In view of the preponderance of refractory/resistant patients in this study, the ORR was low at 4% with a median PFS and OS of 9.1 and 26.9 weeks respectively (85). With respect to adverse events, the most common grade 3/4 toxicities were thrombocytopenia (48%), followed by neutropenia (25%), and anaemia (20%). Significantly, there were no episodes of febrile neutropenia (85).

Both of these aforementioned studies set the foundations for the Study of Picoplatin Efficacy After Relapse (SPEAR) trial (86). This phase II study consisted of 401 patients with relapsed SCLC (<6 months of completing 1st line platinum-based chemotherapy) randomised 2:1 to picoplatin with BSC (n=268) or BSC alone (n=133). Disappointingly, this trial failed to show any survival advantages in the treatment arm over BSC (P=0.09) (86). However, this may be explained by the unbalanced proportion of patients who received post study chemotherapy in the BSC arm. Interestingly, the subset analysis of refractory patients (i.e. no response or relapse <45 days of completing 1st line platinum-based therapy) who did not receive post-study chemotherapy (n=273), revealed statistically significant PFS advantage favouring the picoplatin arm (P=0.03) amounting to just 2 weeks (86). Despite this, it appears curious why a comparator of BSC was chosen over drugs such as topotecan and amrubicin which both have documented activity in the second line setting. However, with the justifiable nihilism generated by the SPEAR trial amongst lung oncologists, it appears unlikely that such a study will ever be realised.

Belotecan

The modest efficacy witnessed in first-line therapy with the novel topoisomerase I inhibitor; belotecan is also mirrored in a few small studies in the relapse setting. Rhee *et al.* published the results of a Phase II trial in 25 patients with relapsed SCLC (sensitivity status unknown) treated with belotecan at an initial dose of 0.5 mg/m^2 i.v.i. d1-5 q21 days (87). In accordance with toxicity, appropriate dose adjustments were only allowed to be implemented once during subsequent cycles. Out of the 21 evaluable patients, 6 had an objective tumour response; i.e. ORR 24% on the intention to treat analysis. Furthermore, the median PFS and OS were 2.2 and 9.9 months respectively with a 1-year survival rate of 38.3% (87). Although the incidence of grade 3/4 neutropenia was particularly high (88%), severe non-haematological toxicities were not commonplace (87). Similarly, another single agent study was executed in 27 patients with refractory disease who had relapsed within 3 months of obtaining response from platinum-irinotecan based first line therapy (88). The ORR was 22%, with median PFS of 4.7 months (95% CI, 3.6-5.8 months) and a reasonable median OS of 13.1 months (95% CI, 10.4-15.8 months) (88). The latter result is of particular interest as it

suggests that belotecan has a role in salvaging patients who are resistant to other topoisomerase I inhibitors.

More recently, Kim *et al*. have published a larger study investigating the efficacy of belotecan monotherapy in 50 patients with sensitive relapsed (n=20) or refractory SCLC (n=30) (89). The ORR was 14% (95% CI, 4-24%) with a median follow up period of 4.2 months (range, 0.1-19.2 months), and median PFS and OS of 1.6 and 4.5 months respectively. As expected, patients with sensitive relapsed disease fared significantly better compared to refractory counterparts for ORR (20% *vs*. 10%), OS (6.5 *vs*. 4.0 months; P=0.003) with a trend towards superior PFS (2.8 *vs*. 1.5 months; P=0.053). Of note, the multivariate analysis confirmed that the type of relapse and prior response to chemotherapy were independent prognostic factors for OS (89). Again, grade 3/4 myelosuppression was evident with the highest rate associated with neutropenia (54%) followed by thrombocytopenia (38%) and anaemia (32%) (89). Furthermore, one treatment-related death secondary to sepsis was documented in this study. Despite the expected deleterious side effects, belotecan has shown modest activity within the second line setting for both sensitive and refractory SCLC and, as with amrubicin, warrants further exploration in this particular domain.

Future directions and closing remarks

The novel chemotherapeutic agents previously highlighted have indeed provided some optimism, albeit short lived. Other drugs have recently come to the fore and similarly demonstrate variable degrees of efficacy. Bendamustine; a bifunctional alkylating agent, has shown activity in combination with carboplatin in chemotherapy-naïve ED-SCLC. Amongst 55 patients, Koster *et al*. documented an ORR of 72.7% which included a single complete responder. In addition median TTP (5.2 months), MST (8.3 months) and toxicity profiles all compared favourably in comparison to other standard 1st line platinum containing regimens (99). Bendamustine also appears effective in sensitive relapsed SCLC (i.e. TFI ≥60 days) with ORR 29% and median PFS and OS of 4 and 7 months respectively (90). In view of this preliminary data, a current phase I/IIa study is actively recruiting 30 patients with chemotherapy-naïve SCLC to be treated with 3 cycles of bendamustine combined with irinotecan followed by 3 cycles of standard carboplatin and etoposide (Clincaltrials.gov identifier: NCT00856830).

Following on from the success of pemetrexed in non-squamous non-squamous NSCLC and mesothelioma (100,101), attempts have been made to add this to the armament of therapeutic regimes in SCLC. However the outcomes of two recent phase II studies using pemetrexed monotherapy (500 and 900 mg/m^2) in patients with sensitive and refractory relapsed SCLC have been inadequate with minimal efficacy seen in this setting (102,103). These damning results are not entirely unexpected. The discrepancies in the efficacy of pemetrexed in non-squamous and squamous NSCLC seen with the seminal Scagliotti study (100), are based on the higher thymidylate synthase (TS; the principal substrate for pemetrexed) expression associated with squamous histotypes (104). Indeed, a subsequent study has further shown that lower TS expression in advanced non squamous NSCLC is associated with longer PFS (105). Moreover, TS expression in SCLC (both from resected tumours and cell lines) is significantly higher than pulmonary squamous and adenocarcinomas (106,107). Hence, it would appear counterintuitive to adopt strategies involving TS inhibitors for SCLC therapy.

This review has attempted to outline the historical and current progress in the chemotherapeutic management of SCLC. Platinum-etoposide doublets still represent the gold standard of first line therapy and attempts to switch the mode of topoisomerase inhibition may prove to be the most strategic method in improving survival. Although the survival advantages garnered from substituting etoposide for irinotecan in the JCOG study were not recapitulated in the subsequent SWOG S0124 trial; current studies comparing the efficacy of amrubicin or belotecan with platinum with EP (52,54) could potentially change practice. Similarly, both of these agents are showing promise as single agents in salvaging patients with either sensitive or refractory relapsed disease. Taking into consideration the dearth of FDA approved 2nd line regimens in SCLC, there is an obvious urge to develop larger clinical trials with these agents. Furthermore, despite the disheartening outcomes in the SPEAR study, picoplatin may still serve as a viable alternative to either cisplatin or carboplatin in its ability to avert the development of resistance. Hence, trials comparing picoplatin doublets with other platinum containing regimens in previously untreated SCLC could also be considered.

As with other solid tumour types, the successful quest in prolonging survival in SCLC will most likely involve appropriate combinations with the novel drugs outlined in this review alongside emerging therapies such as multi-targeted receptor tyrosine kinase inhibitors or other agents

which serve to block signalling cascades inherent to the aggressive tumorigenicity of SCLC (e.g. inhibitors of IGFR, mTOR, MET and hedgehog signalling). Exhaustive preclinical studies with such combinatorial therapies will be required to examine both their efficacy and the inevitable upregulation of resistance pathways that ensue. The development of future clinical trials emanating from these studies will require robust design in order to make significant steps in changing the landscape of this bleak disease.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. van Meerbeeck JP, Fennell DA, De Ruysscher DK. Small-cell lung cancer. Lancet 2011;378:1741-55.
3. Kato Y, Ferguson TB, Bennett DE, et al. Oat cell carcinoma of the lung. A review of 138 cases. Cancer 1969;23:517-24.
4. Pelayo Alvarez M, Gallego Rubio O, Bonfill Cosp X, et al. Chemotherapy versus best supportive care for extensive small cell lung cancer. Cochrane Database Syst Rev 2009;(4):CD001990.
5. Miller AB, Fox W, Tall R. Five-year follow-up of the Medical Research Council comparative trial of surgery and radiotherapy for the primary treatment of small-celled or oat-celled carcinoma of the bronchus. Lancet 1969;2:501-5.
6. Green RA, Humphrey E, Close H, et al. Alkylating agents in bronchogenic carcinoma. Am J Med 1969;46:516-25.
7. Lowenbraun S, Bartolucci A, Smalley RV, et al. The superiority of combination chemotherapy over single agent chemotherapy in small cell lung carcinoma. Cancer 1979;44:406-13.
8. Livingston RB, Moore TN, Heilbrun L, et al. Small-cell carcinoma of the lung: combined chemotherapy and radiation: a Southwest Oncology Group study. Ann Intern Med 1978;88:194-9.
9. Hann CL, Ettinger DS. The change in pattern and pathology of small cell lung cancer. In: Govindan R. eds. American Society of Clinical Oncology. Alexandria, VA, 2009.
10. Chute JP, Chen T, Feigal E, et al. Twenty years of phase III trials for patients with extensive-stage small-cell lung cancer: perceptible progress. J Clin Oncol 1999;17:1794-801.
11. Shepherd FA, Crowley J, Van Houtte P, et al. The International Association for the Study of Lung Cancer lung cancer staging project: proposals regarding the clinical staging of small cell lung cancer in the forthcoming (seventh) edition of the tumor, node, metastasis classification for lung cancer. J Thorac Oncol 2007;2:1067-77.
12. Australian Institute of Health and Welfare & Cancer Australia 2011. Lung cancer in Australia: an overview. Cancer series no. 64. Canberra: AIHW, 2011.
13. Aupérin A, Arriagada R, Pignon JP, et al. Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. Prophylactic Cranial Irradiation Overview Collaborative Group. N Engl J Med 1999;341:476-84.
14. Fox W, Scadding JG. Medical Research Council comparative trial of surgery and radiotherapy for primary treatment of small-celled or oat-celled carcinoma of bronchus. Ten-year follow-up. Lancet 1973;2:63-5.
15. Kalemkerian GP, Akerley W, Bogner P, et al. Small cell lung cancer. J Natl Compr Canc Netw 2013;11:78-98.
16. Byers LA, Wang J, Nilsson MB, et al. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. Cancer Discov 2012;2:798-811.
17. Rosell R, Wannesson L. A genetic snapshot of small cell lung cancer. Cancer Discov 2012;2:769-71.
18. Haddadin S, Perry MC. History of small-cell lung cancer. Clin Lung Cancer 2011;12:87-93.
19. Barnard WG. The nature of the "oat-celled sarcoma" of the mediastinum. J Pathol 1926;29:241-4.
20. Zelen M. Keynote address on biostatistics and data retrieval. Cancer Chemother Rep 3 1973;4:31-42.
21. Lally BE, Urbanic JJ, Blackstock AW, et al. Small cell lung cancer: have we made any progress over the last 25 years? Oncologist 2007;12:1096-104.
22. Johnson DH. Management of small cell lung cancer: current state of the art. Chest 1999;116:525S-530S.
23. Watson WL, Berg JW. Oat cell lung cancer. Cancer 1962;15:759-68.
24. Levine B, Weisberger AS. The response of various types of bronchogenic carcinoma to nitrogen mustard. Ann Intern Med 1955;42:1089-96.
25. Wolf J, Spear P, Yesner R, et al. Nitrogen mustard and the steroid hormones in the treatment of inoperable bronchogenic carcinoma. Am J Med 1960;29:1008-16.
26. Wolf J, Yesner R, Patno ME. Evaluation of nitrogen

mustard in prolonging life of patients with bronchogenic carcinoma. Cancer Chemother Rep 1962;16:473-5.
27. Medical Research Council Lung Cancer Working Party. Radiotherapy alone or with chemotherapy in the treatment of small-cell carcinoma of the lung. Br J Cancer 1979;40:1-10.
28. Joss RA, Cavalli F, Goldhirsch A, et al. New drugs in small-cell lung cancer. Cancer Treat Rev 1986;13:157-76.
29. Goldhirsch A, Joss R, Cavalli F, et al. Etoposide as single agent and in combination chemotherapy of bronchogenic carcinoma. Cancer Treat Rev 1982;9:85-90.
30. Evans WK, Shepherd FA, Feld R, et al. VP-16 and cisplatin as first-line therapy for small-cell lung cancer. J Clin Oncol 1985;3:1471-7.
31. Evans WK, Shepherd FA, Feld R, et al. First-line therapy with VP-16 and cisplatin for small-cell lung cancer. Semin Oncol 1986;13:17-23.
32. Loehrer PJ Sr, Einhorn LH, Greco FA. Cisplatin plus etoposide in small cell lung cancer. Semin Oncol 1988;15:2-8.
33. Cavalli F, Sonntag RW, Jungi F, et al. VP-16-213 monotherapy for remission induction of small cell lung cancer: a randomized trial using three dosage schedules. Cancer Treat Rep 1978;62:473-5.
34. Jett JR, Everson L, Therneau TM, et al. Treatment of limited-stage small-cell lung cancer with cyclophosphamide, doxorubicin, and vincristine with or without etoposide: a randomized trial of the North Central Cancer Treatment Group. J Clin Oncol 1990;8:33-8.
35. Seifter EJ, Ihde DC. Therapy of small cell lung cancer: a perspective on two decades of clinical research. Semin Oncol 1988;15:278-99.
36. Roth BJ, Johnson DH, Einhorn LH, et al. Randomized study of cyclophosphamide, doxorubicin, and vincristine versus etoposide and cisplatin versus alternation of these two regimens in extensive small-cell lung cancer: a phase III trial of the Southeastern Cancer Study Group. J Clin Oncol 1992;10:282-91.
37. Fukuoka M, Furuse K, Saijo N, et al. Randomized trial of cyclophosphamide, doxorubicin, and vincristine versus cisplatin and etoposide versus alternation of these regimens in small-cell lung cancer. J Natl Cancer Inst 1991;83:855-61.
38. Sundstrøm S, Bremnes RM, Kaasa S, et al. Cisplatin and etoposide regimen is superior to cyclophosphamide, epirubicin, and vincristine regimen in small-cell lung cancer: results from a randomized phase III trial with 5 years' follow-up. J Clin Oncol 2002;20:4665-72.
39. Shaw EG, Frytak S, Eagan RT, et al. Etoposide-cisplatin and thoracic radiation therapy salvage of incomplete responders to a noncisplatin induction regimen for limited and extensive small-cell carcinoma of the lung. Am J Clin Oncol 1996;19:154-8.
40. Skarlos DV, Samantas E, Kosmidis P, et al. Randomized comparison of etoposide-cisplatin vs. etoposide-carboplatin and irradiation in small-cell lung cancer. A Hellenic Co-operative Oncology Group study. Ann Oncol 1994;5:601-7.
41. Pujol JL, Carestia L, Daurès JP. Is there a case for cisplatin in the treatment of small-cell lung cancer? A meta-analysis of randomized trials of a cisplatin-containing regimen versus a regimen without this alkylating agent. Br J Cancer 2000;83:8-15.
42. Rossi A, Di Maio M, Chiodini P, et al. Carboplatin- or cisplatin-based chemotherapy in first-line treatment of small-cell lung cancer: the COCIS meta-analysis of individual patient data. J Clin Oncol 2012;30:1692-8.
43. Noda K, Nishiwaki Y, Kawahara M, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 2002;346:85-91.
44. Hanna N, Bunn PA Jr, Langer C, et al. Randomized phase III trial comparing irinotecan/cisplatin with etoposide/cisplatin in patients with previously untreated extensive-stage disease small-cell lung cancer. J Clin Oncol 2006;24:2038-43.
45. Lara PN Jr, Natale R, Crowley J, et al. Phase III trial of irinotecan/cisplatin compared with etoposide/cisplatin in extensive-stage small-cell lung cancer: clinical and pharmacogenomic results from SWOG S0124. J Clin Oncol 2009;27:2530-5.
46. Kim SJ, Kim JS, Kim SC, et al. A multicenter phase II study of belotecan, new camptothecin analogue, in patients with previously untreated extensive stage disease small cell lung cancer. Lung Cancer 2010;68:446-9.
47. Hong J, Jung M, Kim YJ, et al. Phase II study of combined belotecan and cisplatin as first-line chemotherapy in patients with extensive disease of small cell lung cancer. Cancer Chemother Pharmacol 2012;69:215-20.
48. Lim S, Cho BC, Jung JY, et al. Phase II study of camtobell inj. (belotecan) in combination with cisplatin in patients with previously untreated, extensive stage small cell lung cancer. Lung Cancer 2013;80:313-8.
49. Ohe Y, Negoro S, Matsui K, et al. Phase I-II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer. Ann Oncol

2005;16:430-6.
50. Yana T, Negoro S, Takada M, et al. Phase II study of amrubicin in previously untreated patients with extensive-disease small cell lung cancer: West Japan Thoracic Oncology Group (WJTOG) study. Invest New Drugs 2007;25:253-8.
51. Kobayashi M, Matsui K, Iwamoto Y, et al. Phase II study of sequential triplet chemotherapy, irinotecan and cisplatin followed by amrubicin, in patients with extensive-stage small cell lung cancer: West Japan Thoracic Oncology Group Study 0301. J Thorac Oncol 2010;5:1075-80.
52. O'Brien ME, Konopa K, Lorigan P, et al. Randomised phase II study of amrubicin as single agent or in combination with cisplatin versus cisplatin etoposide as first-line treatment in patients with extensive stage small cell lung cancer - EORTC 08062. Eur J Cancer 2011;47:2322-30.
53. Kimura T, Kudoh S, Hirata K. Review of the management of relapsed small-cell lung cancer with amrubicin hydrochloride. Clin Med Insights Oncol 2011;5:23-34.
54. Chonnam National University Hospital. Trial of belotecan/cisplatin in chemotherapy naive small cell lung cancer patient (COMBAT). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited 2013 July 9]. Available online: http://clinicaltrials.gov/show/NCT00826644
55. Hanada M, Mizuno S, Fukushima A, et al. A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. Jpn J Cancer Res 1998;89:1229-38.
56. Metro G, Duranti S, Fischer MJ, et al. Emerging drugs for small cell lung cancer--an update. Expert Opin Emerg Drugs 2012;17:31-6.
57. Higashiguchi M, Suzuki H, Hirashima T, et al. Long-term amrubicin chemotherapy for small-cell lung cancer. Anticancer Res 2012;32:1423-7.
58. Pignon JP, Arriagada R, Ihde DC, et al. A meta-analysis of thoracic radiotherapy for small-cell lung cancer. N Engl J Med 1992;327:1618-24.
59. Noro R, Yoshimura A, Yamamoto K, et al. Alternating chemotherapy with amrubicin plus cisplatin and weekly administration of irinotecan plus cisplatin for extensive-stage small cell lung cancer. Anticancer Res 2013;33:1117-23.
60. Sumitomo Pharmaceutical (Suzhou) Co., Ltd. Phase 3 study of amrubicin with cisplatin versus etoposide-cisplatin for extensive disease small cell lung cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited 2013 July 10]. Available online: http://clinicaltrials.gov/ct2/show/NCT00660504
61. Schiller JH, Adak S, Cella D, et al. Topotecan versus observation after cisplatin plus etoposide in extensive-stage small-cell lung cancer: E7593--a phase III trial of the Eastern Cooperative Oncology Group. J Clin Oncol 2001;19:2114-22.
62. Rossi A, Garassino MC, Cinquini M, et al. Maintenance or consolidation therapy in small-cell lung cancer: a systematic review and meta-analysis. Lung Cancer 2010;70:119-28.
63. Davies AM, Evans WK, Mackay JA, et al. Treatment of recurrent small cell lung cancer. Hematol Oncol Clin North Am 2004;18:387-416.
64. Giaccone G, Donadio M, Bonardi G, et al. Teniposide in the treatment of small-cell lung cancer: the influence of prior chemotherapy. J Clin Oncol 1988;6:1264-70.
65. Postmus PE, Berendsen HH, van Zandwijk N, et al. Retreatment with the induction regimen in small cell lung cancer relapsing after an initial response to short term chemotherapy. Eur J Cancer Clin Oncol 1987;23:1409-11.
66. Giaccone G, Ferrati P, Donadio M, et al. Reinduction chemotherapy in small cell lung cancer. Eur J Cancer Clin Oncol 1987;23:1697-9.
67. Glisson BS. Recurrent small cell lung cancer: update. Semin Oncol 2003;30:72-8.
68. von Pawel J, Schiller JH, Shepherd FA, et al. Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. J Clin Oncol 1999;17:658-67.
69. O'Brien ME, Ciuleanu TE, Tsekov H, et al. Phase III trial comparing supportive care alone with supportive care with oral topotecan in patients with relapsed small-cell lung cancer. J Clin Oncol 2006;24:5441-7.
70. Eckardt JR, von Pawel J, Pujol JL, et al. Phase III study of oral compared with intravenous topotecan as second-line therapy in small-cell lung cancer. J Clin Oncol 2007;25:2086-92.
71. Smit EF, Fokkema E, Biesma B, et al. A phase II study of paclitaxel in heavily pretreated patients with small-cell lung cancer. Br J Cancer 1998;77:347-51.
72. Masuda N, Fukuoka M, Kusunoki Y, et al. CPT-11: a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 1992;10:1225-9.
73. Masters GA, Declerck L, Blanke C, et al. Phase II trial of gemcitabine in refractory or relapsed small-cell lung

cancer: Eastern Cooperative Oncology Group Trial 1597. J Clin Oncol 2003;21:1550-5.
74. Furuse K, Kubota K, Kawahara M, et al. Phase II study of vinorelbine in heavily previously treated small cell lung cancer. Japan Lung Cancer Vinorelbine Study Group. Oncology 1996;53:169-72.
75. Goto K, Sekine I, Nishiwaki Y, et al. Multi-institutional phase II trial of irinotecan, cisplatin, and etoposide for sensitive relapsed small-cell lung cancer. Br J Cancer 2004;91:659-65.
76. Fujita A, Takabatake H, Tagaki S, et al. Combination of cisplatin, ifosfamide, and irinotecan with rhG-CSF support for the treatment of refractory or relapsed small-cell lung cancer. Oncology 2000;59:105-9.
77. Groen HJ, Fokkema E, Biesma B, et al. Paclitaxel and carboplatin in the treatment of small-cell lung cancer patients resistant to cyclophosphamide, doxorubicin, and etoposide: a non-cross-resistant schedule. J Clin Oncol 1999;17:927-32.
78. Garassino MC, Torri V, Michetti G, et al. Outcomes of small-cell lung cancer patients treated with second-line chemotherapy: a multi-institutional retrospective analysis. Lung Cancer 2011;72:378-83.
79. Onoda S, Masuda N, Seto T, et al. Phase II trial of amrubicin for treatment of refractory or relapsed small-cell lung cancer: Thoracic Oncology Research Group Study 0301. J Clin Oncol 2006;24:5448-53.
80. Inoue A, Sugawara S, Yamazaki K, et al. Randomized phase II trial comparing amrubicin with topotecan in patients with previously treated small-cell lung cancer: North Japan Lung Cancer Study Group Trial 0402. J Clin Oncol 2008;26:5401-6.
81. Ettinger DS, Jotte R, Lorigan P, et al. Phase II study of amrubicin as second-line therapy in patients with platinum-refractory small-cell lung cancer. J Clin Oncol 2010;28:2598-603.
82. Jotte R, Conkling P, Reynolds C, et al. Randomized phase II trial of single-agent amrubicin or topotecan as second-line treatment in patients with small-cell lung cancer sensitive to first-line platinum-based chemotherapy. J Clin Oncol 2011;29:287-93.
83. Von Pawel J, Jotte R, Spigel DR, et al. Randomized phase 3 trial of amrubicin versus topotecan as second-line treatment for small cell lung cancer (SCLC). J Clin Oncol 2011;29:abstr 7000.
84. Treat J, Schiller J, Quoix E, et al. ZD0473 treatment in lung cancer: an overview of the clinical trial results. Eur J Cancer 2002; 38 Suppl 8:S13-8.
85. Eckardt JR, Bentsion DL, Lipatov ON, et al. Phase II study of picoplatin as second-line therapy for patients with small-cell lung cancer. J Clin Oncol 2009;27:2046-51.
86. Ciuleanu T, Samarzjia M, Demidchik Y, et al. Randomized phase III study (SPEAR) of picoplatin plus best supportive care (BSC) or BSC alone in patients (pts) with SCLC refractory or progressive within 6 months after first-line platinum-based chemotherapy. J Clin Oncol 2010;28:abstr 7002.
87. Rhee CK, Lee SH, Kim JS, et al. A multicenter phase II study of belotecan, a new camptothecin analogue, as a second-line therapy in patients with small cell lung cancer. Lung Cancer 2011;72:64-7.
88. Jeong J, Cho BC, Sohn JH, et al. Belotecan for relapsing small-cell lung cancer patients initially treated with an irinotecan-containing chemotherapy: a phase II trial. Lung Cancer 2010;70:77-81.
89. Kim GM, Kim YS, Ae Kang Y, et al. Efficacy and toxicity of belotecan for relapsed or refractory small cell lung cancer patients. J Thorac Oncol 2012;7:731-6.
90. Schmittel A, Knödler M, Hortig P, et al. Phase II trial of second-line bendamustine chemotherapy in relapsed small cell lung cancer patients. Lung Cancer 2007;55:109-13.
91. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
92. Ettinger DS. Amrubicin for the treatment of small cell lung cancer: does effectiveness cross the Pacific? J Thorac Oncol 2007;2:160-5.
93. Shibayama T, Hotta K, Takigawa N, et al. A phase I and pharmacological study of amrubicin and topotecan in patients of small-cell lung cancer with relapsed or extensive-disease small-cell lung cancer. Lung Cancer 2006;53:189-95.
94. Nogami N, Hotta K, Kuyama S, et al. A phase II study of amrubicin and topotecan combination therapy in patients with relapsed or extensive-disease small-cell lung cancer: Okayama Lung Cancer Study Group Trial 0401. Lung Cancer 2011;74:80-4.
95. Holford J, Sharp SY, Murrer BA, et al. In vitro circumvention of cisplatin resistance by the novel sterically hindered platinum complex AMD473. Br J Cancer 1998;77:366-73.
96. William WN Jr, Glisson BS. Novel strategies for the treatment of small-cell lung carcinoma. Nat Rev Clin Oncol 2011;8:611-9.
97. Mistry P, Kelland LR, Abel G, et al. The relationships between glutathione, glutathione-S-transferase and

cytotoxicity of platinum drugs and melphalan in eight human ovarian carcinoma cell lines. Br J Cancer 1991;64:215-20.
98. Tang CH, Parham C, Shocron E, et al. Picoplatin overcomes resistance to cell toxicity in small-cell lung cancer cells previously treated with cisplatin and carboplatin. Cancer Chemother Pharmacol 2011;67:1389-400.
99. Köster W, Heider A, Niederle N, et al. Phase II trial with carboplatin and bendamustine in patients with extensive stage small-cell lung cancer. J Thorac Oncol 2007;2:312-6.
100. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26:3543-51.
101. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 2003;21:2636-44.
102. Socinski MA, Raju RN, Neubauer M, et al. Pemetrexed in relapsed small-cell lung cancer and the impact of shortened vitamin supplementation lead-in time: results of a phase II trial. J Thorac Oncol 2008;3:1308-16.
103. Jalal S, Ansari R, Govindan R, et al. Pemetrexed in second line and beyond small cell lung cancer: a Hoosier Oncology Group phase II study. J Thorac Oncol 2009;4:93-6.
104. Ceppi P, Volante M, Saviozzi S, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 2006;107:1589-96.
105. Nicolson MC, Fennell DA, Ferry D, et al. Thymidylate Synthase Expression and Outcome of Patients Receiving Pemetrexed for Advanced Nonsquamous Non-Small-Cell Lung Cancer in a Prospective Blinded Assessment Phase II Clinical Trial. J Thorac Oncol 2013;8:930-939.
106. Ibe T, Shimizu K, Nakano T, et al. High-grade neuroendocrine carcinoma of the lung shows increased thymidylate synthase expression compared to other histotypes. J Surg Oncol 2010;102:11-7.
107. Monica V, Scagliotti GV, Ceppi P, et al. Differential Thymidylate Synthase Expression in Different Variants of Large-Cell Carcinoma of the Lung. Clin Cancer Res 2009;15:7547-7552.

Cite this article as: Chan BA, Coward JI. Chemotherapy advances in small-cell lung cancer. J Thorac Dis 2013;5(S5):S565-S578. doi: 10.3978/j.issn.2072-1439.2013.07.43

Targeted therapy in lung cancer: IPASS and beyond, keeping abreast of the explosion of targeted therapies for lung cancer

Peter Savas[1], Brett Hughes[2,3], Benjamin Solomon[1]

[1]The Peter MaCallum Cancer Centre, East Melbourne, Victoria, Australia; [2]Royal Brisbane and Women's Hospital, Herston, Queensland, Australia; [3]The Prince Charles Hospital, Chermside, Queensland, Australia

Correspondence to: A/Prof Benjamin Solomon. The Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia.
Email: Ben.Solomon@petermac.org.

Abstract: Advances in the treatment of non-small cell lung cancer (NSCLC) over the last decade have predominantly involved the development of therapies directed at molecular targets such as mutations in the epidermal growth factor receptor (EGFR) or rearrangements in the anaplastic lymphoma kinase (ALK) gene. Other targets have been discovered at low frequency, with multiple agents approved or in development for treatment of these rare molecular subtypes. The tumour microenvironment has also provided opportunities for therapies targeting angiogenesis and the host immune response. This review will provide an overview of current targeted therapies in NSCLC and promising treatment approaches on the horizon.

Keywords: Non-small-cell lung carcinoma (NSCLC); molecular targeted therapy; immunotherapy; epidermal growth factor receptor (EGFR); anaplastic lymphoma kinase (ALK)

Submitted Jul 19, 2013. Accepted for publication Aug 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.52
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.52

Introduction

Delivering a high chance of benefit and avoiding futile treatment is crucial in the management of advanced lung cancer where quality of life is constantly at risk from disease progression or treatment toxicity. This ideal is now achievable with the realisation of targeted therapy in non-small cell lung cancer (NSCLC). Targeted therapy refers to pharmaceutical agents that affect a known molecular target in the cancer cell or tumour microenvironment. In some cases, the presence of the target is determined prior to treatment by interrogating tumour samples with a variety of histological and molecular techniques. In other cases, the presence of the target is assumed to be present in the majority of patients on the basis of prior analyses on large numbers of samples. Detectable targets that indicate a high chance of treatment benefit with a given therapy are termed predictive biomarkers. This is in contrast to prognostic biomarkers, which merely indicate an influence on prognosis rather than treatment response. Testing for mutations in the epidermal growth factor receptor (*EGFR*) gene and rearrangements of the anaplastic lymphoma kinase (*ALK*) gene in adenocarcinoma of the lung are now in routine clinical use as predictive genomic biomarkers in the management of advanced lung cancer. The group of patients with lung adenocarcinomas that harbour either of these genomic alterations (15-50% depending on the population studied) are already benefiting from targeted therapy with oral kinase inhibitors such as erlotinib and crizotinib. Other potential predictive genomic biomarkers in known oncogenes such as *BRAF*, *ROS1*, *MET* and *PIK3CA* have been identified in a systematic fashion and efforts are underway to target them with novel drug compounds.

It is clear now that lung cancer represents a constellation of diseases with distinct molecular profiles and sensitivity to treatment. This re-imagining of the classification of lung cancer has been paralleled by the discovery that squamous cell carcinoma and adenocarcinoma of the lung have very different molecular architectures, and distinguishing the two on histological grounds remains a crucial first step to guide subsequent molecular analyses. Determining the molecular subtypes of lung cancer in the clinic requires an ongoing effort to develop reliable molecular diagnostics,

Table 1 Phase III trials of EGFR TKIs in exclusively EGFR-mutant advanced NSCLC

Trial	Patients	Targeted agent	Comparator arm	Primary endpoint
Western Japan Thoracic Oncology Group 3405 (12)	172	Gefitinib	Cisplatin + Docetaxel	Median PFS 9.2 versus 6.3 months (HR 0.49, 95% CI: 0.34-0.71, P<0.0001)
North East Japan Study Group 002 (13)	230	Gefitinib	Carboplatin + Paclitaxel	Median PFS 10.8 versus 5.4 months (HR 0.3, 95% CI: 0.22-0.41, P<0.001)
OPTIMAL (14)	165	Erlotinib	Carboplatin + Gemcitabine	Median PFS 13.1 versus 4.6 months (HR 0.16, 95% CI: 0.1-0.26, P<0.0001)
EURTAC (15)	174	Erlotinib	Cisplatin + Docetaxel or Gemcitabine	Median PFS 9.7 versus 5.2 months (HR 0.37, 95% CI: 0.25-0.54, P<0.0001)
LUX-Lung 3 (16)	345	Afatinib	Cisplatin + Pemetrexed	Median PFS 11.1 versus 6.9 months (HR 0.58, 95% CI: 0.43-0.78, P=0.001)
LUX-Lung 6 (17)	364	Afatinib	Cisplatin + Gemcitabine	Median PFS 11 versus 5.6 months (HR 0.28, P<0.0001)

PFS, Pogression free survival; HR, Hazard ratio; CI, Confidence interval.

as has occurred with testing for *EGFR* mutation and *ALK* rearrangement. Lung cancer therapy is also likely to benefit from the nascent field of cancer immunotherapy, with preliminary evidence that targeting the host immune response to lung cancer will be a successful and versatile treatment modality in the future. This review will summarise the current state of targeted therapy for lung cancer with a focus on NSCLC, and discuss promising agents in development.

Targeting oncogenic mutations and chromosomal aberrations in NSCLC

EGFR-mutant NSCLC

Mutations in the *EGFR* gene found in adenocarcinoma of the lung was the first biomarker predictive of benefit from a targeted therapy in NSCLC, and was exemplary of the impressive efficacy that could be expected from this paradigm. Small molecule inhibitors of EGFR were originally developed and tested in unselected lung cancer populations, where some patients were noted to have dramatic responses (1,2). Subsequent studies revealed that tumours with mutations in the intracellular tyrosine kinase domain that mediates downstream signalling of the *EGFR* gene product had substantial clinical responses to oral tyrosine kinase inhibitors (TKIs) such as gefitinib or erlotinib (3-5).

Before *EGFR* mutation was known to be a predictive biomarker, certain patient populations were seen to benefit more from EGFR TKIs, namely those with lung adenocarcinomas, Asian ethnicity, females and never-smokers. It is now known that the enhanced efficacy in these populations is explained by the greater likelihood that their tumours harbour *EGFR* mutations (5-8) and that such mutations are almost exclusively found in adenocarcinoma of the lung (7-9). There is however no clinical characteristic that can be used in lieu of *EGFR* mutation testing.

The efficacy of EGFR TKIs in advanced *EGFR*-mutant lung cancer has now been established in eight randomised phase III clinical trials. The first of these was the pivotal IPASS study which evaluated the efficacy of gefitinib versus first line chemotherapy with carboplatin and paclitaxel in an Asian population of light or never smokers with advanced lung cancer (10). As part of this study which involved over 1,200 patients, 437 patients had tumour samples assayed for EGFR mutations. In the overall population, the study showed a non-inferior progression free survival for gefitinib compared to chemotherapy. It was also found that *EGFR* mutation was a very strong predictor of improved progression free survival with gefitinib, and that gefitinib was inferior to chemotherapy in patients without *EGFR* mutations. These results were confirmed in the phase III First-SIGNAL study which also compared gefitinib to chemotherapy in never-smokers with advanced lung cancer (11).

In addition to IPASS and First-SIGNAL, there have been six randomised controlled phase III trials comparing the EGFR TKIs gefitinib, erlotinib or afatinib to chemotherapy in patients with exclusively *EGFR*-mutant lung cancer, both in Asian and Caucasian populations. These studies which are summarised in *Table 1* (12-17), uniformly show superior

response rates, progression free survival and quality of life with EGFR TKIs compared to cytotoxic chemotherapy. Despite mature follow up data (18-20), no trial of a first line EGFR TKI has shown an overall survival benefit, most likely explained by the large numbers of patients in the chemotherapy arms of these trials that crossed over to EGFR TKI treatment after progression. Although there has been no direct comparison, the second generation EGFR TKI afatinib appears to have more toxicity compared to gefitinib and erlotinib, with higher rates of severe diarrhoea and skin rash (16).

It is now recommended that all patients with advanced adenocarcinoma of the lung be tested for EGFR mutations (21), which is typically carried out using DNA sequencing of archival formalin fixed tumour tissue obtained at biopsy. The frequency of *EGFR* mutation in current or former smokers is approximately 10%, and in never smokers can be up to 40-50% (8,22). Due to the superior response rates and quality of life seen with erlotinib or gefitinib compared to chemotherapy, it is also recommended that all patients with *EGFR*-mutant NSCLC receive these treatments as first line therapy (23-25).

EGFR TKIs continue to have a role in NSCLC without *EGFR* mutations, where they may inhibit the overexpressed non-mutant protein, so-called wild-type *EGFR*. Erlotinib was found to improve overall survival in advanced NSCLC compared to placebo following progression on second or third line chemotherapy in the NCIC Clinical Trials Group BR.21 phase III study (26). This study was conducted before the link between *EGFR* mutation and EGFR TKI response was known, but subsequent subgroup analysis showed that the benefit was maintained in patients with wild-type *EGFR* and non-adenocarcinoma histology. A similar phase III study comparing gefitinib to placebo in a heavily pre-treated population failed to meet statistical significance, but there was a trend towards improved survival (27) with gefitinib.

Only one phase III study has compared EGFR TKIs to chemotherapy as second line therapy in a population that is specifically *EGFR* wild-type (28). Although this study suggested that docetaxel was a superior treatment in this group, final publication of results is awaited. A variety of studies have been conducted in unselected populations, showing that EGFR TKIs are non-inferior to second line chemotherapy (29), have a role as maintenance therapy after first line chemotherapy (30), and have similar efficacy to second line chemotherapy in patients that have failed to respond to first line treatment (31). There are no data to suggest the use of EGFR TKIs as first line therapy in *EGFR* wild-type disease, and this strategy appeared to be detrimental in IPASS (10) and also in the phase III TORCH study of erlotinib followed by chemotherapy versus chemotherapy followed by erlotinib (32).

Second generation EGFR TKIs are irreversible inhibitors of mutant *EGFR*, and also inhibit other receptors in the epidermal growth factor family. Afatinib, an ErbB receptor family blocker, is one such drug that has progressed furthest in development. In a phase IIb/III study of afatinib versus best supportive care in an unselected population of patients who had progressed on two chemotherapy regimens as well as either erlotinib or gefitinib, there was a modest prolongation of progression free survival by 2 months, but no overall survival benefit (33). Afatinib has also been tested in two phase III randomised trials as first line therapy in patients with *EGFR*-mutant NSCLC (*Table 1*) where it showed superior progression free survival compared to chemotherapy (16,17). It has been approved by the United States Food and Drug Administration (FDA) for this indication. Another second generation EGFR TKI dacomitinib has shown superior progression free survival compared to erlotinib when given after failure of prior chemotherapy in a phase II study of 188 patients (34), and is currently under investigation in two phase III studies compared to erlotinib (ARCHER) or placebo (BR26).

An alternative approach to targeting EGFR in NSCLC has been the use of monoclonal antibodies engineered to have strong affinity for the EGFR protein, such as cetuximab (35). Two randomised phase III trials have been conducted comparing chemotherapy to chemotherapy plus cetuximab in advanced NSCLC. The FLEX study of 1,125 patients with advanced NSCLC showed a modest improvement in overall survival of around 1 month with the addition of cetuximab to chemotherapy (36). A similar study failed to show benefit in the primary endpoint of progression free survival (37). Data about the role of EGFR protein expression in predicting benefit have been conflicting, although a retrospective subgroup analysis showed high EGFR expression was predictive of longer survival with cetuximab in the FLEX study (38,39). The lack of clear benefit and uncertainty over an appropriate biomarker has limited the use of cetuximab.

Acquired treatment resistance to EGFR TKIs

There is now little doubt about the effectiveness of EGFR TKIs in *EGFR*-mutant NSCLC. However, despite high initial response rates, drug resistance and clinical failure is

inevitable with the use of these agents over the course of a patient's treatment, so-called acquired resistance. In contrast to cytotoxic chemotherapy, the well defined mechanism of action of EGFR TKIs means that treatment resistance is a potentially tractable problem. Serial biopsies of tumours before and after treatment with EGFR TKIs have provided insight into the mechanisms of treatment failure (40-43), and have now been performed in sufficient numbers of patients to give an overview of the most common resistance mechanisms. In approximately 60% of cases, treatment failure is mediated by the presence of the secondary *EGFR* mutation T790M that is resistant to inhibition by current EGFR TKIs (40,43). This is presumed to develop from a resistant population of cells already present in low numbers before treatment with EGFR TKIs (44). In another 5-15% of cases, activation of alternative pathways within the cell that free it from dependence on *EGFR* signalling occurs, most commonly involving amplification of the *MET* gene (40-42,45) and mutations in *PIK3CA* (41). Mutations in *BRAF* have also been seen, and confirmed to confer resistance in cell line models (46), as has amplification of *HER2* (47). Activation of the *AXL* kinase appears to be another mechanism of acquired resistance (48). Unexpectedly, transformation to small cell histology has been observed in approximately 5% of cases (41,42) and several of these patients responded to conventional chemotherapy regimens used for small cell lung cancer (41). It is of note that several mechanisms of resistance may co-exist in the same tumour (41-43), such as T790M mutation and *MET* amplification.

The great value in understanding the mechanism of acquired resistance is that it provides a pathway to developing improved therapeutic strategies. Given that T790M mutations are the most common mechanism of acquired resistance, developing EGFR TKIs that inhibit T790M mutant *EGFR* is a logical next step. There is *in vitro* evidence that second generation EGFR TKIs such as afatinib may have better efficacy against T790M mutations (49), although response rates in trials with populations expected to have significant numbers of T790M mutations have been poor (33). A phase II study of afatinib combined with cetuximab has however shown promising results, controlling disease in all 22 patients enrolled with 36% showing partial responses (50). Toxicity has been a problem with this combination however. Finally, third generation mutation-selective EGFR TKIs such as CO-1868 have been developed that specifically inhibit the T790M mutant EGFR protein. CO-1868 is currently being tested in a phase I trial in patients with advanced *EGFR*-mutant NSCLC that have progressed on other EGFR TKIs, where it has shown preliminary evidence of efficacy in resistant disease and a favourable toxicity profile (51). AP26113 is another third generation EGFR TKI with T790M activity that is in phase I/II testing (52).

Targeted therapies already exist or are in development for other molecular pathways that may mediate acquired resistance, such as those involving *HER2*, *BRAF*, *PIK3CA* and *MET*. Combining such therapies with EGFR TKIs may provide an avenue for preventing or delaying acquired resistance. This has been applied *in vitro* where EGFR TKI resistance was reversed by co-administration of a MET inhibitor (53,54). Challenges remain in designing trials of tailored drug combinations in this setting and managing the potential toxicities that arise.

ALK-positive NSCLC

ALK was first detected as a fusion oncogene in lung adenocarcinoma in 2007 (55,56), although it had previously been identified as a fusion oncogene arising from a translocation between chromosome 2p and 5q in a subset of anaplastic large cell lymphomas (57). In the context of NSCLC the most frequent *ALK* gene rearrangement arises due to a short inversion in chromosome 2p where the *ALK* gene is fused with the echinoderm microtubule-associated protein-like 4 gene (*EML4*). The aberrant fusion protein EML4-ALK promotes cell growth, and is sufficient to transform cells into a malignant phenotype *in vitro* (55). *ALK*-positive cells seem to rely almost exclusively on the fusion protein to drive cell growth and survival, a concept termed 'oncogene addiction' that also applies to *EGFR*-mutant NSCLC (58). In this context, inhibition of oncogene function in EML4-ALK addicted tumours should result in growth arrest and cell death, and this was observed in animal models using small molecule kinase inhibitors targeting *ALK* (59,60).

Although developed originally as a small molecule inhibitor of the oncogene *c-MET*, crizotinib was also found to inhibit the *ALK* kinase (61), and was already in phase I trials when *ALK* was discovered to play a role in lung cancer. A reliable diagnostic method was also developed to detect *ALK* fusions in archival lung tissue using fluorescence in situ hybridisation (FISH) with break-apart probes. This enabled patients with advanced *ALK*-positive lung cancer to be enrolled rapidly into a phase I trial of crizotinib, where an impressive response rate of 60% was demonstrated (62,63).

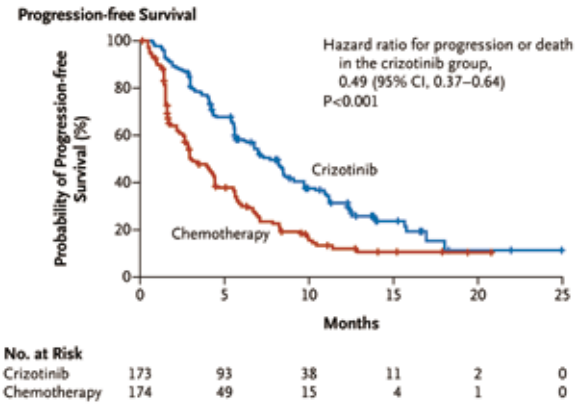


Figure 1 Progression free survival for second line crizotinib versus chemotherapy in ALK-positive NSCLC. From "Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013;368:2385-94. Copyright © 2013 Massachusetts Medical Society". Reprinted with permission.

Most of these patients had received prior chemotherapy. A subsequent report with more mature data compared the overall survival of patients who received crizotinib in the phase I study to *ALK*-positive patients that were not enrolled and also *ALK* negative patients. Although not a randomised comparison, use of crizotinib was associated with improved survival compared to historical cohorts (64). It was also noted that the presence of an *ALK* fusion was not prognostic for survival in the absence of crizotinib.

Of the 1,500 patients screened for *ALK* fusions in the phase I study, only 5% were positive (62). In a similar fashion to *EGFR* mutations, some clinicopathologic characteristics predict a higher likelihood of ALK positivity, including young age, lack of smoking history and adenocarcinoma with solid, acinar or signet-ring histologic patterns. In an unselected population with NSCLC the frequency of *ALK* positivity is approximately 4% (62,65-68). *ALK* fusions are only very rarely found in lung cancers that have mutations in other oncogenes such as *EGFR* or *KRAS* (67).

Crizotinib has since been compared to standard second line chemotherapy in a multi-centre phase III randomised controlled trial in 342 patients with advanced *ALK*-positive lung cancer that had progressed after first line chemotherapy (69). Almost all of the patients in the standard arm received pemetrexed or docetaxel. The study was clearly positive for the primary endpoint with a median progression free survival of 7.7 months in the crizotinib arm and 3.0 months in the chemotherapy arm, shown in *Figure 1* (HR 0.49, 95% CI: 0.37-0.64, P<0.0001) (69). Crizotinib also improved baseline symptoms and delayed subsequent worsening to a greater degree than chemotherapy in quality of life analyses. There was no overall survival benefit seen, most likely because at least 64% of patients in the chemotherapy arm subsequently received crizotinib. A phase III trial of crizotinib as first line treatment for *ALK*-positive lung cancer has recently completed accrual. Crizotinib has received regulatory approval in Europe and the United States. It is recommended by international guidelines that testing for the presence of an ALK fusion be considered for all patients with adenocarcinoma of the lung (23,70).

Crizotinib and *ALK* positive lung cancer is a unique example of the promise of targeted therapy. It has taken only 4 years from the original discovery of the *EML4-ALK* fusion in lung cancer to the FDA approval of crizotinib and its widespread clinical use for this indication.

Acquired resistance to crizotinib

With time, resistance to *ALK* inhibition with crizotinib is inevitable. The median progression free survival in the largest study of crizotinib was 7.7 months (69). In a similar fashion to EGFR TKIs, biopsy of progressing lesions in patients treated with crizotinib has provided insight into resistance mechanisms (71-74). Mutations in the *ALK* gene appear to mediate resistance in around one third of patients, although there is a much wider spectrum of mutations than that seen in *EGFR*-mutant lung cancer where T790M dominates as discussed previously. Activation of alternate signalling pathways involving *EGFR* and *c-KIT* (an oncogene targeted by imatinib) may also play a role in mediating resistance (71). *In vitro* studies suggest that targeting the alternative pathway with existing agents such as gefitinib in the case of *EGFR* or imatinib for *c-KIT* may reverse resistance to crizotinib (71). The mechanism of crizotinib resistance in *ALK* positive tumours currently remains unknown in around one third of cases (75). Of concern, multiple different resistance mechanisms may occur simultaneously in the same patient (71).

Next generation ALK inhibitors with different properties to crizotinib have been developed to have greater potency and potentially target resistance mutations. One agent CH5424802, has been tested in phase I and phase II trials in crizotinib naïve ALK-positive NSCLC, and is notable for the 93% overall response rate seen (76). Another agent LDK378 has shown efficacy in a phase I trial which included both

crizotinib resistant and naïve ALK-positive NSCLC (77), with a response rate of 70%. LDK378 also appeared effective in the presence of resistant *ALK* mutations.

KRAS-mutant NSCLC

KRAS mutations occur in around 30% of NSCLC (73), making them the most common driver mutation seen in an unselected population. Adenocarcinomas make up the majority of NSCLC with *KRAS* mutations (78), and there is a positive association with smoking history (79). *KRAS* mutations may predict a lack of benefit from EGFR TKIs in patient with wild-type *EGFR*, but data have been conflicting (80-82). Despite much research, it has not proved possible to directly target *KRAS*, although recent progress has been made (83). Alternative strategies have involved targeting the down stream signalling pathway of *KRAS* (84), a role fulfilled by the *MEK* inhibitor selumetinib (85). In a randomised phase II trial of second line therapy in *KRAS*-mutant advanced NSCLC, selumetinib plus docetaxel was superior to docetaxel in response rate and progression free survival (86). Other approaches to targeting *KRAS*-mutant NSCLC in early phase trials include PIK3CA/mTOR/AKT pathway inhibitors in combination with *MEK* inhibitors to effectively block downstream *KRAS* signalling (87).

Other oncogenes in NSCLC

With the advent of next generation sequencing technology, driver oncogenes beyond *EGFR*, *ALK* and *KRAS* have been characterised in NSCLC, often at frequencies of less than 5% (88). As targeted therapies already exist for several of these altered genes and are in use in other cancer types, there is currently a focus on identifying lung cancer patients with these alterations and matching them to appropriate therapies within early phase trials (89). There are clear differences between squamous cell and adenocarcinoma histologies in terms of driver oncogenes (9,90), so these will be discussed separately. The pattern and frequency of alterations are summarised in *Figure 2*.

Adenocarcinomas

ROS1 translocation

Fusion genes involving the receptor tyrosine kinase *ROS1* have been found in 1-2% of NSCLC typically in never or light smokers with adenocarcinoma (91,92). This fusion is notable as it appears sensitive to inhibition with crizotinib (91,93), and defines a molecular subclass of lung cancers with clinical similarity to *ALK*-positive cancers.

MET amplification

MET is the gene for the hepatocyte growth factor receptor (HGFR). Activation of *MET* signalling is sufficient to transform cells to a malignant phenotype, and has effects on the cell cycle and survival. NSCLC cells commonly overexpress *MET*, and *MET* amplification is a defined pathway of resistance to EGFR TKIs (40-42,45). The monoclonal antibody onartuzumab (MetMAb) blocks binding of HGF to the *MET* receptor. It was combined with erlotinib in a randomised phase II trial in advanced NSCLC after failure of prior therapy. In patients with *MET* over-expression, combination therapy significantly prolonged overall survival from 4.6 to 12.6 months (HR 0.37, 95% CI: 0.2-0.71, P=0.002) compared to erlotinib alone. Tivantinib, a small molecule MET inhibitor was tested in a phase III trial in combination with erlotinib, but the study was closed early for futility (Press Release, ArQule Inc. and Daiichi Sankyo Co.).

BRAF mutations

BRAF is a well characterised driver mutation in metastatic melanoma, where it is treated with oral *BRAF* inhibitors such as vemurafenib or dabrafenib. A phase II trial of dabrafenib in *BRAF* mutant NSCLC is ongoing, with 7 out of the first 17 patients on trial demonstrating a partial response (94). The frequency of *BRAF* mutation in NSCLC is 1-5% (88,95,96), and appears to be at least equally as common in current or former smokers as non-smokers. The classic sensitising V600E mutation was only found in 50% of the *BRAF* mutant lung cancers, which may limit the use of currently available *BRAF* inhibitors (95).

HER2 amplification and mutations

HER2 amplification or mutation is known to exist in some lung cancers with a frequency of around 3% (97). Attempts at treating HER2 amplified NSCLC with the monoclonal anti-HER2 antibody trastuzumab were unsuccessful (98). *HER2* mutation in exon 20 is a more promising molecular subgroup, and there exist several small molecule inhibitors of the *HER2* tyrosine kinase such as afatinib or dacomitinib (99). There have been early reports of some responses to these drugs in patients with *HER2* mutations (100), and trials are ongoing.

RET translocations

Fusions involving the receptor tyrosine kinase *RET* gene

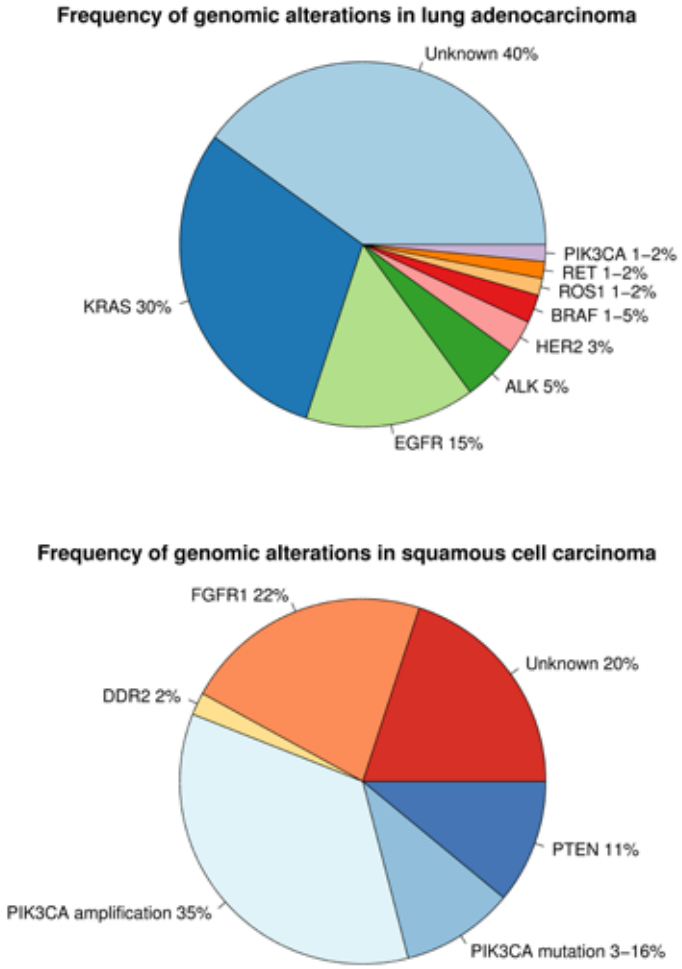


Figure 2 Relative frequency of genomic alterations in adenocarcinoma and squamous cell carcinoma. Data adapted from multiple references (see text) and are estimates only.

have recently been indentified in lung adenocarcinomas, and *in vitro* studies have confirmed the oncogenic potential of at least some of the identified fusions (101). The prevalence of *RET* rearrangements is estimated at between 1-2%, being higher in never or light smokers (92,101). The *RET* kinase inhibitor vandetanib (102) is a well established treatment for medullary thyroid carcinoma and may be a treatment option for *RET* positive adenocarcinoma of the lung.

PIK3CA mutation

PIK3CA is a known oncogene central to the phosphatidylinositide 3-kinase (PI3K) pathway that is deregulated in multiple cancer types (103). *PIK3CA* has been found altered in 1-2% of lung adenocarcinomas, and may co-exist with other mutant oncogenes (104-106). There is considerable effort to target this gene in other cancer types, and early phase trials are underway with *PIK3CA* targeted therapy for lung cancer both as monotherapy and in combination with other targeted agents and chemotherapy.

Squamous cell carcinomas

Recent progress has identified three potential therapeutic targets in squamous cell carcinoma of the lung. The fibroblast growth factor receptor 1 (FGFR1) is one such target, which is amplified in 21-22% of squamous cell carcinomas in recent studies (107,108). These studies also showed that *FGFR1* amplified cells underwent apoptosis when treated with a small molecule FGFR1 inhibitor, and *FGFR1* amplified tumours in mice shrank with inhibitor therapy, suggesting that FGFR1 is an important driver in

some squamous cell carcinomas. Multiple small molecule inhibitors of FGFR1 are in development and entering early phase trials, with promising preliminary activity (109).

Mutations in the receptor tyrosine kinase *DDR2* gene have been seen in 2% of squamous cell carcinomas of the lung (9,110). TKIs widely used in treating chronic myeloid leukaemia such as dasatinib also have activity against *DDR2*. Dasatinib has produced partial responses in some squamous NSCLC patients in phase I trials (111,112). In one of the patients with a response, sequencing of a tumour biopsy revealed a *DDR2* mutation (110). Phase II trials of dasatinib specifically in squamous cell carcinoma of the lung are underway.

Alterations in genes playing a role in the PI3K pathway are present in 30-50% of squamous cell carcinomas, mostly comprising *PIK3CA* amplification and mutation, and deletion of the tumour suppressor gene *PTEN* (9,106). This pathway is important to maintaining cell survival and promoting growth (103), but the relationship between alterations in this pathway and response to inhibitors is complex. Phase I trials of *PIK3CA* inhibitors are underway in squamous NSCLC.

Targeting the tumour microenvironment

Angiogenesis in lung cancer

Angiogenesis has emerged as a broadly available target in multiple cancer types, as any sizeable tumour requires the ability to form a new blood supply to survive (113,114). The most well studied pathway mediating angiogenesis involves the vascular endothelial growth factor (VEGF) family of ligands and associated receptors which have intracellular tyrosine kinase domains that mediate downstream signalling (115). Targeting VEGF receptor tyrosine kinase signalling using small molecule inhibitors has generally proven unsuccessful, despite multiple agents having been tested in phase III trials (116-122). The VEGF and FGF receptor inhibitor nintedanib combined with chemotherapy has shown a marginal benefit of less than one month in progression free survival over chemotherapy alone, as second line treatment of advanced NSCLC in two phase III trials (123,124).

Bevacizumab is the most widely used anti-angiogenic agent in routine practice. It is a recombinant humanised monoclonal antibody that binds to VEGF, specifically the VEGF-A isoform, and prevents activation of the VEGF receptor (125). The Eastern Cooperative Oncology Group E4599 trial was performed in 878 patients with advanced NSCLC, and compared bevacizumab plus chemotherapy with carboplatin and paclitaxel to chemotherapy alone (126). Bevacizumab was continued as maintenance therapy until progression after 6 cycles of chemotherapy. Median overall survival was superior with bevacizumab at 12.3 versus 10.3 months (HR 0.79, 95% CI: 0.67-0.92; P=0.003). Progression free survival and response rate were also superior with bevacizumab in a second phase III trial AVAiL, although overall survival was no different (127). Toxicities of bevacizumab include arterial thromboembolism, hypertension, augmented chemotherapy-related haematological toxicity and bleeding (126). Due to the higher risk of significant haemoptysis, bevacizumab should not be used for squamous cell histology. Bevacizumab has not had widespread uptake as standard first line therapy outside of the United States due to concerns about toxicity, cost and the lack of a biomarker predictive of benefit.

Immunotherapy

Recent advances in tumour immunology have revealed that the immune system plays an important role in controlling malignant growth, and shapes the characteristics of the tumour that eventually manifests clinically (128). Harnessing the immune system as a therapeutic modality has already shown success in advanced melanoma (129) and prostate cancer (130). Although traditionally not considered to be an immunogenic tumour type, there is evidence that markers of a host immune response to lung cancer have a significant prognostic impact in both the adjuvant setting and advanced disease (131-134). Enhancing the immune response may therefore represent a rational therapeutic target. Immunotherapy in lung cancer consists primarily of two approaches: vaccines derived from lung cancer cell lines or tumour associated antigens, and immuno-stimulatory checkpoint antibodies.

Vaccines

Several vaccines have shown promising results in phase II trials, and are currently being evaluated in randomised phase III trials. The largest trials will be discussed here.

Belagenpumatucel-L is an irradiated whole cell product consisting of multiple lung cancer cell lines reflecting adenocarcinoma, large cell carcinoma and squamous cell carcinoma histologies together with an immuno-adjuvant (135). A small single arm phase II trial conducted in a mixed population of early stage and advanced lung cancer demonstrated

radiological responses in 15% of patients with measurable disease and a positive correlation between prolonged overall survival and higher vaccine dose (135). Belagenpumatucel-L is being further evaluated in a phase III trial recruiting patients with stage III-IV disease that is stable or responding after first line therapy.

Other vaccines consist of antigens expressed exclusively or predominantly in lung cancer cells. Melanoma-associated antigen-A3 (MAGE-A3) is expressed in 35% of NSCLC (136), and has been prepared as a mono-antigenic vaccine. This was tested in a randomised placebo-controlled phase II trial following resection of stage I-II NSCLC showing cellular expression of MAGE-A3 (137). Following surgery, the disease free survival and overall survival were no different between vaccine and placebo groups, but there were numerically fewer recurrences in the vaccine group after a median of 44 months post surgery (35% versus 43% in placebo group). 2,270 patients have been recruited to a phase III trial of the MAGE-A3 vaccine, with results awaited.

MUC-1 is an epithelial cell protein that is differentially glycosylated in malignant cells (138) and overexpressed in NSCLC (139,140). The BLP25 vaccine contains the MUC-1 peptide and an immuno-adjuvant encased in a liposomal delivery system (141). In a phase III randomised trial comparing BLP25 to placebo after concurrent or sequential chemoradiotherapy for stage III NSCLC, patients who had received concurrent treatment showed a median overall survival of 30.8 months compared to 20.6 months with placebo (HR 0.78, 95% CI: 0.64-0.95; P=0.016) (142). BLP25 also prolonged survival in a phase II study in advanced NSCLC compared to best supportive care but this was not statistically significant (141). TG4010 is an alternative approach to MUC-1 vaccination, incorporating an attenuated but replication competent vaccinia virus that encodes for the MUC-1 protein and interleukin-2 (143). In a randomised phase II study, cisplatin and gemcitabine plus TG4010 was compared to cisplatin and gemcitabine alone in 148 patients with advanced NSCLC (144). Progression free survival at 6 months was 43% with the vaccine versus 35% without, but this difference was not statistically significant. Further studies with BLP25 and TG4010 are awaited.

Immune checkpoint blockade

Immune checkpoints refer to the molecular mechanisms that control T-cell responses to foreign antigens. Part of the immune checkpoint system encompasses stimulatory or suppressive co-receptors that modulate the interaction of the T-cell receptor (TCR) with human leukocyte antigen (HLA) expressed on the target cell. Two such receptors have emerged as important therapeutic targets in cancer. The cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptor is expressed on T-cells following activation by antigen, and serves to dampen the T-cell response to promote self-tolerance and prevent autoimmune activation. Programmed cell death protein 1 (PD1) is also expressed on T-cells and similarly provides a mechanism for down-regulating the T-cell response if the ligand (programmed cell death 1 ligand 1 or PD-L1, also known as B7) is encountered. Preventing T-cell suppression at the tumour-immune interface by disrupting immunosuppressive signals forms a promising therapeutic strategy for advanced lung cancer that may also extend to adjuvant treatment.

The toxicities of the various immune checkpoint antibodies are similar and relate to autoimmune phenomena such as colitis, skin rash, pneumonitis and endocrinopathies. As these do not overlap with chemotherapy toxicity, combining these treatments with chemotherapy is a feasible approach. Ipilimumab is a humanised IgG1 anti-CTLA-4 receptor antibody, and is already an established therapy for advanced melanoma (129). A randomised placebo controlled trial was conducted comparing ipilimumab plus carboplatin and paclitaxel chemotherapy to placebo plus chemotherapy in 204 patients with advanced NSCLC (145). Ipilimumab was given in two schedules in the treatment arms: concurrent treatment starting from the first cycle of chemotherapy and phased treatment starting after two cycles of chemotherapy. In light of experience with melanoma that ipilimumab may cause an initial worsening in the radiological appearance of lesions used to assess progression free survival, modified immune-related radiological response criteria were used (146). The study was positive for the primary endpoint of immune-related progression free survival, which was 5.7 months in the phased treatment group compared to 4.6 months in the control group (HR 0.72, P=0.05). Efficacy was most pronounced in patients with squamous cell histology. A similar randomised phase II trial was carried out in 130 patients with extensive stage small cell lung cancer, and showed a trend towards improvement in immune-related progression free survival for the phased regimen in combination with chemotherapy compared to chemotherapy alone (6.4 versus 5.3 months; HR 0.64; 95% CI: 0.4-1.02; P=0.03) (147). Further trials for squamous cell lung cancer and small cell lung cancer are planned.

Table 2 Upcoming trials of anti-PD-1 therapy in advanced NSCLC

Population	Treatment arms	Phase
Squamous cell carcinomas of the lung	Nivolumab versus Docetaxel	Phase III
Non-squamous carcinoma of the lung	Nivolumab versus Docetaxel	Phase III
All NSCLC, no previous therapy	Nivolumab monotherapy; Nivolumab + cisplatin/pemetrexed; Nivolumab + carboplatin/paclitaxel; Nivolumab + cisplatin/gemcitabine	Phase I
	Standard first line chemotherapy followed by nivolumab and bevacizumab maintenance	Phase I
	Ipilimumab + nivolumab	Phase I
EGFR-mutant NSCLC	Nivolumab + erlotinib	Phase I
All NSCLC	Lambrolizumab monotherapy; Lambrolizumab + standard chemotherapy; Lambrolizumab in NSCLC overexpressing PD-L1	Phase I

Multiple tumour types express the PD-L1 ligand on their cell surface, highlighting the role of the PD-1 receptor in suppressing anti-tumour T-cell responses (148). Monoclonal antibodies to both PD-1 and PD-L1 have been tested in several phase I trials that enrolled considerable numbers of patients with NSCLC (148,149). In one such trial the anti-PD-1 antibody nivolumab (formerly known as BMS-936558/MDX-1106) produced an unprecedented response rate of 18% amongst 129 NSCLC patients that were heavily pre-treated, with half of these patients having received three or more previous lines of therapy (148). In addition, the anti-PD-L1 antibody BMS-936559 produced response rates of 10% in a phase I trial that included 49 patients with NSCLC (149). The benefit was evident for both squamous cell carcinomas and adenocarcinomas. From these two trials there is early evidence that expression of the PD-L1 ligand in the tumour microenvironment, which can be evaluated with immunohistochemistry, may predict benefit from anti-PD-1/PD-L1 therapies. In addition to nivolumab, lambrolizumab is another anti-PD-1 antibody that has shown efficacy in melanoma and is being evaluated in lung cancer. Upcoming trials involving nivolumab and lambrolizumab are shown in *Table 2*.

Conclusions

The last ten years have seen a revolution in the way that lung cancer is conceptualised and treated, born out by advances in genomics, cell biology and drug development technologies. The same advances that facilitated this revolution will continue to provide a roadmap for ongoing improvements by identifying new targets and defining the mechanisms of treatment failure and resistance. The transition of crizotinib from an investigational compound to an approved therapy in a mere 4 years also provides hope that there will be a rapid expansion in therapeutic options available to patients in the near feature. Similarly, immunotherapy represents an entirely new class of agents with a promising efficacy and toxicity profile. With the arrival of targeted therapy come multiple challenges however. The development of targeted therapies is often at odds with the traditional clinical trial structure required by regulatory authorities, where phase III trials illustrating an overall survival benefit are considered the gold standard. In addition, targeted therapies carry high costs to the patient or funding agency, and the long term economic viability of the current drug development cycle is uncertain. Finally, it is still the case that the majority of patients with advanced lung cancer have no targeted therapy available to them at the current time, either due to a lack of known targets in their tumour or poor access to novel agents. Addressing both these issues will remain a priority if the successes of the past decade are to be maintained.

Acknowledgements

Disclosure: Brett Hughes has served on Advisory Boards for Roche, Pfizer and Boehringer Inglheim. Benjamin Solomon has served on Advisory Boards for Roche, Pfizer, Novartis, Astra Zeneca, Eli Lilly, Clovis Oncology and Boehringer Ingelheim.

References

1. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated

patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. J Clin Oncol 2003;21:2237-46.
2. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 2003;290:2149-58.
3. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
4. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
5. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306-11.
6. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol 2004;22:1103-9.
7. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol 2005;23:857-65.
8. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958-67.
9. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.
10. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
11. Han JY, Park K, Kim SW, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol 2012;30:1122-8.
12. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-8.
13. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
14. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
15. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
16. Sequist LV, Yang JC, Yamamoto N, et al. Phase III Study of Afatinib or Cisplatin Plus Pemetrexed in Patients With Metastatic Lung Adenocarcinoma With EGFR Mutations. J Clin Oncol 2013;31:3327-34.
17. Wu YL, Zhou C, Hu CP, et al. LUX-Lung 6: A randomized, open-label, phase III study of afatinib versus gemcitabine/cisplatin as first-line treatment for Asian patients with EGFR mutation-positive advanced adenocarcinoma of the lung. J Clin Oncol 2013;31:abstr 8016.
18. Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011;29:2866-74.
19. Inoue A, Kobayashi K, Maemondo M, et al. Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naïve non-small cell lung cancer with sensitive EGFR gene mutations (NEJ002). Ann Oncol 2013;24:54-9.
20. Mitsudomi T, Morita S, Yatabe Y, et al. Updated overall survival results of WJTOG 3405, a randomized phase III trial comparing gefitinib with cisplatin plus docetaxel as the first-line treatment for patients with non-small cell lung cancer harboring mutations of the epidermal growth factor rece. J Clin Oncol 2012;30:abstr 7521.
21. Keedy VL, Temin S, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: epidermal growth factor receptor (EGFR) Mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. J Clin Oncol 2011;29:2121-7.
22. Dogan S, Shen R, Ang DC, et al. Molecular epidemiology of EGFR and KRAS mutations in 3,026 lung

adenocarcinomas: higher susceptibility of women to smoking-related KRAS-mutant cancers. Clin Cancer Res 2012;18:6169-77.
23. Peters S, Adjei AA, Gridelli C, et al. Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23 Suppl 7:vii56-64.
24. Ettinger DS, Akerley W, Bepler G, et al. Non-small cell lung cancer. J Natl Compr Canc Netw 2010;8:740-801.
25. Mok T, Yang JJ, Lam KC. Treating patients with EGFR-sensitizing mutations: first line or second line--is there a difference? J Clin Oncol 2013;31:1081-8.
26. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123-32.
27. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527-37.
28. Garassino M, Martelli O, Bettini A, et al. TAILOR: A phase III trial comparing erlotinib with docetaxel as the second-line treatment of NSCLC patients with wild-type EGFR. J Clin Oncol 2012;30:abtract LBA7501.
29. Kim ES, Hirsh V, Mok T, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet 2008;372:1809-18.
30. Cappuzzo F, Ciuleanu T, Stelmakh L, et al. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. Lancet Oncol 2010;11:521-9.
31. Ciuleanu T, Stelmakh L, Cicenas S, et al. Efficacy and safety of erlotinib versus chemotherapy in second-line treatment of patients with advanced, non-small-cell lung cancer with poor prognosis (TITAN): a randomised multicentre, open-label, phase 3 study. Lancet Oncol 2012;13:300-8.
32. Gridelli C, Ciardiello F, Gallo C, et al. First-line erlotinib followed by second-line cisplatin-gemcitabine chemotherapy in advanced non-small-cell lung cancer: the TORCH randomized trial. J Clin Oncol 2012;30:3002-11.
33. Miller VA, Hirsh V, Cadranel J, et al. Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. Lancet Oncol 2012;13:528-38.
34. Ramalingam SS, Blackhall F, Krzakowski M, et al. Randomized phase II study of dacomitinib (PF-00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2012;30:3337-44.
35. Kurai J, Chikumi H, Hashimoto K, et al. Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines. Clin Cancer Res 2007;13:1552-61.
36. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet 2009;373:1525-31.
37. Lynch TJ, Patel T, Dreisbach L, et al. Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter phase III trial BMS099. J Clin Oncol 2010;28:911-7.
38. Pirker R, Pereira JR, von Pawel J, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. Lancet Oncol 2012;13:33-42.
39. Khambata-Ford S, Harbison CT, Hart LL, et al. Analysis of potential predictive markers of cetuximab benefit in BMS099, a phase III study of cetuximab and first-line taxane/carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 2010;28:918-27.
40. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932-7.
41. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
42. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17:1169-80.
43. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 2013;19:2240-7.
44. Chmielecki J, Foo J, Oxnard GR, et al. Optimization

of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. Sci Transl Med 2011;3:90ra59.
45. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
46. Ohashi K, Sequist LV, Arcila ME, et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. Proc Natl Acad Sci U S A 2012;109:E2127-33.
47. Takezawa K, Pirazzoli V, Arcila ME, et al. HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFRT790M mutation. Cancer Discov 2012;2:922-33.
48. Zhang Z, Lee JC, Lin L, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet 2012;44:852-60.
49. Li D, Ambrogio L, Shimamura T, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 2008;27:4702-11.
50. Janjigian YY, Groen HJM, Horn L, et al. Activity and tolerability of afatinib (BIBW 2992) and cetuximab in NSCLC patients with acquired resistance to erlotinib or gefitinib. J Clin Oncol 2011;29:abstr 7525.
51. Sequist L V, Soria JC, Gadgeel SM, et al. First-in-human evaluation of CO-1686, an irreversible, selective, and potent tyrosine kinase inhibitor of EGFR T790M. J Clin Oncol 2013;31:abstr 2524.
52. Rivera VM, Wang F, Anjum R, et al. AP26113 is a dual ALK/EGFR inhibitor: Characterization against EGFR T790M in cell and mouse models of NSCLC. Cancer Res 2012;72:abstr 1794.
53. Wang W, Li Q, Takeuchi S, et al. Met kinase inhibitor E7050 reverses three different mechanisms of hepatocyte growth factor-induced tyrosine kinase inhibitor resistance in EGFR mutant lung cancer. Clin Cancer Res 2012;18:1663-71.
54. Nakagawa T, Takeuchi S, Yamada T, et al. Combined therapy with mutant-selective EGFR inhibitor and Met kinase inhibitor for overcoming erlotinib resistance in EGFR-mutant lung cancer. Mol Cancer Ther 2012;11:2149-57.
55. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
56. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190-203.
57. Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1994;263:1281-4.
58. McDermott U, Iafrate AJ, Gray NS, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res 2008;68:3389-95.
59. Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci U S A 2008;105:19893-7.
60. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008;14:4275-83.
61. Cui JJ, Tran-Dubé M, Shen H, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem 2011;54:6342-63.
62. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
63. Camidge DR, Bang YJ, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. Lancet Oncol 2012;13:1011-9.
64. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol 2011;12:1004-12.
65. Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27:4247-53.
66. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res 2009;15:5216-23.
67. Wong DW, Leung EL, So KK, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. Cancer 2009;115:1723-33.
68. Zhang YG, Jin ML, Li L, et al. Evaluation of ALK rearrangement in Chinese non-small cell lung cancer using FISH, immunohistochemistry, and real-time quantitative RT-PCR on paraffin-embedded tissues. PLoS One 2013 May;8:e64821.

69. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus Chemotherapy in Advanced ALK-Positive Lung Cancer. N Engl J Med 2013;368:2385-94.
70. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular Testing Guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Mol Diagn 2013;15:415-53.
71. Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. Sci Transl Med 2012;4:120ra17.
72. Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 2012;18:1472-82.
73. Gainor JF, Varghese AM, Ou SH, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. Clin Cancer Res 2013;19:4273-81.
74. Kim S, Kim TM, Kim DW, et al. Heterogeneity of genetic changes associated with acquired crizotinib resistance in ALK-rearranged lung cancer. J Thorac Oncol 2013;8:415-22.
75. Shaw AT, Engelman JA. ALK in lung cancer: past, present, and future. J Clin Oncol 2013;31:1105-11.
76. Seto T, Kiura K, Nishio M, et al. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study. Lancet Oncol 2013;14:590-8.
77. Shaw AT, Mehra R, Kim DW, et al. Clinical activity of the ALK inhibitor LDK378 in advanced, ALK-positive NSCLC. J Clin Oncol 2013;31:abstr 8010.
78. Graziano SL, Gamble GP, Newman NB, et al. Prognostic significance of K-ras codon 12 mutations in patients with resected stage I and II non-small-cell lung cancer. J Clin Oncol 1999;17:668-75.
79. Slebos RJ, Kibbelaar RE, Dalesio O, et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med 1990;323:561-5.
80. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol 2008;9:962-72.
81. Mao C, Qiu LX, Liao RY, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. Lung Cancer 2010;69:272-8.
82. Sun JM, Hwang DW, Ahn JS, et al. Prognostic and predictive value of KRAS mutations in advanced non-small cell lung cancer. PLoS One 2013;8:e64816.
83. Zimmermann G, Papke B, Ismail S, et al. Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic KRAS signalling. Nature 2013;497:638-42.
84. Wang Y, Kaiser CE, Frett B, et al. Targeting Mutant KRAS for Anticancer Therapeutics: A Review of Novel Small Molecule Modulators. J Med Chem 2013;56:5219-30.
85. Yeh TC, Marsh V, Bernat BA, et al. Biological characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. Clin Cancer Res 2007;13:1576-83.
86. Jänne PA, Shaw AT, Pereira JR, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol 2013;14:38-47.
87. Khan KH, Yan L, Mezynski J, et al. A phase I dose escalation study of oral MK-2206 (allosteric Akt inhibitor) with oral selumetinib (AZD6244; ARRY-142866) (MEK 1/2 inhibitor) in patients with advanced or metastatic solid tumors. J Clin Oncol 2012;30:abstr e13599.
88. Barlesi F, Blons H, Beau-Faller M, et al. Biomarkers France: Results of routine EGFR, HER2, KRAS, BRAF, PI3KCA mutations detection and EML4-ALK gene fusion assessment on the first 10,000 non-small cell lung cancer patients. J Clin Oncol 2013;31:abstr 8000.
89. Kim ES, Herbst RS, Wistuba II, et al. The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov 2011;1:44-53.
90. Imielinski M, Berger AH, Hammerman PS, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012;150:1107-20.
91. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-70.
92. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med 2012;18:378-81.
93. Awad MM, Katayama R, McTigue M, et al. Acquired resistance to crizotinib from a mutation in CD74-ROS1. N Engl J Med 2013;368:2395-401.
94. Planchard D, Mazieres J, Riely GJ, et al. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation-positive non-small cell lung cancer (NSCLC) patients. J Clin Oncol 2013;31:abstr 8009.
95. Paik PK, Arcila ME, Fara M, et al. Clinical characteristics

of patients with lung adenocarcinomas harboring BRAF mutations. J Clin Oncol 2011;29:2046-51.
96. Marchetti A, Felicioni L, Malatesta S, et al. Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. J Clin Oncol 2011;29:3574-9.
97. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res 2005;65:1642-6.
98. Clamon G, Herndon J, Kern J, et al. Lack of trastuzumab activity in nonsmall cell lung carcinoma with overexpression of erb-B2: 39810: a phase II trial of Cancer and Leukemia Group B. Cancer 2005;103:1670-5.
99. Kwak E. The role of irreversible HER family inhibition in the treatment of patients with non-small cell lung cancer. Oncologist 2011;16:1498-507.
100. De Grève J, Teugels E, Geers C, et al. Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. Lung Cancer 2012;76:123-7.
101. Lipson D, Capelletti M, Yelensky R, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18:382-4.
102. Wells SA Jr, Robinson BG, Gagel RF, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol 2012;30:134-41.
103. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550-62.
104. Garnett MJ, Edelman EJ, Heidorn SJ, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 2012;483:570-5.
105. Chaft JE, Arcila ME, Paik PK, et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. Mol Cancer Ther 2012;11:485-91.
106. Yamamoto H, Shigematsu H, Nomura M, et al. PIK3CA mutations and copy number gains in human lung cancers. Cancer Res 2008;68:6913-21.
107. Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2:62ra93.
108. Dutt A, Ramos AH, Hammerman PS, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 2011;6:e20351.
109. Wolf J, LoRusso PM, Camidge RD, et al. A phase I dose escalation study of NVP-BGJ398, a selective pan FGFR inhibitor in genetically preselected advanced solid tumors. Cancer Res 2012;72:abstr nr LB-122.
110. Hammerman PS, Sos ML, Ramos AH, et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. Cancer Discov 2011;1:78-89.
111. Haura EB, Tanvetyanon T, Chiappori A, et al. Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non-small-cell lung cancer. J Clin Oncol 2010;28:1387-94.
112. Johnson FM, Bekele BN, Feng L, et al. Phase II study of dasatinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:4609-15.
113. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. Ann Surg 1972;175:409-16.
114. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
115. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669-76.
116. Paz-Ares LG, Biesma B, Heigener D, et al. Phase III, randomized, double-blind, placebo-controlled trial of gemcitabine/cisplatin alone or with sorafenib for the first-line treatment of advanced, nonsquamous non-small-cell lung cancer. J Clin Oncol 2012;30:3084-92.
117. Scagliotti G, Novello S, von Pawel J, et al. Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non-small-cell lung cancer. J Clin Oncol 2010;28:1835-42.
118. Scagliotti GV, Krzakowski M, Szczesna A, et al. Sunitinib plus erlotinib versus placebo plus erlotinib in patients with previously treated advanced non-small-cell lung cancer: a phase III trial. J Clin Oncol 2012;30:2070-8.
119. Lee JS, Hirsh V, Park K, et al. Vandetanib Versus placebo in patients with advanced non-small-cell lung cancer after prior therapy with an epidermal growth factor receptor tyrosine kinase inhibitor: a randomized, double-blind phase III trial (ZEPHYR). J Clin Oncol 2012;30:1114-21.
120. De Boer RH, Arrieta Ó, Yang CH, et al. Vandetanib plus pemetrexed for the second-line treatment of advanced non-small-cell lung cancer: a randomized, double-blind phase III trial. J Clin Oncol 2011;29:1067-74.
121. Natale RB, Thongprasert S, Greco FA, et al. Phase III trial of vandetanib compared with erlotinib in patients with previously treated advanced non-small-cell lung cancer. J Clin Oncol 2011;29:1059-66.
122. Scagliotti GV, Vynnychenko I, Park K, et al. International, randomized, placebo-controlled, double-blind phase III study of motesanib plus carboplatin/paclitaxel in patients

with advanced nonsquamous non-small-cell lung cancer: MONET1. J Clin Oncol 2012;30:2829-36.
123. Reck M, Kaiser R, Mellemgaard A, et al. Nintedanib (BIBF 1120) plus docetaxel in NSCLC patients progressing after first-line chemotherapy: LUME Lung 1, a randomized, double-blind phase III trial. J Clin Oncol 2013;31:abstr LBA8011.
124. Hanna NH, Kaiser R, Sullivan RN, et al. Lume-lung 2: A multicenter, randomized, double-blind, phase III study of nintedanib plus pemetrexed versus placebo plus pemetrexed in patients with advanced nonsquamous non-small cell lung cancer (NSCLC) after failure of first-line chemotherapy. J Clin Oncol 2013;31:abstr 8034.
125. Ferrara N, Hillan KJ, Gerber HP, et al. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 2004;3:391-400.
126. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355:2542-50.
127. Reck M, von Pawel J, Zatloukal P, et al. Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). Ann Oncol 2010;21:1804-9.
128. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565-70.
129. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
130. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-22.
131. Al-Shibli KI, Donnem T, Al-Saad S, et al. Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. Clin Cancer Res 2008;14:5220-7.
132. Kawai O, Ishii G, Kubota K, et al. Predominant infiltration of macrophages and CD8(+) T Cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. Cancer 2008;113:1387-95.
133. Petersen RP, Campa MJ, Sperlazza J, et al. Tumor infiltrating Foxp3+ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients. Cancer 2006;107:2866-72.
134. Tao H, Mimura Y, Aoe K, et al. Prognostic potential of FOXP3 expression in non-small cell lung cancer cells combined with tumor-infiltrating regulatory T cells. Lung Cancer 2012;75:95-101.
135. Nemunaitis J, Dillman RO, Schwarzenberger PO, et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. J Clin Oncol 2006;24:4721-30.
136. Sienel W, Varwerk C, Linder A, et al. Melanoma associated antigen (MAGE)-A3 expression in Stages I and II non-small cell lung cancer: results of a multi-center study. Eur J Cardiothorac Surg 2004;25:131-4.
137. Vansteenkiste J, Zielinski M, Linder A, et al. Adjuvant MAGE-A3 Immunotherapy in Resected Non-Small-Cell Lung Cancer: Phase II Randomized Study Results. J Clin Oncol 2013;31:2396-403.
138. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, et al. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem 1990;265:15286-93.
139. Rochlitz C, Figlin R, Squiban P, et al. Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer. J Gene Med 2003;5:690-9.
140. Ho SB, Niehans GA, Lyftogt C, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res 1993;53:641-51.
141. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol 2005;23:6674-81.
142. Butts CA, Socinski MA, Mitchell P, et al. START: A phase III study of L-BLP25 cancer immunotherapy for unresectable stage III non-small cell lung cancer. J Clin Oncol 2013;31:abstr 7500.
143. Ramlau R, Quoix E, Rolski J, et al. A phase II study of Tg4010 (Mva-Muc1-Il2) in association with chemotherapy in patients with stage III/IV Non-small cell lung cancer. J Thorac Oncol 2008;3:735-44.
144. Quoix E, Ramlau R, Westeel V, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled phase 2B trial. Lancet Oncol 2011;12:1125-33.
145. Lynch TJ, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol 2012;30:2046-54.
146. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the

evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412-20.
147. Reck M, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease-small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. Ann Oncol 2013;24:75-83.
148. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443-54.
149. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65.

Cite this article as: Savas P, Hughes B, Solomon B. Targeted therapy in lung cancer: IPASS and beyond, keeping abreast of the explosion of targeted therapies for lung cancer. J Thorac Dis 2013;5(S5):S579-S592. doi: 10.3978/j.issn.2072-1439.2013.08.52

Medical Oncology

Apoptotic agents

Dean A. Fennell

University of Leicester & Leicester University Hospitals, UK

Correspondence to: Prof. Dean A. Fennell, PhD, FRCP, Chair of Thoracic Medical Oncology. University of Leicester & Leicester University Hospitals. Hodgkin Building, PO Box 138, Lancaster Road, Leicester LE1 9HN, UK. Email: df132@le.ac.uk.

Submitted Dec 16, 2012. Accepted for publication Jan 15, 2013.
doi: 10.3978/j.issn.2218-6751.2013.01.03
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.01.03

Activation of apoptosis (programmed cell death) is a highly efficient means of tumour suppression frequently hijacked in lung cancer, and is a major goal of cancer drug therapy. When this can be achieved in the clinic, it is associated with durable disease control. Targeting the core apoptosis pathway has been a research goal since its initial discovery, and outstanding research endeavours have been translated into discovery of a new class of potent, targeted "apoptotic agents". Despite this, early phase II clinical trials have not met with initial expectations. This review addresses the challenges and significant potential, in the light of recent discoveries, for personalising therapy with apoptotic agents as a basis for improving outcomes in lung cancer.

Apoptosis: a primer

The core apoptosis pathway constitutes a genetically hardwired, and highly regulated mechanism for ensuring cellular demise. It plays a critical role in development, but is hijacked by cancer cells, as an essential transforming process during tumor evolution (1). Apoptotic cell death involves three key events; firstly, an initiation phase engaged by a stimulus such as cellular damage, stress, or inhibition of critical growth factor pathways. Secondly, a commitment phase in which an irreversible decision to initiate apoptosis is made. Mitochondria play a critical role in this phase. Thirdly, the execution phase involving cellular demise. Much is understood regarding the regulation of the apoptotic pathways, in particular, the interplay of the BCL2 proteins which orchestrate the signalling of these first and second phases.

The BCL2 family are composed of pro- and antiapoptotic members which physically interact to govern the initiation of apoptosis (2-4). This event is regulated by the oligomerisation of multidomain proapoptotic BCL2 proteins BAK and BAX, which constitutively reside in the outer mitochondrial membrane and/or cytoplasm respectively (2,5,6). The trigger for oligomerisation is the BH3-only domain protein sub-family (which comprises at least 8 proteins—BID, BIM, PUMA, BAD, NOXA, BFM, BNIP3, and HRK). BH3 proteins are activated either by transcriptional upregulation e.g., Death receptor triggered cleavage of BID, P53 driven upregulation of NOXA/PUMA) or post-translational modification of BIM by phosphorylation). These proteins then cause apoptosis by either directly triggering oligomerisation of BAX/BAK (BID,BIM,PUMA) or releasing BAX/BAK from members of the prosurvival BCL2 family (BCLX, BCL2, BCLW, MCL1, A1) (7-12). The propensity of BAX/BAK to oligomerise is governed by the ratio of prosurvival to proapoptotic proteins. Cancer cells appear to constitutely activate BH3 proteins (13-15). In order to protect against apoptosis, selection for amplification of prosurvival BCL2 family proteins BCLX and MCL1 occurs as a common event (16). Amplification is associated with dependency which may be therapeutically tractable as discussed further on.

BAX/BAK oligomerisation causes permeabilisation of the outer mitochondrial membrane, releasing multiple pro-apoptotic factors into the cytosol (17-20). This is the event which constitutes irreversible commitment to death—the beginning of the end for the cancer cell. Cellular demolition is executed by the caspases, a family of zymogens which are post-translationally modified leading to their activation (21,22).

Apoptosis and therapeutic outcomes in lung cancer

In recent years, it has become clear, that to achieve

effective outcomes in cancer therapy, induction of apoptosis appears to be a critical requirement. This is borne out in the dramatic radiological regressions associated with inhibition of non-squamous non-small cell lung cancer, harbouring either somatic mutations of the epidermal growth factor receptor (23,24) or an anaplastic lymphoma kinase fusion protein (EML4-ALK) (25-27). These so-called "driver oncogenes" constitutively activate, and lead to dependency on, growth factor signalling pathways involving phosphoinositide 3 kinase/AKT/mTOR and mitogen activated protein kinase (MAPK) axes (28). As a consequence, these pathways constitutively phosphorylate and suppress the BH3 only protein BIM. Following the inhibition of mutated receptor EGFR or ALK receptor tyrosine kinases, BIM is unleashed, leading to activation of BAX/BAK and apoptosis (29-33). Indeed, BIM expression is a prerequisite for clinical activity (34,35). This new paradigm involving targeting of driver oncogene addiction has shown that the mitochondrial apoptosis pathway is fully functional in NSCLC, and that provided a driver oncogene dependency can be identified, mitochondrial apoptosis can be efficiently activated leading to significant improvement in clinical outcome. With the most comprehensive genomic landscape studies to date having recently defined the extent of common somatic mutations in lung cancer (36-38), it is likely that many more clinically tractable oncogene addictions will be validated as effective targets for inducing apoptosis efficiently.

Personalising anti-apoptotic BCL2 inhibition

Prosurvival BCL2 proteins suppress BAX/BAK activation by sequestering both of these multidomain proteins and/or BH3 only proteins (2). The first and most specific inhibitor of BCL2/X/W was ABT-263 (Abbott) (39). Phase II studies were conducted in small cell lung cancer, based on preclinical evidence of addiction to BCL2. However, limited efficacy was observed (40). Why was this? MCL1 is a widely overexpressed prosurvival protein; indeed it is one of the most commonly amplified genes in cancer (16). MCL1 efficiently overcomes the proapoptotic effects of ABT263 and may play a role in clinical drug resistance (41-45). Importantly, the prosurvival BCL2 family addiction observed in cell lines and xenografts was not borne out in heterotransplants nor patients, suggesting that SCLC may not be "predominantly BCL2/BCLX" addicted in the clinical setting. Furthermore, it is clear in SCLC that the tumour microenvironment could significantly impact cancer cell biology by significantly attenuating apoptotic susceptibility (46,47), something which has been modelled preclinically in NSCLC and mesothelioma (48,49). Nevertheless, patient subgroup analysis showed that in patients with high circulating Pro-GRP, encoded by a gene neighbouring BCL-2 and co-amplified in SCLCs with BCL2 amplification, there was a greater response rate (40). This suggests, that in the context of BCL2 amplified SCLC, AB263 may exhibit single agent activity consistent with a degree of sensitivity. This genetic event exists only in a proportion of patients with SCLC, implicating a need to select patients harbouring BCL-2 amplification. Indeed, in common with other modes of targeted therapy, treating the right target population is likely to be a critical requirement for achieving clinically relevant activity when considered as single agents.

Recent analysis of genome-wide somatic copy number variations in cancer has revealed BCLX encoded by BCL2L2 and MCL-1, as the most frequently amplified genes in the cancer genome, and are encoded at 1q21.2 and 20q21 respectively (16). Where there is evidence of amplification, this appears to be associated with addiction, at least at the preclinical level. A proportion of NSCLCs harbour amplification at these loci, suggesting that addiction could be exploited. One novel approach has been recently reported. A search for transcriptional repressors of MCL1 (which has an exceedingly short protein half-life of around 30 minutes) identified anthracyclines as potent MCL-1 inhibitors (50). These compounds owe their proapoptotic activity to the transcriptional repression of MCL1, leading to its rapid downregulation at protein level. In the context of 1q21.2 amplification, this is associated with induction of apoptosis. This raises the intriguing question as to whether or not anthracyclines may exhibit particularly high activity in the context of 1q21.2 amplification in NSCLC, and deserved to be addressed in a clinical trial. High dose epirubicin has an associated response rate of around 25%, and 1q21.2 amplification occurs in around 25% of patients (36,51). Whether the majority of responders to epirubicin were also 1q21.2 amplified, is as yet, unknown.

Taken together, it appears that addiction to prosurvival BCL2 family proteins is restricted to subsets of lung cancers. These subsets may be identifiable through detection of somatic mutations involving amplification. Apoptotic agents targeting prosurvival BCL2 proteins are, when considered as monotherapy, are likely to be much like any other targeted agent, in that they may only exhibit useful efficacy in restricted subsets of cancers, perhaps

identifiable through individual copy number variations.

Death receptors

The apoptosis pathway can be directly activated through the ligation of cell surface receptors related to the tumour necrosis factor superfamily which include tumour necrosis factor related apoptosis inducing ligand (TRAIL) receptors (52,53). A direct consequence of receptor oligomerisation is the assembly of a cell surface signalling module (the death inducing signalling complex or DISC), which comprises homotypic domain interactions between receptor (TRAIL receptor 1 or 2), an adaptor (FADD), and an apical caspase (8 or 10). The proapoptotic activity of TRAIL ligands is selective for cancer versus normal cells (54). Recently, it has been shown that *in vivo*, disruption of tumour endothelial vasculature by TRAIL causes tumour regression (55). Agonistic antibody based activation of receptors for TRAIL have been explored in a series of recent phase II clinical trials in non-small cell lung cancer (56,57). Preclinical studies demonstrated promising synergy when combined with chemotherapy and other targeted agents (58-61). Unfortunately, predicted improvement in efficacy was not confirmed in unselected patients (62) . Despite this, it has been found that TRAIL monotherapy is potentially very active in a small population of patients with NSCLC. For example, one patient with chemorefractory NSCLC exhibited a confirmed response lasting 96 weeks following the agonistic DR5 antibody (conotumumab, AMG-655) (63). This potentially reflects an underlying "hypersensitive" subgroup for which, there is at present, no validated predictive biomarker. TRAIL agonists are inhibited by the cellular FLICE like inhibitor protein (c-FLIP) which exhibits high expression in non-small cell lung cancer, and the ratio of FLIP to caspase 8 is a potential rheostat, regulating sensitivity to TRAIL receptor agonists. Similarly, O'glycosylation (64) and VDAC1 have been implicated as regulators of TRAIL sensitivity preclinically (65). What role these potential biomarkers have *in vivo*, if any, should be systematically investigated in future studies in order to maximize the likelihood of identifying a TRAIL receptor agonist sensitive population; something which clearly exists, albeit perhaps at low frequency.

Smac'ing lung cancer

During permeabilization of the mitochondrial outer membrane, one of the apoptogenic factors released is the second mitochondria derived activator of apoptosis (SMAC) (20). Since its discovery, SMAC was shown to target inhibitor of apoptosis proteins, which constitutively suppress caspase activation and therefore the execution phase of apoptosis. The conserved tetrapeptide motif AVPI in SMAC interacts with the BIR domain of caspase 3, blocking its activation. Structure based analysis of this interaction led to a rational drug discovery effort to create so-called smac mimetics (66,67). This class of pharmacology however was shown to uncover a programmed necrosis pathway (68,69). In an inflammatory microenvironment, cytokine activation of TNF receptors leads to the assembly of a so called type 1 complex, which is prosurvival, and signals to caspase 8 through NF kappa beta. This signalling is dependent upon bound cIAP1 and cIAP2. SMAC or its mimetics interact with cIAP1/cIAP2 leading to their rapid ubiquitination and degradation. The consequence is the recruitment of TNF receptor with caspase 8 into complex II, comprising RIP kinase which leads to necrotic death of the cell. This death signalling is driven by TNF receptor activation; as such, the conversion of a survival pathway, to a death signalling pathway following IAP degradation, effectively exploits the tumour microenvironment and so constitute a "death switch".

SMAC mimetics are at the earliest stage of development with respect to "apoptotic agents" and are currently under phase 1 evaluation in the clinic (70). At present, there are, as yet no defined molecular biomarkers of clinical sensitivity, however it is clear from preclinical studies that autocrine TNF-alpha activation facilitates the synergistic interaction between SMAC mimetic and chemotherapy (71). Accordingly, there is an expectation that this class of agent might be most effective in highly inflammatory cancers.

Systematic approaches for personalising apoptotic agents

An initiative entitled the genomics of drug sensitivity established as a collaboration between the Wellcome Sanger Institute in the UK and Massachusettes General Hospital/Harvard, in the USA, provides a potentially high throughput platform for identifying genetic biomarkers of sensitivity and/or resistance, that might aid clinical development of apoptotic agents (72,73). Using over 1,000 genetically defined cell lines, a candidate drug is screened for sensitivity. The correlation between sensitivity measured by IC50 and genetic mutations are determined. As such,

this provides a remarkably powerful tool for hypothesis generation, particularly around hitherto unanticipated but statistically robust drug-gene associations. For example, for ABT-263, the CML driver oncogene bcr-abl is highly correlated with *in vitro* activity (72).

Summary

Efficient induction of apoptosis is a prerequisite for effective disease control in the management of lung cancer, exemplified by receptor tyrosine kinase inhibitor efficacy in EGFR and EML4-ALK mutated NSCLC. Decades after the discovery of the core apoptosis signalling pathways, apoptotic agents have finally been developed with potent on-target activity. Population based genetic heterogeneity of lung cancer is now an accepted reality that has underpinned successful stratified therapy. Despite this, development of apoptotic agents has been predominantly conducted in unselected populations. The challenge moving forward will be understand how best to target these drugs using molecular biomarkers, so as to maximize patient benefit in selected subgroups.

Acknowledgements

Disclosure: The author declares no conflicts of interest.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
2. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993;74:609-19.
3. Kuwana T, Mackey MR, Perkins G, et al. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 2002;111:331-42.
4. Llambi F, Moldoveanu T, Tait SW, et al. A unified model of mammalian BCL-2 protein family interactions at the mitochondria. Mol Cell 2011;44:517-31.
5. Yin C, Knudson CM, Korsmeyer SJ, et al. Bax suppresses tumorigenesis and stimulates apoptosis in vivo. Nature 1997;385:637-40.
6. Wei MC, Zong WX, Cheng EH, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 2001;292:727-30.
7. Ren D, Tu HC, Kim H, et al. BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program. Science 2010;330:1390-3.
8. Kim H, Tu HC, Ren D, et al. Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. Mol Cell 2009;36:487-99.
9. Kim H, Rafiuddin-Shah M, Tu HC, et al. Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. Nat Cell Biol 2006;8:1348-58.
10. Letai A, Bassik MC, Walensky LD, et al. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell 2002;2:183-92.
11. Lovell JF, Billen LP, Bindner S, et al. Membrane binding by tBid initiates an ordered series of events culminating in membrane permeabilization by Bax. Cell 2008;135:1074-84.
12. Cheng EH, Wei MC, Weiler S, et al. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell 2001;8:705-11.
13. Certo M, Del Gaizo Moore V, Nishino M, et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell 2006;9:351-65.
14. Vo TT, Ryan J, Carrasco R, et al. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. Cell 2012;151:344-55.
15. Ni Chonghaile T, Sarosiek KA, Vo TT, et al. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. Science 2011;334:1129-33.
16. Beroukhim R, Mermel CH, Porter D, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899-905.
17. Zou H, Henzel WJ, Liu X, et al. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997;90:405-13.
18. Liu X, Kim CN, Yang J, et al. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 1996;86:147-57.
19. Li P, Nijhawan D, Budihardjo I, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91:479-89.
20. Du C, Fang M, Li Y, et al. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000;102:33-42.
21. Salvesen GS, Dixit VM. Caspases: intracellular signaling by proteolysis. Cell 1997;91:443-6.
22. Martin SJ, Green DR. Protease activation during apoptosis: death by a thousand cuts? Cell 1995;82:349-52.

23. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
24. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
25. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
26. Gerber DE, Minna JD. ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. Cancer Cell 2010;18:548-51.
27. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
28. Sordella R, Bell DW, Haber DA, et al. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004;305:1163-7.
29. Deng J, Shimamura T, Perera S, et al. Proapoptotic BH3-only BCL-2 family protein BIM connects death signaling from epidermal growth factor receptor inhibition to the mitochondrion. Cancer Res 2007;67:11867-75.
30. Cragg MS, Kuroda J, Puthalakath H, et al. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med 2007;4:1681-89; discussion 1690.
31. Costa DB, Halmos B, Kumar A, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med 2007;4:1669-79; discussion 1680.
32. Gong Y, Somwar R, Politi K, et al. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. PLoS Med 2007;4:e294.
33. Takezawa K, Okamoto I, Nishio K, et al. Role of ERK-BIM and STAT3-survivin signaling pathways in ALK inhibitor-induced apoptosis in EML4-ALK-positive lung cancer. Clin Cancer Res 2011;17:2140-8.
34. Ng KP, Hillmer AM, Chuah CT, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 2012;18:521-8.
35. Faber AC, Corcoran RB, Ebi H, et al. BIM expression in treatment-naive cancers predicts responsiveness to kinase inhibitors. Cancer Discov 2011;1:352-65.
36. Imielinski M, Berger AH, Hammerman PS, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012;150:1107-20.
37. Cancer Genome Atlas Research Network, Hammerman PS, Hayes DN, et al. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.
38. Govindan R, Ding L, Griffith M, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150:1121-34.
39. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435:677-81.
40. Rudin CM, Hann CL, Garon EB, et al. Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. Clin Cancer Res 2012;18:3163-9.
41. Tahir SK, Yang X, Anderson MG, et al. Influence of Bcl-2 family members on the cellular response of small-cell lung cancer cell lines to ABT-737. Cancer Res 2007;67:1176-83.
42. Chen S, Dai Y, Harada H, et al. Mcl-1 down-regulation potentiates ABT-737 lethality by cooperatively inducing Bak activation and Bax translocation. Cancer Res 2007;67:782-91.
43. Lin X, Morgan-Lappe S, Huang X, et al. 'Seed' analysis of off-target siRNAs reveals an essential role of Mcl-1 in resistance to the small-molecule Bcl-2/Bcl-XL inhibitor ABT-737. Oncogene 2007;26:3972-9.
44. van Delft MF, Wei AH, Mason KD, et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 2006;10:389-99.
45. Konopleva M, Contractor R, Tsao T, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006;10:375-88.
46. Sethi T, Rintoul RC, Moore SM, et al. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance in vivo. Nat Med 1999;5:662-8.
47. Rintoul RC, Sethi T. The role of extracellular matrix in small-cell lung cancer. Lancet Oncol 2001;2:437-42.
48. Barbone D, Yang TM, Morgan JR, et al. Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids. J Biol Chem 2008;283:13021-30.
49. Barbone D, Ryan JA, Kolhatkar N, et al. The Bcl-2 repertoire of mesothelioma spheroids underlies acquired apoptotic multicellular resistance. Cell Death Dis 2011;2:e174.

50. Wei G, Margolin AA, Haery L, et al. Chemical genomics identifies small-molecule MCL1 repressors and BCL-xL as a predictor of MCL1 dependency. Cancer Cell 2012;21:547-62.
51. Smit EF, Berendsen HH, Piers DA, et al. A phase II study of high dose epirubicin in unresectable non small cell lung cancer. Br J Cancer 1992;65:405-8.
52. Hymowitz SG, Christinger HW, Fuh G, et al. Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. Mol Cell 1999;4:563-71.
53. Sheridan JP, Marsters SA, Pitti RM, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 1997;277:818-21.
54. Yada A, Yazawa M, Ishida S, et al. A novel humanized anti-human death receptor 5 antibody CS-1008 induces apoptosis in tumor cells without toxicity in hepatocytes. Ann Oncol 2008;19:1060-7.
55. Wilson NS, Yang A, Yang B, et al. Proapoptotic activation of death receptor 5 on tumor endothelial cells disrupts the vasculature and reduces tumor growth. Cancer Cell 2012;22:80-90.
56. Soria JC, Smit E, Khayat D, et al. Phase 1b study of dulanermin (recombinant human Apo2L/TRAIL) in combination with paclitaxel, carboplatin, and bevacizumab in patients with advanced non-squamous non-small-cell lung cancer. J Clin Oncol 2010;28:1527-33.
57. Greco FA, Bonomi P, Crawford J, et al. Phase 2 study of mapatumumab, a fully human agonistic monoclonal antibody which targets and activates the TRAIL receptor-1, in patients with advanced non-small cell lung cancer. Lung Cancer 2008;61:82-90.
58. Jin H, Yang R, Ross J, et al. Cooperation of the agonistic DR5 antibody apomab with chemotherapy to inhibit orthotopic lung tumor growth and improve survival. Clin Cancer Res 2008;14:7733-40.
59. Chen KF, Chen HL, Shiau CW, et al. Sorafenib and its derivative SC-49 sensitize hepatocellular carcinoma cells to CS-1008, a humanized anti-DR5 antibody. Br J Pharmacol 2013;168:658-72.
60. Chen KF, Yu HC, Liu CY, et al. Bortezomib sensitizes HCC cells to CS-1008, an antihuman death receptor 5 antibody, through the inhibition of CIP2A. Mol Cancer Ther 2011;10:892-901.
61. Broaddus VC, Dansen TB, Abayasiriwardana KS, et al. Bid mediates apoptotic synergy between tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and DNA damage. J Biol Chem 2005;280:12486-93.
62. Soria JC, Márk Z, Zatloukal P, et al. Randomized phase II study of dulanermin in combination with paclitaxel, carboplatin, and bevacizumab in advanced non-small-cell lung cancer. J Clin Oncol 2011;29:4442-51.
63. Herbst RS, Kurzrock R, Hong DS, et al. A first-in-human study of conatumumab in adult patients with advanced solid tumors. Clin Cancer Res 2010;16:5883-91.
64. Wagner KW, Punnoose EA, Januario T, et al. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. Nat Med 2007;13:1070-7.
65. Chacko AD, Liberante F, Paul I, et al. Voltage dependent anion channel-1 regulates death receptor mediated apoptosis by enabling cleavage of caspase-8. BMC Cancer 2010;10:380.
66. Li L, Thomas RM, Suzuki H, et al. A small molecule Smac mimic potentiates TRAIL- and TNFalpha-mediated cell death. Science 2004;305:1471-4.
67. Flygare JA, Beresini M, Budha N, et al. Discovery of a potent small-molecule antagonist of inhibitor of apoptosis (IAP) proteins and clinical candidate for the treatment of cancer (GDC-0152). J Med Chem 2012;55:4101-13.
68. Wang L, Du F, Wang X. TNF-alpha induces two distinct caspase-8 activation pathways. Cell 2008;133:693-703.
69. Vince JE, Wong WW, Khan N, et al. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 2007;131:682-93.
70. Cai Q, Sun H, Peng Y, et al. A potent and orally active antagonist (SM-406/AT-406) of multiple inhibitor of apoptosis proteins (IAPs) in clinical development for cancer treatment. J Med Chem 2011;54:2714-26.
71. Probst BL, Liu L, Ramesh V, et al. Smac mimetics increase cancer cell response to chemotherapeutics in a TNF-α-dependent manner. Cell Death Differ 2010;17:1645-54.
72. Garnett MJ, Edelman EJ, Heidorn SJ, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 2012;483:570-5.
73. Yang W, Soares J, Greninger P, et al. Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res 2013;41:D955-61.

Cite this article as: Fennell DA. Apoptotic agents. Transl Lung Cancer Res 2013;2(3):238-243. doi: 10.3978/j.issn.2218-6751.2013.01.03

Medical Oncology

Adaptive resistance to targeted therapies in cancer

Rafael Rosell[1,2], Niki Karachaliou[2], Daniela Morales-Espinosa[3], Carlota Costa[2], Miguel Angel Molina[2], Irene Sansano[2,4], Amaya Gasco[5], Santiago Viteri[5], Bartomeu Massuti[6], Jia Wei[7], María González Cao[5], Alejandro Martínez Bueno[5]

[1]Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Badalona, Spain; [2]Pangaea Biotech, Dexeus University Institute, Barcelona, Spain; [3]Hospital Medica Sur, Mexico DF, Mexico; [4]Hospital Vall Hebron, Barcelona, Spain; [5]IOR, Dexeus University Institute, Barcelona, Spain; [6]Hospital General de Alicante, Spain; [7]The Comprehensive Cancer Center of Drum Tower Hospital, Nanjing, China

Correspondence to: Rafael Rosell. Catalan Institute of Oncology, Medical Oncology Service, Hospital Germans Trias i Pujol, Ctra Canyet, s/n, 08916 Badalona, Spain. Email: rrosell@iconcologia.net.

Abstract: It is widely acknowledged that there is a need for molecular profiling in non-small-cell lung cancer. For example, treatment based on EGFR mutation status has attained successful results. However, in spite of excellent initial response to oral EGFR tyrosine kinase inhibitors (TKIs), progression-free survival is still limited. Current research has focused mostly on acquired resistance mechanisms, such as overexpression of AXL and loss of the Mediator MED12. In this review, in contrast, we discuss adaptive, rather than acquired, resistance. Adaptive resistance can occur almost immediately after starting targeted therapy through a rapid rewiring of cancer cell signaling. By losing ERK negative feedback on receptor tyrosine kinase (RTK) expression, cancer cells are exposed to the stimuli of several ligands, and the ensuing activation of several RTKs reprograms all the canonical signaling pathways. The overexpression of several RTKs was observed in breast cancer cell lines treated with a MEK inhibitor and in $BRAF^{V600E}$ melanoma cell lines treated with BRAF inhibitors. This rebound effect of overexpression of several RTKs, including ERBB3, also occurs in lung cancers driven by Kras or EGFR mutations when treated with MEK, PI3K or dual PI3K/mTOR inhibitors. Synthetic lethality can be effectively induced by co-targeting these overexpressed RTKs. We speculate that in patients with EGFR mutations, adaptive resistance occurs in a significant proportion of patients. Rebiopsies performed hours after starting treatment with EGFR TKIs can identify which RTKs are overexpressed after treatment. Efficient co-targeting of these RTKs can induce synthetic lethality and help overcome the limited effect of EGFR TKI monotherapy.

Keywords: Adaptive resistance; RTK reprogramming; signalability; synthetic lethality

Submitted Nov 16, 2012. Accepted for publication Dec 21, 2012.
doi: 10.3978/j.issn.2218-6751.2012.12.08
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2012.12.08

Kinase inhibitors have limited benefit in cancer treatment because of a rebound effect, as exemplified by the inhibition of mitogen-activated protein-extracellular signal-regulated kinase (MEK) in triple-negative breast cancer cells. MEK inhibition causes acute loss of extracellular signal-regulated kinase (ERK), resulting in rapid c-Myc degradation, which induces the expression and activation of several receptor tyrosine kinases (RTKs) (1) (*Figure 1*). The transduction of signals from activated RTKs has been termed "signalability". This signalability is markedly suppressed in $BRAF^{V600E}$ melanomas (2), which have high levels of ERK-dependent feedback and markedly decreased sensitivity to extracellular ligands. BRAF-driven tumors are relatively insensitive to secreted growth factors because of the inability of ligands to induce signaling. However, ERK inhibition reduces the ERK-dependent feedback, growth factors can signal, and the antitumor effects of the inhibitor are attenuated. Thus, the signaling network is radically changed and reactivated as

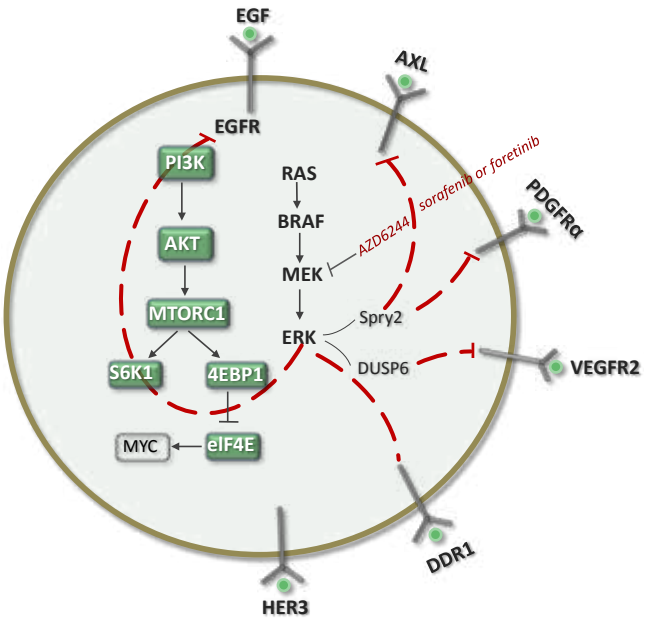


Figure 1 In breast cancer cell lines, MEK inhibition (2), Akt inhibition (3) or mTOR inhibition (4) causes kinome reprogramming in different breast cancer subtypes.

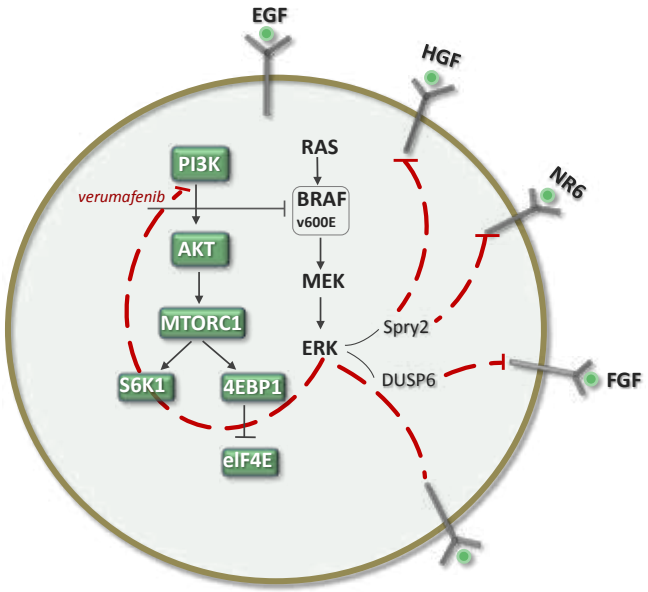


Figure 2 Ligand stimulation of ERK and PI3K signaling in $BRAF^{V600E}$ melanomas. In untreated melanomas, there is low signalability. However, hours after the ERK pathway is inhibited, the transduction of the signal is markedly potentiated (restored signalability). The induction of signalability could be blocked with the addition of an HER kinase inhibitor, such as neratinib (2).

an adaptation to inhibition of ERK signaling (2).

This dynamic reprogramming of signalability was also described in triple-negative breast cancers. MEK inhibition with AZD6244 increased RTK expression and downstream survival signaling, coinciding with the reactivation of RAF-MEK-ERK signaling. AZD6244 induces expression of platelet-derived growth factor receptor beta (PDGFRb), VEGFR2, HER3, AXL and DDR1 (*Figure 1*). Sorafenib inhibits PDGFRa/b, VEGFR2, DDR1/2, BRAF and RAF (1), and the combination of AZD6244 with sorafenib and foratenib caused a synthetic lethal effect. This kinase rewiring has also been observed in EGFR-mutant non-small-cell lung cancer (NSCLC) resistant to erlotinib, where AXL was overexpressed in 20% of cases. The tumor growth was reverted with the addition of an AXL inhibitor to erlotinib (5).

Induction of signalability when ERK-dependent feedback is relieved requires the presence of RTKs. Multiple ligands contribute to ERK rebound in melanomas exposed to BRAF inhibitors, including EGF, hepatocyte growth factor (HGF), NRG and FGF, which antagonized vemurafenib sensitivity in several $BRAF^{V600E}$ melanomas tested (*Figure 2*). In fact, several reports have shown that ligands, particularly HGF, can cause resistance to RAF inhibitors (6,7). In contrast, other growth factors, such as PDGF and insulin growth factor (IGF), have a minimal effect and some, like transforming growth factor beta, accentuated vemurafenib-induced growth inhibition (2). $BRAF^{V600E}$ melanoma cells have high levels of pMEK and pERK and high levels of dual specificity phosphatase (DUSP) 6 and Sprouty (Spry) 2. After 24 hours of exposure to vemurafenib, DUSP6 and Spry2 levels were markedly diminished, leading to relief of ERK-dependent feedback and potentiating a permissive signaling environment. Tumor growth of melanoma-derived xenografts was abrogated with the combination of vemurafenib and either lapatinib or neratinib (2).

In order to understand how EGFR-driven tumors adapt to pathway inhibition and design more effective therapies, it is necessary to identify the pathways that can be reactivated in the patients. This will require comparison of pretreatment biopsies with biopsies obtained hours after treatment and the development of new technologies to determine which ligands are present and which pathways become reactivated. This can permit the rational

combination of therapies aimed at inhibiting adaptive resistance to the targeted therapy.

ERK signaling

ERK signaling plays an important role in regulating pleiotypic cellular functions. Activation of RTKs causes Ras to adopt an active, guanosine triphosphate (GTP)-bound conformation in which it induces the dimerization and activation of members of the RAF kinase family. Activated RAF phosphorylates and activates MEK1/2, which phosphorylate and activate ERK1/2, which in turn regulate cellular function by phosphorylating multiple substrates. A complex network of negative-feedback interactions limits the amplitude and duration of ERK signaling. Negative feedback is mediated directly by ERK-dependent inhibitory phosphorylation of components of the pathway, including EGFR, SOS and RAF. Importantly, ERK activation induces the expression of proteins that negatively regulate the pathway, including members of the Spry and DUSP families. BRAF-mutant colorectal cancers, which express greater levels of pEGFR than BRAF-mutant melanomas, are less sensitive to vemurafenib in spite of transient inhibition of pERK, since rapid ERK reactivation occurs through EGFR-mediated activation of Ras and CRAF. Interestingly, combined RAF and EGFR inhibition blocked reactivation of ERK signaling. Spry4 levels decreased after treatment with vemurafenib, coinciding with induction of pCRAF and pERK. Tumor growth in BRAF colorectal cancer cell lines was abrogated with the combination of vemurafenib and erlotinib. High pEGFR levels were observed in 60% of patients with colorectal cancer, who may benefit from combined RAF/EGFR or RAF/MEK inhibition (8). Similarly, in multiple $BRAF^{V600E}$ colon cancers, the inhibition of EGFR by cetuximab or gefitinib or erlotinib was strongly synergistic with BRAF inhibition both *in vitro* and *in vivo*. BRAF inhibition causes a rapid feedback activation of EGFR, supporting continued proliferation in the presence of BRAF inhibition (9).

In Kras-driven NSCLCs, ERK activity is negatively regulated by DUSP1, which can be abrogated by crosstalk with the Notch pathway. A significant proportion of patients with NSCLC have a hyperactivated Notch pathway, which is evidenced by higher levels of active g-secretase complex, increased levels of NICD (the activated form of Notch1), decreased Numb mRNA (a negative regulator of the Notch pathway), and increased HES1 protein (a downstream target of the Notch pathway). NSCLC patients with high HES1 expression had shorter overall survival. The Notch pathway regulates ERK phosphorylation by abrogating DUSP1 (10). These observations support the therapeutic potential of targeting g-secretase with MEK inhibitors in Kras-driven NSCLCs.

ERK activation is a common feature of tumors with RTK dysregulation stemming from EGFR, Kras or BRAF mutations. MEK inhibitors induced activation of phosphoinositide 3-kinase (PI3K)-Akt in EGFR-mutant cancer models via loss of an inhibitory threonine phosphorylation in the conserved juxtamembrane domains of EGFR and HER2 (11). Phosphorylation of the threonine residue impairs EGFR activation through disruption of receptor dimerization. Recent findings suggest that direct ERK-mediated phosphorylation of EGFR T669 and HER2 T677 suppresses activation of ERBB3. Suppressing ERK with AZD6244 prevented the effects of EGFR T669A on ERBB3/PI3K/Akt signaling in an EGFR-mutant cancer cell line. These findings suggest that combining MEK inhibition with ERBB3 or PI3K inhibitors may be a valuable strategy. The combination of MEK inhibitors with ERBB3 antibodies would block feedback activation of Akt and induce synthetic lethality (*Figure 3*). Interestingly, in Kras-mutant cancers that initially respond to single-agent RAF and MEK inhibitors, chronic inhibition of this pathway may lead to persistent activation of EGFR or HER2 (12).

Other feedback systems involving insulin growth factor receptor (IGFR) and mammalian target of rapamycin (mTOR)

The PI3K, RAF/MEK/ERK and mTORC1 pathways transmit signals from RTKs to downstream effector networks regulating cell growth, metabolism, survival and proliferation. One of the first feedback mechanisms indicating a rewiring phenomenon was the inhibition of mTORC1, which relieves protosomal degradation of IRS1, leading to feedback upregulation of IRS1/PI3K/Akt, reducing the efficacy of mTORC1 inhibitors as single agents and prompting the use of combination therapies (13,14). Briefly, mTORC1 activation leads to PI3K and MAPK inhibition through a negative feedback loop stemming from S6K1, while treatment with mTORC1 inhibitors results in a hyperactive RTK/IRS1/PI3K pathway, increasing the signal toward the Ras-RAF1-MEK1/2-ERK pathway. Hence, the combination of MEK and mTORC1 inhibitors could induce synthetic lethality by attenuating the rebound effect (13).

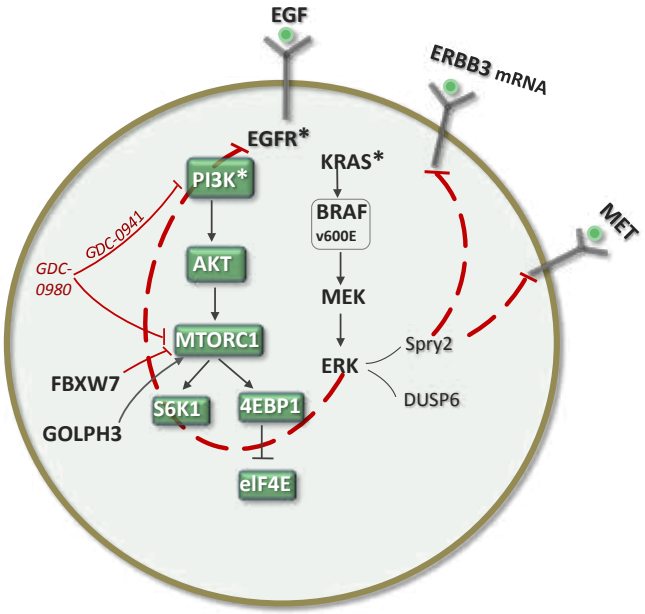


Figure 3 The combination of MEK inhibitors with ERBB3 antibodies blocks feedback activity of Akt in multiple cancer models (12).

mTOR is an intracellular serine threonine kinase involved in the control of translational initiation. PI3K/Akt-dependent phosphorylation signaling occurs through tuberin, the protein product of TSC1/TSC2 complex, which leads to activation of mTOR. mTOR phosphorylates S6 kinase, which phosphorylates the ribosomal protein S6. mTOR also phosphorylates eukaryotic translation initiation factor 4E-binding protein (4E-BP1). This causes 4E-BP1 to disassociate from the eukaryotic translation initiation factor 4E (eIF4E), allowing activation of protein translation. Hyperactivation of mTOR signaling frequently occurs in cancer in more than 70% of patients, making the targeting of mTOR an attractive anticancer therapy. Rapamycin, a naturally occurring allosteric inhibitor of mTORC1, and its analogs (rapalogs) are clinically approved for treatment of renal cell cancer, mantle cell lymphomas and pancreatic neuroendocrine tumors. However, the overall success of rapalog monotherapy is limited. In a feedback mechanism, rapamycin inhibits the function of mTOR, leading to the downregulation of S6 kinase and maintenance of the 4E-BP1 eukaryotic translation initiation factor 4E complex. The inhibition of mTOR leads to downregulation of pS6K and p4E-BP1, leading to a rebound of Akt, since the negative feedback loop is lost. This allows activation of IRS1 (15,16). IGF1 receptor inhibition could prevent rapamycin-induced Akt activation and sensitize tumor cells to inhibition of mTOR (16). Erlotinib also blocked the rapamycin-induced increase in Akt phosphorylation in lung cancer cells lines (15).

About 30% of cancers exhibit elevated eIF4E levels, which correlate with poor prognosis. eIF4E overexpression induces cell transformation by selectively augmenting translation of mRNAs referred to as eIF4E-sensitive mRNAs, which encode proliferation- and survival-promoting proteins (cyclins, c-MYC and Bcl-xL). Multiple factors can induce the expression of eIF4E in cancer cells, including gene amplification and transcriptional upregulation by c-MYC, and lead to an increase in eIF4E mRNA stability by HuR. HuR increases Musashi1 mRNA stability, and high Musashi1 expression is associated with Notch1 expression. Musashi1 represses the regulation of NUMB mRNA, a negative regulator of Notch (17). Resistance to BEZ235, a dual PI3K/mTOR inhibitor) can be acquired through amplification of eIF4E. A model has been proposed whereby an elevated eIF4E/4E-BP ratio renders tumors resistant not only to a TOR inhibitor but also to dual PI3K/mTOR inhibitors. These findings suggest that the eIF4E/4E-BP ratio could serve as a predictive marker for tailoring personalized treatment with TOR inhibitors (18).

PI3K and Akt inhibitors relieve negative feedback on ERBB receptors and other RTKs, leading to partial reactivation of PI3K/Akt signaling, MEK/ERK signaling, and other downstream pathways, potentially limiting the use of PI3K inhibitors as single agents. For example, receptor activation of PI3K-Akt causes Akt-dependent phosphorylation of FOXO proteins, which downregulate the expression of some of the receptors that are tightly coupled to PI3K, including HER3, IGF1R and IR. In addition, Akt activation leads to activation of TORC1 and S6K, which inhibits IRS1 expression, as mentioned above. Akt inhibitors will result in activation of FOXO-dependent transcription of receptors and, importantly, inhibition of S6K-dependent inhibition of signalability with resultant activation of multiple receptors. In fact, all drugs that inhibit components of dysregulated mitogenic signaling pathways would be expected to relieve feedback inhibition of other components of the signaling network. Combined inhibition of the oncoprotein and key pathways reactivated by inhibition of negative feedback would thus enhance antitumor activity. Akt inhibitors cause tumor regression when combined with low doses of HER kinase of HSP90 inhibitors, which prevent or attenuate induction of receptor

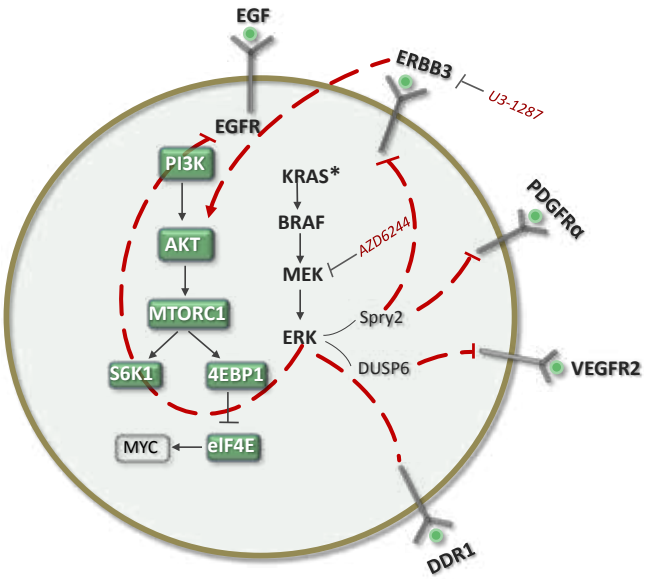


Figure 4 Co-targeting EGFR or downstream at the level of MEK results in synergistic inhibition of cell growth in NSCLC cell lines treated with PI3K inhibitors or dual PI3K/mTOR inhibitors (20).

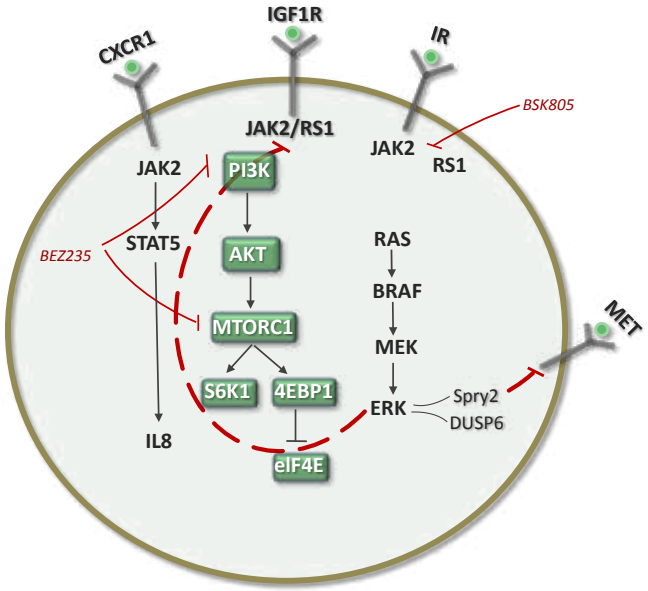


Figure 5 In triple-negative breast cancer, PI3K/mTOR inhibition elicits a positive feedback loop by activating JAK2/STAT5. Inhibition of JAK2 abrogates this feedback and provides a rationale for combining inhibition of PI3K/mTOR and JAK2/STAT5 pathways (23).

phosphorylation (3). The combination of ganetespib, a HSP90 inhibitor, with BEZ235, a dual pan-PI3K/mTOR inhibitor, displayed significant efficacy in a Kras-mutant NSCLC xenograft model (19).

NSCLC cell lines harboring PI3K pathway alterations (RTK activation, PI3K mutation or amplification, or PTEN loss) are sensitive to the PI3K inhibitor GDC0941. Also, a dual PI3K/mTOR inhibitor, GDC0980, has shown even broader activity across cell lines and tumor xenografts. The combination of GDC0941 with paclitaxel, erlotinib, or a MEK inhibitor had greater effects on cell viability than PI3K inhibition alone (20). PIK3CA mutations and PTEN loss are predictors of sensitivity to PI3K inhibitors, as are FBXW7, which encodes a ubiquitin ligase that degrades mTOR, and GOLPH3, which is amplified in NSCLC and associated with mTOR inhibitor sensitivity. Tumor cells harboring deletions or mutations in FBXW7 are sensitive to rapamycin treatment (21). Rapamycin was significantly more effective against xenograft tumors expressing high levels of GOLPH3. GOLPH3, a Golgi oncoprotein, is a bona fide oncoprotein that is amplified in several types of cancers (22). In two EGFR-mutant NSCLC cell lines (HCC827 and H1975), GDC0941 showed greater than 100% tumor growth inhibition. The dual PI3K/mTOR inhibitor GDC0980 had greater antitumor activity than GDC0941 in models harboring Kras mutations or dual PIK3CA/Kras mutations (22). Intriguingly, treatment of inhibitor-sensitive cell lines with GDC0941 and GDC0980 resulted in increased expression of RTKs such as ERBB3 and MET, akin to the abovementioned examples (*Figure 4*). Co-targeting EGFR or downstream at the level of MEK could result in synergistic inhibition of cell growth, consistent with the hypothesis that such co-targeting may block PI3K inhibitor-induced pathway reactivation (*Figure 4*). It is particularly intriguing that the NSCLC cell line H1975 (16 copies of MYC) was very sensitive to GDC0941, which suggests that the biology of PI3K resistance may differ between breast cancer and NSCLC (20). In fact, a new rewiring mechanism of resistance to dual PI3K/mTOR inhibitors has been reported in breast cancer. Following PI3K-mTOR inhibition with BEZ235, increasing IRS1-dependent activation of JAK2/STAT5 and secretion of IL-8 was observed in several cell lines and primary breast tumors (*Figure 5*). Pharmacological inhibition of JAK2 abrogated this feedback loop and combined inhibition of PI3K/mTOR (with BEZ235) and JAK2 (with NVP-BSK805) synergistically reduced cancer cell number and tumor growth. The findings pave the way for co-targeting of the

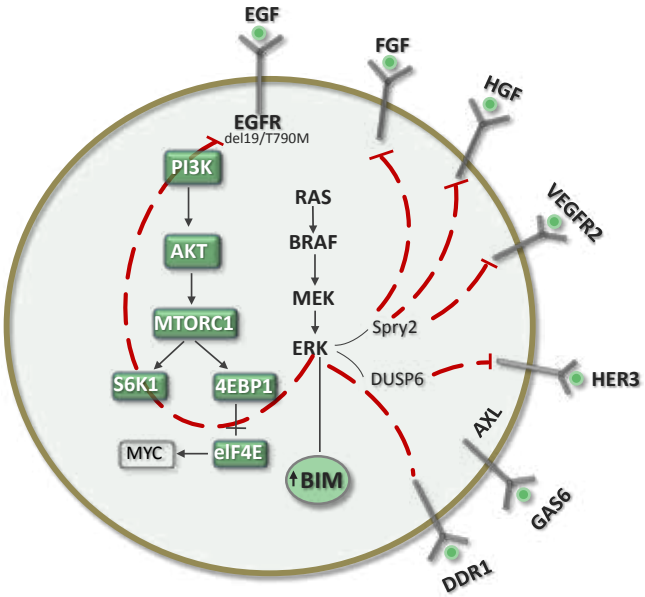


Figure 6 Hypothetical model for tumors with EGFR mutations, where erlotinib can abrogate ERK and adaptive resistance can be expected in tumors expressing BIM. Rebiopsies hours after initiating treatment will help us to understand the correct RTKs for co-targeting.

PI3K/mTOR and JAK2/STAT5 pathways in triple-negative breast cancer (23).

Treatment with erlotinib increased the levels of EGFR/IGF1R heterodimer localized on cell membrane, activated IGF1R and its downstream signaling mediators, and stimulated mTOR-mediated *de novo* protein synthesis of EGFR and survivin in NSCLC cells. These data suggest that enhanced synthesis of survivin protein mediated by the IGFR/EGFR heterodimer counteracts the antitumor action of erlotinib, suggesting the need for integrating IGF1R targeted agents with EGFR tyrosine kinase inhibitors (TKIs) for patients with NSCLC (24). Although this evidence is compelling, no trials have investigated this possibility. What is intriguing is that IGF1R targeted therapy has so far failed to give the expected results. Interestingly, the anti-IGF1R antibody figitumumab induced IGF1R/b-arrestin association, allowing b-arrestin1-dependent activation of ERK signaling. In consequence, the addition of an ERK1/2 inhibitor increased sensitivity to figitumumab (25). b-arrestin acts as an E3 ligase adaptor in response to IGF stimulation. After IGF binds to the tetrameric IGF1R, b-arrestin recruits Mdm2 to the receptor. Mdm2 ubiquitinates IGF1R, causing its internalization. Once internalized, IGF1R is degraded by the protoesome and b-arrestin mediates the activation of ERK from internalized signalosomes. ERK then translocates to the nucleus and activates transcription (26). In short, b-arrestin1 recruitment to IGF1R leads to ERK signaling activation and receptor downregulation. The combination of IGF1R targeting antibodies and MAPK inhibitors could be a new treatment strategy (25). Furthermore, Klotho inhibits the IGF1 pathway. Low Klotho expression has been found in breast cancer. Studies in breast cancer cells revealed increased activation of the FGF pathway following Klotho overexpression. Therefore, Klotho is an inhibitor of the IGF1 pathway and an activator of the FGF pathway in human breast cancer (27).

Adaptive resistance in NSCLC driven by EGFR mutations

EGFR mutant-driven NSCLC responds very well to EGFR TKIs such as erlotinib. However, the response rate is around 60% and progression-free survival around one year or less, and all patients will eventually relapse (28,29). There are thus intrinsic resistance mechanisims, at least for the 30-40% of patients who do not respond initially, which can be attributed to crosstalk with other signaling pathways. For responders, the limited progression-free survival indicates that adaptive mechanisms of resistance can develop. Our hypothesis is that responders have high expression levels of BIM and that attenuation of the ERK pathway caused by erlotinib can lead to an effect similar to that observed with BRAF inhibitors in melanomas driven by $BRAF^{V600E}$ mutations (*Figure 2*) or with MEK inhibitors in triple-negative breast cancers (*Figure 1*). The loss of ERK function can lead to the stimulation of several RTKs and ligands (*Figure 6*). It is thus absolutely vital to perform a rebiopsy several hours following erlotinib treatment in order to understand the dynamic changes that cancer cells can undergo. The experience acquired in breast cancers with MEK inhibitors and in melanoma with BRAF inhibitors can be reproducible in EGFR-mutant NSCLC responders. Understanding the phenomenon of adaptive resistance from the beginning can lead to combining EGFR TKIs with other inhibitors blocking other RTKs, which presumably will include ERBB3. The correct identification of adaptive resistance mechanisms will allow us to implement the correct combination of targeted therapies for a true synthetic lethal effect that will increase the currently limited progression-free survival

times attained with EGFR TKIs alone.

Acknowledgements

Work in Dr Rosell's laboratory is partly supported by a grant from La Caixa Foundation, which had no role in writing the manuscript or in the decision to publish.
Disclosure: This manuscript has not been published or submitted for publication elsewhere. The authors declare no conflicts of interest.

References

1. Duncan JS, Whittle MC, Nakamura K, et al. Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. Cell 2012;149:307-21.
2. Lito P, Pratilas CA, Joseph EW, et al. Relief of Profound Feedback Inhibition of Mitogenic Signaling by RAF Inhibitors Attenuates Their Activity in BRAFV600E Melanomas. Cancer Cell 2012;22:668-82.
3. Chandarlapaty S, Sawai A, Scaltriti M, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell 2011;19:58-71.
4. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, et al. mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. Cancer Discov 2011;1:248-59.
5. Zhang Z, Lee JC, Lin L, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet 2012;44:852-60.
6. Straussman R, Morikawa T, Shee K, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. Nature 2012;487:500-4.
7. Wilson TR, Fridlyand J, Yan Y, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature 2012;487:505-9.
8. Corcoran RB, Ebi H, Turke AB, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. Cancer Discov 2012;2:227-35.
9. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483:100-3.
10. Maraver A, Fernandez-Marcos PJ, Herranz D, et al. Therapeutic effect of γ-secretase inhibition in KrasG12V-driven non-small cell lung carcinoma by derepression of DUSP1 and inhibition of ERK. Cancer Cell 2012;22:222-34.
11. Red Brewer M, Choi SH, Alvarado D, et al. The juxtamembrane region of the EGF receptor functions as an activation domain. Mol Cell 2009;34:641-51.
12. Turke AB, Song Y, Costa C, et al. MEK inhibition leads to PI3K/AKT activation by relieving a negative feedback on ERBB receptors. Cancer Res 2012;72:3228-37.
13. Carracedo A, Ma L, Teruya-Feldstein J, et al. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 2008;118:3065-74.
14. Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene 2008;27:5527-41.
15. Johnson BE, Jackman D, Jänne PA. Rationale for a phase I trial of erlotinib and the mammalian target of rapamycin inhibitor everolimus (RAD001) for patients with relapsed non small cell lung cancer. Clin Cancer Res 2007;13:s4628-31.
16. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 2006;66:1500-8.
17. Vo DT, Abdelmohsen K, Martindale JL, et al. The oncogenic RNA-binding protein Musashi1 is regulated by HuR via mRNA translation and stability in glioblastoma cells. Mol Cancer Res 2012;10:143-55.
18. Alain T, Morita M, Fonseca BD, et al. eIF4E/4E-BP Ratio Predicts the Efficacy of mTOR Targeted Therapies. Cancer Res 2012;72:6468-76.
19. Acquaviva J, Smith DL, Sang J, et al. Targeting KRAS-Mutant Non-Small Cell Lung Cancer with the Hsp90 Inhibitor Ganetespib. Mol Cancer Ther 2012;11:2633-43.
20. Spoerke JM, O'Brien C, Huw L, et al. Phosphoinositide 3-Kinase (PI3K) Pathway Alterations Are Associated with Histologic Subtypes and Are Predictive of Sensitivity to PI3K Inhibitors in Lung Cancer Preclinical Models. Clin Cancer Res 2012;18:6771-83.
21. Mao JH, Kim IJ, Wu D, et al. FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. Science 2008;321:1499-502.
22. Scott KL, Kabbarah O, Liang MC, et al. GOLPH3 modulates mTOR signalling and rapamycin sensitivity in cancer. Nature 2009;459:1085-90.
23. Britschgi A, Andraos R, Brinkhaus H, et al. JAK2/STAT5 Inhibition Circumvents Resistance to PI3K/mTOR Blockade: A Rationale for Cotargeting These Pathways in Metastatic Breast Cancer. Cancer Cell 2012;22:796-811.
24. Morgillo F, Woo JK, Kim ES, et al. Heterodimerization

of insulin-like growth factor receptor/epidermal growth factor receptor and induction of survivin expression counteract the antitumor action of erlotinib. Cancer Res 2006;66:10100-11.
25. Zheng H, Shen H, Oprea I, et al. β-Arrestin-biased agonism as the central mechanism of action for insulin-like growth factor 1 receptor-targeting antibodies in Ewing's sarcoma. Proc Natl Acad Sci U S A 2012;109:20620-5.
26. Kovacs JJ, Hara MR, Davenport CL, et al. Arrestin development: emerging roles for beta-arrestins in developmental signaling pathways. Dev Cell 2009;17:443-58.
27. Wolf I, Levanon-Cohen S, Bose S, et al. Klotho: a tumor suppressor and a modulator of the IGF-1 and FGF pathways in human breast cancer. Oncogene 2008;27:7094-105.
28. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
29. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.

Cite this article as: Rosell R, Karachaliou N, Morales-Espinosa D, Costa C, Molina MA, Sansano I, Gasco A, Viteri S, Massuti B, Wei J, González Cao M, Martínez-Bueno A. Adaptive resistance to targeted therapies in cancer. Transl Lung Cancer Res 2013;2(3):152-159. doi: 10.3978/j.issn.2218-6751.2012.12.08

State of the art of radiotherapy

Pilar Garrido, Mª Eugenia Olmedo

Medical Oncology Department, IRYCIS, Hospital Universitario Ramón y Cajal, Carretera Colmenar Viejo km 9100, 28034, Madrid, Spain
Correspondence to: Pilar Garrido, MD, PhD. Medical Oncology Department, IRYCIS, Hospital Universitario Ramón y Cajal, Carretera Colmenar Viejo km 9100, 28034, Madrid, Spain. Email: pilargarridol@gmail.com.

Abstract: Locally advanced or stage III disease accounts for ~30% of patients with non-small-cell lung cancer (NSCLC), which means only in the United States, more than 50,000 new patients each year. Stage III is a very heterogeneous disease, the management of patients is complex and several conditions (performance status, weight loss, comorbidities, characteristics of nodal involvement or resectability) must be considered before selecting the best treatment, which in most cases is chemotherapy (CT) and radiotherapy (RT). In this article, we will review key changes in the management of unresectable stage III during the last decades. Also we will highlight some challenges and areas of active research.

Keywords: Stage III; chemoradiation; locally advanced disease; concomitant therapy

Submitted Feb 10, 2013. Accepted for publication Mar 06, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.02
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.02

Combined treatment versus radiotherapy alone

Potentially curative treatment of unresectable stage III necessitates adequate locoregional control as well as control of the micrometastatic disease that is likely to be present in most patients. In the 1980s, the standard of care for locally advanced disease was RT alone, led to a median survival time of less than 10 months and 3-year survival rates below 10%. In the early 1990s, a phase III trial conducted by the Cancer and Leukemia Group B (CALGB) group (1) showed a survival advantage using sequential therapy with CT and RT (*Table 1*). The trial randomized patients with unresectable stage III and medically inoperable stage II NSCLC to receive two cycles of cisplatin and vinblastine over 5 weeks followed by RT to 60 Gy versus RT alone. The response rate was 56% for patients receiving chemotherapy and radiation compared with 43% for patients receiving radiation therapy alone; median survival times were 13.7 versus 9.6 months, respectively (P=0.0066). More importantly, there was a 17% survival rate at 5 years in the combined-modality therapy arm versus a 7% rate in the radiation therapy alone arm, with few patients experiencing relapse after 2 to 3 years of follow-up. These results were duplicated in a separate phase III run by the US Intergroup study, reported by Sause *et al.* (2).

Based on the radio sensitizer activity of most CT agent, particularly cisplatin and carboplatin, the concomitant administration of both modalities was also explored and compared with RT alone. Randomized data from Jeremic *et al.* (3,4) confirmed the clinical benefit of concurrent chemoradiotherapy compared with radiation alone by comparing hyperfractionated radiation therapy alone with hyperfractionated radiation therapy and different regimens of carboplatin/etoposide. The concurrent regimens significantly improved 3-year survival [23% *vs.* 6.6% (3), 23% *vs.* 9% (4)]. There was no significant reduction in the rate of distant metastasis with concurrent chemoradiation. Data reported by Shaake-Koning *et al.* (5) in a European Organization for Research and Treatment of Cancer (EORTC) three-arm trial evaluated 331 patients assigned to radiation alone (55 Gy), radiation with weekly cisplatin (30 mg/m^2), or radiation with daily cisplatin (6 mg/m^2). The addition of cisplatin to the thoracic radiation resulted in an improvement in overall survival compared with radiation alone. The 3-year survival rate for radiation alone was 2% compared with 13% for patients receiving radiation with weekly cisplatin and 16% for patients receiving radiation with daily cisplatin. In two-way comparisons, statistical

Table 1 Phase III comparing radiotherapy alone versus combined treatment in stage III NSCLC patients

Study	Arms	Number of patients	Median survival (months)	3-y OS (%)	5-y OS (%)	P
Dillman (1)	CT + RT	155	13.7	24	17	0.012
	RT alone		9.6	10	7	
Sause (2)	CT + RT	490	13.2	17	8	0.04
	HFX		12	14	6	
	RT alone		11.4	11	5	
Jeremic (3)	HFX	169	8	6.6	4.9	0.0027
	HFX + CT wk		18	23	21	
	HFX RT + CT		13	16	16	
Jeremic (4)	HFX	131	14	9		0.021
	HFX + CT		22	23		
Schaake-Koning (5)	RT alone	331		2		0.04
	RT + CT wk			13		
	RT + CT daily			16		

CT, chemotherapy; RT, radiotherapy; HFX, hyperfractionated radiotherapy; OS, overall survival; Wk, weekly.

significance was only achieved for the radiation and daily cisplatin arm (P=0.009). Although other trials have failed to show a survival advantage with concurrent chemoradiation therapy compared with radiation therapy alone, several meta-analyses, focused on adding platinum-containing chemotherapy either at systemic doses preceding or at low radiosensitizing dose concomitant with chest radiotherapy in patients with good performance and no significant weight loss, also showed a significantly improvement on the outcomes as compared with single modality chest radiotherapy with traditional dose and fractionation schedules (1.8-2.0 Gy per fraction per day to 60-70 Gy in 6-7 weeks). The first meta-analysis was published in 1995 (6). The patients treated with chemotherapy and radical radiotherapy experienced a 13% reduction in risk of death with an absolute survival benefit of 4% at 2 years. Studies in which radiotherapy and chemotherapy were given concurrently were specifically excluded from this analysis, so this benefit was observed for sequential chemoradiotherapy. In 2004, an individual patient data metaanalysis (7) was published comparing radiotherapy alone with concurrent chemoradiotherapy based on nine trials and 1,764 patients. It showed an absolute survival advantage of 4% at 2 years when combining radiation therapy with chemotherapy (HR 0.89; 95% CI, 0.81-0.98; P=0.02). Finally, the Cochrane review of 2004 (8) concluded that the addition of concurrent chemotherapy to radical radiation therapy reduced the risk of death at 2 years by 7% [relative risk (RR), 0.93; 95% CI, 0.88-0.98; P=0.01]. The risk of acute esophagitis (grade ≥3) is greater with concurrent treatment (RR, 1.58; 95% CI, 1.19-2.09; P=0.001), but there was no significant difference in the risk of acute pneumonitis. Thus, sequential and concomitant combined-modality strategies were each established to be superior to radiotherapy alone offering a 4% increase in absolute survival at 2 years and confirmed combined modality as the standard of care in the management of locally advanced inoperable NSCLC.

Sequential versus concomitant chemoradiation

The next generation of clinical trials investigated concurrent chemoradiation versus sequential approach, showing in two of three phase III studies favorable results to concomitant therapy (*Table 2*). The West Japan Lung Cancer Group conducted the first published trial about this topic (9). They randomized 320 patients to receive thoracic radiation (56 Gy, split-course) either after or concurrent with cisplatin (80 mg/m^2), vindesine (3 mg/m^2), and mitomycin (8 mg/m^2) chemotherapy. Patients receiving concurrent therapy had a median survival time of 16.5 months compared with 13.3 months for the sequentially treated patients (P=0.03). The 5-year survival rate was also superior for the concurrently treated patients (15.8%) compared with patients receiving sequential therapy (8.9%). However, the study NPC 95-01 run by the Groupe Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Français

Table 2 Phase III trial comparing sequential and concomitant chemoradiotherapy in stage III NSCLC patients

	N	Arm	MST (months)	5-y OS (%)	P
Furuse (9)	320	Sequential (MVdP/RT)	13.3	8.9	0.03
	314	Concomitant (MVdP + RT)	16.5	15.8	
Fournel (10)	205	Sequential (VrbP/RT)	14.5	14.2*	0.24
		Concomitant (PE + RT)	16.3	20.7	
Curran (11)	610	Sequential (PVb/RT)	14.6	10	0.046
		Concomitant (PVb + RT)	17	16	
		Concomitant (PE + HFX)	15.6	13	

N, number of patients; MST, median survival time; OS, overall survival; MVdP, Mytomicin, vindesine and cisplatin; RT, standard radiotherapy; PVd, Cisplatin, vindesin; PE, cisplatin, etoposide; HFX, Hyperfractionated radiotherapy; VrbP, Vinorelbine, cisplatin; *4-years survival.

de Pneumo-Cancérologie (10) did not achieve a statistical significance difference on survival between concomitant and sequential approaches (P=0.24). In this trial, 205 patients were randomized to receive either 3 cycles of cisplatin (120 mg/m^2) and vinorelbine (30 mg/m^2 weekly) followed by thoracic radiation at a dose of 66 Gy or concurrent therapy consisting of cisplatin (20 mg/m^2) and etoposide (50 mg/m^2) for two cycles along with thoracic radiation. Patients in the concurrent arm received 2 further cycles of consolidation chemotherapy that consisted of cisplatin (80 mg/m^2) and vinorelbine (30 mg/m^2 weekly) to match the total cisplatin dose given in the other arm. In spite of similar findings to the West Japan Lung Cancer Group trial, with numerical advantage for the concurrent therapy in median survival time (14.5 versus 16.3 months) and in the 2- and 4-year survival rates (26.5% and 14.2%, versus 39.3% and 20.7%, respectively), the trend toward prolonged survival with concomitant therapy did not achieve statistical significance. One possible explanation is the high number of toxic deaths in the concurrent arm comparing to the sequential one (10 versus 6). Finally, the third and largest randomized phase III trial comes from the Radiation Therapy Oncology Group (RTOG) (11). In RTOG 94-1012, 610 patients were randomly assigned to the following three treatment arms: once-daily radiation (60 Gy) after induction cisplatin (100 mg/m^2) and vinblastine (5 mg/m^2) chemotherapy; once-daily radiation [60] concurrent with the same chemotherapy; or hyperfractionated radiation (69.6 Gy) with concurrent cisplatin (50 mg/m^2) and oral etoposide (50 mg twice daily). The median survival time was superior for patients receiving concurrent therapy with daily radiation (17.0 months) compared with patients receiving sequential treatment (14.6 months); this result was statistically significant (P=0.038). The overall 4-year survival rate was also better for patients on the concurrent arm compared with the sequential arm (21% *vs.* 12%, respectively). Several phase II studies and meta-analysis also supported the benefit of concomitant over sequential therapy. A systematic review and individual patient data meta-analysis including 1,205 patients conducted by the NSCLC Collaborative Group (12) confirmed a significant benefit of concomitant therapy on overall survival (HR, 0.84; 95% CI, 0.74 to 0.95; P=0.004), with an absolute benefit of 5.7% (from 18.1% to 23.8%) at 3 years and 4.5% at 5 years. Notably, although rates of distant failures were equivalent (HR, 1.04; 95% CI, 0.86 to 1.25; P=0.69), concomitant treatment decreased locoregional progression (HR, 0.77; 95% CI, 0.62 to 0.95; P=0.01). Concomitant therapy also increased acute esophageal toxicity (grade 3-4) from 4% to 18% with a relative risk of 4.9 (95% CI, 3.1 to 7.8; P=0.001) but not acute pulmonary toxicity. Authors concluded that concomitant therapy improved survival of patients with locally advanced NSCLC, primarily because of a better locoregional control, but at the cost of manageable increased acute esophageal toxicity.

Chemoradiation plus induction or consolidation strategies

In spite of the advantage in survival demonstrated by the use of concomitant CT and RT, both locoregional and distant failure remain a problem. Following treatment with chemoradiotherapy, 70% to 75% of patients develop recurrent or progressive disease; roughly one third of

patients fail in the radiation field (local failure), one third of patients fail outside the irradiated field (distal failure) and one third of patients fail both locally and distally. Based on this, several trials were focused in adding more CT as induction or consolidation strategies to concomitant therapy.

The Cancer and Leukemia Group B (CALGB) conducted several studies focused on the induction strategy. They published in 2002 (13) the results of a randomized phase II trial comparing efficacy and toxicities of three regimens in which patients were randomized to receive one of these three agents (paclitaxel, gemcitabine, or vinorelbine) in combination with cisplatin for two cycles as induction chemotherapy followed by two additional cycles of these drugs with concurrent standard chest radiotherapy. They postulated that given the encouraging activity of these agents, in the stage III setting might lead to further prolongation of survival times. In addition, all three agents have been demonstrated to act as radiation sensitizers in preclinical models. The primary end points were response to both induction and concomitant chemoradiotherapy. One hundred eighty seven patients were accrued. Total response rates to induction chemotherapy on the three study arms were 40%, 33%, and 44% (gemcitabine, paclitaxel, and vinorelbine) and best overall response rates were 74%, 67%, and 73% with overlapping 95% CIs. The most common toxicities to induction chemotherapy were grade 3 or 4 granulocytopenia on all three arms (observed in approximately 50% of patients) and 25% grade 3 or 4 thrombocytopenia on the gemcitabine arm. However, there were notable differences among the three study arms in the toxicities during concomitant chemoradiotherapy. Grade 3 or 4 granulocytopenia was seen in 51% of patients treated with gemcitabine and 53% of patients treated with paclitaxel, which contrasts with 27% of patients treated with vinorelbine. In addition, thrombocytopenia was seen in 56% of patients on the gemcitabine arm. Grade 3 or 4 esophagitis was most pronounced on the gemcitabine arm (35% of patients grade 3 and 17% of patients grade 4) whereas these numbers were 35% and 4% for paclitaxel and 13% and 12% for vinorelbine. Overall median survival time for all patients was 17 months. For the three study arms, median survival times and 3-year survival rates were 18.3, 14.8 and 17.7 months and 28%, 19% and 23% for gemcitabine, paclitaxel and vinorelbine, respectively. Based on its widespread acceptance by oncologists and general good tolerance they chose carboplatin and paclitaxel as a chemotherapy regimen for the subsequent phase III trial, the CALGB 39-801 (14) study. The primary endpoint was to detect a 40% increase in median survival, from 13 to 18.2 months, with the addition of induction chemotherapy. Three hundred sixty-six patients were randomly assigned to immediate concurrent chemoradiotherapy with weekly carboplatin and paclitaxel during 66 Gy of chest radiotherapy, or induction CT with two cycles of carboplatin and paclitaxel administered every 21 days followed by identical chemoradiotherapy. The study was negative because survival differences were not statistically significant, with a median survival on concomitant arm of 12 versus 14 months on induction CT arm and a 2-year survival of 29% and 31% respectively. However, the toxicity, mainly of neutropenia grade 3 or 4 was superior in the induction CT arm (18% and 20%, respectively). Remarkably, the survival times were at the lower range of reported values for patients with stage III disease treated with concomitant chemoradiotherapy even after adjusting for prognostic factors such as the weight loss. Possible explanations could be the selection of a weekly regimen of CT during RT treatment and/or the use of a carboplatin-based regimen instead of a cisplatin-based one. In any case, this study demonstrated the absence of value to adding induction CT with currently established agents.

Testing the hypothesis of consolidation CT the Southwest Oncology Group (SWOG) run two consecutive phase II studies. In the first one (SWOG-9019) (15), published in 2002, all patients received cisplatin, 50 mg/m^2/d on days 1, 8, 29, and 36; etoposide, 50 mg/m^2/d on days 1 to 5 and 29 to 33; and RT, 1.8 Gy per day, 5 days a week, starting within 24 hours of the first day of chemotherapy followed by two cycles of the same CT regimen. Fifty eligible patients were accrued. Grade 4 neutropenia was the most common toxicity (32%). Grade 3/4 esophagitis occurred in 12% and 8%. Median follow-up was 52 months, and overall median survival was 15 months and 3- and 5-year survivals were 17% and 15%. The second phase II study, S9504 (16), was designed to test the concept of taxane sequencing in combined-modality therapy and patients were selected using identical eligibility, staging criteria, and treatment, excepting docetaxel consolidation that those of the predecessor study (S9019). The primary objective was to estimate, within the limitations of a historical comparison, whether substitution of docetaxel for continued PE during the consolidation phase of treatment would improve survival compared with the predecessor trial S9019, and whether toxicities were acceptable. A sample size of 80 eligible patients with stage IIIB disease confirmed

Table 3 Randomized phase II studies comparing induction versus consolidation chemotherapy in patients receiving concomitant chemoradiotherapy

Study	Scheme	N	MST (months)	2-y OS (%)	P
Belani (20)	CT[1]→RT	276	13	30	NS
	CT→CT/RT		12.7	53	
	CT/RT→CT		16.3	63	
Senan (21)	CT[4]→CT/RT	72	12.8	63*	0.8
	CT/RT→CT		14.8	66	
Fournel (22)	CT[2]→CT/RT[3]	133	19.3	47	NS
	CT/RT→CT		16.9	43	
Garrido (23)	CT[5]→CT[6]/RT	135	13.8	40	0.13
	CT/RT→CT		13	27	

CT, Chemotherapy; RT, radiotherapy; N, number of patients; MST, median survival time; OS, overall survival; *1-year OS; Chemotherapy regimens: [1]Carboplatin and paclitaxel; [2]Cisplatin and paclitaxel; [3]Cisplatin and vinorelbine; [4]Cisplatin and docetaxel; [5]Gemcitabine and docetaxel; [6]Carboplatin and docetaxel.

on central review was required to demonstrate a 6 months increase in median survival compared with that observed in S9019. Concurrent chemoradiotherapy was generally well tolerated, but two patients died from probable radiation-associated pneumonitis. The esophagitis rate was 17% (20% in S9019). Neutropenia during consolidation docetaxel was common (57% with grade 4). At a median follow-up of 71 months, the median progression free survival was 16 months and the median survival 26 months. Overall survival at 3, 4, and 5 years was 37%, 29%, and 29%, respectively. Although the survival results were provocative, particularly the long-term results updated in a second paper (17), confirmation and validation using a phase III design was necessary. Regrettably, when that study was run18, the results did not confirm the survival advantage of using three cycles of docetaxel after concomitant CT/RT versus concomitant CT/RT alone. The Hoosier Oncology Group (18) randomized patients to receive three cycles of docetaxel versus observation after finishing without progression concomitant chemoradiation with the same regimen than previous SWOG phase II studies. The primary objective was overall survival. Based on a data and safety monitoring board recommendation, the trial was closed after an analysis of the initial 203 patients. The grade 3-5 toxicities were clearly superior in the docetaxel arm (febrile neutropenia 10.9%, pneumonitis 9.6%, 5.5% died) as well as the percentage of patients hospitalized (28.8% during docetaxel versus 8.1% in observation arm). Although the MST for all patients was extremely good (21.7 months), no statically differences were found between docetaxel and observation arms (21.2 and 23.2 months, respectively). An update in survival was published (19), adding a retrospective analysis of efficacy and toxicity in older patients included in the trial. The 3-, 4-, and 5-year survival rates for the overall study were 30.7%, 18.0%, and 13.9%, respectively, without differences between docetaxel and observation. Older patients had similar MST but higher rates of grade 3/4 toxicity and hospitalization during induction.

Direct comparison between induction and consolidation strategies

Four randomized phase II study and an early closed phase III trials were focused on directly compared combined chemoradiation plus full doses of CT previously or at the end of concomitant therapy (*Table 3*). Belani and cols (20) published in 2005 a phase II randomized noncomparative trial conducted to determine the optimal sequencing and integration of paclitaxel/carboplatin with radiotherapy in stage III NSCLC patients. Survival data were compared with historical standard sequential chemoradiotherapy data from the RTOG. Patients received two cycles of induction paclitaxel/carboplatin every 21 days followed by RT (arm 1, sequential) or two cycles of induction paclitaxel/carboplatin followed by weekly paclitaxel/carboplatin with concurrent RT (arm 2, induction/concurrent), or weekly paclitaxel/carboplatin/RT followed by two cycles of full doses of paclitaxel/carboplatin (arm 3, concurrent/consolidation). The primary objective was survival. For analyses, each arm was compared with a historical control using the

sequential chemoradiotherapy arm of the RTOG 88-08 trial, for which the available reported median survival time was 13.7 months. The final number of patients enrolled was 276. According to the paper, when the accrual to the phase II study reached the projected number of patients, an interim statistical analysis using the triangle test was applied to all three arms. Arm 2 was closed to accrual due to the low likelihood of benefit compared with historical control. Sample sizes in arms 1 and 3 were expanded to accommodate a phase III design. Subsequently, when data from the RTOG 9410 study became available and confirmed the benefit of concurrent therapy, accrual decreased and the study was permanently closed to accrual. Although the study was not designed to directly compassion among arms, the final results were favorable to consolidation arm with median overall survival of 13.0, 12.7, and 16.3 months for arms 1, 2, and 3, respectively. The most frequent grade 3-4 toxicity during induction chemotherapy was granulocytopenia (32% and 38% of patients on study arms 1 and 2, respectively) and the most common locoregional grade 3/4 toxicity during and after RT was esophagitis, as expected more pronounced with concomitant therapy (arms 2 and 3).

Three European studies have been also designed to compare face-to-face induction and consolidation strategies but using a phase II approach. The Pulmon Art (21) was a multicenter trial conducted in 15 centers from 8 European countries designed to examine the safety and toxicity profile of two sequences cisplatin-docetaxel, either as induction before or consolidation after concurrent CT-RT with radiosensitizing doses of the same doublet in order to identify the most feasible regimen for further studies. The primary end point was the incidence of grade ≥3 esophagitis in the two treatment sequences. They estimated that the maximal rate considered acceptable by clinicians was 25%. Seventy-two patients (36 patients each arm in the intent to treatment design) were randomly allocated but 5 patients were switched from consolidation to induction arm due to higher V_{20} than permitted. The safety population consisted of 41 patients treated in the induction arm and 29 in the consolidation one. Adverse events that were grade ≥3 were reported for 63% of patients and 72%, respectively. The incidence of grade ≥3 esophagitis was not significantly different from the allowable incidence of 25% (Grade 3-4 in 32% and 2% in the induction arm and 21% and 3%, in the consolidation arm). A total of 18 patients developed grade ≥2 radiation pneumonitis but no significant correlation was observed between V_{20} and incidence of grades 2-5 pneumonitis. The authors did not find differences in overall response rate, overall survival (with a median OS of all eligible patients was 28.0 months) or progression free survival between arms. In spite of the selection of patients, 26% discontinued treatment prematurely and only 55-57% received the planned RT dose of 66 Gy.

A French multicenter phase II study included 133 patients in 35 centers (22). It compared 3 cycles of cisplatin every 21 days and reduced vinorelbine doses on day 1 and 8 concomitant with RT plus two cycles of cisplatin and paclitaxel as induction or consolidation. The primary objective was response rate at the end of treatment. Toxicities and response rates are similar in both arms, but induction followed by CT/RT appears to provide a better therapeutic outcome with median survival time of 19.3 versus 16.9 months and 2-year survival rates of 47% versus 43% (induction and consolidation, respectively).

Finally, the SLCG 0008 study conducted by the Spanish Lung Cancer Group (23) initially compared three arms (sequential CT followed by thoracic radiation; concurrent CT/TRT followed by consolidation CT and induction CT followed by concurrent CT/RT). However, based on the preliminary results of the RTOG 9410 trial published at that time, the sequential arm was closed with only 19 patients enrolled. The study continued comparing concomitant arms plus induction or consolidation CT and results of 135 patients from 16 Spanish university hospitals were available. The full dose regimen selected was a non-platinum schema (docetaxel and gemcitabine) based on an expected better tolerability profile. Weekly docetaxel and carboplatin was chosen to receive in combination with RT (60 Gy). The primary endpoint was response rate, with no statistically differences founded between the two arms (56% consolidation and 57% induction). Hematological toxicity was mild but significantly superior with consolidation CT; the esophagitis rate was similar in both arms (16% and 15%). With a median follow-up of 57 months, no statistically significant differences were found between consolidation and induction arm in median survival (13 and 13.8 months) or long-term survival (2-y OS 27% *vs.* 40%, 5-y OS 16% *vs.* 22%). Based on the modest results founded in median survival time and similar toxicities to other platinum regimen, authors concluded that this regimen cannot be recommended as an alternative to platinum-based CT/TRT. A phase III study was designed to directly compare both strategies using a triplet combination of CT with cisplatin, gemcitabine and vinorelbine but it was

prematurely closed for poor accrual due to administrative problems (24). To clarify this question a meta-analysis of the pooled data of the phase II studies should be addressed since they are very similar in design and patient selection.

Studies comparing second and third generation chemotherapy agents

Clinical research efforts have focused on incorporating newer chemotherapeutic agents, either singly or in combination with a platinum compound, into concurrent chemoradiation regimens for locally advanced NSCLC. A large number of pilot studies have been reported, many of which have shown encouraging results. However, for many newer chemotherapeutic agents, dose-limiting toxicities require that lower doses be given during the concurrent phase. Two Japanese phase III studies addressed this topic, being both published in 2010. The Okayama Lung Cancer Study Group run a phase III (OLCSG 0007) (25) comparing the West Japan Lung Cancer Group regimen (cisplatin, vindesine, and mitomycin) with docetaxel and cisplatin administered on a day 1 and 8 regimen for two cycles plus RT, which was not administered in split course in any arms. The primary endpoint was the survival time at 2 years considering on the basis of previous report of an approximately 35% 2-year survival rate for the MVP arm and 55% for the DP arm. Based on this analysis, 96 patients in each arm were required. According to the results, the study was negative because the difference on survival at 2 years did not reached statistical significance (P=0.059) although was numerically superior (78.8% versus 70.3%). Similarly, although the response rate, median survival time, and progression free survival rates tended to be greater in the DP arm than in the MVP arm, the differences were not statistically significant (P>0.05). Authors remarked unpredictably better survival in the MVP arm, possibly related to a better selection of patients as well as the use of a non split course of RT. Based on this, the sample size was small to detect survival differences.

The West Japan Oncology Group conducted other phase III trial (WJTOG0105) with 3 arms (26). Treatment was composed of concurrent chemoradiotherapy and subsequent consolidation chemotherapy. Patients enrolled on arm A received 4 cycles of the MVP regimen. On day 2 of chemotherapy, RT was begun at the dose of 2 Gy/fraction given in 15 fractions over 3 weeks, followed by a rest period of 1 week. Subsequently, radiation was again resumed at the dose of 2 Gy/fraction given in 15 fractions over 3 weeks. The total dose of radiation administered was 60 Gy. In arms B patients received weekly irinotecan and carboplatin during RT followed by two cycles of full dose of both agents. RT was initiated on day 1. The total dose of 60 Gy was given in 30 fractions over a 6-week period. Finally, patients in the arm C were allocated the same schema but irinotecan was substituted by paclitaxel. The primary end point was comparison of the overall survival between the control group (arm A, with an estimated median OS time of 16.5 months) and each of the treatment groups (arm B or C, that would show an increase in the median OS to 20.5 months). A total of 456 patients were registered in a period of 4 years [2001-2005]. Regarding the toxicity, the incidences of grade 3 or worse severe hematologic toxicity, infection, febrile neutropenia, and gastrointestinal toxicity were significantly higher in arm A than in arm B or C. There were no statistically significant differences in the incidences of esophagitis, dyspnea, or pneumonitis. Similarly to the previous study, the differences in survival were not statically significant (arm A *vs.* B, P=0.392; arm A *vs.* C, P =0.876) with median survival time and 3- and 5-year survival rates of 20.5 months, 35.3%, and 17.5% in arm A, 19.8 months, 24.2%, and 17.8% in arm B, and 22.0 months, 26.4%, and 19.5% in arm C. The authors emphasized the more favorable profile of arm C (paclitaxel/carboplatin) to justify their conclusion about that regimen should be considered standard.

Finally, a small trial bi-centric phase II trial designed to assess the activity and safety of weekly paclitaxel-carboplatin versus cisplatin-etoposide (PE) and RT has been recently published (27). Consolidation treatment was delivered as per local protocol considering either platinum-based doublet chemotherapy regimen or single agent chemotherapy regimen both acceptable. The primary endpoint of this trial was 3-year overall survival but only 35 patients in each arm were considerer needed based on an assumption of differences in 3-year survival between 35% for PE regimen and 18% for the weekly paclitaxel/carboplatin schema. The results in terms of median survival time were favorable to PE (20.2 versus 13.5 months) in the PC arm. The 3-year survival rates were 33.1% and 13%, respectively. By contrary, the incidence of Grade 3-4 neutropenia was higher in the PE arm than that in the PC arm (78.1% *vs.* 51.5%, P=0.049). Once again, the total failure, locoregional relapses, and distant metastases were high in both arms (57.6%, 33.3%, and 33.3% in the PE arm and 78.1%, 46.9%, and 40.7% in the PC arm), highlighting the need to explore new strategies.

Table 4 Ongoing phase II studies in stage III NSCLC EGFR mutated patients

Design	Primary objective	Number estimated patients
Erlotinib + RT *vs*. CDDP/Etoposide/RT NCT01714908	PFS	100
Gefitinib + RT NCT01391260	ORR	30
Neoadjuvant Afatinib, then CT, then surgery and adjuvant CT followed by Afatinib ASCENT Trial NCT01553942	ORR	30

RT, radiotherapy; CT, chemotherapy (pemetrexed and cisplatin); PFS, progression free survival; ORR, overall response rate.

New chemotherapy agents

Pemetrexed, a multitargeted antifolate active in advanced non-squamous NSCLC patients have been also tested in stage III. Several phase I studies (28-30) founded that it was feasible to combine pemetrexed/carboplatin or cisplatin at full dose with RT. In addition, phase II (31,32) results showed promising results compared with historical studies, but these results needed to be confirmed in larger trials. Regrettably, the phase III trial comparing the combination of pemetrexed, cisplatin with cisplatin, etoposide concomitant with RT in patients with nonsquamous NSCLC stopped the accrual on September 2012 because the experimental arm has crossed the futility boundary and it is unlike to attend the HR of 0.74 in favor of the pemetrexed arm.

Molecular targeted agents

In non-selected population different types of agents have been evaluated in stage III NSCLC. The SWOG investigated the use of gefitinib as maintenance after maximum cytoreduction with chemoradiotherapy in the phase II study S0023 (33). All patients received cisplatin, etoposide concomitant with radiotherapy followed by 3 cycles of docetaxel. Patients whose disease did not progress were randomly assigned to gefitinib 250 mg/d or placebo until disease progression, intolerable toxicity, or the end of 5 years. The planned sample size was 672 patients to confer power of 0.89 to detect a 33% increase over the expected median survival time of 21 months. However, an unplanned interim analysis rejected the alternative hypothesis of improved survival at the P=0.0015 level for 243 randomly assigned patients and the study was closed. The median survival time was 23 months for gefitinib and 35 months for placebo (P=0.013). Although the reasons for this result remain unclear, it was established that routine use of maintenance EGFR-TKIs in stage III disease outside of a clinical trial should be avoided.

Cetuximab has been also tested in several studies in the stage III setting (34). Blumenschein and colleagues reported a median survival of 22.7 months and 50% 2-year survival in RTOG 0324 (35), adding weekly cetuximab to low-dose weekly paclitaxel-carboplatin with RT, followed by consolidation cetuximab-paclitaxel-carboplatin. On the basis of these results, an intergroup phase III trial (RTOG 0617) was designed to test radiation with carboplatin and paclitaxel, with or without cetuximab. However, on June 2011, two of the four arms in the protocol were closed to accrual when a planned interim analysis showed that the higher radiation dose being tested, 74 Gy, could not produce an overall survival benefit compared with the lower, standard dose of 60 Gy (36). Although data are immature, treatment-related toxicities were not significantly different (37), and differences in local versus distant disease failure have not been reported. The 60 Gy control and cetuximab arms of the study are currently ongoing.

Antiangiogenic therapy has been also tested in stage III. Unfortunately, to date, phase II trials of bevacizumab combined with platinum-based chemoradiotherapy were closed early because of an excess risk of hemorrhage and tracheoesophageal fistulas (38).

Limited data with molecular targeted agents are available in selected population such as the EGFR mutated patients but several phase II studies are ongoing (39-42) (*Table 4*).

Future directions

Unfortunately, despite much effort during the last 20 years, we have witnessed little progress in treating unresectable stage III NSCLC. Treatment failures continue to occur both locoregionally and/or distantly, although radiographic evidence of locoregional failures only account for approximately one third of recurrences, suggesting the urgent need for more adequate systemic control. Similarly, novel approaches to improve radiation

therapy delivery are needed. Strategies such as intensity-modulated radiotherapy, which enhances the radiation oncologist's ability to contour radiation doses around a tumor with selective sparing of adjacent structures, and proton therapy are being investigated but caution should be used when interpreting the results of the trials exploring these modalities due to selection biases inherent in phase II studies and the lack of level 1 evidence. Therefore, outside the context of clinical trials, these techniques cannot be recommended as a standard alternative. Finally, it should be pointed out that therapeutic advances will likely come from a greater understanding of tumor biology and optimal patient selection. Improving our understanding of molecular subtypes will hopefully lead to rational drug design and more precise clinical trial questions. Only through active partnerships between patients and their healthcare providers to enroll patients in appropriate clinical trials will we see significant improvements in outcomes in our patients in a near future.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Dillman RO, Herndon J, Seagren SL, et al. Improved survival in stage III non-small-cell lung cancer: seven-year follow-up of cancer and leukemia group B (CALGB) 8433 trial. J Natl Cancer Inst 1996;88:1210-5.
2. Sause W, Kolesar P, Taylor S IV, et al. Final results of phase III trial in regionally advanced unresectable non-small cell lung cancer: Radiation Therapy Oncology Group, Eastern Cooperative Oncology Group, and Southwest Oncology Group. Chest 2000;117:358-64.
3. Jeremic B, Shibamoto Y, Acimovic L, et al. Randomized trial of hyperfractionated radiation therapy with or without concurrent chemotherapy for stage III non-small-cell lung cancer. J Clin Oncol 1995;13:452-8.
4. Jeremic B, Shibamoto Y, Acimovic L, et al. Hyperfractionated radiation therapy with or without concurrent low-dose daily carboplatin/etoposide for stage III non-small-cell lung cancer: a randomized study. J Clin Oncol 1996;14:1065-70.
5. Schaake-Koning C, van den Bogaert W, Dalesio O, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. N Engl J Med 1992;326:524-30.
6. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899-909.
7. Aupérin A, Le Péchoux C, Pignon JP, et al. Concomitant radio-chemotherapy based on platin compounds in patients with locally advanced non-small cell lung cancer (NSCLC): a meta-analysis of individual data from 1764 patients. Ann Oncol 2006;17:473-83.
8. Rowell NP, O'rourke NP. Concurrent chemoradiotherapy in non-small cell lung cancer. Cochrane Database Syst Rev 2004;(4):CD002140.
9. Furuse K, Fukuoka M, Kawahara M, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. J Clin Oncol 1999;17:2692-9.
10. Fournel P, Robinet G, Thomas P, et al. Randomized phase III trial of sequential chemoradiotherapy compared with concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer: Groupe Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Français de Pneumo-Cancérologie NPC 95-01 Study. J Clin Oncol 2005;23:5910-7.
11. Curran WJ Jr, Paulus R, Langer CJ, et al. Sequential vs. concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. J Natl Cancer Inst 2011;103:1452-60.
12. Aupérin A, Le Péchoux C, Rolland E, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. J Clin Oncol 2010;28:2181-90.
13. Vokes EE, Herndon JE 2nd, Crawford J, et al. Randomized phase II study of cisplatin with gemcitabine or paclitaxel or vinorelbine as induction chemotherapy followed by concomitant chemoradiotherapy for stage IIIB non-small-cell lung cancer: cancer and leukemia group B study 9431. J Clin Oncol 2002;20:4191-8.
14. Vokes EE, Herndon JE 2nd, Kelley MJ, et al. Induction chemotherapy followed by chemoradiotherapy compared with chemoradiotherapy alone for regionally advanced unresectable stage III Non-small-cell lung cancer: Cancer and Leukemia Group B. J Clin Oncol 2007;25:1698-704.
15. Albain KS, Crowley JJ, Turrisi AT 3rd, et al. Concurrent cisplatin, etoposide, and chest radiotherapy in pathologic stage IIIB non-small-cell lung cancer: a Southwest Oncology Group phase II study, SWOG 9019. J Clin Oncol 2002;20:3454-60.

16. Gandara DR, Chansky K, Albain KS, et al. Consolidation docetaxel after concurrent chemoradiotherapy in stage IIIB non-small-cell lung cancer: phase II Southwest Oncology Group Study S9504. J Clin Oncol 2003;21:2004-10.
17. Gandara DR, Chansky K, Albain KS, et al. Long-term survival with concurrent chemoradiation therapy followed by consolidation docetaxel in stage IIIB non-small-cell lung cancer: a phase II Southwest Oncology Group Study (S9504). Clin Lung Cancer 2006;8:116-21.
18. Hanna N, Neubauer M, Yiannoutsos C, et al. Phase III study of cisplatin, etoposide, and concurrent chest radiation with or without consolidation docetaxel in patients with inoperable stage III non-small-cell lung cancer: the Hoosier Oncology Group and U.S. Oncology. J Clin Oncol 2008;26:5755-60.
19. Jalal SI, Riggs HD, Melnyk A, et al. Updated survival and outcomes for older adults with inoperable stage III non-small-cell lung cancer treated with cisplatin, etoposide, and concurrent chest radiation with or without consolidation docetaxel: analysis of a phase III trial from the Hoosier Oncology Group (HOG) and US Oncology. Ann Oncol 2012;23:1730-8.
20. Belani CP, Choy H, Bonomi P, et al. Combined chemoradiotherapy regimens of paclitaxel and carboplatin for locally advanced non-small-cell lung cancer: a randomized phase II locally advanced multi-modality protocol. J Clin Oncol 2005;23:5883-91.
21. Senan S, Cardenal F, Vansteenkiste J, et al. A randomized phase II study comparing induction or consolidation chemotherapy with cisplatin-docetaxel, plus radical concurrent chemoradiotherapy with cisplatin-docetaxel, in patients with unresectable locally advanced non-small-cell lung cancer. Ann Oncol 2011;22:553-8.
22. Fournel P, Vergnenégre A, Robinet G, et al. Induction (ICT) or consolidation chemotherapy (CT) with cisplatin (C) and paclitaxel (P) plus concurrent chemo-radiation (CT/TRT) with cisplatin and vinorelbine (V) for unresectable non-small cell lung cancer (NSCLC) patients (pts): Randomized phase II trial GFPC-GLOT-IFCT 02-01. J Clin Oncol 2006;24:abstr 7048.
23. Garrido P, Rosell R, Massutí B, et al. Predictors of long-term survival in patients with lung cancer included in the randomized Spanish Lung Cancer Group 0008 phase II trial using concomitant chemoradiation with docetaxel and carboplatin plus induction or consolidation chemotherapy. Clin Lung Cancer 2009;10:180-6.
24. Berghmans T, Van Houtte P, Paesmans M, et al. A phase III randomised study comparing concomitant radiochemotherapy as induction versus consolidation treatment in patients with locally advanced unresectable non-small cell lung cancer. Lung Cancer 2009;64:187-93.
25. Segawa Y, Kiura K, Takigawa N, et al. Phase III trial comparing docetaxel and cisplatin combination chemotherapy with mitomycin, vindesine, and cisplatin combination chemotherapy with concurrent thoracic radiotherapy in locally advanced non-small-cell lung cancer: OLCSG 0007. J Clin Oncol 2010;28:3299-306.
26. Yamamoto N, Nakagawa K, Nishimura Y, et al. Phase III study comparing second- and third-generation regimens with concurrent thoracic radiotherapy in patients with unresectable stage III non-small-cell lung cancer: West Japan Thoracic Oncology Group WJTOG0105. J Clin Oncol 2010;28:3739-45.
27. Wang L, Wu S, Ou G, et al. Randomized phase II study of concurrent cisplatin/etoposide or paclitaxel/carboplatin and thoracic radiotherapy in patients with stage III non-small cell lung cancer. Lung Cancer 2012;77:89-96.
28. Brade A, Bezjak A, MacRae R, et al. Phase I trial of radiation with concurrent and consolidation pemetrexed and cisplatin in patients with unresectable stage IIIA/B non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2011;79:1395-401.
29. Cardenal F, Arnaiz MD, Morán T, et al. Phase I study of concurrent chemoradiation with pemetrexed and cisplatin followed by consolidation pemetrexed for patients with unresectable stage III non-small cell lung cancer. Lung Cancer 2011;74:69-74.
30. Li BS, Gong HY, Huang W, et al. Phase I study of pemetrexed, cisplatin, and concurrent radiotherapy in patients with locally advanced non-small cell lung cancer. Am J Clin Oncol 2012;35:115-9.
31. Gadgeel SM, Ruckdeschel JC, Patel BB, et al. Phase II study of pemetrexed and cisplatin, with chest radiotherapy followed by docetaxel in patients with stage III non-small cell lung cancer. J Thorac Oncol 2011;6:927-33.
32. Govindan R, Bogart J, Stinchcombe T, et al. Randomized phase II study of pemetrexed, carboplatin, and thoracic radiation with or without cetuximab in patients with locally advanced unresectable non-small-cell lung cancer: Cancer and Leukemia Group B trial 30407. J Clin Oncol 2011;29:3120-5.
33. Kelly K, Chansky K, Gaspar LE, et al. Phase III trial of maintenance gefitinib or placebo after concurrent chemoradiotherapy and docetaxel consolidation in inoperable stage III non-small-cell lung cancer: SWOG S0023. J Clin Oncol 2008;26:2450-6.

34. Nieder C, Pawinski A, Dalhaug A, et al. A review of clinical trials of cetuximab combined with radiotherapy for non-small cell lung cancer. Radiat Oncol 2012;7:3.
35. Blumenschein GR Jr, Paulus R, Curran WJ, et al. Phase II study of cetuximab in combination with chemoradiation in patients with stage IIIA/B non-small-cell lung cancer: RTOG 0324. J Clin Oncol 2011;29:2312-8.
36. Bradley J, Paulus R, Komaki R, et al. A randomized phase III comparison of standard-dose (60 Gy) versus high-dose (74 Gy) conformal chemoradiotherapy +/- cetuximab for stage IIIa/IIIb non-small cell lung cancer: Preliminary findings on radiation dose in RTOG 0617. The 53rd Annual Meeting of the American Society of Radiation Oncology, October 2-6, 2011, Miami, FL.
37. Cox JD. Are the results of RTOG 0617 mysterious? Int J Radiat Oncol Biol Phys 2012;82:1042-4.
38. Spigel DR, Hainsworth JD, Yardley DA, et al. Tracheoesophageal fistula formation in patients with lung cancer treated with chemoradiation and bevacizumab. J Clin Oncol 2010;28:43-8.
39. Raben D, Bunn PA. Biologically targeted therapies plus chemotherapy plus radiotherapy in stage III non-small-cell lung cancer: a case of the Icarus syndrome? J Clin Oncol 2012;30:3909-12.
40. Rengan R, Maity AM, Stevenson JP, et al. New strategies in non-small cell lung cancer: improving outcomes in chemoradiotherapy for locally advanced disease. Clin Cancer Res 2011;17:4192-9.
41. Stinchcombe TE, Bogart JA. Novel approaches of chemoradiotherapy in unresectable stage IIIA and stage IIIB non-small cell lung cancer. Oncologist 2012;17:682-93.
42. Ausborn NL, Le QT, Bradley JD, et al. Molecular profiling to optimize treatment in non-small cell lung cancer: a review of potential molecular targets for radiation therapy by the translational research program of the radiation therapy oncology group. Int J Radiat Oncol Biol Phys 2012;83:e453-64.

Cite this article as: Garrido P, Olmedo MªE. State of the art of radiotherapy. Transl Lung Cancer Res 2013;2(3):189-199. doi: 10.3978/j.issn.2218-6751.2013.03.02

Recent advances in radiotherapy for thoracic tumours

Michael Fay[1,2,3], Christopher M. Poole[1,4], Gary Pratt[1,2]

[1]Division of Oncology, Royal Brisbane and Women's Hospital, Queensland Health, Brisbane, Australia; [2]School of Medicine, University of Queensland, Brisbane, Australia; [3]Visiting Scientist, Preclinical Molecular Imaging, Eberhard Karls Universität Tübingen, Germany; [4]Medical Physics, Queensland University of Technology, Brisbane, Australia

Correspondence to: Dr. Michael Fay. Division of Oncology, Royal Brisbane and Women's Hospital, Queensland Health, Brisbane, Australia and School of Medicine, University of Queensland, Brisbane, Australia; Currently Visiting Scientist, Preclinical Molecular Imaging, Eberhard Karls Universität Tübingen, Germany. Email: Michael_Fay@health.qld.gov.au.

Abstract: Radiation Oncology technology has continued to advance at a rapid rate and is bringing significant benefits to patients. This review outlines some of the advances in technology and radiotherapy treatment of thoracic cancers including brachytherapy, stereotactic radiotherapy, tomotherapy and intensity modulated radiotherapy. The importance of functional imaging with PET and management of movement are highlighted. Most of the discussion relates to non-small cell lung cancer but management of mesothelioma and small cell lung cancer are also covered. This technology has substantial benefits to patients in terms of decreasing toxicity both in the short and longer term.

Keywords: Radiotherapy; technology; review; advances; thoracic

Submitted Jul 31, 2013. Accepted for publication Aug 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.08.46
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.08.46

Non-small cell lung cancer—staging

One cannot consider radiotherapy advances without first evaluating the impact of imaging. We should note the staging changed with the introduction of TNM version 7 following a large multinational, multi-disciplinary, and international collaboration (1). Clearly, avoiding treating patients with metastatic disease is beneficial and improves the cost effectiveness of treatment (2). However, we have reached another level of PET use with the rational integration of functional imaging data into radiotherapy planning. It makes little sense to try to treat possible subclinical disease when you are unable to control the primary tumour (David Ball—personal communication). Indeed trying to treat larger volumes may actually impair outcomes by compromising dose.

Waiting time continues to receive attention. Earlier studies have shown that delay is associated with larger tumour volumes at treatment (3). Most recently the effect of delay on the extent of the disease on PET volumes has been examined (4). When patients were subjected to a staging PET and an RT planning PET it was evident that the mean tumour volume had almost doubled on PET. 6/82 patients were then unsuitable for radical treatment (4).

EBUS/TBNA has had an effect here as well. While FDG PET is exquisitely sensitive it is not 100% specific; the presence of tuberculosis significantly complicates the analysis for example. Recent studies of regional nodes suggest TBNA in instead of PET may improve staging accuracy (5).

PET-MR is the next major clinically available advance in imaging technology. Concurrent acquisition of PET data and MR imaging has presented significant technical challenges as the whole method of acquiring a PET image has had to be re-engineered (6,7). The presence of a magnetic field however, limits the range of positrons thereby increasing in intrinsic resolution of PET-MR when compared to conventional PET. This technology is in the early stages of clinical adoption.

In-situ disease (CIS)/minimally invasive disease

Bronchial brachytherapy has attracted some interest with advances in bronchoscopic technique and technical improvements such as bronchoscopic ultrasound (EBUS) allowing a unique view of the tumour.

Brachytherapy is being employed using bronchoscopically placed catheters and an iridium HDR source. Bronchoscopic advances such as ultrasound have assisted in the definition of tumour volume and defining the edges of tumour to be treated.

Managing movement of the tumour and organs at risk

Various techniques exist to account for tumour movement, both during planning and treatment. Fiducial markers are one such solution that is useful throughout. Implanted fiducial markers present an opportunity to better define tumour outline at the planning stage and provide a 'geometric fix' on a tumour during therapy, when coupled with real-time imaging modalities. Advances here are happening concurrently with technical developments in bronchoscopy and ultrasound.

A robotically controlled linear accelerator (Cyberknife™, Accuray) solves the online movement problem by moving the radiation source in sync with the target. Diagnostic X-rays are used to close the feedback loop with the linear accelerator, constantly updating it with the position of the target. Another such online target tracking system is Calypso (Calypso Medical), which uses radio frequency transponders as fiducial markers.

Offline gating presents yet another solution to the problem allowing for more precise target definition. Here the CT puts the images into "bins" according to the phase of the breathing cycle. Total scan time is increased but useful position data can be acquired; thus a "4D CT" is generated. Online gating systems are also available for target motion compensation during treatment by tracking the motion of the patients external contour, such as Varian RPM. Systems such as this however, track a surrogate of the target motion, not the target motion itself.

Another example of motion compensation is to use a PET fused to the planning CT. As the PET is acquired over about 20 mins it smears out the tumour volume effectively defining a region in which the tumour is most likely to reside.

4D CT (and 4D-PET) have been looked at as a way of defining tumour motion which may be more accurate than our usual geometric expansions. Finally coaching of patients using some form of bio-feedback is finding increasing clinical application with the same aim.

Volume definition

With better technology telling us where to treat; so have come RT advances allowing us to treat small and moving targets. The concept of "volumetric conformity" still has significant difficulties with implementation. At present automated methods for tumour delineation have not proven robust enough for clinical use.

PET imaging with 18FDG has revolutionised both the staging and treatment volume definition but problems remain. Standardised uptake values (SUV) are not standardised between machines and edges of the tumour remain difficult to define (8-10). Modelling has been undertaken looking at the changes in dose to critical normal tissues. This shows PET decreases the dose delivered to normal tissue; while improving the tumour control probability (11).

Cone beam CT (or tomotherapy megavoltage CT) present the possibility of adjusting tumour volume definitions during treatment as the tumour shrinks (12). Such approaches appear to decrease the volume of normal tissue irradiated (13).

Finally automated target volume definition, again with FDG, has been attempted. As yet there is little agreement with "manually derived" contours (14). The technology is still immature but may one day allow daily changes in treatment volume without prohibitive cost.

Treatment response

Advances in imaging have allowed changes to the irradiated volume to occur even on a daily basis (15).

A pilot study of interval PET has shown that a PET two weeks into treatment can be useful in terms of defining response to radiotherapy (16). Further modifications will no doubt examine dose painting to boost areas of greater tumour activity. While we doubt any oncologist would stop treatment early in the course of treatment it may be a prompt to increase dose or intensify treatment.

Intensity modulated radiotherapy (IMRT) techniques such as helical tomotherapy have allowed us to "bend" the dose cloud around critical structures. The role of tomotherapy would seem to be particularly in central and posteriorly placed tumours where the radiation oncologist is trying to avoid critical structures. It may have a role in treating multiple primary tumours or RT plans in which very large volumes of normal tissue are being irradiated. The role of dose painting and dose escalation continues to receive research attention.

Planning studies suggest the dose uniformity and homogeneity may be better with tomotherapy™ but the

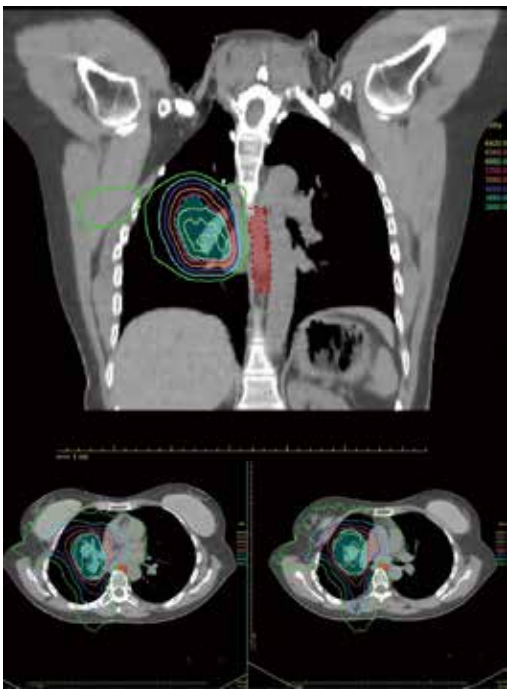


Figure 1 Tomotherapy™ plan of a non-small cell lung cancer avoiding the oesophagus (shown in red).

area irradiated to low dose is probably increased (13). Tomotherapy™ has been evaluated in studies looking at integral dose and the risk of induced malignancies (17). This is thought to be no greater than other highly conformal techniques (*Figure 1*).

Hypofractionation using stereotactic body radiotherapy

Probably the largest clinical impact has come from hypofractionating treatment. The biological effect of RT is significantly increased by giving a small number of large fractions (so called hypofractionation). Often this is less than five fractions. The work of Timmerman and others have highlighted the importance of a biologically equivalent dose (BED) of at least 150 Gy (18,19). Given the significant changes in patterns of care which are happening, it is surprising there is not a wealth of randomised clinical trials (20). The practical implications of implementing such a change to treatment paradigms should not be forgotten (21).

Central tumours have caused some concern that toxicity would be increased but a recent systematic review has not borne this out (22). As long as an appropriate fractionation schedule is employed the toxicity appears manageable and efficacy maintained. Imaging is even more important for hypofractionation, and especially with regard to motion compensation.

Protons have been employed in hypofractionated lung treatment although cost remains prohibitive in many countries (23).

Investigators have looked at minimally invasive disease treated with stereotactic radiotherapy (24). Fitting with the concept of tumours formerly referred to as bronchioloalveloar carcinoma (BAC) as a field change there were concerns that there would be potential difficulties with defining the edges of the tumour. Interestingly however, the there was no significant difference noted in three year regional failure.

Tumour volume

Tumour volume has been investigated by the Trans-Tasman Radiation Oncology Group (TROG). Previously tumour size was not shown to correlate with clinical stage (25) and more recent work has shown the relationship to prognosis is complex. Indeed the new staging system makes little mention of tumour size (26). The prognostic significance of tumour size changes over time—in the first 18 months the larger the tumour the higher risk of dying. Beyond 18 months the association is weak and the authors suggest size alone should not be a reason to deny a patient potentially curative treatment (26).

Locally advanced disease

The advances here are likely to be from combinations of chemotherapy or combinations with molecular agents. We need better tools to quantify the effect of low doses of radiation on normal lung tissue. The high dose region is usually able to be smaller and more conformal but problems still remain.

In locally advanced disease the challenge lies in minimising volumes of normal lung irradiated while covering all the tumour and doing so at a dose high enough to sterilise the area. New approaches to advanced disease include the addition of biologic agents such as cetuximab (27,28) as they have in other sites. This is based on preclinical models suggesting a radiosensitisation effect (27). This treatment has modest additional benefit.

Molecular markers

The molecular revolution has not escaped this corner of

medicine. TGF-beta isoforms are thought to be related to the risk of radiation pneumonitis. The relationship between TGF-beta and the development of pneumonitis appears complex and ongoing efforts aim to refine predictors of radiation pneumonitis (29).

Radiation pneumonitis has also been associated with genetic variation in the form of single nucleotide polymorphisms (SNPs) for certain genotypes of heat shock proteins associated with a greatly increased risk of radiation pneumonitis in non small cell lung cancers treated with chemoradiation (30).

Radiogenomic studies (31) ave pointed to promising areas of research aimed at predicting response to combined modality therapy. Early *in vitro* early evidence has emerged of anaplastic lymphoma kinase (ALK) inhibitors enhancing radiotherapy response (32). *In vivo* models show some promise with concurrent use of hedgehog pathway inhibitors (33).

Small cell lung cancer

PET imaging with ^{18}FDG for small cell lung cancer has been examined in a systematic review (34). Cost appeared comparable—at least in the Australian context. Radiotherapy changes, such as changed field borders, resulted in changes in about 28% of patients. About 6% of small cell lung cancer patients would be offered RT after a PET who would not have been offered RT prior to PET. A further 9% of patients with occult metastatic disease would be spared radical treatment.

We have phase III evidence supporting the benefit of hyperfractionated accelerated radiotherapy but the uptake of this seems slow in practice—perhaps reflecting the difficulty in getting patients though such a regimen. Perhaps here is a further application of IMRT treatment.

Mesothelioma—pleural radiotherapy

IMRT techniques have been widely used in management of resected and unresected malignant pleural mesothelioma (35-37). There is as yet no consensus on its role as a routine standard of care (38).

There are some reports that conventional lung normal tissue constraints using V20 and Mean Lung Dose MLD are not appropriate after extrapleural pneumonectomy and that more conservative constraints using V5 are needed (39).

An Italian study has reported in abstract form reporting the use of accelerated hypofractionation over 5 fractions with helical Tomotherapy for unresected mesothelioma with acceptable toxicity (40). An Australian study reported 71% infield local control included PET based Total Glycolytic Volume as well as survival outcome data using IMRT (41).

Conclusions

It is fortunate that emerging health technologies are set to change the way we implement radiation oncology practice to achieve the best outcomes for our patients with lung cancer. Nonetheless, despite the hope and promise of new technologies we should not forget the effect that our treatments have on our patient's quality of life. As these new tools allow us to do more—we hope that we will be better able to choose patients for treatment, adapt that treatment to them and that with more conformal treatment related toxicity will reduce (42).

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Goldstraw P, Ball D, Jett JR, et al. Non-small-cell lung cancer. Lancet 2011;378:1727-40.
2. Kalff V, Hicks RJ, MacManus MP, et al. Clinical impact of (18)F fluorodeoxyglucose positron emission tomography in patients with non-small-cell lung cancer: a prospective study. J Clin Oncol 2001;19:111-8.
3. O'Rourke N, Edwards R. Lung cancer treatment waiting times and tumour growth. Clin Oncol (R Coll Radiol) 2000;12:141-4.
4. Everitt S, Plumridge N, Herschtal A, et al. The impact of time between staging PET/CT and definitive chemo-radiation on target volumes and survival in patients with non-small cell lung cancer. Radiother Oncol 2013;106:288-91.
5. Kuo CH, Chen HC, Chung FT, et al. Diagnostic value of EBUS-TBNA for lung cancer with non-enlarged lymph nodes: a study in a tuberculosis-endemic country. PLoS One 2011;6:e16877.
6. Chandarana H, Heacock L, Rakheja R, et al. Pulmonary Nodules in Patients with Primary Malignancy: Comparison of Hybrid PET/MR and PET/CT Imaging. Radiology 2013;268:874-81.
7. Schwenzer NF, Schraml C, Müller M, et al. Pulmonary lesion assessment: comparison of whole-body hybrid

MR/PET and PET/CT imaging--pilot study. Radiology 2012;264:551-8.
8. Suzawa N, Ito M, Qiao S, Uchida K, et al. Assessment of factors influencing FDG uptake in non-small cell lung cancer on PET/CT by investigating histological differences in expression of glucose transporters 1 and 3 and tumour size. Lung Cancer 2011;72:191-8.
9. MacManus M, Nestle U, Rosenzweig KE, et al. Use of PET and PET/CT for radiation therapy planning: IAEA expert report 2006-2007. Radiother Oncol 2009;91:85-94.
10. Yaremko B, Riauka T, Robinson D, et al. Thresholding in PET images of static and moving targets. Phys Med Biol 2005;50:5969-82.
11. van Der Wel A, Nijsten S, Hochstenbag M, et al. Increased therapeutic ratio by 18FDG-PET CT planning in patients with clinical CT stage N2-N3M0 non-small-cell lung cancer: a modeling study. Int J Radiat Oncol Biol Phys 2005;61:649-55.
12. Knap MM, Hoffmann L, Nordsmark M, et al. Daily cone-beam computed tomography used to determine tumour shrinkage and localisation in lung cancer patients. Acta Oncol 2010;49:1077-84.
13. Meng LL, Feng LC, Wang YL, et al. Dosimetric comparison between helical tomotherapy and intensity-modulated radiation therapy plans for non-small cell lung cancer. Chin Med J (Engl)2011;124:1667-71.
14. Niyazi M, Landrock S, Elsner A, et al. Automated biological target volume delineation for radiotherapy treatment planning using FDG-PET/CT. Radiat Oncol 2013;8:180.
15. Dobbs HJ. Defining the radiation target on a daily basis. Cancer Imaging 2006;6:30-2.
16. Kong FM, Frey KA, Quint LE, et al. A pilot study of [18F] fluorodeoxyglucose positron emission tomography scans during and after radiation-based therapy in patients with non small-cell lung cancer.J Clin Oncol 2007;25:3116-23.
17. Kim DW, Chung WK, Shin D, et al. Risk of second cancer from scattered radiation of intensity-modulated radiotherapies with lung cancer. Radiat Oncol 2013;8:47.
18. Timmerman R, Papiez L, McGarry R, et al. Extracranial stereotactic radioablation: results of a phase I study in medically inoperable stage I non-small cell lung cancer. Chest 2003;124:1946-55.
19. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. JAMA 2010;303:1070-6.
20. Senan S. Surgery versus stereotactic radiotherapy for patients with early-stage non-small cell lung cancer: More data from observational studies and growing clinical equipoise. Cancer 2013;119:2668-70.
21. Dahele M, Pearson S, Purdie T, et al. Practical considerations arising from the implementation of lung stereotactic body radiation therapy (SBRT) at a comprehensive cancer center. J Thorac Oncol 2008;3:1332-41.
22. Senthi S, Haasbeek CJ, Slotman BJ, et al. Outcomes of stereotactic ablative radiotherapy for central lung tumours: a systematic review. Radiother Oncol 2013;106:276-82.
23. Hata M, Tokuuye K, Kagei K, et al. Hypofractionated high-dose proton beam therapy for stage I non-small-cell lung cancer: preliminary results of a phase I/II clinical study. Int J Radiat Oncol Biol Phys 2007;68:786-93.
24. Badiyan SN, Bierhals AJ, Olsen JR, et al. Stereotactic body radiation therapy for the treatment of early-stage minimally invasive adenocarcinoma or adenocarcnioma in situ (formerly bronchioloalveolar carcinoma): a patterns of failure analysis. Radiat Oncol 2013;8:4.
25. Ball DL, Fisher R, Burmeister B, et al. Stage is not a reliable indicator of tumor volume in non-small cell lung cancer: a preliminary analysis of the Trans-Tasman Radiation Oncology Group 99-05 database. J Thorac Oncol 2006;1:667-72.
26. Ball DL, Fisher RJ, Burmeister BH, et al. The complex relationship between lung tumor volume and survival in patients with non-small cell lung cancer treated by definitive radiotherapy: a prospective, observational prognostic factor study of the Trans-Tasman Radiation Oncology Group (TROG 99.05). Radiother Oncol 2013;106:305-11.
27. Blumenschein GR Jr, Paulus R, Curran WJ, et al. Phase II study of cetuximab in combination with chemoradiation in patients with stage IIIA/B non-small-cell lung cancer: RTOG 0324. J Clin Oncol 2011;29:2312-8.
28. Jensen AD, Münter MW, Bischoff H, et al. Treatment of non-small cell lung cancer with intensity-modulated radiation therapy in combination with cetuximab: the NEAR protocol (NCT00115518). BMC Cancer 2006;6:122.
29. Vujaskovic Z, Groen HJ. TGF-beta, radiation-induced pulmonary injury and lung cancer. Int J Radiat Biol 2000;76:511-6.
30. Pang Q, Wei Q, Xu T, et al. Functional promoter variant rs2868371 of HSPB1 is associated with risk of radiation pneumonitis after chemoradiation for non-small cell lung cancer. Int J Radiat Oncol Biol Phys 2013;85:1332-9.
31. Das AK, Bell MH, Nirodi CS, et al. Radiogenomics

predicting tumor responses to radiotherapy in lung cancer. Semin Radiat Oncol 2010;20:149-55.
32. Dai Y, Melzig C, Hanne J, et al. Combined ALK-inhibition and Radiation Therapy in Lung Cancer. International Journal of Radiation Oncology*Biology*Physics. Elsevier Inc, 2012;84:S706.
33. Zeng J, Aziz K, Chettiar ST, et al. Hedgehog pathway inhibition radiosensitizes non-small cell lung cancers. Int J Radiat Oncol Biol Phys 2013;86:143-9.
34. Ruben JD, Ball DL. The efficacy of PET staging for small-cell lung cancer: a systematic review and cost analysis in the Australian setting. J Thorac Oncol 2012;7:1015-20.
35. Rosenzweig KE, Zauderer MG, Laser B, et al. Pleural intensity-modulated radiotherapy for malignant pleural mesothelioma. Int J Radiat Oncol Biol Phys 2012;83:1278-83.
36. Patel PR, Yoo S, Broadwater G, et al. Effect of increasing experience on dosimetric and clinical outcomes in the management of malignant pleural mesothelioma with intensity-modulated radiation therapy. Int J Radiat Oncol Biol Phys 2012;83:362-8.
37. Ebara T, Kawamura H, Kaminuma T, et al. Hemithoracic intensity-modulated radiotherapy using helical tomotherapy for patients after extrapleural pneumonectomy for malignant pleural mesothelioma. J Radiat Res 2012;53:288-94.
38. Chapman E, Berenstein EG, Diéguez M, et al. Radiotherapy for malignant pleural mesothelioma. Cochrane Database Syst Rev 2006;(3):CD003880.
39. Allen AM, Czerminska M, Jänne PA, et al. Fatal pneumonitis associated with intensity-modulated radiation therapy for mesothelioma. Int J Radiat Oncol Biol Phys 2006;65:640-5.
40. Parisi E, Sarnelli A, Giannini M, et al. Accelerated Hypofractionated Radiation Therapy Using Helical Tomotherapy for the Treatment of Medically Inoperable Pleural Mesothelioma: IRST Preliminary Data. International Journal of Radiation Oncology*Biology*Physics. Elsevier Inc, 2012;84:S578-S9.
41. Feigen M, Lee ST, Lawford C, et al. Establishing locoregional control of malignant pleural mesothelioma using high-dose radiotherapy and (18) F-FDG PET/CT scan correlation. J Med Imaging Radiat Oncol 2011;55:320-32.
42. McCloskey P, Balduyck B, Van Schil PE, et al. Radical treatment of non-small cell lung cancer during the last 5 years. Eur J Cancer 2013;49:1555-64.

Cite this article as: Fay M, Poole CM, Pratt G. Recent advances in radiotherapy for thoracic tumours. J Thorac Dis 2013;5(S5):S551-S555. doi: 10.3978/j.issn.2072-1439.2013.08.46

Alternatives to surgery in early stage disease—stereotactic body radiotherapy

Meredith Elana Giuliani[1,2], Andrea Bezjak[1,2]

[1]Radiation Medicine Program, Princess Margaret Hospital, Toronto, Ontario, Canada; [2]Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada

Correspondence to: Dr Andrea Bezjak, Professor, Radiation Oncologist. Department of Radiation Oncology, University of Toronto, Princess Margaret Cancer Centre, 610 University Ave, Toronto, ON, M5G2M9, Canada. Email: Andrea.Bezjak@rmp.uhn.on.ca.

Abstract: The management of early stage non-small cell lung carcinoma (NSCLC) has been revolutionized by the introduction of stereotactic body radiotherapy (SBRT). SBRT is now the standard of care for medically inoperable patients with early stage NSCLC. However, the role of SBRT in medically operable patients remains controversial. This article will review the indications, the technical considerations, image guidance principles, potential toxicities and special circumstances in lung SBRT.

Keywords: Local control; non-small cell lung carcinoma; stereotactic body radiotherapy; stereotactic ablative radiotherapy; toxicity

Submitted Oct 05, 2013. Accepted for publication Oct 13, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.10
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.10

Introduction

The strictest definition of early stage non-small cell lung carcinoma (NSCLC) refers to patients with T1-2aN0 tumors (1). This chapter will focus on the management of these early stage NSCLC with radiotherapy, and specifically with high dose high precision stereotactic body radiotherapy (SBRT), also known as stereotactic ablative radiotherapy (SABR).

Currently the standard or care for early stage NSCLC is lobectomy in patients who are suitable candidates (2). However, many patients are not suitable for lobectomy due to medical co-morbidities, pulmonary function or in some circumstances patient preference. The surgical alternatives to lobectomy, in the form of sublobar resections, are being explored in such patients. Radiotherapy is an option for patients who are not able to undergo surgical resection. We do not recommend observation in this patient population, unless the patient is estimated to have an extremely limited life expectancy from comorbidities, as the median survival in patients with untreated stage I NSCLC is 14 months and the majority die of lung cancer (3). In a population based study, the introduction of SBRT lead to a reduction in the proportion of patients receiving no treatment for their early stage lung cancer, and also significantly improved the survival of patients with early stage lung cancer at the population level (4).

Prior to the widespread use of SBRT, radiotherapy involved 6 to 7 weeks of treatment with standard dose fractionation of 2 Gy per fraction daily; typical doses were 60 Gy in 30 fractions or more, to the primary tumor and surrounding lung ("involved field") and occasionally to the lymph node regions deemed at risk of harboring microscopic disease. These regimens have the advantage of conventional dose per fraction, with potentially less late normal tissue injury (although these doses are well above radiation tolerance of lung, and some amount of lung fibrosis is to be expected), but a lower biological dose. With lower biological doses there is an expected lower rate of long-term local control (5). Clinical outcomes were generally poor with local failures occurring in approximately 40% of patients (6). The focus of therapy turned to dose escalation in the hope of improving clinical outcomes, specifically local control in this patient population.

Dose escalation strategies occurred in the form of hypofractionated regimens. Common regimens used at our institution which have acceptable efficacy, 20% local failure at 5 years, and are well tolerated are 60 Gy in 20 fractions or 50 Gy in 20 fractions (7). A Canadian national phase II study in peripheral tumors using 60 Gy in 15 fractions reported 2-year actuarial local control of 88% and 2-year overall survival of 69%. The most frequent toxicities were fatigue, cough and dyspnea. Radiation pneumonitis occurred in 10% of patients (8).

Stereotactic body radiotherapy (SBRT)

Lung SBRT or SABR involves using few high dose fractions to treat small target volume (9) guided by a set of coordinates (thus the term "stereotactic"). These coordinates are set in relationship to the precise location of the tumor, rather than a set of external marks (tattoos) or anatomical landmarks (such as bony structures), which is typical for conventional RT. The principles of body SBRT are an adaptation of the principles and experience gained from stereotactic brain RT, a well-established high-precision RT technique that uses a set of coordinates on a stereotactic frame afixed to the patient's head, to direct multiple beams to a well-defined intracranial target. This allows the delivery of high doses of RT to the target while minimizing the exposure of normal tissue. In the case of lung cancer, the coordinates are set in relationship to the tumor itself, which can be visualized either directly with volumetric imaging such as cone-beam CT which is part of a linear accelerator, or localized through use of implanted fiducial markers, akin to what has been used with gold seed implants for prostate radiotherapy.

In addition to the use of tumor localization in the three dimensions, other important principles of stereotactic RT that need to be applied to lung SBRT are the precise outline (contouring) of a well-defined target (tumor), identification of a relatively tight (small) planning target volume (PTV) by minimizing target motion and set-up variation, conformal RT planning, using multiple small beams coming from various directions and planes, daily set-up verification prior to each treatment and the use of high RT doses that can ensure high rates of tumor cell kill.

Several single center and multicenter prospective studies, as well as numerous retrospective reports have established the safety and efficacy of lung SBRT for early stage lung cancer. There are many dose and fractionation schedules used. Local control in the order of 85-90% has been reported with most dose-fractionation schedules that provide a biologic effective dose (BED) of 100 Gy or more (10). Those schedules include 48 Gy in 4 fractions (of 12 Gy each), 55 Gy in 5 fractions (of 11 Gy each), 60 Gy in 8 fractions (of 7.5 Gy each), and 54-60 Gy in 3 fractions (of 18-20 Gy per fraction). The choice of schedule and dose depends on tumor size, location and institutional experience/preference.

In the context of lung SBRT tumors are generally <5 cm. SBRT may be considered for T1-2N0M0 and select <5 cm T3N0M0 chest wall NSCLC (11). It is our practice to deliver 54 Gy in 3 fractions for larger peripheral tumors, away from organs at risk (OAR), 48 Gy in 4 fractions for peripheral tumors <3 cm in diameter and 60 Gy in 8 fractions for centrally located tumors (i.e., tumors within a 2 cm radius of the airway or great vessels). The optimal dose for centrally located tumors is controversial and is awaiting analysis and reporting of the phase I/II RTOG study 0813 (12). In the phase II multicenter RTOG 0236 study, SBRT for early stage NSCLC in medically inoperable patients, with 60 Gy/3 fractions (equivalent to 54 Gy/3 fractions when corrected for lung tissue heterogeneity) was associated with a 3-year 98% tumor control, 91% local control and 56% overall survival (OS) (13).

Accurate mediastinal staging in potential candidates from SBRT is essential. Traditionally, patients who receive surgical resection for early stage NSCLC would have invasive mediastinal staging, either preoperative or intraoperative. In surgical patients staged preoperatively with PET/CT as N0, the occult node positivity rate at the time of surgery is 18%. Patients with tumors >3 cm or high SUVmax are at higher risk of occult nodal metastasis (14). Thus, before proceeding with SBRT, patients should at a minimum have PET staging and biopsy of any enlarged or suspicious nodes, and there may be merit in EBUS staging of other SBRT candidates who are at a high risk occult nodal disease. However, despite the absence of rigorous staging, the incidence of nodal relapse following SBRT is low, 5-10% in most series; low dose irradiation to first eschalon nodal regions has been postulated as one possible cause and immune effect of SBRT to the primary lesion in causing a presentation of antigens and resultant immune response that may control other areas of micro-metastatic disease (15), have been postulated as explanations, both have some evidence supporting them.

Technological considerations

As described above, SBRT is a technically rigorous treatment

Figure 1 Abdominal compression plate as used in lung SBRT.

which requires precise tumor localization and treatment delivery to minimize the potential for significant toxicity to normal structures or organs at risk (OARs) (16). To accomplish this one must consider immobilization strategies, respiratory motion control, accurate target delineation, advanced planning algorithms and image guidance (17). We will briefly review the major technological considerations for the planning and delivery of SBRT focusing on motion management and image guidance.

Motion management

All intrathoracic tumors are affected by respiratory movement. Respiratory motion management is an essential component for the successful delivery of lung SBRT (17). There are two major strategies to manage motion in lung SBRT. The first involves reducing respiratory excursion, typically either through abdominal compression or active breathing control (ABC) (*Figure 1*). In some institutions tumor motion is restricted in all patients, in other institutions it is restricted in select circumstances and some institutions employ no motion restriction. When motion restriction is used selectively, a threshold is selected, commonly 1 cm (17). In our institution, using that threshold, less than 25% of patients, require abdominal compression to manage respiratory motion (17).

Figure 2 Stereotactic body frame.

The second method of motion management involves using real-time tumor tracking to intermittently delivery radiotherapy when the target is in the treatment position, this is referred to as "gating". Regardless of the technique used to manage tumor motion, accurate analysis and interpretation of the motion observed on the 4D planning CT scan and accurate localization of the tumor at the time of SBRT delivery is essential to ensure ablation of the tumor and sparing of critical structures.

Target localization

The Stereotactic Body Frame (SBF) was the immobilization strategy used in the earliest reports of extracranial SBRT (18,19) (*Figure 2*). Those early reports emphasized the importance of patient immobilization and accurate repositioning for multi-fraction treatments (9). Clinical outcomes with frame-based SBRT strategies were acceptable (20) however this technique requires a significant amount of treatment unit time and special equipment had to be purchased with staff trained to use it. Now, image guided strategies have been widely implemented to replace the SBF. Continued improvements in the delivery of frameless SBRT offer potential improvements in clinical outcome. Patients with poorer performance status drift more in position during SBRT (21). A change in the delivery of SBRT from multiple static beams to more contemporary volumetric modulated arc therapy (VMAT) affords a faster treatment time which may improve position accuracy by affording less time for patients to drift out of position.

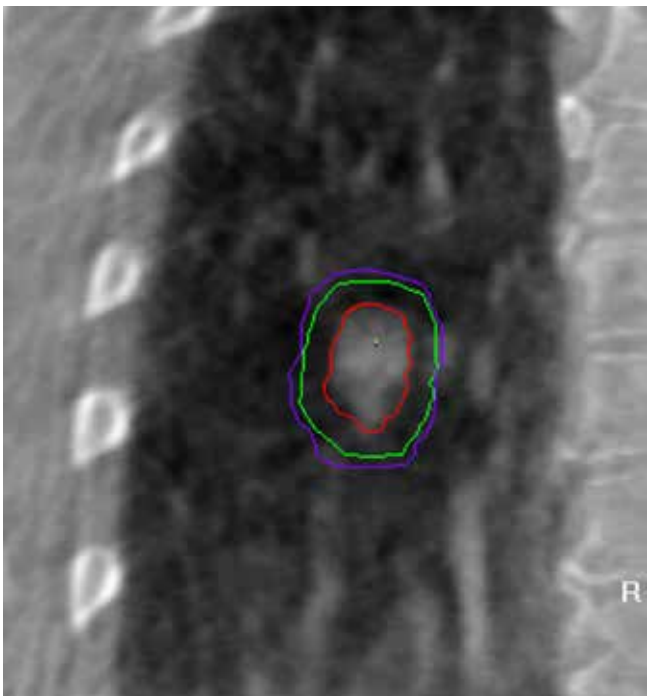


Figure 3 Cone beam CT images taken prior to SBRT. Red line represents the internal target volume (ITV), the green line represents the planning target volume (PTV) and the purple line represents the 95% isodose line from the radiotherapy plan included as a reference.

Several techniques can be used to confirm the tumor location just before or during radiotherapy. These techniques include: CT-on-rails (22), real-time tumor gating (23), TomoTherapy (24), CBCT (25), and Cyberknife (real-time tumor tracking using a robotic system) (26). The conceptual principles are as discussed above, the practical details differ depending on the system. *Figure 3* demonstrates how cone beam images on the treatment unit can be used to position the patient more accurately and guide the radiation beams directly onto the tumor target.

Patient selection for SBRT

SBRT has most widely been adopted for tumors located in the periphery of the lung. In a prospective phase II study conducted by the RTOG the 3-year primary tumor control for stage I/II NSCLC treated with 18 Gy ×3 fractions was 97.6% with only 1 local failure in 55 patients. The lobar control rate at 3 years was 90.6% and the 3-year disease free survival was 48.3% (27). Overall the regimen was well tolerated with 7 patients with grade 3 toxicity and 2 patients with grade 4 toxicity. There were no grade 5 toxicities (27).

SBRT is most commonly used for patients with tumors <5 cm however some centers do deliver SBRT to larger tumors. In our experience, larger tumors still had comparable rates of local control but had higher rates of regional and distant failures, and somewhat higher rates of grade 2 pneumonitis (28).

SBRT toxicity

The rate of adverse events following SBRT is low, however in some circumstances has been severe or fatal (16). The most common side effect in the acute phase is fatigue which is typically mild (grade 1) and seen in approximately 50% of patients (11). Radiation pneumonitis can occur in the 6 weeks to 9 months following SBRT. More uncommon but worrisome due to the catastrophic nature of the outcomes are toxicities related to the central mediastinal structures such as the major vessels (aorta, vena cava etc.) and the proximal airways. Rarely, grade 4 and 5 toxicities such as massive hemoptysis have been reported following SBRT, almost exclusively in the cases of central tumors (29).

Rib fractures and chest wall pain are two side-effects that are almost never reported after conventional fractionated radiotherapy, but have become widely reported and recognized to be associated with SBRT (30). Rib fractures are often asymptomatic and should not be mistaken for bone metastases (*Figure 4*). In a dosimetric and clinical multivariate analysis age, female gender and D0.5 were significantly associated with rib fractures following SBRT (31).

Radiation pneumonitis, a limiting toxicity with conventional RT for lung cancer, and associated with the volume of lung being treated (32) is less commonly reported in patients treated with SBRT, likely due to much smaller volumes treated, even though most patients treated with SBRT have limited lung function. One series reported that grade ≥2 pneumonitis occurred in 11% of patients (29). The risk of radiation pneumonitis is associated with increasing mean lung dose (29).

Similarly, there is minimal reduction of pulmonary function after SBRTand this treatment is suitable even for patients with severe COPD who are oxygen-dependent. At our institution we do not have a minimum cut-off for FEV1 or DLCO. All patients are considered on an individual basis for suitability for SBRT. The only group of patients who are at a higher risk of pulmonary toxicity are patients with idiopathic pulmonary fibrosis.

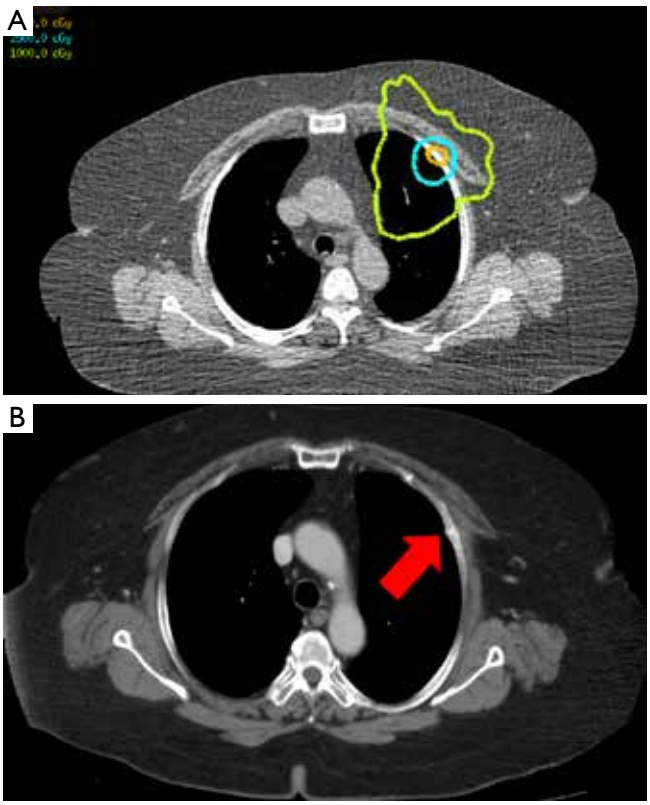


Figure 4 Rib fracture and dosimetric overlay from a Lung SBRT Plan. (A) The orange line represents the 4,320 cGY isodose line, the blue line represents with 2,500 cGy isodose line and the green line represents the 1,000 cGy isodose line; (B) the red arrow indicates the rib fracture.

Radiographic changes following SBRT

The majority of patients have significant radiographic changes in their lung parenchyma following SBRT. These changes gradually develop in the 6 to 12 months following SBRT. Although the majority of patients have developed some degree of radiographic changes 12 months following SBRT the nature of these changes continue to evolve over time. There is no consensus as to how best to categorize these changes however work by Dahele *et al.* proposes a 4 category classification system for late post-SBRT radiographic changes. These categories are: modified conventional pattern, Mass-like fibrosis, Scar-like fibrosis and No evidence of increased density.

These radiographic changes make assessment of local control of the treated tumor following SBRT challenging. Several authors have proposed CT characteristic which may be associated with tumor recurrence as opposed to benign radiographic changes however these have not been independently validated.

The ability to accurately identify patients with residual or recurrent tumors is increasingly important as SBRT is used in operable patients where surgical salvage for a local recurrence may be an option. Further work on other imaging modalities such as MRI, perfusion CT or FLT-PET may be of clinical benefit.

Central tumors

Centrally located tumors require careful consideration when treated with SBRT. Two criteria are currently applied to identify tumors as central: the RTOG 0236 study defined them as tumors that are "within or touching the zone of the proximal bronchial tree defined as a volume 2 cm in all directions around the proximal bronchial tree (carina, right and left main bronchi, right and left upper lobe bronchi, intermedius bronchus, right middle lobe bronchus, lingular bronchus, right and left lower lobe bronchi)" (33). The RTOG 0813 trial in addition also defined as central those "tumors that are immediately adjacent to mediastinal or pericardial pleura (PTV touching the pleura)" (12). Some institutions consider central tumors to also be any tumor within 2 cm of any mediastinal structure (34) although with careful planning, avoidance of mediastinal structures should be possible in most of the latter group.

Timmerman *et al.* reported an excess of respiratory events in patients who received 60 Gy in 3 fractions to centrally located tumors (16). Patients with central tumors had a 2-year freedom from severe toxicity of 54%, significantly lower than patients with peripheral tumors (84%) (16). Thus lead to the introduction of modified fractionations schedules for central tumors. There is significant heterogeneity in institutional practices in that regard, and most try to achieve a BED of 100 or greater. In a patterns-of-practice survey the majority of clinicians preferred a slightly more protracted fractionation schedule (≥4 fractions) for centrally located tumors (35). It is our institutional practice to deliver 60 Gy in 8 fractions; this is supported by data from the NKI group (11,34). Other institutions have reported 50 Gy in 4 fractions (36,37), 48 Gy in 4 fractions (38), 48 Gy in 6 fractions (39), or 60 Gy in 5 fractions (39).

The RTOG phase I/II trial in patients with centrally located tumors has reached the highest planned dose level of 60 Gy in 5 fractions (12) although analysis needs to await the full one year follow-up to determine whether this is

indeed the maximum tolerated dose. The hope is that this study will establish a safe and efficacious dose fractionation for central tumors and will also provide novel data on the radiation tolerance of mediastinal structures.

Medically operable patients

SBRT is now the standard of care in the majority of centers for patients who cannot have surgery for early stage NSCLC. The role of SBRT in patients who are surgical candidates remains controversial. The RTOG has completed accrual to a phase II study exploring the 2-year local control rate in medically operable patients treated with SBRT (40). A review by Onishi *et al.* of SBRT in medically operable patients who refused surgery reported a promising 5-year local control rate of 92% for T1 tumors and 73% for T2 tumors. The 5 year overall survival was 72% for T1 and 62% for T2 tumors (41). However, to conclusively assess the efficacy and safety of SBRT in operable patients compared to surgical resection, randomized data is needed. It is challenging to randomize patients to such different treatment modalities however, several phase III trials have been opened but all had to close due to poor accrual (42). Case-control studies that have included propensity matching (43) have demonstrated that SBRT results are at least equivalent and quite possibly superior to surgery, especially if compared to wedge resection. This is indeed intriguing and provides a solid foundation to offer SBRT even to surgical candidates.

Conclusions

SBRT is a safe and effective treatment for patients with early stage NSCLC who cannot undergo surgical resection. Further studies are needed to determine the safe standard of practice for centrally located tumors and to determine the role of SBRT in medically operable patients.

Acknowledgements

Disclosure: Drs Bezjak and Giuliani have received travel funding from Elekta Inc and Dr Bezjak has received research support from Elekta Inc in the past.

References

1. Edge SB, Byrd DR, Compton CC, et al. AJCC Cancer Staging Manual. Chicago, IL, American Joint Committee on Cancer, 2010
2. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
3. Raz DJ, Zell JA, Ou SH, et al. Natural history of stage I non-small cell lung cancer: implications for early detection. Chest 2007;132:193-9.
4. Wisnivesky JP, Bonomi M, Henschke C, et al. Radiation therapy for the treatment of unresected stage I-II non-small cell lung cancer. Chest 2005;128:1461-7.
5. Fang LC, Komaki R, Allen P, et al. Comparison of outcomes for patients with medically inoperable Stage I non-small-cell lung cancer treated with two-dimensional vs. three-dimensional radiotherapy. Int J Radiat Oncol Biol Phys 2006;66:108-16.
6. Qiao X, Tullgren O, Lax I, et al. The role of radiotherapy in treatment of stage I non-small cell lung cancer. Lung Cancer 2003;41:1-11.
7. Yung T, Giuliani ME, Le LW, et al. Outcomes of accelerated hypofractionated radiotherapy in stage i non-small-cell lung cancer. Curr Oncol 2012;19:e264-9.
8. Cheung P, Faria S, Ahmed S, et al. A Phase II Study of Accelerated Hypofractionated 3-Dimensional COnfirmal Radiotherapy for Inoperable T1-3N0M0 Non-Small Cell Lung Cancer; NCIC CTG BR.25. Radiotherapy & Oncology 2012;104:S67.
9. Wulf J, Hädinger U, Oppitz U, et al. Stereotactic radiotherapy of extracranial targets: CT-simulation and accuracy of treatment in the stereotactic body frame Radiother Oncol 2000;57:225-36.
10. Onishi H, Araki T, Shirato H, et al. Stereotactic hypofractionated high-dose irradiation for stage I nonsmall cell lung carcinoma: clinical outcomes in 245 subjects in a Japanese multiinstitutional study. Cancer 2004;101:1623-31.
11. Taremi M, Hope A, Dahele M, et al. Stereotactic body radiotherapy for medically inoperable lung cancer: prospective, single-center study of 108 consecutive patients. Int J Radiat Oncol Biol Phys 2012;82:967-73.
12. Bezjak A. RTOG 0813: Seamless Phase I/II Study of Stereotactic Lung Radiotherapy (SBRT) for Early Stage, Centrally Located, Non-Small Cell Lung Cancer (NSCLC) in Medically Inoperable Patients. Available online: http://www.rtog.org/ClinicalTrials/ProtocolTable/StudyDetails.aspx?study=0813
13. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer.

JAMA 2010;303:1070-6.
14. Li L, Ren S, Zhang Y, et al. Risk factors for predicting the occult nodal metastasis in T1-2N0M0 NSCLC patients staged by PET/CT: potential value in the clinic. Lung Cancer 2013;81:213-7.
15. Lee Y, Auh SL, Wang Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. Blood 2009;114:589-95.
16. Timmerman R, McGarry R, Yiannoutsos C, et al. Excessive toxicity when treating central tumors in a phase II study of stereotactic body radiation therapy for medically inoperable early-stage lung cancer. J Clin Oncol 2006;24:4833-9.
17. Dahele M, Pearson S, Purdie T, et al. Practical considerations arising from the implementation of lung stereotactic body radiation therapy (SBRT) at a comprehensive cancer center. J Thorac Oncol 2008;3:1332-41.
18. Lax I, Blomgren H, Näslund I, et al. Stereotactic radiotherapy of malignancies in the abdomen. Methodological aspects. Acta Oncol 1994;33:677-83.
19. Blomgren H, Lax I, Näslund I, et al. Stereotactic high dose fraction radiation therapy of extracranial tumors using an accelerator. Clinical experience of the first thirty-one patients. Acta Oncol 1995;34:861-70.
20. Yoon SM, Choi EK, Lee SW, et al. Clinical results of stereotactic body frame based fractionated radiation therapy for primary or metastatic thoracic tumors. Acta Oncol 2006;45:1108-14.
21. Li W, Purdie TG, Taremi M, et al. Effect of immobilization and performance status on intrafraction motion for stereotactic lung radiotherapy: analysis of 133 patients. Int J Radiat Oncol Biol Phys 2011;81:1568-75.
22. Uematsu M, Shioda A, Suda A, et al. Intrafractional tumor position stability during computed tomography (CT)-guided frameless stereotactic radiation therapy for lung or liver cancers with a fusion of CT and linear accelerator (FOCAL) unit. Int J Radiat Oncol Biol Phys 2000;48:443-8.
23. Shirato H, Shimizu S, Shimizu T, et al. Real-time tumour-tracking radiotherapy. Lancet 1999;353:1331-2.
24. Mackie TR, Kapatoes J, Ruchala K, et al. Image guidance for precise conformal radiotherapy. Int J Radiat Oncol Biol Phys 2003;56:89-105.
25. Jaffray DA, Drake DG, Moreau M, et al. A radiographic and tomographic imaging system integrated into a medical linear accelerator for localization of bone and soft-tissue targets. Int J Radiat Oncol Biol Phys 1999;45:773-89.
26. Gibbs IC. Frameless image-guided intracranial and extracranial radiosurgery using the Cyberknife robotic system. Cancer Radiother 2006;10:283-7.
27. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. JAMA 2010;303:1070-6.
28. Allibhai Z, Hope A, Sun A, et al. The impact of tumour size on outcomes following stereotactic body radiotherapy for medically inoperable early-stage non-small cell lung cancer. International Journal of Radiation Oncology, Biology & Physics 2013. (In Press).
29. Borst GR, Ishikawa M, Nijkamp J, et al. Radiation pneumonitis in patients treated for malignant pulmonary lesions with hypofractionated radiation therapy. Radiother Oncol 2009;91:307-13.
30. Voroney JP, Hope A, Dahele MR, et al. Chest wall pain and rib fracture after stereotactic radiotherapy for peripheral non-small cell lung cancer. J Thorac Oncol 2009;4:1035-7.
31. Taremi M, Hope A, Lindsay P, et al. Predictors of radiotherapy induced bone injury (RIBI) after stereotactic lung radiotherapy. Radiat Oncol 2012;7:159.
32. Graham MV, Purdy JA, Emami B, et al. Clinical dose-volume histogram analysis for pneumonitis after 3D treatment for non-small cell lung cancer (NSCLC). Int J Radiat Oncol Biol Phys 1999;45:323-9.
33. RTOG 0236. A Phase II Trial of Stereotactic Body Radiation Therapy (SBRT) in the Treatment of Patients with Medically Inoperable Stage I/II Non-Small Cell Lung Cancer, Available online: http://intranet.rmp.uhn.on.ca/AssetFactory.aspx?did=9471. January 2013, 2004.
34. Haasbeek CJ, Lagerwaard FJ, Slotman BJ, et al. Outcomes of stereotactic ablative radiotherapy for centrally located early-stage lung cancer. J Thorac Oncol 2011;6:2036-43.
35. Daly ME, Perks JR, Chen AM. Patterns-of-care for thoracic stereotactic body radiotherapy among practicing radiation oncologists in the United States. J Thorac Oncol 2013;8:202-7.
36. Chang JY, Balter PA, Dong L, et al. Stereotactic body radiation therapy in centrally and superiorly located stage I or isolated recurrent non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2008;72:967-71.
37. Rowe BP, Boffa DJ, Wilson LD, et al. Stereotactic body radiotherapy for central lung tumors. J Thorac Oncol 2012;7:1394-9.
38. Stauder MC, Macdonald OK, Olivier KR, et al. Early pulmonary toxicity following lung stereotactic body

radiation therapy delivered in consecutive daily fractions. Radiother Oncol 2011;99:166-71.
39. Nuyttens JJ, van der Voort van Zyp NC, Praag J, et al. Outcome of four-dimensional stereotactic radiotherapy for centrally located lung tumors. Radiother Oncol 2012;102:383-7.
40. Timmerman RD. RTOG 0618: A Phase II Trial of Stereotactic Body Radiation Therapy (SBRT) in the Treatment of Patients with Operable Stage I/II Non-Small Cell Lung Cancer, Available online: http://www.rtog.org/ClinicalTrials/ProtocolTable/StudyDetails.aspx?study=0618, 2010.
41. Onishi H, Shirato H, Nagata Y, et al. Stereotactic body radiotherapy (SBRT) for operable stage I non-small-cell lung cancer: can SBRT be comparable to surgery? Int J Radiat Oncol Biol Phys 2011;81:1352-8.
42. Hurkmans CW, Cuijpers JP, Lagerwaard FJ, et al. Recommendations for implementing stereotactic radiotherapy in peripheral stage IA non-small cell lung cancer: report from the Quality Assurance Working Party of the randomised phase III ROSEL study. Radiat Oncol 2009;4:1.
43. Grills IS, Mangona VS, Welsh R, et al. Outcomes after stereotactic lung radiotherapy or wedge resection for stage I non-small-cell lung cancer. J Clin Oncol 2010;28:928-35.

Cite this article as: Giuliani ME, Bezjak A. Alternatives to surgery in early stage disease—stereotactic body radiotherapy. Transl Lung Cancer Res 2013;2(5):332-339. doi: 10.3978/j.issn.2218-6751.2013.10.10

Post-operative radiation therapy

Amaury Paumier[1], Cécile Le Péchoux[2]

[1]Radiation Oncology Department, Institut de Cancérologie de l'Ouest, Angers-Nantes, France; [2]Radiation Oncology Department, Thoracic Oncology Unit, Gustave Roussy-Hôpital Universitaire, France

Correspondence to: Dr Cécile Le Péchoux. Radiation Oncology Department, Gustave Roussy-Hôpital Universitaire, 114 Rue Edouard Vaillant, Villejuif 94800, France. Email: cecile.lepechoux@gustaveroussy.fr.

Abstract: In completely resected non-small-cell lung cancer (NSCLC) patients with pathologically involved mediastinal lymph nodes (N2), administration of adjuvant platinum-based chemotherapy is now considered the standard of care, based on level 1 evidence. The role of post-operative radiotherapy (PORT) in this group of patients remains controversial. In the PORT meta-analysis published in 1998, the conclusions were that if adjuvant radiotherapy was detrimental to patients with early-stage completely resected NSCLC, the role of PORT in the treatment of tumours with N2 involvement was unclear and further research was warranted. Recent retrospective and non-randomized studies as well as subgroup analyses of recent randomized trials evaluating adjuvant chemotherapy, provide evidence of the possible benefit of PORT in patients with mediastinal nodal involvement. The question of PORT indication is also valid for those patients with proven N2 disease who undergo neo-adjuvant chemotherapy followed by surgery. The risk of local recurrence for N2 patients varies between 20% and 60%. Based on currently available data, PORT should be discussed for fit patients with completely resected NSCLC with N2 nodal involvement, within a multidisciplinary setting, preferably after completion of adjuvant chemotherapy or after surgery if patients have had neo-adjuvant chemotherapy. There is need for new randomized evidence to reassess PORT using modern three-dimensional conformal radiation technique, with attention to normal organ sparing, particularly lung and heart, to reduce the possible additional toxicity. Randomized evidence is needed. A new large international multi-institutional randomized trial Lung ART evaluating PORT in this patient population is now underway, as well as a Chinese study comparing postoperative sequential chemotherapy followed by radiotherapy versus adjuvant chemotherapy alone.

Keywords: Non-small cell lung cancer (NSCLC); complete resection; post-operative radiotherapy (PORT); conformal radiotherapy; adjuvant treatment

Submitted Aug 28, 2013. Accepted for publication Oct 07, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.03
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.03

Introduction

Even after complete resection of operable non-small cell lung cancer (NSCLC), patients are at high risk of recurrence (1). This risk of relapse is both distant and local, so that adjuvant chemotherapy as well as radiotherapy have been evaluated in randomized, though often underpowered, trials.

Concerning adjuvant chemotherapy, the results of the first published meta-analysis were updated in 2010 (2) including a total of 8,447 patients with both data from the old trials, and from all recent trials, showing an absolute increase in survival of 4% at 5 years (from 60% to 64%, P<0.0001). The beneficial effect of adjuvant chemotherapy was also observed in the LACE meta-analysis, which only included trials with cisplatin-based regimen (3), showing an absolute survival benefit at five years of 5.4% (P=0.005). After complete resection, adjuvant chemotherapy is now a standard of care for stage II and III NSCLC patients, even for elderly patients (4), but is controversial in stage I patients (5). Nonetheless, even among these patients, local control remains an important issue as 20-40% of patients

Table 1 Details and results of certain phase III studies

Trial	Recruitment	Stage	N patients	Total dose/fraction size (Gy)	RT technique	Local recurrence rate (%)	P	5-year survival rate (%)	P
Belgium (6)*	1966-1977	I, II (N0)	104	–	–	10.90	ns	43	<0.05
			98	60/2	Cobalt	1.20		24	(no PORT)
LCSG (12)	1978-1985	II, III	120	–	–	41	0.001	40	ns
			110	50.4/1.8	Cobalt and Linac	3		40	
CAMS (11)	1981-1995	II, III	182	–	–	33.20	0.01	40.5	ns
			183	60/2	Cobalt and Linac	12.70		42.9	
Lille (10)*	1985-1991	I	72	–	–	na	ns	51.6	ns
			60	45-60/2	Cobalt and Linac	na		35.2	
GETCB (7) [86 and 88]	1986-1994	I, II, III	355	–	–	34	ns	43	0.002
			373	60/2-2.5	Cobalt and Linac	28		30	(no PORT)
Italy (15)*	1989-1997	I	53	–	–	23	0.019	58	0.048
			51	50.4/1.8	Linac	2.20		67	(PORT)**
Austria (16)		I, II, III	72	–	–	20	<0.01	20.4	ns
			83	50-56/2	Linac	7		29.7	

*, pN0 patients; **, this result was no longer significant when updated (14).

will suffer from loco-regional relapse.

Concerning post-operative radiotherapy (PORT), it has been evaluated for decades, and, despite several trials and meta-analysis, it is still debated.

PORT through the prism of evidence-based medicine

The PORT meta-analysis which initially included 9 randomized trials (6-12) is a landmark study published in 1998 (13) and updated in 2005 (14) with a 10th study (15). The conclusions of this meta-analysis are well known among clinicians: PORT is detrimental to patients with early stage (I or II), whereas for those with N2 disease there was no significant adverse effect. The details of the 10 trials are much less known than the conclusions of the meta-analysis, however, these details are of paramount importance to better understand the possible role of PORT in the N2 subgroup of patients which is not clear as shown in *Tables 1,2*. It should be outlined that none of these trials used adjuvant chemotherapy which is now a standard (2,3).

Three randomised trials were dedicated to early stage (pN0) patients. The first trial was performed by Van Houtte *et al.* (6) and included 175 N0 patients from 1966 to 1977. PORT was delivered with a cobalt 60 unit (Co). The 5-year survival rate was respectively 24% in the RT arm and 43% in the control arm. The deleterious effect of RT was even more pronounced after pneumonectomy with a survival rate of 16% with PORT and 43% in the control arm. A study performed a decade later, by the same team (17), has highlighted the potential benefit of modern facilities (linear accelerator and computed tomography-based treatment planning): the 5-year survival rate was only 8% among patients treated with Co, whereas it was 30% in patients treated with more modern radiotherapy. The second trial dedicated to pN0 patients is the study of Lafitte *et al.* (10) which found no significant difference in overall survival or local control between surgery and PORT versus surgery alone. The authors pointed out that the main pattern of relapse was distant recurrence and that systemic adjuvant therapy should be considered. The third study performed by Trodella *et al.*, also focused on pN0 patients (15). PORT was delivered at the dose of 50.4 Gy in 28 fractions of 1.8 Gy, using modern facilities as described above. The first

Table 2 Updated survival of trials included in PORT meta-analysis (14)

Trial	Recruitment	Total dose/fraction size (Gy)	RT technique	No deaths/no patients		
				S+PORT	S	P
Belgium (6)*	1966-1977	60/2	Cobalt	88/98	80/104	0.012 (no PORT)
LCSG (12)	1978-1985	50.4/1.8	Cobalt and Linac	84/110	81/120	0.457
CAMS (11)	1981-1995	60/2	Cobalt and Linac	83/153	100/164	0.874
Lille (10)*	1985-1991	45-60/2	Cobalt and Linac	59/81	45/82	0.032
EORTC 08861	1986-1990	56/2	Linac	26/52	20/54	0.098
MRC LU11 (9)	1986-1993	40/2.6	Cobalt and Linac	116/154	123/154	0.748
Slovenia (8)	1988-1992	30/2.5-3	Cobalt and Linac	30/35	33/39	0.517
GETCB-86 (7)	1986-1994	60/2-2.5	Cobalt and Linac	69/99	59/90	0.378
GETCB-88 (7)	1988-1994	60/2-2.5	Cobalt and Linac	152/274	120/265	0.002 (no PORT)
Italy (15)*	1989-1997	50.4/1.8	Linac	23/51	30/53	0.215
Metaanalysis (14)				730/1,107	691/1,125	0.002 (no PORT)

*, pN0 patients.

results published in 2002 showed a positive trend in overall 5-year survival in favor of PORT (67% versus 58% in the control arm, P=0.046), but this trend was not confirmed when data were reanalyzed for the update of PORT meta-analysis (14). Even if this trend was unconfirmed, the authors highlighted, that the treatment fields were very limited, and that there was no detrimental effect related to PORT in this trial which used modern radiotherapy. However, it is now generally considered that such pN0 patients are more at risk of distant failure than local failure.

Three randomized studies included stage II and III, or pN1 and pN2 patients, excluding thus pN0 patients. In the randomized study conducted by the Lung Cancer Study Group (LCSG) (12), 230 patients with stage II or III resected squamous cell carcinoma were enrolled. There was no significant difference in overall survival, with a 5-year survival rate of about 40% in both arms, although PORT reduced significantly the rate of local recurrence (1% with PORT and 41% in the control arm, P<0.001). Moreover, subgroup analysis suggested that disease free survival (DFS) could be prolonged by PORT for N2 patients. The study of the Medical Research Council (MRC) (9) had a similar design to the LCSG trial, but it also included patients with adenocarcinoma. The results were quite similar with better local control that did not translate into a significant overall survival benefit. Once again, subgroup analysis revealed a trend for better overall survival in N2 patients. A phase III Chinese trial involving 366 N1 or N2 resected patients, came to the same conclusions: they found a lower rate of local recurrence (12.7% with PORT and 33.2% in the control arm, P=0.01) with no impact on survival (5-year survival rate was respectively 42.9% with PORT and 40.5% in the control arm, P=0.56).

Finally, the study of Dautzenberg *et al*. (7) which is the largest trial included in the meta-analysis on PORT, included 728 patients: 221 with stage I, 180 patients with stage II and 327 patients with stage III. The authors observed a detrimental effect of PORT on survival: 5-year overall survival rate was 30% with PORT versus 43% in the control arm (P=0.002). Once again for N2 patients, there was a trend in favor of PORT in decreasing loco-regional relapse. The excess of deaths among patients treated with PORT was due to a high incidence of cardiac and respiratory complications (such as cardiorespiratory failure, radiation pneumonitis, and massive haemoptysis). These non-cancer-related deaths seemed correlated with fractionation: they were much more frequent among patients who had received a daily fraction of 2 Gy or more (26% with daily fraction >2 Gy versus 16-18% in case of daily fraction ≤2 Gy). In the Mayer study (16), which was not included in the meta-analysis, 155 completely resected patients with T1-3 N0-2 NSCLC were randomly assigned to observation or PORT. The results (16) were similar to those of the LCSG (12), CAMS (11) or MRC (9): a significant increase of local control could be observed among patients who had PORT but with no impact in overall survival.

In the early 90s, for operable patients with small N2 nodal involvement, surgery and PORT was considered as a

standard of care, before publication of several studies and meta-analyses (18-20) had proven the beneficial effect of adjuvant (or neo-adjuvant) chemotherapy in this group of patients [IALT, JBR, ANITA, LACE, Meta-analyses 2010, 30]. Therefore, the Eastern Cooperative Oncology Group (ECOG) proposed a randomised phase III study which allocated patients who had complete surgery to PORT (50.4 Gy in 1.8 Gy fractions), which was considered as the reference arm, or to chemo-radiotherapy (CPORT: cisplatin and etoposide regimen administered concurrently with PORT) (21). There was no significant difference between the 2 arms, neither in terms of survival (3-year survival rates respectively of 52% with PORT and 50% with CPORT, P=0.56), nor local recurrences (13% with PORT and 12% with CPORT, P=0.84). Interestingly, the authors performed a retrospective analysis in order to compare the impact of a simple systematic sampling versus a complete mediastinal lymph node dissection (22) and found a survival advantage among patients who had a mediastinal lymph node dissection. Nonetheless, the result of this nonrandomized and non-planned comparison should be interpreted with caution. They outline that the modalities of surgery and most importantly nodal exploration are also important to consider, in order to evaluate PORT. Finally in this chapter it seems important to mention that a group of the studies included in the meta-analysis published in 2010, based on individual data from 13 randomised trials (2,660 patients; 63% being stage III) has evaluated the combination of surgery plus PORT which was the control arm versus surgery plus PORT and adjuvant chemotherapy which was the investigational arm (2). An absolute and significant survival benefit of 4% in favor of adjuvant chemotherapy was found (the 5-year survival rates were 29% in the surgery plus PORT and 33% with surgery plus PORT and chemotherapy, P=0.009). This survival benefit was similar to that observed with surgery plus chemotherapy compared to surgery alone. The authors concluded that the benefit of chemotherapy was similar irrespective of whether PORT was added to surgery or not. So adjuvant chemotherapy has become the standard of care, for stages II and III patients. The question would now concern PORT which should be in the investigational arm, whereas the control arm would include surgery plus chemotherapy.

Could an increase of loco-regional control be beneficial to high risk patients?

Four randomised trials including N2 patients [LCSG (12), CAMS (11), MRC (9) and the Austrian trial (16)] found PORT to be associated with a significant decrease of local failure but with no impact on survival. The study of Dautzenberg *et al*. (7) suggested that PORT could improve local control only in N2 patients. It should be outlined that these studies were performed at an era where staging evaluation did not comprise PET-CT scan and brain imaging, so that several patients included in these trials, especially those with N2 disease, might have been metastatic at the time they were included. Thus, the potential effect of PORT on local control may also have been diluted by the occurrence of distant metastases. As adjuvant chemotherapy is now part of the standard treatment in these patients, PORT needs to be reevaluated in the subgroup of patients who, after a complete staging evaluation with PET-CT and brain imaging, are found to be pN2. The question of PORT may also be valid in high risk patients who have pre-operative chemotherapy whether or not they have nodal downstaging. As shown in a Swiss phase II study which evaluated neo-adjuvant chemotherapy in stage IIIA patients with proven pathological N2 disease, the rate of local relapse can be high as it reached 60% at 5 years (23). Recently, Mauguen *et al*. (24) have found that disease free survival seemed to be a valid surrogate endpoint for overall survival. This finding also suggests that improving local control and disease free survival might improve survival.

Within the Surveillance, Epidemiology, and End Results (SEER) database (25), 7,465 patients treated from 1988 to 2002 (a time period in which linear accelerators were already common in clinical treatment) were retrospectively analysed. The same conclusions were drawn than those suggested by PORT meta-analysis (13,14): a survival benefit for N2 patients and a detrimental effect on survival for N0 and N1, even if it can be extrapolated that patients were treated with more modern radiotherapy techniques. Among the 840 patients included in the ANITA trial (20), 232 received PORT. Survival of patients with and without PORT in each arm (adjuvant chemotherapy or observation) was well described (26): in univariate analysis, PORT had a detrimental effect on survival, but, in the subgroup of patients with N2 disease, survival was improved with PORT both in the chemotherapy (median survival of 23.8 months without PORT and 47.4 months with PORT) and the observation arm (median survival of 12.7 months without PORT and 22.7 months with PORT). The author thereby advocated that further evaluation of PORT in completely resected pN2 NSCLC should be performed in randomized trials. Scotti *et al*. have retrospectively reviewed the data of

175 patients with completely resected N2 disease (27). Local failure rates were 15.1% in the PORT group and 32.1% in the no-PORT group (P=0.009), but overall survival was similar in both groups. For these patients treated between 1988 and 2004, radiotherapy has resulted in mild toxicity.

Patients with ipsilateral mediastinal lymph node involvement are a heterogeneous subgroup. From a retrospective study involving 702 patients who underwent surgery in 6 French centers, Andre *et al.* (28) have used a subclassification taking into account 2 criteria concerning nodal involvement: minimal (mN2: no preoperative detection of N2 disease) or clinical (cN2: enlarge lymph node on CT scan) disease, and single (L1) or multiple (L2) lymph node involvement. The 5-year survival rates for patients treated with primary surgery were dramatically different within subgroups: mN2 L1 (244 patients): 34%, mN2 L2 (78 patients): 11%, cN2 L1 (118 patients): 8% and cN2 L2 (122 patients): 3%. Unfortunately, no data were available concerning the pattern of relapse. For the authors, the poor prognosis of cN2 patients leads to propose multimodality treatment, such as peri-operative (neoadjuvant and adjuvant) chemotherapy and PORT. We have seen that surgery and adjuvant chemotherapy is now the standard of care (2,29), thus, neoadjuvant chemotherapy is the preferred sequence by some clinicians. However, a recent meta-analysis didn't find any survival difference between pre and post-operative chemotherapy (30). As mediastinal downstaging after induction treatment is a strong and a relevant prognostic factor (31,32), one option is to refer operable patients with cytologically or pathologically proven ipsilateral mediastinal node involvement to surgeons in cases of response to preoperative chemotherapy.

Ichinose *et al.* were able to retrospectively assess 332 completely resected N2 patients between 1992 and 1993 in Japan (33). Out of these 332 patients, 130 (39.2%) experienced local failure. The number of N2 stations was found to be a prognostic factor for local recurrence. Another Japanese study has retrospectively assessed PORT according the number of lymph node stations involved (34). PORT had no significant effect on overall survival, but significantly improved disease free-survival (by decreasing local recurrence) in patients with multiple N2 involvement. In this subgroup of patients, the 5-year disease-free survival rates were 41% in the PORT group and 5.9% in the non-PORT group. The same concept was tested by Urban *et al.* who analysed 11,324 patients from the SEER database (35). Their results suggest, once again, that PORT is beneficial to patients with pN2 disease, when the lymph node ratio (number of positive nodes/number of resected nodes) is at least 50% or more.

Is the detrimental effect of PORT still an issue with modern radiotherapy?

All patients randomised in the 11 phase 3 studies evaluating PORT were treated before 2000; some were even included as early as 1966. It must be emphasized that only 3 out of the 11 randomized trials evaluating PORT used exclusively modern radiotherapy, i.e., computed tomography based treatment planning and linear accelerator delivering high energy [(15,16) and the unpublished EORT 08861 trial], and a substantial number of patients enrolled in these studies were treated with Co unit. As shown in *Tables 1,2*, a worse survival was found in the Belgian (6) and the GETCB studies (7), which use Co, high total dose (60 Gy) and high dose per fraction (>2 Gy). It has been demonstrated that using more "modern" radiotherapy, resulted in lower morbidity than treatment with cobalt unit (17). Moreover, the use of a 2-dimensional technique, instead of CT-based 3 dimensional conformal radiotherapy, leads to an underdosage in the area at risk (36). The total dose, the dose per fraction and the treated volume are also of major concern when considering toxicity. Firstly, the total dose delivered to the majority of patients included in the meta-analysis (6,7,10,11) was as high as 60 Gy, whereas 54 Gy would be sufficient in a prophylactic setting and less harmful (37). Secondly, it is well demonstrated that fractionation schedules with more than 2.5/3 Gy per fraction leads to a higher rate of cardiac (38) and pulmonary (39) injury. Dautzenberg *et al.* (7) highlighted the detrimental effect of large doses per fraction in PORT. In most studies included in the meta-analysis, the irradiated volume was usually quite large and included most of the mediastinum (both the ipsi and contra lateral side of the mediastinum) and the supraclavicular area. Incidence of nodal involvement derived from surgical series (40) and the pattern of relapse after surgery without PORT (41) may help to define higher risk areas. To further improve the definition of the nodal areas at risk, a CT-based node map, derived from the classification proposed by Mountain and Dresler (42), has been defined (43). However there have been changes concerning the delimitations of nodal stations and introduction of the nodal zone concept in the new TNM classification (44).

Breast cancer provides an interesting model of benefit/risk balance with adjuvant radiotherapy: despite a decreased risk of local recurrence with radiotherapy, no significant impact on

overall survival has been proven until the end of the 90s (45,46), due to an increased risk of mortality from ischemic heart disease. With modern radiotherapy, a significant benefit in favor of radiotherapy has finally be highlight (45,46), and it has been shown that the risk of death from heart disease has substantially decreased more contemporary techniques of radiotherapy (47).

For patients suffering from NSCLC, some retrospective data suggest that PORT-related toxicity might have also decreased with time. Rate of death from intercurrent disease (DID) of the patients included in the ECOG study, which evaluated PORT versus CPORT (21), was compared to the expected rate of DID calculated from United States vital statistics (48). The 4-year rate of DID was 12.9% for patients treated in the ECOG study and was not significantly different from the 10.1% 4-year expected rate of DID (P=0.16). Data concerning 6,148 patients treated from 1983 to 1993 were obtained from the SEER program (49): 3,589 received PORT (58%) and 2,559 did not (42%). PORT was significantly associated with an increased risk of death from heart disease. However, this excess of cardiovascular toxicities after PORT was only observed in the cohort of patients treated between 1983 and 1988 but not in the cohort of patients diagnosed from 1989 to 1993. The authors hypothesize that the decrease of cardiac toxicity related to PORT was due to improvements of the thoracic radiotherapy (treatment planning with computed tomography allowing 3 dimensional conformal radiotherapy and high energy delivered by linear accelerator instead of Cobalt).

Overall, radiotherapy modalities used in the randomized trials included in the meta-analysis (13) appear now outdated, especially when compared to the recommendations for planning and delivery of thoracic radiotherapy that have been recently published (50). Latest techniques could further decrease PORT-related toxicity. Image-guided radiotherapy (IGRT) offers several ways to deal with respiratory motion (51,52), such as the deep-inspiration breath-hold radiotherapy (53), the breathing-synchronized radiotherapy or the 4-dimensional CT scan (54) which allows to generate a personalized treatment volume. The clinical results are encouraging (55,56), notably concerning toxicity. Intensity modulated radiotherapy (IMRT) allows better dose distribution compared to conformal radiotherapy (57), with interesting clinical results in inoperable NSCLC (58,59).

Tobacco use seems to be associated with poorer outcome to patients treated by surgery and PORT (60). Gareen *et al.* (61) have suggested that clinicians have to help patients to quit smoking by giving them specific advice and follow-up instead of a brief injunction.

So, a randomized trial testing PORT for N2 patient was urgently needed...

The ongoing phase III Lung Adjuvant Radiotherapy Trial (ART) is randomizing completely resected patients with cytologically or pathologically proven N2 mediastinal disease between PORT and observation (62) (*Figure 1*). Patients may have had neo-adjuvant chemotherapy, or adjuvant chemotherapy. At initial staging, 18-fluorodeoxyglucose positron emission tomography scanning is recommended (63). In case of pre-operative chemotherapy, the ipsilateral mediastinal involvement has to be pathologically proven before any treatment, so that even in case of mediastinal downstaging (N2 to N1 or N0), the patient can enter the study. Even if induction chemotherapy produces a good response, up to one third of these patients may eventually suffer from local relapse (64), thus it seems interesting to evaluate PORT in this subgroup of patients. There has been a proposal for the definition of complete resection by a group of surgeons of the IASLC (65). Lymph node exploration is mandatory, however the surgeon might choose to use either a simple node sampling or a complete systematic nodal dissection, because the role of these 2 approaches is still debated (66-68). Three-dimensional conformal radiotherapy is, of course, mandatory, together with the use of high-energy photon (6-10 MV) delivered by a linear accelerator. The planned total dose is 54 Gy (37) in fractions of 1.8 or 2 Gy, and the dose per fraction should never exceed 2 Gy (7,38,39). Elective nodal irradiation, which means to treat the whole mediastinum, including the ipsi and contra lateral side of the mediastinum down to the pillars of the diaphragm, and the supra clavicular areas, is not allowed. Treated volume is now limited to involved node station(s) and stations at high risk according to tumor location (40,41,69). This contouring protocol has been evaluated and was able to reduce variability of the treated volume among clinicians (70). Quality assurance procedures, such as a dummy run before any inclusion in a center, aim to verify the compliance to the Lung ART protocol (volume definition, dose to organ at risk, etc.). Indeed, it has been demonstrated that compliance to radiotherapy protocol mustn't be neglected because it can dramatically impact outcome (71). The main end point of this study is disease free survival (DFS) (24). The 3-year DFS among pN2 patients is about 30%, and the 3-year local recurrence rate is also about 30% (18). In order to observe a 10% absolute improvement of the DFS (from 30% to 40%), the inclusion of 700 patients is planned in the Lung ART study.

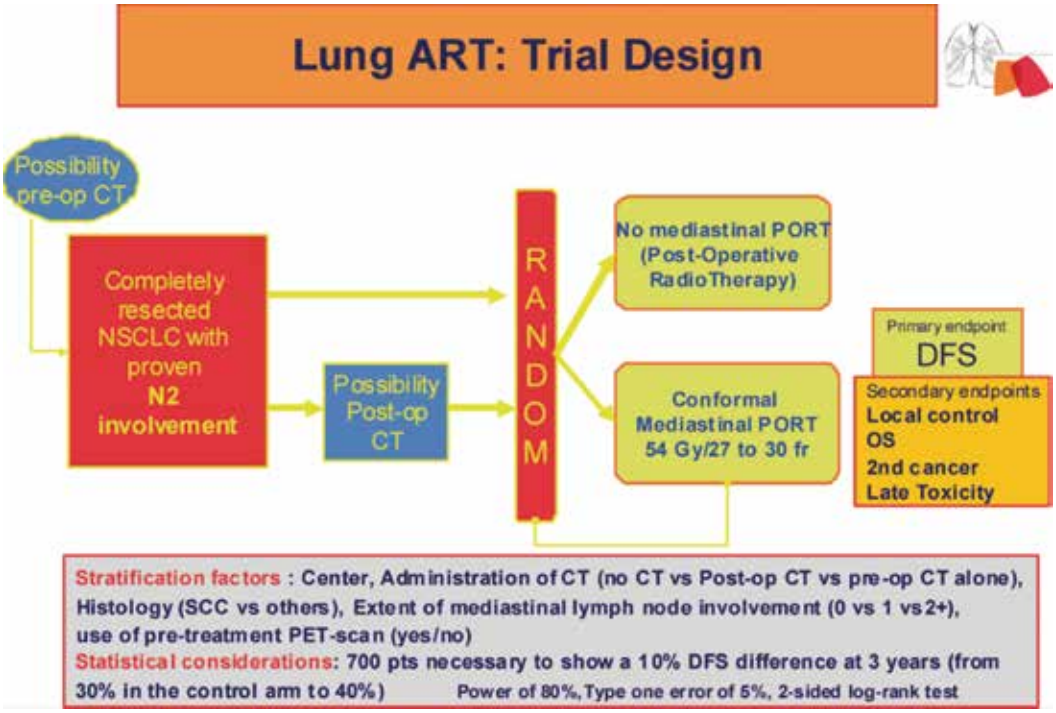


Figure 1 Lung adjuvant radiotherapy trial (ART) design. CT, chemotherapy; DFS, disease-free survival; NSCLC: non-small cell lung cancer; PET, positron emission tomography; PORT, postoperative radiation therapy; post-op, postoperative; pre-op, preoperative; SCC, squamous cell carcinoma.

This study, involving the Intergroupe Francophone de Cancerologie Thoracique (IFCT 0503), has accrued more than 200 patients in France, and has been joined recently by a large national group from the United Kingdom, and the Lung Group, as well as the Radiation Oncology Group from the European Organisation for Research and Treatment of Cancer (EORTC 22055-08053). Another trial is on going in China comparing 4 cycles of adjuvant chemotherapy to 4 cycles of chemotherapy followed by radiotherapy, after complete resection of NSCLC.

In the pre-PET era, the high rate of distant metastases diluted any real effect of local control on overall outcome. As the population of resected N2 patients has changed, because of better selection (more accurate staging with PET CT, brain imaging), better surgery (lung sparing techniques, pre-op and post-op care…), administration of systematic adjuvant or neo-adjuvant chemotherapy which has become standard of care, the major technical advances of radiotherapy may enhance the ability of PORT to improve local relapse free survival and possibly overall survival but this has to be proven. The results of these randomized trials could change the standard care in resected N2 patients.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111:1710-7.
2. NSCLC Meta-analyses Collaborative Group, Arriagada R, Auperin A, et al. Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. Lancet 2010;375:1267-77.
3. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
4. Früh M, Rolland E, Pignon JP, et al. Pooled analysis of the effect of age on adjuvant cisplatin-based chemotherapy for completely resected non-small-cell lung cancer. J Clin Oncol 2008;26:3573-81.
5. Kalemkerian GP. Adjuvant therapy for non-small-cell lung cancer. Lancet 2010;375:1230-1.
6. Van Houtte P, Rocmans P, Smets P, et al. Postoperative

radiation therapy in lung caner: a controlled trial after resection of curative design. Int J Radiat Oncol Biol Phys 1980;6:983-6.
7. Dautzenberg B, Arriagada R, Chammard AB, et al. A controlled study of postoperative radiotherapy for patients with completely resected nonsmall cell lung carcinoma. Groupe d'Etude et de Traitement des Cancers Bronchiques. Cancer 1999;86:265-73.
8. Debevec M, Bitenc M, Vidmar S, et al. Postoperative radiotherapy for radically resected N2 non-small-cell lung cancer (NSCLC): randomised clinical study 1988-1992. Lung Cancer 1996;14:99-107.
9. Stephens RJ, Girling DJ, Bleehen NM, et al. The role of post-operative radiotherapy in non-small-cell lung cancer: a multicentre randomised trial in patients with pathologically staged T1-2, N1-2, M0 disease. Medical Research Council Lung Cancer Working Party. Br J Cancer 1996;74:632-9.
10. Lafitte JJ, Ribet ME, Prévost BM, et al. Postresection irradiation for T2 N0 M0 non-small cell carcinoma: a prospective, randomized study. Ann Thorac Surg 1996;62:830-4.
11. Feng QF, Wang M, Wang LJ, et al. A study of postoperative radiotherapy in patients with non-small-cell lung cancer: a randomized trial. Int J Radiat Oncol Biol Phys 2000;47:925-9.
12. Effects of postoperative mediastinal radiation on completely resected stage II and stage III epidermoid cancer of the lung. The Lung Cancer Study Group. N Engl J Med 1986;315:1377-81.
13. Postoperative radiotherapy in non-small-cell lung cancer: systematic review and meta-analysis of individual patient data from nine randomised controlled trials. PORT Meta-analysis Trialists Group. Lancet 1998;352:257-63.
14. Burdett S, Stewart L, PORT Meta-analysis Group. Postoperative radiotherapy in non-small-cell lung cancer: update of an individual patient data meta-analysis. Lung Cancer 2005;47:81-3.
15. Trodella L, Granone P, Valente S, et al. Adjuvant radiotherapy in non-small cell lung cancer with pathological stage I: definitive results of a phase III randomized trial. Radiother Oncol 2002;62:11-9.
16. Mayer R, Smolle-Juettner FM, Szolar D, et al. Postoperative radiotherapy in radically resected non-small cell lung cancer. Chest 1997;112:954-9.
17. Phlips P, Rocmans P, Vanderhoeft P, et al. Postoperative radiotherapy after pneumonectomy: impact of modern treatment facilities. Int J Radiat Oncol Biol Phys 1993;27:525-9.
18. Arriagada R, Bergman B, Dunant A, et al. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351-60.
19. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005;352:2589-97.
20. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719-27.
21. Keller SM, Adak S, Wagner H, et al. A randomized trial of postoperative adjuvant therapy in patients with completely resected stage II or IIIA non-small-cell lung cancer. Eastern Cooperative Oncology Group. N Engl J Med 2000;343:1217-22.
22. Keller SM, Adak S, Wagner H, et al. Mediastinal lymph node dissection improves survival in patients with stages II and IIIa non-small cell lung cancer. Eastern Cooperative Oncology Group. Ann Thorac Surg 2000;70:358-65; discussion 365-6.
23. Betticher DC, Hsu Schmitz SF, Tötsch M, et al. Prognostic factors affecting long-term outcomes in patients with resected stage IIIA pN2 non-small-cell lung cancer: 5-year follow-up of a phase II study. Br J Cancer 2006;94:1099-106.
24. Mauguen A, Pignon JP, Burdett S, et al. Surrogate endpoints for overall survival in chemotherapy and radiotherapy trials in operable and locally advanced lung cancer: a re-analysis of meta-analyses of individual patients' data. Lancet Oncol 2013;14:619-26.
25. Lally BE, Zelterman D, Colasanto JM, et al. Postoperative radiotherapy for stage II or III non-small-cell lung cancer using the surveillance, epidemiology, and end results database. J Clin Oncol 2006;24:2998-3006.
26. Douillard JY, Rosell R, De Lena M, et al. Impact of postoperative radiation therapy on survival in patients with complete resection and stage I, II, or IIIA non-small-cell lung cancer treated with adjuvant chemotherapy: the adjuvant Navelbine International Trialist Association (ANITA) Randomized Trial. Int J Radiat Oncol Biol Phys 2008;72:695-701.
27. Scotti V, Meattini I, Saieva C, et al. Post-operative radiotherapy in N2 non-small cell lung cancer: a retrospective analysis of 175 patients. Radiother Oncol

2010;96:84-8.
28. Andre F, Grunenwald D, Pignon JP, et al. Survival of patients with resected N2 non-small-cell lung cancer: evidence for a subclassification and implications. J Clin Oncol 2000;18:2981-9.
29. Burdett SS, Stewart LA, Rydzewska L. Chemotherapy and surgery versus surgery alone in non-small cell lung cancer. Cochrane Database Syst Rev 2007;(3):CD006157.
30. Lim E, Harris G, Patel A, et al. Preoperative versus postoperative chemotherapy in patients with resectable non-small cell lung cancer: systematic review and indirect comparison meta-analysis of randomized trials. J Thorac Oncol 2009;4:1380-8.
31. Betticher DC, Hsu Schmitz SF, Tötsch M, et al. Mediastinal lymph node clearance after docetaxel-cisplatin neoadjuvant chemotherapy is prognostic of survival in patients with stage IIIA pN2 non-small-cell lung cancer: a multicenter phase II trial. J Clin Oncol 2003;21:1752-9.
32. van Meerbeeck JP, Kramer GW, Van Schil PE, et al. Randomized controlled trial of resection versus radiotherapy after induction chemotherapy in stage IIIA-N2 non-small-cell lung cancer. J Natl Cancer Inst 2007;99:442-50.
33. Ichinose Y, Kato H, Koike T, et al. Overall survival and local recurrence of 406 completely resected stage IIIa-N2 non-small cell lung cancer patients: questionnaire survey of the Japan Clinical Oncology Group to plan for clinical trials. Lung Cancer 2001;34:29-36.
34. Matsuguma H, Nakahara R, Ishikawa Y, et al. Postoperative radiotherapy for patients with completely resected pathological stage IIIA-N2 non-small cell lung cancer: focusing on an effect of the number of mediastinal lymph node stations involved. Interact Cardiovasc Thorac Surg 2008;7:573-7.
35. Urban D, Bar J, Solomon B, et al. Lymph node ratio may predict the benefit of postoperative radiotherapy in non-small-cell lung cancer. J Thorac Oncol 2013;8:940-6.
36. DiBiase SJ, Werner-Wasik M, Croce R, et al. Standard off-cord lung oblique fields do not include the entire mediastinum: a computed tomography simulator study. Am J Clin Oncol 2000;23:249-52.
37. Machtay M, Lee JH, Shrager JB, et al. Risk of death from intercurrent disease is not excessively increased by modern postoperative radiotherapy for high-risk resected non-small-cell lung carcinoma. J Clin Oncol 2001;19:3912-7.
38. Cosset JM, Henry-Amar M, Girinski T, et al. Late toxicity of radiotherapy in Hodgkin's disease. The role of fraction size. Acta Oncol 1988;27:123-9.
39. Dubray B, Henry-Amar M, Meerwaldt JH, et al. Radiation-induced lung damage after thoracic irradiation for Hodgkin's disease: the role of fractionation. Radiother Oncol 1995;36:211-7.
40. Watanabe Y, Shimizu J, Tsubota M, et al. Mediastinal spread of metastatic lymph nodes in bronchogenic carcinoma. Mediastinal nodal metastases in lung cancer. Chest 1990;97:1059-65.
41. Kelsey CR, Light KL, Marks LB. Patterns of failure after resection of non-small-cell lung cancer: implications for postoperative radiation therapy volumes. Int J Radiat Oncol Biol Phys 2006;65:1097-105.
42. Mountain CF, Dresler CM. Regional lymph node classification for lung cancer staging. Chest 1997;111:1718-23.
43. Chapet O, Kong FM, Quint LE, et al. CT-based definition of thoracic lymph node stations: an atlas from the University of Michigan. Int J Radiat Oncol Biol Phys 2005;63:170-8.
44. Rusch VW, Asamura H, Watanabe H, et al. The IASLC lung cancer staging project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2009;4:568-77.
45. Overgaard M, Hansen PS, Overgaard J, et al. Postoperative radiotherapy in high-risk premenopausal women with breast cancer who receive adjuvant chemotherapy. Danish Breast Cancer Cooperative Group 82b Trial. N Engl J Med 1997;337:949-55.
46. Ragaz J, Jackson SM, Le N, et al. Adjuvant radiotherapy and chemotherapy in node-positive premenopausal women with breast cancer. N Engl J Med 1997;337:956-62.
47. Giordano SH, Kuo YF, Freeman JL, et al. Risk of cardiac death after adjuvant radiotherapy for breast cancer. J Natl Cancer Inst 2005;97:419-24.
48. Wakelee HA, Stephenson P, Keller SM, et al. Post-operative radiotherapy (PORT) or chemoradiotherapy (CPORT) following resection of stages II and IIIA non-small cell lung cancer (NSCLC) does not increase the expected risk of death from intercurrent disease (DID) in Eastern Cooperative Oncology Group (ECOG) trial E3590. Lung Cancer 2005;48:389-97.
49. Lally BE, Detterbeck FC, Geiger AM, et al. The risk of death from heart disease in patients with nonsmall cell lung cancer who receive postoperative radiotherapy: analysis of the Surveillance, Epidemiology, and End Results database. Cancer 2007;110:911-7.
50. De Ruysscher D, Faivre-Finn C, Nestle U, et al. European Organisation for Research and Treatment of Cancer

recommendations for planning and delivery of high-dose, high-precision radiotherapy for lung cancer. J Clin Oncol 2010;28:5301-10.
51. Verellen D, De Ridder M, Linthout N, et al. Innovations in image-guided radiotherapy. Nat Rev Cancer 2007;7:949-60.
52. Paumier A, Crespeau A, Krhili S, et al. Dosimetric study of the different techniques to deal with respiratory motion for lung stereotactic radiotherapy. Cancer Radiother 2012;16:263-71.
53. Hanley J, Debois MM, Mah D, et al. Deep inspiration breath-hold technique for lung tumors: the potential value of target immobilization and reduced lung density in dose escalation. Int J Radiat Oncol Biol Phys 1999;45:603-11.
54. Hof H, Rhein B, Haering P, et al. 4D-CT-based target volume definition in stereotactic radiotherapy of lung tumours: comparison with a conventional technique using individual margins.Radiother Oncol 2009;93:419-23.
55. Giraud P, Djadi-Prat J, Morvan E, et al. Dosimetric and clinical benefits of respiratory-gated radiotherapy for lung and breast cancers: results of the STIC 2003. Cancer Radiother 2012;16:272-81.
56. Giraud P, Morvan E, Claude L, et al. Respiratory gating techniques for optimization of lung cancer radiotherapy. J Thorac Oncol 2011;6:2058-68.
57. Rousseau D, Autret D, Krhili S, et al. Are there any dosimetric advantages in using VMAT for treatment of locally advanced non-small cell lung cancer? Cancer Radiother 2012;16:619-26.
58. Scorsetti M, Navarria P, Mancosu P, et al. Large volume unresectable locally advanced non-small cell lung cancer: acute toxicity and initial outcome results with rapid arc. Radiat Oncol 2010;5:94.
59. Liao ZX, Komaki RR, Thames HD Jr, et al. Influence of technologic advances on outcomes in patients with unresectable, locally advanced non-small-cell lung cancer receiving concomitant chemoradiotherapy. Int J Radiat Oncol Biol Phys 2010;76:775-81.
60. Nguyen SK, Masson-Côté L, Fortin A, et al. Influence of smoking status on treatment outcomes after post-operative radiation therapy for non-small-cell lung cancer. Radiother Oncol 2010;96:89-93.
61. Gareen IF, Park ER, Gorelick J, et al. The effects of physician-delivered brief smoking cessation on ACRIN/NLST participants' smoking behaviors. J Clin Oncol 2013;13:abstr 7525.
62. Le Péchoux C, Dunant A, Pignon JP, et al. Need for a new trial to evaluate adjuvant postoperative radiotherapy in non-small-cell lung cancer patients with N2 mediastinal involvement. J Clin Oncol 2007;25:e10-1.
63. Ung YC, Maziak DE, Vanderveen JA, et al. 18Fluorodeoxyglucose positron emission tomography in the diagnosis and staging of lung cancer: a systematic review. J Natl Cancer Inst 2007;99:1753-67.
64. Amini A, Lou F, Correa AM, et al. Predictors for locoregional recurrence for clinical stage III-N2 non-small cell lung cancer with nodal downstaging after induction chemotherapy and surgery. Ann Surg Oncol 2013;20:1934-40.
65. Rami-Porta R, Wittekind C, Goldstraw P, et al. Complete resection in lung cancer surgery: proposed definition. Lung Cancer 2005;49:25-33.
66. Izbicki JR, Passlick B, Pantel K, et al. Effectiveness of radical systematic mediastinal lymphadenectomy in patients with resectable non-small cell lung cancer: results of a prospective randomized trial. Ann Surg 1998;227:138-44.
67. Wu Yl, Huang ZF, Wang SY, et al. A randomized trial of systematic nodal dissection in resectable non-small cell lung cancer. Lung Cancer 2002;36:1-6.
68. Wright G, Manser RL, Byrnes G, et al. Surgery for non-small cell lung cancer: systematic review and meta-analysis of randomised controlled trials. Thorax 2006;61:597-603.
69. Kiricuta IC, Mueller G, Stiess J, et al. The lymphatic pathways of non-small cell lung cancer and their implication in curative irradiation treatment. Lung Cancer 1994;11:71-82.
70. Spoelstra FO, Senan S, Le Péchoux C, et al. Variations in target volume definition for postoperative radiotherapy in stage III non-small-cell lung cancer: analysis of an international contouring study. Int J Radiat Oncol Biol Phys 2010;76:1106-13.
71. Peters LJ, O'Sullivan B, Giralt J, et al. Critical impact of radiotherapy protocol compliance and quality in the treatment of advanced head and neck cancer: results from TROG 02.02. J Clin Oncol 2010;28:2996-3001.

Cite this article as: Paumier A, Le Péchoux C. Post-operative radiation therapy. Transl Lung Cancer Res 2013;2(5):423-432. doi: 10.3978/j.issn.2218-6751.2013.10.03

Proton radiotherapy in the treatment of lung cancer

Heath Devin Skinner, Ritsuko Komaki

Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Correspondence to: Heath Devin Skinner. The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 97, Houston, TX 77030, USA. Email: hskinner@mdanderson.org.

Abstract: Radiation therapy for lung cancer often leads to treatment-related pneumonitis or lung fibrosis, especially when given with concurrent chemotherapy. These side effects can impair quality of life and negatively affect treatment outcomes. With the advent of proton radiotherapy comes the possibility of reducing these toxicities by minimizing the amount of non-target tissue that is irradiated. Protons have significantly different physical characteristics that can make their use advantageous over standard photon radiotherapy. Multiple retrospective reviews and phase I/II studies have linked the use of protons with fewer side effects in at least some patients with lung cancer, and randomized trials comparing proton therapy with photon treatments are ongoing. Technologic advances may allow for even further minimization of toxicity associated with radiation therapy. In this review, we discuss the current state of proton therapy for the management of lung cancer as well as challenges and opportunities for further development of this treatment modality.

Keywords: Radiation therapy; lung cancer; protons

Submitted Nov 05, 2012. Accepted for publication Dec 06, 2012.
doi: 10.3978/j.issn.2218-676X.2012.12.02
View this article at: http://dx.doi.org/10.3978/j.issn.2218-676X.2012.12.02

Introduction

Ever since the first attempts to treat malignancy with radiotherapy were made in the early 1900s, delivering a tumorcidal dose of radiotherapy while minimizing toxicity to nearby normal tissues has always been a challenge. Initially, tumors could be targeted only via direct or near-direct contact with a radiotherapy source. With the advent of Cobalt-60 radiation sources and, later, linear accelerators, therapeutic radiation could be delivered to virtually any site in the body. However, the dose that can be delivered to the tumor continues to be limited by normal tissue constraints. Fundamentally, this is determined by the physical characteristics of standard photon or electron radiotherapy. Photons, which include standard X-rays, and electrons deposit the radiation dose over the entire track of the beam; after peaking at a physically determined depth in water (or tissue), the deposited dose decreases slowly. For example, as shown in *Figure 1*, the maximum dose of radiation delivered by a standard 6 MV photon beam is at a depth of 1.5 cm in water. For electrons of similar energies, the depth at which maximum dose is delivered (D_{max}) is even less. This dose distribution is reasonable for superficial tumors, but for tumors more than 1.5 cm below the surface of the skin, for one radiation beam, the normal tissue proximal to the tumor will be treated to a higher dose than the tumor itself. This physical reality is compensated for in standard radiotherapy by the use of multiple beams that converge at the level of the tumor. With more advanced planning techniques, such as intensity-modulated radiotherapy (IMRT), the intensity of each beam can be altered by using a computer-determined "best solution" for all beams to maximize tumor dose while sparing surrounding normal tissue. Despite these significant advances, standard radiotherapy continues to be limited by the generally inalterable physical characteristics of a photon (or electron) beam. This has led to interest in other forms of radiotherapy with different beam characteristics. Here we focus primarily on proton radiotherapy, the most common charged particle therapy in clinical use for lung cancer in the United States.

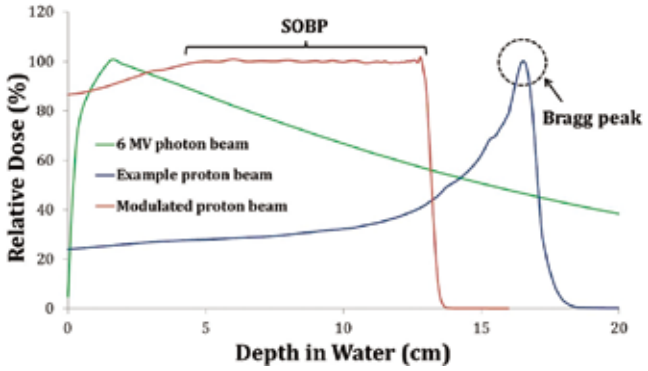


Figure 1 Depth-dose characteristics of proton and photon beams. The example proton beam is of a higher energy than the SOBP for clarity.

Physical characteristics of proton beams

As previously stated, photon beams reach their maximum dose (D_{max}) at a known depth in tissue, a known physical property determined by the beam energy. Higher photon energies lead to greater D_{max} at the expense of increased fall-off dose as well as increased possibility of neuton scattering. However, charged particles such as protons have minimal ionization along their beam path, meaning that the dose delivered to any point along the beam path is minimal and the entrance dose for any particular proton beam is less than that for a comparable photon beam. Instead, the vast majority of dose in a charged particle beam is deposited near the end of the beam path, when the particles have nearly stopped (*Figure 1*). This phenomenon was initially observed as early as 1904, and has been dubbed the "Bragg peak" for its discoverer William Henry Bragg (1). By modulating the proton energy, the depth of the Bragg peak, or point of maximal radiation dose delivery, can be altered. However, the area of the Bragg peak for any one proton energy is too narrow for clinical use, requiring the use of a summed proton beam of multiple energies, resulting in the so-called "spread-out Bragg peak" (*Figure 1*).

A concept useful in comparing forms of radiotherapy is that of relative biologic effectiveness (RBE). Simply stated, this is a ratio between a standard dose of radiation (typically 250 kVp X-rays) and the dose of the test radiation required to produce the same biologic effect. Although the concept is fairly simple, derivation of the RBE is a complex process, depending upon a number of variables including the type of tissue being studied, the degree of hypoxia within the tissue, the type of radiation being used, the dose delivered, and the energy lost over the beam path (linear energy transfer or LET). Historically, the RBE for a variety of different types of radiation has been determined primarily by *in vitro* and preclinical studies. For clinical use, the RBE for a proton beam (within the Bragg peak) is generally assumed to be 1.1 (2,3), meaning that for every 1 Grey (Gy), the biological effectiveness of a proton beam is similar to what is seen with 1.1 Gy of standard X-rays. This has led to the use of the term cobalt-Grey equivalent (CGE) when describing doses or proton therapy. Thus 74 CGE is equivalent to 67.3 Gy delivered by protons. Although the RBE/CGE concept provides a clinically useful value, several caveats must be borne in mind. The RBE is thought to vary slightly over the breadth of a Bragg peak. Specifically, the experimentally determined RBE values within a proton beam generally increase over the Bragg peak and are highest in the final millimeters (4-8). This effect is recognized in the course of routine clinical treatments by the recommendation that no proton beam should terminate in a critical normal structure. RBE also varies as a function of the tissue irradiated; *in vivo* preclinical models have predicted average values ranging from 0.7 to 1.6 (2). Examination of this variation in the RBE of a proton beam has led to attempts to integrate this factor into treatment planning (9-11).

Clinical use of proton therapy in lung cancer

The use of proton radiotherapy has grown substantially, particularly over the past decade, with 10 facilities using this modality in the United States alone. The unique characteristics of proton radiotherapy has led to its use being championed to allow both sparing of normal tissue and increasing the radiation dose delivered to targets heretofore limited by proximity to adjacent normal surrounding structures. Particular interest in proton radiotherapy has been expressed for the treatment of lung cancer. The standard therapy for locally advanced lung cancer involves a combination of radiation and chemotherapy delivered concurrently, typically to radiation doses of 60-70 Gy. However, treatment in this dose range can be quite toxic, leading to significant pulmonary injury (mainly pneumonitis and fibrosis) as well as esophagitis and other toxic effects (12). Any damage to the lungs in patients with lung cancer tends to be exacerbated by a lack of pulmonary reserve, as many patients present with some form of chronic obstructive disease from cigarette smoking and many require supplemental oxygen even before radiotherapy. The findings regarding the value of dose-escalation in these patients is somewhat conflicting (13,14).

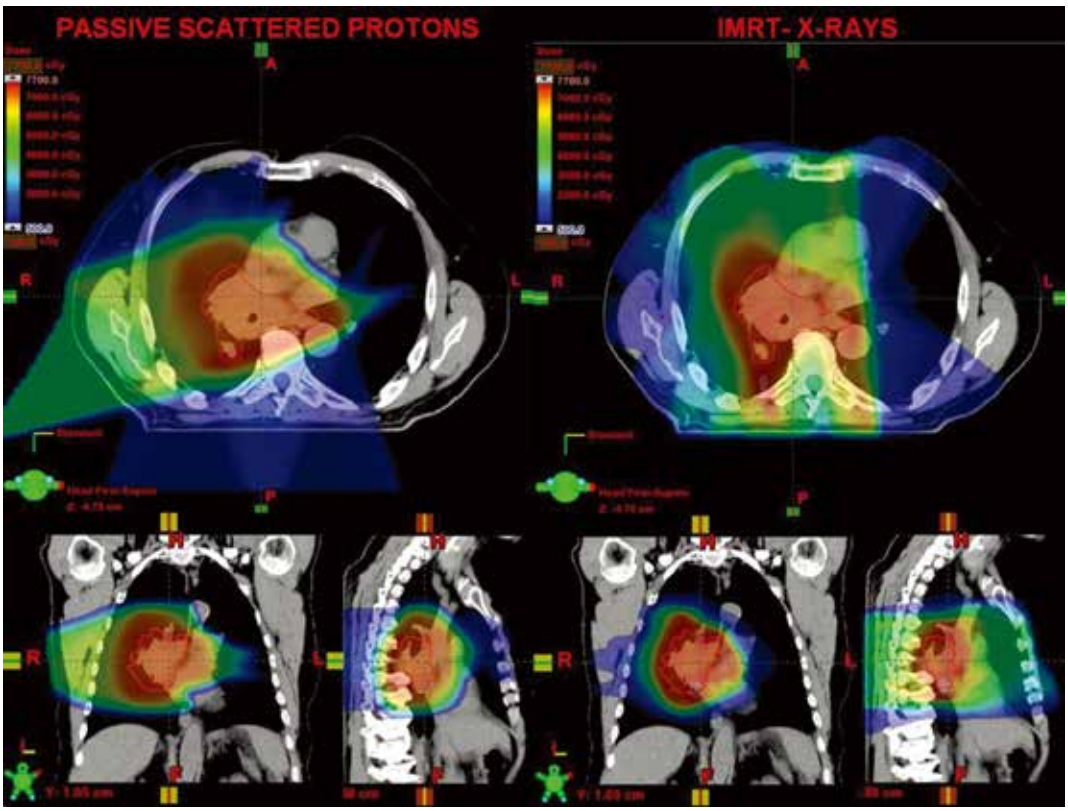


Figure 2 Example of comparison plans for the treatment of lung cancer between passive scatter proton radiotherapy and IMRT.

One possibility for this disparity could be treatment-related toxicity; in other words, although tumor control may be increased in patients treated with higher radiation doses, the commensurate increase in toxicity and toxicity-related death can mask any potential benefit. Hence the desire for a radiation treatment modality that can minimize radiation dose to critical structures (e.g., the lungs) while allowing the possibility of dose escalation to the target. One such modality that attempts to achieve this goal is proton beam therapy.

Several planning studies have been done to compare the dose to normal surrounding structures associated with either photon or proton radiotherapy. Generally, in these studies, proton beam therapy has shown benefits over standard conformal radiotherapy; specifically, the dose to the uninvolved lung can in some cases be superior to that provided via conventional radiotherapy (15-17) or IMRT (15,17). Examples of a typical plan for passive scatter proton radiotherapy and one for IMRT are shown in *Figure 2*. Proton radiotherapy may also have advantages over photon-based stereotactic radiotherapy for smaller tumors in terms of sparing normal tissue (15,18-20). However, the benefit from the use of protons from the dosimetric perspective is not universal. Because of the uncertainty of the exact range of the Bragg peak, particularly in hypodense tissues such as lung, the use of additional margin of high-dose radiation may be required, leading to a higher dose to critical structures, particularly when they are close to the target (21). Further, because many tumors have irregular borders and involve the mediastinum, highly conformal IMRT may provide an advantage in regard to normal tissue sparing compared with the traditional passive-scatter approach to proton radiotherapy (22).

Dosimetric studies aside, a growing body of literature details the clinical experience of using charged particle therapy for lung cancer. Several institutions have generated significant data from the use of proton radiotherapy as monotherapy. One of the earliest published studies reported the investigators' experience in treating mainly early-stage non-small cell lung cancer (NSCLC) with a proton boost after traditional photon radiotherapy. In that study of 37 patients, the local control rate was 87%, and only 2 patients developed symptomatic pneumonitis (23). Studies of stereotactic or hypofractionated proton-based radiotherapy

for early-stage lung cancer have shown similar local control rates for small, peripheral lesions (24-27). However, in the same studies, local control rates for larger lesions have been less favorable, falling in the range of 40% to 60%. Toxicity in these studies has been minimal; in a phase I/II trial recently completed at MD Anderson Cancer Center involving a dose of 87.5 CGE, the rates of symptomatic pneumonitis and esophagitis were 11% and 6% (27).

Less information is available regarding combinations of proton radiotherapy with concurrent chemotherapy for locally advanced lung cancer. The guiding principle for radiotherapy to the lung has been to increase the dose to the point of maximum tolerability, as a radiation dose-response relationship has been observed for locally advanced lung cancer (13). However, any dose escalation must take into account the significant toxicity associated with thoracic radiotherapy. In fact, the most recent national trial of dose-escalated thoracic radiotherapy initiated by the RTOG led to the premature closure of the high-dose treatment group (74 Gy) because of the absence of any observed survival benefit (14). Although the final toxicity data from this trial were not available when this review was written, it is worth noting that 7 patients died in the high-dose group versus 3 in the control group (treated to 60 Gy). Thus, it seems that significant caution should be observed in attempting dose escalation of thoracic radiotherapy when that therapy involves conventional methods. However, a recent retrospective review of concurrent platinum-based chemotherapy and proton radiotherapy noted particularly low rates of pneumonitis (2%) and esophagitis (5%) compared with those rates in a similar group of patients treated to a lower dose (63 Gy) by either 3-dimensional conformal radiotherapy or IMRT (28). Further investigation of these results in a phase II trial showed similarly positive results, with local control rates of close to 80% and pneumonitis and esophagitis rates of around 2% and 11% (29). These results are also being evaluated further in a Bayesian randomized trial of image-guided proton radiotherapy compared with photon radiotherapy for patients with locally advanced lung cancer.

Despite the dosimetric evidence and some clinical data supporting the use of proton radiotherapy for the treatment of lung cancer, significant challenges remain. First, treatment planning using proton radiotherapy is complicated by the inherent motion of the lung. Unlike photon radiotherapy, protons are drastically affected by the material through which they pass. Thus tissue densities must be accounted for during treatment planning. However the motion of the lung - and consequently the motion of the tumor - during the respiratory cycle can make this challenging, particularly in light of the finite range of protons. Although proton radiotherapy is appealing in the context of sparing normal structures, any changes in the path of the beams during respiration could change the range of the proton beam significantly, leading to marginal misses of the target or increased dose to surrounding normal structures. This problem has been addressed in several planning studies [reviewed in (30)], and a variety of different approaches are being used to minimize this problem. In one such approach, "smearing" the target volume artificially increases the volume targeted in an attempt to ensure good coverage despite small changes arising from motion during the respiratory cycle. This problem of appropriate targeting is further amplified by changes in the tumor itself during radiotherapy: tumors can shrink or become more cavitary in response to radiotherapy, which again changes the density of the tissue traversed by the proton beam and altering its range. At MD Anderson Cancer Center, we have tried to minimize this problem by obtaining images throughout the course of the radiotherapy and modifying the plans ("adaptive planning") if the tumor responds significantly.

Further difficulties arise from highly irregular targets. As noted previously, IMRT can in many cases provide more conformal treatment for large irregular lesions. In an initial dosimetric comparison between IMRT and proton radiotherapy as part of the above-mentioned randomized protocol, IMRT was found to have a dosimetric advantage in many cases (31). One possible solution to the problem of conformality is the use of some form of modulated proton radiotherapy. Conventional proton radiotherapy ("passive scatter") uses material to scatter the beam over a large area, with a rotating wheel placed in the beam path to allow generation of a spread-out Bragg peak. This approach basically delivers a uniform dose over the extent of the target, but does not allow generation of irregular contours for the dose to be delivered. The concept of "pencil beam" proton radiotherapy is being investigated to improve upon this dose distribution; in this technique, the dose can be "painted" over any particular target by the use of pencil beams of protons directed at small segments of an individual target. Although this approach can improve the conformal coverage of irregular targets, in some situations it can be less robust than use of a passive scatter beam, because the accuracy of scanning beam proton radiotherapy is affected to an even greater extent by organ motion (32). Studies of the use of scanning beam technology for the treatment of

lung cancer are ongoing.

Finally, cautions have been raised regarding the problem of unintended neutron dose when using proton radiotherapy. The production of secondary neutrons may be of particular concern for passive scatter beams, in which a physical component is placed in the beam path, because these scattered neutrons may themselves be carcinogenic. Although this could be of significant import for younger populations, patients with lung cancer tend to be older, and risk estimates for carcinogenesis are highest for young patients (33). Further, the magnitude of neutrons generated by passive scatter beams is debated in the literature (34). Regardless, the advent of scanning beam technology has greatly reduced the possible risk of neutron scatter in the use of proton beam radiotherapy (33).

Future challenges and opportunities

The advent of proton radiotherapy for lung cancer brings with it an opportunity to minimize the toxicity of current standard-of-care therapy. At present, this possibility is being investigated in at least one randomized trial in which the benefits of passive scatter proton radiotherapy are being compared with those of IMRT. Further prospective studies are also ongoing to compare the benefits of stereotactic proton radiotherapy with those of standard 3D-conformal stereotactic radiotherapy. So, what further challenges and opportunities remain? One major criticism of proton radiotherapy for the treatment of lung cancer is its cost. The development of proton radiotherapy capability requires a significant cost outlay for any institution. Moreover, a treatment course of proton radiotherapy is significantly more expensive at the current time than is a comparable course of IMRT. Although this cost will likely decrease with time, proton radiotherapy remains an expensive treatment option. However, the costs of proton radiotherapy for lung cancer must be weighed against the costs of toxicity associated with therapy. In fact, in one cost-effectiveness model involving only recent studies, proton radiotherapy was found to be cost-effective for the management of selected cases of lung cancer (35). This finding underscores the idea that merely calculating treatment costs does not completely measure the value of any particular therapy.

With regard to technology, the current "cutting edge" in proton radiotherapy delivery is the development of intensity modulation. As noted previously, one of the disadvantages of passive beam proton radiotherapy is the inability to conform to a highly irregular target or to allow dose-painting within the irradiated field. Scanning beam technology removes this disadvantage. Moreover, one could conceivably generate treatment plans that take advantage of the increased RBE at the Bragg peak by deliberately encompassing a radioresistant area of a tumor (e.g., an hypoxic area) within the Bragg peak of each scanning beam. Theoretically, this approach would lead to improved response without incurring any toxicity associated with dose escalation. However, as noted previously, scanning beam technology for proton delivery is highly dependent on precise planning software and improved motion management. As these technologies improve, true intensity modulation of protons in the treatment of lung cancer will become a reality.

In summary, the current state of proton beam radiotherapy or intensity-modulated proton beam radiotherapy for lung cancer is one of optimism. Prospective trials of proton radiotherapy are ongoing, and those findings, as they mature, will be valuable in further clarifying the role of proton radiotherapy for the management of this deadly disease.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Bragg W. On the ionization of various gasses by the alpha particles of radium. Proc Phys Soc 1907;20:523-50.
2. Paganetti H, Niemierko A, Ancukiewicz M, et al. Relative biological effectiveness (RBE) values for proton beam therapy. Int J Radiat Oncol Biol Phys 2002;53:407-21.
3. International Commission on Radiation Units and Measurements. Prescribing, recording, and reporting proton-beam therapy (ICRU report 78). Bethesda, MD.
4. Wouters BG, Lam GK, Oelfke U, et al. Measurements of relative biological effectiveness of the 70 MeV proton beam at TRIUMF using Chinese hamster V79 cells and the high-precision cell sorter assay. Radiat Res 1996;146:159-70.
5. Bettega D, Calzolari P, Chauvel P, et al. Radiobiological studies on the 65 MeV therapeutic proton beam at Nice using human tumour cells. Int J Radiat Biol 2000;76:1297-303.
6. Tang JT, Inoue T, Inoue T, et al. Comparison of radiobiological effective depths in 65-MeV modulated proton beams. Br J Cancer 1997;76:220-5.
7. Courdi A, Brassart N, Hérault J, et al. The depth-

dependent radiation response of human melanoma cells exposed to 65 MeV protons. Br J Radiol 1994;67:800-4.
8. Matsuura T, Egashira Y, Nishio T, et al. Apparent absence of a proton beam dose rate effect and possible differences in RBE between Bragg peak and plateau. Med Phys 2010;37:5376-81.
9. Frese MC, Wilkens JJ, Huber PE, et al. Application of constant vs. variable relative biological effectiveness in treatment planning of intensity-modulated proton therapy. Int J Radiat Oncol Biol Phys 2011;79:80-8.
10. Carabe A, Moteabbed M, Depauw N, et al. Range uncertainty in proton therapy due to variable biological effectiveness. Phys Med Biol 2012;57:1159-72.
11. Jones B, Underwood TS, Dale RG. The potential impact of relative biological effectiveness uncertainty on charged particle treatment prescriptions. Br J Radiol 2011;84:S61-9.
12. O'Rourke N, Roqué I Figuls M, et al. Concurrent chemoradiotherapy in non-small cell lung cancer. Cochrane Database Syst Rev 2010;(6):CD002140.
13. Partridge M, Ramos M, Sardaro A, et al. Dose escalation for non-small cell lung cancer: analysis and modelling of published literature. Radiother Oncol 2011;99:6-11.
14. Bradley JD, Paulus R, Komaki R, et al. A randomized phase III comparison of standard-dose (60 Gy) versus high-dose (74 Gy) conformal chemoradiotherapy +/– cetuximab for stage IIIa/IIIb non-small cell lung cancer: Preliminary findings on radiation dose in RTOG 0617. Int J Radiat Oncol Biol Phys 2011;81: abstr LBA2.
15. Chang JY, Zhang X, Wang X, et al. Significant reduction of normal tissue dose by proton radiotherapy compared with three-dimensional conformal or intensity-modulated radiation therapy in Stage I or Stage III non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2006;65:1087-96.
16. Lee CH, Tait D, Nahum AE, et al. Comparison of proton therapy and conformal X-ray therapy in non-small cell lung cancer (NSCLC). Br J Radiol 1999;72:1078-84.
17. Nichols RC, Huh SN, Henderson RH, et al. Proton radiation therapy offers reduced normal lung and bone marrow exposure for patients receiving dose-escalated radiation therapy for unresectable stage iii non-small-cell lung cancer: a dosimetric study. Clin Lung Cancer 2011;12:252-7.
18. Macdonald OK, Kruse JJ, Miller JM, et al. Proton beam radiotherapy versus three-dimensional conformal stereotactic body radiotherapy in primary peripheral, early-stage non-small-cell lung carcinoma: a comparative dosimetric analysis. Int J Radiat Oncol Biol Phys 2009;75:950-8.
19. Hoppe BS, Huh S, Flampouri S, et al. Double-scattered proton-based stereotactic body radiotherapy for stage I lung cancer: a dosimetric comparison with photon-based stereotactic body radiotherapy. Radiother Oncol 2010;97:425-30.
20. Wang C, Nakayama H, Sugahara S, et al. Comparisons of dose-volume histograms for proton-beam versus 3-D conformal x-ray therapy in patients with stage I non-small cell lung cancer. Strahlenther Onkol 2009;185:231-4.
21. Seco J, Panahandeh HR, Westover K, et al. Treatment of non-small cell lung cancer patients with proton beam-based stereotactic body radiotherapy: dosimetric comparison with photon plans highlights importance of range uncertainty. Int J Radiat Oncol Biol Phys 2012;83:354-61.
22. Zhang X, Li Y, Pan X, et al. Intensity-modulated proton therapy reduces the dose to normal tissue compared with intensity-modulated radiation therapy or passive scattering proton therapy and enables individualized radical radiotherapy for extensive stage IIIB non-small-cell lung cancer: a virtual clinical study. Int J Radiat Oncol Biol Phys 2010;77:357-66.
23. Bush DA, Slater JD, Bonnet R, et al. Proton-beam radiotherapy for early-stage lung cancer. Chest 1999;116:1313-9.
24. Bush DA, Slater JD, Shin BB, et al. Hypofractionated proton beam radiotherapy for stage I lung cancer. Chest 2004;126:1198-203.
25. Iwata H, Murakami M, Demizu Y, et al. High-dose proton therapy and carbon-ion therapy for stage I nonsmall cell lung cancer. Cancer 2010;116:2476-85.
26. Nihei K, Ogino T, Ishikura S, et al. High-dose proton beam therapy for Stage I non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2006;65:107-11.
27. Chang JY, Komaki R, Wen HY, et al. Toxicity and patterns of failure of adaptive/ablative proton therapy for early-stage, medically inoperable non-small cell lung cancer. Int J Radiat Oncol Biol Phys 2011;80:1350-7.
28. Sejpal S, Komaki R, Tsao A, et al. Early findings on toxicity of proton beam therapy with concurrent chemotherapy for nonsmall cell lung cancer. Cancer 2011;117:3004-13.
29. Chang JY, Komaki R, Lu C, et al. Phase 2 study of high-dose proton therapy with concurrent chemotherapy for unresectable stage III nonsmall cell lung cancer. Cancer 2011;117:4707-13.
30. Liao Z, Lin SH, Cox JD. Status of particle therapy for

lung cancer. Acta Oncol 2011;50:745-56.
31. Mohan R, Zhang X, Matney J, et al. IMRT vs. passively scattered proton therapy (PSPT) for locally advanced non-small cell lung cancer (LA NSCLC) randomized trial - is there equipoise? Int J Radiat Oncol Biol Phys 2010;78:S201-2.
32. Schippers JM, Lomax AJ. Emerging technologies in proton therapy. Acta Oncol 2011;50:838-50.
33. Brenner DJ, Hall EJ. Secondary neutrons in clinical proton radiotherapy: a charged issue. Radiother Oncol 2008;86:165-70.
34. Paganetti H, Bortfeld T, Delaney TF. Neutron dose in proton radiation therapy: in regard to Eric J. Hall (Int J Radiat Oncol Biol Phys 2006;65:1-7). Int J Radiat Oncol Biol Phys 2006;66:1594-5; author reply 1595.
35. Grutters JP, Pijls-Johannesma M, Ruysscher DD, et al. The cost-effectiveness of particle therapy in non-small cell lung cancer: exploring decision uncertainty and areas for future research. Cancer Treat Rev 2010;36:468-76.

Cite this article as: Skinner HD, Komaki R. Proton radiotherapy in the treatment of lung cancer. Transl Cancer Res 2012;1(4):264-270. doi: 10.3978/j.issn.2218-676X.2012.12.02

Prognostic and predictive biomarkers in early stage non-small cell lung cancer: tumor based approaches including gene signatures

Simona Carnio, Silvia Novello, Mauro Papotti, Marco Loiacono, Giorgio Vittorio Scagliotti

University of Torino, Department of Oncology, Torino, Italy

Correspondence to: Giorgio Vittorio Scagliotti. University of Torino, Department of Oncology, S. Luigi Hospital, Regione Gonzole 10, 10043 Orbassano (Torino), Italy. Email: giorgio.scagliotti@unito.it.

Abstract: In early stage non-small cell lung cancer (NSCLC) large randomized trials have demonstrated that in patients with radically resected disease adjuvant chemotherapy improves 5-year survival rates. However, a customization of systemic treatment is needed to avoid treatments in patients cured by surgery alone or to justify the use of adjuvant chemotherapy in high risk patients, including those in stage IA. Recently, the possibility of identifying prognostic and predictive factors related to the genetic signatures of the tumor that could affect adjuvant and neo-adjuvant treatment choices for resectable non-small cell lung cancer (NSCLC) has been of interest. This review summarizes the current status and future opportunities for clinical application of genotyping and genomic tests in early NSCLC.

Keywords: Prognostic and predictive biomarkers; early stages; non-small cell lung cancer (NSCLC); gene signatures

Submitted Sep 15, 2013. Accepted for publication Oct 10, 2013.
doi: 10.3978/j.issn.2218-6751.2013.10.05
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.10.05

Introduction

Surgery remains the only potentially curative treatment for early-stage non-small cell lung cancer patients (NSCLC) resulting in 5-year survival rates ranging from 77% in pathological stage IA to 23% in stage IIIA tumors (1).

Clinical trials and meta-analyses have demonstrated that in patients with early-stage NSCLC adjuvant chemotherapy improves survival (2-6) with an average benefit of 5% at 5 years and, consequently, adjuvant chemotherapy is recommended for patients with resected stage II-III NSCLC (7-9). Nevertheless, a proportion of stage I patients have poor prognosis and may benefit significantly from adjuvant chemotherapy, while some relatively good prognosis stage II patients may not share similar benefits. Therefore, new diagnostic paradigms are urgently needed to select stage I-II subjects who may take advantage from adjuvant chemotherapy and clinical trials.

The strongest clinical prognostic factors in NSCLC include stage, sex, age, and performance status (10-12), but a better individualization of treatment approaches requires a more precise understanding of the molecular features of lung cancer.

A wide array of individual molecular markers have been tested in advanced as well as early stage NSCLC for prognostic and predictive value and this review will focus on the current existing evidence to support their investigational value. Most of these molecular markers have been found to be either prognostic and/or predictive at the same time. One of the first and largest retrospective biomarker studies in 515 resected stage I NSCLC patients failed to show any significant association between survival and the expression of an extensive panel of biomarkers, including epidermal growth factor receptor (EGFR), HER2/neu, bcl-2, p53 and angiogenesis markers (13).

Lastly, the human genome project, allowed the development and clinical applications of genomic-based assays, including increasingly dense microarray platforms for global analyses of gene expression, copy number

variation, DNA methylation and microRNA and several genomic signatures have been identified and tested in early stage NSCLC for their prognostic value.

Excision repair cross-complementation group 1

Cisplatin inhibits replication by binding to DNA and forming platinum-DNA adducts causing strand breaks when the DNA helices unwind in preparation for replication. The nuclear excision repair (NER) family of genes is involved in repair of these DNA strand breaks (14). The excision repair cross-complementation group 1 (ERCC1) enzyme is involved in the final step of the NER pathway that recognizes and removes cisplatin-induced DNA adducts, therefore, leading to cisplatin resistance. High tumoral ERCC1 expression, therefore, predicts for cisplatin resistance.

ERCC1 activity may be assessed as protein by standard immunohistochemistry (IHC) or automated AQUA technology (15). Alternatively, it can be assessed at the mRNA level through quantitative real-time polymerase chain reaction (qRT-PCR). Currently, there is no consensus about the superiority of one approach versus the other because both techniques are rarely assessed concomitantly (16,17).

In resected NSCLC, patients with high ERCC1 expression (>50 unitless ratio) had a better survival outcome (median OS, 94.6 *vs.* 35.5 months; P=0.01) when compared to patients with low ERCC1 expression generating the hypothesis that an intact DNA repair mechanism may reduce the accumulation of genetic aberrations that are thought to contribute to malignant potential phenotype and therefore the risk of relapse after definitive treatment (18).

The predictive role of ERCC1 was initially assessed by RT-PCR in a series of small retrospective studies in advanced NSCLC (19,20). The median overall survival (OS) was significantly longer in patients with low ERCC1 compared to patients with high ERCC1. Subsequently, ERCC1 was investigated in a subgroup of patients enrolled in a large adjuvant chemotherapy trial (21) and, by standard immunohistochemistry, in 761 paraffin-embedded tumor samples (22). A benefit from cisplatin-based adjuvant chemotherapy was associated with the absence or low expression of ERCC1 (test for interaction, P=0.009) with a significantly prolonged disease-free survival and OS among patients with ERCC1-negative tumors (HR for death, 0.65; 95% CI, 0.50-0.86; P=0.002) as opposed to ERCC-1 positive tumors. Morever, the prognostic value of ERCC1 was confirmed in the control group with a significantly higher 5-year OS among patients with ERCC1-positive tumors than negative ones (HR, 0.66; 95% CI, 0.49-0.90; P=0.009). The same tumors were also scored by AQUA and low ERCC1 scores were marginally prognostic (HR =0.77 for high versus low scores, P=0.10) (23) while in an additional study, ERCC1 was exclusively predictive in squamous cell carcinoma (24).

The specificity of the commonly used mouse monoclonal antibody 8F1 to ERCC1 has been extensively debated (25,26). It was also found that none of the 16 antibodies tested could distinguish among the four known ERCC1 protein isoforms (27). In the neo-adjuvant setting, mRNA ERCC1 levels in pretreatment tissue samples were correlated with the capacity to achieve an objective response following platinum-based chemotherapy (P<0.05), but not with the formation of local or distant metastases (28).

The predictive value of ERCC1 was enhanced by the concurrent evaluation of MutS homolog 2 (MSH2), a major active component of the mismatch repair system. Patients with double-negative tumors experienced a greater benefit from chemotherapy (29).

Overall these data indicate a prognostic role of ERCC1 expression while its predictive role remains to be further assessed and additional validation studies are needed.

Breast cancer susceptibility gene 1

The protein encoded by breast cancer susceptibility gene 1 (BRCA1) has a crucial role in DNA repair as well as in cell-cycle checkpoints and mitotic spindle assembly (30). BRCA1 sensitizes cancer cells to apoptosis induced by antimicrotubule drugs, such as taxanes and vinca alkaloids, while conferring resistance to DNA-damaging agents, including platinum agents. The potential prognostic role of a panel of nine candidate biomarkers including BRCA1 was investigated in two independent cohorts of chemotherapy naive patients with early-stage NSCLC. BRCA1 was the only independent factor affecting OS (31). In a group of patients with locally advanced NSCLC, treated with neo-adjuvant cisplatin and gemcitabine followed by surgery, those with the lowest levels of BRCA1 mRNA expression had significantly greater benefit from chemotherapy in terms of clinical and pathological downsizing and OS (32).

For its localization to sites of DNA double strand breaks, the upstream activity of the receptor-associated protein 80 (RAP-80) is required for BRCA1. In a first line study in advanced NSCLC patients with the lowest expression of both BRCA1 and RAP-80 receiving cisplatin

plus gemcitabine it was shown that RAP-80 can modulate the effect of BRCA1. In addition to a close correlation with BRCA1, RAP-80 expression was identified as an independent predictor for OS (33). In a phase II feasibility study of adjuvant chemotherapy in patients with stage II-IIIA NSCLC, those with high BRCA1 transcriptional levels received single agent docetaxel, whereas those with intermediate and low BRCA1 expression were treated with cisplatin-doublets and OS did not differ between the treatment arms (34).

A recent meta-analysis of 23 studies assessed the role of BRCA1 as a predictor of clinical outcome in platinum- and paclitaxel-based chemotherapy in NSCLC patients. In 17 platinum-based studies, low/negative BRCA1 was associated with better objective response rate [ORR] (OR =1.70, 95% CI, 1.32-2.18), longer OS and event-free survival [EFS] (HR =1.58, 95% CI, 1.27-1.97, and HR =1.60, 95% CI, 1.07-2.39 for OS and EFS, respectively). In 4 paclitaxel-based chemotherapy studies, patients with high/positive BRCA1 had better ORR (OR =0.41, 95% CI, 0.26-0.64) while OS and EFS were not evaluated because of the insufficient data available (35). Some studies reported that ERCC1 expression is closely linked to RRM1 and BRCA1 levels (32,36,37), with concordant levels in 70-80% of cases (20,38).

Ribonucleotide reductase M1

Ribonucleotide reductase M1 (RRM1) is the regulatory component of an essential enzyme that catalyzes the reduction of ribonucleoside diphosphates to the corresponding deoxyribonucleotides. A role for ribonucleotide reductase in DNA repair has been proposed, given the capacity of RRM1 to bind a p53-regulated paralog of RRM2 called p53R2 (39,40). Increased RRM1 predicts for decreased tumor invasiveness and metastatic potential, therefore predicting for more indolent behavior, perhaps mediated through its direct correlation with phosphatase and PTEN (phosphatase and tensin homolog) protein expression (41,42). In resected NSCLC, RRM1 protein proved prognostic, with low levels associated with a median OS of 60.2 months, compared to more than 120 months in high RRM1 tumors (43). RRM1 is a major predictor of disease response to gemcitabine, being its predominant target, as well as platinum (44). Several studies investigated the predictive value of RRM1 in patients treated with gemcitabine plus cisplatin (45,46) demonstrating that RRM1 expression was significantly and inversely correlated with disease response, though not with survival (47). A recent meta-analysis in 1,243 patients with advanced NSCLC treated with gemcitabine-based regimens concluded that low tumor RRM1 was associated with a better response rate and longer survival (48).

Thymidylate synthase

Thymidylate synthase (TS) catalyzes the conversion of deoxyuridine monophosphate (dUMP) to (deoxy) thymidine monophosphate (TMP), which requires oxidization of tetrahydrofolate to dihydrofolate. High tumoral levels of TS have been associated with resistance to 5-FU (49-51). Retrospective and prospective data from phase III trials in advanced NSCLC have established the favorable predictive value of TS in non-squamous NSCLC treated with pemetrexed with mRNA and protein TS expression lower in non-squamous compared to squamous histology and small cell lung cancer (52,53).These findings have been recently confirmed in a large retrospective study, although there was a wide range between individual patients (54).

Similarly distinct TS expression patterns among NSCLC subtypes were observed in stage I-IIIA NSCLC (55). In two different cohorts of chemotherapy naive patients with resected early-stage NSCLC, TS was a prognostic factor with mixed results. In one study high TS mRNA (but not protein) expression, was significantly associated with adverse disease free survival (DFS) and in the other study, high TS expression as determined by AQUA but not by qRT-PCR, predicted improved OS (55,56).

ITACA (International TAilored Chemotherapy Adjuvant) (57) trial is a randomized phase III trial comparing adjuvant pharmacogenomic-driven chemotherapy based on ERCC-1 and TS assessment by qRT-PCR versus standard adjuvant chemotherapy in completely resected stage II-IIIA NSCLC. The molecular assessment groups patients into four different genetic profiles with patients dichotomized by high versus low expression of both ERCC-1 and TS. Within 30 to 45 days post-surgery, patients in each genetic profile are randomized to receive either a standard adjuvant chemotherapy doublet (control arm) or an experimental treatment guided by molecular determinants (tailored chemotherapy arm). The study is currently accruing patients in Italy and in Germany and more than 600 patients have been already randomized (see *Figure 1*).

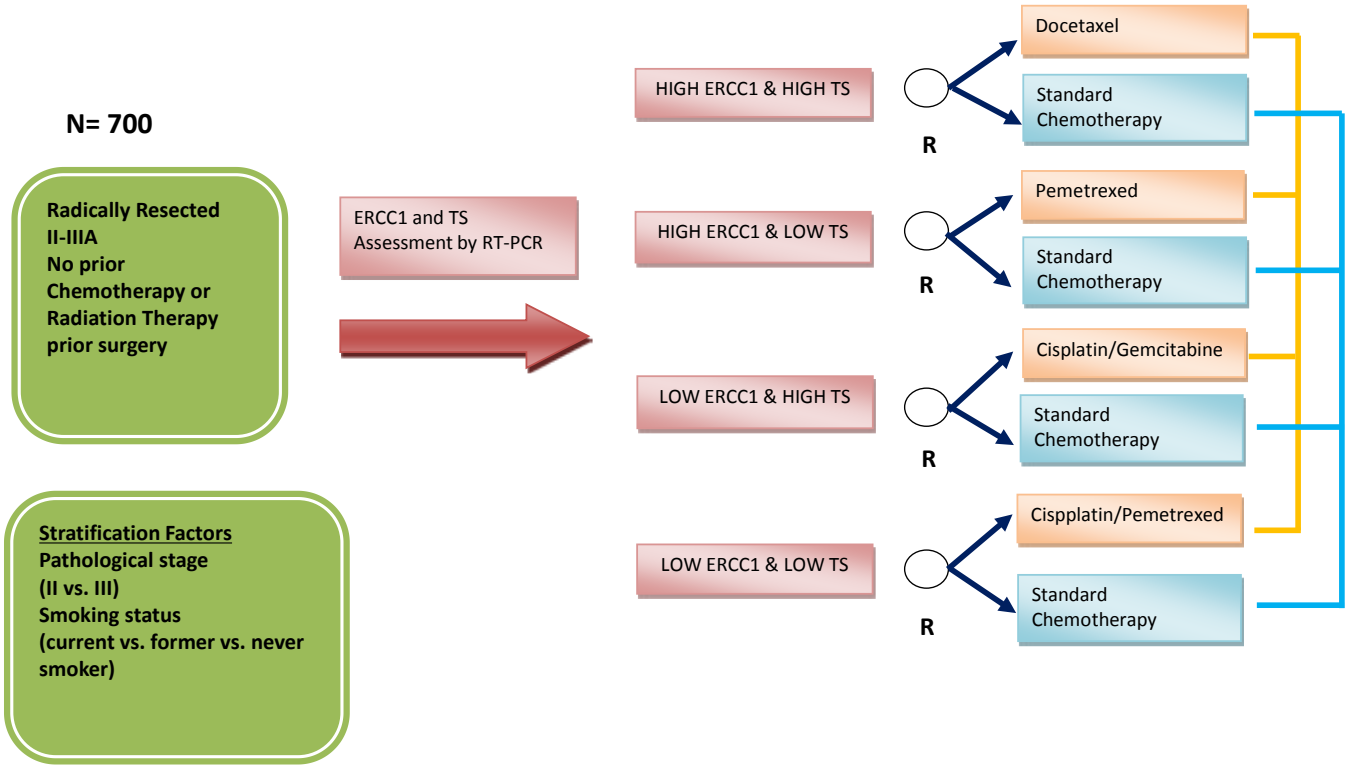


Figure 1 Study Design of The ITACA Adjuvant Trial. Randomization is performed according to each genetic profile. Cisplatin-doublet, investigator choice is the Control Arm of the study. Chemotherapy is either Vinorelbine 25-30 mg/m^2 IV over 10 minutes, days 1 and 8 & Cisplatin 75 mg/m^2 IV over 60 minutes, day 1, immediately following Vinorelbine or Docetaxel 75 mg/m^2 IV over 60 minutes, day 1 & Cisplatin 75 mg/m^2 IV over 60 minutes, day 1, immediately following Docetaxel or Gemcitabine 1,200 mg/m^2 IV over 30 minutes, days 1 and 8 & Cisplatin 75 mg/m^2 IV over 60 minutes day 1, immediately following gemcitabine. For the purpose of the final statistical analysis at the end of the study all controls will be grouped together in one single group (control group, blu boxes and line) and all tailored chemotherapies will be assembled together (experimental group, orange boxes and line).

Cyclin-dependent kinase inhibitor 1B

The cyclin-dependent kinase inhibitor 1B, p27^{Kip1}, is a tumor-suppressor protein that induces cell-cycle arrest in phase G1. Despite its anti-proliferative properties, p27^{Kip1} up-regulation leads to de novo resistance to platinum agents by allowing cancer cells to repair DNA damage and avoid apoptosis. A survival benefit from cisplatin-based chemotherapy was only demonstrated in patients with p27^{Kip1} negative tumors (58,59). Cyclin D2 has been associated with poor recurrence-free survival in patients in stage III NSCLC treated with surgery with or without adjuvant chemotherapy (60).

β-tubulin

β-tubulin (βTubIII) is an essential element of microtubules, which serves as a cellular structural component involved in vital processes, including mitosis. Class IIIβ-tubulin (βTUBIII) corresponds to an isotype with enhancing impact on microtubule dynamics, contributing to intrinsic cancer cell resistance to antimitotic agents. Several studies have shown that βTubIII expression may predict response and outcome in patients with advanced NSCLC receiving tubulin binding agents (61,62). In patients with early stage NSCLC treated with an adjuvant vinorelbine-based regimen (2), high βTUBIII expression was shown to be an independent adverse predictor of recurrence-free survival (63). The prognostic value was confirmed retrospectively in patients enrolled in another adjuvant study (64) and more recently in a neo-adjuvant study (65). In patients with β-tubulin positive immunostaining, median PFS was 30.6 versus 60.1 months (HR =1.46; 95% CI, 1.08-1.99) for those negative.

RAS oncogene and p53

K*RAS* mutation was associated in some studies with a poor prognosis in early NSCLC patients (66) but not in others. In the phase III NCIC JBR.10 adjuvant trial, where patients with stage IB-II disease were prospectively stratified by the presence of mutation in any of the *RAS* genes, the effect of *RAS* mutation status on the treatment outcome and prognosis was not significant. Nevertheless, the lack of benefit from the cisplatin/vinorelbine combination in patients with *RAS* mutations, in contrast to the total study population, may suggest a possible negative predictive role (67). Similarly, another adjuvant study in stage IB patients showed that, the presence K*RAS* mutations trended for an inferior survival of patients with tumors larger than 4 cm (68). Consistent data were reported in an Italian adjuvant study where in a subgroup of 227 patients the presence of K-*RAS* mutation, but not p53 and Ki67, in the univariate, but not in the multivariate analysis, was associated with shorter survival (69).

p53 nuclear immunoreactivity is considered a surrogate marker of *TP53* gene mutations. However, the sensitivity and positive predictive value of p53 IHC expression for *TP53* mutation status are estimated to be only 75% and 65%, respectively (70). A retrospective analysis of the phase III NCIC-JBR.10 adjuvant trial showed p53 IHC overexpression to be an independent unfavorable prognostic factor among patients in the observation arm. In contrast to p53 expression, *TP53* mutation status was neither prognostic for survival, nor predictive for efficacy of adjuvant chemotherapy (71).

Gene expression signatures

Microarray technologies allow exploration of the prognostic significance of thousands of markers using high-throughput and computational approaches. To date, in lung cancer more than 30 studies have been reported (72) a large number showing that gene expression signature may stratify early stage NSCLC patients with different prognosis or survival outcome.

Although most of these signatures have been validated in one or more independent patient cohorts, microarray dataset overlaps between the genes sets have consistently been minimal. Thus there is a strong possibility that sample collection methods, processing protocols, single-institution subject cohorts, small sample sizes, and peculiarities of the different microarray platforms are contributing significantly to the results. To address these issues, a multi-institutional collaborative study was conducted to generate gene expression profiles from a large number of samples with *a priori* determined clinical features, useful to evaluate proposed prognostic models for potential clinical implementation. A large series of lung adenocarcinomas were tested for whether microarray measurements of gene expression either alone or combined with basic clinical covariates (stage, age, sex) can be used to predict overall survival in lung cancer subjects. Risk scores were produced substantially correlated with actual subject outcome, especially when clinical and molecular information are combined to build prognostic models for early stage lung cancer (73).

A malignancy-risk gene signature composed of several genes associated with proliferative activity has been successfully applied to predict breast cancer risk (74,75) and also tested for prognostic and predictive value in an early-stage NSCLC patients. The malignancy-risk gene signature was tested using a large NSCLC microarray dataset from the Director's Challenge Consortium (n=442) and two independent NSCLC microarray datasets (n=117 and 133 datasets, respectively). The malignancy-risk gene signature was significantly associated with OS (P<0.001) in two independent datasets and also with adjuvant chemotherapy (P=0.02) (76). Xu *et al.* developed an empirical model which is not based on the knowledge of patients' survival time for determining the lung cancer biomarker signature. It has been hypothesized that instead of an individual gene, two functionally imbalanced groups of genes (Yin and Yang) determine the fate of the tumor cells, which ultimately determines patient's survival time. The Yin and Yang genes were selected by comparing expression data from normal lung and lung cancer tissue samples using both unsupervised clustering and pathways analyses. The model was tested in four independent lung cancer datasets and significantly stratified patients into high- and low-risk survival groups and predicted chemotherapy outcomes for stages II and III (77).

A 14-gene expression assay that uses quantitative PCR, runs on formalin-fixed paraffin-embedded tissue samples, and differentiates patients with heterogeneous statistical prognoses was developed in a cohort of 361 resected patients with non-squamous NSCLC and validated in 2 different cohorts of 433 patients with resected stage I non-squamous NSCLC and 1,006 patients with stage I-III non-squamous NSCLC resected in several leading Chinese cancer centres. The signature significantly segregated patients in low-, intermediate- and high-risk patients with relevant differences in 5-year survival rate. Multivariate

analysis in both cohorts indicated that no standard clinical risk factors could account for, or provide, the prognostic information derived from tumour gene expression. This quantitative-PCR-based assay reliably identified patients with early-stage non-squamous NSCLC at high risk for mortality after surgical resection (78).

Other biomarkers in early NSCLC

Insulin receptor (IR) and Insulin-like growth factor receptor (IGF1R) are implicated in the development and progression of NSCLC, either by interacting with the EGFR pathway or independently. In patients with resected NSCLC, IGF1R amplification determined by FISH was an independent favorable prognostic factor, unlike IGF1R protein (79). It has been observed that in early stage NSCLC, IGF1R/EGFR FISH+ and IGF1R/EGFR IHC+ were associated with shorter disease-free survival (P=0.05 and P=0.05, respectively). Patients with concomitant IGF1R/EGFR FISH+/IHC+ had a worse DFS and OS (P=0.005 and P=0.01, respectively) (80).

Hepatocyte growth factor receptor (c-MET) is a proto-oncogene associated with tumor invasive growth. In patients with resected stage I-III carcinomas, not treated with adjuvant chemotherapy, a high MET gene copy number was an independent adverse prognostic factor mainly in squamous histotype (81). Similarly, in patients with early-stage NSCLC HER2 expression was associated with poorer prognosis especially in stages IB and IIA diseases (82).

Higher expression of CXCR7 is associated with metastatic progression and poor DFS in patients with stage I NSCLC (83). In a retrospective analysis of completely resected stage I tumors, patients with CXCR4-positive tumors had a significantly longer survival than patients with CXCR4-negative tumors (P=0.039). Interestingly, the 5-year metastasis rates were 23.5% and 34.1% in patients with CXCR4-positive and CXCR4-negative expression, respectively (P=0.2) (84).

Recently, a set of genes with altered methylation status were identified in stage I NSCLCs, some of which associated with survival. Such newly identified potential candidates for a NSCLC molecular screening need further analysis in order to determine their clinical utility (85,86).

Studies in early stage NSCLC have reported an association between vascular endothelial growth factor (VEGF) over-expression and progression or poor survival (87,88), but overall the prognostic role of VEGF expression in NSCLC remains undetermined.

Conclusions

Currently, tumor stage remains the strongest predictor of survival in NSCLC. Early-stage patients are treated primarily by surgical resection. However, 30% to 55% of these patients develop recurrence and die of their disease. The current staging system is inadequate for predicting the outcome of treatment and the prognosis in individual patients. For this reason there is an urgent need to search for new individual biomarkers and gene signatures in the tumor tissue. Many studies have investigated several molecular alterations in early lung cancer and their predictive and prognostic implications are still a matter of clinical research and even for the most promising are not yet ready for prime time application. Currently, randomized clinical trials are exploring the real value of these new diagnostic tools.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
2. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005;352:2589-97.
3. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719-27.
4. Arriagada R, Bergman B, Dunant A, et al. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351-60.
5. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
6. NSCLC Meta-analyses Collaborative Group, Arriagada R, Auperin A, et al. Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell

lung cancer: two meta-analyses of individual patient data. Lancet 2010;375:1267-77.
7. NCCN Clinical practice guidelines in oncologyTM. Non Small Cell Lung Cancer.V.1.2011. Available online: http://www.nccn.org/professionals/physician gls/PDF.nscl.pdf
8. Vansteenkiste J, De Ruysscher D, Eberhardt WEE, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;00:1-10.
9. Pisters KM, Evans WK, Azzoli CG, et al. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non small-cell lung cancer guideline. J Clin Oncol 2007;25:5506-18.
10. Lau CL, D'Amico DA, Harpole DH Jr. Clinical and molecular prognostic factors and models for non-small cell lung cancer. In: Pass HI, Mitchell JB, Johnson DH, et al. eds. Lung Cancer Principles and Practice (ed 2). Philadelphia, PA, Lippincott Williams & Wilkins 2000:602-11.
11. Graziano SL. Non-small cell lung cancer: clinical value of new biological predictors. Lung Cancer 1997;17:S37-S58.
12. Takise A, Kodama T, Shimosato Y, et al. Histopathologic prognostic factors in adenocarcinomas of the peripheral lung less than 2 cm in diameter. Cancer 1988;61:2083-8.
13. Pastorino U, Andreola S, Tagliabue E, et al. Immunocytochemical markers in stage I lung cancer: relevance to prognosis. J Clin Oncol 1997;15:2858-65.
14. Altaha R, Liang X, Yu JJ, et al. Excision repair cross complementing-group 1: gene expression and platinum resistance. Int J Mol Med 2004;14:959-70.
15. Metro G, Zheng Z, Fabi A, et al. In situ protein expression of RRM1, ERCC1, and BRCA1 in metastatic breast cancer patients treated with gemcitabine-based chemotherapy. Cancer Invest 2010;28:172-80.
16. Doll CM, Prystajecky M, Eliasziw M, et al. Low ERCC1 mRNA and protein expression are associated with worse survival in cervical cancer patients treated with radiation alone. Radiother Oncol 2010;97:352-9.
17. De Castro G Jr, Pasini FS, Siqueira SA, et al. ERCC1 protein, mRNA expression and T19007C polymorphism as prognostic markers in head and neck squamous cell carcinoma patients treated with surgery and adjuvant cisplatin-based chemoradiation. Oncol Rep 2011;25:693-9.
18. Simon GR, Sharma S, Cantor A, et al. ERCC1 expression is a predictor of survival in resected patients with non-small cell lung cancer. Chest 2005;127:978-83.
19. Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. Clin Cancer Res 2002;8:2286-91.
20. Ceppi P, Volante M, Novello S, et al. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. Ann Oncol 2006;17:1818-25.
21. Arriagada R, Dunant A, Pignon JP, et al. Long-term results of the international adjuvant lung cancer trial evaluating adjuvant Cisplatin-based chemotherapy in resected lung cancer. J Clin Oncol 2010;28:35-42.
22. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983-91.
23. Bepler G, Olaussen KA, Vataire AL, et al. ERCC1 and RRM1 in the international adjuvant lung trial by automated quantitative in situ analysis. Am J Pathol 2011;178:69-78.
24. Pierceall WE, Olaussen KA, Rousseau V, et al. Cisplatin benefit is predicted by immunohistochemical analysis of DNA repair proteins in squamous cell carcinoma but not adenocarcinoma: theranostic modeling by NSCLC constituent histological subclasses. Ann Oncol 2012;23:2245-52.
25. Niedernhofer LJ, Bhagwat N, Wood RD. ERCC1 and non-small-cell lung cancer. N Engl J Med 2007;356:2538-40; author reply 2540-1.
26. Ma D, Baruch D, Shu Y, et al. Using protein microarray technology to screen anti-ERCC1 monoclonal antibodies for specificity and applications in pathology. BMC Biotechnol 2012;12:88.
27. Friboulet L, Olaussen KA, Pignon JP, et al. ERCC1 isoform expression and DNA repair in non-small-cell lung cancer. N Engl J Med 2013;368:1101-10.
28. Marra A, Kemming D, Krueer, et al. ERCC1 as a predictor of response to induction therapy for stage III non-small cell lung cancer. J Clin Oncol 2013;31:abstr e18511.
29. Kamal NS, Soria JC, Mendiboure J, et al. MutS homologue 2 and the long-term benefit of adjuvant chemotherapy in lung cancer. Clin Cancer Res 2010;16:1206-15.
30. Deng CX. BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. Nucleic Acids Res 2006;34:1416-26.
31. Rosell R, Skrzypski M, Jassem E, et al. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. PLoS One 2007;2:e1129.

32. Taron M, Rosell R, Felip E, et al. BRCA1 mRNA expression levels as an indicator of chemoresistance in lung cancer. Hum Mol Genet 2004;13:2443-9.
33. Rosell R, Perez-Roca L, Sanchez JJ, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. PLoS One 2009;4:e5133.
34. Cobo M, Massuti B, Moran T, et al. Spanish customized adjuvant trial (SCAT) based on BRCA1 mRNA levels. J Clin Oncol 2008;26;abstr 7533.
35. Yang Y, Xie Y, Xian L. Breast cancer susceptibility gene 1 (BRCA1) predict clinical outcome in platinum- and toxal-based chemotherapy in non-small-cell lung cancer (NSCLC) patients: a system review and meta-analysis. J Exp Clin Cancer Res 2013;32:15.
36. Rosell R, Felip E, Taron M, et al. Gene expression as a predictive marker of outcome in stage IIB-IIIA-IIIB non-small cell lung cancer after induction gemcitabine-based chemotherapy followed by resectional surgery. Clin Cancer Res 2004;10:4215s-4219s.
37. Zhang GB, Chen J, Wang LR, et al. RRM1 and ERCC1 expression in peripheral blood versus tumor tissue in gemcitabine/carboplatin-treated advanced non-small cell lung cancer. Cancer Chemother Pharmacol 2012;69:1277-87.
38. Gandara DR, Grimminger PP, Mack PC, et al. Histology- and gender-related associations of ERCC1, RRM1, and TS biomarkers in 1,802 patients with NSCLC: Implications for therapy. J Clin Oncol 2010;28:7513.
39. Smith P, Zhou B, Ho N, et al. 2.6 A X-ray crystal structure of human p53R2, a p53-inducible ribonucleotide reductase. Biochemistry 2009;48:11134-41.
40. Rosell R, Danenberg KD, Alberola V, et al. Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. Clin Cancer Res 2004;10:1318-25.
41. Gautam A, Li ZR, Bepler G. RRM1-induced metastasis suppression through PTEN-regulated pathways. Oncogene 2003;22:2135-42.
42. Bepler G, Sharma S, Cantor A, et al. RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. J Clin Oncol 2004;22:1878-85.
43. Zheng Z, Chen T, Li X, et al. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. N Engl J Med 2007;356:800-8.
44. Fairman JW, Wijerathna SR, Ahmad MF, et al. Structural basis for allosteric regulation of human ribonucleotide reductase by nucleotide-induced oligomerization. Nat Struct Mol Biol 2011;18:316-22.
45. Bepler G, Kusmartseva I, Sharma S, et al. RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. J Clin Oncol 2006;24:4731-7.
46. Bepler G, Sommers KE, Cantor A, et al. Clinical efficacy and predictive molecular markers of neoadjuvant gemcitabine and pemetrexed in resectable non-small cell lung cancer. J Thorac Oncol 2008;3:1112-8.
47. Reynolds C, Obasaju C, Schell MJ, et al. Randomized phase III trial of gemcitabine-based chemotherapy with in situ RRM1 and ERCC1 protein levels for response prediction in non-small-cell lung cancer. J Clin Oncol 2009;27:5808-15.
48. Gong W, Zhang X, Wu J, et al. RRM1 expression and clinical outcome of gemcitabine-containing chemotherapy for advanced non-small-cell lung cancer: a meta-analysis. Lung Cancer 2012;75:374-80.
49. Johnston PG, Lenz HJ, Leichman CG, et al. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res 1995;55:1407-12.
50. Leichman CG, Lenz HJ, Leichman L, et al. Quantification of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. J Clin Oncol 1997;15:3223-3229.
51. Shirota Y, Stoehlmacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 2001;19:4298-304.
52. Scagliotti G, Hanna N, Fossella F, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two Phase III studies. Oncologist 2009;14:253-63.
53. Ceppi P, Volante M, Saviozzi S, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 2006;107:1589-96.
54. Maus MK, Mack PC, Astrow SH, et al. Histology-related associations of ERCC1, RRM1, and TS biomarkers in patients with non-small-cell lung cancer: implications for therapy. J Thorac Oncol 2013;8:582-6.
55. Shintani Y, Ohta M, Hirabayashi H, et al. New prognostic indicator for non-small-cell lung cancer, quantitation of thymidylate synthase by real-time reverse transcription

polymerase chain reaction. Int J Cancer 2003;104:790-5.
56. Zheng Z, Li X, Schell MJ, et al. Thymidylate synthase in situ protein expression and survival in stage I nonsmall-cell lung cancer. Cancer 2008;112:2765-73.
57. Novello S, Manegold C, Grohe C, et al. International tailored chemotherapy adjuvant trial: Itaca trial. J Clin Oncol 2012;30:abstr TPS7109.
58. Rekhtman N, Azzoli CG, Kris MG, et al. Patterns of co-expression of ERCC1 and P27 in resected non-small cell lung cancer by immunohistochemistry. J Clin Oncol 2008;26;abstr 7595.
59. Filipits M, Pirker R, Dunant A, et al. Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program. J Clin Oncol 2007;25:2735-40.
60. Ko E, Kim Y, Park SE, et al. Reduced expression of cyclin D2 is associated with poor recurrence-free survival independent of cyclin D1 in stage III non-small cell lung cancer. Lung Cancer 2012;77:401-6.
61. Dumontet C, Isaac S, Souquet PJ, et al. Expression of class III beta tubulin in non-small cell lung cancer is correlated with resistance to taxane chemotherapy. Bull Cancer 2005;92:E25-30.
62. Sève P, Mackey J, Isaac S, et al. Class III beta-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel. Mol Cancer Ther 2005;4:2001-7.
63. Sève P, Lai R, Ding K, et al. Class III beta-tubulin expression and benefit from adjuvant cisplatin/vinorelbine chemotherapy in operable non-small cell lung cancer: analysis of NCIC JBR.10. Clin Cancer Res 2007;13:994-9.
64. Reiman T, Seve P, Vataire A, et al. Prognostic value of class III B-tubulin (Tubb3) in operable non-small cell lung cancer (NSCLC) and predictive value for adjuvant cisplatin-based chemotherapy (CT): A validation study on three randomized trials. J Clin Oncol 2008;26;abstr 7506.
65. Zalcman G, Levallet G, Bergot E, et al. Evaluation of class III beta-tubulin (bTubIII) expression as a prognostic marker in patients with resectable non-small cell lung cancer (NSCLC) treated by perioperative chemotherapy (CT) in the phase III trial IFCT-0002. J Clin Oncol 2009;27:abstr 7526.
66. Kosaka T, Yatabe Y, Onozato R, et al. Prognostic implication of EGFR, KRAS, and TP53 gene mutations in a large cohort of Japanese patients with surgically treated lung adenocarcinoma. J Thorac Oncol 2009;4:22-9.
67. Tsao MS, Aviel-Ronen S, Ding K, et al. Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. J Clin Oncol 2007;25:5240-7.
68. Capelletti M, Wang XF, Gu L, et al. Impact of KRAS mutations on adjuvant carboplatin/paclitaxel in surgically resected stage IBm NSCLC: CALGB 9633. J Clin Oncol 2010;28;abstr 7008.
69. Scagliotti GV, Fossati R, Torri V, et al. Randomized study of adjuvant chemotherapy for completely resected stage I, II, or IIIA non-small-cell Lung cancer. J Natl Cancer Inst 2003;95:1453-61.
70. Greenblatt MS, Bennett WP, Hollstein M, et al. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994;54:4855-78.
71. Schiller JH, Adak S, Feins RH, et al. Lack of prognostic significance of p53 and K-ras mutations in primary resected non-small-cell lung cancer on E4592: a Laboratory Ancillary Study on an Eastern Cooperative Oncology Group Prospective Randomized Trial of Postoperative Adjuvant Therapy. J Clin Oncol 2001;19:448-57.
72. Zhu CQ, Pintilie M, John T, et al. Understanding prognostic gene expression signatures in lung cancer. Clin Lung Cancer 2009;10:331-40.
73. Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma, Shedden K, Taylor JM, et al. Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. Nat Med 2008;14:822-7.
74. Liu R, Wang X, Chen GY, et al. The prognostic role of a gene signature from tumorigenic breast-cancer cells. N Engl J Med 2007;356:217-26.
75. Wan YW, Qian Y, Rathnagiriswaran S, et al. A breast cancer prognostic signature predicts clinical outcomes in multiple tumor types. Oncol Rep 2010;24:489-94.
76. Chen DT, Hsu YL, Fulp WJ, et al. Prognostic and predictive value of a malignancy-risk gene signature in early-stage non-small cell lung cancer. J Natl Cancer Inst 2011;103:1859-70.
77. Xu W, Banerji S, Davie JR, et al. Yin Yang gene expression ratio signature for lung cancer prognosis. PLoS One 2013;8:e68742.
78. Kratz JR, He J, Van Den Eeden SK, et al. A practical molecular assay to predict survival in resected non-squamous, non-small-cell lung cancer: development and international validation studies. Lancet 2012;379:823-32.
79. Dziadziuszko R, Merrick DT, Witta SE, et al. Insulin-like growth factor receptor 1 (IGF1R) gene copy number is associated with survival in operable non-small-cell lung

cancer: a comparison between IGF1R fluorescent in situ hybridization, protein expression, and mRNA expression. J Clin Oncol 2010;28:2174-80.
80. Ludovini V, Flacco A, Bianconi F, et al. Concomitant high gene copy number and protein overexpression of IGF1R and EGFR negatively affect disease-free survival of surgically resected non-small-cell-lung cancer patients. Cancer Chemother Pharmacol 2013;71:671-80.
81. Go H, Jeon YK, Park HJ, et al. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol 2010;5:305-13.
82. Xia Q, Zhu Z, Wang J, et al. Expression and association of HER2 with prognosis in early-stage (T1-T2N0M0) non-small cell lung cancer. Tumour Biol 2012;33:1719-25.
83. Iwakiri S, Mino N, Takahashi T, et al. Higher expression of chemokine receptor CXCR7 is linked to early and metastatic recurrence in pathological stage I nonsmall cell lung cancer. Cancer 2009;115:2580-93.
84. Spano JP, Andre F, Morat L, et al. Chemokine receptor CXCR4 and early-stage non-small cell lung cancer: pattern of expression and correlation with outcome. Ann Oncol 2004;15:613-7.
85. Lokk K, Vooder T, Kolde R, et al. Methylation markers of early-stage non-small cell lung cancer. PLoS One 2012;7:e39813.
86. de Fraipont F, Levallet G, Creveuil C, et al. An apoptosis methylation prognostic signature for early lung cancer in the IFCT-0002 trial. Clin Cancer Res 2012;18:2976-86.
87. Liao M, Wang H, Lin Z, et al. Vascular endothelial growth factor and other biological predictors related to the postoperative survival rate on non-small cell lung cancer. Lung Cancer 2001;33:125-32.
88. Baillie R, Carlile J, Pendleton N, et al. Prognostic value of vascularity and vascular endothelial growth factor expression in non-small cell lung cancer. J Clin Pathol 2001;54:116-20.

Cite this article as: Carnio S, Novello S, Papotti M, Loiacono M, Scagliotti GV. Prognostic and predictive biomarkers in early stage non small-cell lung cancer: tumor based approaches including gene signatures. Transl Lung Cancer Res 2013;2(5):372-381. doi: 10.3978/j.issn.2218-6751.2013.10.05

Prognostic and predictive biomarkers in early stage NSCLC: CTCs and serum/plasma markers

Philip A. J. Crosbie[1,2], Rajesh Shah[3], Yvonne Summers[4,5], Caroline Dive[2], Fiona Blackhall[2,5]

[1]North West Lung Centre, University Hospital of South Manchester NHS Foundation Trust, University of Manchester, Manchester Academic Health Science Centre, Wythenshawe, M23 9LT, UK; [2]Clinical and Experimental Pharmacology Group, Paterson Institute for Cancer Research, University of Manchester, M20 4BX, UK; [3]Department of Thoracic Surgery, [4]Pulmonary Oncology Unit, University Hospital of South Manchester NHS Foundation Trust, Wythenshawe, M23 9LT, UK; [5]Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, M20 4BX, UK

Correspondence to: Dr. Fiona Blackhall. Department of Medical Oncology, The Christie NHS Foundation Trust, Wilmslow Road, Manchester, M20 4BX, UK. Email: fiona.blackhall@christie.nhs.uk.

Abstract: Resection of early stage non-small cell lung cancer (NSCLC) offers patients the best hope of cure, however recurrence rates post-resection remain high suggesting the presence of micro-metastatic disease at the time of surgery undetected by standard staging methods. A critical step in the metastatic cascade is the entry of tumor cells into the circulation enabling their distribution to and seeding of distant organs. This review explores the evidence for predictive and prognostic circulating biomarkers in the early stage NSCLC population. We summarize studies that have explored a variety of targets including circulating proteins, nucleic acids and more recently circulating tumor cells (CTCs) as potentially clinically relevant biomarkers in the early stage setting. Circulating biomarkers may add clinically relevant information about the biological behavior of tumors over and above that provided by pathological staging. Improvement in the stratification of patients according to the likelihood of metastatic relapse after radical treatments such as surgical resection could allow more effective targeting of systemic therapies such as adjuvant chemotherapy.

Keywords: Early stage; non-small cell lung cancer (NSCLC); prognostic; predictive; circulating; biomarker

Submitted Sep 08, 2013. Accepted for publication Sep 27, 2013.
doi: 10.3978/j.issn.2218-6751.2013.09.02
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.09.02

Introduction

Lung cancer is the most common cause of cancer related mortality in the world, responsible for 1.4 million deaths/year (1). A striking feature of lung cancer is its poor survival, with 5-year survival less than 10% in the UK and less than 15% in the USA. One major contributing factor to poor survival is the late clinical presentation of the majority of patients, 80% present with locally advanced or distant metastatic disease at which stage treatments are generally much less effective. The benefit of early diagnosis was underlined by the National Lung Screening Trial which investigated the effect of low dose CT screening in an at risk population and reported a 20% reduction in lung cancer specific mortality (2). However, achieving long-term survival even after curative intent surgery is a major challenge with recurrence occurring in 50% of cases overall and five-year survival rates of 58% to 73% for stage I, 36% to 46% for stage II and 24% for stage IIIA reported (3). Recurrence most commonly occurs at distant sites indicating the presence of micro-metastatic disease undetected by current staging strategies (4,5). Trials of platinum based adjuvant chemotherapy to treat micro-metastases have shown increases in 5-year survival in the order of 5% in patients with stage II-IIIA disease (6). The adjuvant treatment of stage I is more controversial as definitive evidence of efficacy is lacking (7). There is therefore a pressing clinical need to develop both more effective adjuvant therapy but also target the current therapies in a more effective

manner. Pathological stage is the most robust methodology of selecting patients for adjuvant chemotherapy however recurrence rates even in stage I disease are in the order of 25% to 40% suggesting that an additional marker/markers that enable accurate stratification of recurrence risk over and above that provided by pathological stage are necessary for more accurate prognostication. In such a way it may be possible to stratify high-risk stage I patients who may benefit from adjuvant chemotherapy and low risk stage II who may avoid chemotherapy. Furthermore, lung cancer is a heterogenous disease and the molecular profile and/or biological behavior of disease recurring after surgery may be different to that of the primary tumor. Consequently, there may be added benefit of examining circulating factors, which may reflect the behavior and molecular profile of metastatic disease more accurately than primary tumor sampling, which could result in sampling error because of tumor heterogeneity.

Method

A literature search was performed using PubMed/Medline. Search limits set included human studies, 1990 to the present day (June 2013) and articles written in English. Initial searches using the terms: circulating biomarkers, non-small cell lung cancer (NSCLC) and prognostic/predictive were followed by more targeted searches. For circulating tumor cells (CTCs) additional search terms included: CTCs, CellSearch (CS), ISET and pulmonary vein. Nucleic acid searches used additionally: circulating nucleic acids, or DNA, or RNA or microRNA and protein searches included specific proteins including: carcinoembryonic antigen (CEA), or CYFRA 21-1, or neuron specific enolase and other identified proteins. Finally reference lists were screened for additional studies.

Results

Standard clinical measures in blood

Standard biochemical and hematological measures, taken routinely as part of the assessment for radical treatment, may in themselves provide additional prognostic information. The results of several trials are discussed below.

Hemoglobin

Tomita *et al.* studied 240 patients who underwent surgical resection of NSCLC (8). Classification of low hemoglobin level was <13.0 g/dL in men and <12.0 g/dL in women. Overall, 88 patients (36.7%) were classified as having a low pre-operative hemoglobin. Five-year survival was significantly lower in patients with a low hemoglobin (43.0% *vs.* 73.5%; P<0.0001). After multivariate analysis pre-operative hemoglobin level remained a significant poor prognostic factor.

White blood cells

The impact of peripheral pre-operative white blood cell count has been investigated in several reports (9-12). A total white blood cell count above the median has been reported to be a poor prognostic factor (9). Kobayashi *et al*. examined the outcomes of 237 patients with resected node negative NSCLC (10). Raised neutrophil count and low lymphocyte count were associated with survival after univariate analysis; however only a low lymphocyte count remained an indicator of poor prognosis after multivariate analysis. Similarly Zhang *et al*. reported elevation of peripheral lymphocytes to be a favorable prognostic factor (11). Sarraf *et al*. examined the ratio of neutrophil and lymphocyte counts as a prognostic marker. This measure was significantly associated with stage, however after multivariate analysis the ratio remained an independent prognostic factor (12).

Platelets

A study of 510 patients by Yu *et al*. examined pre-operative platelet count and outcomes in patients newly diagnosed with NSCLC (13). All patients were treated with surgical resection [clinical stage I n=234 (45.9%), stage II n=128 (25.1%) and stage III n=148 (29.0%)]. Three-year overall survival (OS) was 75.3% for patients with a normal count ($\leq 300\times10^9$/L, n=449; 88.0%) and 59.2% for those with an elevated count ($>300\times10^9$/L, n=61; 12.0%); the risk of disease progression was also increased (HR 1.57, 1.02-2.45). Multivariate analysis showed age and platelet count to be the only independent prognostic markers. Tomita *et al*. also reported that pre-operative thrombocytosis ($>40\times10^4$/mm^3) was a poor prognostic marker in a study of 289 patients undergoing resection for NSCLC. Five-year survival was 30.8% in patients with elevated platelets (n=13/289) compared with 68.7% in patients with a normal count (276/289) (14).

These studies suggest that routinely measured clinical parameters in blood may provide prognostic information. The advantages of this approach are that no additional cost is required for the assays and the assays themselves are

robust. The disadvantages are that measured changes are not necessarily specific to the cancer but may be reflective of other co-morbidities or co-existing acute illnesses such as infection.

Circulating proteins

Historically circulating proteins have been most intensely investigated as prognostic biomarkers in the early lung cancer setting. The most commonly investigated proteins are CEA and CYFRA 21-1 and these studies are reviewed below.

CEA

CEA is a cell adhesion glycoprotein that is expressed in a limited number of tissues and at very low levels in healthy individuals (15). A large number of studies over the past two decades have examined the prognostic value of CEA in serum/plasma in early stage NSCLC [reviewed (16) and summarized in *Table 1*]. A majority of studies have found elevated levels of CEA to be associated with poor prognosis in resected NSCLC including stage I (17-21,23,27,28,30,32-35,37,40-47). A limited number of studies have reported no association (25,29,31,38,48,49). The largest study by Okada *et al*. examined pre and post-operative serum CEA levels in 1,000 patients undergoing resection for NSCLC (33). Patients with elevated levels of pre-operative CEA (368/1,000) had significantly lower 5-year survival compared to patients with normal levels (53.8% *vs*. 75.2%; P<0.0001). In patients with resected pathological stage I disease, those with persistently elevated post-operative CEA levels had significantly worse 5-year survival (48.6%) than patients whose CEA level normalized post-operatively (74.2%) and those who had normal pre-operative levels (84.2%) (34). Several studies have confirmed the association with persistently elevated CEA in the post-operative period and marked reduction in survival (17,18,30,33,39). As an example, Kosu *et al*. retrospectively analyzed pre and post plasma CEA levels in 263 patients with resected pathological stage I NSCLC (18). A majority had adenocarcinoma and half the population had less than 5 pack years smoking history. Patients with normal CEA before and after surgery had a 5-year survival of 95.5% compared with 85.5% (4-year survival) in patients with a pre-operative high CEA that normalized post-operatively and 59.3% in patients whose CEA was elevated both pre and post-operatively. After multivariate analysis tumor diameter greater than 30 mm, the presence of visceral pleural invasion and persistent CEA elevation were independent poor prognostic indicators. The authors conclude that patients with high post-operative CEA may benefit from adjuvant chemotherapy.

One important aspect of many of the studies investigating CEA as a prognostic marker is that the prevalence of smoking is relatively low with upwards of half the lung cancer cases being diagnosed in never smokers. Significantly smoking may increase CEA expression in lung tissue (50). In the study by Okada *et al*. CEA measurement was not prognostic in current smokers potentially reducing the clinical use of CEA in populations with a higher burden of smoking induced lung carcinogenesis (34). In a majority of studies it is also unclear what role co-existing inflammatory conditions such as chronic obstructive pulmonary disease may have in modifying CEA measurements.

CYFRA 21-1 (serum cytokeratin 19 fragment)

Cytokeratins are filamentous proteins ubiquitously expressed by epithelial cells which can be used as markers of epithelial origin (51). Cytokeratin 7, 8, 18 and 19 are the most commonly seen types associated with carcinomas and these proteins can leak into the circulation and be detected as degraded complexes and as such may act as circulating biomarkers of malignancy (52,53). Degradation fragments of cytokeratin 19 can be detected in the circulation, referred to as CYFRA 21-1, released from tumor cells by necrosis or cell lysis. This protein fragment has been assessed as a prognostic marker in several studies (25,32,38,45,46,54-58). A meta-analysis of 2,063 patients with newly diagnosed NSCLC showed an elevated pre-treatment CYFRA 21-1 fragment level (>3.6 ng/mL) to be an independent poor prognostic factor at 12 months (HR 1.88, 1.64-2.15) (56). Raised CYFRA 21-1 was of borderline significance in a subpopulation of patients treated with surgical resection (survival HR 1.41, 0.99-2.03; n=437). In a study of 85 patients with squamous cell carcinoma (stages I-IIIA), the risk of death after five years of follow up was doubled in patients with CYFRA 21-1 levels above 3.6 ng/mL (HR 2.05; 1.09-3.83) (54).

Several studies have examined the combined prognostic value of pretreatment CEA and CYFRA 21-1 as a 'tumor marker index' (TMI) (25,32,57). Blankenburg *et al*. reported no prognostic value of either single or combined measures in a study of 240 patients. In contrast, studies by Tomita (57) and Muley (32) both reported improved prognostic value to the combined data. Muley *et al*. reported reduced 3-year survival in 153 stage I patients with raised CYFRA 21-1

Table 1 Summary table of studies that have investigated the prognostic value of circulating CEA levels in patients undergoing radical lung cancer surgery

Author	No. patients	Stage [%]	Pathology[1] [%]	CEA level ng/mL	Smoking status [% NS][2]	CEA result[3] n [%]	Outcome[4]	Prognostic
Kato 2013 (17)	177	I	Ad [80]; Sq [20]	>2.5 Ad; >3.0 Sq	Ad [50]; Sq [3]	Ad: HH 29 [23.1], HN 36 [28.6], N 61 [48.4], Sq: H 15 [41.7]	5 ys DFS: Ad: HH 49.4%, HN 65.1%, N 93.4%, Sq: H 51.9%, N 81.0%	Yes
Kozu 2013 (18)	263	I	Ad [81]; Sq [18]	>3.5	<5 pack yr [49]	N 198 [75.3], HN 44 [16.7], HH 21 [8.0]	5 ys: N 95.5%, HN 85.5%, HH 59.3%	Yes
Lin 2012 (19)	169	IB-IIIA after 2 cycles adj chemo	Ad [37]; Sq [49]	>4.7	–	H 63[37.3], N 98 [58.0]	Median DFS: H 34 *vs.* N 53 months	Yes
Nagashima 2012 (20)	71	I [70]	Sq [100]	>4	SI low [44]	H 19 [26.8], N 52 [73.2]	DFS: H 63.5%, N 88.2%	Yes
Tomita 2012 (21)	197	I [75]; II-III [24]	Ad [80]	>5	–	H 48 [24.3], N 149 [75.6]	5 ys: H 51.1%, N 82.7%	Yes
Hanagiri 2011 (22)	341	I	Ad [77]; Sq [16]	>2.5	–	H 88 [25.8], N 253 [74.2]	5 ys: H 76.3%, L 88.3%	Yes
Takahashi 2011 (23)	649	I [76]; II [10]	Ad [71]; Sq [24]	>3	–	HH 145 [22.3], HN 149 [23.0], N 297 [45.8]	5 ys: HH 51.1%, HN 85.5%, N 78.4%	Yes
Tomita 2009 (24)	220	I [70]; II [15]	Ad [75]	>2.5	NS [43]	–	5 ys all: H 62%, N 79.6%; 5 ys Ad: H 71.1%, N 88.2%	Yes
Blankenburg 2008 (25)	240	I	Ad [38]; Sq [42]	>6.7	–	–	5 ys: H 49% *vs.* N 66%	No
Kashiwabara 2008 (26)	136	I	Ad [100]	>2.5	NS [65]	H 16 [11.8], N 120 [88.2]	DFS: H 43.8%, N 95%	Yes
Matsuguma 2008 (27)	455	I	Ad [69]; Sq [25]	>5	NS [35] FS [25]	HH 20 [4.4], HN 112 [24.6], N 323 [71.0]	5 ys: HH 43.1%, HN 56.2%, N 85.9%	Yes
Hsu 2007 (28)	163-f	I	Ad [83]	>6	NS [93]	H 47 [29], N 115 [71]	5 ys: H 59.6%, N 75.7%	Yes
Kobayashi 2007 (29)	163	I <2 cm	Ad [83]	>5.0	NS [48]	H 20 [12.5], N 140 [87.5]	HR 1.03 (0.3-3.1)	No
Matsuoka 2007 (30)	275	I	Ad [70]; Sq [26]	>5	NS [38]	H Ad 51, Sq 13, N Ad 133, Sq 50	5 ys Ad: H 54.6%, N 86.9%; Sq: H 82.1%, N 78.5%	Yes
Mizuguchi 2007 (31)	137	I	Ad [63]; Sq [34]	>7.8	–	–	Not prognostic after multivariate analysis	No
Muley 2004 (32)	153	I	Ad [49]; Sq [39]	>9.8	–	–	3 ys: H 41.6% *vs.* N 79.2%	Yes

Table 1 (*continued*)

Table 1 (*continued*)

Author	No. patients	Stage [%]	Pathology[1] [%]	CEA level ng/mL	Smoking status [% NS][2]	CEA result[3] n [%]	Outcome[4]	Prognostic
Okada 2004 (33)	1,000	I [70]	Ad [69]; Sq [26]	>5	–	HH 126 [12.6], HN 242 [24.2], N 632 [63.2]	5 ys: HH 35.2%, HN 62.6%, N 75.2%	Yes
Okada 2004 (34)	954	I [69]	Ad [73]; Sq [27]	>5	Ad [50]; Sq [8]	smoking increases CEA	5 ys: Ad 83.7/57.4, Sq 76.6/28.0	Yes
Sakao 2004 (35)	100		Ad [100]	>5	–	H 24 [24], N 76 [76]	DFS: H 33.3%, N 63.2%	Yes
Tomita 2004 (36)	313	I-II [70]	Ad [70]; Sq [30]	>5	–	–	5 ys: Ad H 42.5%, N 77.6%; Sq H 57.8% N 63.3	Ad-yes, Sq-no
Buccheri 2003 (37)	118	I [57]; II [14]	Ad [49]; Sq [40]	>10	–	H [55]	Stage I + II 1yr rec: H 67%, N 12%	Yes
Reinmuth 2002 (38)	67	I [64]; II-III [36]	Ad [25]; Sq [46]; Lg [24]	>5	–	–	No significant difference in survival	No
Sawabata 2002 (39)	297	I	–	>7	–	HH 15 [5.1%], HN 41 [13.9%] Ad, N 241 [81%]	5 ys: HH 18%, HN 68%, N 72%	Yes
Hotta 2000 (40)	39	I	Ad [67]; Sq [28]	>6.7	SI low [49]	H 9 [23.1], N 30 [76.9]	H: Lower OS + DFS	Yes
Suzuki 1999 (41)	365	I	Ad [100]	>5	<5 pack yr [40]	H 89, N 180	5 ys: H 53%, N 78.5%	Yes
Rubins 1998 (42)	50	I [56]; II [14]; III [30]	Ad [42]; Sq [44]	variable	–	–	Reduced survival with increasing CEA level	Yes
Icard 1994 (43)	152	I [34]; II [23]; IIIa [36]	Ad [43]; Sq [28]	all >10; Int 10-30; H >30	–	Stage I: Int 35, H 7; stage II: Int 17, H 12; stage IIIa: Int 28, H 17	5 ys: stage I: H 0%, Int 40%; stage II: H O%, Int 44%; stage IIIa: H 9%, Int 0%	Yes

[1]Ad, adenocarcinoma; Sq, squamous cell carcinoma; Lg, large cell carcinoma; [2]NS, non-smoker; FS, former smoker; SI, smoking index; [3]H, high preoperatively; HH, high pre and post-operatively; N, normal pre-operatively; HN, high pre-operatively and normal post-operatively; Int, intermediate; [4]abbreviations as per [3], DFS, disease free survival; OS, overall survival; 5 ys, 5-year survival.

baseline levels prior to surgical resection (3-year survival: high 60.2% *vs.* normal 78.4%). Patients were categorized according to high, intermediate and low levels of geometric mean CEA and CYFRA 21-1 and 3-year survival analyzed. Survival was 96.7% in patients with low levels, 77.2% in the intermediate group and 55.7% in patients with high levels. Tomita *et al*. reported reduced 5-year survival in patients with elevated pre-treatment CYFRA 21-1 (5-year survival: high 40% *vs.* normal 67%), in a cohort of 291 patients of stage I-III. An analysis of 5-year survival by TMI, defined as positive by the presence of elevated CYFRA 21-1 and or CEA, showed reduced survival with TMI positive patients (37.1%) compared to TMI negative patients (72.3%).

A study by Mizuguchi *et al.* measured Sialyl Lewis^x (an important cell surface carbohydrate antigen) in addition to CYFRA 21-1 and CEA pre-operatively in 137 patients with completely resected stage I NSCLC (31). Thirty of the patients recurred and after multivariate analysis pre-operative CYFRA 21-1 and SXL levels, but not CEA, were independent prognostic markers. Patients with high

Table 2 Summary table of studies to have combined CYFRA 21-1 and CEA/SLX to determine a tumor measure index (TMI)

Author	No. patients	Stage [%]	Pathology[1] [%]	Measure	Level ng/mL	Smoking status (% NS)[2]	Result[3] n [%]	Outcome[4]	Prognostic
Tomita 2010 (57)	291	I [64], II-III [36]	Ad [72]	CYFRA 21-1	>2.4	–	H 58, N 233	5 ys: H 40%, N 67%	Yes
				CEA + CYFRA = TMI	<1= both N, >1=1or 2 H		<1=202, >1=89	5 ys: <1 72.3%, >1 37.1%	Yes
Blankenburg 2008 (25)	240	I	Ad [38], Sq [42]	CYFRA 21-1	>3.3	–	–	5 ys: H 64% N 64%	No
				CEA + CYFRA = TMI	Geometric mean		H 71 [30], Int 87 [36], N 82 [34]	5 ys: H 63.1%, Int 63.5%, N 65.1%	No
Mizuguchi 2007 (31)	137	I	Ad [63], Sq [34]	CYFRA 21-1	>3.2	–	–	OS: HR 3.5 (2.9-4.1)	Yes
				SLX	>36 U/mL			OS: HR 4.1 (3.4-4.8)	Yes
				SLX + CYFRA	<1= both N, 1=1H +1N, >1= both H		>1 [6], 1 [31], <1 [63]	5 ys: >1-13%, 1-52%, <1-80%	Yes
Muley 2004 (32)	153	I	Ad [49], Sq [39]	CYFRA 21-1	>3.3	–	H [21], N [79]	3 ys: H 60.2% *vs.* N 78.4%	Yes
				NSE	>14.5		–	Not related to outcome	No
				CEA + CYFRA = TMI	Geometric mean		H 49 [23], Int 60 [42], N 32 [35]	3 ys: H 55.7%, Int 77.2%, N 96.7%	Yes

[1]Ad, adenocarcinoma; Sq, squamous cell carcinoma; Lg, large cell carcinoma; [2]NS, non-smoker; FS, former smoker; SI, smoking index; [3]H, high preoperatively; HH, high pre and post-operatively; N, normal pre-operatively; HN, high pre-operatively and normal post-operatively; Int, intermediate; [4]abbreviations as per [3], DFS, disease free survival; OS, overall survival; 5 ys, 5-year survival.

levels of both markers were five times more likely to recur than those with normal values (*Table 2*).

Neuron specific enolase

Enolases are integral to the glycolysis pathway by linking 2-phosphoglycerate and phosphor-enolpyruvate metabolism. Neuron specific enolase has been associated with small cell lung cancer as a clinical biomarker (59). However several studies have examined NSE as a circulating biomarker in NSCLC (38,49,54,55,60,61). In three of the studies NSE was not prognostic (38,49), including a study of 164 patients with resected stage I disease (60). Two studies reported an association with poor prognosis in advanced disease (54,55). However, the largest study to date by Yu *et al.* looked at 481 patients with operable NSCLC and measured pre-operative serum levels of NSE, SCC and carbohydrate antigen 125 (CA125) (61). Both elevated levels of NSE and CA125 were associated with reduced disease free survival (DFS) and OS. In a multivariable Cox regression model advanced clinical stage and both elevation of CA125 and NSE were independent prognostic factors associated with reduced survival. Elevation of more than one circulating biomarker was associated with a worse outcome.

C-reactive protein (CRP)

Elevated CRP has been associated with poorer outcomes after surgery in two studies (62,63). O'Dowd *et al.* reported elevated CRP but not total white cell count to be an independent prognostic factor after multivariate analysis (63). Median survival was 75.9 months in the normal CRP group

and 26.2 months in the high CRP group. Similarly Hara *et al.* showed reduction in 5-year disease specific (56.2% *vs.* 77.6%; P=0.003) and OS (50.2% *vs.* 74.2%; P=0.001) in the high CRP arm.

Fibrinogen

Sheng *et al.* report the prognostic significance of fibrinogen, an important protein in the clotting cascade, in a study of patients with operable NSCLC (n=567; 69.3% were stage I + II) (64). Normal serum fibrinogen levels (<4 g/L) were recorded in 343 patients (60.4%) compared with 224 patients (39.5%) with elevated fibrinogen levels (>4 g/L). Patients with higher baseline fibrinogen levels had lower 3-year progression-free survival (49.2% *vs.* 63.3%) and lower OS rates (66.0% *vs.* 80.9%) than patients with normal serum fibrinogen concentrations. Fibrinogen level was an independent poor prognostic marker after Cox proportional regression analysis.

Other circulating proteins

Squamous cell carcinoma antigen has been investigated in several studies without showing prognostic significance (31,54,60,65). CA125 is a standard clinical measure used in the diagnosis and monitoring of patients with ovarian cancer, but its prognostic potential has also been investigated in a small number of studies looking at patients undergoing curative resection of NSCLC. Three studies have reported elevation of pre-operative CA125 to be associated with a worse prognosis (65-67) with one study reporting no association (60).

Despite the multiple studies of circulating proteins described above none have proved sufficiently robust to incorporate into routine practice either to alter the intensity of follow up or direct adjuvant therapy.

Circulating nucleic acids

Circulating DNA can be detected in healthy individuals and in significantly higher concentrations in patients with cancer (68). Tumor specific RNA and DNA can enter the circulation through processes including necrosis and apoptosis from both primary and metastatic sites and may also be detected in plasma/serum (69).

Total circulating free DNA

Total circulating plasma DNA concentration was investigated as a potential biomarker in a population of 1,035 heavy smokers over the age of 50 who were taking part in an annual low dose CT screening study (70). A total of 38 patients were diagnosed with lung cancer during the study period. Study participation also included annual blood tests and no difference in median DNA concentration was seen between cases and controls at baseline. However, in 33 lung cancer cases who went on to have surgical resection, and who also had repeat blood samples, median DNA concentration was significantly higher within the 12-month period immediately prior to diagnosis and surgery (4.6 ng/mL) compared with samples taken over a year prior to diagnosis (2.4 ng/mL). This finding suggests that longitudinal changes in DNA concentration may predict for the development of malignancy in smokers rather than a fixed threshold concentration. In addition, five-year survival was noted to be significantly worse in patients with DNA concentrations in the highest tertile (33%) when compared to the lowest tertile. A similar association with outcome was reported by van der Drift *et al.*, in a study of 46 patients with newly diagnosed NSCLC (29/46 were stage I to III). Significantly reduced survival was seen in patients with DNA concentrations in the highest tertile (median survival 11.8 months) compared to the lowest tertile (median survival 21.5 months) (71).

Conversely, baseline circulating free DNA concentration was not correlated with prognosis, either DFS or OS, in a study of 76 patients undergoing curative resection (stage I-II n=60, stage III n=16) (72). However in patients who relapsed early (within 3 months; n=9) increasing circulating DNA concentration was seen in a repeat blood sample taken 3 months post-operatively. By comparison patients who did not relapse showed a reduction in DNA concentration by the same time point. A similar finding was reported by Szpechcinski *et al.* who demonstrated reduction (n=11) or stability (n=7) in circulating DNA levels in patients who had undergone resection of NSCLC between 3 to 6 months post-surgery except for 2 individuals with early relapse whose DNA concentrations increased significantly (73). On this basis cfDNA might have an application to monitor patients for relapse prior to symptom development or radiological change and thereby inform on the intensity of follow up.

Circulating microRNAs

MicroRNAs are small non-coding single stranded RNA molecules that serve a regulatory purpose in controlling the function of messenger RNA (74,75). Tissue expression of microRNAs has been shown to be prognostic in NSCLC (76). MicroRNAs can also be detected in the

circulation, where they are stable (77), and several studies have examined their prognostic significance in early stage lung cancer. Hu *et al*. developed a panel of 4 miRNAs (high miR-486 and miR30d; low miR1 and miR499) whose differential expression was associated with poor survival (78). Testing of the panel in 243 patients with stage I-IIIa NSCLC treated with both surgery and adjuvant chemotherapy showed the miRNAs to be an independent predictor of survival. Indeed the authors reported a dose effect dependent on how many markers in the panel were affected and increasing risk of cancer death (2 measures: HR 3.14, 1.7-6.0; 3 measures HR 16.5, 8.6-31.7; 4 measures 34.1, 16.3-71.6).

Boeri *et al*. examined the predictive value of miRNAs in plasma taken from two CT screening trials (79). Patients who attended for annual CT screening also had an annual blood test. A panel of 15miRNAs (mir-660, mir-140-5p, mir-451, mir-28-3p, mir-30c, and mir-92a most commonly deregulated) was able to correctly categorize 30 out of 35 patients who developed lung cancer, using plasma taken over 12 months and upwards of 28 months prior to diagnosis. Misclassification occurred in 1 out of 5 control pools. A further signature was developed (containing mir-486-5p) to explore the use of miRNA expression and prognosis at the time of diagnosis. The panel correctly classified 9 out of 11 patients with poor prognosis but misclassified 2 out of 10 patients with good prognosis.

Chen *et al*. examined the prognostic significance of a specific miRNA (miR-17-5p) in the serum of 221 patients newly diagnosed with lung cancer (80). Aberant expression of miR-17-5p has previously been demonstrated in lung cancer and serum miR-17-5p levels were significantly higher in this study than normal controls. Patients were stratified according to expression levels (stage I-III: high expression n=99 and low expression n=109) and survival compared. High expression was associated with a lower median survival of 33 months compared with 40 months. This difference remained after a Cox proportional hazard model (HR 1.8, 1.04-3.01). Reduced survival in patients with stage I disease (n=180) was also reported by Heegaard *et al*. when the population was stratified according to miR-233 levels (reduced levels associated with worse survival) (81). Silva *et al*. demonstrated a reduction in DFS for patients undergoing surgery (n=37) who had low levels of miR-30e-3p (DFS rate at 50 months =13%, 14-52) compared with high levels (DFS rate 50%, 23-77) (82). Measures of plasma let-7f and miR-20b were not associated with DFS. Survival was associated with differential expression of three miRNAs (miR-96, miR-182, and miR-183) in a study of 70 patients with NSCLC treated with surgical resection (76).

Circulating mRNA

Cheng *et al*. explored the prognostic value of relative levels of circulating cMET mRNA in blood from 45 patients undergoing resection using RT-PCR (83). Over-expression of cMET, a proto-oncogene implicated in angiogenesis and metastasis, was recorded in 23 patients and after a mean follow up period of 23 months 18 (78.3%) of these patients had recurred and 8 died. This compared with 4 (18.2%) recurrences and no deaths in the cMET negative group. After multivariate analysis cMET positivity was the strongest predictor of recurrence (HR 3.9, 1.2-13.3). Hepatocyte growth factor (HGF), the ligand for cMET, has been variably associated with outcome in early stage NSCLC; reported to be prognostic in studies with small samples sizes (84,85) but not in a much larger study of 196 patients (86).

CTCs

CTCs or Circulating Tumor Microemboli (CTMs, cells in contiguous groups) are postulated to have a critical role in the development of metastatic disease (87). The detection of CTCs in patients with lung cancer has been described in numerous clinical studies and has in general been associated with a poorer prognosis (88). A number of different methods have been used [reviewed (89)] and the field is rapidly evolving; current methodologies can be broadly divided into assays that physically isolate individual cells/groups of cells for further characterization and nucleic acid based techniques, which infer the presence of CTCs.

Cell based detection

The most robustly developed platform is CellSearch (CS), which is fully validated and approved for clinical use in advanced breast, bowel and prostate cancer. CTC enrichment from blood is performed automatically (CellTracks AutoPrep) through immunomagnetic selection of epithelial cell adhesion molecule (EpCAM) expressing cells, using ferrofluid particles coated with an anti-EpCAM antibody, which are then separated from blood by magnets. Selected cells are then stained with phycoerythrin-conjugated anti-cytokeratin (8,40,44) antibodies (epithelial cell marker), allophycocyanin-conjugated anti-CD45 antibodies (white blood cell marker) and a nuclear stain (4',6-diamidino-2-phenylindole;

DAPI). Analysis is undertaken using a semi-automatic fluorescence microscope (CellTracks Analyser II) and CTCs categorised using morphological (round or oval, visible nucleus in cytoplasm, cells at least 4 μm in diameter) and immunofluorescent criteria (CK^+, $DAPI^+$ and $CD45^-$). The automated processing and semi-automated analysis produce low inter and intra-assay variability (90). Using this method, our group has shown that CTC number is an independent prognostic marker in advanced NSCLC; in univariate analysis, patients ≥5 CTCs had an OS of 4.3 months compared with 8.1 months in those with <5 CTCs (P<0.001). Indeed, CTC number was the strongest predictor of OS (HR 7.9, 2.9-22.0; P<0.001) after multivariate analysis. However, ≥2 CTCs were detected in only 32% of stage IV patients (19/60) and rarely in patients with stage IIIB (7%, 2/27) and not at all in stage IIIA (0%, 0/14) (91). One possible disadvantage of the CS approach is the reliance on EpCAM positive selection. EpCAM expression is commonly found in tumours of epithelial origin (92), but not necessarily on all CTCs (93).

The theory of epithelial to mesenchymal transition proposes that cells develop a more metastatic phenotype, reflected by down regulation of epithelial markers and upregulation of mesenchymal markers, that facilitates migration from the primary tumour into the circulation (94). Selection of CTCs based purely on epithelial markers may underestimate the CTC burden and therefore lower the sensitivity of this approach; this may be especially relevant in NSCLC (95). Selection of CTCs using non-epithelial based markers may therefore have advantages; one example is ISET (Isolation by Size of Epithelial Tumour cells) (96). ISET technology involves the filtering of blood through a membrane with 8 μm pores to isolate cells or groups of cells larger than this size independently of cellular protein expression. Cells may then be characterized morphologically and by protein expression. Using this method in a direct comparison with CS we have shown that ISET identifies significantly more CTCs and CTMs in a study of 40 patients with advanced lung cancer (97). In this study ISET demonstrated CTCs in 80% (32/40) of patients compared with 23% (9/40) using CS; CTCs were detected by both methods in only 7 patients and although CTMs were detected in 38% (15/50) by ISET, CS identified no CTMs.

In the early non-small cell cancer setting, Hofman *et al.* investigated the prognostic value of CTCs detected in peripheral blood drawn from 210 patients prior to surgical resection of NSCLC using both CS and ISET (98,99). Pathological stage was I or II in 62% (131/210) and III or IV in the remainder (38%, 79/210). CTCs were standardly defined (CK^+, $DAPI^+$, $CD45^-$) with CS and with ISET as cells with positive immunocytochemical staining for cytokeratin and/or vimentin with morphological features of non-hematological cells. CTCs were detected in 69% of patients overall [ISET 50% + ve (104/210); CS + ve: ≥1 in 39% (82/210) and ≥2 in 21% (44/210)]. CTC number was not related to disease stage or histological subtype. CTC counts were higher using ISET (mean 34, range, 1 to 23) compared with CS (mean 12, range, 1 to 150). In the 144 patients where CTCs were detected only one in five (20%, 42/210) were detected using both CS and ISET. ISET cells stained with cytokeratin alone in 26.0% (27/104), cytokeratin and vimentin in 52.9% (55/104) and vimentin alone in 22.1% (23/104). Multivariate Cox proportional hazard regression analysis showed the presence of CTCs to be a poor prognostic factor, associated with reduced DFS after a median follow up of 15 months, irrespective of method used for CTC detection (CS: HR 1.6, 1.3-4.7; P=0.008. ISET: HR 1.4, 1.1-3.3; P=0.006).

A small study by Sawabata *et al.* examined CTC count using CS in peripheral blood immediately pre and post operatively and 10 days after surgical resection of NSCLC. CTCs were detected in 1 of 9 before, 3 out of nine patients immediately after and in no patients 10 days after surgery. After a median follow up of 14 months no patient had evidence of relapse (100).

Nucleic acid based detection

Nucleic acid based methodologies to detect CTCs have been used in the early NSCLC setting. A study by Yie *et al.* applied a RT-PCR ELISA technique to the cellular fraction of peripheral blood (2 mL) taken from patients with NSCLC to determine the concentration of survivin mRNA (101). Survivin is a protein commonly overexpressed in malignancy and has a role in tumor progression (102), a recent meta-analysis reported expression of survivin in NSCLC to be a poor prognostic factor (103). The authors determined an upper limit of normal for survivin mRNA concentration in blood by examining 172 healthy volunteers. Levels above the highest in this cohort were then classed as abnormal and designated as CTC positive. Using this classification, 44.1% of patients with NSCLC taking part in the study had detectable CTCs (n=63/143), corresponding to 26% of stage I + II (n=13/50) and 47.9% (n=35/73) of stage III patients. Follow up for a median period of 36 months was performed on 67 patients who were treated with surgical resection; of these 26 were

CTC positive (38.8%). A total of 18 relapses occurred in the CTC positive cohort (n=18/26; 60% stage I + II, 75% stage III) compared with 4 in the CTC negative cohort (n=4/41; 15.9% stage I + II, 8.3% stage III). The likelihood of relapse was significantly higher in CTC positive patients (RR 43.5, 2.7-70.9; P=0.008).

A panel of 4 marker genes (homo sapiens keratin 19, ubiquitin thiolesterase, highly similar to HSFIB1 for fibronectin, tripartite motif-containing 28) was developed by Sher *et al*. and tested (RT-PCR) in a cohort of 54 patients undergoing curative resection of NSCLC (104). The panel was determined by examining genes with large differential expression ratios between lung cancer cell lines and white blood cells. Detection of any of the marker genes classified a sample as CTC positive; the detection rate was 72% (39/54). The method was further developed to allow semi-quantitative measurement of the relative expression of marker genes to determine a circulating tumor cell load; which was validated using spiked tumor cell experiments. Patients were categorized into high and low cancer cell load for analysis and those with a low cancer cell load had better prognosis when matched for stage.

Yamashita *et al*. examined the blood of 103 patients with NSCLC pre and post-surgery (105). CTCs were defined by the detection of mRNA for CEA, which is expressed in epithelial cells. Patients with detectable CEA in the pre-operative blood sample had a significantly worse prognosis. Median survival in patients with a positive pre-operative test was 14 months compared to over 26 months in those with a negative test. Multivariate analysis showed that stage and CEA mRNA detection were independent prognostic factors (P=0.0004, RR 0.21). Yoon *et al*. sampled blood from patients before and after resection of NSCLC (n=79) and defined CTC status by the presence of thyroid transcription factor-1 (TTF-1) and cytokeratin19 (CK19) mRNA using real time RT-PCR (106). TTF-1 was detected in 36.1% (22/61) and 37.5% of patients before and after surgery respectively. CK19 mRNA positive samples were detected in 42.6% (26/61) and 25.0% (12/48) before and after surgery. Post-surgery positivity for both measures was most strongly correlated with shorter disease-free survival (P=0.006). Patients who only had post-surgery positive TTF-1 samples had a significantly shorter disease-free survival (P<0.001); this was not the case for CK19, which was not prognostic when measured independently.

Pulmonary vein sampling

In addition to analysis of peripheral blood, a small number of studies using various methodologies have explored the presence of CTCs within the pulmonary vein at the time of surgical resection. The pulmonary veins drain blood directly from the lungs and deliver oxygenated blood to the heart for distribution to the systemic circulation; their proximity to the primary tumor and the ability to take blood before the filtering effect of the capillary bed, which has been reported to remove 90% of CTCs (107), makes pulmonary vein sampling potentially advantageous. In a study of 30 patients undergoing resection of lung cancer, Okumura *et al*. detected CTCs in 96% (n=29/30) of pulmonary compared with 17% (n=5/30) of matched peripheral vein samples (using CS) (108). Pulmonary vein CTC count was not prognostic after a median follow-up period of 13 months (2 deaths from lung cancer-one patient with high and one with low pulmonary vein CTC count).

A novel method of CTC detection, involving a CD45 negative enrichment step followed by density gradient centrifugation, was developed by Funaki *et al*. who reported a pulmonary vein CTC prevalence of 72% (n=68/94) (109). Circulating tumor micro-emboli were detected in over half of patients with a CTC positive blood sample (n=35/68). Recurrence occurred in a total of 16 patients at local (n=4), local and distant (n=7) and distant only (n=5) sites after a median follow up period of 13 months. Of these 16 patients 15 had detectable CTCs or CTMs in the pulmonary vein at the time of surgery. Multivariate analysis showed the presence of pulmonary vein CTMs (RR 8.9, 1.7-21.0; P=0.006) and tumor stage III and IV (RR 9.8, 3.4-40.4; P=0.002) to be the only significant predictors of relapse.

Sienel *et al*., using an anti-cytokeratin antibody to define CTCs, detected pulmonary vein CTCs in 18% of patients (11/62) (110). There was an overall trend towards poor prognosis in patients with detectable CTCs, 7/11 died from lung cancer compared to 13/39 patients with no CTCs detected (P=0.054). Subgroup analysis of patients with no mediastinal nodal involvement (i.e., N0 or N1) showed CTC detection to be a significantly adverse prognostic marker (RR 4.2, 1.6-11.1; P=0.004) after multivariate analysis. Pulmonary vein CTCs were also shown to have adverse prognostic significance by Dong *et al*. using flow cytometry to detect CTCs (defined as $CD45^-$, CK^+, $2F7/S5A^+$) (111). Of 31 patients included in the study 15 had detectable CTCs (48.4%). Median follow up was 30 months and patients with CTCs in the pulmonary vein had significantly worse prognosis [2-year survival 62.5% (CTC – ve) *vs*. 26.7% (CTC + ve); P=0.023]. Multivariate analysis showed that only disease stage and CTC positive test (RR 2.8, 1.1-7.2;

P=0.03) were the only independent prognostic tests. By contrast, Franco *et al*. reported the presence of CTCs in the pulmonary vein of 23.9% of patients undergoing resection of NSCLC (n=11/45) but CTC number was not related to prognosis (112).

In these studies the prevalence of pulmonary vein CTCs was markedly different (18% to 96%). The interpretation of these results needs to take into account not only methodological differences in CTC isolation and characterization but also the exact timing of pulmonary vein sampling with respect to the operation itself. Surgical manipulation of lungs peri-operatively has been investigated as a potential cause of increased CTCs. Two studies examining the sequence of vessel ligation at the time of surgery concluded that initial pulmonary vein ligation was associated with a lower post-operative release of CTCs than pulmonary artery ligation (113,114). However, studies by Yamashita *et al*. and Kozak *et al*. reported that the sequence of vessel ligation had no significant impact on prognosis (105,115). In three of the studies pulmonary vein sampling was performed after lung resection, potentially artificially elevating CTC numbers. Nevertheless, collectively the results are provocative for CTC analysis from pulmonary vein samples to inform on prognosis and thereby therapeutic decision making.

In summary, these studies overall have demonstrated the presence of CTCs in the surgical setting and have associated the finding of CTCs with a poorer prognosis. The utility of CTC detection has not yet been developed to a point that could guide clinical decision making and further prospective studies are required for this purpose.

Conclusions and future directions

Achieving long-term survival in patients with radically treated early stage lung cancer remains a major challenge with recurrence rates overall of 50%. Several different circulating biomarkers show promise as indicators of prognosis in patients with resected NSCLC. As an example, CEA has been studied most extensively and recent studies measuring pre and post-operative CEA levels have identified a small proportion of stage I adenocarcinoma patients with a particularly poor prognosis who may benefit from adjuvant chemotherapy. Evidence is lacking however for the predictive value of CEA in this setting. Some studies were performed prior to the routine use of adjuvant therapy or in stage I patients who are not routinely treated. In addition, the prognostic value of CEA may be more applicable to countries with a significant proportion of non-smoking lung cancer cases (up to 50% in some reports). The clinical value in European and American populations is less clear-cut, where there is a much heavier burden of smoking induced lung cancer and as a consequence common co-existence of inflammatory lung conditions such as chronic obstructive pulmonary disease.

A panel of biomarkers may be more reliable in predicting prognosis than a single measure, e.g., combined CYFRA 21-1 and CEA, which have been shown in several studies to be more strongly associated with prognosis than each individual measure taken alone. Newer markers such as circulating nucleic acids (DNA or microRNAs) or CTCs have the potential to reflect directly the biological behavior and provide molecular insights into the tumor biology itself. However, due to the ready availability and low cost of protein based markers in clinical laboratories, it would be prudent to include for example CEA or CYFRA 21-1 in any prospective study of a novel biomarker requiring more sophisticated and costly technology.

There are a multitude of studies that have examined circulating biomarkers and prognosis post-surgical resection. Results do indicate that it may be possible to differentiate patients with similar pathologies and stage into high and low risk categories based on the probability of recurrence on the basis of a convenient blood based test. Adjuvant therapy trials of the late 1990s/early 2000s did not incorporate translational studies of circulating biomarkers; focusing instead on tissue markers in resected tumor tissue. With the availability of new technologies and the opportunities they provide it may be opportune to revisit adjuvant trials with prospective evaluation of circulating biomarkers.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395-409.
3. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision

of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
4. Choi YS, Shim YM, Kim K, et al. Pattern of recurrence after curative resection of local (stage I and II) non-small cell lung cancer: difference according to the histologic type. J Korean Med Sci 2004;19:674-6.
5. Boyd JA, Hubbs JL, Kim DW, et al. Timing of local and distant failure in resected lung cancer: implications for reported rates of local failure. J Thorac Oncol 2010;5:211-4.
6. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
7. NSCLC Meta-analyses Collaborative Group, Arriagada R, Auperin A, et al. Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. Lancet 2010;375:1267-77.
8. Tomita M, Shimizu T, Hara M, et al. Impact of preoperative hemoglobin level on survival of non-small cell lung cancer patients. Anticancer Res 2008;28:1947-50.
9. Carus A, Ladekarl M, Hager H, et al. Tumor-associated neutrophils and macrophages in non-small cell lung cancer: No immediate impact on patient outcome. Lung Cancer 2013;81:130-7.
10. Kobayashi N, Usui S, Kikuchi S, et al. Preoperative lymphocyte count is an independent prognostic factor in node-negative non-small cell lung cancer. Lung Cancer 2012;75:223-7.
11. Zhang J, Huang SH, Li H, et al. Preoperative lymphocyte count is a favorable prognostic factor of disease-free survival in non-small-cell lung cancer. Med Oncol 2013;30:352.
12. Sarraf KM, Belcher E, Raevsky E, et al. Neutrophil/lymphocyte ratio and its association with survival after complete resection in non-small cell lung cancer. J Thorac Cardiovasc Surg 2009;137:425-8.
13. Yu D, Liu B, Zhang L, et al. Platelet count predicts prognosis in operable non-small cell lung cancer. Exp Ther Med 2013;5:1351-54.
14. Tomita M, Shimizu T, Ayabe T, et al. Prognostic significance of the combined use of preoperative platelet count and serum carcinoembryonic antigen level in non-small-cell lung cancer. Gen Thorac Cardiovasc Surg 2010;58:573-6.
15. Hammarstrom S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 1999;9:67-81.
16. Grunnet M, Sorensen JB. Carcinoembryonic antigen (CEA) as tumor marker in lung cancer. Lung Cancer 2012;76:138-43.
17. Kato T, Ishikawa K, Aragaki M, et al. Optimal predictive value of preoperative serum carcinoembryonic antigen for surgical outcomes in stage I non-small cell lung cancer: Differences according to histology and smoking status. J Surg Oncol 2013;107:619-24.
18. Kozu Y, Maniwa T, Takahashi S, et al. Prognostic significance of postoperative serum carcinoembryonic antigen levels in patients with completely resected pathological-stage I non-small cell lung cancer. J Cardiothorac Surg 2013;8:106.
19. Lin XF, Wang XD, Sun DQ, et al. High serum CEA and CYFRA21-1 levels after a two-cycle adjuvant chemotherapy for NSCLC: possible poor prognostic factors. Cancer Biol Med 2012;9:270-3.
20. Nagashima T, Sakao Y, Mun M, et al. A clinicopathological study of resected small-sized squamous cell carcinomas of the peripheral lung: prognostic significance of serum carcinoembryonic antigen levels. Ann Thorac Cardiovasc Surg 2013;19:351-7.
21. Tomita M, Shimizu T, Ayabe T, et al. Maximum SUV on positron emission tomography and serum CEA level as prognostic factors after curative resection for non-small cell lung cancer. Asia Pac J Clin Oncol 2012;8:244-7.
22. Hanagiri T, Sugaya M, Takenaka M, et al. Preoperative CYFRA 21-1 and CEA as prognostic factors in patients with stage I non-small cell lung cancer. Lung Cancer 2011;74:112-7.
23. Takahashi N, Suzuki K, Takamochi K, et al. Prognosis of surgically resected lung cancer with extremely high preoperative serum carcinoembryonic antigen level. Gen Thorac Cardiovasc Surg 2011;59:699-704.
24. Tomita M, Shimizu T, Hara M, et al. Serum carcinoembryonic antigen level in non-small-cell lung cancer patients with preoperative normal serum level. Gen Thorac Cardiovasc Surg 2009;57:303-6.
25. Blankenburg F, Hatz R, Nagel D, et al. Preoperative CYFRA 21-1 and CEA as prognostic factors in patients with stage I non-small cell lung cancer: external validation of a prognostic score. Tumour Biol 2008;29:272-7.
26. Kashiwabara K, Saeki S, Sasaki J, et al. Combined evaluation of postoperative serum levels of carcinoembryonic antigen less than or equal to 2.5 ng/ml and absence of vascular invasion may predict no recurrence

of stage I adenocarcinoma lung cancer. J Thorac Oncol 2008;3:1416-20.
27. Matsuguma H, Nakahara R, Igarashi S, et al. Pathologic stage I non-small cell lung cancer with high levels of preoperative serum carcinoembryonic antigen: clinicopathologic characteristics and prognosis. J Thorac Cardiovasc Surg 2008;135:44-9.
28. Hsu WH, Huang CS, Hsu HS, et al. Preoperative serum carcinoembryonic antigen level is a prognostic factor in women with early non-small-cell lung cancer. Ann Thorac Surg 2007;83:419-24.
29. Kobayashi N, Toyooka S, Soh J, et al. Risk factors for recurrence and unfavorable prognosis in patients with stage I non-small cell lung cancer and a tumor diameter of 20 mm or less. J Thorac Oncol 2007;2:808-12.
30. Matsuoka K, Sumitomo S, Nakashima N, et al. Prognostic value of carcinoembryonic antigen and CYFRA21-1 in patients with pathological stage I non-small cell lung cancer. Eur J Cardiothorac Surg 2007;32:435-9.
31. Mizuguchi S, Nishiyama N, Iwata T, et al. Serum Sialyl Lewis x and cytokeratin 19 fragment as predictive factors for recurrence in patients with stage I non-small cell lung cancer. Lung Cancer 2007;58:369-75.
32. Muley T, Dienemann H, Ebert W. CYFRA 21-1 and CEA are independent prognostic factors in 153 operated stage I NSCLC patients. Anticancer Res 2004;24:1953-6.
33. Okada M, Nishio W, Sakamoto T, et al. Prognostic significance of perioperative serum carcinoembryonic antigen in non-small cell lung cancer: analysis of 1,000 consecutive resections for clinical stage I disease. Ann Thorac Surg 2004;78:216-21.
34. Okada M, Nishio W, Sakamoto T, et al. Effect of histologic type and smoking status on interpretation of serum carcinoembryonic antigen value in non-small cell lung carcinoma. Ann Thorac Surg 2004;78:1004-9; discussion 1009-10.
35. Sakao Y, Tomimitsu S, Takeda Y, et al. Carcinoembryonic antigen as a predictive factor for postoperative tumor relapse in early-stage lung adenocarcinoma. Eur J Cardiothorac Surg 2004;25:520-2.
36. Tomita M, Matsuzaki Y, Edagawa M, et al. Prognostic significance of preoperative serum carcinoembryonic antigen level in lung adenocarcinoma but not squamous cell carcinoma. Ann Thorac Cardiovasc Surg 2004;10:76-80.
37. Buccheri G, Ferrigno D. Identifying patients at risk of early postoperative recurrence of lung cancer: a new use of the old CEA test. Ann Thorac Surg 2003;75:973-80.
38. Reinmuth N, Brandt B, Semik M, et al. Prognostic impact of Cyfra21-1 and other serum markers in completely resected non-small cell lung cancer. Lung Cancer 2002;36:265-70.
39. Sawabata N, Ohta M, Takeda S, et al. Serum carcinoembryonic antigen level in surgically resected clinical stage I patients with non-small cell lung cancer. Ann Thorac Surg 2002;74:174-9.
40. Hotta K, Segawa Y, Takigawa N, et al. Evaluation of the relationship between serum carcinoembryonic antigen level and treatment outcome in surgically resected clinical-stage I patients with non-small-cell lung cancer. Anticancer Res 2000;20:2177-80.
41. Suzuki K, Nagai K, Yoshida J, et al. Prognostic factors in clinical stage I non-small cell lung cancer. Ann Thorac Surg 1999;67:927-32.
42. Rubins JB, Dunitz J, Rubins HB, et al. Serum carcinoembryonic antigen as an adjunct to preoperative staging of lung cancer. J Thorac Cardiovasc Surg 1998;116:412-6.
43. Icard P, Regnard JF, Essomba A, et al. Preoperative carcinoembryonic antigen level as a prognostic indicator in resected primary lung cancer. Ann Thorac Surg 1994;58:811-4.
44. Ford CH, Stokes HJ, Newman CE. Carcinoembryonic antigen and prognosis after radical surgery for lung cancer: immunocytochemical localization and serum levels. Br J Cancer 1981;44:145-53.
45. Kao CH, Hsieh JF, Ho YJ, et al. Cytokeratin fragment 19 (CYFRA 21-1) and carcinoembryonic antigen for early prediction of recurrence of lung adenocarcinoma. Lung 1999;177:333-7.
46. Nisman B, Amir G, Lafair J, et al. Prognostic value of CYFRA 21-1, TPS and CEA in different histologic types of non-small cell lung cancer. Anticancer Res 1999;19:3549-52.
47. Niklinski J, Furman M, Laudanski J, et al. Prognostic value of pretreatment CEA, SCC-Ag and CA 19-9 levels in sera of patients with non-small cell lung cancer. Eur J Cancer Prev 1992;1:401-6.
48. Buccheri G, Ferrigno D, Vola F. Carcinoembryonic antigen (CEA), tissue polypeptide antigen (TPA) and other prognostic indicators in squamous cell lung cancer. Lung Cancer 1993;10:21-33.
49. Foa P, Fornier M, Miceli R, et al. Tumour markers CEA, NSE, SCC, TPA and CYFRA 21.1 in resectable non-small cell lung cancer. Anticancer Res 1999;19:3613-8.
50. Ohwada A, Takahashi H, Nagaoka I, et al. Effect

of cigarette smoke on the mRNA and protein expression of carcinoembryonic antigen (CEA), a possible chemoattractant for neutrophils in human bronchioloalveolar tissues. Thorax 1995;50:651-7.
51. Buccheri G, Ferrigno D. Lung tumor markers of cytokeratin origin: an overview. Lung Cancer 2001;34 Suppl 2:S65-9.
52. Sundstrom BE, Stigbrand TI. Cytokeratins and tissue polypeptide antigen. Int J Biol Markers 1994;9:102-8.
53. Broers JL, Ramaekers FC, Rot MK, et al. Cytokeratins in different types of human lung cancer as monitored by chain-specific monoclonal antibodies. Cancer Res 1988;48:3221-9.
54. Kulpa J, Wojcik E, Reinfuss M, et al. Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. Clin Chem 2002;48:1931-7.
55. Pujol JL, Boher JM, Grenier J, et al. Cyfra 21-1, neuron specific enolase and prognosis of non-small cell lung cancer: prospective study in 621 patients. Lung Cancer 2001;31:221-31.
56. Pujol JL, Molinier O, Ebert W, et al. CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. Br J Cancer 2004;90:2097-105.
57. Tomita M, Shimizu T, Ayabe T, et al. Prognostic significance of tumour marker index based on preoperative CEA and CYFRA 21-1 in non-small cell lung cancer. Anticancer Res 2010;30:3099-102.
58. Brechot JM, Chevret S, Nataf J, et al. Diagnostic and prognostic value of Cyfra 21-1 compared with other tumour markers in patients with non-small cell lung cancer: a prospective study of 116 patients. Eur J Cancer 1997;33:385-91.
59. Pujol JL, Quantin X, Jacot W, et al. Neuroendocrine and cytokeratin serum markers as prognostic determinants of small cell lung cancer. Lung Cancer 2003;39:131-8.
60. Ma S, Shen L, Qian N, et al. The prognostic values of CA125, CA19.9, NSE, AND SCC for stage I NSCLC are limited. Cancer Biomark 2011;10:155-62.
61. Yu D, Du K, Liu T, et al. Prognostic Value of Tumor Markers, NSE, CA125 and SCC, in Operable NSCLC Patients. Int J Mol Sci 2013;14:11145-56.
62. Hara M, Matsuzaki Y, Shimuzu T, et al. Preoperative serum C-reactive protein level in non-small cell lung cancer. Anticancer Res 2007;27:3001-4.
63. O'Dowd C, McRae LA, McMillan DC, et al. Elevated preoperative C-reactive protein predicts poor cancer specific survival in patients undergoing resection for non-small cell lung cancer. J Thorac Oncol 2010;5:988-92.
64. Sheng L, Luo M, Sun X, et al. Serum fibrinogen is an independent prognostic factor in operable nonsmall cell lung cancer. Int J Cancer 2013;133:2720-5.
65. Diez M, Gomez A, Hernando F, et al. Serum CEA, CA125, and SCC antigens and tumor recurrence in resectable non-small cell lung cancer. Int J Biol Markers 1995;10:5-10.
66. Diez M, Torres A, Pollan M, et al. Prognostic significance of serum CA 125 antigen assay in patients with non-small cell lung cancer. Cancer 1994;73:1368-76.
67. Gaspar MJ, Diez M, Rodriguez A, et al. Clinical value of CEA and CA125 regarding relapse and metastasis in resectable non-small cell lung cancer. Anticancer Res 2003;23:3427-32.
68. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011;11:426-37.
69. Bremnes RM, Sirera R, Camps C. Circulating tumour-derived DNA and RNA markers in blood: a tool for early detection, diagnostics, and follow-up? Lung Cancer 2005;49:1-12.
70. Sozzi G, Roz L, Conte D, et al. Plasma DNA quantification in lung cancer computed tomography screening: five-year results of a prospective study. Am J Respir Crit Care Med 2009;179:69-74.
71. van der Drift MA, Hol BE, Klaassen CH, et al. Circulating DNA is a non-invasive prognostic factor for survival in non-small cell lung cancer. Lung Cancer 2010;68:283-7.
72. Ludovini V, Pistola L, Gregorc V, et al. Plasma DNA, microsatellite alterations, and p53 tumor mutations are associated with disease-free survival in radically resected non-small cell lung cancer patients: a study of the perugia multidisciplinary team for thoracic oncology. J Thorac Oncol 2008;3:365-73.
73. Szpechcinski A, Chorostowska-Wynimko J, Kupis W, et al. Quantitative analysis of free-circulating DNA in plasma of patients with resectable NSCLC. Expert Opin Biol Ther 2012;12 Suppl 1:S3-9.
74. Mallick R, Patnaik SK, Yendamuri S. MicroRNAs and lung cancer: Biology and applications in diagnosis and prognosis. J Carcinog 2010;9:8.
75. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259-69.
76. Yu SL, Chen HY, Chang GC, et al. MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell 2008;13:48-57.

77. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513-8.
78. Hu Z, Chen X, Zhao Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. J Clin Oncol 2010;28:1721-6.
79. Boeri M, Verri C, Conte D, et al. MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer. Proc Natl Acad Sci U S A 2011;108:3713-8.
80. Chen Q, Si Q, Xiao S, et al. Prognostic significance of serum miR-17-5p in lung cancer. Med Oncol 2013;30:353.
81. Heegaard NH, Schetter AJ, Welsh JA, et al. Circulating micro-RNA expression profiles in early stage nonsmall cell lung cancer. Int J Cancer 2012;130:1378-86.
82. Silva J, Garcia V, Zaballos A, et al. Vesicle-related microRNAs in plasma of nonsmall cell lung cancer patients and correlation with survival. Eur Respir J 2011;37:617-23.
83. Cheng TL, Chang MY, Huang SY, et al. Overexpression of circulating c-met messenger RNA is significantly correlated with nodal stage and early recurrence in non-small cell lung cancer. Chest 2005;128:1453-60.
84. Hosoda H, Izumi H, Tukada Y, et al. Plasma hepatocyte growth factor elevation may be associated with early metastatic disease in primary lung cancer patients. Ann Thorac Cardiovasc Surg 2012;18:1-7.
85. Ujiie H, Tomida M, Akiyama H, et al. Serum hepatocyte growth factor and interleukin-6 are effective prognostic markers for non-small cell lung cancer. Anticancer Res 2012;32:3251-8.
86. D'Amico TA, Brooks KR, Joshi MB, et al. Serum protein expression predicts recurrence in patients with early-stage lung cancer after resection. Ann Thorac Surg 2006;81:1982-7; discussion 1987.
87. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011;331:1559-64.
88. Ma XL, Xiao ZL, Liu L, et al. Meta-analysis of circulating tumor cells as a prognostic marker in lung cancer. Asian Pac J Cancer Prev 2012;13:1137-44.
89. Allan AL, Keeney M. Circulating tumor cell analysis: technical and statistical considerations for application to the clinic. J Oncol 2010;2010:426218.
90. Riethdorf S, Fritsche H, Muller V, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res 2007;13:920-8.
91. Krebs MG, Sloane R, Priest L, et al. Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol 2011;29:1556-63.
92. Went PT, Lugli A, Meier S, et al. Frequent EpCam protein expression in human carcinomas. Hum Pathol 2004;35:122-8.
93. Hou JM, Krebs M, Ward T, et al. Circulating tumor cells as a window on metastasis biology in lung cancer. Am J Pathol 2011;178:989-96.
94. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442-54.
95. Farace F, Massard C, Vimond N, et al. A direct comparison of CellSearch and ISET for circulating tumour-cell detection in patients with metastatic carcinomas. Br J Cancer 2011;105:847-53.
96. Vona G, Sabile A, Louha M, et al. Isolation by size of epithelial tumor cells: a new method for the immunomorphological and molecular characterization of circulatingtumor cells. Am J Pathol 2000;156:57-63.
97. Krebs MG, Hou JM, Sloane R, et al. Analysis of circulating tumor cells in patients with non-small cell lung cancer using epithelial marker-dependent and -independent approaches. J Thorac Oncol 2012;7:306-15.
98. Hofman V, Ilie MI, Long E, et al. Detection of circulating tumor cells as a prognostic factor in patients undergoing radical surgery for non-small-cell lung carcinoma: comparison of the efficacy of the CellSearch Assay and the isolation by size of epithelial tumor cell method. Int J Cancer 2011;129:1651-60.
99. Hofman V, Bonnetaud C, Ilie MI, et al. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. Clin Cancer Res 2011;17:827-35.
100. Sawabata N, Okumura M, Utsumi T, et al. Circulating tumor cells in peripheral blood caused by surgical manipulation of non-small-cell lung cancer: pilot study using an immunocytology method. Gen Thorac Cardiovasc Surg 2007;55:189-92.
101. Yie SM, Lou B, Ye SR, et al. Clinical significance of detecting survivin-expressing circulating cancer cells in patients with non-small cell lung cancer. Lung Cancer 2009;63:284-90.
102. Duffy MJ, O'Donovan N, Brennan DJ, et al. Survivin: a promising tumor biomarker. Cancer Lett 2007;249:49-60.
103. Huang LN, Wang DS, Chen YQ, et al. Expression of survivin and patients survival in non-small cell lung cancer:

a meta-analysis of the published studies. Mol Biol Rep 2013;40:917-24.
104. Sher YP, Shih JY, Yang PC, et al. Prognosis of non-small cell lung cancer patients by detecting circulating cancer cells in the peripheral blood with multiple marker genes. Clin Cancer Res 2005;11:173-9.
105. Yamashita J, Matsuo A, Kurusu Y, et al. Preoperative evidence of circulating tumor cells by means of reverse transcriptase-polymerase chain reaction for carcinoembryonic antigen messenger RNA is an independent predictor of survival in non-small cell lung cancer: a prospective study. J Thorac Cardiovasc Surg 2002;124:299-305.
106. Yoon SO, Kim YT, Jung KC, et al. TTF-1 mRNA-positive circulating tumor cells in the peripheral blood predict poor prognosis in surgically resected non-small cell lung cancer patients. Lung Cancer 2011;71:209-16.
107. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2002;2:563-72.
108. Okumura Y, Tanaka F, Yoneda K, et al. Circulating tumor cells in pulmonary venous blood of primary lung cancer patients. Ann Thorac Surg 2009;87:1669-75.
109. Funaki S, Sawabata N, Nakagiri T, et al. Novel approach for detection of isolated tumor cells in pulmonary vein using negative selection method: morphological classification and clinical implications. Eur J Cardiothorac Surg 2011;40:322-7.
110. Sienel W, Seen-Hibler R, Mutschler W, et al. Tumour cells in the tumour draining vein of patients with non-small cell lung cancer: detection rate and clinical significance. Eur J Cardiothorac Surg 2003;23:451-6.
111. Dong Q, Huang J, Zhou Y, et al. Hematogenous dissemination of lung cancer cells during surgery: quantitative detection by flow cytometry and prognostic significance. Lung Cancer 2002;37:293-301.
112. Franco R, Pirozzi G, Scala S, et al. CXCL12-binding receptors expression in non-small cell lung cancer relates to tumoral microvascular density and CXCR4 positive circulating tumoral cells in lung draining venous blood. Eur J Cardiothorac Surg 2012;41:368-75.
113. Kurusu Y, Yamashita J, Hayashi N, et al. The sequence of vessel ligation affects tumor release into the circulation. J Thorac Cardiovasc Surg 1998;116:107-13.
114. Ge MJ, Shi D, Wu QC, et al. Observation of circulating tumour cells in patients with non-small cell lung cancer by real-time fluorescent quantitative reverse transcriptase-polymerase chain reaction in peroperative period. J Cancer Res Clin Oncol 2006;132:248-56.
115. Kozak A, Alchimowicz J, Safranow K, et al. The impact of the sequence of pulmonary vessel ligation during anatomic resection for lung cancer on long-term survival--a prospective randomized trial. Adv Med Sci 2013;58:156-63.

Cite this article as: Crosbie PA, Shah R, Summers Y, Dive C, Blackhall F. Prognostic and predictive biomarkers in early stage NSCLC: CTCs and serum/plasma markers. Transl Lung Cancer Res 2013;2(5):382-397. doi: 10.3978/j.issn.2218-6751.2013.09.02

Copy number gains of FGFR1 and 3q chromosome in squamous cell carcinoma of the lung

Pedro Mendez[1,2], Jose Luis Ramirez[1,2]

[1]Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Badalona, Spain; [2]Health Sciences Research Institute Germans Trias i Pujol, Badalona, Spain

Correspondence to: Jose Luis Ramirez. Molecular Biology Laboratory, Medical Oncology Service-ICO, Hospital Germans Trias i Pujol, Ctra Canyet s/n, 08916-Badalona, Spain. Email: jlramirez@iconcologia.net; Pedro Mendez. Molecular Biology Laboratory, Medical Oncology Service-ICO, Hospital Germans Trias i Pujol, Fundació Investigació en Ciències de la Salut Germans Trias i Pujol, Ctra Canyet s/n, 08916-Badalona, Spain. Email: pmendez00@gmail.com.

Abstract: Squamous cell carcinoma of the lung (SQCCL) remains a leading cause of cancer-related death. Unlike non-smoker adenocarcinoma of the lung, where highly efficient tyrosine kinase inhibitors are available for treating mutant EGFR or ALK-rearranged, no targetable biomarkers are available for SQCCL. The frequent and focal amplification of FGFR1 has generated great expectations in offering new therapeutical options in case of 16-22% of SQCCL patients. Broad 3q chromosome amplification is widely recognized as the most common chromosomal aberration found in SQCCL, where PIK3CA, SOX2, ACK1, PRKCI, TP63, PLD1, ECT2, and others genes are located. Although SOX2 has been postulated as a key regulator of basal stem cells transformation and tumor progression, it seems to confer a good prognosis in SQCCL. It is known that each patient might carry a different length of 3q chromosome amplicon. Thus, we suggest that the number and the biological importance of the genes spanned along each patient's 3q amplicon might help to explain inter-individual outcome variations of the disease and its potential predictive value, especially when relevant oncogenes such as those mentioned above are implicated. Currently, there is no clinical predictive data available from clinical trials. In this review, we have focused on the potential role of FGFR1 in SQCCL prognosis. Additionally, we have explored recently available public data on the comprehensive genomic characterization of SQCCL, in relation to the protein-coding genes that have a strong gene copy number - mRNA correlation in 3q chromosome, that were previously described as potential driver oncogenes or its modifiers in SQCCL.

Keywords: FGFR1; Squamous cell carcinoma; 3q chromosome; amplification; lung cancer

Submitted Feb 10, 2013. Accepted for publication Mar 08, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.05
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.05

Despite the decrease in the incidence of squamous cell carcinoma of the lung (SQCCL) in the last decades, it still represents 20-30% of non-small cell lung cancer (NSCLC) (1). Unlike non-smoker lung adenocarcinoma, where strong biomarkers of response to specific tyrosine kinase inhibitors (TKI) (such as activating mutations of EGFR or ALK rearrangements) are available, in SQCCL actionable alterations have only been partially characterized in recent years, without any breakthrough in treating such tumor entities (2).

Gene copy number (GCN), like other genetic structural variations, represents an event of strong evolutionary pressure within both normal cells and particularly in cancer cells, where genomic instability it is a hallmark. GCN gains, such as gene duplication or amplification, can cause an increase in protein levels. Nowadays, there are three

molecular mechanisms that can potentially produce a gene amplification, including the double-stranded DNA repair pathways: non-homologous end-joining (NHEJ), non-allelic homologous recombination (NAHR) (3,4), and DNA re-replication. In DNA re-replication, license control of replication is lost and a single DNA molecule is replicated more than once, triggering GCN gains, amplification, genomic instability and tumorigenesis (5).

Fibroblast growth factor receptor 1 (FGFR1) is one of the four family members of the FGFR of transmembrane tyrosine kinase receptors (TKR) involved in regulation of embryonic development, differentiation and cell proliferation (6-8). The functional validation of FGFR1 gene amplification in SQCCL was initially described by Weiss *et al.* Their work placed this histological-tumor subtype on the edge of the wave, identifying new therapeutic options that could change the management of SQCCL patients (9).

Broad amplification at 3q chromosome is the most frequent chromosomal alteration in SQCCL tumors. It was initially reported using fluorescent in situ hybridization (FISH) (10). It is known that increasing frequency of 3q amplification can be found from dysplasia to metastatic squamous lesions (11). Moreover, the potential epidemiological relationship of 3q amplification and tobacco consumption has been suggested (2). A recent comprehensive genomic characterization of SQCCL reported that 3q amplicon covers 3q13 to 3q29 (12). They also showed a correlation of GCN and mRNA levels at single-gene resolution.

This review highlights the recent findings on the prognostic and/or predictive value of FGFR1, as well as other important genes targeted by the 3q chromosome amplification in SQCCL.

FGFR1 amplification

FGFR is a family of receptors tyrosine kinases (RTK) consisting of four family members (FGFR1-4). FGFR1, like other RTKs, has an extracellular domain, a transmembrane domain and an intracellular domain, where the catalytic tyrosine kinase domain is located. The FGFR1 gene resides at 8p12 cytoband and spans a genomic DNA fragment of 57.7 Kb in length. Upon receptor activation, it promotes cell proliferation, angiogenesis, survival and apoptotic resistance through the PLCγ/PKC, RAS/MAPK and PI3K-AKT pathways (13). The oncogenic potential of activated FGFR1 represents an attractive therapeutic target that is currently being clinically tested.

The seminal work of Weiss *et al.* (9), demonstrated a growth dependency of a subset of SQCCL based on FGFR1 amplification that was abrogated both in lung cancer cell lines and in NCI-H1581 mice xenografts by PD173074, a specific TKI. No activating mutations were found. Twenty-two percent of squamous lung cancer tumors were carriers of FGFR1 focal amplification, as detected by FISH. Further studies confirmed that the percentage of amplification ranges from 16-22% (14-16) and independent *in vitro* studies confirmed that FGFR1-amplified cells are vulnerable when treated with a specific TKI (17). FGFR1 has also been reported to be amplified in other cancers, including 17.4% oral squamous cell carcinoma (18), 6% of esophageal squamous cell carcinoma (19), 10-17% of breast (20,21), 7.8% of ovarian (22,23), 3.4% of bladder (24) and 9% of prostate cancer (25).

Heist *et al.*, in a retrospective cohort of 226 SQCLC, where almost 70% of the patients were staged as IA-IIB, detected 16% of FGFR1 amplification. They measured gene copy number by FISH , using for the threshold of gene amplification a FISH ratio equal to 2.2 or higher (14). In this study, amplification of FGFR1 was not correlated with age, sex, stage or smoking history. They found no correlation with overall survival. On the other hand, Weiss *et al.* reported a trend towards inferior survival among patients amplified for FGFR1 (9). In a recent work carried out by Kim *et al.* reported that patients, carriers of FGFR1 amplification, had significantly shorter disease-free survival and overall survival than diploid patients (wild type), regardless of sex, smoking status, adjuvant therapy and pathologic stage. These findings are in contradiction to those previously published by Heist and Weiss, and suggest FGFR1 amplification is an independent prognostic marker in this cohort of patients. Furthermore, in the same study, a positive association of FGFR1 amplification and smoking habit, in a dose-dependent manner, was reported. An interesting observation is that none of the 37 patients classified as never-smokers were carriers of amplified FGFR1 (26). Recently, a 100% concordance of FGFR1 amplification between primary SQCCL tumors and their lymph node metastatic tissue was described, suggesting an important role for FGFR1 in tumor prognosis and progression. So further studies are needed to validate whether the prognostic impact of FGFR1 amplification is a population-based phenomena or not (16).

Due to the important biological impact of FGFR

Table 1 Selected FGFR inhibitors currently used in clinical development and/or evaluation

DRUG	Company	TARGETS	Clinical development stage
Small-Molecule TKIs			
Vargalef (BIBF1120)	Boehringer Ingelheim, Novartis	FGFRs, VEGFR and PDGFR	III
Ponatinib (AP24534)	Ariad	FGFR, VEGFR and IGF-1R	I
Dovotinib (TKI258)	Novartis	FGFRs, VEGFRs, KIT, FLT3, CSFR and PDGFRs	III
Brivanib (BMS582664)	Bristol Myers Squib	VEGFRs and FGFRs	II
AZD4547	Astra Zeneca	FGFRs	I/II
Cediranib (AZ2171)	Astra Zeneca	VEGFRs, FGFRs and KIT	III
TSU68 (SU668)	Taiho Pahrmace	FGFRs, VEGFR and PDGFR	II
E7080	Eisai	FGFRs, VEGFR and PDGFR	II
E3810	Ethical Oncology Science	FGFRs, VEGFR	I
BGJ398	Novartis	FGFRs	I
RG1507	Roche, Genmab	FGFRs, VEGFR and PDGFR	II
LY2874455	Lilly	FGFRs	n/a
FGFR antibodies			
Figitumumab	Pfizer	FGFR, VEGFR and IGF-1R	III
Cixutumumab	ImClone Systems	FGFR, VEGFR and IGF-1R	II
AMG479	Amgen	FGFR, VEGFR and IGF-1R	II/III
BIIB022	Biogen Idec	FGFR, VEGFR and IGF-1R	I/II
FP1039 (Fusion protein)	Five Prime	FGFR1	I/II
R3Mab	Genectech	FGFR3	n/a

Abbreviations: FGFRs, fibroblast growth factor receptors; VEGFRs, vascular endothelial growth factor receptor; PDFRs, platelet derived growth factor receptor; IGF-1R, Insulin Growth factor-1 receptor; KIT, mast/stem cell growth factor receptor; FLT3, fms-like tyrosine Kinase receptor 3; CSFR, colony stimulating factor receptor; n/a, not applicable.

activation in tumor cell growth, survival, tumor angiogenesis, progression and metastasis, the development and clinical testing of anti-FGFR compounds are currently major areas of research. There has been a great expectation as some reports have suggested FGFR1 amplification as a predictive biomarker of specific TKIs. There are two different types of FGFR inhibitors under development: small TKI molecules and ligand-competitor antibodies (see *Table 1*). Most of the small molecules exert their biological activity by binding into the ATP-binding pocket. This prevents either auto-phosphorylation of the receptor or proliferative signal transduction through transphosphorylation of receptor-dimers and their downstream adaptor proteins such as FSR2 (17,27). A clinical trial with BIBF1120, which inhibits FGFR1, will be developed in the Netherlands and in Spain in the second line treatment of SQCCL patients with FGFR1 amplification. Double methodological validation of FGFR1-amplified tumors will be carried out by FISH and multiplex ligation-dependent probe amplification (MLPA).

Taking advantage of what we have learned from gastrointestinal stromal tumors treated with imatinib/sunitinib (28,29), as well as from the history of erlotinib/gefitinib or crizotinib in lung cancers carriers of mutant EGFR (30,31) or ALK-rearrangements (32) respectively, we will need to identify the mechanisms of intrinsic, adaptive and acquired resistance to TKI treatment, as quickly as possible, and how to revert them clinically. The priority should be to analyze the presence of gain-of-functional mutations, amplification or overexpression of RTKs that activates redundant pro-survival pathways which bypass the drugged one (33,34). In addition, alterations in apoptotic pathways have also been demonstrated a key role in TKI resistance, and thus need to be analyzed (35-38).

Currently, fluorescent in situ hybridization (FISH) is the standard method available for identification of gene amplification among cancer patients. The previous

experience from ERBB2 in breast cancer has shown that a key point was the inter-laboratories standardization of FISH criteria (39,40). Recently it has been reported in a cohort of 307 squamous lung carcinomas a reference guide to classify the tumor entities with respect to their FGFR1 gene status by FISH (41). The authors defined low-level amplification by ≥5 FGFR1 signals in ≥50% of tumor cells, whereas high-level amplification is defined by an FGFR1/centromere 8 (CEN8) ratio ≥2.0, or by an average number of FGFR1 signals per tumor cell nucleus ≥6, or by the percentage of tumor cells containing ≥15 FGFR1 signals or large clusters ≥10%.

In order to establish an appropriate GCN threshold correlation between FGFR1 gene dosage and drug response in SQCCL patients, we propose to measure FGFR1 gene status by FISH along with, a secondary independent quantification of FGFR1 gene copy number by MLPA. In addition to clarify how FGFR1 amplification is translated at active-protein levels, we recommend measuring phospho FGFR1 and phospho FSR2 as indicators of FGFR1 signal transduction activity (17,27).

3q amplification

Over the recent decades, due to the great technical advancement in the field of molecular biology, there has been vast improvement towards the genetic characterization of tumors, in an effort to understand how their biology can be targeted to improve cancer patient care. One of the most frequent chromosomal aberrations found in NSCLC is the amplification at 3q chromosome, which can be present in up to 43% of cases. It can be found in squamous dysplasia, established carcinoma and also in metastatic tissue (42) and is suggested that 3q amplification frequency increases as disease progresses (43). It is known that each patient carries a different length of 3q chromosome amplicon (see *Figure 1*). We hypothesized that the number and the biological importance of the trapped genes in each patient's 3q amplicon might be helpful to explain the inter-individual differences in disease outcome or its response towards specific targeted therapy.

Only a few genes that are targeted by the 3q chromosome amplicon have been functionally validated as prognosis modifiers of cancer disease, and even fewer as biomarkers of cancer therapy. Among these genes are phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) (44-46), SRY-related HMG-box (SOX2) (47-50), tumor protein 63 (TP63) (42), atypical Protein kinase C iota (aPKCι) (51,52), eukaryotic translation initiation factor 4 gamma 1 (EIF4G1) (53,54), member of RAS oncogene family RAP2B, and others.

PIK3CA encodes for the p110α catalytic subunit of phosphatidylinositol (PI) 3-kinase. A broad range of cancer-related functions have been associated with its activation, such as cell proliferation, survival, oncogenic RAS signaling and transformation, making this an attractive target for therapeutic intervention. PIK3CA was found to be amplified in up to 45% of SQCCL cancer patients (55-59) and, due the strong correlation between PIK3CA amplification and its increased activity through its downstream effectors such as AKT and mTOR, this gene also appeared as an oncogene candidate (44). Abnormalities including mutations and amplification of PIK3CA/AKT/mTOR/PTEN are more common in SQCCL than in adenocarcinoma of the lung (60-62). Similar results have been showed by Spoerke *et al.* in their study where they have evaluated the candidate predictive biomarkers of sensitivity to select PI3K/mTOR pathway inhibitors in lung cancer patients. They suggests that different predictive biomarker strategies might be needed for both squamous and non-squamous patient populations, due to their alteration patterns and frequency (46).

The transcription factor TP63 (TP73L) is a homologue of p53 that functions by transactivating p53-targeted genes. The TP63 gene is expressed as multiple isoforms with different functions, including a full length (TAp63) and a truncated amino-deleted isoform ΔNp63, also called p40 (63). TAp63 can induce cell cycle arrest and apoptosis in response to DNA damage (64), whereas ΔNp63 has opposite functions because of its competition with p53, with respect to cell cycle arrest, mobility, invasion (epithelial–mesenchymal transition) and senescence. The ratio of TAp63 and ΔNp63 regulates chemosensitivity. ΔNp63a is the most commonly expressed TP63 isoform in squamous cell carcinoma together with TP63 amplification (65). Massion *et al.* reported that 88% of SQCCLs have TP63 amplification by FISH (42). As an interesting finding, they observed that TP63 amplification was an early event in the development of squamous carcinoma along with overexpression by IHC which results in better survival. ΔNp63 has been demonstrated as a more specific maker of squamous cell carcinoma than full length TP63, in the differential diagnosis in comparison with other lung histologies (66,67).

The SOX_2 gene is a key transcription factor that coordinates embryonic development, differentiation and self-renewal of normal non-alveolar epithelium of

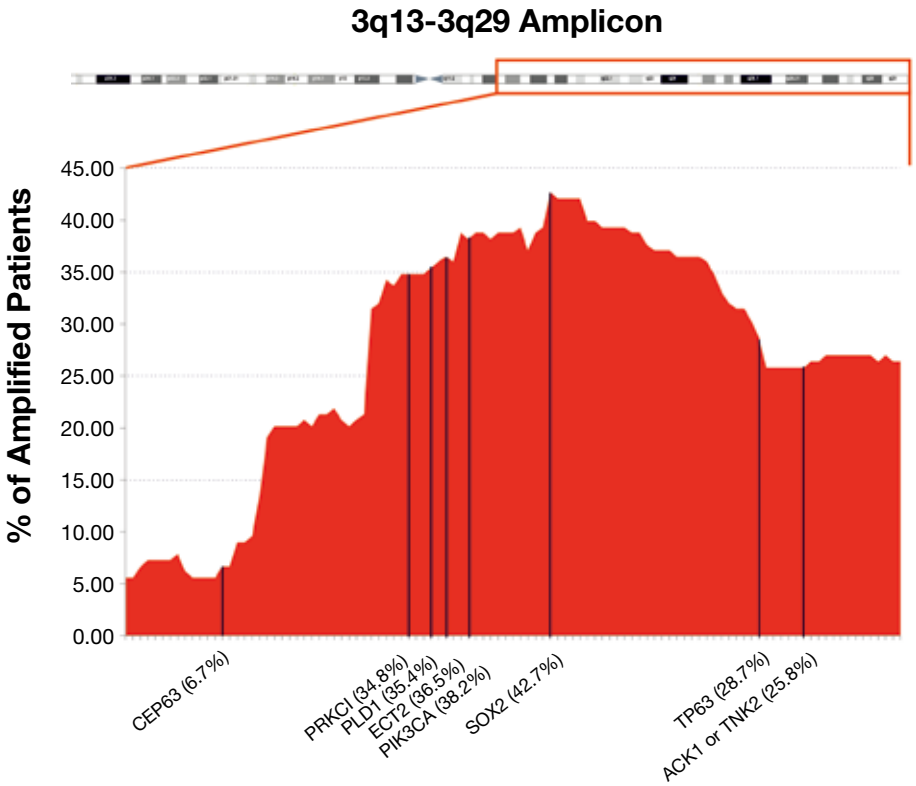


Figure 1 Percentage of patients, carriers of 3q chromosome amplification for each gene. A representative list of 3q chromosome region was sorted by chromosome position from 3q13 to 3q29.

the airway. SOX_2 amplification has been reported in 43-60% (11,48,50,68) of SQCCL and in 27% of SCLC (69). The biological and clinical impact of SOX_2 in lung cancer is reviewed by Karachaliou *et al.* (doi: 10.3978/j.issn.2218-6751.2013.01.01).

CEP63 (centrosomal protein 63 kDa) plays a role in DNA damage response. Following DNA double strand breaks (DSBs) formation, it is delocalized from centrosomes and recruits CDK1, a regulator mitotic entry of the cell (70,71).

We took advantage of a recent report where authors performed a high resolution genomic characterization of SQCCL by RNA-seq, gene copy number and mRNA expression analysis (12) that is publicly available at the cBio cancer genomics portal (72). In this section, we will summarize the recent evidence of selected 3q-resident genes, where gene amplification might explain its contribution to malignant transformation, tumor progression or its role as a biomarker for targeted therapies. From protein-coding genes located at 3q, we only selected those were having strong correlation of GCN and mRNA. We defined strong GCN-mRNA correlation for a given gene, when at least 50% of the amplified tumors expressed higher levels of mRNA than diploid tumors (see *Figure 2*).

Atypical protein kinase C iota (aPKCι)

aPKCι belongs to the atypical subgroup within the protein kinase C family of structurally related serine/threonine kinases. Different PKC isoenzymes are involved in different functions, such as: cellular differentiation, proliferation, polarity and apoptosis. Atypical PKCs, unlike most of the members of the family, can be activated independently of Ca^{2+}, diacyglycerol or phosphatidylserine (73). High aPKCι expression has been found in several human tumors, including squamous carcinomas of head and neck (64), esophageal (74,75) and lung (52), but also in lung adenocarcinoma (76). Recent data suggests that aPCKι activity is required by the oncogenic RasG12D mice model to progress from bronchial hyperplasia to lung tumor (77). In the same study, bronchoalveolar stem cells that lacked Prkci, the mice gene that encodes for aPKCι, were unable to transform neither *in vitro* nor *in vivo*.

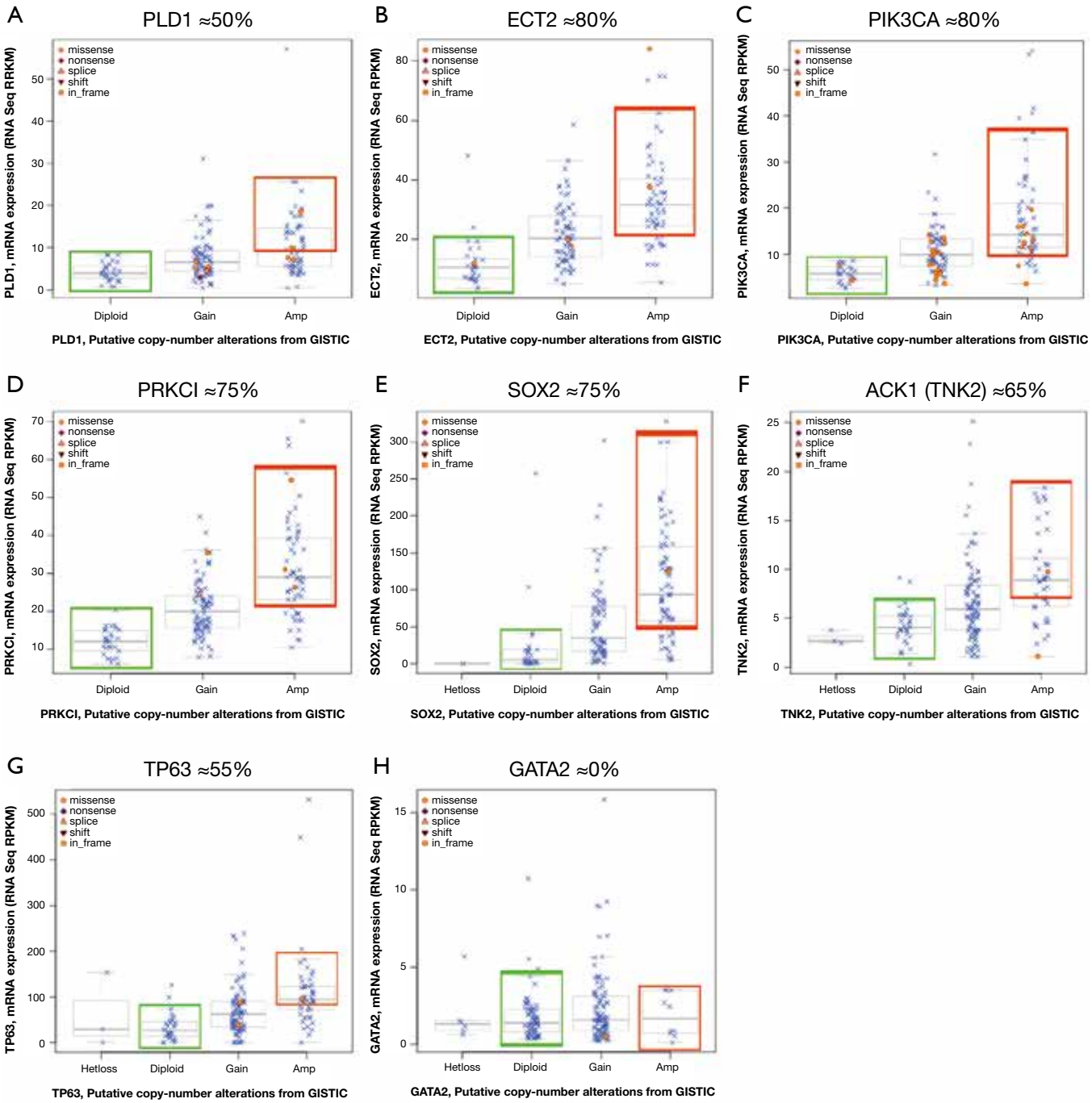


Figure 2 Boxplots of mRNA expression vs. gene copy number in SQCCL selected oncogenes. Only protein-coding genes were considered for screening as candidate genes. Gene data was retrieved from the cBio portal (http://www.cbioportal.org/public-portal/) based on their GCN- mRNA correlation and previous available bibliography. Those genes in which ≥50% of the amplified tumors expressed higher mRNA levels than diploid were selected for further bibliographic review. A-G, selected genes showing an strong correlation of GCN and mRNA expression levels. H, an example of a discarded gene due to its low correlation value.

aPKCι interacts with PAR6α, forming a complex that triggers the activation of RAC1-PAK-MEK-ERK pro-survival pathway. Interestingly in NSCLC, the ECT2 oncogene, which also localizes at 3q amplicon, is mislocalized in the cytoplasm, where it is a target of phosphorylation at Thr-328 by aPKCι (78) for a proper oncogenic signaling through the RAC1 small GTPase pathway (79).

Taking into consideration, the importance of aPKCι in KRAS-mediated lung tumors, the prognostic and/or predictive role of PRKCI amplification and aPKCι overexpression needs to be evaluated in oncogene "addicted" lung tumors, such as lung adenocarcinoma induced by EGFR-activating mutations or oncogenic rearrangements of ALK, where targeted therapies have a strong impact on patient survival and quality of care. Of course, it might be also interested to address the same question in SQCCL carriers of FGFR1 amplification treated with FGFRs inhibitors.

Activated CDC42 kinase 1 (ACK1)

ACK1, also known as TNK2, is a non-receptor tyrosine and serine/threonine protein kinase which functions as transducer of multiple ligand-activated RTKs including EGFR (80,81), AXL (82), MERTK (53), HER2 (83) and PDGFR (84) by activating cytosolic or nuclear effectors such as AKT and AR respectively to promote cell growth and survival (85,86). EGF ligand stimulation activates the ACK1 activity, which at the same time prevents EGFR from ubiquitination (87). AKT activation by ACK1 happens in a PI3K-independent manner. When phosphorylated by ACK1 at Tyr-176, unlike the PI3K-activated AKT, it is confined to the membrane phosphatidic acid phospholipid. Once the phospho-activated AKT/ACK1 complex is located at the plasma membrane, it then translocates into the nucleus where it phosphorylates FoxO3a, preventing the expression of the BIM-1 pro-apoptotic gene, the GADD45 DNA repair gene and p21 and p27 inducers of cell cycle arrest. Moreover it can also activate the mitotic progression (88). In addition, the E3 ubiquitin ligase Nedd4-2 is a negative regulator of ACK1 when co-expressed (87,89), and can be rescued by treatment with MG132, a proteasomal inhibitor. Xenografts of prostate LNCaP cells are usually poorly tumorigenic in nude mice. But when LNCaP cells expressing a constitutively activated ACK1 were engrafted into nude mice, they rendered very large tumors within the first 24 days after injection. In prostate cancer, activated ACK1, phosphorylates androgen receptor (AR) either at Tyr-267 or Tyr-363 led to the nuclear translocation of AR/ACK1 complex, thus activating the transcription of AR target genes such as prostate-cancer proteins: prostate-specific antigen (PSA) and HK2, independently of androgen or testosterone, the Androgen receptor ligands (83). Interestingly, a hallmark of prostate cancer progression implies the acquisition of an androgen-resistant phenotype, which might be explained in some cases by the AR estrogen-independent activation by ACK1.

Conclusions

Taking into consideration, the potential biological and medical impact of FGFR1, its activation turned to be a major area of research interest. Although prognostic data on FGFR1 has only recently been reported, the results are contradictory. Larger studies are needed to clarify its prognostic role. Furthermore, FGFR1 inhibitors have entered clinical trials, and over the next few years its predictive role with targeted TKIs will be definitely clarified.

On the other hand, finding new predictive biomarkers in highly genetic heterogeneous tumors such as SQCCL might be challenging because of the coexistence of multiple driver oncogenes, both in the same cellular clone or in different ones. An example might be the 3q chromosome amplification in SQCCL.

Acknowledgements

Work in Dr. Rosell's laboratory is partly supported by a grant from La Caixa Foundation, which had no role in writing the manuscript or in the decision of publication.

Disclosure: This manuscript has not been published so far or submitted for publication elsewhere. The authors declare no conflicts of interest.

References

1. Travis WD. Pathology of lung cancer. Clin Chest Med 2011;32:669-92.
2. Drilon A, Rekhtman N, Ladanyi M, et al. Squamous-cell carcinomas of the lung: emerging biology, controversies, and the promise of targeted therapy. Lancet Oncol 2012;13:e418-26.
3. Zhang F, Gu W, Hurles ME, et al. Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet 2009;10:451-81.

4. Shlien A, Malkin D. Copy number variations and cancer. Genome Med 2009;1:62.
5. Truong LN, Wu X. Prevention of DNA re-replication in eukaryotic cells. J Mol Cell Biol 2011;3:13-22.
6. Itoh N, Ornitz DM. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. J Biochem 2011;149:121-30.
7. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010;10:116-29.
8. Heist RS, Sequist LV, Engelman JA. Genetic changes in squamous cell lung cancer: a review. J Thorac Oncol 2012;7:924-33.
9. Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2:62ra93.
10. Kettunen E, el-Rifai W, Björkqvist AM, et al. A broad amplification pattern at 3q in squamous cell lung cancer--a fluorescence in situ hybridization study. Cancer Genet Cytogenet 2000;117:66-70.
11. McCaughan F, Pole JC, Bankier AT, et al. Progressive 3q amplification consistently targets SOX2 in preinvasive squamous lung cancer. Am J Respir Crit Care Med 2010;182:83-91.
12. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.
13. Mason I. Initiation to end point: the multiple roles of fibroblast growth factors in neural development. Nat Rev Neurosci 2007;8:583-96.
14. Heist RS, Mino-Kenudson M, Sequist LV, et al. FGFR1 amplification in squamous cell carcinoma of the lung. J Thorac Oncol 2012;7:1775-80.
15. Dutt A, Ramos AH, Hammerman PS, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 2011;6:e20351.
16. Göke F, Franzen A, Menon R, et al. Rationale for treatment of metastatic squamous cell carcinoma of the lung using fibroblast growth factor receptor inhibitors. Chest 2012;142:1020-6.
17. Guagnano V, Kauffmann A, Wöhrle S, et al. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. Cancer Discov 2012;2:1118-33.
18. Freier K, Schwaenen C, Sticht C, et al. Recurrent FGFR1 amplification and high FGFR1 protein expression in oral squamous cell carcinoma (OSCC). Oral Oncol 2007;43:60-6.
19. Ishizuka T, Tanabe C, Sakamoto H, et al. Gene amplification profiling of esophageal squamous cell carcinomas by DNA array CGH. Biochem Biophys Res Commun 2002;296:152-5.
20. Turner N, Pearson A, Sharpe R, et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 2010;70:2085-94.
21. Courjal F, Cuny M, Simony-Lafontaine J, et al. Mapping of DNA amplifications at 15 chromosomal localizations in 1875 breast tumors: definition of phenotypic groups. Cancer Res 1997;57:4360-7.
22. Gorringe KL, Jacobs S, Thompson ER, et al. High-resolution single nucleotide polymorphism array analysis of epithelial ovarian cancer reveals numerous microdeletions and amplifications. Clin Cancer Res 2007;13:4731-9.
23. Theillet C, Adelaide J, Louason G, et al. FGFRI and PLAT genes and DNA amplification at 8p12 in breast and ovarian cancers. Genes Chromosomes Cancer 1993;7:219-26.
24. Simon R, Richter J, Wagner U, et al. High-throughput tissue microarray analysis of 3p25 (RAF1) and 8p12 (FGFR1) copy number alterations in urinary bladder cancer. Cancer Res 2001;61:4514-9.
25. Edwards J, Krishna NS, Witton CJ, et al. Gene amplifications associated with the development of hormone-resistant prostate cancer. Clin Cancer Res 2003;9:5271-81.
26. Kim HR, Kim DJ, Kang DR, et al. Fibroblast growth factor receptor 1 gene amplification is associated with poor survival and cigarette smoking dosage in patients with resected squamous cell lung cancer. J Clin Oncol 2013;31:731-7.
27. Marek L, Ware KE, Fritzsche A, et al. Fibroblast growth factor (FGF) and FGF receptor-mediated autocrine signaling in non-small-cell lung cancer cells. Mol Pharmacol 2009;75:196-207.
28. Agulnik M, Giel JL. Understanding Rechallenge and Resistance in the Tyrosine Kinase Inhibitor Era: Imatinib in Gastrointestinal Stromal Tumor. Am J Clin Oncol 2014;37:417-22.
29. Joensuu H, DeMatteo RP. The management of gastrointestinal stromal tumors: a model for targeted and multidisciplinary therapy of malignancy. Annu Rev Med 2012;63:247-58.
30. Mayo C, Bertran-Alamillo J, Molina-Vila MÁ, et al. Pharmacogenetics of EGFR in lung cancer: perspectives and clinical applications. Pharmacogenomics 2012;13:789-802.

31. Santarpia M, Altavilla G, Salazar MF, et al. Tyrosine kinase inhibitors for non-small-cell lung cancer: finding patients who will be responsive. Expert Rev Respir Med 2011;5:413-24.
32. Camidge DR, Doebele RC. Treating ALK-positive lung cancer--early successes and future challenges. Nat Rev Clin Oncol 2012;9:268-77.
33. Yano S, Takeuchi S, Nakagawa T, et al. Ligand-triggered resistance to molecular targeted drugs in lung cancer: roles of hepatocyte growth factor and epidermal growth factor receptor ligands. Cancer Sci 2012;103:1189-94.
34. Wilson TR, Fridlyand J, Yan Y, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature 2012;487:505-9.
35. Costa DB, Halmos B, Kumar A, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med 2007;4:1669-79; discussion 1680.
36. Garofalo M, Romano G, Di Leva G, et al. EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers. Nat Med 2011;18:74-82.
37. Ng KP, Hillmer AM, Chuah CT, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 2012;18:521-8.
38. Okamoto K, Okamoto I, Okamoto W, et al. Role of survivin in EGFR inhibitor-induced apoptosis in non-small cell lung cancers positive for EGFR mutations. Cancer Res 2010;70:10402-10.
39. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 2007;131:18-43.
40. Gruver AM, Peerwani Z, Tubbs RR. Out of the darkness and into the light: bright field in situ hybridisation for delineation of ERBB2 (HER2) status in breast carcinoma. J Clin Pathol 2010;63:210-9.
41. Schildhaus HU, Heukamp LC, Merkelbach-Bruse S, et al. Definition of a fluorescence in-situ hybridization score identifies high- and low-level FGFR1 amplification types in squamous cell lung cancer. Mod Pathol 2012;25:1473-80.
42. Massion PP, Taflan PM, Jamshedur Rahman SM, et al. Significance of p63 amplification and overexpression in lung cancer development and prognosis. Cancer Res 2003;63:7113-21.
43. Pelosi G, Del Curto B, Trubia M, et al. 3q26 Amplification and polysomy of chromosome 3 in squamous cell lesions of the lung: a fluorescence in situ hybridization study. Clin Cancer Res 2007;13:1995-2004.
44. Massion PP, Kuo WL, Stokoe D, et al. Genomic copy number analysis of non-small cell lung cancer using array comparative genomic hybridization: implications of the phosphatidylinositol 3-kinase pathway. Cancer Res 2002;62:3636-40.
45. Massion PP, Taflan PM, Shyr Y, et al. Early involvement of the phosphatidylinositol 3-kinase/Akt pathway in lung cancer progression. Am J Respir Crit Care Med 2004;170:1088-94.
46. Spoerke JM, O'Brien C, Huw L, et al. Phosphoinositide 3-kinase (PI3K) pathway alterations are associated with histologic subtypes and are predictive of sensitivity to PI3K inhibitors in lung cancer preclinical models. Clin Cancer Res 2012;18:6771-83.
47. Rodriguez-Pinilla SM, Sarrio D, Moreno-Bueno G, et al. Sox2: a possible driver of the basal-like phenotype in sporadic breast cancer. Mod Pathol 2007;20:474-81.
48. Bass AJ, Watanabe H, Mermel CH, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41:1238-42.
49. Gen Y, Yasui K, Zen Y, et al. SOX2 identified as a target gene for the amplification at 3q26 that is frequently detected in esophageal squamous cell carcinoma. Cancer Genet Cytogenet 2010;202:82-93.
50. Wilbertz T, Wagner P, Petersen K, et al. SOX2 gene amplification and protein overexpression are associated with better outcome in squamous cell lung cancer. Mod Pathol 2011;24:944-53.
51. Regala RP, Weems C, Jamieson L, et al. Atypical protein kinase C iota plays a critical role in human lung cancer cell growth and tumorigenicity. J Biol Chem 2005;280:31109-15.
52. Regala RP, Weems C, Jamieson L, et al. Atypical protein kinase C iota is an oncogene in human non-small cell lung cancer. Cancer Res 2005;65:8905-11.
53. Mahajan NP, Whang YE, Mohler JL, et al. Activated tyrosine kinase Ack1 promotes prostate tumorigenesis: role of Ack1 in polyubiquitination of tumor suppressor Wwox. Cancer Res 2005;65:10514-23.
54. van der Horst EH, Degenhardt YY, Strelow A, et al. Metastatic properties and genomic amplification of the tyrosine kinase gene ACK1. Proc Natl Acad Sci U S A 2005;102:15901-6.
55. Shayesteh L, Lu Y, Kuo WL, et al. PIK3CA is implicated as

an oncogene in ovarian cancer. Nat Genet 1999;21:99-102.
56. Ma YY, Wei SJ, Lin YC, et al. PIK3CA as an oncogene in cervical cancer. Oncogene 2000;19:2739-44.
57. Redon R, Muller D, Caulee K, et al. A simple specific pattern of chromosomal aberrations at early stages of head and neck squamous cell carcinomas: PIK3CA but not p63 gene as a likely target of 3q26-qter gains. Cancer Res 2001;61:4122-9.
58. Massion PP, Taflan PM, Rahman SM, et al. Role of p63 amplification and overexpression in lung cancer development. Chest 2004;125:102S.
59. Tonon G, Wong KK, Maulik G, et al. High-resolution genomic profiles of human lung cancer. Proc Natl Acad Sci U S A 2005;102:9625-30.
60. Ji M, Guan H, Gao C, et al. Highly frequent promoter methylation and PIK3CA amplification in non-small cell lung cancer (NSCLC). BMC Cancer 2011;11:147.
61. Okudela K, Suzuki M, Kageyama S, et al. PIK3CA mutation and amplification in human lung cancer. Pathol Int 2007;57:664-71.
62. Yamamoto H, Shigematsu H, Nomura M, et al. PIK3CA mutations and copy number gains in human lung cancers. Cancer Res 2008;68:6913-21.
63. Melino G. p63 is a suppressor of tumorigenesis and metastasis interacting with mutant p53. Cell Death Differ 2011;18:1487-99.
64. Frederick MJ, VanMeter AJ, Gadhikar MA, et al. Phosphoproteomic analysis of signaling pathways in head and neck squamous cell carcinoma patient samples. Am J Pathol 2011;178:548-71.
65. Hibi K, Trink B, Patturajan M, et al. AIS is an oncogene amplified in squamous cell carcinoma. Proc Natl Acad Sci U S A 2000;97:5462-7.
66. Bishop JA, Teruya-Feldstein J, Westra WH, et al. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol 2012;25:405-15.
67. Nonaka D. A study of ΔNp63 expression in lung non-small cell carcinomas. Am J Surg Pathol 2012;36:895-9.
68. Hussenet T, Dali S, Exinger J, et al. SOX2 is an oncogene activated by recurrent 3q26.3 amplifications in human lung squamous cell carcinomas. PLoS One 2010;5:e8960.
69. Rudin CM, Durinck S, Stawiski EW, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 2012;44:1111-6.
70. Löffler H, Fechter A, Matuszewska M, et al. Cep63 recruits Cdk1 to the centrosome: implications for regulation of mitotic entry, centrosome amplification, and genome maintenance. Cancer Res 2011;71:2129-39.
71. Smith E, Dejsuphong D, Balestrini A, et al. An ATM- and ATR-dependent checkpoint inactivates spindle assembly by targeting CEP63. Nat Cell Biol 2009;11:278-85.
72. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.
73. Murray NR, Kalari KR, Fields AP. Protein kinase Cι expression and oncogenic signaling mechanisms in cancer. J Cell Physiol 2011;226:879-87.
74. Liu SG, Wang BS, Jiang YY, et al. Atypical protein kinase Cι (PKCι) promotes metastasis of esophageal squamous cell carcinoma by enhancing resistance to Anoikis via PKCι-SKP2-AKT pathway. Mol Cancer Res 2011;9:390-402.
75. Yang YL, Chu JY, Luo ML, et al. Amplification of PRKCI, located in 3q26, is associated with lymph node metastasis in esophageal squamous cell carcinoma. Genes Chromosomes Cancer 2008;47:127-36.
76. Erdogan E, Klee EW, Thompson EA, et al. Meta-analysis of oncogenic protein kinase Ciota signaling in lung adenocarcinoma. Clin Cancer Res 2009;15:1527-33.
77. Regala RP, Davis RK, Kunz A, et al. Atypical protein kinase C{iota} is required for bronchioalveolar stem cell expansion and lung tumorigenesis. Cancer Res 2009;69:7603-11.
78. Justilien V, Jameison L, Der CJ, et al. Oncogenic activity of Ect2 is regulated through protein kinase C iota-mediated phosphorylation. J Biol Chem 2011;286:8149-57.
79. Justilien V, Fields AP. Ect2 links the PKCiota-Par6alpha complex to Rac1 activation and cellular transformation. Oncogene 2009;28:3597-607.
80. Kelley LC, Weed SA. Cortactin is a substrate of activated Cdc42-associated kinase 1 (ACK1) during ligand-induced epidermal growth factor receptor downregulation. PLoS One 2012;7:e44363.
81. Lin Q, Wang J, Childress C, et al. The activation mechanism of ACK1 (activated Cdc42-associated tyrosine kinase 1). Biochem J 2012;445:255-64.
82. Pao-Chun L, Chan PM, Chan W, et al. Cytoplasmic ACK1 interaction with multiple receptor tyrosine kinases is mediated by Grb2: an analysis of ACK1 effects on Axl signaling. J Biol Chem 2009;284:34954-63.
83. Mahajan NP, Liu Y, Majumder S, et al. Activated Cdc42-associated kinase Ack1 promotes prostate cancer progression via androgen receptor tyrosine phosphorylation. Proc Natl Acad Sci U S A 2007;104:8438-43.
84. Galisteo ML, Yang Y, Ureña J, et al. Activation of the

nonreceptor protein tyrosine kinase Ack by multiple extracellular stimuli. Proc Natl Acad Sci U S A 2006;103:9796-801.
85. Mahajan K, Mahajan NP. Shepherding AKT and androgen receptor by Ack1 tyrosine kinase. J Cell Physiol 2010;224:327-33.
86. Mahajan K, Mahajan NP. PI3K-independent AKT activation in cancers: a treasure trove for novel therapeutics. J Cell Physiol 2012;227:3178-84.
87. Chan W, Tian R, Lee YF, et al. Down-regulation of active ACK1 is mediated by association with the E3 ubiquitin ligase Nedd4-2. J Biol Chem 2009;284:8185-94.
88. Mahajan K, Coppola D, Challa S, et al. Ack1 mediated AKT/PKB tyrosine 176 phosphorylation regulates its activation. PLoS One 2010;5:e9646.
89. Lin Q, Wang J, Childress C, et al. HECT E3 ubiquitin ligase Nedd4-1 ubiquitinates ACK and regulates epidermal growth factor (EGF)-induced degradation of EGF receptor and ACK. Mol Cell Biol 2010;30:1541-54.

Cite this article as: Mendez P, Ramirez JL. Copy number gains of FGFR1 and 3q chromosome in squamous cell carcinoma of the lung. Transl Lung Cancer Res 2013;2(2):101-111. doi: 10.3978/j.issn.2218-6751.2013.03.05

Predictive models for customizing chemotherapy in advanced non-small cell lung cancer (NSCLC)

Laura Bonanno

Medical Oncology 2, Istituto Oncologico Veneto I.R.C.C.S., Padova, Italia

Correspondence to: Laura Bonanno. Medical Oncology 2, Istituto Oncologico Veneto I.R.C.C.S., Padova, Italia. Email: laura.bonanno@ioveneto.it.

Abstract: The backbone of first-line treatment for Epidermal Growth Factor (EGFR) wild-type (wt) advanced Non-small cell lung cancer (NSCLC) patients is the use of a platinum-based chemotherapy combination. The treatment is characterized by great inter-individual variability in outcome. Molecular predictive markers are extremely needed in order to identify patients most likely to benefit from platinum-based treatment and resistant ones, thus optimizing chemotherapy approach in NSCLC. Several components of DNA repair response (DRR) have been investigated as potential predictive markers. Among them, high levels of expression of ERCC1, both at protein and mRNA levels, have been associated with resistance to cisplatin in NSCLC. In addition, low levels of expression of RRM1, a target for gemcitabine, have been associated with improved OS in advanced NSCLC patients treated with cisplatin and gemcitabine. Preclinical data and retrospective analyses showed that BRCA1 is able to induce resistance to cisplatin and sensitivity to antimicrotubule agents. In addition, the mRNA levels of expression of *RAP80*, encoding for a protein cooperating with BRCA1 in homologous recombination (HR), have demonstrated to further sub-classify low BRCA1 NSCLC tumors, improving the predictive model. On the basis of biological knowledge on DNA repair pathway and recent controversial results from clinical validation of potential molecular markers, integrated analysis of multiple DNA repair components could improve predictive information and pave the way to a new approach to customized chemotherapy clinical trials.

Keywords: Platinum; ercc1; brca1; dna repair; predictive modeling

Submitted Feb 10, 2013. Accepted for publication Mar 12, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.07
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.07

Introduction

The most important progresses in the field of advanced NSCLC disease are related to the capacity of individuating so-called driver-mutations, that is molecular alterations able to render the tumors specifically sensitive to targeted therapy. The first and best known example is the discovery of *EGFR* mutations, characterizing a subgroup of tumors in which the treatment with EGFR selective inhibitors (*gefitinib*, *erlotinib*, *afatinib*) significantly improves survival and quality of life (1-6). Currently, we also know that patients carrying *ALK* rearrangements could significantly benefit from *crizotinib* treatment (7) and that we can individuate several other subgroups of patients with lung adenocarcinoma characterized by dysregulation of main oncogenic pathways induced by a specific genetic alteration (8,9). Finally, a series of potentially targetable molecular alterations have been recently found also in squamous cell carcinoma (SCC) (10). Nevertheless, still about 80% of advanced NSCLC patients receive standard first-line chemotherapy treatment and their best therapeutic option is considered platinum-based chemotherapy, when clinically feasible. Clinical and radiological responses are obtained only in a subgroup of these patients and the median overall survival (OS) of the chemotherapy-treated population is still inferior to one year. Moreover, platinum-based chemotherapy is currently the standard second-line treatment after progression to an EGFR-inhibitor in EGFR-mutated patients.

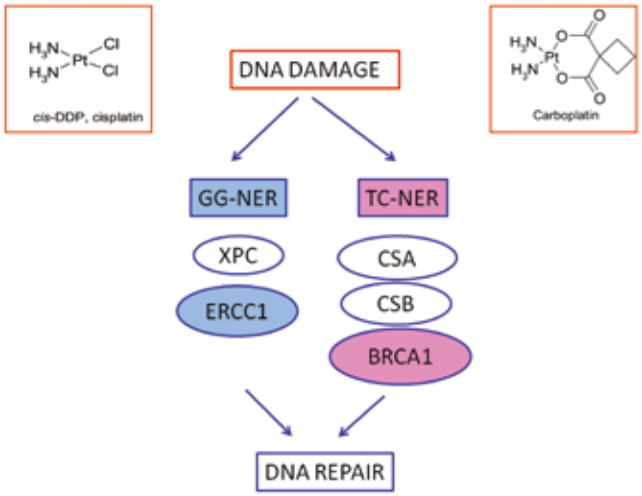


Figure 1 The figure shows the two simplified sub-pathways of nucleotide excision repair (NER): Global-genome (GG)-NER targets the whole genome sequences, while transcription-coupled (TC)-NER recognizes specifically lesions involving actively transcribed DNA. ERCC1 is a fundamental element in GG-NER, while BRCA1 is mainly involved in TC-NER.

In this clinical context, the aim of the research concerning molecular predictive markers of platinum sensitivity is to optimize chemotherapy approach and provide more precise information to patient at diagnosis.

Biological rationale for predictive models in NSCLC

Cisplatin and carboplatin act as DNA-damaging agents and have largely overlapping resistance mechanisms. For this reason, defective DNA repair capacity, one of the main factors responsible for carcinogenesis, may contribute to the cytotoxic effect of the drugs. On the other hand, DNA repair capacity, contributing to genome stability, is one of the most studied mechanisms of platinum resistance.

Cellular DNA repair capacity depends on complex inter-related mechanisms, also interacting with cell cycle control and apoptotic pathways. For this reason, considerable efforts have been made to validate predictive markers as surrogate of DNA repair capacity and, in particular, of the capacity of repairing the lesions induced by platinum on DNA.

Cisplatin and carboplatin inhibit DNA replication mainly acting as cross-links inducing agents. They bind DNA, and in prevalence nucleophilic N7-sites on purine bases, leading to the generation of protein-DNA and DNA-DNA intra- and, less commonly, interstrand adducts. Platinum-induced lesions cause distortions in DNA structure that are recognized by multiple DNA repair pathways. These DNA distortions are mainly repaired by the nucleotide excision repair (NER) system. NER is a pathway involved in DRR specifically targeting DNA helix-distorting lesions, including cisplatin- and ultraviolet-induced lesions. It functions as a so-called "cut-and-paste" mechanism including different sequential steps: DNA damage recognition, local opening of the DNA helix around the lesion, damage excision and gap filling. It consists of two sub-pathways: global genome NER (GG-NER) and transcription-coupled NER (TC-NER), sharing the same core mechanism but differing in the way that DNA lesions are recognized and in the target DNA sequences. TC-NER specifically recognizes actively transcribed DNA sequences (*Figure 1*).

The structure-specific endonuclease excision repair cross-complementing 1 (ERCC1) is a protein playing pivotal role in GG-NER. It is thus involved in the rate-limiting step of the pathway, that is incision process. Together with its XP group F (XPF) partner, it cuts the damaged DNA strand at the 5 site of the helix-distorting lesion. In addition, the ERCC1/XPF complex is also involved in the homologous recombination (HR) repair of platinum-induced DNA damage. In tumor experimental models cisplatin exposure is able to increase *ERCC1* mRNA expression levels. The mRNA expression of *ERCC1* correlates with the capacity of DNA adducts repair (11,12) while higher activation of ERCC1 is associated with platinum resistance in several tumor models (13).

RRM1 is the regulatory subunit of ribonucleotide reductase and controls the function of the enzyme involved in deoxynucleotide production. Deoxynucleotide availability is essential to conclude NER and this could explain a potential predictive role for ribonucleotide reductase subunit M1 (RRM1) in patients treated with platinum, in addition to known data about gemcitabine sensitivity. Gemcitabine is an inhibitor of ribonucleotide reductase and increased RRM1 expression has been associated with gemcitabine resistance (14,15). In clinical setting, low mRNA levels of *RRM1* have been associated with improved outcome of patients treated with platinum and gemcitabine, showing a sort of synergism in the DNA-repair-linked resistance mechanisms of the two drugs (16-18).

Replication blocks induced by cisplatin lead to activation of HR, creating the so-called "stalled replication forks" and, in this way, the sequential coordinated action of NER and

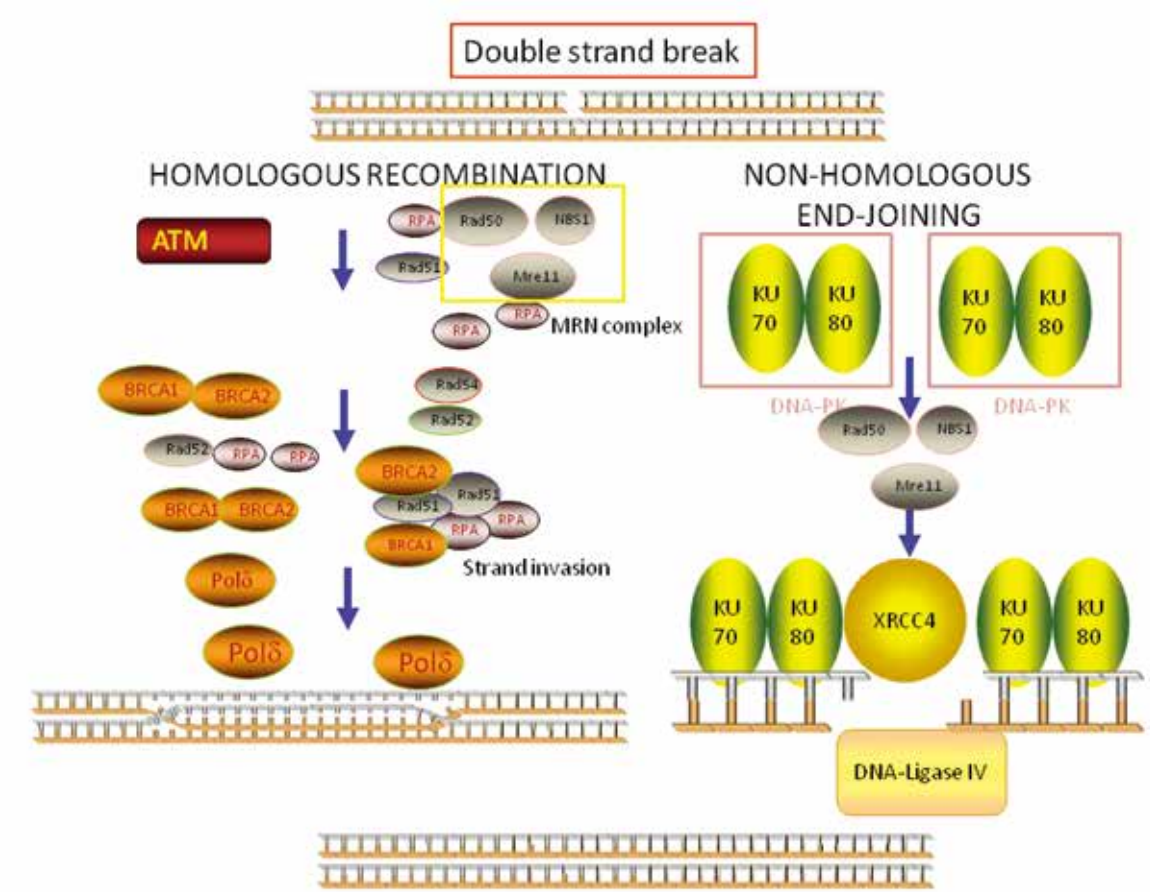


Figure 2 The figure shows the two main pathways involved in the repair of double strand breaks: homologous recombination, an error-free mechanism, using undamaged strand as template, and non-homologous end-joining, an error-prone mechanism.

HR is required for repairing the platinum-induced DNA damage. HR is one of the major pathways involved in DNA double strand breaks (DSBs) repair. It acts using the non-damaged strand as a template and so it is considered an "error-free" system (*Figure 2*). The role of HR in the repair of platinum-induced lesions is at the basis of the potential role of Breast cancer susceptibility gene 1 (BRCA1), one main component of HR, in predicting resistance to platinum.

HR is a complex mechanism initiating with the recognition of DSBs by the multifunctional protein sensor complex (MRE11-RAD50-NBS1), the activation of the check-point phosphoinositide 3-kinase related ATM, ATR and DNA-PK and the subsequent phosphorylation of histone H2AX proteins (19). ATM phosphorylates the mediator of DNA checkpoint 1 (MDC1) at the region surrounding the DSB and in this way triggers the recruitment of DNA repair effectors (*Figure 3*). The assembly process requires a series of post-translational modifications of DNA repair components. In particular, the E3 ubiquitin-ligase RNF8 recognizes the phosphorylated MDC1 and creates a complex with the E3 ubiquitin-ligase RNF168 and the E2 ubiquitin-conjugating enzyme UBC13, leading to the recruitment of BRCA1 (20). The assembly of the RNF8-UBC13 complex is facilitated by the activity of the HECT type E3 ligase (HERC2) (21), an E3 ubiquitin-ligase which can also target BRCA1 for degradation (22). A large proportion of BRCA1 present at DSBs co-localizes with a group of proteins forming a complex called BRCA1-A complex, including also BARD1, BRCC36, ABRAXAS and *RAP80* (*Figure 3*). In particular, RAP80, an ubiquitin-interaction motif (UIM) protein, recognizes the histones ubiquitinated by RNF8/UBC13 and triggers the formation of the complex. In experimental model, the presence of RAP80 is essential for the recruitment of the BRCA1-A complex at DNA damage sites, while its loss

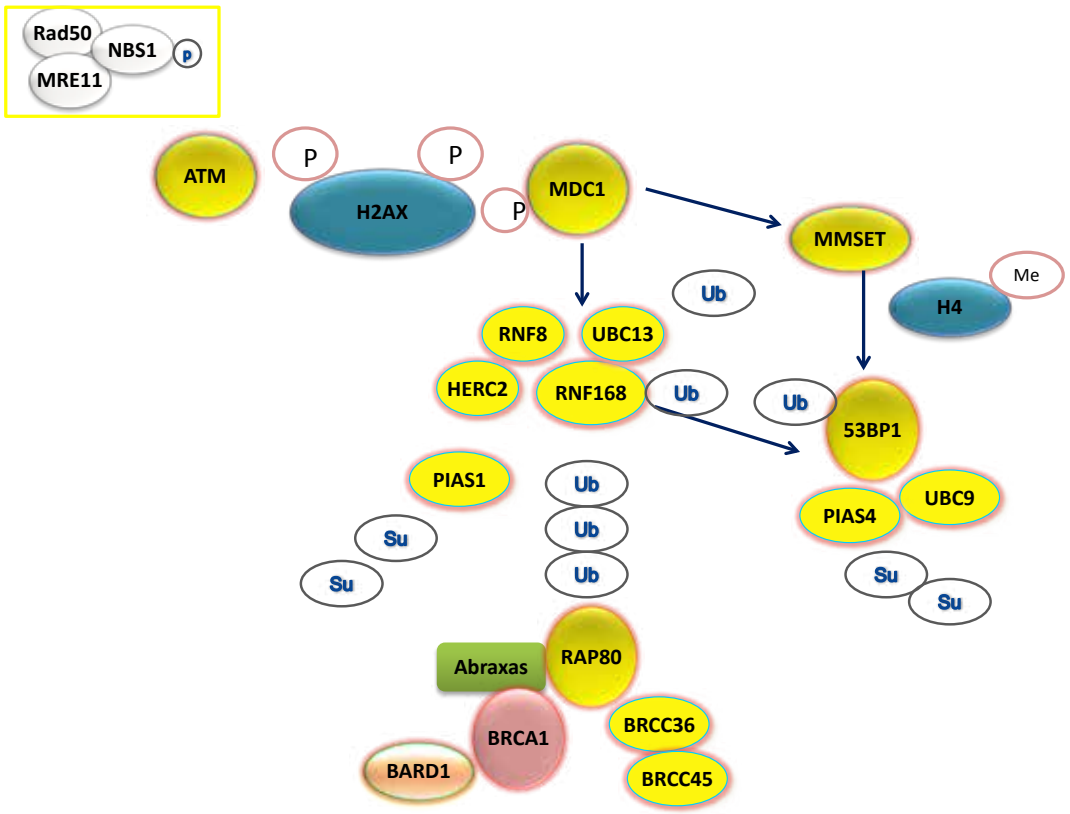


Figure 3 The recruitment of BRCA1-A complex and of 53BP1 at DNA damage sites after double strand breaks. The detailed molecular processes are described in the text.

abrogates the repair response (23-27). On the other hand, BRCA1 forms also other complexes at DNA damage sites, increasing the level of complexity.

Notably, BRCA1 is considered to play a fundamental role also in TC-NER (28,29) (*Figure 1*). This point could be particularly relevant, since GG-NER could have low affinity for cisplatin-induced DNA adducts, while TC-NER is specifically initiated by cisplatin cross-links and an experimental model of TC-NER deficient cells demonstrated hypersensitivity to cisplatin (30-32). The effect of the BRCA1 in determining resistance to platinum has been also directly demonstrated in preclinical models and, in parallel, BRCA1-deficiency has been associated with platinum-resistance (33-35).

BRCA1 is also involved in Non homologous end-joining (NHEJ), an error-prone pathway involved in DSBs repair and in mismatch repair (MMR), which can recognize cisplatin-induced DNA lesions, while normally dealing with erroneous insertions, deletions and mis-incorporations of bases during DNA replication (36).

The role of BRCA1 in HR and NHEJ response to DSBs (*Figure 2*) implies inter-relation with other DNA repair components. In particular, recent findings show a complex functional interplay with 53BPB1, a protein acting as an activator of P53, but also involved in NHEJ and HR. The protein 53BP1 modulates the chromatin structure at DNA damage sites and contributes in maintaining genomic stability (37). In addition, it is able to negatively regulate HR repair, by inhibiting CTIP, a protein that creates a complex with BRCA1 (BRCA1-C complex), thus supporting HR (38). Interestingly, 53BP1 function could contribute to the expression of BRCA1-loss phenotype and, in the absence of 53BP1, HR capacity could be maintained independently on BRCA1 (38). Finally, BRCA1-independent HR capacity can be suppressed by abrogating RNF8, consistently with the complexity of the pathway (39). The protein 53BP1 is recruited at DNA damage sites through two different mechanisms, activated after MDC1 recruitment at DNA damage sites. The first mechanism of 53BP1 recruitment depends on RNF8/UBC13 complex formation and subsequent histone ubiquitination (20). The second mechanism of 53BP1 localization at DSBs is driven

by histone methylation, regulated by the methyltransferase MMSET (40) (*Figure 3*).

In addition to ubiquitination process, the assembly of DNA repair effectors at DSB requires also another post-translational modification process called sumoylation. For this reason, specific small ubiquitin-related modifier (SUMO)-conjugating systems are required. In particular, the E3 ligase PIAS1 and PIAS4 are recruited at DSBs and their depletion reduces BRCA1 accumulation at DSBs (41). PIAS4 forms a complex with E2 ligase UBC9, positively modulating 53BP1 function (42) (*Figure 3*).

The complex and inter-related pathways involved in the repair of platinum-induced DNA lesions explain the difficulty in finding one single marker measurable in patients' blood or tumor biopsies able to effectively predict resistance to platinum in clinical setting.

Strategies for building predictive models in lung cancer

The biological observation that tumor cells with defective capacity of removing cisplatin-DNA adducts are hypersensitive to platinum and the increasing knowledge about DNA repair pathways, forming a complex integrated network, carries great potential clinical application. In translational application of this knowledge the issue is to find a reliable marker able to measure the cellular capacity of repairing platinum-induced DNA damage and clinically performable in samples from patients' blood or small biopsies.

The most direct method of quantifying DNA repair capacity would be to measure and to monitor the rate of unrepaired DNA-adducts in tumor cells following platinum exposure directly (*Table 1*). For practical application, it is possible to measure DNA repair capacity *in vitro* by culturing patients' peripheral lymphocytes and measuring the unrepaired DNA adducts induced by a cross-links inducing agent. Recently, it has been suggested that the DNA repair capacity, quantified with this method, could predict the patients' outcome to platinum-based chemotherapy. A retrospective analysis in a large but heterogeneous population of NSCLC showed a trend for improved overall survival (OS) in patients with the lowest rate of DNA repair capacity measured in peripheral lymphocytes. This trend was higher in the subgroup of patients with stage I-IIIA disease and in adenocarcinoma histology (43).

Anyway, the most studied way to translate preclinical findings about DNA repair and platinum efficacy into clinical benefit is based on the idea that one single protein could lead the repair of DNA-induced damage and that its activity could mirror global capacity of repairing platinum-induced lesions and, consequently, predict resistance to platinum. Actually, most of the clinical data available and currently under prospective evaluation concerns the use of a molecular marker, considered the main protagonist of a DNA repair pathway, as potential predictive marker of platinum resistance (*Table 1*). In particular the most studied molecular markers are the aforementioned ERCC1, BRCA1 and RRM1. These markers have been studied both at protein levels, with immunoistochemistry (ICH), and at mRNA level, through quantitative reverse transcriptase PCR. From the technical point of view, quantitative reverse transcriptase shows high sensitivity and reproducibility. The use of mRNA measurement could be suitable for screening a series of markers in retrospective analyses, and could be successfully performed also in small biopsies and formalin-fixed paraffin-embedded tissues (51), even though it could have some limits in quantifying differences in the expression of very low expressing genes, using this kind of samples. In addition, it could require centralized evaluation in laboratories with long-term experience in the specific field. On the other hand, IHC is a really cost-effective method, although sometimes appearing less reproducible and objective. Once a marker is validated, IHC testing has the potentiality of being performed at each pathology centre providing rapid predictive information, if sufficient tumor material is available. Particularly interesting is also the possibility of studying DNA repair components expression in circulating tumor cells (CTCs), which are present in peripheral blood of patients with metastatic disease and can be analyzed quantitatively and qualitatively with several techniques. The most important point is the possibility to analyze the expression of specific biomarkers in CTCs, as surrogate of tumor samples, and to monitor functional changes of these biomarkers induced by treatment. Preliminary promising data are available from a small cohort of patients and relevant difference in progression free survival (PFS) to platinum-based chemotherapy was described favoring the group of patients lacking ERCC1 expression. Unfortunately, serial evaluation at multiple time points was missing for most of the patients (54) (*Table 1*).

On the basis of DNA repair complexity, some retrospective data are also already available about the possibility that integrated analysis of more than one component of a DNA repair pathway could provide more precise predictive information (51) (*Table 1*).

Table 1 Main clinical data depicted in the text about potential predictive biomarkers in platinum-treated NSCLC patients. In the table the data are summarized according to the methodology used

Methodology	Clincal study type	Clinical setting	Biomarker	Results	Reference
DNA repair capacity in peripheral lymphocytes	Retrospective evaluation	Platinum-treated NSCLC	DNA repair capacity	Trend for improved OS in patients with low DNA repair capacity	(43)
Single DNA repair component at protein level	Retrospective evaluation	Adjuvant	ERCC1	OS benefit from platinum-treatment only in low/negative expressing ps	(44)
	Retrospective evaluation	Adjuvant	ERCC1; BRCA1	Improved DFS for SCC with low/ negative expression of ERCC1	(45)
Single DNA repair component at mRNA level	Retrospective evaluation	Advanced disease	ERCC1; RRM1	Improved OS for low-expressing ps	(18)
	Retrospective evaluation	Advanced disease	ERCC1	Improved OS for low-expressing ps	(46)
	Retrospective evaluation	Advanced disease (second-line)	ERCC1; BRCA1	Higher RR, PFS, OS for low-expressing ps	(47)
	Retrospective evaluation	Advanced disease	RRM1	Improved OS for low-expressing ps	(16)
	Retrospective evaluation	Advanced disease	RRM1	Improved OS for low-expressing ps	(17)
	Individual patient analysis	Advanced disease	ERCC1; RRM1	Improved RR and OS in ps treated with customized CT according to ERCC1 and RRM1	(48)
	Phase III trial	Advanced disease	ERCC1	Improved RR in ps treated with customized CT according to ERCC1	(49)
	Retrospective evaluation	Neoadjuvant	BRCA1	Improved OS for low-expressing ps	(50)
	Phase II	Advanced disease	BRCA1	Feasibility of customized CT according to BRCA1 mRNA	(51)
	Retrospective evaluation	Advanced disease	ERCC1	Negative	(52)
	Retrospective evaluation	Advanced disease	ERCC1; BRCA1	Improved RR, PFS, OS for ps expressing low ERCC1	(53)
CTCs evaluation of a single DNA repair component	Retrospective evaluation	Advanced disease	ERCC1	Improved PFS in ps expressing low ERCC1	(54)
Integrated DNA repair components analysis	Retrospective evaluation	Advanced disease	BRCA1; RAP80	Trend for Improved PFS and OS for ps expressing low BRCA1 and RAP80	(51)

Abbreviations: CTCs, circulating tumor cells; DFS, disease free survival; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression free survival; ps, patients; RR, response rate; SCC, squamous cell carcinoma.

Clinical data available about DNA repair components as molecular predictive markers in NSCLC

Several potential molecular markers have been suggested for predicting the efficacy of platinum-based treatment and some of them are still under clinical evaluation.

ERCC1 and RRM1

In advanced NSCLC samples low *ERCC1* mRNA expression levels have been associated with improved survival after first-line treatment with cisplatin and gemcitabine, thus seemingly confirming the biological resistance model (18,46). Recently, a predictive value for *ERCC1* (and *BRCA1*) mRNA expression

has been confirmed also in a series of patients treated with a platinum-based combination in second-line setting (47) (*Table 1*).

A meta-analysis including the results of 12 selected studies confirmed the potential predictive value of ERCC1, either at mRNA or at protein level, showing that negative/low ERCC1 expressing NSCLC patients treated with platinum-based combinations achieve higher response rate (RR) and improved median OS. In parallel, it indicated little difference favoring IHC in the results according to the technique used. The predictive effect seemed higher in the Asiatic population, compared to the Caucasian one and in patients treated with a combination including cisplatin, while it decreased when considering also carboplatin-based chemotherapy (55).

In advanced patients treated with this platinum and gemcitabine, also low RRM1 expression levels correlate with improved outcome (14,16-17). On the other hand, RRM1 is known to be associated with resistance to gemcitabine even when administered in non-platinum containing combinations (56-58). A meta-analysis, including the results of 18 selected trials, indicated an improved RR, PFS and OS for NSCLC expressing negative/low RRM1 treated with gemcitabine-containing chemotherapy (59).

A recently published individual patient analysis suggested that customizing chemotherapy according to ERCC1 and RRM1 expression could be translated into improved outcome for advanced NSCLC patients. The analysis included a comparison between two study populations, including four prospectively accrued cohorts of patients: the "personalized treatment" group and the "standard treatment" one (48). In the "personalized" group of treatment, ERCC1 and RRM1 were measured at mRNA level through real-time reverse transcriptase; patients whose tumors expressed low levels of both genes received carboplatin and gemcitabine, those with high levels of both genes received docetaxel and vinorelbine, patients with high *ERCC1* and low *RRM1* received gemcitabine and docetaxel, those with low *ERCC1* but high *RRM1* were treated with carboplatin and docetaxel (60). Even though the design of the study implies only hypothesis-generating results, it is encouraging to see that patients accrued in a phase II trial (NCT00215930) and thus treated according to the levels of expression of *ERCC1* and *RRM1* showed statistically significant improvement, both in terms of RR and of OS, when compared with patients receiving a "non-molecularly-driven" treatment (48) (*Table 1*).

In the adjuvant setting the predictive role of ERCC1 was suggested for the first time by an important retrospective analysis of tumor samples from patients enrolled in the International Adjuvant Lung Cancer Trial (IALT). The benefit of cisplatin-based adjuvant chemotherapy in terms of OS was limited to patients whose tumors did not express ERCC1. On the contrary, the expression of the marker was associated with improved prognosis in the group of patients who did not receive adjuvant chemotherapy. In this large study, ERCC1 was investigated at protein level using ICH (44) (*Table 1*).

Only the results of prospective phase III trials will let us draw definitive conclusions. Prospective results on the predictive role of ERCC1 are available in advanced disease setting. Standard chemotherapy with cisplatin and docetaxel was compared with customized treatment according to the mRNA levels of expression of *ERCC1* measured in pretreatment biopsies. In the experimental arm, patients with low levels of *ERCC1* received cisplatin and docetaxel, patients with high *ERCC1* received docetaxel and gemcitabine. The patients allocated to the experimental arm demonstrated a significantly improved response rate (RR), not mirrored by increase of OS (49). This was the first prospective phase III clinical trial with available results in the field of customized chemotherapy and raised many questions about the methods to use to customize chemotherapy and the best chemotherapy combination for studying predictive models. Currently, several prospective trials are ongoing to validate ERCC1 predictive role both in early stage (phase III: TASTE, NCT00775385; ITACA) and in advanced stage disease (phase III: NCT00801736; NCT00499109; phase II: NCT01648517, NCT01356368; NCT00736814) (*Table 2*). In most of the studies the predictive value of ERCC1 is analyzed in conjuction with RRM1 evaluation, in particular when considering chemotherapy first-line combination including cis- or carboplatin and gemcitabine.

BRCA1

Clinical investigation concerning the predictive role of BRCA1 expression in NSCLC has retrospectively confirmed that low mRNA expression is correlated with good prognosis and increased sensitivity to cisplatin (50,61). A retrospective analysis in paraffin-embedded NSCLC samples, collected before neoadjuvant treatment with cisplatin and gemcitabine, demonstrated that the group of tumors with the lowest expression of *BRCA1* obtained the greatest benefit from cisplatin-gemcitabine treatment (50) (*Table 1*). On the basis of this finding, a prospective phase II trial was planned. In this

Table 2 Ongoing prospective clinical trial evaluating the predictive role of ERCC1, RRM1 and BRCA1

Trial number	Phase	Clinical setting	Biomarker	Methodology
NCT00775385	Phase II-III	Adjuvant (II-IIIA)	ERCC1	IHC
NCT00801736	Phase III	Advanced disease	ERCC1	NA
NCT00499109	Phase III	Advanced disease	ERCC1; RRM1	NA
NCT01648517	Phase II	Advanced disease	ERCC1; RRM1	mRNA
NCT01356368	Phase II	Advanced disease	ERCC1; RRM1	NA
NCT00736814	Phase II	Advanced disease	ERCC1; RRM1	mRNA
NCT00617656	Phase III	Advanced disease	BRCA1; RAP80	mRNA
NCT00478699	Phase III	Adjuvant (II-IIIA)	BRCA1	mRNA
Eudra-ct: 2008-001764-36	Phase III	Adjuvant (II-IIIA)	ERCC1; TS	IHC and mRNA

Abbreviations: IHC, immunohistochemistry; NA, not available.

trial, the treatment of advanced non-squamous NCSLCs was customized according to mRNA expression levels of *BRCA1* measured by reverse transcriptase PCR. Patients whose tumors expressed low levels of *BRCA1* received cisplatin and gemcitabine in first line setting; patients with high levels of the two received docetaxel alone, while patients with intermediate expression of BRCA1 were treated with cisplatin and docetaxel. The study demonstrated the feasibility of *BRCA1* expression analysis in clinical practice and the median OS achieved was similar in the three genotyped groups. The samples from patients enrolled in the prospective trial were retrospectively analyzed to measure mRNA expression of *RAP80* and *ABRAXAS*, as main components of BRCA1-A complex (*Figure 3*). The mRNA expression of RAP80 resulted as potential new predictive marker, able to further subclassify the outcome of low-*BRCA1* expressing patients. In the study population, patients with low levels of both *BRCA1* and *RAP80* obtained an impressive median PFS of 14 months (51).

Consequently, a multi-centric phase III trial has been coordinated in order to confirm the predictive value of integrated *BRCA1-RAP80* analysis and their applicability in clinical practice (BREC, NTC00617656) (*Table 2*). In the trial, the outcome of advanced NSCLC patients treated with non-personalized chemotherapy (cisplatin-docetaxel) in first-line setting is compared to the one of patients receiving customized chemotherapy. The primary end-point is time to progression (TTP). All the samples of patients allocated to the experimental arm are analyzed for mRNA expression of *BRCA1* and *RAP80* through real-time PCR and the levels of expression of the two genes are categorized using tertiles as cut-off points. Patients in the experimental arm receive cisplatin and gemcitabine if *RAP80* is low, independently on the levels of *BRCA1*, cisplatin and docetaxel if *RAP80* is intermediate or high in the presence of low or intermediate BRCA1, docetaxel alone when *BRCA1* is high and *RAP80* intermediate or high. The accrual has been completed and interim analysis results will be soon available. Another phase III prospective trial is ongoing to test the predictive value of *BRCA1* in the adjuvant setting (GEPC-SCAT, NCT00478699) (*Table 2*). Patients with stage II or IIIA NSCLCs, after complete surgical resection, are randomized to receive adjuvant chemotherapy with cisplatin and docetaxel or adjuvant chemotherapy customized according to *BRCA1* mRNA levels. Patients allocated to the experimental arm receive cisplatin and gemcitabine if *BRCA1* is low, cisplatin and docetaxel if *BRCA1* is intermediate, docetaxel alone if *BRCA1* is high.

New perspectives: integrated analysis of multiple DNA repair components and histology-driven analyses

While prospective trials are ongoing, controversial data about DNA repair components as predictive markers of platinum-based chemotherapy efficacy are available. In other words, not all the retrospective series confirmed the predictive role of ERCC1 and BRCA1 and several are the possible explanations. Among the most recent retrospective series, an analysis of mRNA expression levels of *ERCC1* in formalin-fixed paraffin embedded tumor samples did not confirm the predictive role of *ERCC1*. The mRNA expression of *ERCC1* was neither correlated to RR nor to OS in advanced NSCLC patients prospectively recruited in a phase III trial and treated with platinum-

based chemotherapy (52). In a more recent retrospective evaluation, tumor tissues from patients prospectively enrolled in a phase III trial and treated with platinum and gemcitabine were analyzed for the expression of six DNA repair components. In this case, the authors found no predictive value for BRCA1 and RAP80 mRNA, whereas low ERCC1 and ABRAXAS levels were associated with increased RR and improved OS mRNA and PFS. Notably, in this study only 45 patients out of 137 had sufficient tumor material to perform planned analyses (53). These results are also in contrast with the data of the retrospective analysis published in 2009, showing potential predictive value for RAP80, but not for ABRAXAS (51). In a larger series of patients, treated with platinum-based chemotherapy in adjuvant setting, protein expression of seven DNA repair components has been analyzed using IHC. The number of cases with evaluable results was variable according to the different biomarkers, with a range of 550-716 cases. Despite the large study population, neither tested biomarker was able to predict the benefit from adjuvant chemotherapy with statistical significance. The analyzed DNA repair components were generally expressed at higher levels in squamous cell carcinomas, with respect to adenocarcinoma histology. In the analysis by histology, higher benefit from platinum-based adjuvant chemotherapy was demonstrated in squamous cell carcinoma patients with low expression of ERCC1 and ATM. This difference in outcome was measured in terms of disease free survival (DFS) (45). Actually, histology has gained increasing role in NSCLC definition and treatment decision making in the latest years and we know that different biology characterizes adenocarcinoma *versus* squamous cell carcinoma.

All mentioned controversial results open new questions and suggest a new approach to predictive modeling in lung cancer.

Probably analysis by histology will help in the interpretation of retrospective analyses and ongoing prospective validation of potential predictive markers. Squamous cell carcinoma should probably be analyzed separately for non-completely known biological reason. One possible explanation is that the pattern of expression of DRR genes is different according to histology. Recently we have confirmed higher levels of expression of BRCA1, but also 53BP1 and UBC9, in squamous histology in a retrospective series of 115 advanced NSCLCs (62). Also in our series the differential expression according to histology was not mirrored by differential sensitivity to platinum-based chemotherapy according to histology. Another point to take into account is the differential sensitivity to different platinum-based chemotherapy according to histology. It is possible that the chemotherapy combinations could influence the outcome of patients differently in different histological subtypes, increasing the level of complexity. We already know that the partner for platinum in chemotherapy combinations could influence predictive modeling interpretation. As a matter of fact, platinum and gemcitabine seem to show a sort of synergism in DNA repair-associated resistance mechanisms (16-18), whereas taxanes and vinca alkaloids could not be the best partner for platinum in customized chemotherapy approach. BRCA1 is modulator of cellular response to chemotherapy drugs, inducing resistance to platinum and sensitivity to antimicrotubule agents (63). Finally, when interpreting the results in retrospective series treated with platinum-based combinations also containing pemetrexed, the well-known differential sensitivity to the drugs should be taken into account according to histology (64) and the molecular modulators of pemetrexed sensitivity (65).

In addition to histology-driven analyses, predictive information in lung cancer could be improved by building predictive models able to integrate the influence of several DNA repair components, contributing to cellular response to DNA repair damage. Actually, cellular response to DNA damage includes redundant mechanisms normally contributing to genome stability and interacting in sometimes unexpected ways to chemotherapy-induced DNA damage. We know that *RAP80* expression could improve *BRCA1* predictive information, when analyzed at mRNA levels in low *BRCA1* expressing NSCLCs (51). One of the most interesting inter-relation is the one between BRCA1 and 53BP1. We have recently explored the predictive value of integrated BRCA1 and 53BP1 analysis in advanced NSCLC patients treated with a platinum-based combination not including antimicrotubules agents in first-line setting. In this series, we measured *BRCA1*, 53BP1 and other six components of 53BP1 pathway at mRNA level using real-time PCR. The levels of mRNA expression were considered as categorical variables using median values as cut-off points. *BRCA1* was not confirmed as predictive marker, when considered in isolation. Among patients expressing low levels of *BRCA1* mRNA, patients with low levels of *53BP1* obtained an impressive median OS of more than 19 months and a PFS of 10 months, in sharp contrast with patients with low BRCA1 and high 53BP1 (median OS: 8.2 months, median PFS: 5.9 months; P=0.01 for OS, P<0.0001 for PFS). In patients with high levels of

BRCA1 the median OS was 10 months, independently on 53BP1 (62). These results demonstrate the potentiality of integrated predictive modeling in lung cancer.

Conclusions

Current clinical data concerning the potential predictive role of DDR components require confirmation by large prospective randomized phase III trials but highlight the possibility of significant improvement in outcome of advanced NSCLC patients treated with chemotherapy. Available data suggest that different platinum-based doublets should be analyzed separately, considering the possible influences of different predictive markers and that histology-driven analysis could improve predictive modeling interpretation. In addition, only optimal clinical stratification of patients will permit correct interpretation of results concerning predictive biomarkers. Finally, current results demonstrate that it is difficult to identify a single marker able to predict response to a drug or a combination of drugs. Integrated analysis of several potential biomarkers based on the study of DNA repair pathways biology will probably provide more insight in predictive modeling in lung cancer.

Acknowledgements

The author thanks Martina Boscaro for reviewing the language of the article.
Disclosure: The author declares no conflict of interest.

References

1. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer 2010;10:760-74.
2. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
3. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
4. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-8.
5. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
6. Yang JC, Shih JY, Su WC, et al. Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): a phase 2 trial. Lancet Oncol 2012;13:539-48.
7. Camidge DR, Bang YJ, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. Lancet Oncol 2012;13:1011-9.
8. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455:1069-75.
9. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011;12:175-80.
10. Drilon A, Rekhtman N, Ladanyi M, et al. Squamous-cell carcinomas of the lung: emerging biology, controversies, and the promise of targeted therapy. Lancet Oncol 2012;13:e418-26.
11. Li Q, Gardner K, Zhang L, et al. Cisplatin induction of ERCC-1 mRNA expression in A2780/CP70 human ovarian cancer cells. J Biol Chem 1998;273:23419-25.
12. Li Q, Yu JJ, Mu C, et al. Association between the level of ERCC-1 expression and the repair of cisplatin-induced DNA damage in human ovarian cancer cells. Anticancer Res 2000;20:645-52.
13. Arora S, Kothandapani A, Tillison K, et al. Downregulation of XPF-ERCC1 enhances cisplatin efficacy in cancer cells. DNA Repair (Amst) 2010;9:745-53.
14. Bepler G, Kusmartseva I, Sharma S, et al. RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. J Clin Oncol 2006;24:4731-7.
15. Davidson JD, Ma L, Flagella M, et al. An increase in the expression of ribonucleotide reductase large subunit 1 is associated with gemcitabine resistance in non-small cell lung cancer cell lines. Cancer Res 2004;64:3761-6.
16. Rosell R, Scagliotti G, Danenberg KD, et al. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. Oncogene 2003;22:3548-53.
17. Rosell R, Danenberg KD, Alberola V, et al. Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. Clin Cancer Res 2004;10:1318-25.

18. Ceppi P, Volante M, Novello S, et al. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. Ann Oncol 2006;17:1818-25.
19. Williams RS, Williams JS, Tainer JA. Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. Biochem Cell Biol 2007;85:509-20.
20. Yan J, Jetten AM. RAP80 and RNF8, key players in the recruitment of repair proteins to DNA damage sites. Cancer Lett 2008;271:179-90.
21. Bekker-Jensen S, Rendtlew Danielsen J, Fugger K, et al. HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes. Nat Cell Biol 2010;12:80-6;sup pp 1-12.
22. Wu W, Sato K, Koike A, et al. HERC2 is an E3 ligase that targets BRCA1 for degradation. Cancer Res 2010;70:6384-92.
23. Wang B, Matsuoka S, Ballif BA, et al. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science 2007;316:1194-8.
24. Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science 2007;316:1202-5.
25. Sobhian B, Shao G, Lilli DR, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science 2007;316:1198-202.
26. Yan J, Kim YS, Yang XP, et al. The ubiquitin-interacting motif containing protein RAP80 interacts with BRCA1 and functions in DNA damage repair response. Cancer Res 2007;67:6647-56.
27. Wu J, Liu C, Chen J, et al. RAP80 protein is important for genomic stability and is required for stabilizing BRCA1-A complex at DNA damage sites in vivo. J Biol Chem 2012;287:22919-26.
28. Le Page F, Randrianarison V, Marot D, et al. BRCA1 and BRCA2 are necessary for the transcription-coupled repair of the oxidative 8-oxoguanine lesion in human cells. Cancer Res 2000;60:5548-52.
29. Abbott DW, Thompson ME, Robinson-Benion C, et al. BRCA1 expression restores radiation resistance in BRCA1-defective cancer cells through enhancement of transcription-coupled DNA repair. J Biol Chem 1999;274:18808-12.
30. Lainé JP, Egly JM. Initiation of DNA repair mediated by a stalled RNA polymerase IIO. EMBO J 2006;25:387-97.
31. Tremeau-Bravard A, Riedl T, Egly JM, et al. Fate of RNA polymerase II stalled at a cisplatin lesion. J Biol Chem 2004;279:7751-9.
32. Furuta T, Ueda T, Aune G, et al. Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. Cancer Res 2002;62:4899-902.
33. Bhattacharyya A, Ear US, Koller BH, et al. The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. J Biol Chem 2000;275:23899-903.
34. Husain A, He G, Venkatraman ES, et al. BRCA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum(II). Cancer Res 1998;58:1120-3.
35. Tassone P, Di Martino MT, Ventura M, et al. Loss of BRCA1 function increases the antitumor activity of cisplatin against human breast cancer xenografts in vivo. Cancer Biol Ther 2009;8:648-53.
36. Kennedy RD, Quinn JE, Johnston PG, et al. BRCA1: mechanisms of inactivation and implications for management of patients. Lancet 2002;360:1007-14.
37. Bothmer A, Robbiani DF, Di Virgilio M, et al. Regulation of DNA end joining, resection, and immunoglobulin class switch recombination by 53BP1. Mol Cell 2011;42:319-29.
38. Bunting SF, Callén E, Wong N, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 2010;141:243-54.
39. Nakada S, Yonamine RM, Matsuo K. RNF8 regulates assembly of RAD51 at DNA double-strand breaks in the absence of BRCA1 and 53BP1. Cancer Res 2012;72:4974-83.
40. Pei H, Zhang L, Luo K, et al. MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature 2011;470:124-8.
41. Morris JR, Boutell C, Keppler M, et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 2009;462:886-90.
42. Galanty Y, Belotserkovskaya R, Coates J, et al. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 2009;462:935-9.
43. Wang LE, Yin M, Dong Q, et al. DNA repair capacity in peripheral lymphocytes predicts survival of patients with non-small-cell lung cancer treated with first-line platinum-based chemotherapy. J Clin Oncol 2011;29:4121-8.
44. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983-91.
45. Pierceall WE, Olaussen KA, Rousseau V, et al. Cisplatin benefit is predicted by immunohistochemical analysis

of DNA repair proteins in squamous cell carcinoma but not adenocarcinoma: theranostic modeling by NSCLC constituent histological subclasses. Ann Oncol 2012;23:2245-52.
46. Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. Clin Cancer Res 2002;8:2286-91.
47. Papadaki C, Sfakianaki M, Ioannidis G, et al. ERCC1 and BRAC1 mRNA expression levels in the primary tumor could predict the effectiveness of the second-line cisplatin-based chemotherapy in pretreated patients with metastatic non-small cell lung cancer. J Thorac Oncol 2012;7:663-71.
48. Simon GR, Schell MJ, Begum M, et al. Preliminary indication of survival benefit from ERCC1 and RRM1-tailored chemotherapy in patients with advanced nonsmall cell lung cancer: evidence from an individual patient analysis. Cancer 2012;118:2525-31.
49. Cobo M, Isla D, Massuti B, et al. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. J Clin Oncol 2007;25:2747-54.
50. Taron M, Rosell R, Felip E, et al. BRCA1 mRNA expression levels as an indicator of chemoresistance in lung cancer. Hum Mol Genet 2004;13:2443-9.
51. Rosell R, Perez-Roca L, Sanchez JJ, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. PLoS One 2009;4:e5133.
52. Booton R, Ward T, Ashcroft L, et al. ERCC1 mRNA expression is not associated with response and survival after platinum-based chemotherapy regimens in advanced non-small cell lung cancer. J Thorac Oncol 2007;2:902-6.
53. Joerger M, deJong D, Burylo A, et al. Tubulin, BRCA1, ERCC1, Abraxas, RAP80 mRNA expression, p53/p21 immunohistochemistry and clinical outcome in patients with advanced non small-cell lung cancer receiving first-line platinum-gemcitabine chemotherapy. Lung Cancer 2011;74:310-7.
54. Das M, Riess JW, Frankel P, et al. ERCC1 expression in circulating tumor cells (CTCs) using a novel detection platform correlates with progression-free survival (PFS) in patients with metastatic non-small-cell lung cancer (NSCLC) receiving platinum chemotherapy. Lung Cancer 2012;77:421-6.
55. Chen S, Zhang J, Wang R, et al. The platinum-based treatments for advanced non-small cell lung cancer, is low/negative ERCC1 expression better than high/positive ERCC1 expression? A meta-analysis. Lung Cancer 2010;70:63-70.
56. Nakano Y, Tanno S, Koizumi K, et al. Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. Br J Cancer 2007;96:457-63.
57. Bepler G, Sommers KE, Cantor A, et al. Clinical efficacy and predictive molecular markers of neoadjuvant gemcitabine and pemetrexed in resectable non-small cell lung cancer. J Thorac Oncol 2008;3:1112-8.
58. Souglakos J, Boukovinas I, Taron M, et al. Ribonucleotide reductase subunits M1 and M2 mRNA expression levels and clinical outcome of lung adenocarcinoma patients treated with docetaxel/gemcitabine. Br J Cancer 2008;98:1710-5.
59. Gong W, Zhang X, Wu J, et al. RRM1 expression and clinical outcome of gemcitabine-containing chemotherapy for advanced non-small-cell lung cancer: a meta-analysis. Lung Cancer 2012;75:374-80.
60. Simon G, Sharma A, Li X, et al. Feasibility and efficacy of molecular analysis-directed individualized therapy in advanced non-small-cell lung cancer. J Clin Oncol 2007;25:2741-6.
61. Rosell R, Skrzypski M, Jassem E, et al. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. PLoS One 2007;2:e1129.
62. Bonanno L, Costa C, Majem M, et al. The predictive role of the 53BP1 pathway in advanced Non-small-cell lung cancer (NSCLC) patients (p) treated with first-line platinum-based chemotherapy. Ann Oncol 2012;23:abstr 1183.
63. Quinn JE, Kennedy RD, Mullan PB, et al. BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. Cancer Res 2003;63:6221-8.
64. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26:3543-51.
65. Wu MF, Hsiao YM, Huang CF, et al. Genetic determinants of pemetrexed responsiveness and nonresponsiveness in non-small cell lung cancer cells. J Thorac Oncol 2010;5:1143-51.

Cite this article as: Bonanno L. Predictive models for customizing chemotherapy in advanced non-small cell lung cancer (NSCLC). Transl Lung Cancer Res 2013;2(3):160-171. doi: 10.3978/j.issn.2218-6751.2013.03.07

Are we ready to use biomarkers for staging, prognosis and treatment selection in early-stage non-small-cell lung cancer?

Bartomeu Massuti[1], Jose Miguel Sanchez[2], Florentino Hernando-Trancho[3], Niki Karachaliou[4], Rafael Rosell[4,5]

[1]Hospital General de Alicante, Alicante, Spain; [2]Hospital La Princesa, Madrid, Spain; [3]Hospital Clinico Universitario San Carlos, Madrid, Spain; [4]Breakthrough Cancer Research Unit, Pangaea Biotech S.L, Barcelona, Spain; [5]Catalan Institute of Oncology, Badalona, Spain

Correspondence to: Niki Karachaliou, MD. Breakthrough Cancer Research Unit, Pangaea Biotech S.L, C/Sabino de Arana, 5-19, 08028 Barcelona, Spain. Email: nkarachaliou@pangaeabiotech.com.

Abstract: Lung cancer accounts for the majority of cancer-related deaths worldwide. At present, platinum-based therapy represents the standard of care in fit stage II and IIIA non-small cell lung cancer (NSCLC) patients following surgical resection. In advanced disease, personalized chemotherapy and targeted biologic therapy based on histological and molecular tumor profiling have already shown promise in terms of optimizing treatment efficacy. While disease stage is associated with outcome and is commonly used to determine adjuvant treatment eligibility, it is known that a subset of patients with early stage disease experience shorter survival than others with the same clinicopathological characteristics. Improved methods for identifying these individuals, at or near the time of initial diagnosis, may inform the decision to pursue adjuvant therapy options. Among the numerous candidate molecular biomarkers, only few gene-expression profiling signatures provide clinically relevant information, while real-time quantitative polymerase-chain reaction (RT-qPCR) strategy involving relatively small numbers of genes offers a practical alternative with high cross-platform performance. mRNA and/or protein expression levels of excision repair cross-complementation group 1 (ERCC1), ribonucleotide reductase M subunit 1 (RRM1) and breast cancer susceptibility gene 1 (BRCA1) are among the most promising potential biomarkers for early disease and their clinical utility is currently being evaluated in randomized phase II and III clinical trials. This review describes the most promising clinicopathological and molecular biomarkers with predictive and prognostic significance in lung cancer that have been identified through advanced research and which could influence adjuvant and neoadjuvant chemotherapy decisions for operable NSCLC in routine clinical practice.

Keywords: Non-small cell lung cancer; adjuvant therapy; biomarkers; individualized therapy

Submitted Feb 10, 2013. Accepted for publication Mar 11, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.06
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.06

Introduction

Approximately 25-30% of patients with non-small cell lung cancer (NSCLC) present with early stage disease and undergo surgery with curative intent. Despite complete tumor resection, many of these patients will develop systemic relapses with or without local relapses and will eventually die. A meta-analysis of early trials indicated a trend towards improved survival for adjuvant platinum-based chemotherapy and led to a re-evaluation of adjuvant treatment in clinical trials in large patient populations. Several of these trials demonstrated an improved survival with the use of adjuvant chemotherapy. The survival benefit was then further confirmed in a meta-analysis that included all five cisplatin-based trials (*Table 1*) (1-7). Early NSCLC comprises a heterogeneous group of diseases, with diverse innate aggressiveness and degree of response to cytotoxic agents. For instance, some patient subsets with stage II or even stage IIIA have excellent prognosis

Table 1 Adjuvant chemotherapy of completely resected NSCLC

	N	Stage	CT	5-year survival (%)		HR (95% CI)	P
				CT	Control		
ALPI-EORTC (1)	1,088	I-IIIA	MVP	49	48	0.96 (0.81-1.13)	NS
IALT (2)	1,867	I-III	Cis/Vinca	44.5	40.4	0.86 (0.76-0.98)	<0.03
NCIC CTG-JBR.10 (3)	482	IB-II	Cis/Vinorelbine	69	54	0.69 (0.52-0.91)	0.04
ANITA (4)	840	IB-IIIA	Cis/Vinorelbine	51.2	42.6	0.80 (0.66-0.96)	0.02
CALGB 9633 (5)	344	IB	Carbo/Paclitaxel	57	5	0.80 (0.60-1.07)	0.1
BLT (6)	381					1.0	NS
LACE meta-analysis (7)	4,584	I-IIIA	Cisplatin-based	48.8	43.5	0.89 (0.82-0.96)	0.004

ALPI, adjuvant lung project Italy; NCIC CTG-JBR.10, National Cancer Institute of Canada Clinical Trials Group-JBR.10 trial; ANITA, Adjuvant Navelbine International Trialist Association; CALGB, Cancer and Leukemia Group B; BLT, Big Lung Trial; IALT, International Adjuvant Lung Cancer Trial; LACE meta-analysis, Lung Adjuvant Cisplatin Evaluation meta-analysis; CT, chemotherapy; MVP, mitomycin C, vindesine and cisplatin; Vinca, etoposide or vinorelbine or vinblastine or vindesine; HR, hazard ratio.

and could be spared the toxicity of unnecessary therapy. Others, such as the elderly or less fit, as well as those with stage I disease, remain undertreated despite potential benefit from adjuvant chemotherapy. This underscores the need for a customized approach to tailor adjuvant chemotherapy according to patient characteristics and tumor characteristics. Personalizing therapy based on an individual patient's molecular profile is a potentially promising approach to optimize efficacy with available agents. Prognostic biomarkers indicate the natural course of disease, irrespective of treatment, while those defined as predictive can foresee differential therapeutic outcomes. However, some biomarkers combine both of these functions, such as ERCC1 and RRM1. Identification and application of the appropriate biomarkers would enable selection of only high-risk patients to receive the most effective treatment. In this review we describe potential predictive and prognos-tic markers and their current role, benefit, and possible future use in the management of patients with early stage NSCLC.

Clinicopathological prognostic and predictive factors in early stage NSCLC

It has long been recognized that differences in clinical factors such as stage, sex, and tumor factors such as cellular differentiation, vascularity, and vascular invasion, are prognostic of outcome and important in determining adjuvant therapy decisions for early stage NSCLC. To date, pathological stage, as defined by tumor size and nodal status, is the only prospectively validated clinicopathological biomarker with both prognostic and predictive value. According to guidelines from the European Society of Medical Oncology (ESMO), American Society of Clinical Oncology (ASCO), National Comprehensive Cancer Network (NCCN) and the American College of Chest Physicians (ACCP), platinum-based adjuvant chemotherapy is considered standard treatment for resected stage II-IIIA disease with an estimated survival benefit of 4-5% at 5 years (8-11). Although pre hoc subgroup analyses have shown no benefit for patients with pathological stage IB disease, interpretation of these results should be cautious, since the test for interaction between treatment effect and stage was not significant in any of the phase III trials with platinum-based regimens (2-4,12). Only one of the available meta-analyses did demonstrate a significant differential treatment effect, largely in the stage IA subgroup, suggesting that patients with stages II and IIIA have greatest benefit (2). The reported 11% 5 years survival gain of adjuvant tegafur-uracil in stage I NSCLC cannot be directly extended to Western countries where tegafur-uracil has not yet been reliably tested (13). At the same time, the assumption of therapeutic benefit for stage IB disease with tumor size larger than 4 cm is based on an unplanned subgroup analysis (5). Nonetheless, worse prognosis observed with increasing T size has been recognized in the 7th TNM edition. T2 was divided into T2a (3-5 cm) and T2b (5-7 cm), with 5 year overall survival of 58% and 49%, respectively (P<0.0001); T2bN0 was upstaged to stage IIA. Correlation with the

new staging system failed to validate the 5 cm cut-off in the 9-year update of CALGB 9633, showing a trend towards a significant benefit for adjuvant treatment for patients with tumors >7 cm (HR=0.53; P=0.051), although this interaction should be investigated further (14-16).

With regard to the importance of accurate staging, a recent retrospective analysis of lymph node dissection in more than 20,000 patients with pathological stage I NSCLC implied that the number of recovered lymph nodes might be predictive for survival outcomes, although this could well be attributed to a direct therapeutic effect (17). Furthermore, a large retrospective analysis from the SEER database showed that the increasing number of resected positive nodes and a higher ratio between metastatic and overall resected nodes has an independent negative prognostic impact for overall survival in N1 patients (18,19). Recent studies investigated high tumor grade, vascular invasion and visceral pleural infiltration as poor prognostic determinants, based on mostly retrospective cohort studies, and are commonly recommended as adjunct selection criteria for patients who are borderline candidates for adjuvant chemotherapy (20,21).

Other clinicopathological features prospectively shown to be independent, unfavorable prognostic factors in early NSCLC include older age, male sex and non-squamous-cell histology. Only performance status was likely to predict therapeutic effect (3,4,7). Likewise, certain histological subtypes, such as large-cell neuroendocrine carcinoma and pure bronchioloalveolar carcinoma which confer, respectively, worse or better outcome, could also guide treatment strategy (22,23). Interestingly, a high correlation between gene expression profile signatures and tumor histological phenotype has been shown for early NSCLC (24). It seems that even a thoroughly validated molecular signature does not outperform combined conventional clinical and pathologic variables in predicting survival of NSCLC patients (24). Therefore, incorporating the subtype and grade into conventional clinical models could provide predictive accuracy similar to that of well validated gene panels (24).

Molecular prognostic and predictive markers in early stage NSCLC

Gene expression profiling signatures

Gene expression profiles may facilitate treatment decisions in lung cancer, similar to their use to predict chemotherapy benefit in early stage breast cancer. Several groups have developed prognostic signatures based on mRNA, microRNA or proteomic profiles in order to better define patients with good prognosis who could potentially be spared adjuvant treatments, and those with poor prognosis who may benefit from successful adjuvant therapies. However, most prognostic signatures have some limitations that should be taken into account when analyzing their potential clinical utility. For instance, survival of NSCLC patients depends to a large extent on co-morbidity factors and the impact of this cannot be accounted for by prognostic tests based on tumor molecular profiling. At the same time, methodological or statistical data analyses have often been insufficient in the original studies proposing the signature for clinical use. Consider the recent example of the phase III Cancer and Leukemia Group B (CALGB) 30506 trial that was originally designed to validate the potential utility of a lung cancer metagene model in selecting patients with pT1 -T2N0 tumors for adjuvant chemotherapy. The study was recently amended as the original authors failed to replicate their own results. This highlights the need for very careful large-scale validation of prognostic signatures before they can be prospectively tested in clinical studies with adjuvant therapies for lung cancer (25).

Subramanian and Simon have recently published an elegant review of 16 published studies involving the analysis of gene expression data for developing prognostic signatures in NSCLC, in which they report serious methodological flaws in design and analysis, including inappropriate patient dataset selection, lack of independent validation, biased reporting of re-substitution statistics, incomplete protocol specification and use of statistical methods (26). Indeed, only a few gene signature studies have yielded data specifically referring to NSCLC stages IA, IB, or II that warrant further prospective validation (27-33). Among them it is worth mentioning the prognostic 15-gene signature for early NSCLC that was recently reported as the first deriving from prospectively collected tumor samples from patients enrolled in a phase III adjuvant trial. Gene expression profiling was conducted on mRNA from 133 frozen tumor samples from the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG)-JBR.10 trial (28). The prognostic value of this gene signature was tested in four independent published microarray data sets and by quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR). Among these genes were

nuclear proteins or transcription regulators such as mouse double minute 2 homolog (MDM2), zinc finger protein 236 (ZNF236), fos-related antigen 2 (FOSL2), hexamethylene bis-acetamide (HMBA)-inducible protein 1 (HEXIM1), myelin transcription factor 1-like (MYT1L) and inhibitor of kappa light polypeptide *gene* enhancer in B-cells (IKBKAP). The second subset of genes included protein melan-A (*MLANA*), ATPase subunit beta-1 (*ATP1B1*), L1 cell adhesion molecule (*L1CAM*), and stathmin-2 (*STMN2*), which encode for transmembrane- or membrane-associated proteins, potentially involved in signaling pathways and, finally, sodium/potassium-transporting ATPase subunit beta-1 (*ATP1B1*) and uridine monophosphate synthetase (UMPS) which are involved in purine and pyramidine metabolism, respectively, suggesting dependency of NSCLC on these pathways (28). This 15-gene expression profile was unique in that it could also predict response to systemic chemotherapy, whereas most other gene profiles have served only as prognostic markers following surgery. The signature was shown to interact significantly with the effect of cisplatin plus vinorelbine chemotherapy, with high-risk patients benefiting the most, although its potential predictive role requires independent validation. Also clinically relevant to the adjuvant strategy, this signature was able to assign, separately, stage IB and II patients to high- and low-risk subgroups with significantly different overall survival (28). When the predictive value of previously published prognostic signatures, applied to treated and untreated patients in the JBR.10 data set was evaluated, only the six-gene signature identified by Boutros was proved to be both significantly prognostic and predictive (27) . The six-gene model comprised: syntaxin 1A (STX1A), hypoxia inducible factor 1A (HIF1A), chaperonin containing TCP1 subunit 3 (CCT3), MHC Class II DP beta 1 (HLA-DPB1), v-maf musculoaponeurotic fibrosarcoma onco-gene homolog K (MAFK), and ring finger protein 5 (RNF5) (27).

Finally, a 14-gene assay that uses RT-qPCR analysis of formalin-fixed, paraffin-embedded tissues was developed with a cohort of 361 patients with non-squamous NSCLC resected at the University of California, San Francisco, by Kratz, He, *et al.* from Michael Mann and David Jablons' group (34). The investigators developed a 14-gene signature panel, consisting of 11 cancer-related genes: BCL2-associated athanogene (BAG1), breast cancer susceptibility gene 1 (BRCA1), cell division control protein 6 homolog (CDC6), cyclin-dependent kinase 2 associated protein 1 (CDK2AP1), receptor tyrosine-protein kinase erbB-3 (ERBB3), galactoside 3(4)-L-fucosyltransferas (FUT3), interleukin 11 (IL11), lymphocyte-specific protein tyrosine kinase (LCK), Rho family GTPase 3 (RND3), SH3 domain-binding glutamic acid-rich protein (SH3BGR), and wingless-type MMTV integration site family, member 3A (WNT3A) together with 3 reference genes, esterase D (ESD), TATA box binding protein (TBP) and Yes-associated protein 1 (YAP1). They validated the candidate gene signatures in 2 different populations: a community-based series of 433 resections for stage I non-squamous NSCLC from Northern California, and a cohort of 1,006 resections for stage IA-IIIB non-squamous NSCLC from the China Clinical Trials Consortium (34). The combination of gene signatures proved to be independently prognostic, irrespective of TNM stage grouping (34), in stage I, II and III patients. The prognostic value was significantly greater than certain clinical risk stratification criteria proposed by the US National Comprehensive Cancer Network for stage I resections (34). Furthermore, it was similarly effective in the Northern California and Chinese validation populations (34). However, there are some limitations to this study that should be pointed out. For instance, patients with squamous cell histology were excluded and there was poor overall quality of pathologic nodal staging, bearing in mind that 18% of resections for NSCLC in United States have no lymph nodes examined. As with all studies so far, this is another retrospective series, albeit the largest and most rigorously validated one performed to date.

Individual prognostic and predictive biomarkers in early-stage NSCLC

There are several candidate markers for sensitivity or resistance to chemotherapy identified in retrospective analyses of tumor biopsies from phase III clinical trials testing the value of adjuvant chemotherapy (*Tables 2,3*).

Excision repair cross complementation group 1 (ERCC1)
ERCC1 is a rate limiting enzyme in the nucleotide excision repair (NER) and interstrand cross-link repair pathways, which recognizes and repairs platinum induced adducts. Cancer cells overexpressing ERCC1 are more likely to have *de novo* resistance to cisplatin and a growing list of reports links cisplatin, carboplatin, and oxaliplatin resistance to ERCC1 mRNA levels in tumors. This relationship has been suggested for patients with gastric, bladder, ovarian, colorectal, and lung cancer. It was shown that ERCC1 levels evaluated by immunohistochemistry (IHC) are also

Table 2 Prognostic biomarkers in early stage non-small cell lung cancer

Study	Stage of disease	Number of patients	Study design	Biomarker (assay)	Biomarker status	HR for overall survival (P value)
Olaussen *et al.*, 2006 (35)	I-III	1867/761	Retrospective analysis within IALT study	ERCC1 (IHC)	Positive expression (H-score > median value)	0.66 (0.009)
Kamal *et al.*, 2010 (36)	I-III	1867/673	Retrospective analysis within IALT study	MSH2 (IHC)	High expression (H-score=3)	0.66 (0.01)
Tsao *et al.*, 2007 (37)	IB-II	482/253	Retrospective analysis within NCIC CTGJBR.10	p53 (IHC)	Positive expression (staining score ≥15%)	1.89 (0.03)
Graziano *et al.*, 2010 (38)	IB	344/250	Retrospective analysis within CALBG 9633	p53 (IHC)	Positive expression	2.30 (0.0005)
Seve *et al.*, 2007 (39)	IB-II	482/265	Retrospective analysis within NCIC CTGJBR.10	βTUBIII (IHC)	High expression (H-score > median value)	1.72 (0.04)
Rosell *et al.*, 2007 (40)	I-IIIA; IB-IIB	126; 58 (validation cohort)	Retrospective analysis of cohort data	BRCA1 (RT-qPCR)	High expression (relative gene Expression > median value)	1.98 (0.02); 2.4 (0.04)

HR, hazard ratio.

Table 3 Predictive biomarkers in early stage non-small cell lung cancer

Study	Stage of disease	Number of patients	Study design	Biomarker (assay)	Biomarker status	HR for overall survival (P value); P value for interaction test
Olaussen *et al.*, 2006 (35)	I-III	1867/761	Retrospective analysis within IALT study	ERCC1 (IHC)	Negative expression *vs.* positive expression (H score> median value)	0.65 (0.002) *vs.* 1.14 (0.40); 0.009
Kamal *et al.*, 2010 (36)	I-III	1867/658	Retrospective analysis within IALT study	MSH2/ ERCC1 (IHC)	Both low *vs*. both high	0.65 (0.01) *vs.* 1.32 (0.19); 0.01
Kamal *et al.*, 2010 (36)	I-III	1867/not defined	Retrospective analysis within IALT study	MSH2/p27 (IHC)	Both low *vs.* both high	0.65 (0.01) *vs.* 1.31 (0.22); 0.01
Kamal *et al.*, 2010 (36)	I-III	1867/673	Retrospective analysis within IALT study	MSH2 (IHC)	Low expression *vs.* high expression (H score =3)	0.76 (0.03) *vs.* 1.12 (0.48); 0.06
Tsao *et al.*, 2007 (37)	IB-II	482/253	Retrospective analysis within NCIC CTGJBR.10	p53 (IHC)	Positive expression (staining score≥15%) *vs.* negative	0.54 (0.02) *vs.* 1.40 (0.26); 0.02
Filipits *et al.*, 2007b (41)	I-III	1867/778	Retrospective analysis within IALT study	p27 (IHC)	Negative expression *vs.* positive expression (H score> median value)	0.66 (0.006) *vs.*1.09 (0.54); 0.02
Pirker *et al.*, 2007 (42)	I-III	1867/778	Retrospective analysis within IALT study	ERCC1/p27 (IHC)	Both negative *vs.* both positive	0.52 (95% CI: 0.36-0.74) *vs.* 1.27 (95% CI: 0.87-1.84); not specified

HR, hazard ratio; *vs.*, versus.

predictive for the survival benefit afforded by adjuvant cisplatin-based chemotherapy in patients with totally resected stage I to IIIA NSCLC (35).

The International Adjuvant Lung Cancer Trial (IALT)-Bio translational research project aimed to study molecular biomarkers of tumors for their potential predictive values with regard to the effect of adjuvant chemotherapy on survival in IALT patients. Five groups of molecular biomarkers (19 markers in total) were studied by IHC: drug transporters, DNA repair, cell cycle regulators, signal transduction and apoptosis. Both ERCC1 and cyclin-dependent kinase inhibitor 1B (CDKN1B or p27) were found to have predictive value in patients with completely resected NSCLC undergoing adjuvant cisplatin-based chemotherapy (35). Interestingly, in patients randomly assigned to the observation arm, the subgroup with ERCC1-positive tumors had better survival compared with those with ERCC1-negative tumors (35). The paradoxical status of ERCC1, which was found to be a good prognostic marker in untreated resected NSCLC patients but a poor predictor of efficient adjuvant chemotherapy, was also confirmed in another study by Zheng et al, where the concomitant high expression of RRM1 and ERRC1 delineated a subgroup of *chemonaïve* patients with stage I disease with excellent survival outcomes (43). p27 is a tumor-suppressor protein that induces cell-cycle arrest in phase G1. Overexpression of p27 may confer de novo resistance to cisplatin by giving necessary time to repair cisplatin-induced DNA damage. In retrospective analysis of the IALT, patients with p27-negative tumors had longer survival after chemotherapy compared with surgery alone (41). Among six cell cycle regulators evaluated by IHC within the IALT-bio project, only p27 was identified to significantly correlate with treatment effect. Its predictive ability was independent from ERCC1 expression and, as anticipated, only patients with p27-negative tumors had survival benefit of cisplatin-based chemotherapy. Furthermore, when combining the IHC features of ERCC1 and p27, patients with tumors negative for both biomarkers seemed to benefit most from adjuvant chemotherapy (41).

In a pharmacogenomic trial with a biomarker-strategy design in advanced NSCLC by Cobo *et al.*, ERCC1 mRNA expression was evaluated prospectively in an attempt to predict response to cisplatin-based or cisplatin-free chemotherapy regimens in stage IIIB or IV in NSCLC patients (44). Overall response rate was significantly higher in the genotypic arm, where chemotherapy regimen was tailored by ERCC1 mRNA expression (44). Patients in the control arm were not evaluated for the biomarker and received standard platinum-based combination (44). Within the customized arm, patients with low ERCC1 levels were treated with the same regimen as the control arm, whereas those with high levels received a non platinum regimen (44). Most importantly, however, clinical relevance remained limited, given that there was no difference between the two arms in either progression-free survival or overall survival. This paradox of favorable long term outcome despite cisplatin chemoresistance probably indicates that, by preventing mutagenesis, DNA repair may not only prevent cancer but may retard molecular events related to progression in established tumors. Thus, high expression of ERCC1 may indicate a favorable outcome in these untreated patients by identifying tumors that have progressed relatively little at the molecular level. Intact DNA repair mechanisms prevent accumulation of genetic aberrations that confer a high malignant potential (45). A recent meta-analysis failed to support two common ERCC1 gene polymorphisms *ERCC1* C118T/C8092A and *ERCC2* Lys751Gln/Asp312Asn as useful prognostic factors for assessing treatment response to platinum-based chemotherapies in NSCLC patients (46).

Additional biomarkers related to the repair of cisplatin-induced DNA damage have been included in the IALT bio project in order to enhance the predictive power of ERCC1. MutS homolog 2 (MSH2) is a major active component of the mismatch repair machinery; IHC expression of MSH2 displayed a very similar pattern of significance to ERCC1. Specifically, patients with low MSH2 levels had markedly better survival with adjuvant chemotherapy. Those with high levels seemed to have no benefit and in fact had a median survival 9 months shorter than those who did not receive chemotherapy, although this was not statistically significant. Similar to the prognostic role of other DNA-excision-repair proteins, high MSH2 levels predicted significantly longer survival in patients in the observation arm. When MSH2 and ERCC1 expression patterns were combined to form four phenotypes, the benefit from chemotherapy was significantly greater for patients with double-negative tumors. This was also noted when MSH2 expression was combined with that of p27, suggesting that MSH2 immunostaining was a superior predictive biomarker when considered jointly with either of the two other variables (36).

Ribonucleotide reductase subunit M1 (RRM1)

RRM1 is a regulatory component of ribonucleoside-

diphosphate reductase, a key enzyme in DNA synthesis that catalyzes the formation of deoxyribonucleotides, by reducing ribonucleotides. The reaction requires generation of a radical allowing the 2'-hydroxyl of ribose to be reduced, which is carried out by the RRM1 enzyme (47). The antimetabolite gemcitabine interferes with the function of RRM1 by reducing the pool of deoxyribonucleotide-5'-diphosphate available for DNA synthesis (45). Although relevant data for the adjuvant setting are lacking, correlative studies within randomized clinical trials in advanced NSCLC have shown that RRM1 overexpression, either at the mRNA or protein level, predicts poor response to gemcitabine-based chemotherapy (48-50). It is somewhat surprising that RRM1 protein expression has recently predicted outcome in patients treated with cisplatin and vinorelbine in a biomarker study. In this treatment arm, patients without RRM1 protein expression showed improved disease control rates, progression-free survival and overall survival, while RRM1 had no predictive impact in patients treated with cisplatin, paclitaxel and gemcitabine (51).

In contrast to predicting chemoresistance, RRM1 is a biologically and clinically important determinant of malignant behavior in NSCLC whose overexpression seems to confer favorable outcome. RRM1 suppresses cell migration and metastasis, which is at least partially mediated through induction of the phosphatase and tensin homolog gene (PTEN). RRM1 is in a region of frequent loss of heterozygosity (LOH), and LOH at this locus was found to be an independent determinant of poor survival in a large cohort of patients with stage I and II NSCLC (52). High RRM1 transcriptional expression, defined as mRNA levels above the median value, was favorably prognostic of survival in two independent cohorts of patients with resected NSCLC, most of who were diagnosed at early stage and treated with surgery only. In this study, RRM1 overexpression was a stage-independent predictor of survival, albeit highly correlated with PTEN expression (53). Longer overall survival was recently found in another group of NSCLC patients with high RRM1 mRNA expression who had undergone curative lung resection (54). The prognostic role of RRM1 was also confirmed by Zheng *et al.* who measured RNA expression of RRM1 and ERCC1 using RT-qPCR in fresh frozen and formalin fixed paraffin-embedded tumor samples (43). This study showed that RRM1 expression correlated with ERCC1 expression and that patients whose tumors had high expression of RRM1 had superior survival compared with the low expression group (43). In contrast to the previous study, there was no correlation with PTEN expression at the protein level. Interestingly, the concomitant high expression of RRM1 and ERCC1 delineated a subgroup of patients with excellent survival outcomes, accounting for 30% of the cohort (43).

Breast cancer susceptibility gene 1 (BRCA1)

BRCA1 is a multifunctional nuclear phosphoprotein which is ubiquitously expressed in all tissues and serves in part as a tumor suppressor, a "caretaker" and a "gatekeeper" in preserving genomic stability. BRCA1 has recently emerged as one of the most appealing biomarkers for personalizing chemotherapy in NSCLC. It has been implicated in normal cellular functions including cell cycle regulation, replication, mitotic spindle assembly, transcription regulation and higher chromatin hierarchical control (55). Also, BRCA1 has a crucial role in DNA repair as a component of the transcription-coupled NER and the homologous recombinant repair pathways. BRCA1 functions as a sensitizer to apoptosis induced by antimicrotubulin agents, such as taxanes and vinca alkaloids and also abrogates apoptosis induced by a range of DNA-damaging agents, including cisplatin and etoposide. Upstream activity of the receptor-associated protein 80 (RAP-80) is required for localization of BRCA1 to sites of DNA double-strand breaks (55).

In a recently reported feasibility study, adjuvant chemotherapy was customized based on BRCA1 mRNA levels in 84 patients with completely resected NSCLC. Patients with higher BRCA1 transcriptional levels were treated with single agent docetaxel, whereas those with intermediate and low BRCA1 expression received cisplatin-based doublets. Interim analyses showed that single-agent docetaxel was not inferior to cisplatin/docetaxel in terms of survival in patients with high BRCA1 levels (56). Therefore, high BRCA1 predicts resistance to cisplatin and possibly sensitivity to docetaxel. Expression levels of BRCA1, divided in quartiles, were assessed in a cohort of 55 patients with stage II to IIIA NSCLC who received neoadjuvant chemotherapy with cisplatin/gemcitabine followed by complete resection. Those with the lowest levels of BRCA1 mRNA expression had significantly greater benefit from chemotherapy in terms of clinical and pathological downsizing as well as overall survival (57).

The potential prognostic role of BRCA1 was investigated in two independent cohorts of chemonaïve patients with early-stage NSCLC analyzed by RT-qPCR.

In the study by Rosell *et al.*, expression level of nine genes involved in DNA repair, including BRCA1, were correlated with overall survival in 126 NSCLC patients who had undergone complete resection and did not receive adjuvant chemotherapy (40). In a univariate analysis, three genes appeared to influence relapse: myeloid zinc finger 1 (MZF1), thioredoxin-1 (TRX1), and BRCA1. However, only BRCA1 and stage III disease remained significant predictors of survival in the multivariate analysis (40). For the 40 patients with a high level of BRCA1 expression, median survival was 29 months while median survival was not reached for the 83 patients with low BRCA1 expression (40). The striking lack of prognostic significance of other biomarkers included in this study may be partially due to the strong intergene coexpression, such as that between BRCA1 and ERCC1, observed. The independent adverse prognostic effect of high BRCA1 expression was confirmed in another cohort of patients with early stage NSCLC also evaluated for ERCC1 and RRM1 mRNA levels. In this study, xeroderma pigmentosum complementation group G (XPG), a key gene for the NER system, was identified as an independent favorable predictor of survival outcome, as well as a potential modulator of recurrence risk among patients with BRCA1 overexpression (58).

Thymidylate synthase (TS)

Thymidylate synthase (TS) and methylenetetrahydrofolate reductase (MTHFR) play important roles in folate metabolism. TS is an enzyme involved in purine synthesis and, as an anti-cancer chemothera¬py target, can be inhibited by TS inhibitors such as fluorinated pyrimidine fluorouracil or certain folate analogues, most notably pemetrexed. Consistent findings across phase III trials in advanced NSCLC have established the favorable predictive effect of non-squamous cell histology on treatment with pemetrexed (59). Differentially high TS expression in squamous cell NSCLC represents the main molecular basis underlying this treatment by histology interaction. Data from a current study indicate higher *TS* expression levels in squamous cell and in high-grade carcinomas (60). No clinical data exist to confirm the predictive role of either histology or TS expression in the adjuvant setting. However an independent prognostic effect for *TS* has been revealed in chemonaïve patients with resected early stage NSCLC. *TS* protein expression was correlated significantly with higher proliferative activity of NSCLC cells and, consequently, with poor prognosis in patients with NSCLC who had higher *TS* level (61). Two other different studies of chemonaïve patients with resected early stage NSCLC revealed an independent prognostic effect for TS, but with conflicting qualitative results. High TS expression at the mRNA, but not IHC level, was significantly associated with adverse disease-free survival in the study from Shintani *et al.* High TS expression as determined by automated in situ protein quantification, but not by RT-qPCR, predicted improved overall survival in the latter study, in which also TS protein levels did not correlate with those of ERCC1 and RRM1 (62,63). No correlations between intratumoral *TS* levels and any known clinicopathological variables were reported, with the exception of a recently published article in which *TS* gene expression was associated with disease stage, lymph node metastasis, tumor differentiation, prognosis, and tumor cell proliferation in patients with lung adenocarcinoma (64).

Kirsten-rous avian sarcoma (KRAS) and p53

The Kirsten-rous avian sarcoma (KRAS) protein is a member of the RAS family of proteins that encode small GTPases involved in cellular signal transduction. Activation of Ras signalling causes cell growth, differentiation, and survival, by transmitting signal downstream from growth factor receptors, including epidermal growth factor receptor (EGFR). From the three RAS genes, KRAS contains > 90% of the mutations detectable in almost 10-25% of NSCLC and mostly affecting codon 12 and 13 (65). Recent data suggested that KRAS mutations may affect outcome of NSCLC patients receiving chemotherapy. In the adjuvant setting, data from the JBR10 trial suggested no benefit from adjuvant chemotherapy in KRAS mutated patients (3,37). Similarly, a retrospective analysis of patients with stage IB disease enrolled in the phase III CALGB-9633 study showed that, among those with tumors larger than 4 cm, KRAS mutations may predict less overall survival benefit from the combination of carboplatin plus paclitaxel (66). In the LACE-BIO pooled analysis, the prognostic and predictive role of KRAS mutations was investigated in 1,751 patients treated with adjuvant chemotherapy. Among evaluable patients, KRAS mutations had no effect on survival (67). It should be stressed that the formal test for interaction between the biomarker and treatment effect was not statistically significant in any of the above studies. KRAS mutation status is associated with cigarette smoking and adenocarcinoma histology. The role of KRAS mutations as a prognostic factor in NSCLC remains controversial. Although some studies suggested a potential

negative prognostic effect, other studies did not confirm any negative impact on survival for individuals harboring KRAS mutation (68-71).

The TP53 gene, located on the short arm of human chromosome 17, encodes for a nuclear phosphoprotein involved in the regulation of cell proliferation. The tumor suppressor protein, p53, has a wide range of functions, most of which are mediated via regulation of gene transcription. Commonly described as 'the genome guardian', p53 is involved in important cellular processes, such as stress response, cell-cycle control, DNA repair and apoptosis. The mutant gene product, which tends to accumulate to high levels in cancer cells, is believed to exert a dominant negative effect over coexpressed normal TP53. In resected lung cancers, point mutations of the TP53 gene have been found in all histologic types, including approximately 45% of resected NSCLC and, even more frequently, in small-cell lung cancer. Similar to observations with *KRAS* mutations, p53 mutations have been retrospectively correlated with clinical features such as younger age and squamous histology, but not sex, tumor stage, nodal status, neuroendocrine differentiation, or prior chemotherapy. Many retrospective studies have examined the prognostic role of p53 gene mutations in NSCLC. However, most of these studies have been limited by small size, heterogeneous patient samples, potential selection biases, and/or insensitive p53 mutation detection techniques, leading to inconsistent results. p53 mutations have been associated with decreased survival, no statistically significant change in survival, or improved survival in NSCLC. Previous meta-analyses have indicated that TP53 mutations and p53 expression are weak predictors of outcome in NSCLC (72,73). In contrast, the first published prospective trial examining the prognostic role of p53 mutations in NSCLC demonstrated that neither p53 expression nor TP53 mutations were shown to have predictive value. However, this should be interpreted taking into consideration the different study design and the use of an old-generation regimen (74). However, in the retrospective companion analysis of the phase III NCIC CTG-JBR.10 adjuvant trial p53 IHC overexpression was found to be an independent unfavorable prognostic factor among patients in the observation arm. In addition, only patients with p53-positive tumors derived benefit from the cisplatin plus vinorelbine combination. In contrast to p53 expression, TP53 mutation status was neither prognostic for survival, nor predictive for efficacy of adjuvant chemotherapy (37). This suggests that the biological effects of TP53 mutations and p53 protein overexpression are not equal, highlighting their complex role in tumor aggressiveness and chemosensitivity. Finally, a recent biomarker study as part of the phase III CALGB- 9633 adjuvant trial identified p53 and mucin overexpression as independent adverse prognostic factors for stage IB patients (38).

β-TUBULIN and epidermal growth factor receptor

β-tubulin is an essenti al element of microtubules, which, in turn, serve as cellular structural components involved in vital processes, including mitosis. Among described mechanisms of resistance to anti-tubulin agents, class III β-tubulin (βTUBIII) overexpression is of particular interest. To assess whether βTUBIII might be a useful marker in early NSCLC patients undergoing adjuvant chemotherapy with a vinorelbine-based regimen, levels of βTUBIII were measured in tumor samples from patients treated in the NCIC CTG JBR.10 study. No significant interaction between the biomarker and the effect of cisplatin plus vinorelbine combination was found. Subgroup analysis suggested that high, rather than low, βTUBIII levels were predictive for chemotherapy benefit. However, high βTUBIII expression was shown to be an independent adverse predictor of recurrence-free survival (39). Its prognostic value was retrospectively confirmed in patients enrolled in the IALT study (75). The adverse prognostic significance of high βTUBIII expression is consistent with prior published reports in the setting of advanced NSCLC. Rosell *et al.* correlated high βTUBIII mRNA levels with inferior outcome in advanced NSCLC patients treated with anti-tubulin agents (76). It has also been shown that high level of expression of βTUBIII in tumor cells, assessed by a semiquantitative IHC assay, was associated with a lower response rate and poor prognosis in advanced NSCLC patients receiving vinorelbine-based chemotherapy (77). In a recent study, high tumor expression of βTUBIII, assessed by IHC in 47 NSCLC patients receiving a paclitaxel-based regimen, was predictive of lower response to therapy and inferior survival (78).

Epidermal growth factor receptor (EGFR) status, defined by mutation analysis or amplification by fluorescent in situ hybidization (FISH), was recently explored in correlation with the results of the phase III NCIC CTG-JBR.10 adjuvant trial. Neither sensitizing mutations nor high gene copy were significantly prognostic in the observation arm. Similarly, although there was a trend toward greater benefit from the cisplatin plus vinorelbine

combination, interaction between the biomarkers and treatment effect was not significant (79). After adjusting for covariates, a recent large, prospective, cohort study of patients with stage I-III adenocarcinoma, 20% of whom had received perioperative chemotherapy, failed to show any significant association between overall survival and mutation status of either EGFR or KRAS (80). In conclusion, the potential prognostic and predictive effect of EGFR amplification and the two most prominent mutations, del 19 and L858R, with regard to chemotherapy effect in the adjuvant setting remains undefined.

Conclusions

Finally, are we ready to adopt the use of biomarkers into early stage NSCLC staging, prognosis and treatment selection? Survival amongst cancer patients has improved in recent decades with the availability and application of various treatment modalities. Tumor classification, stage and, sometimes, grade are used to assess prognosis. Although adjuvant chemotherapy has been well established for patients with early stage NSCLC, stage alone is not an ideal biomarker to predict the utility of chemotherapy as the vast majority of patients derive no benefit from treatment. The discovery of molecular biomarkers with the potential to select high-risk patients and predict drug efficacy is essential, especially in controversial fields such as treatment of elderly patients and stage I disease. Biomarker expression often supplants or complements tumor classification, stage or grade. In recent years, a widespread search for new, tumor biology-driven therapeutics has begun, especially in advanced NSCLC. However in the adjuvant setting, it seems that discovery of so-called promising markers translates rather slowly into clinical applicability and few markers have so far been integrated into clinical practice. There are many practical issues, such as the pharmaceutical companies concerns regarding fractionation of markets and medico-legal fears surrounding generation and possession of information. Furthermore, it can take time for physicians and patients to accept and adopt customizing adjuvant chemotherapy. However, as therapies become increasingly target specific, biomarkers will inevitably develop in tandem to play greater roles in staging, grading, and selection of adjuvant therapy; the practical hurdles are many and complex. As already mentioned, the RT-qPCR strategy involving a relatively small number of gene biomarkers and the use of paraffin-embedded specimens seems to outperform wide-genome profiling, although cutoff point definition for continuous variables, such as transcript levels, is particularly challenging because of the great inter-individualization variation of gene expression. In addition, the limited size of most studies and variable techniques used for marker determination plays a role. Often, initially promising results are not reproducible. Another important point is the possible discordance of biomarker status between different types of assays and the corresponding differences in association with clinical outcomes. As already discussed, mRNA expression of a biomarker gene does not necessarily correlate with protein levels as determined by IHC. Apart from multiple technical issues that potentially affect the results of each method, biomarker expression at the protein level depends on additional translational factors, such as microRNA, posttranslational modifications and degradation.

Although the cisplatin plus vinorelbine doublet is currently the standard option for adjuvant chemotherapy, use of appropriate surrogate biomarkers would facilitate randomized clinical trials to establish alternative or superior regimens with smaller sample sizes and shorter follow-up time. The prognostic and/or predictive role of many of the aforementioned biomarkers has been strongly supported by retrospective translational studies. Ideally, biomarkers should be validated analogously in prospective, well-controlled clinical studies of diverse patients across multiple institutions, with well-established standards for all steps of the process. We are anxiously awaiting the results of four prospective multicentre clinical trials of customized adjuvant strategy currently underway (*Table 4*). ERCC1, RRM1 and BRCA1 are considered to be among the most promising biomarkers with stage-independent, combined prognostic and predictive value, the clinical utility of which is being validated in the ongoing large-scale, randomized phase II and III trials. Until the, highly anticipated, results are in, neither these nor other candidate biomarkers should be used in daily clinical practice as decision-making criteria.

These steps towards personalized medicine will hopefully represent a shift in the management of early staged NSCLC. Indeed, NSCLC should no longer be viewed as one common generic disease, but rather as a collection of rarer tumors with differing biological behaviors and sensitivities to various systemic treatments.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

Table 4 Ongoing prospective biomarker studies in early stage non-small cell lung cancer

Trial	Stage/histology	Biomarker(s)	Study design	Treatment arms
SWOG-S0720	I (pT ≥2 cm)	ERCC1, RRM1 (AQUA ± RTqPCR ± protein polymorphisms)	Phase II	Cisplatin/gemcitabine (↓RRM1 and/or ERCC1) versus observation (↑RRM1 and ERCC1)
ITACA	II-III	ERCC1,TS (RT-qPCR)	Phase III	Pharmacogenomic-guided chemotherapy regimen versus control*
TASTE	II-IIIA (pN1)/ non squamous	ERCC1, EGFR (mutations)	Randomized phase II	Customized treatment versus cisplatin/pemetrexed**
GECP-SCAT	pN1-N2	BRCA1 (RT-qPCR)/EGFR, KRAS (mutations)	Phase III	Customized chemotherapy*** versus cisplatin/ docetaxel±RT (pN2)
RADIANT	IB-IIIA	EGFR (IHC±FISH)/ EGFR, KRAS (mutations)	Phase III	Erlotinib versus placebo ± adjuvant chemotherapy (up to 4 cycles)

AQUA, automated quantitative analysis; SWOG, Southwest Oncology Group; ITACA, International Tailored Chemotherapy Adjuvant; TASTE, Tailored Post-Surgical Therapy in Early Stage NSCLC; GECP-SCAT, Spanish Lung Cancer Group (Grupo Espanol de Cancer de Pulmon)-Spanish Customized Adjuvant Trial; RADIANT, Randomized Double-blind Trial in Adjuvant NSCLC with Tarceva; * taxane (↑ERRC1 and TS mRNA levels), or pemetrexed (↑ERRC1, ↓TS mRNA levels), or cisplatin/gemcitabine (↓ERRC1, ↑TS mRNA levels), or cisplatin/pemetrexed (↓ERRC1 and TS mRNA levels) versus standard chemotherapy with a platinum-based doublet chosen by the investigator; **Customized treatment according to EGFR and ERCC1 status: erlotinib (EGFR-mutant tumors) or observation (EFGR-wild type and ERCC1-positive tumors) or cisplatin/pemetrexed (EGFR-wild type and ERCC1-negative tumors); ***Customized chemotherapy: docetaxel (high BRCA1 levels) or cisplatin/docetaxel (intermediate BRCA1 levels) or cisplatin/gemcitabine (low BRCA1 levels).

References

1. Scagliotti GV, Fossati R, Torri V, et al. Randomized study of adjuvant chemotherapy for completely resected stage I, II, or IIIA non-small-cell Lung cancer. J Natl Cancer Inst 2003;95:1453-61.
2. Arriagada R, Dunant A, Pignon JP, et al. Long-term results of the international adjuvant lung cancer trial evaluating adjuvant Cisplatin-based chemotherapy in resected lung cancer. J Clin Oncol 2010;28:35-42.
3. Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005;352:2589-97.
4. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association (ANITA)): a randomised controlled trial. Lancet Oncol 2006;7:719-27.
5. Strauss GM, Herndon JE 2nd, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer: CALGB 9633 with the Cancer and Leukemia Group B, Radiation Therapy Oncology Group, and North Central Cancer Treatment Group Study Groups. J Clin Oncol 2008;26:5043-51.
6. Waller D, Peake MD, Stephens RJ, et al. Chemotherapy for patients with non-small cell lung cancer: the surgical setting of the Big Lung Trial. Eur J Cardiothorac Surg 2004;26:173-82.
7. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
8. Crinò L, van Meerbeeck J. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v103-15.
9. Pisters KM, Evans WK, Azzoli CG, et al. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non small-cell lung cancer guideline. J Clin Oncol 2007;25:5506-18.
10. Robinson LA, Ruckdeschel JC, Wagner H Jr, et al. Treatment of non-small cell lung cancer-stage IIIA: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 2007;132:243S-265S.
11. Scott WJ, Howington J, Feigenberg S, et al. Treatment

of non-small cell lung cancer stage I and stage II: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 2007;132:234S-242S.
12. Pocock SJ, Assmann SE, Enos LE, et al. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. Stat Med 2002;21:2917-30.
13. Hamada C, Tanaka F, Ohta M, et al. Meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small-cell lung cancer. J Clin Oncol 2005;23:4999-5006.
14. Rami-Porta R, Ball D, Crowley J, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. J Thorac Oncol 2007;2:593-602.
15. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
16. Strauss GM, Wang XF, Maddaus M, et al. Adjuvant chemotherapy (AC) in stage IB non-small cell lung cancer (NSCLC): Long-term follow-up of Cancer and Leukemia Group B (CALGB) 9633. ASCO Meeting Abstracts 29:7015. OpenURL.
17. Varlotto JM, Recht A, Nikolov M, et al. Extent of lymphadenectomy and outcome for patients with stage I nonsmall cell lung cancer. Cancer 2009;115:851-8.
18. Jonnalagadda S, Arcinega J, Smith C, et al. Validation of the lymph node ratio as a prognostic factor in patients with N1 nonsmall cell lung cancer. Cancer 2011;117:4724-31.
19. Jonnalagadda S, Smith C, Mhango G, et al. The number of lymph node metastases as a prognostic factor in patients with N1 non-small cell lung cancer. Chest 2011;140:433-40.
20. Ruffini E, Asioli S, Filosso PL, et al. Significance of the presence of microscopic vascular invasion after complete resection of Stage I-II pT1-T2N0 non-small cell lung cancer and its relation with T-Size categories: did the 2009 7th edition of the TNM staging system miss something? J Thorac Oncol 2011;6:319-26.
21. Maeda R, Yoshida J, Ishii G, et al. Poor prognostic factors in patients with stage IB non-small cell lung cancer according to the seventh edition TNM classification. Chest 2011;139:855-61.
22. Travis WD, Garg K, Franklin WA, et al. Bronchioloalveolar carcinoma and lung adenocarcinoma: the clinical importance and research relevance of the 2004 World Health Organization pathologic criteria. J Thorac Oncol 2006;1:S13-19.
23. Iyoda A, Hiroshima K, Nakatani Y, et al. Pulmonary large cell neuroendocrine carcinoma: its place in the spectrum of pulmonary carcinoma. Ann Thorac Surg 2007;84:702-7.
24. Sun Z, Yang P. Gene expression profiling on lung cancer outcome prediction: present clinical value and future premise. Cancer Epidemiol Biomarkers Prev 2006;15:2063-8.
25. Potti A, Mukherjee S, Petersen R, et al. Retraction: A genomic strategy to refine prognosis in early-stage non-small-cell lung cancer. N Engl J Med 2006;355:570-80. N Engl J Med 2011;364:1176.
26. Subramanian J, Simon R. Gene expression-based prognostic signatures in lung cancer: ready for clinical use? J Natl Cancer Inst 2010;102:464-74.
27. Boutros PC, Lau SK, Pintilie M, et al. Prognostic gene signatures for non-small-cell lung cancer. Proc Natl Acad Sci U S A 2009;106:2824-8.
28. Zhu CQ, Ding K, Strumpf D, et al. Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. J Clin Oncol 2010;28:4417-24.
29. Kadara H, Behrens C, Yuan P, et al. A five-gene and corresponding protein signature for stage-I lung adenocarcinoma prognosis. Clin Cancer Res 2011;17:1490-501.
30. Broët P, Camilleri-Broet S, Zhang S, et al. Prediction of clinical outcome in multiple lung cancer cohorts by integrative genomics: implications for chemotherapy selection. Cancer Res 2009;69:1055-62.
31. Roepman P, Jassem J, Smit EF, et al. An immune response enriched 72-gene prognostic profile for early-stage non-small-cell lung cancer. Clin Cancer Res 2009;15:284-90.
32. Bianchi F, Nuciforo P, Vecchi M, et al. Survival prediction of stage I lung adenocarcinomas by expression of 10 genes. J Clin Invest 2007;117:3436-44.
33. Lu Y, Lemon W, Liu PY, et al. A gene expression signature predicts survival of patients with stage I non-small cell lung cancer. PLoS Med 2006;3:e467.
34. Kratz JR, He J, Van Den Eeden SK, et al. A practical molecular assay to predict survival in resected non-squamous, non-small-cell lung cancer: development and international validation studies. Lancet 2012;379:823-32.
35. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983-91.

36. Kamal NS, Soria JC, Mendiboure J, et al. MutS homologue 2 and the long-term benefit of adjuvant chemotherapy in lung cancer. Clin Cancer Res 2010;16:1206-15.
37. Tsao MS, Aviel-Ronen S, Ding K, et al. Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. J Clin Oncol 2007;25:5240-7.
38. Graziano SL, Gu L, Wang X, et al. Prognostic significance of mucin and p53 expression in stage IB non-small cell lung cancer: a laboratory companion study to CALGB 9633. J Thorac Oncol 2010;5:810-7.
39. Sève P, Lai R, Ding K, et al. Class III beta-tubulin expression and benefit from adjuvant cisplatin/vinorelbine chemotherapy in operable non-small cell lung cancer: analysis of NCIC JBR.10. Clin Cancer Res 2007;13:994-9.
40. Rosell R, Skrzypski M, Jassem E, et al. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. PLoS One 2007;2:e1129.
41. Filipits M, Pirker R, Dunant A, et al. Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program. J Clin Oncol 2007;25:2735-40.
42. Pirker R, Filipits M, Dunant A, et al. Ialt-Bio: A challenging research to improve adjuvant chemotherapy of completely resected NSCLC: D3-03. J Thorac Oncol 2007;2:S397-S398.
43. Zheng Z, Chen T, Li X, et al. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. N Engl J Med 2007;356:800-8.
44. Cobo M, Isla D, Massuti B, et al. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. J Clin Oncol 2007;25:2747-54.
45. Gazdar AF. DNA repair and survival in lung cancer--the two faces of Janus. N Engl J Med 2007;356:771-3.
46. Yin M, Yan J, Voutsina A, et al. No evidence of an association of ERCC1 and ERCC2 polymorphisms with clinical outcomes of platinum-based chemotherapies in non-small cell lung cancer: a meta-analysis. Lung Cancer 2011;72:370-377.
47. Poole AM, Logan DT, Sjoberg BM. The evolution of the ribonucleotide reductases: much ado about oxygen. J Mol Evol 2002;55:180-96.
48. Reynolds C, Obasaju C, Schell MJ, et al. Randomized phase III trial of gemcitabine-based chemotherapy with in situ RRM1 and ERCC1 protein levels for response prediction in non-small-cell lung cancer. J Clin Oncol 2009;27:5808-15.
49. Rosell R, Danenberg KD, Alberola V. et al. Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. Clin Cancer Res 2004;10:1318-25.
50. Boukovinas I, Papadaki C, Mendez P, et al. Tumor BRCA1, RRM1 and RRM2 mRNA expression levels and clinical response to first-line gemcitabine plus docetaxel in non-small-cell lung cancer patients. PLoS One 2008;3:e3695.
51. Vilmar AC, Santoni-Rugiu E, Sorensen JB. Predictive impact of RRM1 protein expression on vinorelbine efficacy in NSCLC patients randomly assigned in a chemotherapy phase III trial. Ann Oncol 2013;24:309-14.
52. Bepler G, Gautam A, McIntyre LM, et al. Prognostic significance of molecular genetic aberrations on chromosome segment 11p15.5 in non-small-cell lung cancer. J Clin Oncol 2002;20:1353-60.
53. Bepler G, Sharma S, Cantor A, et al. RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. J Clin Oncol 2004;22:1878-85.
54. Pesta M, Kulda V, Fiala O, et al. Prognostic significance of ERCC1, RRM1 and BRCA1 in surgically-treated patients with non-small cell lung cancer. Anticancer Res 2012;32:5003-10.
55. Reguart N, Cardona AF, Carrasco E, et al. BRCA1: a new genomic marker for non-small-cell lung cancer. Clin Lung Cancer 2008;9:331-9.
56. Cobo M, Massuti B, Moran T, et al. Spanish customized adjuvant trial (SCAT) based on BRCA1 mRNA levels. ASCO Meeting Abstracts 2008;26:7533.
57. Taron M, Rosell R, Felip E, et al. BRCA1 mRNA expression levels as an indicator of chemoresistance in lung cancer. Hum Mol Genet 2004;13:2443-9.
58. Bartolucci R, Wei J, Sanchez JJ, et al. XPG mRNA expression levels modulate prognosis in resected non-small-cell lung cancer in conjunction with BRCA1 and ERCC1 expression. Clin Lung Cancer 2009;10:47-52.
59. Scagliotti G, Hanna N, Fossella F, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two Phase III studies. Oncologist 2009;14:253-63.
60. Ceppi P, Volante M, Saviozzi S, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer 2006;107:1589-96.
61. Nakagawa T, Otake Y, Yanagihara K, et al. Expression of thymidylate synthase is correlated with proliferative activity in non-small cell lung cancer (NSCLC). Lung

Cancer 2004;43:145-9.
62. Shintani Y, Ohta M, Hirabayashi H, et al. New prognostic indicator for non-small-cell lung cancer, quantitation of thymidylate synthase by real-time reverse transcription polymerase chain reaction. Int J Cancer 2003;104:790-5.
63. Zheng Z, Li X, Schell MJ, et al. Thymidylate synthase in situ protein expression and survival in stage I nonsmall-cell lung cancer. Cancer 2008;112:2765-73.
64. Nakagawa T, Tanaka F, Otake Y, et al. Prognostic value of thymidylate synthase expression in patients with p-stage I adenocarcinoma of the lung. Lung Cancer 2002;35:165-70.
65. Karachaliou N, Mayo C, Costa C, et al. KRAS Mutations in Lung Cancer. Clin Lung Cancer 2013;14:205-14.
66. Capelletti M, Wang XF, Gu L, et al. Impact of KRAS mutations on adjuvant carboplatin/paclitaxel in surgically resected stage IB NSCLC: CALGB 9633. J Clin Oncol (Meeting Abstracts) 2010;28:7008.
67. M.S. Tsao PH, Bourredjem A, et al. LACE-bio pooled analysis of the prognostic and predictive value of KRAS mutation in completely resected non-small cell lung cancer (NSCLC).Ann Oncol 2010;21 supple 8, abstract 4218.
68. Slebos RJ, Kibbelaar RE, Dalesio O, et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med 1990;323:561-5.
69. Mascaux C, Iannino N, Martin B, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 2005;92:131-9.
70. Mitsudomi T, Steinberg SM, Oie HK, et al. ras gene mutations in non-small cell lung cancers are associated with shortened survival irrespective of treatment intent. Cancer Res 1991;51:4999-5002.
71. Grossi F, Loprevite M, Chiaramondia M, et al. Prognostic significance of K-ras, p53, bcl-2, PCNA, CD34 in radically resected non-small cell lung cancers. Eur J Cancer 2003;39:1242-50.
72. Steels E, Paesmans M, Berghmans T, et al. Role of p53 as a prognostic factor for survival in lung cancer: a systematic review of the literature with a meta-analysis. Eur Respir J 2001;18:705-19.
73. Mitsudomi T, Hamajima N, Ogawa M, et al. Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analysis. Clin Cancer Res 2000;6:4055-63.
74. Schiller JH, Adak S, Feins RH, et al. Lack of prognostic significance of p53 and K-ras mutations in primary resected non-small-cell lung cancer on E4592: a Laboratory Ancillary Study on an Eastern Cooperative Oncology Group Prospective Randomized Trial of Postoperative Adjuvant Therapy. J Clin Oncol 2001;19:448-57.
75. Reiman T, Seve P, Vataire A, et al. Prognostic value of class III B-tubulin (Tubb3) in operable non-small cell lung cancer (NSCLC) and predictive value for adjuvant cisplatin-based chemotherapy (CT): A validation study on three randomized trials. ASCO Meeting Abstracts 2008;26:7506.
76. Rosell R, Scagliotti G, Danenberg KD, et al. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. Oncogene 2003;22:3548-53.
77. Sève P, Isaac S, Trédan O, et al. Expression of class III {beta}-tubulin is predictive of patient outcome in patients with non-small cell lung cancer receiving vinorelbine-based chemotherapy. Clin Cancer Res 2005;11:5481-6.
78. Seve P, Mackey J, Isaac S, et al. Class III beta-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel. Mol Cancer Ther 2005;4:2001-7.
79. Tsao MS, Sakurada A, Ding K, et al. Prognostic and predictive value of epidermal growth factor receptor tyrosine kinase domain mutation status and gene copy number for adjuvant chemotherapy in non-small cell lung cancer. J Thorac Oncol 2011;6:139-47.
80. D'Angelo SP, Janjigian YY, Kris MG, et al. Impact of EGFR and KRAS mutations on survival in 1,000 patients with resected lung adenocarcinoma. J Clin Oncol (Meeting Abstracts) 2010;28:7011.

Cite this article as: Massuti B, Sanchez JM, Hernando-Trancho F, Karachaliou N, Rosell R. Are we ready to use biomarkers for staging, prognosis and treatment selection in early-stage non-small-cell lung cancer? Transl Lung Cancer Res 2013;2(3):208-221. doi: 10.3978/j.issn.2218-6751.2013.03.06

Tumor heterogeneity: evolution through space and time in EGFR mutant non small cell lung cancer patients

Margarita Majem[1], **Jordi Remon**[2]

[1]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; [2]Hospital de Mataró, Mataró, Spain

Correspondence to: Margarita Majem. Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Sant Antoni Maria Claret 167, 08025 Barcelona, Spain. Email: mmajem@santpau.cat.

Abstract: NSCLC patients with mutations in epidermal growth factor receptor (EGFR) gene have dramatic responses with the EGFR tyrosine kinase inhibitors (TKI) in the majority of patients. However, all patients will eventually present progression of disease because of both primary and acquired resistance to EGFR TKI. In the recent years several studies have identified mechanisms involved in primary and secondary resistance to EGFR TKI treatment that can also be potential therapeutic strategies, although up to 30% of cases of acquired resistance to EGFR TKI are still unexplained. In this review we describe the mechanisms of resistance to EGFR TKIs in NSCLC patients that have been discovered and potential therapeutic strategies to overcome EGFR TKI resistance. Additionally we highlight the importance of performing additional biopsies not only at time of acquired resistance to EGFR TKI but also immediately after initiation of therapy to discover the remaining unknown mechanisms of acquired resistance to EGFR TKI as well as the underlying molecular basis of the heterogeneity in response to EGFR TKI.

Keywords: Primary resistance; acquired resistance; epidermal growth factor receptor; non-small cell lung cancer

Submitted Feb 10, 2013. Accepted for publication Mar 13, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.09
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.09

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer mortality worldwide, and traditional chemotherapeutic drugs are only modestly effective. Most lung cancer patients usually present with advanced stage disease, where the efficacy of chemotherapy is low, with a 5-year survival rate lower than 15% (1).

The discovery of mutated oncogenes encoding activated signaling molecules that drive cellular proliferation and promote tumor growth has led to the development of more effective and less toxic targeted therapies for NSCLC patients. Particularly, NSCLC patients with mutations in epidermal growth factor receptor (EGFR) gene have dramatic responses and better outcome with the EGFR tyrosine kinase inhibitors (TKI) gefitinib and erlotinib (1-9).

The EGFR is a well characterized mutated oncogene in NSCLC that is associated predominantly with adenocarcinoma histology. EGFR-mutated tumors depend to EGFR signaling for their proliferation and survival. Nearly 90% of lung-cancer-specific EGFR mutations comprise a leucine-to-arginine substitution at position 858 (L858R) and deletion in exon 19 that affect the conserved sequence LREA (delE746-A750) (3,8,10,11).

Unfortunately, despite the dramatic efficacy of EGFR TKI in NSCLC patients with EGFR activating mutations, all patients eventually acquire resistance, with progression of disease occurring in patients around 10-13 months after starting treatment (2,7,12). There are two main mechanisms of resistance to EGFR TKI: the lack of an initial response to therapy, also called de novo or primary resistance to EGFR TKI, and resistance that develops following an initial response to EGFR TKI, also called acquired resistance to EGFR TKI.

To discover those mechanisms involved in EGFR TKI

resistance is a significant challenge in order to develop more effective targeted therapies alone or in combination with EGFR TKI for patients with NSCLC and EGFR mutations. In this article we review the molecular basis of resistance of EGFR mutant NSCLC patients to EGFR TKI and rebiopsy strategies to better understand the underlying molecular basis of resistance.

Primary resistance to EGFR TKIs

Patients with NSCLC and EGFR activating mutation will experience significant tumor regression with EGFR TKI in approximately 70% of cases (5), which means a lack of an initial response in about 30% of patients. Those patients will present primary or de novo resistance to EGFR TKI.

To date, two main mechanisms of primary resistance to EGFR TKI in EGFR mutant NSCLC patients have been described: first, the presence of secondary alterations in EGFR that prevent inhibition of EGFR by an EGFR TKI (also known drug resistant EGFR mutation), and second, the presence of additional genetic alternations that occur together with EGFR mutation.

Secondary alterations in EGFR

EGFR exon 20 insertions

EGFR Exon 20 insertions comprise approximately 4% of all EGFR mutant NSCLC (13) and are associated with lower sensitivity to the reversible EGFR TKIs both in preclinical models and in patients that have experienced a lack of response when treated with gefitinib or erlotinib (14-16). The irreversible EGFR TKIs could be more effective in these mutations (15,17-19).

EGFR T790M (c.2369C>T) mutation in non-small cell lung cancer

The T790M mutation results in an amino acid substitution at position 790 in EGFR, from a threonine (T) to a methionine (M). This gatekeeper mutation also occurs within exon 20, which encodes part of the kinase domain EGFR and alters the binding of EGFR TKI to the ATP-binding pocket, and therefore EGFR TKI are unable to block EGFR signalling (20-22). These pretreatment T790M mutations generally occur together with another EGFR sensitizing mutation and have been found to be associated with decreased sensitivity to EGFR TKIs (16). Additionally, the baseline T790M mutations may be present as an underlying germline mutation at a low frequency (0.5% of never smokers with lung cancer) (23) and may be associated with familial cancer syndromes (24).

Rosell *et al*. assessed the T790M mutation in pretreatment diagnostic specimens from 129 EGFR TKI treated advanced NSCLC patients with EGFR mutations, and found that EGFR T790M mutation was present in 45 of 129 patients (35%). Progression-free survival was 12 months in patients with and 18 months in patients without the T790M mutation (P=0.05). Additionally, it was found that low BRCA-1 levels neutralized the negative effect of the T790M mutation and were associated with longer progression-free survival to erlotinib, whereas high levels of BRCA-1 may lead to de novo resistance through increased DNA damage repair capacity, suggesting that pretreatment assessment of both T790M mutation and BRCA1 expression could be useful to predict outcome (25). Additionally, in the EURTAC trial the T790M mutation was detected in 38% of the pre-treatment specimens analysed (26).

Fujita *et al*. evaluated the incidence of T790M in pretreatment tumor specimens using highly sensitive colony hybridization technique and was detected in 30/38 resected tumor tissues of patients with the EGFR mutation (79%). The median time to treatment failure was 9 months for the patients with pretreatment T790M and 7 months for the patients without the T790M mutation (P=0.44), and suggested that patients with high proportion of T790M allele may have a relatively favorable prognosis (27).

In addition to EGFR T790M, primary EGFR TKI resistance may also be due to other secondary mutations in EGFR (e.g., D761Y) that can occur concurrent with an activating EGFR kinase domain mutation (e.g., L858R) (28).

Genetic alternations with EGFR mutations

Other genetic alterations may occur together with EGFR mutation causing EGFR TKI resistance by preserving cell survival even with EGFR inhibition. These additional genetic alterations that promote EGFR pathway include:

Activation of phosphoinositide-3-kinase (PI3K)/AKT signaling

Phosphatase and tensine homolog (PTEN) acts as a tumor suppressor by negatively regulating the PI3K/AKT signaling pathway. In preclinical studies, loss of PTEN was associated with decreased sensitivity of EGFR mutant lung

tumors to EGFR TKI by increased activity of the PI3K-AKT pathway, and degradation of activated EGFR (29,30).

Somatic mutations in PIK3CA have been found in 1-3% of all NSCLC (31,32). These mutations usually occur within two "hotspot" areas within exon 9 (the helical domain) and exon 20 (the kinase domain). Preclinical data has shown that introduction of activating PIK3CA mutants into EGFR mutant lung cancer cell lines confers resistance to EGFR TKI (33).

Crosstalk with the IGF1R pathway

Resistance to EGFR TKI in cell lines with EGFR activating mutations through crosstalk with the IGF1R pathway has been observed through in preclinical models. For example, some EGFR-mutant cells undergo only G1 cell cycle phase arrest in the presence of erlotinib, but undergo apoptosis when co-treated with an IGF1R-specific antibody (34). In another study, EGFR mutant NSCLC cell lines persisting after EGFR TKI treatment were enriched for a drug-tolerant subpopulation that may have existed prior to treatment that showed a distinct chromatin state that is regulated by IGF1R signalling (35).

Activation of NFκB signaling

NFκB is a protein complex that controls the transcription of DNA. NFκB signaling has been associated with cancer and inflammation (36), and it has also been suggested that activation of NFκB signaling may cause primary resistance to EGFR TKI treatment in EGFR mutant lung cancer patients.

Bivona *et al*. used a cell line (H1650) with EGFR mutation but resistant to EGFR TKI and showed that inhibition of the NFκB pathway enhanced cell death by EGFR TKI whereas activation of NFκB rescued EGFR-mutant lung cancer cells from EGFR TKI treatment. Additionally, genetic or pharmacologic inhibition of NFκB enhanced erlotinib-induced apoptosis in erlotinib-sensitive and erlotinib-resistant EGFR-mutant lung cancer models, and increased expression of the NFκB inhibitor IκB, predicted for improved response and survival in EGFR-mutant lung cancer patients treated with EGFR TKI. Importantly, IκB status was not predictive of outcomes in EGFR mutant lung cancer patients treated with surgery or chemotherapy, indicating NFκB signaling is specific biomarker of EGFR TKI response in this patient population (37). These data identify NFκB as a potential drug target, together with EGFR, in EGFR-mutant lung cancers.

High BIM expression levels

BIM, also known as BCL2-like 11, is a proapoptotic protein that is overexpressed in different malignancies (38,39). Various chemotherapeutic agents use BIM as a mediating executioner of cell death. Hence, BIM suppression supports metastasis and chemoresistance. BIM upregulation is required for apoptosis induction by EGFR-TKIs in EGFR-mutant NSCLC. Low BIM mRNA levels could lead to gefitinib resistance in NSCLC with EGFR mutations and could be a marker of primary resistance. The extracellular regulated kinase (ERK) pathway also negatively regulates BIM expression in NSCLC with EGFR mutations (40-42). Components that cause induction of BIM may have a role to overcome resistance to EGFR TKI in NSCLC with EGFR mutations. Recent studies have showed that HDAC inhibition can epigenetically restore BIM function in vitro and death sensitivity of EGFR-TKI, in cases of EGFR mutant NSCLC where resistance to EGFR-TKI is associated with a common BIM polymorphism (43).

Treatment approaches to overcome primary resistance

For lung cancer patients harboring secondary alterations in EGFR, more effectively EGFR TKI is needed. Second-generation irreversible EGFR TKI have shown to be more active targeting T790M or EGFR exon 20 insertion mutation than gefitinib or erlotinib (44-46). Additionally, the Spanish Lung Cancer Group is conducting a phase Ib/IIb Study to evaluate the role of gefitinib in combination with olaparib in NSCLC patients with EGFR mutation to overcome primary resistance in those patients with high BRCA1 levels (NCT01513174). For lung cancer patients harboring other genetic alterations with EGFR mutation the use of polytherapy could overcome primary resistance. For example, a phase II trial of erlotinib and AT-101 (BCL-2 pan inhibitor) in NSCLC patients with EGFR mutations has been performed, although no results have been presented, yet (NCT00988169).

Additionally, a combination of an EGFR TKI with PI3K-AKT, IGFR, NFκB or BIM inhibitors could also play a role in those alterations co-occur causing EGFR TKI resistance.

Acquired resistance to EGFR TKI

Several mechanisms of acquired resistance to EGFR TKI in EGFR mutant NSCLC patients have been reported, which

could be grouped in four main categories: first, the presence of secondary mutations in EGFR; second, the presence by-pass tracks activation; third a phenotypic transformation; and fourth, additional genetic alternations that occur together with EGFR mutation. Up to 30% of cases are still unexplained.

Second-site mutations in EGFR

Approximately 50-60% of cases with acquired resistance to EGFR TKI therapy have a second-site mutation T790M ("gatekeeper mutation") in the kinase domain of EGFR that coexists with the EGFR activating mutation (21,47). Conversely to primary T790M mutation, acquired resistance by T790M mutation identifies a subset of EGFR-mutant lung cancers with indolent growth in preclinical (48) and clinical set (49).

The subclonal populations of EGFR mutant tumor cells with and without the EGFR T790M can coexist in an EGFR mutant NSCLC with acquired resistance to EGFR TKI. This heterogeneity would explain both the "flare" phenomenon (rapid tumor regrowth upon withdrawal of an EGFR TKI) observed upon discontinuation of an EGFR TKI and also the finding that EGFR mutant NSCLC patients may respond to subsequent EGFR TKI treatment after initial discontinuation of therapy (50-53).

In addition to EGFR T790M mutation, there are other mutations that have been associated with acquired EGFR TKI resistance: T854A in exon 21 (54), L747S (55), and D761Y (28), both in exon 19. However, the frequency of all such mutation appears to be very low in comparison with the T790M mutation.

By-pass tracks activation

Other mechanism of acquired resistance to EGFR TKI is the activation of parallel pathways in which the key downstream targets of EGFR are activated independently of EGFR. These mechanisms include MET amplification and HGF overexpression. Amplification of the receptor tyrosine kinase MET leads EGFR inhibitor resistance by causing phosphorylation of ERBB3, which in turn sustains the activation of the PI3K/Akt signal downstream, providing a bypass signalling even in the presence of EGFR inhibitor. MET amplification was detected in 22% of lung cancer specimens that developed acquired resistance to EGFR TKI and inhibition of both EGFR and MET was required to kill the resistant cells, suggesting a persistent oncogenic addiction to EGFR pathway beyond to acquired resistance to EGFR TKI (56-58). In the clinic, MET amplification was reported in 4% of patients. The prevalence of MET-dependent resistance may depend upon the assay used (59).

Although MET amplification can occur with the EGFR T790M mutation, about 60% of MET amplification is independent of T790M mutation. There is an inverse relationship between the presence of T790M and MET gene copy number, suggesting a complementary role of the two mechanisms in the acquisition of resistance. In preclinical models, MET inhibitors may be able to overcome MET-mediated resistance, even in cells that harbour the T790M mutation (60). Concurrent inhibition therapy might be essential for outcome improvement (61). MET activation by overexpression of its ligand, HGF, also induced drug resistance *in vitro* and *in vivo* through GAB1 signalling, which directly activates PI3K/Akt pathway (62). In patients with paired tumor specimens, HGF expression was higher in drug-resistant specimens than in the pretreatment specimens (P=0.025) (63) and in other study with 23 acquired resistance tumors, high-level HGF expression was detected in higher proportion than T790M mutation (62). Japanese patients with weak HGF expression by immunohistochemistry tend to have lower 5-year OS than those with overexpression (22.2% *vs.* 75%, P=0.259) (64). Of note, MET amplification has also been observed in EGFR mutant NSCLC patients prior to EGFR TKI and was associated with the development of acquired resistance to EGFR TKIs (60), suggesting that EGFR TKI may select for preexisting cells with MET amplification during the acquisition of EGFR TKI resistance.

Phenotypic transformation

This acquired resistance mechanism includes the histological transformation to small cell lung cancer (SCLC) and the epithelial to mesenchymal transition (EMT), with an incidence of 14% and 5%, respectively (58). These new SCLC retain the original EGFR-sensitizing mutation and respond to standard small cell carcinoma chemotherapy, but the exact mechanism for this histological transformation is unknown.

EMT is a phenomenon characterized in which the cancer cell looses its epithelial morphology and develops a more spindle-like mesenchymal morphology with often associated with a shift in expression of specific proteins (for example,

loss of E-cadherin and gain of vimentin) resulting in a more invasiveness phenotype (65). The exact mechanism for the acquisition of the EMT phenotype remains unclear; some studies have found an upregulation of NOTCH-1 expression (66), the aberrant expression of transforming growth factor (TGF)-β (67,68), and phosphorylation of MEK (69). Increased expression of E-cadherin, has been associated with clinical activity of EGFR TKI in NSCLC patients (70,71). EMT has been also associated with acquired resistance to EGFR TKI in preclinical models (65,71) as well as in several studies (58). It is unknown if mesenchymal-like cells in the acquired resistant tumors are exist prior to therapy or are induced upon drug treatment. It has been recently described that activation of the AXL receptor tyrosine kinase by overexpression or upregulation of its ligand GAS6 confers acquired resistance to EGFR TKI in preclinical models, and the inhibition of AXL restored erlotinib sensitivity. Upregulation of AXL was associated with the development of an EMT in EGFR mutant NSCLC with acquired resistance. Approximately 20% of the EGFR TKI resistant tumors showed increased AXL expression (72).

Additional genetic alternations

PIK3CA mutation

Mutation in PIK3CA was identified in 5% of EGFR mutant lung cancers that developed acquired EGFR TKI resistance as well as in preclinical models (58).

PTEN mutation

In preclinical models, loss of PTEN expression contributes to TKI resistance in NSCLC (73). Cells with knockdown of PTEN, with constitutive PI3KCA activation, have a deficient homologous recombinant DNA repair and increased sensitivity to cisplatin and PARP inhibitors (74).

HER2 amplification

HER2 amplification has been recently detected in 12% of tumors with acquired resistance to EGFR TKI, and only in 1% of untreated EGFR mutant NSCLC cells. This new mechanism of acquired resistance was exclusive with T790M mutation (75). Interestingly, in preclinical models the combination of afatinib plus cetuximab significantly inhibited HER2 phosphorylation. These results implicate HER2 as a novel protein involved in the sensitivity or resistance of EGFR mutant NSCLC providing a rationale to assess its status and target HER2 in such tumors.

MAPK1 amplification

MAPK1 amplification was described in approximately 5% of clinical specimens from patients with acquired resistance to EGFR TKI treatment and was mutually exclusive with the T90M mutation or MET amplification (76).

BRAF mutation

RAS pathway mutations are rare, but BRAF mutations (V600E, G469A) can occur in 1% of tumors with acquired resistance to EGFR TKI (77).

JAK2

In a preclinical cell line model, the activation of JAK2 (an upstream STAT signal pathway) caused acquired EGFR TKI resistance. Combined treatments of erlotinib plus a JAK2 inhibitor (JSI-124) restored sensitivity to erlotinib in PC-9/ERB3 cells and reduced tumors in a murine xenograft model (78).

IGFR

In vitro data showed that the increased IGF-1R signalling through the loss of IGF inhibitory proteins may also mediate resistance to EGFR TKI by activating downstream targets that bypass dependency in EGFR (79).

Loss of activating EGFR mutant gene

Loss of activating EGFR mutant gene contributes to acquire resistance to EGFR TKI in lung cancer cells. This loss of addiction to mutant EGFR resulted in gain of addiction to both HER2/HER3 and PI3K/AKT signalling to acquire EGFR TKI resistance (80).

Treatment approaches to overcome acquired resistance

Given this role of persistent EGFR signalling in causing resistance to TKI, a second generation irreversible EGFR TKI bind to a different EGFR tyrosine kinase domain have shown activity against lung cancer cells harboring both EGFR activation mutations and the T790M resistance-mutation (17,45,81,82). A phase III trial of afatinib versus placebo in patients with acquired resistance to EGFR TKI demonstrated a 2-month improvement in progression free survival; although no significant benefit in overall survival was observed (83).

A more recent strategy for intensification of EGFR inhibition has been the addition of monoclonal antibodies targeting EGFR, such as cetuximab. Combined treatment

Table 1 Summary of rebiopsy studies and the molecular and histological alterations

Rebiopsy studies	Mechanisms of resistance to EGFR inhibitors analyzed	Histological alterations in the resistant tumor
Arcilla *et al*.	Pretreatment *EGFR* mutation: 100%	
	T790M:	
	Standard sequencing: 49%	
	Fragment Analysis 53%	Not performed
	Combined standard and LNA-PCR/sequencing: 70%	
	MET amplification: 11%	
Sequist *et al*.	Pretreatment *EGFR* mutation: 100%	
	T790M: 49%	SCLC transformation: 14%
	MET amplification: 5%	
	PIK3CA mutation: 5%	ETM: 8%
	β-catenin mutations: 5% (all with T790M mut)	
Oxnard *et al*.	T790M: 62%	Not performed
Ohashi *et al*.	B-RAF: 1%	Not performed

SCLC, small cell lung cancer; EMT, Epithelial to mesenchymal transition.

with afatinib and cetuximab induced regression in T790M transgenic murine and mice models with erlotinib resistant lung tumors (84). This synergistic activity has been confirmed in phase I/II clinical trial, with a response rate of 32% in heavily pre-treated population with T790M-positive and T790M-negative tumors and a median Progression free survival of 4.67 months (85). Erlotinib plus cetuximab has showed to overcome T790M-mediated drug resistance in preclinical data (86). However, this strategy did not show significant activity in a phase I/II trial in patients with acquired resistance to erlotinib (85). The new T790M specific inhibitor WZ-4002 is also under investigation, and has demonstrated to induce greater growth inhibition *in vitro* and *in vivo* against T790M than against WT EGFR (87). Indeed, the FLT3 inhibitor, an indolocarbazole compounds, is under investigation as potent and reversible inhibitor of EGFR T790M that spare wild-type EGFR in the context of T790M-mediated drug resistance in NSCLC (88).

Combined treatments of erlotinib plus therapies targeting compensatory pathways that lead to acquired EGFR TKI resistance may overcome resistance. The addition of a MET inhibitor may benefit those patients with EGFR mutant NSCLC and MET amplification. Antibodies targeting the MET ligand HGF (AMG102), MET itself (MetMAb), and small molecule inhibitors against MET are in clinical development. The combination of AXL inhibitors, such as XL880, MP-470 or SGI-7079, with an EGFR TKI is also a potential approach to overcome resistance associated with EMT (89).

Furthermore, inhibition of NOTCH-1 can be a novel strategy for the reversal of the EMT phenotype thereby potentially increasing therapeutic drug sensitivity to lung cancer cells. BEZ235, a dual inhibitor of PI3K and mTOR, would overcome EGFR-TKI resistance induced by HGF in an EGFR mutant lung cancer cell lines (90).

Finally, combination therapy with EGFR TKI and PI3KCA inhibitor, PARP inhibitors (in PTEN mutant patients), HER2 inhibitors, B-RAF inhibitors or IGFR inhibitors could have a therapeutic effect in tumors with acquired resistant to EGFR TKI by those mechanisms and some of them are being investigated in clinical trials (91).

Strategies to determine molecular basis of resistance to EGFR TKI in NSCLC with EGFR mutations

As commented previously, the biological basis underlying acquired EGFR TKI resistance is unknown in approximately 30% of patients. Some of these previously described mechanisms of resistance to EGFR TKI that have been identified in preclinical models and have not been validated in patients with acquired resistance. The analysis of clinical specimens is crucial to discover the remaining unknown mechanisms of EGFR TKI resistance. In the last years many authors have published their own experience with rebiopsies on patients with EGFR mutant NSCLC at the time of progression in order to identify how EGFR mutant NSCLC acquire resistance to EGFR TKI (*Table 1*).

Arcila *et al*. undertook a rebiopsy study to determine the feasibility of rebiopsy in patients with EGFR mutant NSCLC with acquired resistance to EGFR TKI and to evaluate the spectrum of EGFR mutations and MET amplification in tumors at progression. One hundred and fifty three samples were obtained from 121 patients including frozen samples, fresh fluids, FFPE tissue and cytologies from fine needle aspirates (FNA); eighty-two per cent were successfully analyzed. Biopsies provided the highest success rate followed by FNA and pleural fluids. Pathologic confirmation was performed in 106 resistant tumors: one hundred and two adenocarcinomas, one squamous cell carcinoma, two small cell carcinomas and 1 with a mixed histology (combined large cell carcinoma/adenocarcinoma in one sample and a high grade neuroendocrine carcinoma in a second). EGFR mutations (exons 19 and 21) were found in 100/104 in resistant samples, seventy-one per cent had EGFR exon 19 deletions, one per cent had an insertion in exon 18 and 28% had an exon 21 point mutation. Of note, patients with multiple tissue sampling had the same mutation in all tumor sites, and all patients maintained the baseline sensitizing mutation. The T790M mutation was detected in 51% of mutant samples by standard analysis, and the retest of 30 negative patients by the LNA-PCR/sequencing method detected 11 additional mutants, raising the T790M mutation rate to 70%. MET amplification was found in 11% (4 patients), three of them also harbored the EGFR T790M mutation (57).

Sequist *et al*. performed rebiopsies on 37 EGFR mutant NSCLC patients with acquired resistance to identify the mechanisms of resistance to EGFR inhibitors. Pre- and post-EGFR TKI tumor samples were analyzed for the presence of genetic alterations with a genotyping platform (SNaPshot assay), and EGFR and MET amplification with fluorescence in situ hybridization (FISH). Eighteen (49%) patients acquired the T790M mutation, and two (5%) patients developed MET amplification, which was not present in the pretreatment specimen. Two (5%) patients showed acquired PIK3CA mutations, two (5%) cases had β-catenin mutations (together with the T790M mutation). Fifteen (41%) rebiopsies didn't reveal any new mutations. The authors also found significant histological alterations in the resistant tumor; five patients (14%) had a diagnosis of SCLC, all maintaining the original EGFR mutation. Additionally, three resistant specimens had phenotypic changes consistent with a mesenchymal, supporting an ETM, none showed another identified resistance mechanism while maintained their original EGFR mutation. Of note, EMT or SCLC were not observed in biopsies from EGFR wild-type tumors resistant to chemotherapy (58).

Interestingly, multiple biopsies over the course of the disease were performed in 3 patients showing gain and loss of the T790M mutation in multiple biopsies from the same anatomical location during the clinical course in two of them at time of progression or when de EGFR TKI was interrupted. The rebiopsy from the third patient showed SCLC transformation with the original EGFR L858R mutation plus an acquired PIK3CA mutation. However, those changes were not observed at progression to treatment for SCLC, where adenocarcinoma histology with EGFR L858R mutation was again demonstrated (58). These results explain why retreatment of NSCLC patients with EGFR TKI who had experienced favorable results from their initial treatment could benefit some patients (53,92).

Oxnard *et al*. performed a rebiopsy protocol in EGFR mutant lung cancer patients with acquired resistance to EGFR TKI comparing for the presence of the T790M. T790M was identified in 62% of patients in the rebiopsy specimens with longer survival after progression than patients without T790M (49,59).

Finally, Ohashi *et al*. systematically screened for recurrent mutations in *RAS/NRAS/BRAF/MEK1* in nearly 200 tumor samples from patients with acquired resistance to EGFR TKI. They found two BRAF mutations: one case with concurrent *EGFR* exon19 deletion and *EGFR* T790M and *BRAF* V600E mutations and another case with *EGFR* exon19 deletion and the *BRAF* G469A mutation (2/195, 1.0%). They studied further the biological and therapeutic consequences of acquired *NRAS* and *BRAF* mutations in *EGFR*-mutant lung tumor cells and showed that these tumor cells were resistant to erlotinib alone but were sensitive to combination treatment with EGFR and MEK inhibition (77).

There is no doubt that identifying the molecular mechanisms underlying variable response and resistance to EGFR TKI in EGFR mutant NSCLC is a major obstacle to optimize EGFR TKI therapy. A more comprehensive analysis of clinical specimens from EGFR TKI-treated patients should offer a better knowledge about if known mechanisms of resistance occur exclusively and concomitantly to promote clinical resistance. This is a key issue to resolve because we will need to determine whether to target individual or multiple drivers of resistance with targeted therapies in patients according to their molecular alterations present in their tumors.

Additionally, multiple rebiopsy studies also suggest that genetic mechanisms of resistance are potentially reversible, and therefore, a static diagnostic biopsy may be insufficient to guide therapeutic decision making throughout the course of a patient's disease (58). To perform a rebiopsy at time of progression in EGFR mutant NSCLC patients is becoming more and more standard.

However, the underlying molecular basis of the heterogeneity in response to EGFR TKI has never been explored in patients immediately after initiation of therapy. This information would be crucial to study the early changes that can compromise response and progression and would help to uncover the molecular causes of treatment resistance and optimize the EGFR TKI therapy. Characterizing the complete molecular landscape of response to EGFR TKI in EGFR mutant NSCLC specimens from patients before and serially during treatment would reveal not only novel biomarkers of response to therapy but also potential new therapeutic targets to prevent or overcome resistance to EGFR TKI in NSCLC patients.

Summary

Several studies have showed that rebiopsy of EGFR mutant NSCLC patients with acquired resistance to EGFR TKI is feasible and provides sufficient material for mutation analysis in most patients. Interestingly, a wide heterogeneity in resistance mechanisms has been observed, each of which may require its own therapeutic strategy.

Indeed, it is becoming crucial the need of continuous assessment of each tumor evolution during the course of treatment not only to determine how it became resistant to therapy but also to allow us to design rational strategies to overcome resistance or to prevent acquired resistance in patients.

Since many patients do not undergo rebiopsy at progression, the lack of available resistant tumor tissue limits the molecular guided stratification of patients and negatively affects further investigation of acquired resistance. Of note, mechanisms of primary resistance are not usually analyzed in rebiopsy protocols in EGFR mutant NSCLC patients receiving EGFR TKI after the initiation of EGFR TKI which compromises a better understanding of how to prevent resistance to therapy.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007;2:706-14.
2. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
3. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
4. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306-11.
5. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958-67.
6. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-8.
7. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
8. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
9. Inoue A, Kobayashi K, Maemondo M, et al. Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naïve non-small cell lung cancer with sensitive EGFR gene mutations (NEJ002). Ann Oncol 2013;24:54-9.
10. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
11. Sharma SV, Bell DW, Settleman J, et al. Epidermal growth

factor receptor mutations in lung cancer. Nat Rev Cancer 2007;7:169-81.
12. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
13. Mitsudomi T, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. FEBS J 2010;277:301-8.
14. Wu JY, Wu SG, Yang CH, et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. Clin Cancer Res 2008;14:4877-82.
15. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. Lancet Oncol 2012;13:e23-31.
16. Wu JY, Yu CJ, Chang YC, et al. Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. Clin Cancer Res 2011;17:3812-21.
17. Engelman JA, Zejnullahu K, Gale CM, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. Cancer Res 2007;67:11924-32.
18. Li D, Ambrogio L, Shimamura T, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 2008;27:4702-11.
19. Yuza Y, Glatt KA, Jiang J, et al. Allele-dependent variation in the relative cellular potency of distinct EGFR inhibitors. Cancer Biol Ther 2007;6:661-7.
20. Yun CH, Mengwasser KE, Toms AV, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 2008;105:2070-5.
21. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786-92.
22. Inukai M, Toyooka S, Ito S, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res 2006;66:7854-8.
23. Girard N, Lou E, Azzoli CG, et al. Analysis of genetic variants in never-smokers with lung cancer facilitated by an Internet-based blood collection protocol: a preliminary report. Clin Cancer Res 2010;16:755-63.
24. Bell DW, Gore I, Okimoto RA, et al. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. Nat Genet 2005;37:1315-6.
25. Rosell R, Molina MA, Costa C, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. Clin Cancer Res 2011;17:1160-8.
26. Rosell R, Molina-Vila M, Taron M, et al. EGFR compound mutants and survival on erlotinib in non-small cell lung cancer (NSCLC) patients (p) in the EURTAC study. J Clin Oncol 2012;30:abstr 7522.
27. Fujita Y, Suda K, Kimura H, et al. Highly sensitive detection of EGFR T790M mutation using colony hybridization predicts favorable prognosis of patients with lung cancer harboring activating EGFR mutation. J Thorac Oncol 2012;7:1640-4.
28. Balak MN, Gong Y, Riely GJ, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 2006;12:6494-501.
29. Sos ML, Koker M, Weir BA, et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. Cancer Res 2009;69:3256-61.
30. Vivanco I, Rohle D, Versele M, et al. The phosphatase and tensin homolog regulates epidermal growth factor receptor (EGFR) inhibitor response by targeting EGFR for degradation. Proc Natl Acad Sci U S A 2010;107:6459-64.
31. Kawano O, Sasaki H, Endo K, et al. PIK3CA mutation status in Japanese lung cancer patients. Lung Cancer 2006;54:209-15.
32. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
33. Engelman JA, Mukohara T, Zejnullahu K, et al. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. J Clin Invest 2006;116:2695-706.
34. Gong Y, Yao E, Shen R, et al. High expression levels of total IGF-1R and sensitivity of NSCLC cells in vitro to an anti-IGF-1R antibody (R1507). PLoS One 2009;4:e7273.
35. Sharma SV, Lee DY, Li B, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141:69-80.
36. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-κB as the matchmaker. Nat Immunol 2011;12:715-23.
37. Bivona TG, Hieronymus H, Parker J, et al. FAS and NF-

κB signalling modulate dependence of lung cancers on mutant EGFR. Nature 2011;471:523-6.
38. Akiyama T, Dass CR, Choong PF. Bim-targeted cancer therapy: a link between drug action and underlying molecular changes. Mol Cancer Ther 2009;8:3173-80.
39. Wang Z, Zhang B, Yang L, et al. Constitutive production of NF-kappaB2 p52 is not tumorigenic but predisposes mice to inflammatory autoimmune disease by repressing Bim expression. J Biol Chem 2008;283:10698-706.
40. Cragg MS, Kuroda J, Puthalakath H, et al. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med 2007;4:1681-89; discussion 1690.
41. Gong Y, Somwar R, Politi K, et al. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. PLoS Med 2007;4:e294.
42. Costa DB, Halmos B, Kumar A, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med 2007;4:1669-79; discussion 1680.
43. Nakagawa T, Takeuchi S, Yamada T, et al. EGFR-TKI resistance due to BIM polymorphism can be circumvented by in combination with HDAC inhibition. Cancer Res 2013;73:2428-34.
44. Carter TA, Wodicka LM, Shah NP, et al. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. Proc Natl Acad Sci U S A 2005;102:11011-6.
45. Majem M, Pallarès C. An update on molecularly targeted therapies in second- and third-line treatment in non-small cell lung cancer: focus on EGFR inhibitors and anti-angiogenic agents. Clin Transl Oncol 2013;15:343-57.
46. Ramalingam SS, Blackhall F, Krzakowski M, et al. Randomized phase II study of dacomitinib (PF-00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2012;30:3337-44.
47. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.
48. Chmielecki J, Foo J, Oxnard GR, et al. Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. Sci Transl Med 2011;3:90ra59.
49. Oxnard GR, Arcila ME, Sima CS, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res 2011;17:1616-22.
50. Riely GJ, Kris MG, Zhao B, et al. Prospective assessment of discontinuation and reinitiation of erlotinib or gefitinib in patients with acquired resistance to erlotinib or gefitinib followed by the addition of everolimus. Clin Cancer Res 2007;13:5150-5.
51. Milton DT, Riely GJ, Pao W, et al. Molecular on/off switch. J Clin Oncol 2006;24:4940-2.
52. Kurata T, Tamura K, Kaneda H, et al. Effect of re-treatment with gefitinib ('Iressa', ZD1839) after acquisition of resistance. Ann Oncol 2004;15:173-4.
53. Yano S, Nakataki E, Ohtsuka S, et al. Retreatment of lung adenocarcinoma patients with gefitinib who had experienced favorable results from their initial treatment with this selective epidermal growth factor receptor inhibitor: a report of three cases. Oncol Res 2005;15:107-11.
54. Bean J, Riely GJ, Balak M, et al. Acquired resistance to epidermal growth factor receptor kinase inhibitors associated with a novel T854A mutation in a patient with EGFR-mutant lung adenocarcinoma. Clin Cancer Res 2008;14:7519-25.
55. Costa DB, Schumer ST, Tenen DG, et al. Differential responses to erlotinib in epidermal growth factor receptor (EGFR)-mutated lung cancers with acquired resistance to gefitinib carrying the L747S or T790M secondary mutations. J Clin Oncol 2008;26:1182-4; author reply 1184-6.
56. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
57. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17:1169-80.
58. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
59. Oxnard GR, Arcila ME, Chmielecki J, et al. New strategies in overcoming acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer. Clin Cancer Res 2011;17:5530-7.
60. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib

or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932-7.
61. Suda K, Murakami I, Katayama T, et al. Reciprocal and complementary role of MET amplification and EGFR T790M mutation in acquired resistance to kinase inhibitors in lung cancer. Clin Cancer Res 2010;16:5489-98.
62. Yano S, Wang W, Li Q, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res 2008;68:9479-87.
63. Turke AB, Zejnullahu K, Wu YL, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 2010;17:77-88.
64. Uramoto H, Yamada T, Yano S, et al. Prognostic value of acquired resistance-related molecules in Japanese patients with NSCLC treated with an EGFR-TKI. Anticancer Res 2012;32:3785-90.
65. Suda K, Tomizawa K, Fujii M, et al. Epithelial to mesenchymal transition in an epidermal growth factor receptor-mutant lung cancer cell line with acquired resistance to erlotinib. J Thorac Oncol 2011;6:1152-61.
66. Xie M, Zhang L, He CS, et al. Activation of Notch-1 enhances epithelial-mesenchymal transition in gefitinib-acquired resistant lung cancer cells. J Cell Biochem 2012;113:1501-13.
67. Shan B, Yao TP, Nguyen HT, et al. Requirement of HDAC6 for transforming growth factor-beta1-induced epithelial-mesenchymal transition. J Biol Chem 2008;283:21065-73.
68. Serizawa M, Takahashi T, Yamamoto N, et al. Combined Treatment with Erlotinib and a Transforming Growth Factor-β Type I Receptor Inhibitor Effectively Suppresses the Enhanced Motility of Erlotinib-Resistant Non-Small-Cell Lung Cancer Cells. J Thorac Oncol 2013;8:259-69.
69. Morgillo F, Cascone T, D'Aiuto E, et al. Antitumour efficacy of MEK inhibitors in human lung cancer cells and their derivatives with acquired resistance to different tyrosine kinase inhibitors. Br J Cancer 2011;105:382-92.
70. Yauch RL, Januario T, Eberhard DA, et al. Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. Clin Cancer Res 2005;11:8686-98.
71. Coldren CD, Helfrich BA, Witta SE, et al. Baseline gene expression predicts sensitivity to gefitinib in non-small cell lung cancer cell lines. Mol Cancer Res 2006;4:521-8.
72. Zhang Z, Lee JC, Lin L, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet 2012;44:852-60.
73. Yamamoto C, Basaki Y, Kawahara A, et al. Loss of PTEN expression by blocking nuclear translocation of EGR1 in gefitinib-resistant lung cancer cells harboring epidermal growth factor receptor-activating mutations. Cancer Res 2010;70:8715-25.
74. Mendes-Pereira AM, Martin SA, Brough R, et al. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol Med 2009;1:315-22.
75. Takezawa K, Pirazzoli V, Arcila ME, et al. HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFRT790M mutation. Cancer Discov 2012;2:922-33.
76. Blakely CM, Bivona TG. Resiliency of lung cancers to EGFR inhibitor treatment unveiled, offering opportunities to divide and conquer EGFR inhibitor resistance. Cancer Discov 2012;2:872-5.
77. Ohashi K, Sequist LV, Arcila ME, et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. Proc Natl Acad Sci U S A 2012;109:E2127-33.
78. Harada D, Takigawa N, Ochi N, et al. JAK2-related pathway induces acquired erlotinib resistance in lung cancer cells harboring an epidermal growth factor receptor-activating mutation. Cancer Sci 2012;103:1795-802.
79. Guix M, Faber AC, Wang SE, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest 2008;118:2609-19.
80. Tabara K, Kanda R, Sonoda K, et al. Loss of activating EGFR mutant gene contributes to acquired resistance to EGFR tyrosine kinase inhibitors in lung cancer cells. PLoS One 2012;7:e41017.
81. Kwak EL, Sordella R, Bell DW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. Proc Natl Acad Sci U S A 2005;102:7665-70.
82. Sequist LV, Besse B, Lynch TJ, et al. Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:3076-83.
83. Miller VA, Hirsh V, Cadranel J, et al. Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. Lancet Oncol 2012;13:528-38.
84. Regales L, Gong Y, Shen R, et al. Dual targeting of EGFR can overcome a major drug resistance mutation in

mouse models of EGFR mutant lung cancer. J Clin Invest 2009;119:3000-10.
85. Janjigian YY, Azzoli CG, Krug LM, et al. Phase I/II trial of cetuximab and erlotinib in patients with lung adenocarcinoma and acquired resistance to erlotinib. Clin Cancer Res 2011;17:2521-7.
86. Wang M, Zhao J, Zhang LM, et al. Combined Erlotinib and Cetuximab overcome the acquired resistance to epidermal growth factor receptors tyrosine kinase inhibitor in non-small-cell lung cancer. J Cancer Res Clin Oncol 2012;138:2069-77.
87. Zhou W, Ercan D, Chen L, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. Nature 2009;462:1070-4.
88. Lee HJ, Schaefer G, Heffron TP, et al. Noncovalent Wild-type-Sparing Inhibitors of EGFR T790M. Cancer Discov 2013;3:168-81.
89. Byers LA, Diao L, Wang J, et al. An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. Clin Cancer Res 2013;19:279-90.
90. Sano T, Takeuchi S, Nakagawa T, et al. The novel phosphoinositide 3-kinase-mammalian target of rapamycin inhibitor, BEZ235, circumvents erlotinib resistance of epidermal growth factor receptor mutant lung cancer cells triggered by hepatocyte growth factor. Int J Cancer 2013;133:505-13.
91. Gadgeel SM, Wozniak A. Preclinical Rationale for PI3K/Akt/mTOR Pathway Inhibitors as Therapy for Epidermal Growth Factor Receptor Inhibitor-Resistant Non-Small-Cell Lung Cancer. Clin Lung Cancer 2013;14:322-32.
92. Yoshimoto A, Inuzuka K, Kita T, et al. Remarkable effect of gefitinib retreatment in a patient with nonsmall cell lung cancer who had a complete response to initial gefitinib. Am J Med Sci 2007;333:221-5.

Cite this article as: Majem M, Remon J. Tumor heterogeneity: evolution through space and time in EGFR mutant non small cell lung cancer patients. Transl Lung Cancer Res 2013;2(3):226-237. doi: 10.3978/j.issn.2218-6751.2013.03.09

EGFR molecular testing in African-American non-small cell lung cancer patients - a review of discrepant data

Bradley T. Clifford[1], Pingfu Fu[1], Nathan A. Pennell[2], Balazs Halmos[3], Rom S. Leidner[1]

[1]Departments of Medicine and Biostatistics, University Hospitals - Case Medical Center, Case Western Reserve University, Cleveland, OH, USA; [2]Cleveland Clinic, Cleveland, OH, USA; [3]Department of Medicine, New York Presbyterian Hospital - Columbia University Medical Center, Columbia College of Physicians and Surgeons, USA

Correspondence to: Bradley T. Clifford, MD. Division of Hematology/Oncology, Case Western Reserve University, University Hospitals of Cleveland, 11100 Euclid Ave, WRB 3-126, Cleveland, OH 44106, USA. Email: bradley.clifford@uhhospitals.org.

Abstract: Substantial discrepancy in the literature has recently emerged regarding epidermal growth factor receptor (EGFR) mutational frequency in African American (AA) NSCLC. The first wave of tissue profiling studies, including by our group in 2009, consistently observed a significantly lower frequency of EGFR mutation in AA *vs*. White NSCLC, whereas three recent reports appear to directly contradict these findings. Reasons for this discrepancy are unclear, but one plausible explanation arises from Simpson's paradox, the consequence of aggregating heterogeneous study cohorts (in this case, the proportion of never-smokers in the study cohort). Our review of all prior studies (combined total 386 AA NSCLC cases) underscores the wide variation in the proportion of AA never-smokers among various studies (13-57%), calling inter-study comparisons into question. In parallel, we assessed objective response by RECIST to EGFR targeted therapy for AA NSCLC in the community setting, prior to the advent of routine EGFR testing. We observed a trend toward reduced response for community-based treatment of unselected AA NSCLC (5%; 3/57) as compared to overall response rates of 10% reported by large North American trials of primarily White NSCLC patients, but this was not significant (P=0.223).

Keywords: African-American; epidermal growth factor receptor (EGFR); non-small cell lung cancer; Simpson's paradox

Submitted Dec 16, 2012. Accepted for publication Jan 21, 2013.
doi: 10.3978/j.issn.2218-6751.2013.01.04
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.01.04

Substantial discrepancy has recently emerged regarding the true frequency of activating epidermal growth factor receptor (EGFR) mutations in African American (AA) NSCLC subpopulations. Three recent reports appear to contradict earlier, and generally consistent, findings of a low mutation frequency in AA patients. The first round of sequencing studies by our group and others (2005 to 2009, *Table 1*) indicated that the frequency of activating EGFR mutations was significantly lower in African Americans (AA), ranging from 2-3% (4 of 160 cases combined), compared to a White cohort (1-5). However, in 2011 two large studies (*Table 1*: Reinersman, Cote) observed a much higher frequency of activating EGFR mutations in AA NSCLC cohorts, ranging from 12-19% (31 of 188 cases combined), without significant differences compared to White NSCLC cohorts. A third 2011 study (*Table 1*: Harada) reported an even higher EGFR mutation frequency of 31% for AA NSCLC, though this is based on a limited cohort of only sixteen cases (6-8).

What could account for these discordant results? One explanation may lie in the heterogenous study cohorts. While not all prior studies have provided data on smoking status—and this is somewhat surprising given the disease at hand—comparison of the studies which do report this information, demonstrates that the proportion of never-smokers in the AA NSCLC cohorts varies widely from 13% to 57% (see *Table 1*). We would argue that the lower end of

Table 1 Summary of literature on EGFR in AA NSCLC

Study	Yang [2005] (1)	Riely [2006] (2)	Tsao [2006] (3)	Krishnaswamy [2009] (4)	Leidner [2009] (5)	Harada [2011] (6)	Reinersman [2011] (7)	Cote [2011] (8)
Sequencing Method	Sanger	Sanger (del19 and L858R only)	Sanger	Sanger	Sanger	Sanger	Sanger (del19 and L858R only)	Sequenom Mass Spec.
P-value (AA *vs.* White)	0.03	–	–	–	0.02	–	0.11	0.53
African-American (*n*)	41	14	8	66	53	16	121	67
Mutation frequency	3% (1/41)	43% (6/14)	0% (0/8)	3% (2/66)	2% (1/53)	31% (5/16)	19% (23/121)	12% (8/67)
95% CI	0-13%	n/a	0-40%	n/a	0-11%	n/a	13-27%	n/a
% Never smokers	n/a	57% (8/14)	n/a	n/a	13% (7/53)	50% (8/16)	n/a	16% (11/67)
Mutations in never smokers	0%	n/a	0%	0%	0%	100% (5/5)	n/a	88% (7/8)
EGFR mutation [*n*]	del 19 [1]	del 19 (NR) L858R (NR)	–	del 19 [1] S768I [1]	S768N [1]	del 19 [2] L858R [1] N771GY [1] A767-V769 dup [1]	del 19 [18] L858R [5]	del 19 [8]
White (*n*)	177	259	139	76	89	–	476	77
Mutation frequency	14% (25/177)	21% (55/259)	7% (10/139)	3% (2/76)	17% (15/89)	–	13% (61/476)	16% (12/77)
95% CI	9-20%	n/a	4-13%	n/a	11-26%	–	10-16%	n/a
% Never Smokers	n/a	n/a	n/a	n/a	15% (13/89)	–	n/a	27% (21/77)
Mutations in never smokers	48% (12/25)	n/a	n/a	0%	40% (6/15)	–	n/a	66% (8/12)
EGFR mutation [*n*]	del 19 [10] L858R [11] G778F [2] D770 del [2] G719R [1] H772L/ V773M [1]	del 19 (NR) L858R (NR)	n/a	P858L [1] P733T [1]	del 19 [7] L858R [8]	–	n/a	del 19 [7] L858R [3] E709A [1] G719S [1]

this range (13% Leidner) is more informative to community practice, and is more congruent with previously reported smoking rates in a large AA NSCLC cohort (7% in a series of 1,288 patients) (9); than the high proportion of never-smokers in recent studies indicating a higher frequency of EGFR mutation for AA NSCLC (57% Cote, 50% Harada). This skew may be due to archival specimen sourcing from referral-based tertiary/quaternary centers, and also points to the likely explanation for discrepancy—Simpson's paradox—a statistical phenomenon in which a correlation observed between heterogeneous groups in the aggregate, is reversed when groups are disaggregated (10-12).

As an illustration of Simpson's paradox, we can disaggregate smoking status and EGFR mutation findings based on results from the only two studies which published sufficient data to allow for this analysis (see *Table 2*, Leidner, Cote). Notably, these two studies had similar sized cohorts of AA NSCLC cases, but arrived at divergent conclusions regarding EGFR mutation frequency. *Table 2* presents *disaggregated* results according to ever/never smoking status for each study. In both studies, the ever-smoker group represents the large majority of the AA NSCLC cohort, (87% Leidner 46/53 and 84% Cote 56/67), as would be expected. Restricting analysis to AA ever-smokers, demonstrates good agreement between studies at 2% EGFR mutation frequency (1/46 Leidner and 1/56 Cote).

Table 2 Disaggregation of ever *vs.* never smokers to illustrate Simpson's paradox

Study	Ever smokers		Never smokers		Combined	
	Leidner [2009]	Cote [2011]	Leidner [2009]	Cote [2011]	Leidner [2009]	Cote [2011]
Mutation% AA NSCLC	2% (1/46)	2% (1/56)	0% (0/7)	64% (7/11)	2% (1/53)	12% (8/67)

In contrast, when the small AA never-smoker groups are compared, the real driver of divergence comes sharply into view, with a 64% discrepancy in EGFR mutation frequency between studies (0/7 Leidner 0% and 7/11 Cote 64%). Strikingly, 7 of the 8 total EGFR mutations identified by Cote *et al.*, were detected in the very limited AA never-smoker subgroup (n=11).

The discrepancy between the two studies above, which disappears for ever-smokers when data are disaggregated, illustrates of Simpson's paradox—a statistical phenomenon with implications both in the theoretical context and in practical application. In the famous Berkeley court case, alleged bias favoring males over females was claimed, based on analysis of admissions data *in the aggregate*. The alleged bias was subsequently shown to be driven by consistent and disproportionately higher rates of female applicants to more competitive departments, which came into clear view only after disaggregated analysis at the departmental level (13). This is also the likely explanation for apparent discrepancy between EGFR tissue profiling studies in AA NSCLC. In this case, disaggregation of the data according to smoking status (for the two studies where this is possible) demonstrates that the apparent divergence is driven entirely by results from very small never-smoker subgroups.

Additional discrepancies between the various studies which should be mentioned, include differences in the specific EGFR mutations being reported and the varying sensitivity of detection for the sequencing methods employed. Two activating EGFR mutations are routinely tested in clinical practice: a short oligonucleotide deletion in exon 19 (del 19) and a non-synonymous point mutation in exon 21 leading amino acid substitution at residue 858 (L858R). Together, these two mutations (del 19 and L858R) account for up to 90% of identified EGFR mutations. Several, much rarer mutations have been reported, including prior reports in the AA NSCLC tissue profiling literature (*Table 1*). Because these rare mutations are not routinely tested in clinical practice, the actual sensitivity they may, or may not, confer to EGFR targeted therapy is not known. In fact, there is evidence to suggest that some EGFR mutations may actually confer resistance. In the recent study by Harada *et al.*, five EGFR mutations were observed in a cohort of 16 AA NSCLC cases, and notably, all were observed in AA never-smokers (6). Two of these five mutations represented rare insertions in exon 20 (N771GY and 767A-769V dup). Subsequent laboratory modeling using YFP-tagged MCF-7 cells expressing these mutations actually showed increased resistance to erlotinib *in vitro*, which may open an intriguing line of future investigation surrounding mechanisms of proclivity to rare variant EGFR mutations in specific population groups.

A further source of potential discrepancy between studies bears mention—the use of different sequencing technologies, which could influence the scope and threshold of mutations being detected. While most prior studies relied on standard Sanger sequencing for mutational profiling, the study by Cote *et al.* used a higher sensitivity platform (Sequenom mass spectrometry) which can detect mutation in as few as 5-10% of tumor cells (14). Whether response to EGFR TKI in a tumor consisting of >90% wild-type EGFR cells is clinically meaningful, remains to be determined, but may ultimately reveal that higher sensitivity is not a sine qua non of clinical benefit.

A final consideration must be given to patient self-reporting for determination of race which may lead to selection bias. Objective measure of genetic admixture is now theoretically possible, for example using ancestry SNP genotyping panels (15). The complex interaction of race and genetics in somatic tumorigenesis is not mechanistically well characterized or clinically interpretable at the present time. However, as hinted at by clustering of rare variant EGFR mutations in NSCLC, this may be an area ripe for future investigations as genomic advances proceed apace.

In the end, tissue-based EGFR mutational analysis is only a surrogate for what is actually of primary clinical interest: a reasonable prediction of treatment efficacy. In order to further assess the treatment effects of EGFR TKI's, we reviewed treatment response in an unselected AA NSCLC patient population treated in the community setting (Cleveland, OH). If indeed activating EGFR mutations are significantly rarer among AA NSCLC patients, a significantly lower rate of objective response would be expected in comparison to a White, North American counterpart where previous objective response

Table 3 Patient characteristics	
Age, years	
Mean	64
Range	45-83
Gender, no of patients	
Female	37 (65%)
Male	20 (35%)
Histology	
Adenocarcinoma	65% (37/57)
Squamous	12% (7/57)
Large Cell	2% (1/57)
Undifferentiated	12% (7/57)
Unknown	9% (5/57)
Stage at time of EGFR TKI therapy	
IIIB	3% (2/57)
IV	97% (55/57) *
Smoking status	
Never smoker	11% (6/57)
Ever smoker (current or previous)	89% (51/57)
EGFR TKI therapy	
Erlotinib	93% (53/57)
Gefitinib	7% (4/57)
First line EGFR TKI therapy	37% (21/57)
*21% with brain metastases (12/57).	

rates of roughly 10% have been observed (12.3% Perez-Soler *et al.* 2004 and 8.9% Shepherd *et al.* 2005) (16,17). Self-identified AA patients with advanced NSCLC treated with empiric EGFR TKI (either erlotinib or gefitinib) prior to widespread mutation testing, were evaluated for radiographic response by RECIST criteria (18). Patient characteristics are summarized in *Table 3*.

We observed a 5% rate of response to EGFR TKI among unselected AA NSCLC patients treated in the community setting prior to the advent of routine EGFR mutation screening, using objective RECIST criteria and chart review of 57 cases. While this result did not reach significance (P=0.223), when compared against a 10% response rate as reported in large North American trials of unselected primarily White NSCLC patients (16,17), it represents a trend toward reduced rate of response. This trend is more in line with the results of early tissue profiling studies, including our own, which pointed to a lower frequency of activating EGFR mutations in AA *vs.* White NSCLC, and is consistent with current clinical practice of limiting EGFR TKI to patients with activating EGFR mutations by tissue analysis, regardless of race. As EGFR mutations are more frequent in never-smokers, a true evaluation of mutational frequency by race should be studied in a never-smoker cohort to help clarify the relationship between race and EGFR mutational status.

Acknowledgements

Funding: R. Leidner, NCI 5K12 CA076917-12.
Disclosure: B. Clifford, None; P. Fu, None; N. Pennell: Teva, Oncogenex; B. Halmos: Eli-Lilly, Pfizer, Oncothyreon, Daiichi and OSI; R. Leidner, None.

References

1. Yang SH, Mechanic LE, Yang P, et al. Mutations in the tyrosine kinase domain of the epidermal growth factor receptor in non-small cell lung cancer. Clin Cancer Res 2005;11:2106-10.
2. Riely GJ, Pao W, Pham D, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 2006;12:839-44.
3. Tsao AS, Tang XM, Sabloff B, et al. Clinicopathologic characteristics of the EGFR gene mutation in non-small cell lung cancer. J Thorac Oncol 2006;1:231-9.
4. Krishnaswamy S, Kanteti R, Duke-Cohan JS, et al. Ethnic differences and functional analysis of MET mutations in lung cancer. Clin Cancer Res 2009;15:5714-23.
5. Leidner RS, Fu P, Clifford B, et al. Genetic abnormalities of the EGFR pathway in African American Patients with non-small-cell lung cancer. J Clin Oncol 2009;27:5620-6.
6. Harada T, Lopez-Chavez A, Xi L, et al. Characterization of epidermal growth factor receptor mutations in non-small-cell lung cancer patients of African-American ancestry. Oncogene 2011;30:1744-52.
7. Reinersman JM, Johnson ML, Riely GJ, et al. Frequency of EGFR and KRAS mutations in lung adenocarcinomas in African Americans. J Thorac Oncol 2011;6:28-31.
8. Cote ML, Haddad R, Edwards DJ, et al. Frequency and type of epidermal growth factor receptor mutations in African Americans with non-small cell lung cancer. J Thorac Oncol 2011;6:627-30.
9. 9. Schwartz AG, Swanson GM. Lung carcinoma in African Americans and whites. A population-based study in metropolitan Detroit, Michigan. Cancer 1997;79:45-52.
10. Simpson EH. The Interpretation of Interaction in

Contingency Tables. J Roy Stat Soc B 1951;13:238-41.
11. Mittal Y. Homogeneity of subpopulations and Simpson's paradox. J Am Stat Assoc 1991;86:167-72.
12. Fu P, Panneerselvam A, Clifford B, et al. Simpson's paradox - aggregating and partitioning populations in health disparities of lung cancer patients. Stat Methods Med Res 2012. [Epub ahead of print].
13. Bickel PJ, Hammel EA, O'connell JW. Sex bias in graduate admissions: data from berkeley. Science 1975;187:398-404.
14. MacConaill LE, Campbell CD, Kehoe SM, et al. Profiling critical cancer gene mutations in clinical tumor samples. PLoS One 2009;4:e7887.
15. Via M, Ziv E, Burchard EG. Recent advances of genetic ancestry testing in biomedical research and direct to consumer testing. Clin Genet 2009;76:225-35.
16. Pérez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. J Clin Oncol 2004;22:3238-47.
17. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123-32.
18. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.

Cite this article as: Clifford BT, Fu P, Pennell NA, Halmos B, Leidner RS. EGFR molecular testing in African-American non-small cell lung cancer patients - a review of discrepant data. Transl Lung Cancer Res 2013;2(3):251-255. doi: 10.3978/j.issn.2218-6751.2013.01.04

Prognosis

International trial of adjuvant therapy in high risk stage I non-squamous cell carcinoma identified by a 14-gene prognostic signature

Johannes R. Kratz[1], Michael J. Mann[2], David M. Jablons[2]

[1]Massachusetts General Hospital, GRB 425, Boston, MA 02114, USA; [2]UCSF Thoracic Surgery, MU W424, San Francisco, CA 94143-0118, USA
Correspondence to: David M. Jablons, M.D. UCSF Thoracic Surgery, 500 Parnassus Ave, MU W424, San Francisco, CA 94143-0118, USA. Email: David.Jablons@ucsfmedctr.org.

Abstract: There is widespread agreement amongst clinical oncologists that more refined risk-stratification in early-stage lung cancer patients beyond conventional TNM staging is needed. Over the past decade, a number of molecular prognostic signatures have been designed to meet this need by correlating patterns in the differences in gene expression or modification to patient prognosis. Unfortunately, the majority of proposed signatures are not amenable to practical widespread implementation or have not yet undergone large-scale, rigorous clinical validation. A practical 14-gene prognostic signature that has undergone large-scale blinded independent validation is now ready for widespread clinical use. An international clinical trial is underway that has been designed to document the precise degree of benefit derived from adjuvant therapy in high-risk stage I patients identified by the 14-gene prognostic assay.

Keywords: Non-small cell lung cancer; molecular; prognostic; predictive; biomarker; signature; personalized therapy; adjuvant therapy

Submitted May 13, 2013. Accepted for publication May 17, 2013.
doi: 10.3978/j.issn.2218-6751.2013.05.01
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.05.01

Introduction

Surgical resection remains the gold standard of treatment for patients with stage I non-small cell lung cancer (NSCLC) and essentially represents the hope for cure (1,2). As compared to the high 5-year survival rates experienced by patients with the other prevalent, localized solid tumors such as breast cancer (98%), colorectal cancer (91%), and prostate cancer (100%), the 5-year survival rate after diagnosis of "localized" lung cancer is only 53% (3). Although some of these survival differences may be attributed to differences in patient demographics associated with each of these solid tumors, the wide discrepancy in outcomes also indicates that the label of "localized" disease conferred by our current TNM staging system does not adequately predict outcome or response to "complete" resection.

Current surgical standard of care dictates the complete removal of an affected lobe as opposed to sublobar resection for patients with stage I disease whenever possible (4). The poorer outcomes associated with sublobar resections is thought to be due to the presence of micrometastatic disease that has spread either locally or via the lymphatic system past the detectable margins of surgical resection. The prevalence of micrometastatic disease in lung cancer not only explains the poor outcomes associated with sublobar resections, but also the inadequacy of our current staging system that relies on tumor size, and nodal status alone to predict the likelihood of distant occult metastasis or micrometastasis and therefore patient outcome.

Molecular prognostic signatures

The desire to improve risk stratification beyond TNM staging has led to the recent development of molecular

prognostic signatures in lung cancer. A number of biomarker signatures prognostic of survival in NSCLC have been proposed (5,6). After a decade of research and dozens of proposed signatures, proof-of-principle is no longer the objective. Clinicians are now looking for a meaningful way to integrate data from a prognostic gene signature into the standard care of NSCLC patients. More accurate prognostic information, such as may be accessible through gene signatures, could contribute in a very important way to this current clinical decision making. That decision making often involves suboptimal choices that are often being weighed largely based on the current level of inadequately precise prognostic information based on TNM staging alone. For example, a more accurate prognostic picture may influence the approach toward surgical resection. Achieving complete oncologic lobar resection at the expense of pulmonary function is often a challenging balance to strike in patients with limited cardiopulmonary reserve. Often, such patients with stage IA lung cancer undergo wedge resection rather than anatomic resection because of the estimated risk of greater pulmonary volume loss. Subsequent risk analysis of the resected tumors could allow a more precise risk-benefit ratio to be estimated in the consideration of subsequent re-operation for completion lobectomy. Prognostic signatures may also contribute to decisions regarding post-operative care. Patients with high-risk tumors could be followed closely with more frequent imaging and be considered for adjuvant chemotherapy after surgical resection. Although the NCCN currently recommends administration of adjuvant chemotherapy in "high-risk" stage I patients [defined as stage IB disease and one of the following features: poorly differentiated tumors, vascular invasion, wedge resection, tumors larger than 4 cm, visceral pleural involvement, or undetermined lymph node status (Nx) (7)], these collective criteria based on conventional clinicopathologic features have never been validated to predict benefit from adjuvant chemotherapy. Conversely, patients with low-risk tumors could potentially be spared from toxic chemotherapy regimens that may be more likely to harm than help.

Until recently, the successful translation of prognostic signature bench research to the bedside has been challenging. One major barrier has been the lack of development of a prognostic signature using practical laboratory technology. Most proposed prognostic signatures have been developed using microarray technology (5,6). While microarrays are extremely powerful at surveying multiple potential prognostic gene candidates, the practical applicability of microarrays for a clinically rigorous test remains largely unproven. Microarrays typically require fresh-frozen tissue that has been snap-frozen immediately upon surgical resection (6). This creates a logistical barrier that may prove extremely difficult or even impossible to overcome in a community non-academic based setting. While new reagents such as RNAlater (Life Technologies, Foster City, CA) that preserve RNA at room temperature may one day provide a possible alternative to snap freezing, the overall robustness of such a platform, which involves a microarray approach that relies inherently upon extremely pure RNA, remains to be seen. In addition, many microarray-based prognostic signatures are platform dependent and based on complex algorithms (6), making them difficult for other groups to interpret, understand, and independently validate. A clinical trial based on the "metagene" prognostic model which utilized a complex microarray-based algorithm, for example, was recently stopped and the research paper describing the algorithm was recently retracted because of the inability of other groups to independently validate the model (8).

The lack of rigorous clinical validation has been the second major barrier to clinical adoption. The majority of proposed prognostic gene signatures lack clinically relevant validation. While an attempt has been made by many groups to develop prognostic algorithms using large patient cohorts, the necessity of validating these algorithms on equally large independent cohorts in a blinded fashion has been underemphasized (5,6,9). As a result, it is difficult to convince the clinician that a prognostic gene signature that was developed on a specific population is universally applicable to the patient in front of them. This is an even bigger problem in tests that rely upon a large amount of quantitative data to generate a result due to statistical "overfitting". Overfitting leads to quantitative coefficients and cut-off points that are too specific to the training cohort; validation on independent datasets typically fails in these cases. As a result, tests developed by "overfitting" data to the study population cannot be generalized to other patient populations (6).

Development and validation of a 14-gene prognostic algorithm using paraffin-embedded tissues

In light of the technical challenges posed by use of a microarray-based platform and objection from the clinical community about the lack of rigorous clinical validation,

our group recently developed a quantitative PCR-based assay that measures gene expression in formalin-fixed paraffin-embedded (FFPE) lung tumor specimens (10). Quantitative PCR is robust, inexpensive, widely available, easy to interpret, and highly reproducible. Special techniques were developed to extract RNA from 361 FFPE specimens from patients who had undergone resection of stage I-IV non-squamous NSCLC at UCSF (10). The expression levels of 14 genes were measured using quantitative PCR and correlated to patient outcomes using penalized cox proportional hazards modeling (10). Three of these genes were housekeeping genes; eleven of these genes are intricately related to known canonical lung cancer pathways such as *KRAS* and *eGFR* (10). Risk scores were divided into terciles in the UCSF training cohort to yield low-, intermediate, and high-risk categories.

Once the prognostic algorithm was derived on this UCSF cohort, blinded, independent validation was performed using two large international cohorts. The first validation cohort consisted of 433 patients who underwent resection of pathologic stage I disease in the Kaiser-Permanente Northern California healthcare system. The second cohort consisted of over 1,000 patients who underwent resection of pathologic stage I-III disease at major centers of cancer care excellence that belong to the China Clinical Trials Consortium (CCTC).

Kaplan-Meier analysis demonstrated that the assay was able to successfully risk stratify patients at low, intermediate, and high-risk of mortality within 5 years of surgical resection. This risk stratification was successful not only in the Kaiser stage I validation cohort, but also within each of the stages of the CCTC validation cohort (10). Risk category was the strongest predictor of morality after adjusting for age, sex, smoking history, histology, and stage. In addition, the assay improved risk discrimination in all stage I patients (stage IA and IB) beyond the NCCN criteria (6) currently used to identify high-risk stage I patients for adjuvant chemotherapy. Furthermore, the assay was able to successfully risk-stratify patients with node-negative tumors less than 2 cm, identifying patients with almost 50% 5-year mortality despite surgical resection of these small T1a tumors (11). The numbers of small T1a tumors is expected to rapidly increase as more and more institutions and providers adopt the new lung cancer screening guidelines (6).

Of note, only one other group attempted blinded, large-scale independent validation of a molecular prognostic signature. A multi-center effort was made as part of the National Cancer Institute Directors Challenge to develop and validate a microarray-based signature using fresh frozen tissue samples collected at multiple institutions (12). Eight signatures were submitted by the participating institutions for validation. None of these signatures, however, was able to risk stratify stage I patients better than clinical covariates alone (12), highlighting the difficulty of achieving successful blinded validation of molecular prognostic signatures.

Worldwide trial of adjuvant therapy in patients with high risk stage I non-squamous cell carcinoma

The NCCN currently recommends administration of adjuvant chemotherapy in "high-risk" stage I patients. The following criteria define "high-risk" patients according to the NCCN (7):

Patients with stage IB disease and one of the following features:

(I) poorly differentiated tumors;
(II) vascular invasion;
(III) wedge resection;
(IV) tumors larger than 4 cm;
(V) visceral pleural involvement;
(VI) insufficient lymph node staging (Nx).

Notably, with the exception of tumor size, none of the above criteria have been validated to predict benefit from adjuvant chemotherapy. They have been adopted by the NCCN with the thought that these criteria are correlated with more aggressive tumors and worse survival. It has already been shown, however, that the 14-gene assay far outperforms the above NCCN criteria in identifying high-risk patients (10). The new gene signature may therefore represent a more rigorously validated tool for implementation of this current published guideline. A randomized clinical trial that has been designed to document the exact degree of benefit derived from adjuvant chemotherapy in high-risk patients identified by the assay is underway.

Conclusions

There is widespread acknowledgment of the need for more refined risk-stratification in early-stage lung cancer patients beyond conventional TNM staging. Although the development of molecular prognostic signatures has met this need, the majority of proposed signatures lack clinical relevance because they are impractical or have not yet

undergone rigorous clinical validation. A practical 14-gene prognostic signature that has undergone large-scale blinded independent validation is now ready for widespread clinical use. An ongoing international clinical trial is expected to provide additional documentation of the degree of benefit derived from this personalization of lung cancer care that is already being integrated into current practice.

Acknowledgements

The authors would like to thank Christina Pham for her helpful assistance with editing this manuscript.
Disclosure: The authors have a consulting relationship with Pinpoint Genomics Inc./Life Technologies Corporation, the company that established a CLIA-certified laboratory and developed the molecular assay based on UCSF technology. The authors are also inventors of related technology owned by the University of California, for which the University has submitted patent applications and which has been licensed to Pinpoint Genomics/Life Technologies Corporation.

References

1. Goldstraw P, Ball D, Jett JR, et al. Non-small-cell lung cancer. Lancet 2011;378:1727-40.
2. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med 2008;359:1367-80.
3. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
4. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg 1995;60:615-22; discussion 622-3.
5. Subramanian J, Simon R. Gene expression-based prognostic signatures in lung cancer: ready for clinical use? J Natl Cancer Inst 2010;102:464-74.
6. Kratz JR, Jablons DM. Genomic prognostic models in early-stage lung cancer. Clin Lung Cancer 2009;10:151-7.
7. NCCN clinical practice guidelines in oncology non-small cell lung cancer 2012.
8. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
9. Goldstraw P, Shepherd FA, Pass HI. The International Staging System for lung cancer. Am Soc Clin Oncol Ed Book 2009;29:462-8.
10. Kratz JR, He J, Van Den Eeden SK, et al. A practical molecular assay to predict survival in resected non-squamous, non-small-cell lung cancer: development and international validation studies. Lancet 2012;379:823-32.
11. Kratz JR, Van den Eeden SK, He J, et al. A prognostic assay to identify patients at high risk of mortality despite small, node-negative lung tumors. JAMA 2012;308:1629-31.
12. Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma, Shedden K, Taylor JM, et al. Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. Nat Med 2008;14:822-7.

Cite this article as: Kratz JR, Mann MJ, Jablons DM. International trial of adjuvant therapy in high risk stage I non-squamous cell carcinoma identified by a 14-gene prognostic signature. Transl Lung Cancer Res 2013;2(3):222-225. doi: 10.3978/j.issn.2218-6751.2013.05.01

Impact of EGFR mutation status on tumor response and progression free survival after first-line chemotherapy in patients with advanced non-small-cell lung cancer: a meta-analysis

Wenhua Liang[1,2]*, Yaxiong Zhang[1,2]*, Shiyang Kang[1,2]*, Hui Pan[1,2], Wenlong Shao[1,2], Qiuhua Deng[1,2], Xiaoshun Shi[1,2], Wei Wang[1,2], Jianxing He[1,2]

[1]Department of Cardiothoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; [2]Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease, Guangzhou 510120, China

*These authors contributed equally to this work.

Correspondence to: Jianxing He, MD, PhD, FACS. No. 151, Yanjiang Rd, Guangzhou 510120, China. Email: drjianxing.he@gmail.com.

Objectives: Non-small-cell lung cancer (NSCLC) patients harboring sensitive epidermal growth factor receptor (EGFR) mutations derive greater benefits from EGFR-tyrosine kinase inhibitors (EGFR-TKIs) than those with wild type tumors. However, whether EGFR mutation status is associated with the efficacy of cytotoxic chemotherapy or prognosis in advanced NSCLC patients remained controversial. Thus, we sought to conduct a meta-analysis to answer this question.

Methods: Electronic databases were searched for eligible literatures. The primary outcomes were objective response rate (ORR) and 6-month progression-free survival (PFS) rate. The pooled odds ratio (OR) was calculated using random-effects model. Subgroup analyses stratified by study types, EGFR mutation detection methods, chemotherapy regimens, and patient origins were proposed.

Results: A total of 14 studies involving 1,772 advanced NSCLC patients with known EGFR mutation status who had received first-line chemotherapy were included. Patients with positive EGFR mutation had numerically higher ORR than wild type patients (36.2% *vs*. 30.1%) without significant differences (OR 1.24, 95% CI, 0.90 to 1.70; P=0.19). However, patients with EGFR mutants had significantly superior 6-month PFS rate than wild-type patients (58.6% *vs*. 47.2%; OR 1.88, 95% CI, 1.33 to 2.65; P=0.0003). Results of the subgroup analyses were concordant with the overall ones.

Conclusions: This comprehensive analysis revealed that advanced NSCLC patients with sensitivity EGFR mutation had higher 6-month PFS rate and potentially greater ORR compared with wild-type patients after first-line chemotherapy. It suggested that EGFR mutation status should be considered a significant factor for patient stratification in evaluating the efficacy of antitumor agents in addition to EGFR-TKIs.

Keywords: Non-small-cell lung cancer (NSCLC); epidermal growth factor receptor (EGFR) mutation; first-line chemotherapy; meta-analysis

Submitted on Jul 17, 2014. Accepted for publication Jul 23, 2014.
doi: 10.3978/j.issn.2072-1439.2014.07.33
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.07.33

Introduction

Lung cancer, predominantly non-small-cell lung cancer (NSCLC), is the leading cause of cancer-related mortality worldwide (1). The majority of patients are diagnosed at advanced stages in which there are few treatment options (2). Despite the limited efficacy, platinum-based doublet chemotherapy remains the standard first-line treatment for advanced NSCLC in recent years (3,4). Advances in genetic testing allowed the discovery of existence and clinical significance of driver oncogenes which could be selected as a therapeutic target, such as activated epidermal growth factor receptor (EGFR) mutations (5). It has been

extensively proved that NSCLC patients who harbor sensitive EGFR mutations (exon 19 deletion or L858R mutation in exon 21) derive greater benefits from EGFR-tyrosine kinase inhibitors (EGFR-TKIs), such as erlotinib and gefitinib, than those with wild type tumors (6,7). The predictive value of EGFR mutation status for EGFR-TKIs efficacy has been substantially confirmed.

In contrast, people used to believe there is no correlation between EGFR mutation status and cytotoxic chemotherapy. Data from some previous studies suggested that Asians represented higher response rate than Caucasians in receiving chemotherapy (8). From the present point of view, the most prominent intrinsic genetic variance between these two races is the proportion of patients with EGFR mutations. Considering the huge differences in tumor biology between EGFR mutation-positive and -negative NSCLC, it is interesting to investigate whether EGFR mutation status also influence chemotherapy efficacy. Several recent studies revealed that advanced NSCLC patients with positive EGFR mutation had favorable response to first-line cytotoxic chemotherapy compared with wild type patients (9,10), while another study showed contrary results (11). In addition, another clinical research reported that there was no obvious association between EGFR mutation status and first-line chemotherapy response in NSCLC (12). Therefore, whether EGFR mutation status is associated with responsiveness to front-line chemotherapy in advanced NSCLC is still not clear. A comprehensive analysis of the various outcomes is warranted. Thus, we sought to perform a meta-analysis incorporating all available evidences to evaluate the clinical outcome according to the EGFR mutation status in patients with advanced NSCLC treated with front-line conventional chemotherapy.

Methods

Literature search

All relevant articles were retrieved by searching PubMed, Embase and the Central Registry of Controlled Trials of the Cochrane Library using a combination of the terms "EGFR", "epidermal growth factor receptor", "mutation", "lung", "non-small-cell lung cancer", "NSCLC" and "chemotherapy". An additional search through Google Scholar and a manual search through reference lists of relevant reviews and included studies were additionally performed. Two authors (ZY and KS) carried out the search independently. No restriction by language or year was set in the search.

Inclusion and exclusion criteria

Eligible studies should meet the following criteria: (I) studies which investigate or report a subset of patients with first-line chemotherapy without combination of EGFR inhibitors (e.g., TKIs or monoclonal antibodies) or other agents potentially targeting the EGFR pathway (e.g., multitargeted antiangiogenic TKIs) in patients with local advanced or metastatic (IIIB or IV) NSCLC; (II) prior neoadjuvant or adjuvant chemotherapy in patients with recurrence after surgery was permitted if it had elapsed from last administration to relapse at least 6 months; (III) EGFR mutation analysis was performed on available tumor tissue samples instead of circulating free DNA in serum in first-line chemotherapy treatment cohort; (IV) at least one primary outcomes was available. Studies failed to meet the inclusion criteria will be excluded.

Outcomes measures, data extraction and quality assessment

Primary outcomes for this meta-analysis were objective response rate (ORR), namely partial response (PR) plus complete response (CR), and 6-month progression-free survival (PFS) rate. The data collection and assessment of methodological quality followed the QUORUM and the Cochrane Collaboration guidelines (http://www.cochrane.de). The data on study type, treatment regimens, major clinical features, ORR and 6-month PFS rate were extracted by two investigators (FW and PH) independently. Figures were electronically digitized and Kaplan-Meier curves were downloaded by appropriate software (Engauge Digitizer, ver 2.12, Mark Mitchell, 2002, free software down loaded from http://sourceforge.net). Two reviewers (SW and DQ) used a JADAD score to evaluate the quality of randomized controlled trials (RCTs) and a modified Newcastle-Ottawa scale to assess the quality of non-RCT studies (13). Discrepancies were discussed by all investigators to reach consensus.

Statistical analysis

In consideration of any potential heterogeneity, we conducted this meta-analysis with a random-effect model in order to avoid any potential heterogeneity. The results were reported as pooled odds radios (ORs) with the corresponding 95% confidence interval (CI). Subgroup and sensitivity analysis were stratified for literature type, EGFR mutation analysis method, therapeutic regimen, patient

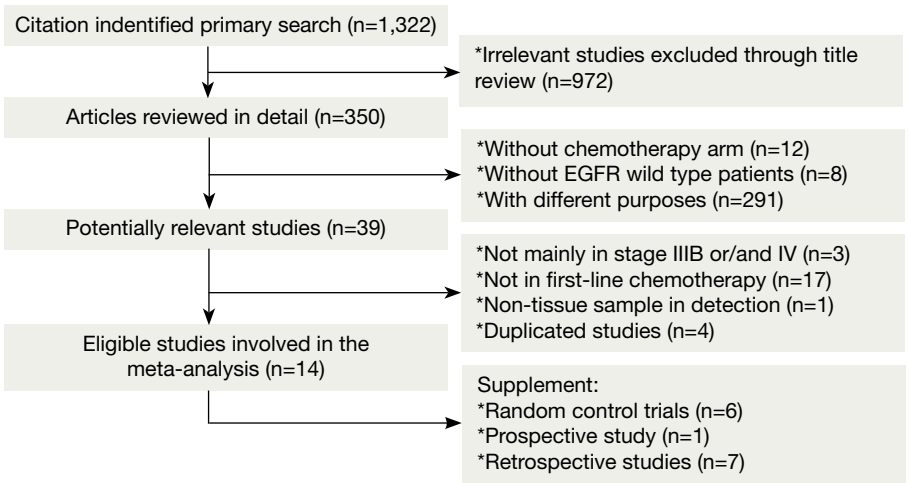


Figure 1 Profile summarizing the trial flow.

origins. An OR greater than one reflected a better ORR or 6-month PFS rate in the EGFR mutant arm. Statistical heterogeneity across studies was assessed with a forest plot and the inconsistency statistic (I^2). Statistical significance was considered at $P<0.05$. All calculations were performed using REVIEW MANAGER (version 5.0 for Windows; the Cochrane Collaboration, Oxford, UK).

Publication bias

An extensive search strategy was made to minimize the potential for publication bias. Graphical funnel plots were generated to visually assess a publication bias (14). The statistical methods to detect funnel plot asymmetry were the rank correlation test of Begg and Mazumdar and the regression asymmetry test of Egger (14,15).

Results

Eligible studies

We identified 1,322 records according to the search strategy and finally included 14 studies (six RCTs, one prospective study and seven retrospective studies) involving 1,772 advanced NSCLC patients who had been tested for EGFR mutations in first-line chemotherapy treatment cohort (9-12,16-25). *Figure 1* summarized the flow chart. Among these studies, chemotherapy regimens were platinum-based doublets at standard dose, namely cisplatin/carboplatin plus one of the third generation agents (including gemcitabine, paclitaxel, docetaxel, vinorelbine, and pemetrexed), or some non-platinum based regimens. Regimens were not specific in five retrospective studies (10,21-24) so that they were excluded in subgroup analysis stratified for therapeutic regimen. Detecting approaches for EGFR mutation included direct sequencing, nested polymerase chain reaction (PCR), amplification refractory mutation system (ARMS), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP); real time-quantitative PCR (RT-qPCR), denaturing high-performance liquid chromatography (DHPLC), which were also a sub-grouping factor. We considered time to progression (TTP) as PFS in studies by Eberhard (11) and Lee (21). *Table 1* summarized the characteristics of all involved studies.

Objective response rate and six-month PFS rate

According to all literature with available data, patients with positive EGFR mutation had higher pooled ORR than wild type patients (35.8% *vs.* 30.1%), but there was no significant difference between the two groups (OR 1.24, 95% CI, 0.90 to 1.70; P=0.19; heterogeneity: Chi^2 =17.47, P=0.13, I^2 =31%; *Figure 2A*). Subgroup analyses stratified by study type (RCT *vs.* non-RCT), EGFR mutation detecting method (direct sequencing *vs.* non-sequencing methods), therapeutic regimen (gemcitabine-based *vs.* non-gemcitabine-based regimens and cisplatin-based *vs.* carboplatin-based regimens) and patient origin (Asians *vs.* non-Asians) consistently revealed no significant difference between the mutant group and wild type group (*Table 2*). EGFR mutants had higher 6-month PFS rate than wild type patients (62.1%

Table 1 Characteristics of included studies

Lead author [year]	Country	Study category (phase)	Therapeutic regimen [cases in total]	Age, median [range] [y]	Female (%)	Non-smoker (%)	Adenocarcinoma (%)	Evaluable cases for EGFR mutation	EGFR mutation analysis method	EGFR exons identified as mutant	EGFR mutation status	ORR (%)	Six-month PFS rate (%)
David A. Eberhard [2005]	USA	RCT (III)	Paclitaxel 200 mg/m^2 BSA, d1, q3w + carboplatin (AUC =6), d1, q3w ×6 cycles [540]	NA	100 (18.5)	20 (3.7)	105 (19.4)	113	Nested PCR	18, 19, 20, 21	Positive	3/14 (21.4)	10/14 (71.43)
											Negative	27/99 (27.3)	78/99 (78.79)
Tony S. Mok [2009]	Asia	RCT (III)	Paclitaxel 200 mg/m^2 BSA, d1, q3w + carboplatin (AUC =5-6) d1, q3w ×6 cycles [608]	57.0 [25-84]	481 (79.1)	569 (93.6)	591 (97.2)	214	DxS ARMS	18, 19, 20, 21	Positive	61/129 (47.3)	64/129 (49.61)
											Negative	20/85 (23.5)	35/85 (41.18)
Shirin Khambata-Ford [2010]	USA	RCT (III)	Paclitaxel 225 mg/m^2 BSA or docetaxel 75 mg/m^2 BSA, d1, q3w + carboplatin (AUC =6) d1, q3w ×6 cycles [338]	65.0 [34-85]	134 (39.6)	25 (7.4)	NA	87	Direct sequencing	18, 19, 20, 21	Positive	1/9 (11.1)	7/9 (77.78)
											Negative	17/78 (21.8)	25/78 (32.05)
Ji-Youn Han [2012]	Asia	RCT (III)	Gemcitabine 1,250 mg/m^2 on d1 and 8+ cisplatin 80 mg/m^2 on d1 q3w × ≤9 cycles [150]	56.5 [19-74]	134 (89.3)	150 (100.0)	NA	43	Direct sequencing	19, 20, 21	Positive	6/16 (37.5)	9/16 (56.25)
											Negative	14/27 (51.9)	15/27 (55.56)
Cesare Gridelli [2012]	International	RCT (III)	Gemcitabine 1,200 mg/m^2 BSA, d1, 8, q3w + cisplatin 80 mg/m^2 BSA, d1, q3w × ≤6 cycles [380]	62.0 [34-81]	128 (33.7)	79 (20.8)	212 (55.8)	137	PCR-RFLP	19, 21	Positive	5/20 (25.0)	14/20 (70.00)
											Negative	NA/117 (NA)	45/117 (38.46)
Yi-Long Wu [2013]	Asia	RCT (III)	Gemcitabine 1,250 mg/m^2 BSA, d1, 8, q4w + carboplatin (AUC =5) or cisplatin 75 mg/m^2 BSA, d1, q4w + placebo d15-28, q4w ×6 cycles [225]	57.3 [37-88]	85 (38.0)	107 (48.0)	168 (75.0)	115	RT-qPCR	18, 19, 21	Positive	7/48 (14.6)	27/48 (56.25)
											Negative	13/67 (19.4)	26/67 (38.81)
Yuko Kawano [2013]	Japan	Prospective (II)	Pemetrexed 500 mg/m^2 BSA, d1, q3w + cisplatin 75 mg/m^2, d1, q3w × ≤4 cycles [50]	60.0 [28-74]	14 (28.0)	14 (28.0)	41 (82.0)	33	RT-qPCR	19, 21	Positive	6/9 (66.7)	3/9 (33.33)
											Negative	11/24 (45.8)	5/24 (20.83)
Kyung-Hun Lee [2006]	Korea	Retrospective	Platinum-based regimen [75]	NA	NA	NA	NA	75	Direct sequencing	18, 19, 21	Positive	6/14 (42.9)	11/14 (78.57)
											Negative	21/61 (34.4)	31/61 (50.82)
Katsuyuki Hotta [2007]	Japan	Retrospective	Platinum-based regimen [35] or non-platinum-based regimen [19]	NA	NA	NA	NA	54	Direct sequencing	19, 21	Positive	3/14 (21.4)	7/14 (45.80)
											Negative	6/40 (15.0)	9/40 (21.90)

Table 1 (*continued*)

Table 1 (*continued*)

Lead author [year]	Country	Study category (phase)	Therapeutic regimen [cases in total]	Age, median [range] [y]	Female (%)	Non-smoker (%)	Adenocarcinoma (%)	Evaluable cases for EGFR mutation	EGFR mutation analysis method	EGFR exons identified as mutant	EGFR mutation status	ORR (%)	Six-month PFS rate (%)
Meina Wu [2009]	China	Retrospective	Platinum-based regimen [132] or non-platinum-based regimen [13]	61.0 [21-78]	66 (45.5)	NA	106 (73.1)	145	DHPLC	19, 21	Positive	19/55 (34.5)	NA
											Negative	30/90 (33.3)	NA
Aristea Kalikaki [2010]	Greece	Retrospective	Platinum-based regimen [79]	NA	30 (23.4)	39 (30.5)	96 (75.0)	79	Direct sequencing	18, 19, 20, 21	Positive	5/8 (62.5)	NA
											Negative	17/71 (23.9)	NA
			Non-platinum-based regimen [49]					49			Positive	0/1 (-)	NA
											Negative	9/48 (18.8)	NA
Jin Hyun Park [2012]	Korea	Retrospective	Gemcitabine + cisplatin/ carboplatin [131]	59.0 [26-82]	120 (55.3)	144 (66.4)	174 (80.2)	131	RT-qPCR	18, 19, 20, 21	Positive	26/85 (30.6)	23/85 (27.06)
											Negative	16/46 (34.8)	13/46 (28.26)
			Paclitaxel + cisplatin/ carboplatin [86]					86			Positive	20/52 (38.5)	25/52 (48.08)
											Negative	12/34 (35.3)	15/34 (44.12)
M. Takeda [2012]	Japan	Retrospective	Platinum-based regimen [200]	63.0 [29-81]	73 (36.5)	65 (32.5)	178 (89.0)	182	ARMS/the PCR-Invader method	NA	Positive	14/31 (45.2)	16/31 (51.61)
											Negative	59/151 (39.1)	57/151 (37.75)
Xiao-Peng Dong [2013]	China	Retrospective	Gemcitabine + cisplatin [81]	57.0	17 (42.5)	57	24 (60.0)	81	Direct sequencing	18, 19, 20, 21	Positive	13/40 (32.5)	36/40 (90.00)
				61.0	19 (46.3)	61	21 (51.2)				Negative	13/41 (31.7)	35/41 (85.37)
			Docetaxel + cisplatin [77]	62.0	19 (43.2)	62	24 (54.5)	77			Positive	16/44 (36.4)	42/44 (95.45)
				58.0	15 (45.5)	58	19 (57.6)				Negative	12/33 (36.4)	28/33 (84.85)
			Vinorelbine + cisplatin [71]	60.0	16 (44.3)	60	22 (61.1)	71			Positive	13/36 (36.1)	35/36 (97.22)
				63.0	16 (45.7)	63	20 (57.1)				Negative	13/35 (37.1)	26/35 (74.29)

RCT, random control trial; AUC, area under the concentration time curve; BSA, body-surface area; ADK, adenocarcinoma; EGFR, epidermal growth factor receptor; ORR, objective response rate; TTP, time to progression; PFS, progression-free survival; PCR, polymerase chain reaction; ARMS, amplification refractory mutation system; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; RT-qPCR, real time-quantitative PCR; DHPLC, denaturing high-performance liquid chromatography; NA, not available.

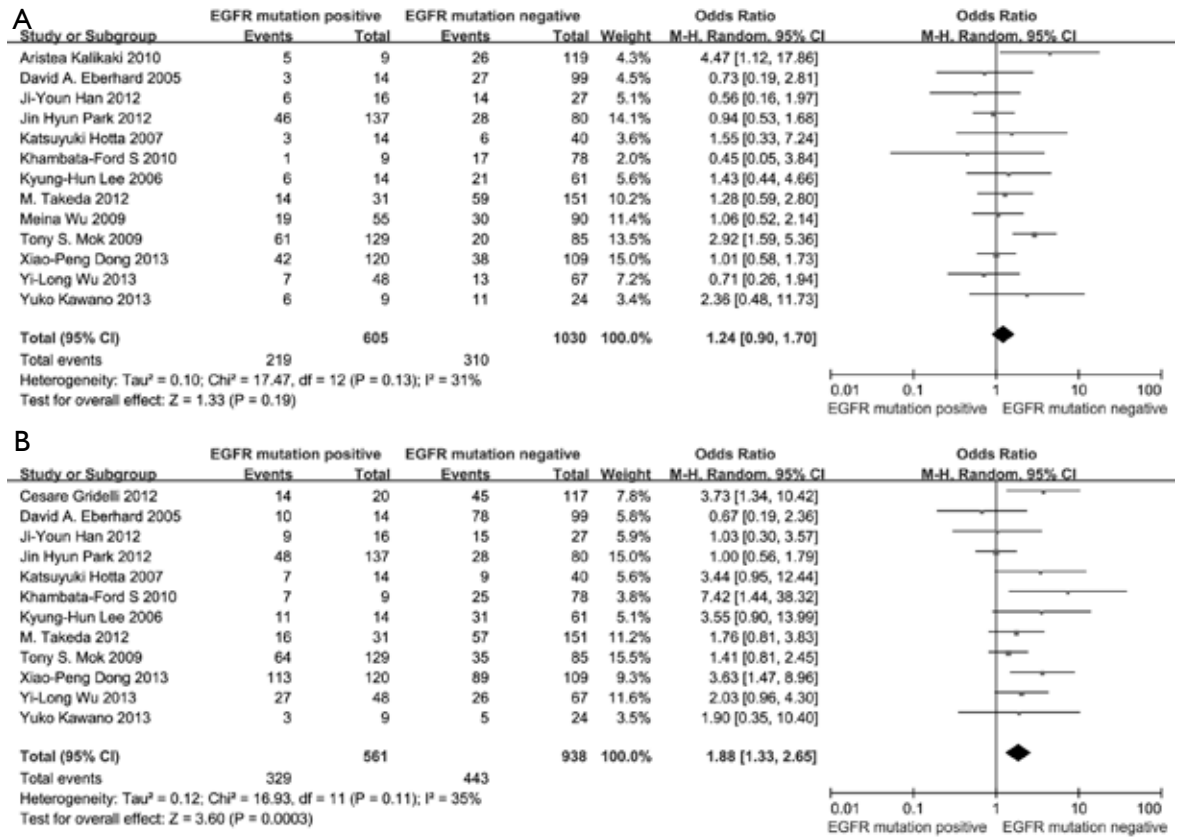


Figure 2 (A) Meta-analysis on objective response rate among advanced NSCLC patients receiving first-line chemotherapy according to EGFR mutation status; (B) meta-analysis on 6-month PFS rate among patients receiving first-line chemotherapy according to EGFR mutation status. NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; PFS, progression-free survival; CI, confidence interval; I^2, inconsistency statistic.

vs. 45.1%) with significance (OR 1.88, 95% CI, 1.33-2.65; P=0.0003; heterogeneity: Chi^2 =16.93, P=0.11, I^2 =35%; *Figure 2B*). Subgroup analyses also revealed similar tendency of significantly superior 6-month PFS of EGFR mutants, regardless of study types, methods of EGFR mutation detection, chemotherapy regimens and patient origins (*Table 3*). Additionally, we pooled the results of DCR although only five studies reported this data. No differences between EGFR mutation positive and negative groups were observed (OR 1.33, 95% CI, 0.93-1.91; P=0.11; heterogeneity: Chi^2 =2.23, P=0.69, I^2 =0%; *Figure 3*).

Assessment of heterogeneity and publication bias

As described above, the statistical heterogeneity was moderate. Any potential clinical heterogeneity was examined and subsequently excluded by subgroup analyses. In addition, sensitivity analysis by leaving any study out did not alter the general results. There was no publication bias for both outcome measures, with asymmetrical appearance on funnel plot analysis (*Figure 4*) and all P values greater than 0.05 in Begg's test and Egger's test.

Discussion

The association of EGFR mutation status with the responsiveness or prognosis in patients with advanced NSCLC after first-line chemotherapy was controversial based on previous small-size reports. A meta-analysis that could incorporate all available results, including subgroup data from RCTs as well, is a good way to address our concerns. In the current study, we found that 6-month PFS rate was significantly higher in EGFR mutants than in wild type patients after first-line chemotherapy, while the ORR and DCR appeared to be higher but the difference did not reach significance. These results admit of two

Table 2 Subgroup analysis on objective response rate among advanced NSCLC patients receiving first-line chemotherapy according to EGFR mutation status

Categories of included studies	Number of included studies	Objective response rate (event/total)		Test of heterogeneity			Test of effect size	
		EGFR mutation positive	EGFR mutation negative	Chi^2	P value	I^2 (%)	OR (95% CI)	P value
Total	13	219/605	310/1,030	17.47	0.13	31	1.24 (0.90-1.70)	0.19
Literature type								
Random control trial	5	78/216	91/356	11.36	0.02	65	0.97 (0.41-2.29)	0.94
Non-random control trial	8	141/389	219/674	5.56	0.59	0	1.17 (0.88-1.56)	0.28
EGFR mutation analysis method								
Direct sequencing method	6	63/182	122/434	6.20	0.29	19	1.17 (0.70-1.96)	0.56
Non-direct sequencing methods[1]	7	156/423	188/596	10.95	0.09	45	1.27 (0.83-1.95)	0.28
Therapeutic regimen								
Gemcitabine based regimens	4	52/189	56/181	0.67	0.88	0	0.80 (0.50-1.28)	0.36
Non-gemcitabine based regimens	6	120/293	112/388	9.11	0.10	45	1.36 (0.78-2.38)	0.28
Therapeutic regimen								
Cisplatin based regimens	3	54/145	63/160	1.93	0.38	0	1.00 (0.62, 1.61)	0.99
Carboplatin based regimens	3	65/152	64/262	5.49	0.06	64	1.27 (0.38, 4.32)	0.70
Patient origin								
Asia	10	210/573	240/734	12.72	0.18	29	1.22 (0.89-1.68)	0.21
Non-Asia area	3	9/32	70/296	4.75	0.09	58	1.27 (0.31-5.20)	0.74

[1]Non-direct sequencing methods included Nested PCR, ARMS, PCR-RFLP, RT-qPCR, and DHPLC. NSCLC, non-small-cell lung cancer; PCR, polymerase chain reaction; ARMS, amplification refractory mutation system; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; RT-qPCR, real time-quantitative PCR; DHPLC, denaturing high-performance liquid chromatography; EGFR, epidermal growth factor receptor; I^2, inconsistency statistic; OR, odds radio; CI, confidence interval.

Table 3 Subgroup analysis on six-month PFS rate among advanced NSCLC patients receiving first-line chemotherapy according to EGFR mutation status

Categories of included studies	Number of included studies	Six-month PFS rate (event/total)		Test of heterogeneity			Test of association	
		EGFR mutation positive	EGFR mutation negative	Chi^2	P value	I^2 (%)	OR (95% CI)	P value
Total	12	329/561	443/938	16.93	0.11	35	1.88 (1.33-2.65)	0.0003
Literature type								
Random control trial	6	131/236	224/473	8.74	0.12	43	1.80 (1.06-3.05)	0.0300
Non-random control trial	6	198/325	219/465	8.18	0.15	39	2.01 (1.20-3.36)	0.0080
EGFR mutation analysis method								
Direct sequencing method	5	147/173	169/315	4.27	0.37	6	3.04 (1.73-5.37)	0.0001
Non-direct sequencing methods[1]	7	182/388	274/623	7.37	0.29	19	1.50 (1.07-2.11)	0.0200
Therapeutic regimen								
Gemcitabine[2] based regimens	5	109/209	134/298	5.16	0.27	23	1.63 (0.99-2.68)	0.0500
Non-gemcitabine[3] based regimens	6	186/293	212/388	10.66	0.06	53	1.88 (0.97-3.62)	0.0600
Therapeutic regimen								
Cisplatin based regimens	4	178/286	167/330	3.26	0.35	8	2.61 (1.44, 4.71)	0.0020
Carboplatin based regimens	3	81/152	138/262	5.27	0.07	62	1.65 (0.59, 4.65)	0.3400
Distribution area of patients								
Asia	9	298/518	295/644	9.46	0.31	15	1.71 (1.25-2.33)	0.0008
Non-Asia area	3	31/43	148/294	6.48	0.04	69	2.53 (0.66-9.63)	0.1700

[1]Non-direct sequencing methods included Nested PCR, ARMS, PCR-RFLP, RT-qPCR, and DHPLC; [2]We defined gemcitabine + platinum-based regimen as gemcitabine + cisplatin/carboplatin; [3]We defined non-gemcitabine + platinum-based regimen as taxane/paclitaxel/docetaxel + cisplatin/carboplatin or vinorelbine/pemetrexed + cisplatin. NSCLC, non-small-cell lung cancer; PCR, polymerase chain reaction; ARMS, amplification refractory mutation system; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; RT-qPCR, real time-quantitative PCR; DHPLC, denaturing high-performance liquid chromatography; EGFR, epidermal growth factor receptor; PFS, progression-free survival; I^2, inconsistency statistic; OR, odds radio; CI, confidence interval.

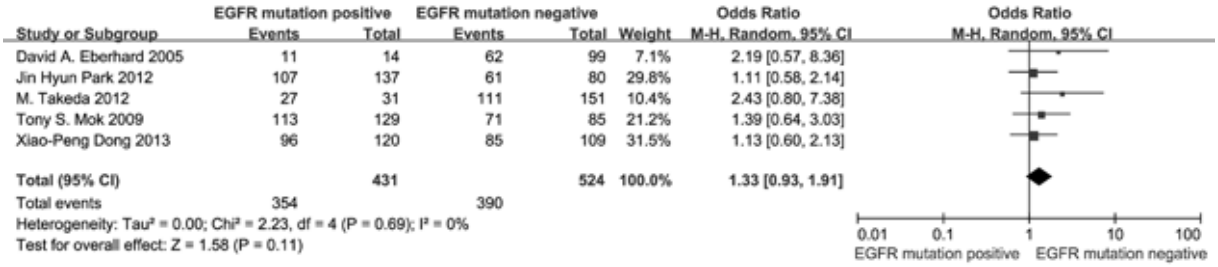


Figure 3 Meta-analysis on disease control rate among advanced NSCLC patients receiving first-line chemotherapy according to EGFR mutation status. NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; CI, confidence interval; I^2, inconsistency statistic.

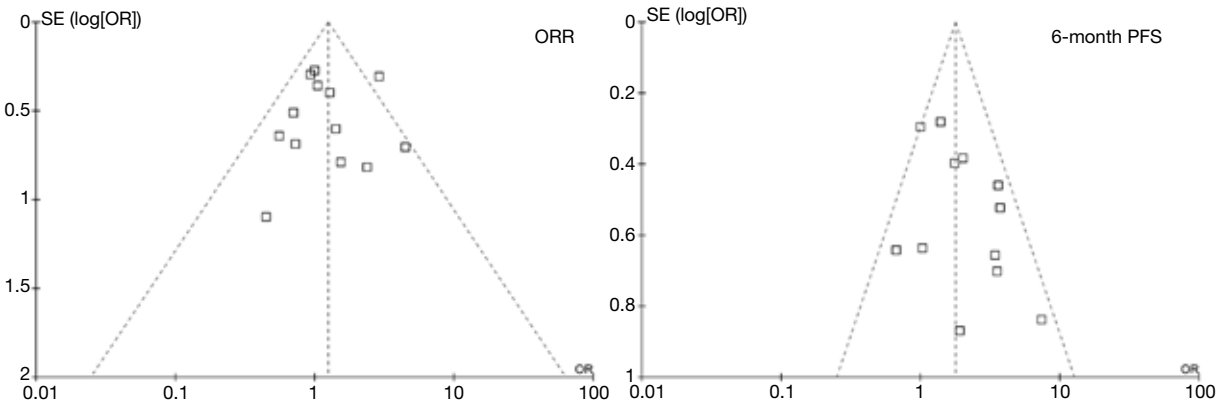


Figure 4 Funnel plots of ORR and 6-month PFS. OR, odds radio; ORR, objective response rate; PFS, progression-free survival.

interpretations.

Firstly, EGFR mutation might indeed be a predictor to the efficacy of cytotoxic chemotherapy. Activation of EGFR-dependent pathway plays an important role in the proliferation and aggressive phenotype transition of epithelial cells especially EGFR-mutated tumors (26,27). Moreover, a prior research indicated that a critical level of EGFR signaling was necessary for cisplatin-mediated apoptosis in tumor cells and suggested an inhibitory effect of this pathway on the repair of cisplatin-damaged DNA (28). Therefore, it was reasonable to hypothesize that tumor cells harboring EGFR mutation are more sensitive to cytotoxic chemotherapy. The hypothesis for selective killing of EGFR+ cells was supported by a clinical observation which showed a reduced plasma EGFR mutation frequency after chemotherapy in patients with NSCLC (29). By selectively eliminating or suppressing the 'seeds', tumor growths were persistently restricted, which translated into prolonged PFS as our result indicated. On the other hand, EGFR mutants did have higher pooled response rate although the magnitude of benefit was not as great as that of PFS. We suspected that the magnitude difference was attributed to the intratumoral heterogeneity. A recent study demonstrated that approximately 30% of patients presented intratumoral EGFR mutational heterogeneity through microdissection of the tumor samples (30). Therefore, tumors detected as EGFR mutated not necessarily contain pure EGFR+ cells. In other words, the intratumoral abundace of EGFR+ cells might be small in some patients. Thus, selective killing of EGFR+ cells was probably not associated with significant tumor shrinkage. As a result, patients intrinsically 'responded' to the chemotherapy might fail to meet the criteria for ORR (at least a 30% decrease in the sum of diameters of target lesions) according to Recist 1.1 criteria (31). However, direct evidence to confirm this mechanism requires real-time re-biopsy after treatments, which seems to be an impossible mission considering ethics. Secondly, we can not rule out the possibility that the improved PFS was merely the underlying prognostic effect of EGFR mutation since there was evidence showing that EGFR mutation was likely to be a favorable prognostic factor (32). However, the prognostic value of EGFR

mutation itself in NSCLC was still controversial (33).

Nonetheless, regardless of what the true causes are, this comprehensive analysis confirmed the association between EGFR mutation and PFS. This was highly concordant with an important report this year that among the patients treated with non-targeted therapy, those with a driver mutation detected had a longer median overall survival than those without identified driver mutations (2.4 *vs.* 2.1 years) (34). All these results gave us some important hints. Firstly, we strongly suggested that investigators should consider the proportion of EGFR mutation patients as a stratification factor in designing or reviewing clinical studies regarding chemotherapy regimen or other non-targeted agents. Second, it might partially explain why some clinical trials on chemotherapy in Asia reported higher response rate than those in Europe-American, and similarly, explain the negative results of combination of gefitinib with chemotherapy in patients with EGFR mutation compared with chemotherapy alone in some previous studies (35). In addition, the response to chemotherapy in EGFR wild type patients or projectively driven mutation 'pan-negative' patients was worse than what we acknowledged. Therefore, more efforts should be made to improve the prognosis of this population.

Notably, we only focused on first-line chemotherapy in this analysis in order to minimize the crossover effects. Some previous investigations suggested an inferior response from EGFR-TKIs following treatment of chemotherapy (36). Consistently, the study by Bai *et al*. also showed that the overall incidence of EGFR mutation was lower in plasma DNA after first-line chemotherapy (29). Thus, getting second-line or third-line chemotherapy involved will tangle the discussion.

This is the first study to comprehensively answer the impact of EGFR mutation on chemotherapy, addressing the confusion from inconsistent conclusions of current studies. However, there are several limitations. First, our meta-analysis was based on non-randomized studies and sub-group data extracted from RCTs, which somehow compromised the evidence level. Second, EGFR exons identified as mutant were heterogeneous among included articles but we were unable to assess whether 19 or 21 exon alterations had different impact on chemotherapy. Finally, we failed to investigate different first-line regimens separately with limited data. In addition, we cannot differentiate the respective impact of EGFR mutation on cell-cycle nonspecific antineoplastic agents (platinum) and specific agents (third-generation agents). For clinical practice, after all, it is essential to determine the optimal regimen for EGFR mutant NSCLC patients, especially who have failed front-line EGFR-TKIs or have no access to these agents. Further studies are warranted.

In conclusion, this meta-analysis showed that advanced NSCLC patient with EGFR mutation had significantly higher 6-month PFS rate and potentially higher ORR than wild type patients after first-line chemotherapy. We suggest that EGFR mutation status should be considered a stratification factor not only in studies regarding EGFR-targeted agents but also in those regarding non-EGFR-targeted drugs.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
2. Wakelee H, Belani CP. Optimizing first-line treatment options for patients with advanced NSCLC. Oncologist 2005;10 Suppl 3:1-10.
3. Scagliotti GV, De Marinis F, Rinaldi M, et al. Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. J Clin Oncol 2002;20:4285-91.
4. Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. J Clin Oncol 2004;22:330-53.
5. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
6. Lee CK, Brown C, Gralla RJ, et al. Impact of EGFR inhibitor in non-small cell lung cancer on progression-free and overall survival: a meta-analysis. J Natl Cancer Inst 2013;105:595-605.
7. Paz-Ares L, Soulières D, Melezínek I, et al. Clinical outcomes in non-small-cell lung cancer patients with EGFR mutations: pooled analysis. J Cell Mol Med 2010;14:51-69.
8. Soo RA, Loh M, Mok TS, et al. Ethnic differences in survival outcome in patients with advanced stage non-small cell lung cancer: results of a meta-analysis of randomized controlled trials. J Thorac Oncol 2011;6:1030-8.
9. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N

Engl J Med 2009;361:947-57.
10. Hotta K, Kiura K, Toyooka S, et al. Clinical significance of epidermal growth factor receptor gene mutations on treatment outcome after first-line cytotoxic chemotherapy in Japanese patients with non-small cell lung cancer. J Thorac Oncol 2007;2:632-7.
11. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005;23:5900-9.
12. Park JH, Lee SH, Keam B, et al. EGFR mutations as a predictive marker of cytotoxic chemotherapy. Lung Cancer 2012;77:433-7.
13. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomized studies in meta-analysis. Ottawa, Ontario: The Ottawa Health Research Institute. Available online: http://www.ohri.ca/programs/clinical_epidemiology/nosgen.doc
14. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
15. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50:1088-101.
16. Khambata-Ford S, Harbison CT, Hart LL, et al. Analysis of potential predictive markers of cetuximab benefit in BMS099, a phase III study of cetuximab and first-line taxane/carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 2010;28:918-27.
17. Han JY, Park K, Kim SW, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol 2012;30:1122-8.
18. Gridelli C, Ciardiello F, Gallo C, et al. First-line erlotinib followed by second-line cisplatin-gemcitabine chemotherapy in advanced non-small-cell lung cancer: the TORCH randomized trial. J Clin Oncol 2012;30:3002-11.
19. Wu YL, Lee JS, Thongprasert S, et al. Intercalated combination of chemotherapy and erlotinib for patients with advanced stage non-small-cell lung cancer (FASTACT-2): a randomised, double-blind trial. Lancet Oncol 2013;14:777-86.
20. Kawano Y, Ohyanagi F, Yanagitani N, et al. Pemetrexed and cisplatin for advanced non-squamous non-small cell lung cancer in Japanese patients: phase II study. Anticancer Res 2013;33:3327-33.
21. Lee KH, Han SW, Hwang PG, et al. Epidermal growth factor receptor mutations and response to chemotherapy in patients with non-small-cell lung cancer. Jpn J Clin Oncol 2006;36:344-50.
22. Wu M, Zhao J, Song SW, et al. EGFR mutations are associated with prognosis but not with the response to front-line chemotherapy in the Chinese patients with advanced non-small cell lung cancer. Lung Cancer 2010;67:343-7.
23. Kalikaki A, Koutsopoulos A, Hatzidaki D, et al. Clinical outcome of patients with non-small cell lung cancer receiving front-line chemotherapy according to EGFR and K-RAS mutation status. Lung Cancer 2010;69:110-5.
24. Takeda M, Okamoto I, Sakai K, et al. Clinical outcome for EML4-ALK-positive patients with advanced non-small-cell lung cancer treated with first-line platinum-based chemotherapy. Ann Oncol 2012;23:2931-6.
25. Dong X, Zhao X, Hao Y, et al. Response to first-line chemotherapy in patients with non-small-cell lung cancer according to epidermal growth factor receptor and K-RAS mutation status. Clin Lung Cancer 2013;14:680-7.
26. Ji H, Li D, Chen L, et al. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. Cancer Cell 2006;9:485-95.
27. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37 Suppl 4:S9-15.
28. Dixit M, Yang JL, Poirier MC, et al. Abrogation of cisplatin-induced programmed cell death in human breast cancer cells by epidermal growth factor antisense RNA. J Natl Cancer Inst 1997;89:365-73.
29. Bai H, Wang Z, Chen K, et al. Influence of chemotherapy on EGFR mutation status among patients with non-small-cell lung cancer. J Clin Oncol 2012;30:3077-83.
30. Bai H, Wang Z, Wang Y, et al. Detection and clinical significance of intratumoral EGFR mutational heterogeneity in Chinese patients with advanced non-small cell lung cancer. PLoS One 2013;8:e54170.
31. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
32. Izar B, Sequist L, Lee M, et al. The impact of EGFR mutation status on outcomes in patients with resected stage I non-small cell lung cancers. Ann Thorac Surg 2013;96:962-8.
33. Selvaggi G, Novello S, Torri V, et al. Epidermal growth factor receptor overexpression correlates with a poor

prognosis in completely resected non-small-cell lung cancer. Ann Oncol 2004;15:28-32.
34. Johnson BE, Kris MG, Berry LD, et al. A multicenter effort to identify driver mutations and employ targeted therapy in patients with lung adenocarcinomas: The Lung Cancer Mutation Consortium (LCMC). J Clin Oncol 2013;31:490s.
35. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol 2005;23:8081-92.
36. Chin TM, Quinlan MP, Singh A, et al. Reduced Erlotinib sensitivity of epidermal growth factor receptor-mutant non-small cell lung cancer following cisplatin exposure: a cell culture model of second-line erlotinib treatment. Clin Cancer Res 2008;14:6867-76.

Cite this article as: Liang W, Zhang Y, Kang S, Pan H, Shao W, Deng Q, Shi X, Wang W, He J. Impact of EGFR mutation status on tumor response and progression free survival after first-line chemotherapy in patients with advanced non-small cell lung cancer: a meta-analysis. J Thorac Dis 2014;6(9):1239-1250. doi: 10.3978/j.issn.2072-1439.2014.07.33

FGFR1 amplifications in squamous cell carcinomas of the lung: diagnostic and therapeutic implications

Hans-Ulrich Schildhaus[1,2*], Lucia Nogova[2,3*], Jürgen Wolf[2,3], Reinhard Buettner[1,2]

[1]Institute of Pathology, University Hospital Cologne, Cologne, Germany; [2]Center for Integrated Oncology Cologne/Bonn, Germany; [3]Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany

*Both authors contributed equally to this work.

Correspondence to: Professor Reinhard Buettner, M.D. Institute of Pathology, University Hospital Cologne, Kerpener Straße 62, D-50937 Köln, Germany. Email: reinhard.buettner@uk-koeln.de.

Abstract: Fibroblast growth factor receptor 1 (FGFR1) is a type 4 receptor tyrosine kinase. The receptor and its ligands play an important role in development and physiology. However, constitutive activation of FGFR1 by gene amplification, translocation or mutation is associated with various malignancies as, for example, breast cancer or myeloproliferative diseases. We have recently reported that *FGFR1* amplification occurs in 20% of pulmonary squamous cell carcinomas, and preclinical tests have shown that these alterations are therapeutically tractable. These findings make *FGFR1* amplification a potential biomarker for lung cancer treatment. Squamous cell carcinomas of the lung are characterized by an uneven *FGFR1* gene copy number distribution. Therefore, fluorescence in situ hybridization assays need to address focality and heterogeneity of *FGFR1* in these tumors. Here, we review our proposal for a reading and evaluation strategy. Furthermore, we highlight the emerging landscape of clinical trials with selective and unselective FGFR inhibitors and provide first response data from early clinical trials.

Keywords: Lung cancer; squamous cell carcinoma; *fibroblast growth factor receptor 1* (*FGFR1*); FISH

Submitted Feb 10, 2013. Accepted for publication Mar 07, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.03
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.03

Introduction

Fibroblast growth factor receptor 1 (FGFR1) amplification represents now one of the most promising predictive biomarkers in lung cancer. This alteration seems to become the first therapeutically relevant genetic change in pulmonary squamous cell carcinomas, which occurs frequently in these tumors. In contrast to adenocarcinomas of the lung, squamous cell carcinomas do not significantly harbor *EGFR* mutations or *ALK*, *ROS1* or *RET* translocations, which are therapeutically tractable. Therefore, *FGFR1* amplifications in pulmonary squamous cell carcinomas are currently in the focus of many researchers and various ongoing clinical trials.

Squamous cell carcinoma is a common subgroup of lung cancer, which is strongly associated with smoking. The estimated annual incidence is approximately 123 newly diagnosed cases per 100,000 inhabitants in Europe (1,2). It is suspected that both incidence and prevalence will still increase, especially among female patients. The current therapeutic regimen for locally advanced or metastatic tumors consists of conventional platinum based chemotherapy and radiation (3). Very recently, data from our group indicated, however, that a focal amplification of chromosome band 8p12, representing the second most common genetic alteration, occurs in pulmonary squamous cell carcinomas which was proven to be related to *FGFR1* amplification (4). Subsequently, we could confirm this finding in a large cohort of 420 clinical lung cancer samples by fluorescence *in situ* hybridization (5). Furthermore, data from *in vitro* studies provided first evidence that *FGFR1*

amplified squamous cell lines are in fact exploitable by FGFR inhibitors (4).

The FGFR family of receptor tyrosine kinases

FGFR1 is a member of the type 4 family of receptor tyrosine kinases, which consists of the closely related and highly conserved FGFRs 1 to 4. All these proteins are transmembrane receptors which are composed of an extracellular ligand binding domain, a transmembrane domain and an intracellular part which contains the functionally relevant tyrosine kinase domain. Three immunoglobulin-like loops (IgI-III) build the extracellular part, with IgI and II being separated by a so-called acid box of few amino acid residues. IgII and III form the ligand binding site. The binding specificity of the receptors is regulated by alternative splicing of the IgIII portion as exons 8 and 9 build alternatively the C-terminal part of this domain, thus forming the IIIb or IIIc variant of the receptor, respectively (6). Epithelial tissues express mostly the IIIb variant, whereas IIIc predominates in mesenchymal cells (7). This alternative splicing is, however, restricted to *FGFR1-3* with *FGFR4* being expressed always in the IIIc form.

FGFRs are activated by binding of their specific ligands - the fibroblast growth factors (FGFs) of which 18 different types are currently known. FGFs can function in an autocrine or paracrine manner and may even have hormonal long-distance effects. Furthermore, FGFs can also be liberated from the stroma, for instance during invasive tumor growth. FGFs bind with high specificity to FGFRs and form a complex with dimerized receptor molecules and a heparan sulphate proteoglycan chain. These activated complexes undergo conformation change and activation of the tyrosine kinase domains which finally trans-phosphorylate. After binding and phosphorylation of adapter proteins FGF signaling functions via different downstream effectors, e.g., the signal transducer and activator of transcription (STAT) pathway. Another signaling axis consists of phospholipase Cγ, protein kinase C and ends in the RAS - MAP kinase pathway. An important regulator of FGFR signaling is FGFR substrate 2 (FRS2) which binds to the juxtamembrane domain of activated FGF receptors and which recruits GRB2 and other downstream molecules finally leading again to an activation of the RAS - RAF - MAP kinase pathway as well as the PI3K - AKT pathway. Among others proteins FGFR-like 1 (FGFRL1 or FGFR5) functions as a negative regulator. FGFRL1 has the capability of binding (or "trapping") FGFs without subsequent tyrosine kinase activity.

FGFR activity and FGF signaling play a major role in development, proliferation, differentiation and survival. Thus, FGFRs are crucial for embryogenesis, e.g., for limb development and organogenesis, and are highly important for many physiological processes including wound healing. In this context, FGFRs can act even as tumor suppressors.

The role of *FGFR1* in oncogenesis

Gains of function of the FGF receptors were found to be associated with various malignancies. Constitutive activation of FGFR1 occurs basically by three major mechanisms: gene amplification, translocation or activating mutations (for overview and selected references see *Table 1*). *FGFR1* mutations have been reported in melanomas but this appears to be a rather rare event. *FGFR1* amplification, however, belongs to the most frequent genetic changes in breast cancer. Amplification of *FGFR1* has additionally been reported in squamous cell carcinomas of the head and neck as well as from the esophagus. Translocations of *FGFR1* have originally been described in a myeloproliferative hematological disorder which has now been referred to as "8p11 myeloproliferative syndrome characterized by *FGFR1* translocation" by the current WHO classification system. Very recently *FGFR1* translocations were additionally found in a subset of glioblastoma multiforme and in a rhabdomyosarcoma.

Altered FGF receptor activity contributes to cancer development by regulating different key processes. Meanwhile, there is clear evidence that unscheduled FGFR activation leads to an increase in cell proliferation and prolonged survival but also cell migration and angiogenesis are stimulated.

FGFR1 amplification in lung cancer - epidemiology

Very recently we have reported on the frequency of *FGFR1* amplifications in pulmonary carcinomas. In the so far largest series we found 20% *FGFR1* amplified tumors among squamous cell carcinomas (5) which was recently confirmed in a second independent study (19). Therefore, *FGFR1* amplification represents one of the most frequent driver lesions in lung cancer next to *EGFR* mutations, and far more often than *ALK*, *ROS1* or *RET* rearrangements or other therapeutically targetable alterations. The high frequency as well as the large list of potential inhibitors which are currently in early or advanced clinical trials make

Table 1 *FGFR1* alterations in malignancies

FGFR1 alterations	Tumor entities (estimated frequency)	Selected references
Amplification	Breast cancer (10%)	Reis-Filho *et al.* [2006] (8)
	Ovarian cancer (5%)	Gorringe *et al.* [2007] (9)
	Bladder cancer (3%)	Simon *et al.* [2001] (10)
	Rhabdomyosarcoma (3%)	Missiaglia *et al.* [2009] (11)
	Squamous cell carcinoma of the lung (20%)	Schildhaus *et al.* [2012] (5)
	Small cell carcinoma of the lung (5%)	Peifer *et al.* [2012] (12)
	Head and neck squamous cell carcinomas (17%)	Freier *et al.* [2007] (13)
	Esophageal squamous cell carcinoma	Randla *et al.* [2012] (14)
Translocation	8p11 myeloproliferative syndrome [*ZNF198-FGFR1*] (100%, entity defining alteration as part of myeloid and lymphoid neoplasms with abormalities of *FGFR1* and various translocation partners of *FGFR1*)	WHO classification [2008] (15)
	Chronic myeloid leukemia (rare)	WHO classification [2008] (15)
	Rhabdomyosarcoma [*FOXO1-FGFR1*] (one case)	Liu *et al.* [2011] (16)
	Glioblastoma multiforme [*TACC-FGFR1/3*] (3%)	Singh *et al.* [2012] (17)
Activating mutation	Melanoma (rare)	Lin *et al.* [2008] (18)

FGFR1 amplification one of the most promising biomarkers for lung cancer treatment.

It is, however, noteworthy that - at least until now - no convincing case of an adenocarcinoma has been proven to be *FGFR1* amplified. In our series of nearly one hundred pulmonary adenocarcinomas all cases were clearly negative for gene amplifications whereas polysomic cases were frequently noticed (5). This finding seems to be restricted to pure adenocarcinomas as we have seen *FGFR1* amplification occasionally in adeno-squamous carcinomas. Furthermore, we have found additionally a pulmonary large cell carcinoma *FGFR1* amplified (5). This might reflect the fact that emerging data from expression profiles provide evidence that some pulmonary large cell carcinomas represent a dedifferentiation endpoint of squamous carcinomas. Taken together, among non small cell carcinomas, *FGFR1* amplification seems to be strongly associated with squamous morphology.

Very recently, we further reported that also small cell carcinomas of the lung can harbor *FGFR1* amplifications (12). Preliminary and not yet published data from our screening program provide first evidence that this is a reproducible finding which can be confirmed in clinical routine cohorts. The frequency of FGFR1 amplifications among small cell carcinomas seems to be lower than in squamous cell carcinomas. Based on our current and still ongoing epidemiologic studies we estimate the frequency of *FGFR1* amplifications among small cell carcinomas around 5%.

Detection of *FGFR1* amplifications by fluorescence *in situ* hybridization

Therapeutic effects of *FGFR1* tyrosine kinase inhibitors seem to be dependent on significantly increased *FGFR1* gene copy numbers. It still needs to be clarified whether *FGFR1* amplification only serves as a surrogate marker for receptor protein overexpression since the receptor itself should represent obviously the therapeutic target. There are currently no validated antibody assays on the market, which could reliably detect *FGFR1* expression levels quantitatively or semiquantitatively by using paraffin embedded tumor samples. Thus, comprehensive studies on the correlation between *FGFR1* gene copy numbers and protein expression levels are still missing. Therefore, current clinical trials with *FGFR1* inhibitors enroll patients who are found to be "*FGFR1* amplified". Reliable *FGFR1* FISH probes are now commercially available. Therefore, fluorescence *in situ* hybridization assays on formalin fixed and paraffin embedded material are carried out to screen patients for clinical trials. This is an important fact as lung cancer samples *per se* are often hard to diagnose. Biopsy samples, which are obtained endoscopically or by transthoracic CT-guided biopsy are often very small and contain only little tumor tissue. Tumor cells are frequently damaged by manipulations and show often crushing artifacts. Surgical samples contain often large tumor areas of necrosis or dense fibrosis which regularly influence hybridization quality.

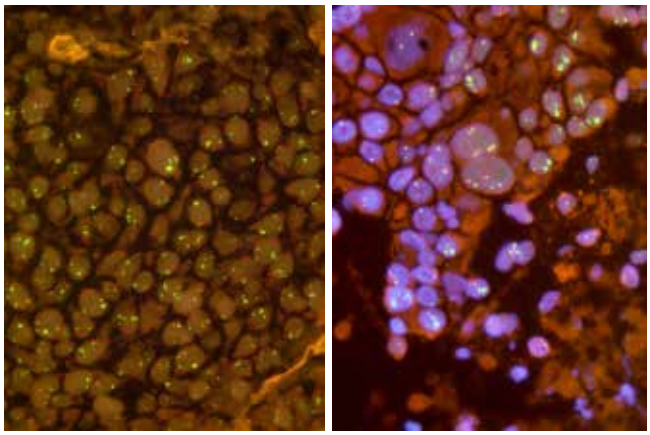


Figure 1 Fluorescence in situ hybridization for detection of FGFR1 amplification in pulmonary squamous cell carcinomas. A. In this tumor, FGFR1 (green) and chromosome 8 (CEN8, orange) copies are more or less evenly distributed. Only occasionally, microclusters are seen (arrow); B. Heterogeneity occurs frequently in these tumors. Beside tumor cells with normal or only slight increase in copy numbers, there are many tumor cell nuclei with tight clusters of amplified FGFR1, some of which are indicated by arrows. Note that the occurrence of FGFR1 clusters is not necessarily related to the size of the nuclei.

Therefore, lung cancer tissue is basically a challenge for FISH. Despite this fact we were able to establish a robust and reliable FISH assay, and we have noticed a drop out rate below 5% in our laboratory by using our protocol (5).

FGFR1 amplification is not yet convincingly defined. Some authors have simply applied the criteria which are commonly used for detection of *her2* amplifications in breast cancer. From our experience, however, these criteria are not useful to evaluate *FGFR1* FISH assays for squamous cell carcinomas of the lung. *FGFR1* in these tumors is characterized by some unique features which make *FGFR1* FISH assays challenging. One major issue is heterogeneity and focality of gene amplifications (*Figure 1*). Thus, adequate screening for amplification hot spots is a prerequisite for reliable *FGFR1* evaluation. Based on our reading and evaluation strategy (5), we recommend careful scanning of the entire tumor area by using a 40× or 63× oil objective. *FGFR1* and centromer 8 (CEN8) signals should be counted for individual tumor cells (63× or 100× oil objective). We suggest counting of 20 tumor cell from three areas, resulting in a total of 60 nuclei. Counting areas should be selected from prior screening as the hot spot areas containing the highest number of *FGFR1* copies. If the signals are found to be evenly distributed random areas should be used. Another important phenomenon is focality of *FGFR1* gene copy distribution. Very often isolated tumor cells with a very high number of *FGFR1* gene copies occur which are surrounded by tumor cells with normal or only slightly increased gene copy numbers. Therefore, it turned out to be mandatory to count contiguous and cohesive tumor cells from each area. It should be avoided to pick only suspicious tumor cell nuclei with increased *FGFR1* and/or CEN8 copy numbers because this approach might lead to an overestimation of gene copy numbers.

Furthermore, the gene copy distribution in pulmonary squamous cell carcinomas is different from e.g., *her2* in breast cancer. A significant proportion of tumors show colocalized clusters with numerically balanced increase in both *FGFR1* and CEN8 copy numbers. Another more or less specific feature represents so-called microclusters which consist of a tight accumulation of more than three *FGFR1* signals. We have proposed to regard these microclusters as 5 signal copies.

Having counted 60 tumor cells a final decision has to be made whether a given sample is "amplified" or not. As already mentioned there are until now no convincing criteria to judge *FGFR1* FISH assays in squamous cell carcinomas. From our point of view this FISH assay should finally serve as a predictive biomarker which should be capable to predict response to anti-FGFR treatment. However, criteria for thresholds and cut-off values still have to be determined retrospectively after finishing the currently ongoing clinical trials individually for each compound. Therefore, we have developed evaluation criteria ("Cologne Score") which are suitable to detect patients with the highest gene copy numbers and to enroll them in clinical trials. In this context, it seems to be important to us not only to use *FGFR1*/CEN8 ratio as criterion for FISH positivity since we have noticed tumors with an enormous increase in *FGFR1* gene copy numbers in a background of co-localized clusters, i.e. an additional increase of centromeric DNA material. These cases would finally result in a ratio of nearly 1.0 and would be considered negative if the decision would rest solely on *FGFR1*/CEN8 ratio. Therefore, it appears useful also to consider average gene copy number as a criterion for positivity. Furthermore, we have seen isolated tumor cells with high level cluster amplification which were surrounded by lesional cells with normal or only slightly increased gene copy numbers. Thus, we proposed to include additionally the percentage of tumor cell with gene clusters of at least 15 gene copies in the catalogue of FISH criteria.

Considering all these items we have proposed to diagnose *FGFR1* amplification ("high level amplification") if one of the following criteria is fulfilled: (I) *FGFR1*/CEN8 ratio is ≥2.0, (II) average gene copy per nucleus is ≥6.0, and (III) percentage of tumor cells containing ≥15 gene copies or large cluster is ≥10%.

Beside unamplified tumors with nearly normal gene count and amplified cases as defined above we became aware of a third category of squamous cell carcinomas which is characterized by a moderate increase of *FGFR1* gene copies. For these more or less polysomic cases we have defined a "low level amplification" category which we defined by a percentage of ≥50% of tumor cells containing ≥5 *FGFR1* gene copies. This criterion was derived and adapted from previous studies on squamous cell carcinomas of the head and neck as well as from reports on breast cancer (8,13). Since polysomy is a common phenomenon in cancer, one might expect that many squamous cell lung cancers might fall into this category. It is, however, important to emphasize the fact that this low level amplification contributes for only one fifth of the amplified pulmonary squamous carcinomas. Low level amplification is found in only 4% of these tumors.

Therapeutic implications resulting from oncogenic FGFR dependence

The identification of FGFR alteration in various types of human cancer led to rapid development of compounds targeting FGFR. As described above, the FGFR family comprises 4 members (*FGFR1*, *2*, *3* and *4*). The small molecules act either as selective pan inhibitors of the FGFR family or as non-selective inhibitors, which usually target not only FGFR but also other intracellular proteins. *Table 2* summarizes FGFR inhibitors in current clinical development.

Currently ongoing phase Ia/Ib and phase II studies recruit patients either with diagnosed *FGFR* alterations only or include unselected patient populations [for overview of FGFR trials see ref (20).].

The phase I/II studies with AZD4547, BGJ398, E-3810 and dovitinib include only patients with genetic *FGFR* alterations. The phase II with ponatinib is recruiting patients with squamous cell carcinoma with retrospective outcome analysis of *FGFR* altered patients (20).

Nintedanib and XL999 were investigated in advanced non small cell lung cancer (NSCLC) without further selection (1,21). All other trials recruited patients with different solid tumors without any further molecular analysis.

Trials recruiting patients with FGFR alterations

BGJ398 is a selective FGFR inhibitor which blocks FGFR1, FGFR2 and FGFR3. The drug is supposed to be effective in tumors with activated FGFR axis due to activating mutations or gene amplification. The BGJ398 phase Ia (first in man) trial recruited patients with solid tumors in an escalating dose schedule starting from 5 mg daily. After cohort 3, only patients with *FGFR1* or *FGFR2* amplification or *FGFR3* mutation are included. Preliminary analysis was conducted after 26 recruited patients including 10 patients with *FGFR1* amplified breast cancer and 3 patients with *FGFR1* amplified squamous cell carcinomas of the lung. One patient with *FGFR1* amplified squamous cell lung cancer with an *FGFR1*/CEP8 ratio of 2.6 by FISH analysis treated with 100 mg BGJ398 showed partial response in CT scan at 8 weeks, confirmed at 12 weeks with an substantial SUV decrease on PET scan at week 4 (22). The trial is currently treating patients with *FGFR* alterations on maximal tolerated dose in the expansion part of the phase I.

The phase I study with the selective FGFR inhibitor AZD4547 (FGFR 1, 2, 3 inhibitor) is currently recruiting patients with *FGFR* amplified tumors at a maximal tolerated dose.

Dovitinib - as an unselective FGFR inhibitor - blocks also VEGFR 1, 2, 3 and PDGFR β besides FGFR 1, 2, 3. First phase I/II studies were conducted in patients with metastatic melanoma without any pre-selection according to genetic alterations. A moderate clinical benefit of stable disease was reached in 12 from 47 enrolled patients (23). The phase I/II study in patients with advanced or metastatic renal cell cancer showed 2 partial responses from 20 recruited patients (24). A phase II study treating 77 metastatic breast cancer patients showed 13% partial responses in the group of patients who were *FGFR1* amplified and hormone positive (25). Phase II studies in patients with metastatic gastric cancer and *FGFR2* amplification and in patients with advanced endometrium cancer and *FGFR2* mutation (stratified to non-mutated patients) are currently ongoing (20).

The expansion part of the phase I study with E-3810, a combined inhibitor of FGFR 1 and VEGFR 1, 2, 3, is recruiting patients with *FGFR1* amplification and patients relapsing after response or long stable disease after anti-angiogenic treatment (26).

Ponatinib is a multikinase inhibitor of FGFR-1, 2, 3, 4, Abl, Src, FLT-3 and c-KIT showing high clinical activity in heavily pretreated patients with chronic myeloid leukemia resistant

Table 2 Selective and non-selective FGFR inhibitors in current clinical development

FGFR inhibition	Drugs	Receptor targets	Provider
Selective			
	AZD4547	FGFR 1, 2, 3	Astra Zeneca
	BGJ398	FGFR 1, 2, 3	Novartis
	JNJ-42756493	pan FGFR	Janssen Research & Development
Non-selective			
	ARQ087	pan FGFR	ArQuele
	Brivanib	FGFR 1 VEGFR 2	Bristol-Meyers-Squibb
	Danusertib	FGFR 1 Aurora kinase A, B, C Abl, Ret, TrkA	Nerviano Medical Sciences
	Dovitinib	FGFR 1, 2, 3 VEGFR 1, 2, 3 PDGFR β	Novartis
	E-3810	FGFR 1 VEGFR 1, 2, 3	Ethical Oncology Science (EOS)
	FP-1039	FGF ligand trap	Five Prime Therapeutics
	LY2874455	FGFR 1, 2, 3, 4 VEGFR 2	Eli Lilly
	MK-2461	FGFR 1, 2, 3 c-Met, Ron Flt-1, 3, Mer PDGFR β	Chemfun Medical technology
	Nintedanib	FGFR 1, 2, 3 VEGFR 1, 2, 3 PDGFR α/β	BoehringerIngelheim
	Orantinib	FGFR 1 VEGFR 2 PDGFR β	TaihoPharmaceutical Co., Ltd.
	Ponatinib	FGFR 1, 2, 3, 4 Abl, Src FLT-3, c-KIT	Ariad Pharmaceuticals
	XL228	FGFR 1 IGF1R Aurora A, B FAK, Src BCR-Abl	Exelixis
	XL999	FGFR 1, 3 VEGFR 2 PDGFR α/β FLT-3, Src	Exelixis

to other tyrosine kinase inhibitors (27). The phase II study enrolling squamous cell lung cancer patients with retrospective analysis of FGFR alterations is currently ongoing (20).

Trials recruiting NSCLC patients without molecular testing

Nintedanib, a FGFR 1, 2, 3, VEGFR 1, 2, 3 and PDGFR α/β inhibitor showed clinical activity in phase I in advanced solid tumors with one complete and two partial responses occurred in patients with renal (n=2) and colorectal cancer (n=1) among 61 recruited patients (28). The phase II study in unselected patient population with NSCLC showed one partial response and 35 stable diseases in 73 treated patients (21).

XL999 is a multikinase inhibitor of FGFR 1, 3, VEGFR 2, PDGFR α/ß, FLT-3 and Src. The preliminary results of a phase II study in nine NSCLC patients showed one partial response (29).

Trials recruiting other cancer entities without molecular testing

Brivanib (FGFR 1 and VEGFR 2 inhibitor) is one of the few FGFR compounds in late clinical development. The phase I was performed in an unselected patient population. In a dose finding part, the best response was stable disease in 1 patient with NSCLC from 5 patients with different tumor entities (30). Another large phase I study recruited 68 patients with different tumor entities. Two patients achieved partial response, one with renal cell carcinoma and one with a carcinoma of Vaters's ampulla (31). The phase II study on brivanib in 55 patients with hepatocellular carcinoma (HCC) showed one complete and 3 partial responses. The median progression free survival (PFS) and overall survival (OS) were 2.7 and 10 months, respectively (32).

In the phase II discontinuation trial, patients with various tumors and stable disease after initial treatment with brivanib was stratified according to FGF-2 expression and randomized to receive either brivanib or placebo. Fifty-three patients with FGF-2 expression and soft tissue sarcoma showed a PFS of 2.8 months in a brivanib group comparing to 1.4 months in the placebo group (33). Regarding the phase III trials, the study in patients with HCC after failure on sorafenib did not meet its primary endpoint in improving of OS (34).

Phase I study with JNJ-42756493, a selective pan FGFR inhibitor is currently ongoing in an unselected patient population with solid tumors and lymphomas after standard treatment (20). Similarly, phase I study with ARQ087 recruiting unselected patients with solid tumors is currently ongoing (20).

The phase I study with danusertib, which inhibits besides FGFR 1 also Aurora kinase A, B, C, Abl, Ret and TrkA was perfomed in unselected patient population. Although the compound showed some clinical benefit in small cell lung, colorectal, breast and ovarian cancer, the adverse effect were characterized due to pronounced hematological toxicity with febrile neutropenia (35). The phase II study on danusertib in metastatic castration resistant prostate cancer showed moderate clinical activity with median PFS of 3 months (36).

FP-1039 is a ligand trap, which binds to FGF ligands and prevents them from binding to FGFRs. A phase I study recruiting patients with all solid tumors showed moderate clinical activity with tumor shrinkage of 20% in a patient with a prostate cancer (37). Phase II study enrolling patients with *FGFR2* mutated endometrial cancer is currently ongoing (20).

LY2874455 is an inhibitor of all FGF receptors with low VEGFR 2 activity (38). The phase I study recruiting patients with all types of advanced cancer (20).

MK-2461 is a common inhibitor of FGFR 1, 2, 3, c-Met, Ron, Flt-1, 3 and PDGFR β. The phase I in solid tumors showed mild clinical activity (39).

Orantinib, a multikinase inhibitor of FGFR 1, VEGFR 2 and PDGFR ß was investigated mainly in hepatocellular carcinoma (HCC). A phase I/II study in 35 patients with HCC showed one complete and two partial responses (40).

XL228 is a multikinase inhibitor of *FGFR1*, IGF1R, Aurora A, B, FAK, Src and bcr-abl. The phase I study in advanced solid tumors and lymphoma showed one partial response in a patient with adenocarcinoma of the lung (41).

Conclusions

A great variety of FGFR inhibitors is currently in clinical development. First results show that these drugs basically have therapeutic effects on solid and hematologic tumors. Data from trials enrolling lung cancer patients indicate that genetic prescreening increases antitumoral efficacy since we could provide first evidence for therapeutic response of a pulmonary *FGFR1* amplified squamous cell carcinoma patient to treatment with a selective FGFR inhibitor. Therefore, FISH is currently the method of choice to detect squamous cell carcinomas of the lung with *FGFR1* amplification.

Acknowledgements

Disclosure: HUS has received honoraria and reimbursements from Pfizer, Abbott Molecular, Zytomed Systems and Roche Pharma, and grants, honoraria and reimbursements from Novartis Oncology. LN has received honoraria from Roche and Novartis, and travel support from Pfizer, Bayer, Novartis, Roche and Lilly. JW has served as an advisory board member of Novartis and Astra Zeneca and has received research support from Novartis and honoraria from Novartis and Astra Zeneca. RB declares no conflict of interest.

References

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer 2013;49:1374-403.
2. Perez-Moreno P, Brambilla E, Thomas R, et al. Squamous cell carcinoma of the lung: molecular subtypes and therapeutic opportunities. Clin Cancer Res 2012;18:2443-51.
3. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92-8.
4. Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2:62ra93.
5. Schildhaus HU, Heukamp LC, Merkelbach-Bruse S, et al. Definition of a fluorescence in-situ hybridization score identifies high- and low-level FGFR1 amplification types in squamous cell lung cancer. Mod Pathol 2012;25:1473-80.
6. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer 2010;10:116-29.
7. Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 2005;16:139-49.
8. Reis-Filho JS, Simpson PT, Turner NC, et al. FGFR1 emerges as a potential therapeutic target for lobular breast carcinomas. Clin Cancer Res 2006;12:6652-62.
9. Gorringe KL, Jacobs S, Thompson ER, et al. High-resolution single nucleotide polymorphism array analysis of epithelial ovarian cancer reveals numerous microdeletions and amplifications. Clin Cancer Res 2007;13:4731-9.
10. Simon R, Richter J, Wagner U, et al. High-throughput tissue microarray analysis of 3p25 (RAF1) and 8p12 (FGFR1) copy number alterations in urinary bladder cancer. Cancer Res 2001;61:4514-9.
11. Missiaglia E, Selfe J, Hamdi M, et al. Genomic imbalances in rhabdomyosarcoma cell lines affect expression of genes frequently altered in primary tumors: an approach to identify candidate genes involved in tumor development. Genes Chromosomes Cancer 2009;48:455-67.
12. Peifer M, Fernández-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012;44:1104-10.
13. Freier K, Schwaenen C, Sticht C, et al. Recurrent FGFR1 amplification and high FGFR1 protein expression in oral squamous cell carcinoma (OSCC). Oral Oncol 2007;43:60-6.
14. Bandla S, Pennathur A, Luketich JD, et al. Comparative genomics of esophageal adenocarcinoma and squamous cell carcinoma. Ann Thorac Surg 2012;93:1101-6.
15. Swerdlow SH, Campo E, Harris NL, et al. eds. WHO classification of tumours of haematopietic and lymphoid tissues. Lyon: IARC, 2008.
16. Liu J, Guzman MA, Pezanowski D, et al. FOXO1-FGFR1 fusion and amplification in a solid variant of alveolar rhabdomyosarcoma. Mod Pathol 2011;24:1327-35.
17. Singh D, Chan JM, Zoppoli P, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. Science 2012;337:1231-5.
18. Lin WM, Baker AC, Beroukhim R, et al. Modeling genomic diversity and tumor dependency in malignant melanoma. Cancer Res 2008;68:664-73.
19. Heist RS, Mino-Kenudson M, Sequist LV, et al. FGFR1 amplification in squamous cell carcinoma of the lung. J Thorac Oncol 2012;7:1775-80.
20. Available online: www.clinicaltrials.gov
21. Reck M, Kaiser R, Eschbach C, et al. A phase II double-blind study to investigate efficacy and safety of two doses of the triple angiokinase inhibitor BIBF 1120 in patients with relapsed advanced non-small-cell lung cancer. Ann Oncol 2011;22:1374-81.
22. Wolf J, Lorusso PM, Camidge RD, et al. A phase I dose escalation study of NVP-BGJ398, a selective pan FGFR inhibitor in genetically preselected advanced solid tumors. Cancer Res 2012;72:abstr LB122.
23. Kim KB, Chesney J, Robinson D, et al. Phase I/II and pharmacodynamic study of dovitinib (TKI258), an inhibitor of fibroblast growth factor receptors and VEGF receptors, in patients with advanced melanoma. Clin Cancer Res 2011;17:7451-61.

24. Angevin E, Lin C, Pande AU, et al. A phase I/II study of dovitinib (TKI258), a FGFR and VEGFR inhibitor, in patients (pts) with advanced or metastatic renal cell cancer: Phase I results. J Clin Oncol 2010;28:abstr 3057.
25. Andre F, Bachelot TD, Campone M, et al. A multicenter, open-label phase II trial of dovitinib, an FGFR1 inhibitor, in FGFR1 amplified and non-amplified metastatic breast cancer J Clin Oncol 2011;29:abstr 289.
26. Soria J, De Braud FG, Cereda R, et al. First-in-man study of E-3810, a novel VEGFR and FGFR inhibitor, in patients with advanced solid tumors. J Clin Oncol 2011;29:abstr TPS149.
27. Cortes JE, Kantarjian H, Shah NP, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. N Engl J Med 2012;367:2075-88.
28. Mross K, Stefanic M, Gmehling D, et al. Phase I study of the angiogenesis inhibitor BIBF 1120 in patients with advanced solid tumors. Clin Cancer Res 2010;16:311-9.
29. March RJ, Mirtsching B, Modi S, et al. A phase II study of XL999 in patients (pts) with NSCLC. J Clin Oncol 2007;25:abstr 18112.
30. Mekhail T, Masson E, Fischer BS, et al. Metabolism, excretion, and pharmacokinetics of oral brivanib in patients with advanced or metastatic solid tumors. Drug Metab Dispos 2010;38:1962-6.
31. Jonker DJ, Rosen LS, Sawyer MB, et al. A phase I study to determine the safety, pharmacokinetics and pharmacodynamics of a dual VEGFR and FGFR inhibitor, brivanib, in patients with advanced or metastatic solid tumors. Ann Oncol 2011;22:1413-9.
32. Park JW, Finn RS, Kim JS, et al. Phase II, open-label study of brivanib as first-line therapy in patients with advanced hepatocellular carcinoma. Clin Cancer Res 2011;17:1973-83.
33. Chou T, Finn RS. Brivanib: a review of development. Future Oncol 2012;8:1083-90.
34. Llovet JM, Decaens T, Raoul JL, et al. Brivanib versus placebo in patients with advanced hepatocellular carcinoma (HCC) who failed or were intolerant to sorafenib. Results from the phase 3 BRISK-PS study. J Hepatol 2012; 56:S549.
35. Cohen RB, Jones SF, Aggarwal C, et al. A phase I dose-escalation study of danusertib (PHA-739358) administered as a 24-hour infusion with and without granulocyte colony-stimulating factor in a 14-day cycle in patients with advanced solid tumors. Clin Cancer Res 2009;15:6694-701.
36. Meulenbeld HJ, Bleuse JP, Vinci EM, et al. Randomized phase II study of danusertib in patients with metastatic castration-resistant prostate cancer after docetaxel failure. BJU Int 2013;111:44-52.
37. Tolcher A, Papadopoulos K, Patniak A, et al. Preliminary results of a dose escalation study of the fibroblast growth factor (FGF)"trap" FP-1039 (FGFR1: Fc) in patients with advanced malignancies. Eur J Cancer 2010;8:abstr 381.
38. Zhao G, Li WY, Chen D, et al. A novel, selective inhibitor of fibroblast growth factor receptors that shows a potent broad spectrum of antitumor activity in several tumor xenograft models. Mol Cancer Ther 2011;10:2200-10.
39. Camacho LH, Moulder SL, LoRusso PM, et al. First in human phase I study of MK-2461, a small molecule inhibitor of c-Met, for patients with advanced solid tumors. J Clin Oncol 2008;26;abstr 14657.
40. Kanai F, Yoshida H, Tateishi R, et al. A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma. Cancer Chemother Pharmacol 2011;67:315-24.
41. Smith DC, Britten C, Clary DO, et al. A phase I study of XL228, a potent IGF1R/AURORA/SRC inhibitor, in patients with solid tumors or hematologic malignancies. J Clin Oncol 2009;27;abstr 3512.

Cite this article as: Schildhaus HU, Nogova L, Wolf J, Buettner R. *FGFR1* amplifications in squamous cell carcinomas of the lung: diagnostic and therapeutic implications. Transl Lung Cancer Res 2013;2(2):92-100. doi: 10.3978/j.issn.2218-6751.2013.03.03

Translational Medicine

ALK and ROS1 as a joint target for the treatment of lung cancer: a review

Raimon Puig de la Bellacasa[1], Niki Karachaliou[2], Roger Estrada-Tejedor[1], Jordi Teixidó[1], Carlota Costa[2], José I. Borrell[1]

[1]Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, E-08017 Barcelona, Spain; [2]Breakthrough Cancer Research Unit, Pangaea Biotech S.L, Barcelona, Spain

Correspondence to: José I. Borrell. Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, E-08017 Barcelona, Spain. Email: j.i.borrell@iqs.url.edu.

Abstract: Rearrangements of the anaplastic lymphoma kinase (ALK) have been described in multiple malignancies, including non-small cell lung cancer (NSCLC). ALK fusions have gain of function properties while activating mutations in wild-type ALK can also occur within the tyrosine kinase domain. ALK rearrangements define a new molecular subtype of NSCLC that is exquisitely sensitive to ALK inhibition. Crizotinib, an orally available small molecule ATP-mimetic compound which was originally designed as a MET inhibitor, was recognized to have "off-target" anti-ALK activity and has been approved in the USA for the treatment of patients with ALK-positive NSCLC. Chromosomal rearrangements involving the ROS1 receptor tyrosine kinase have also been recently described in NSCLC, while crizotinib is currently under clinical trial in this molecular subset of NSCLC patients. The basic approaches of any computer aided drug design work in terms of structure and ligand based drug design. Details of each of these approaches should be covered with an emphasis on utilizing both in order to develop multi-targeted small-molecule kinase inhibitors. Such multi-targeted tyrosine kinase inhibitors can have antiproliferative activity against both ROS1and ALK rearranged NSCLC. Herein, we highlight the importance of targeting these proteins and the advances in optimizing more potent and selective ALK and ROS1 kinase inhibitors.

Keywords: Anaplastic lymphoma kinase (ALK); drug design; kinase inhibitors; non-small cell lung cancer; proto-oncogene tyrosine-protein kinase ROS (ROS1)

Submitted Feb 20, 2013. Accepted for publication Mar 25, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.11
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.11

Lung cancer remains the leading cause of cancer mortality in the world with non-small cell lung cancer (NSCLC) accounting for 80% of cases (1). Conventional chemotherapeutic regimens only marginally improve the outcome of NSCLC patients at advanced stages of disease, with median survival time less than one year after diagnosis (2). Protein kinase activation by somatic mutation or chromosomal alteration is a common mechanism of tumorigenesis. The discovery of a number of these molecular alterations underlying lung cancer has led to uniquely targeted therapies with specific inhibitor drugs such as erlotinib and gefitinib for mutations in the epidermal growth factor receptor (EGFR), or crizotinib for the gene translocation resulting in the echinoderm microtubule associated protein like 4 (EML4)-anaplastic lymphoma kinase (ALK) oncogene (3-5). EML4-ALK was the first targetable fusion oncokinase to be identified in 4-6% of lung adenocarcinomas. EML4-ALK generates a transforming tyrosine kinase with as many as nine different variants identified (6,7) and represents a novel molecular target in a small subset of NSCLCs. Patients with EML4-ALK positive tumors are characteristically younger age, female, and never to light smokers (5,8,9). The fusion gene has been observed predominantly in adenocarcinomas (4-7%) (5,9). Based on data from a phase I clinical trial which showed an overall response rate of 57% and a probability of progression-free

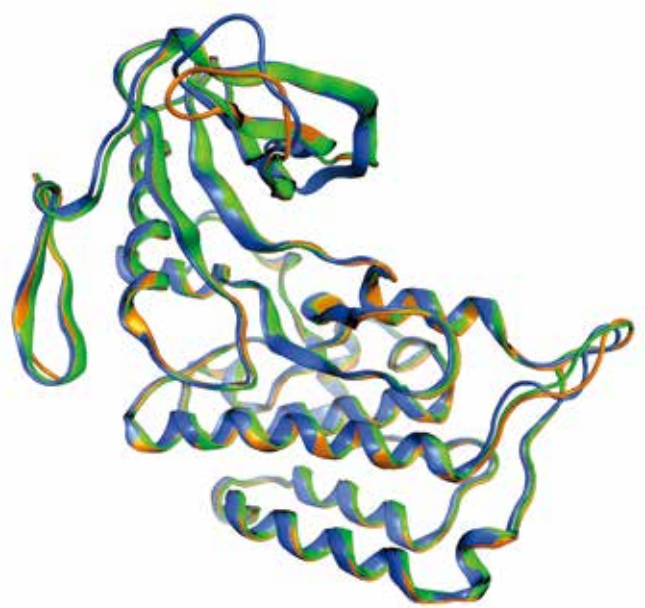


Figure 1 Superposition of the three structures available in the PDB of ALK's catalytic domain. Structure has been colored according to its resolution: 3LCT (2.10Å) in orange, 3LCS (1.95Å) in blue and 3L9P (1.80Å) in green. The loop regions show the main differences between structures.

survival at 6 months of 72%, crizotinib has been approved by the U.S. Food and Drug Administration for the treatment of NSCLC patients with ALK transforming rearrangements (5).

Similarly, proto-oncogene tyrosine-protein kinase ROS (ROS1) is an orphan receptor tyrosine kinase (RTK) that forms fusions and defines another clinically actionable oncogenic driver mutation in NSCLC (10). It has been recently reported that approximately 1.4% of NSCLCs harbor ROS1 rearrangements (11,12). Of the ROS1 fusion-positive tumors, 30% are known to harbor a recurrent translocation t[5;6][q32;q22], which creates the CD74 molecule, major histocompatibility complex, class II invariant chain (CD74)-ROS fusion kinase (13). In fact, ROS1 is evolutionarily related to ALK. Patients with ROS1 rearrangements are also significantly younger, more likely to be never-smokers and are more often diagnosed with the histological subtype of adenocarcinoma with wide distribution of tumor grade (11). Although ROS1 shares only 49% amino acid sequence homology with ALK in the kinase domains, several ALK inhibitors have demonstrated *in vitro* inhibitory activity against ROS1 (14). Recently, a report from investigators at the Massachusetts General Hospital Cancer Center has showed that ROS1-driven tumors can be treated with crizotinib and describes the remarkable response of one patient to crizotinib treatment (11). Interestingly, in this study ROS1 rearrangements were found to be mutually exclusive to ALK rearrangements (11). Preliminary results of a phase I trial of ROS1-positive advanced-stage NSCLC patients treated with crizotinib reported a response rate of 57% and a disease control rate of 79% at 8 weeks (15).

The discovery of new selective and potent inhibitors of ALK and ROS1 kinase raises the importance of using these drugs as a new method for treatment of ALK- and ROS1-derived lung cancer. This review focuses on the importance of targeting these proteins and describes the advances in optimizing more potent and selective ALK and ROS1 kinase inhibitors that have an optimal pharmacokinetic profile and the capacity to inhibit acquired resistant mutations. We aim to stimulate interest and encourage of researchers from different disciplines to learn about new therapeutic avenues following the development of compounds targeting ALK and ROS1 kinases with the aim of increasing survival to these lethal forms of lung cancer.

Structural insights and computational simulations

Receptor Tyrosine kinases (RTK) are transmembrane glycoproteins where the domain responsible to the tyrosine kinase activity is located in the cytoplasm. Although extracellular domain shows remarkable structural differences between TK families, the intracellular region is sensibly conserved.

Although a few years ago there was no resolved three-dimensional structure of ALK, similarity between its sequence permitted to predict its folding from a known RTK structure used as a template, using homology models. Thus, the human ALK receptor was modeled from mouse c-Abl (16), activated insulin receptor tyrosine kinase (InsR) (17,18) or insulin-like growth factor-1 receptor (IGF-1R) (19).

Fortunately, recently some crystal structures of the catalytic domain of ALK have been reported in literature at different resolution levels. All of them are available in the Protein Data Bank (PDB) (20) with ID entries 3L9P, 3LCS, 3LCT (*Figure 1*) (21).

Once the three-dimensional structure of the ALK receptor is available, biological processes related to its structure can be studied virtually, as for example substrate affinity (22), receptor autoactivation or resistant mutations. It can even be used as a structural template in order to predict the structure of RTK homologues by homology modeling (23).

However, crystallographic data should be taken carefully: the combination of crystallographic and biochemical studies reveals that the active conformation of ALK protein requires the phosphorylation of specific residues (24,25) and some ALK crystal structures published to date correspond to unphosphorylated proteins (26).

Additionally, some ALK molecular structures have also been resolved including a bound ligand (*Table 1*). These co-crystallized structures reveal the active site of the protein against one giving compound and describe the interaction within the protein-ligand complex. This information is relevant to assist structure-aided molecular design of ALK inhibitors (i.e. Structure-Based Drug Design, SBDD), providing valuable information about how to improve ALK selectivity.

From SBDD models rises the first approved drug for the treatment of ALK-positive NSCLC, crizotinib (29). By the time diaminopyrimidine (DAP) derivatives were consolidated as bioactive motives, great efforts were done in order to increase its selectivity (31), to optimize their activity (32) or to propose new scaffolds that mimic them. Evaluation of receptor-ligand interactions using docking techniques has become the most preferred SBDD method to study small-molecule ALK inhibitors, not only specific compounds as Novartis NVP-TAE684 (17) but also families including pyridine (33), pyrrolotriazine (19), 2-acyliminobenzimidazole (28), and tetrahydropyrido-[2,3-*b*]pyrazine (34) derivatives. Besides specific chemical families, commercial or public chemical libraries can also be screened using docking techniques in order to identify new candidates (18,33).

Most of the SBDD results agree to consider the targeting of the ATP binding site of the tyrosine kinase domain as a good approach to design RTK inhibitors (35). The analysis of docking results may help to understand the inhibition mechanism, according to the interaction between candidates and the hydrophobic region and/or the gatekeeper. This helps to identify e.g., that preferred interactions of 2-aminopyridines within the ATP binding pocket include hydrogen bonds of pyridine and amino nitrogens with residues close to the gatekeeper (i.e. Met1184 and Glu1182) (34).

Ligand-based drug design (LBDD) has also been applied in the development of new ALK inhibitors. Structure-Activity Relationship (SAR) models correlate the chemical structure of inhibitors with their biological activity. They have been applied to screen series of piperidine carboxamides (27), 2-acyliminobenzimidazoles (28), macrocycles (36), 7-amino-1,3,4,5-tetrahydrobenzo[*b*]azepin-2-ones (37), 2,3,4,5-tetrahydro-benzo[*d*]azepines (38), 2,7-disubstituted pyrrolo[2,1-*f*][1,2,4]triazines (39), diaminocyclohexane methanesulfonamides (40) or tetracyclic derivatives (41,42), tetrahydropyrido[2,3-*b*]pyrazines (34), 3,5-diamino-1,2,4-triazole ureas (43), aryloxy oxo pyrimidinones (44), identifying which substituents confer high ALK-response and proposing chemical modifications of hit compounds.

Far from what it seems, SBDD and LBDD are not exclusive. Most of published LBDD studies include modeling of candidates with the receptor using docking. LBDD and SBDD information can be combined to create pharmacophore models where chemical features (identified from LBDD methods) are used to generate structural keys that active ALK inhibitors must fulfill, taking into account receptor-ligand interactions (identified from SBDD methods) (19,45).

Since mechanisms to drug resistance have been experimentally related to mutations in the ALK amino acid sequence, many efforts have been done in order to obtain resolved crystal structures of ALK mutants (*Table 2*).

Ligand co-crystallizations are also available in mutated structures (mainly F1174L and R1275Q) (26), highlighting structural changes responsible of such behavior. Although resistance can be well correlated with structural changes in some cases (e.g., ALK^{R1275Q}) (46), other mutations exhibit a more subtle behavior.

It is well know that ALK^{L1196M} mutants are crizotinib resistant (47). However, it is interesting to note that the comparison between co-crystal structure of crizotinib with ALK^{wt} (2XP2) (29) and ALK^{L1196M} (2YFX, McTigue M, 2012, unpublished data) reveals that L1196M mutation has little effect on crizotinib's binding mode (*Figure 2*).

It has been recently demonstrated that at least four mutations could be involved in crizotinib's resistance (*Figure 3*): L1196M acts as a gatekeeper and modifies crizotinib's binding site by steric interference (49); G1202R and S1206Y are both located in the solvent-exposed region near to the binding site but G1202R blocks sterically the binding site whereas S1206Y could destabilize electrostatic interaction (50); 1151T insertion is furthest from N terminus Cα-helix but confers a high-level crizotinib resistance probably derived from the modification of ATP affinity (51). This knowledge has been used to design new compounds against gatekeeper mutants (52).

However, other mutations have also been identified. Most of them are abutting the Cα-helix and activation loop, becoming responsible for the modification of the kinase active site. On the basis of the crystal structures, these

Table 1 List of the available ligands co-crystallized with ALK receptor

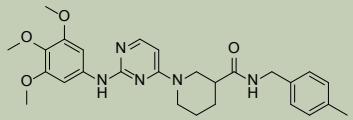
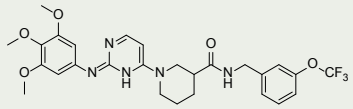
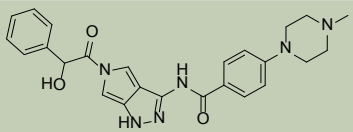
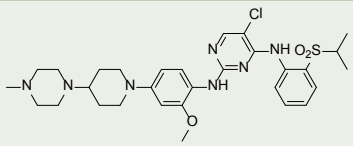
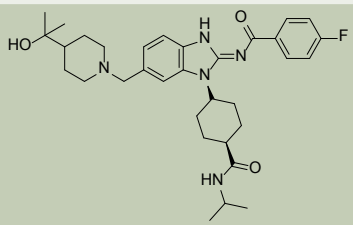
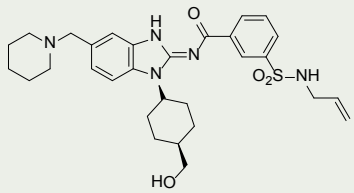
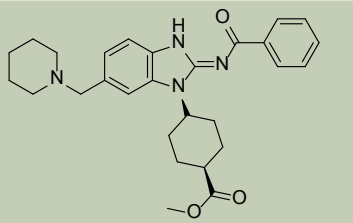
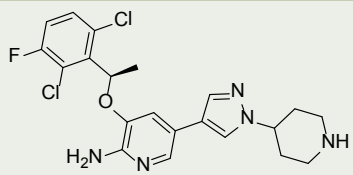
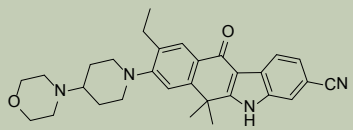
Molecular Structure	Ligand name	PDB ID (resolution)	Reference
	piperidine-carboxamide	4DCE (2.03Å)	(27)
	piperidine-carboxamide	4FNZ (2.60Å)	(26)
	PHA-E429	2XBA (1.95Å)	(22)
	NVP-TAE684	2XB7 (2.50Å)	(22)
	acyliminobenzimidazole inhibitor 36	4FOD (2.00Å)	(28)
	acyliminobenzimidazole inhibitor 1	4FOB (1.90Å)	(28)
	acyliminobenzimidazole inhibitor 2	4FOC (1.70Å)	(28)
	crizotinib	2XP2(1.90Å)	(29)
	CH5424802	3AOX(1.75Å)	(30)

Table 2 List of resolved structures of mutated ALK receptor

Mutation	PDB ID	Reference
F1174L	2YJR (1.90Å)	McTigue M, 2012, unpublished data
F1174L catalytic domain	4FNW (1.75Å)	(26)
C1156Y	2YJS	McTigue M, 2012, unpublished data
L1196M	2YHV	McTigue M, 2012, unpublished data
L1196M+crizotinib	2YFX	McTigue M, 2012, unpublished data
R1275Q catalytic domain	4FNX (1.70Å)	(26)
R1275Q cd + benzoxazole	4FNY (2.45Å)	(26)

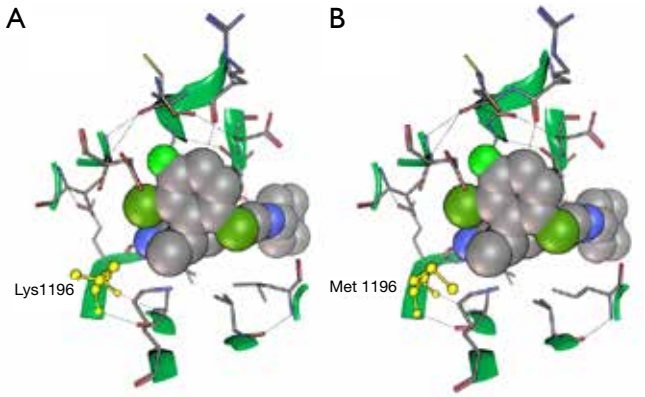


Figure 2 Molecular structure of crizotinib co-crystalized with (A) ALK^{wt} receptor, PDB ID: 2XP2; and (B) ALK^{L1196M} mutant, PDB ID: 2YFX. Although Lys-Met exchange the binding mode of crizotinib remains unchanged. Structures analysis has been performed and rendered using MOE software (48).

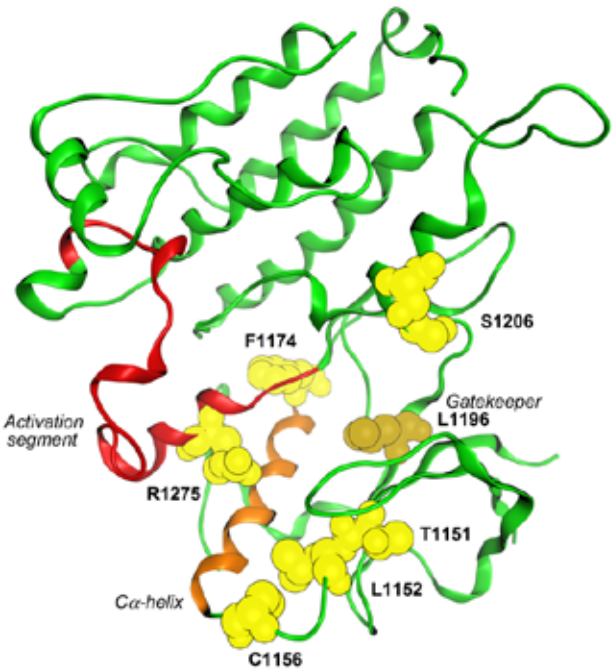


Figure 3 Graphical representation of key mutations related with ALK resistance.

mutations can be related with formation or alteration of hydrogen bonds (e.g., L1152R and C1156Y mutations) (53) or conformational changes (e.g., F1174L induces structural changes leading to an increment of ATP affinity which requires of irreversible inhibitors to block it) (35).

Thus, ligand co-crystallizations can not only help to the understanding of the structural basis of mutations related to drug resistance, but also to identify the binding mode of the second generation ALK inhibitors (30). When no crystallographic data is available, binding mode of new inhibitors with ALK mutants are computationally modeled using docking techniques (54) or molecular dynamic simulations (55). These interaction models provide a useful tool to screen drug candidates (after their validation with experimental evidences).

However, specificity for one receptor could be undesirable in a so complex biological context. For this reason, promiscuous inhibitors (e.g., dual inhibitors) have become gradually more interesting. When a set or library of knowing active ligands for one target is available, the specificity for the second target can be predicted by screening the previous library applying either SBDD or LBDD (56). In this respect, the ALK inhibitor TAE684 has been experimentally evaluated on ROS1, given the high homology between ALK and ROS1 human receptors (57-59).

Unfortunately, tridimensional structure of ROS1 receptor is not available and studies of ligand-receptor interactions have been always performed using ROS1 homology models using IGF-1R (60) or ALK itself (14). Homology models can be later used to study molecular interactions with ligands using docking methods in order to

elucidate their binding mode (60).

In contrast to homology models, other studies consider the structurally related MET receptor as a reference to find inhibitors which are able to interact with kinase domain. Given the receptor, homology docking studies on MET receptors could be therefore extended to structurally similar receptors like ROS1 (61). In fact, ALK inhibitors can also be used on ROS1 according to their similarity (62).

ALK and ROS1 small molecule inhibitors under development

In the last ten years a lot of efforts have been devoted to the development of compounds active against the ALK and ROS1 kinases. The principal reported inhibitors, the companies involved in the development, the core scaffold present in such compounds, the mutation against which the compounds are effective, their off-targets, and the clinical stage achieved are summarized in *Table 3*. On the other hand, the structures of the disclosed compounds are included in *Figure 4*.

ALK inhibition

One of the first discovered ALK inhibitors was NVP-TAE684, a small molecule with a dianilinopyridine scaffold which targets competitively the ATP in its binding site. Firstly, it was showed that NVP-TAE684 could block growth in cell lines and in a mouse model of ALCL (17). Besides to inhibit the proliferation of neuroblastoma and NSCLC cell lines, this ALK inhibitor reduced tumors expressing variants of ALK or EML4-ALK fusion proteins, confirming the oncogenic activity of the fusion kinase and consequently the therapeutic potential of targeted inhibitors (62,63). Despite several studies have reported the effectiveness of NVP-TAE684 against tumors induced by constitutively active ALK or against cell lines with ALK translocations and point mutations, this compound is not currently in any clinical trial (64-68).

Crizotinib (PF-02341066), which was originally developed to inhibit hepatocyte growth factor receptor (c-MET) but a few time later showed inhibitory activity against ALK, is an ATP-competitive small molecule like NVP-TAE684 (69,70). Crizotinib, with an aminopyridine as a core, was described for first time in 2007 and only three years later were reported the first promising clinical trials in NSCLC patients with EML4-ALK fusion genes (5). Phase I study concluded the benefits of the treatment with crizotinib of patients with advanced EML4-ALK-positive NSCLC. Among the 119 patients enrolled, after the treatment with crizotinib, 2 displayed a total recovery, 69 had a partial response and 31 were considered to have stable disease (71). The result of this inhibitory activity and the response of patients to the treatment with crizotinib, motivated its FDA approval under the trade name of Xalkori® (Pfizer). A Subsequent study in phase III trials reported crizotinib resistance in EML4-ALK positive NSCLC patients with some ALK mutations, especially secondary mutations, indicating a 64% overall survival in response to crizotinib treatment of EML4-ALK positive NSCLC patients after two years (49,53,72-74).

After the clinical trials of crizotinib, ALK was established as a drug target in cases of NSCLC, but the discovery of resistance related to mutations created the need to develop a second-generation of ALK inhibitors with the capability to overcome mutation-mediated drug resistance. Some pharmaceutical companies and research groups have reported different new promising candidate drugs to inhibit mutated ALK.

One of these second-generation ALK inhibitors is AP26113, whose chemical structure has not been disclosed but it is believed to be a member of a family compounds described in a patent of ARIAD based on a diaminopyrimidine structure bearing pendant phosphinoyl groups (75). This product is a multikinase inhibitor which shows more selectivity and inhibitory potency against ALK (IC_{50} =0.62 nM) than crizotinib (IC_{50} =3.6 nM). Apart from showing growth inhibition against EML4-ALK and nucleophosmin-ALK fusion gene (NPM-ALK) positive cells, the most interesting feature of AP26113 is that could inhibit some EML4-ALK mutated forms, therefore it could be a useful alternative in cases of crizotinib resistance. Currently, AP26113 is in phase I/II clinical trials (67,76-78).

In order to inhibit ALK mutations, another family of compounds was developed by Xcovery including X-276, X-376 and X-396 (79,80). There is not so much information in the literature about X-276 but it is considered a more selective and potent ALK inhibitor than crizotinib (81). X-376 and X-396 are members of the same family based on aminopyridazine scaffold, despite X-396 showed better results than X-376. X-396 was approximately 10-fold more potent than crizotinib in front of H3122 (EML4-ALK positive E13; A20 NSCLC), H2228 (EML4-ALK positive E6a/b; A20 NSCLC), SU-DHL-1 (NPM-ALK positive), SY5Y (ALK^{F1174L}) cell lines. As an example, in H3122 cell line, X-396 showed an IC_{50} value of 15 nM H3122

Table 3 List of ALK inhibitors under development

Chemical scaffold	Therapeutics	Company	ALK activity	ALK secondary mutations	Other targets	Clinical stage
Aminopyridine	Crizotinib PF-02341066 Xalkori®	Pfizer	24 nM	--	c-MET ROS1	FDA approved
Dianilinopyridine	NVP-TAE684	Novartis	<10 nM	L1196M F1174L	IR, IGF-1R, FLT3, TIE2 LRRK2, ROS1	Not a clinical candidate
Structure undisclosed	AP26113	ARIAD	0.62 nM	L1196M F1174C I1171T F1245C E1210K S1206R G1269S	Multiple kinases ROS1	Phase I/II
Structure undisclosed	X-276	Xcovery	--	--	--	preclinical
Aminopyridazine	X-376	Xcovery	--	--	--	preclinical
Structure undisclosed aminopyridazine	X-396	Xcovery	<0.4 nM	L1196M C1156Y	--	Phase I
Tetracyclic indole	CH5424802 AF-802	Chugai	1.9 nM	L1196M F1174L R1275Q C1156Y	GAK, LTK	Phase I/II
Pyrrolopyrimidine	GSK1838705A	GlaxoSmithKline	0.5 nM	--	IR	Preclinical
Triazinediamine	ASP3026	Astellas	--	--	ROS1	Phase I
Indolocarbazole	CEP-14083	Cephalon	2 nM	--	IR, VEGFR2, TIE2, DLK	Not a clinical candidate
Indolocarbazole	CEP-14513	Cephalon	4 nM	--	IR, VEGFR2, TIE2, DLK	Not a clinical candidate
Tetrahydropyrazine	--	Cephalon	10 nM	--	--	preclinical
Diaminopyridine	CEP-28122	Cephalon	1.9 nM	F1174L R1275Q	--	Phase I
Structure undisclosed	CEP-37440	Cephalon/Teva		--	--	Phase I
Pyrrolotriazine	Compound 32	Cephalon	6 nM	--	--	preclinical
Tetraazatetracyclo-docosanonaene	Macrocycle 2m	Cephalon	0.5 nM	--	--	preclinical
Pyrrolopyrazole	PHA-E429	Nerviano Medical	91 nM	--	Mutiple kinases	preclinical
Indazole	NMS-E628	Nerviano Medical	55 nM	L1196M C1156Y	IGF-1R, Aurora B	preclinical
Structure undisclosed pyridone	CRL151104A	Chembridge St Jude	9.75 nM	F1174L R1275Q	--	preclinical
Pyridone	Pyridone 1	Chembridge St Jude	380 nM	--	--	preclinical
Structure undisclosed	WZ-5-126	--	3.4 nM	--	--	preclinical
Structure undisclosed	LDK378	Novartis	0.15 nM	--	ROS1	Phase I
2,4-pyrimidinediamine	3-39	Novartis	--	--	--	preclinical
Pyridoisoquinoline	F91873 and F91874	Institut de Recherche Pierre Fabre	--	--	Multiple kinases	preclinical
Structure undisclosed	TSR-011	Tesaro	--	--	--	preclinical

c-MET, hepatocyte growth factor receptor; IR, insulin receptor; IGF-1R, insulin-like growth factor 1 receptor; FLT3, FMS-like tyrosine kinase 3; TIE2, angiopoietin-1 receptor; LRRK2, leucine-rich repeat kinase 2; GAK, cyclin G-associated kinase; LTK, leukocyte tyrosine kinase; VEGFR2, vascular endothelial growth factor receptor 2; DLK, dual leucine zipper kinase.

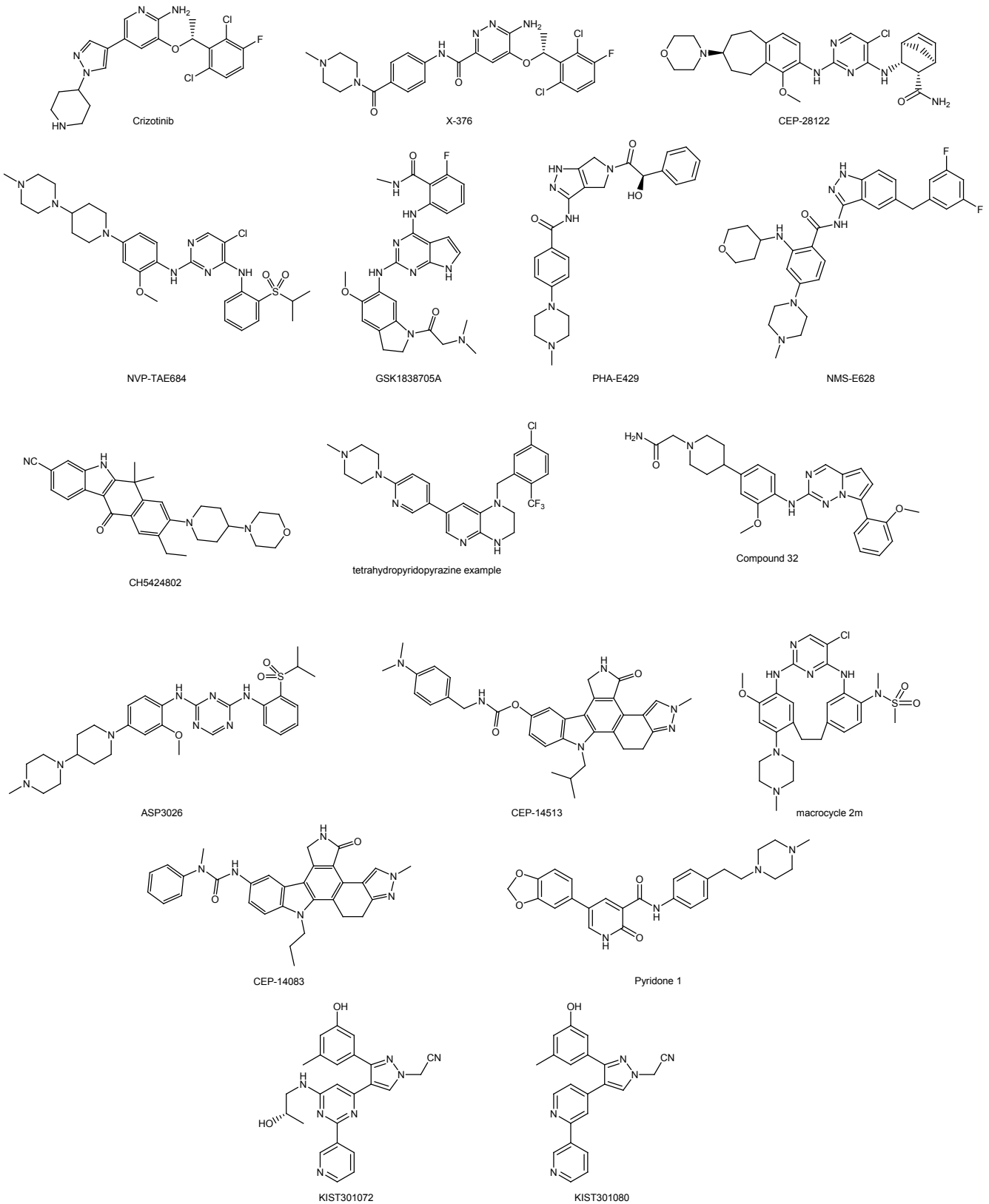


Figure 4 Structures of reported ALK and ROS1 inhibitors.

(EML4-ALK positive NSCLC) cell lines against 180 nM of crizotinib. Furthermore X-396 displayed good inhibition in crizotinib resistant cell lines (Ba/F3-EML4-ALKL1196M, IC_{50} =106 nM; Ba/F3-EML4-ALK^{C1156Y}, IC_{50} =48 nM). Thus, X-396 initiated phase I clinical trials in June 2012 (82).

CH5424802, also known as AF-802, is a tetracyclic indole which has a high ALK^{wt} inhibitory activity in *in vitro* assays (IC_{50} =1.9 nM) as well in front of mutated ALKs, such as ALK^{L1196M} (K_i =1.56 nM), ALK^{F1174L} (IC_{50} =1.0 nM) and ALK^{R1275Q} (IC_{50} =3.5 nM). These results were also reproduced *in vivo* with the treatment in different cell lines: H2228 (EML4-ALK positive E6a/b;A20; IC_{50} =53 nM), KARPAS-299 (NPM-ALK positive ALCL; IC_{50} =3.0 nM), SR-786 (IC_{50}=6.9 nM), NB-1 (ALK amp, IC_{50} =4.5 nM); KELLY (IC_{50} =62 nM) and Ba/F3 (EML4-ALK^{L1196M}) that allowed to show that CH5424802 is a potent inhibitor for a therapy with capacity to overcome the acquired resistance to crizotinib (30,41,83). Due to these promising results, CH5424802 is in phase I/II of clinical trials.

GSK1838705A, developed by GlaxoSmithKline and currently in preclinical phase, contains a pyrrolopyrimidine scaffold and has showed to be selective IGF-1R, insulin receptor (IR) and ALK inhibitor (IC_{50} =1.2, 2 and 0.5 nM respectively). Furthermore, the inhibition of the proliferation of different ALCL cell lines, such as L-82 (IC_{50} =24 nM), SUP-M2 (IC_{50} =28 nM), SU-DHL-1 (IC_{50} =31 nM), Karpas-299 (IC_{50} =52 nM) and SR-786 (IC_{50} =88 nM), has also been described. Besides the potent inhibition of ALK by GSK1838705A, such compound also inhibit cell lines harboring ALK fusion genes in different ALCL cell lines expressing NPM-ALK (EC_{50} =24-88 nM) and in H2228 NSCLC cells expressing EML4-ALK (IC_{50} =191 nM). In addition, it was proved that GSK1838705A inhibits the EML4-ALK phosphorylation (84,85).

There are not so many details about ASP3026, a triazinediamine developed by Astellas which is in Phase I in clinical trials. This compound showed potent and selective activity against EML4-ALK driven tumors with gatekeeper mutation, therefore it is able to overcome crizotinib resistance (86).

Based on the structure of two natural products, staurosporine and 7-hydroxystaurosporine which are able to inhibit ALK (IC_{50} =150 nM and 5 μM respectively in the presence of 30 μM ATP in an ELISA-based ALK assay) (87), Cephalon developed some compounds targeted to inhibit ALK. CEP-14083 and CEP-14513 have showed ATP-competitive activity in ALK, displaying IC_{50} values in enzymatic assays of 2 and 4 nM, respectively, and 10-30 nM in cellular assays (88). The capability of CEP-14083 to inhibit cell lines and animal models harboring ALK alterations was also described (89). Furthermore, CEP-14083 is able to inhibit other kinases, such as IR, vascular endothelial growth factor receptor 2 (VEGFR2), angiopoietin-1 receptor (TIE2), and dual leucine zipper kinase (DLK) but, due to unfavorable physicochemical properties, CEP-14083 and CEP-14513 were discarded for *in vivo* studies (88). Then, Cephalon developed a second generation of ALK inhibitors with different scaffolds, some of them being currently in preclinical studies. Thus, they reported tetrahydropyrazines with IC_{50} around 10 nM and 150 nM in enzyme and in Karpas-299 cell line, respectively (34). They also described 2,4-diaminopyrimidines, the most representative compound is CEP-28122, showing a high selectivity (600-fold with respect to IR, a closely related kinase family member) and a high potency (IC_{50} =1.9 and 20 nM in enzyme and in Karpas-299 cell line, respectively). CEP-28122 also induced growth inhibition in NPM-ALK positive ALCL and EML4-ALK positive NSCLC tumor xenografts in mice. In addition, inhibited growth of neuroblastoma cell lines harboring ALK activating mutants, such as F1174L in NB-1643 cells and R1275Q in SH-SY5Y cells, but not in cell lines which expresses ALK^{wt} (SKNAS cells) (32,37,90). Cephalon also described pyrrolotriazines among which compound 32 displayed an IC_{50} =6 nM in an enzymatic assay and 100 nM against NPM-ALK cells, showing high selectivity in a test against 256 kinases (39). Finally, they reported tetraazatetracyclo docosanonaene macrocycles, Macrocycle 2m showing a high inhibitory potency *in vitro* (IC_{50} =0.5 nM) as well as in a cell-based assay (IC_{50} =10 nM) and high selectivity (173-fold with respect to IR) (36).

PHA-E429 is a compound developed by Nerviano with a pyrrolopyrazole scaffold which is considered as a multikinase inhibitor that also is able to inhibit ALK (IC_{50} =91 nM) (51,91). NMS-E628, an indazole compound, developed by the same company, inhibits ALK (IC_{50} =55 nM against NMP-ALK cells) but also IGF-1R and Aurora B (92). NMS-E628 has showed a high efficiency inhibiting the growth of ALCL cells and NSCLC cells bearing EML4-ALK (H2228) rearrangement and in addition was able to overcome the L1196M and C1156Y mediated TKI resistance (93,94).

CRL151104A, developed by ChemBridge Research Laboratories and St Jude Children's Research Hospital, whose structure is not available, is a third-generation ATP

competitor pyridine compound with capacity to block the cellular phosphorylation. This compound showed a high activity against ALK *in vitro* (IC_{50} =9.75 nM) and also in an *in vivo* assay (IC_{50} ≤100 nM and 2.5 μM against NPM-ALK positive and NPM-ALK negative cells, respectively). CRL151104A has also demonstrated the capability to overcome F1174L and R1275Q ALK mutations in neuroblastoma cell lines (95). There is not so much information about a pyridone compound developed by ChemBridge Research Laboratories and St Jude Children's Research Hospital which is able to inhibit ALK in an enzymatic assay (IC_{50} =380 nM) and in cell-based assays (IC_{50} =18.0 μM and 750 nM against BaF3/NPM-ALK and Karpas-299/NPM-ALK ALCL cell lines, respectively) (33).

WZ-5-126 is a potent ALK small molecule inhibitor with an IC_{50} of 3.4 nM *in vitro*, capable of inhibiting the growth of two ALK positive NSCLC cell lines (62).

Novartis has also been involved in the research of ALK inhibitors such as LDK-378, a potent and selective candidate (IC_{50} =0.15 nM). This compound, in Phase I trials nowadays, is able to inhibit the growth of genetically abnormal ALK-driven NSCLC tumors (96). Furthermore, compound 3-39 described in a Novartis patent, including a 2,4-pyrimidinediamine scaffold, showed brilliants results against EML4-ALK transgenic mouse models (63,97).

On the other hand, F91873 and F91874 are pyridoisoquinolines with multikinase inhibitory activity described by Institut de Recherche Pierre Fabre which are able to inhibit ALK and probably behave as non-ATP competitors (98).

Another ALK inhibitor in preclinical development is TSR-011, developed by Tesaro, but unfortunately the information about it is very scarce (99).

Finally, some other compounds have been reported that are not able to directly inhibit ALK but are able to inhibit the tumor growth in ALK-driven NSCLC models, such as EML4-ALK^{L1196M}, therefore being capable to overcome crizotinib resistance. Two examples of such compounds are the Hsp90 inhibitors retaspimycin (IPI-504) and ganetespib (STA-9090) (67,100-102).

ROS1 inhibitors

Treatments targeting EGFR, in cases of NSCLC patients in which mutant ROS1 kinase is expressed, might be partially or totally ineffective. The lack of knowledge about ROS1 renders important to discover selective compounds in order to confirm theoretical speculations (103).

Some kinase inhibitors were assayed against ROS1 and one of them, staurosporine, showed high inhibitory activity (IC_{50} =0.9 nM) (61). On the other hand, several heterocyclic compounds, such as AST-487, PP 2, AG 1487, PDGFR I-III and D-64406, showed moderate to low inhibitory activities (IC_{50} =1,700, 5,200, 13,600, 48,000 and 365,000 nM respectively) (103-105).

Due to the high homology between the kinase domains of ROS1 and ALK, some ALK inhibitors were assayed against ROS1-driven cells and tumors. NVP-TAE684 showed *in vitro* activity against HCC78 cell lines expressing ROS1 and inhibition of signaling downstream of ROS1 inducing apoptosis in BaF3/FIG-ROS positive cells (IC_{50} =10 nM) (57,79). Furthermore, a computational study of NVP-TAE684 showed its higher affinity for the ROS1 kinase domain with respect to the ALK kinase domain (14).

Apart from being the first ALK inhibitor approved by FDA, crizotinib is also able to inhibit ROS1 with an IC_{50} value of 1.7 nM in an *in vitro* assay. This high inhibition in an enzymatic-based assay was not confirmed in a cell-based assay (IC_{50} =1.4 μM against the HCC78 ROS1-rearranged NSCLC cell line) (79). The inhibition of the ROS1 phosphorylation by crizotinib in the HCC78 cell line in a moderate manner was also described (106). Although only 2% of NSCLC cases harbor ROS1 rearrangements, it is described the case of a patient having a ROS1 positive tumor (without EGFR mutations nor ALK rearrangements) who did not responded to erlotinib treatment (an EGFR inhibitor) but was totally recovered in 12 weeks after crizotinib treatment. This case is the proof that ROS1 is a prime target for the development of new inhibitors for the treatment of NSCLC (11).

Some other ALK inhibitors were assayed against ROS1 with promising results. Thus AP26113 (IC_{50} =1.9 nM) (67) and WZ-5-126 (IC_{50} =8.2 nM) (51), together with crizotinib and ASP3026, have entered clinical trials (59).

Finally, there are only two pyrazole ROS1 selective compounds (KIST301072 and KIST301080), which showed good ROS1 inhibitory activity (IC_{50} =199 and 209 nM, respectively) when tested against 45 kinases (60,106,107).

Conclusions

Traditionally, depending on the type of tumor, therapeutic approaches include different combinations of surgery, radiation therapy, and chemotherapy. However, alternative therapies using receptor tyrosine kinase (RTK) as targets started to be introduced in the beginning of this century. In

the case of lung cancer, such approach has led to discovery of a number of these targeted therapies with specific inhibitor drugs such as erlotinib and gefitinib for mutations in the epidermal growth factor receptor (EGFR). However, in a 4-7% of the of lung adenocarcinomas these treatments are ineffective due to the presence of ALK and/or ROS1 rearrangements. This problem has been partially overcome due to the development of crizotinib (Xalkori®, Pfizer) but the discovery of some crizotinib resistant ALK mutations has forced the research of new inhibitors. Furthermore, the implication of ROS1 protooncogene in this kind of tumors has rendered the situation even more complicated. The present review has tried to show the importance of ALK and ROS1 as combined targets for the development of new treatments for non-small cell lung cancer (NSCLC) when the most common approaches fail and the efforts carried out in this field to date.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10-29.
2. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92-8.
3. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
4. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
5. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
6. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
7. Zhang X, Zhang S, Yang X, et al. Fusion of EML4 and ALK is associated with development of lung adenocarcinomas lacking EGFR and KRAS mutations and is correlated with ALK expression. Mol Cancer 2010;9:188.
8. Wong DW, Leung EL, So KK, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. Cancer 2009;115:1723-33.
9. Takahashi T, Sonobe M, Kobayashi M, et al. Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. Ann Surg Oncol 2010;17:889-97.
10. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190-203.
11. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-70.
12. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med 2012;18:378-81.
13. Jun HJ, Johnson H, Bronson RT, et al. The oncogenic lung cancer fusion kinase CD74-ROS activates a novel invasiveness pathway through E-Syt1 phosphorylation. Cancer Res 2012;72:3764-74.
14. Ou SH, Tan J, Yen Y, et al. ROS1 as a 'druggable' receptor tyrosine kinase:lessons learned from inhibiting the ALK pathway. Expert Rev Anticancer Ther 2012;12:447-56.
15. Shaw AT, Camidge DR, Engelman JA, et al. Clinical activity of crizotinib in advanced non-small cell lung cancer (NSCLC) harboring ROS1 gene rearrangement. J Clin Oncol 2012;30:abstr 7508.
16. Gunby RH, Ahmed S, Sottocornola R, et al. Structural Insights into the ATP Binding Pocket of the Anaplastic Lymphoma Kinase by Site-Directed Mutagenesis, Inhibitor Binding Analysis, and Homology Modeling. J Med Chem 2006;49:5759-68.
17. Galkin AV, Melnick JS, Kim S, et al. Identification of NVP-TAE684, a potent, selective, and efficacious inhibitor of NPM-ALK. Proc Natl Acad Sci U S A 2007;104:270-5.
18. Okamoto M, Kojima H, Saito N, et al. Virtual screening and further development of novel ALK inhibitors. Bioorg Med Chem 2011;19:3086-95.
19. Ott GR, Wells GJ, Thieu TV, et al. 2,7-disubstituted-pyrrolo[2,1-f][1,2,4]triazines:new variant of an old template and application to the discovery of anaplastic lymphoma kinase (ALK) inhibitors with in vivo antitumor activity. J Med Chem 2011;54:6328-41.
20. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. Nucleic Acids Res 2000;28:235-42.
21. Lee CC, Jia Y, Li N, et al. Crystal structure of the ALK (anaplastic lymphoma kinase) catalytic domain. Biochem J 2010;430:425-37.

22. Bossi RT, Saccardo MB, Ardini E, et al. Crystal Structures of Anaplastic Lymphoma Kinase in Complex with ATP Competitive Inhibitors. Biochemistry 2010;49:6813-25.
23. Park H, Chi O, Kim J, et al. Identification of novel inhibitors of tropomyosin-related kinase A through the structure-based virtual screening with homology-modeled protein structure. J Chem Inf Model 2011;51:2986-93.
24. Donella-Deana A, Marin O, Cesaro L, et al. Unique substrate specificity of anaplastic lymphoma kinase (ALK):development of phosphoacceptor peptides for the assay of ALK activity. Biochemistry 2005;44:8533-42.
25. Tartari CJ, Gunby RH, Coluccia AM, et al. Characterization of some molecular mechanisms governing autoactivation of the catalytic domain of the anaplastic lymphoma kinase. J Biol Chem 2008;283:3743-50.
26. Epstein LF, Chen H, Emkey R, et al. The R1275Q neuroblastoma mutant and certain ATP-competitive inhibitors stabilize alternative activation loop conformations of anaplastic lymphoma kinase. J Biol Chem 2012;287:37447-57.
27. Bryan MC, Whittington DA, Doherty EM, et al. Rapid development of piperidine carboxamides as potent and selective anaplastic lymphoma kinase inhibitors. J Med Chem 2012;55:1698-705.
28. Lewis RT, Bode CM, Choquette DM, et al. The discovery and optimization of a novel class of potent, selective, and orally bioavailable anaplastic lymphoma kinase (ALK) inhibitors with potential utility for the treatment of cancer. J Med Chem 2012;55:6523-40.
29. Cui JJ, Tran-Dubé M, Shen H, et al. Structure Based Drug Design of Crizotinib (PF-02341066), a Potent and Selective Dual Inhibitor of MesenchymalEpithelial Transition Factor (c-MET) Kinase and Anaplastic Lymphoma Kinase (ALK). J Med Chem 2011;54:6342-63.
30. Sakamoto H, Tsukaguchi T, Hiroshima S, et al. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. Cancer Cell 2011;19:679-90.
31. Baskaran C, Ramachandran M. Computational molecular docking studies on anticancer drugs. Asian Pac Trop Dis 2012;2:S734-S738.
32. Gingrich DE, Lisko JG, Curry MA, et al. Discovery of an orally efficacious inhibitor of anaplastic lymphoma kinase. J Med Chem 2012;55:4580-93.
33. Li R, Xue L, Zhu T, et al. Design and synthesis of 5-aryl-pyridone-carboxamides as inhibitors of anaplastic lymphoma kinase. J Med Chem 2006;49:1006-15.
34. Milkiewicz KL, Weinberg LR, Albom MS, et al. Synthesis and structure-activity relationships of 1,2,3,4-tetrahydropyrido[2,3-b]pyrazines as potent and selective inhibitors of the anaplastic lymphoma kinase. Bioorg Med Chem 2010;18:4351-62.
35. Ardini E, Galvani A. ALK Inhibitors, a Pharmaceutical Perspective. Front Oncol 2012;2:17.
36. Breslin HJ, Lane BM, Ott GR, et al. Design, synthesis, and anaplastic lymphoma kinase (ALK) inhibitory activity for a novel series of 2,4,8,22-te traazatetracyclo[14.3.1.13,7.19,13]docosa-1(20),3(22),4,6,9(21),10,12,16,18-nonaene macrocycles. J Med Chem 2012;55:449-64.
37. Ott GR, Tripathy R, Cheng M, et al. Discovery of a Potent Inhibitor of Anaplastic Lymphoma Kinase with in Vivo Antitumor Activity. ACS Med Chem Lett 2010;1:493-8.
38. Mesaros EF, Burke JP, Parrish JD, et al. Novel 2,3,4,5-tetrahydro-benzo[d]azepine derivatives of 2,4-diaminopyrimidine, selective and orally bioavailable ALK inhibitors with antitumor efficacy in ALCL mouse models. Bioorg Med Chem Lett 2011;21:463-6.
39. Mesaros EF, Thieu TV, Wells GJ, et al. Strategies to mitigate the bioactivation of 2-anilino-7-aryl-pyrrolo[2,1-f] [1,2,4]triazines:identification of orally bioavailable, efficacious ALK inhibitors. J Med Chem 2012;55:115-25.
40. Zificsak CA, Theroff JP, Aimone LD, et al. Methanesulfonamido-cyclohexylamine derivatives of 2,4-diaminopyrimidine as potent ALK inhibitors. Bioorg Med Chem Lett 2011;21:3877-80.
41. inoshita K, Ono Y, Emura T, et al. Discovery of novel tetracyclic compounds as anaplastic lymphoma kinase inhibitors. Bioorg Med Chem Lett 2011;21:3788-93.
42. Kinoshita K, Asoh K, Furuichi N, et al. Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802). Bioorg Med Chem 2012;20:1271-80.
43. Deng X, Wang J, Zhang J, et al. Discovery of 3,5-Diamino-1,2,4-triazole Ureas as Potent Anaplastic Lymphoma Kinase Inhibitors. ACS Med Chem Lett 2011;2:379-84.
44. Slavish PJ, Price JE, Jiang Q, et al. Synthesis of an aryloxy oxo pyrimidinone library that displays ALK-selective inhibition. Bioorg Med Chem Lett 2011;21:4592-6.
45. Xie HZ, Lan H, Pan YL, et al. Identification of novel anaplastic lymphoma kinase (ALK) inhibitors using a common feature pharmacophore model derived from known ligands crystallized with ALK. Chem Biol Drug Des 2013;81:175-84.
46. Bresler SC, Wood AC, Haglund EA, et al. Differential inhibitor sensitivity of anaplastic lymphoma kinase variants

found in neuroblastoma. Sci Transl Med 2011;3:108ra114.
47. Shiao H, Chiang NJ, Hsieh HP. Anaplastic Lymphoma Kinase (ALK) Inhibitors: New Cancer Breakthroughs for Lung Cancer. J Cancer Res Pract 2011;27:143-56.
48. Chemical Computing Group Inc.; Molecular Operating Environment (MOE), 2012.10;1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2012.
49. Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734-9.
50. Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. Sci Transl Med 2012;4:120ra17.
51. Bossi RT, Saccardo MB, Ardini E, et al. Crystal structures of anaplastic lymphoma kinase in complex with ATP competitive inhibitors. Biochemistry 2010;49:6813-25.
52. Katayama R, Khan TM, Benes C, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proc Natl Acad Sci U S A 2011;108:7535-40.
53. Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res 2011;71:6051-60.
54. Lu L, Ghose AK, Quail MR, et al. ALK mutants in the kinase domain exhibit altered kinase activity and differential sensitivity to small molecule ALK inhibitors. Biochemistry 2009;48:3600-9.
55. Sun HY, Ji FQ. A molecular dynamics investigation on the crizotinib resistance mechanism of C1156Y mutation in ALK. Biochem Biophys Res Commun 2012;423:319-24.
56. Tripathy R, McHugh RJ, Ghose AK, et al. Pyrazolone-based anaplastic lymphoma kinase (ALK) inhibitors:control of selectivity by a benzyloxy group. Bioorg Med Chem Lett 2011;21:7261-4.
57. Gu TL, Deng X, Huang F, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. PLoS One 2011;6:e15640.
58. Doebele RC, Camidge DR. Targeting ALK, ROS1, and BRAF kinases. J Thorac Oncol 2012;7:S375-S376.
59. Chin LP, Soo RA, Soong R, et al. Targeting ROS1 with anaplastic lymphoma kinase inhibitors:a promising therapeutic strategy for a newly defined molecular subset of non-small-cell lung cancer. J Thorac Oncol 2012;7:1625-30.
60. El-Deeb IM, Park BS, Jung SJ, et al. Design, synthesis, screening, and molecular modeling study of a new series of ROS1 receptor tyrosine kinase inhibitors. Bioorg Med Chem Lett 2009;19:5622-6.
61. Peach ML, Tan N, Choyke SJ, et al. Directed discovery of agents targeting the Met tyrosine kinase domain by virtual screening. J Med Chem 2009;52:943-51.
62. McDermott U, Iafrate AJ, Gray NS, et al. Genomic Alterations of Anaplastic Lymphoma Kinase May Sensitize Tumors to Anaplastic Lymphoma Kinase Inhibitors. Cancer Res 2008;68:3389-95.
63. Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci U S A 2008;105:19893-7.
64. Wang YW, Tu P, Lin K, et al. Identification of oncogenic point mutations and hyperphosphorylation of anaplastic lymphoma kinase in lung cancer. Neoplasia 2011;13:704-15.
65. George RE, Sanda T, Hanna M, et al. Activating mutations in ALK provide a therapeutic target in neuroblastoma. Nature 2008;455:975-8.
66. Cerchietti L, Damm-Welk C, Vater I, et al. Inhibition of anaplastic lymphoma kinase (ALK) activity provides a therapeutic approach for CLTC-ALK-positive human diffuse large B cell lymphomas. PLoS One 2011;6:e18436.
67. Katayama R, Khan TM, Benes C, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proc Natl Acad Sci U S A 2011;108:7535-40.
68. Duijkers FA, Gaal J, Meijerink JP, et al. Anaplastic lymphoma kinase (ALK) inhibitor response in neuroblastoma is highly correlated with ALK mutation status, ALK mRNA and protein levels. Cell Oncol (Dordr) 2011;34:409-17.
69. Christensen JG, Zou HY, Arango ME, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. Mol Cancer Ther 2007;6:3314-22.
70. Zou HY, Li Q, Lee JH, et al. An Orally Available Small-Molecule Inhibitor of c-Met, PF-2341066, Exhibits Cytoreductive Antitumor Efficacy through Antiproliferative and Antiangiogenic Mechanisms. Cancer Res 2007;67:4408-17.
71. Camidge DR, Bang Y, Kwak EL, et al. Progression-free survival (PFS) from a phase I study of crizotinib (PF-02341066) in patients with ALK-positive non-small cell lung cancer (NSCLC). J Clin Oncol 2011;29:abstr 2501.
72. Sasaki T, Okuda K, Zheng W, et al. The neuroblastoma-associated F1174L ALK mutation causes resistance to an ALK kinase inhibitor in ALK-translocated cancers. Cancer Res 2010;70:10038-43.
73. Sasaki T, Rodig SJ, Chirieac LR, et al. The biology and

treatment of EML4-ALK non-small cell lung cancer. Eur J Cancer 2010;46:1773-80.
74. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement:a retrospective analysis. Lancet Oncol 2011;12:1004-12.
75. Wang Y, Huang W, Liu S, et al. inventors. Ariad Pharmaceuticals I, USA, assignee. Preparation of phosphorus derivatives as kinase inhibitors. WO2009143389. Patent Application Date: May 21, 2009.
76. Shakespeare WC, Fantin VR, Wang F, et al. Discovery of potent and selective orally active inhibitors of anaplastic lymphoma kinase (ALK). American Association for cancer research annual meeting (AACR) 2009;abstr 3738.
77. Rivera VM, Anjum R, Wang F, et al. Efficacy and pharmacodynamic analysis of AP26113, a potent and selective orally active inhibitor of anaplastic lymphoma kinase (ALK). Cancer Res 2010;70:abstr 3623.
78. Zhang S, Wang F, Keats J, et al. AP26113, a potent ALK inhibitor, overcomes mutations in EML4-ALK that confer resistance to PF-02341066 (PF1066). Cancer Res 2010;70:abstr LB-298.
79. Lovly CM, Heuckmann JM, de Stanchina E, et al. Insights into ALK-Driven Cancers Revealed through Development of Novel ALK Tyrosine Kinase Inhibitors. Cancer Res 2011;71:4920-31.
80. Liang C, Li Z. inventors. Xcovery I, USA, assignee. Substituted pyridazine carboxamides as kinase inhibitors. WO2009154769. Patent Application Date: June 18, 2009.
81. Lovly CM, de Stanchina E, Liang C, et al. Preclinical development of a selective, potent small molecule ALK inhibitor. Cancer Res 2010;70:abstr 1788.
82. Xcovery. Targeted therapeutics. Available online: http://www.xcovery.com/alk (accessed Mar 13, 2013).
83. Kinoshita K, Kobayashi T, Asoh K, et al. 9-Substituted 6,6-Dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazoles as Highly Selective and Potent Anaplastic Lymphoma Kinase Inhibitors. J Med Chem 2011;54:6286-94.
84. Sabbatini P, Korenchuk S, Rowand JL, et al. GSK1838705A inhibits the insulin-like growth factor-1 receptor and anaplastic lymphoma kinase and shows antitumor activity in experimental models of human cancers. Mol Cancer Ther 2009;8:2811-20.
85. Chamberlain SD, Redman AM, Wilson JW, et al. Optimization of 4,6-bis-anilino-1H-pyrrolo[2,3-d] pyrimidine IGF-1R tyrosine kinase inhibitors towards JNK selectivity. Bioorg Med Chem Lett 2009;19:360-4.
86. Kuromitsu S, Mori M, Shimada I, et al. Antitumor activities of ASP3026 against EML4-ALK-dependent tumor models. Mol Cancer Ther 2011;10:abstr A227.
87. Gunby RH, Tartari CJ, Porchia F, et al. An enzyme-linked immunosorbent assay to screen for inhibitors of the oncogenic anaplastic lymphoma kinase. Haematologica 2005;90:988-90.
88. Wan W, Albom MS, Lu L, et al. Anaplastic lymphoma kinase activity is essential for the proliferation and survival of anaplastic large-cell lymphoma cells. Blood 2006;107:1617-23.
89. Ambrogio C, Martinengo C, Voena C, et al. NPM-ALK oncogenic tyrosine kinase controls T-Cell identity by transcriptional regulation and epigenetic silencing in lymphoma cells. Cancer Res 2009;69:8611-9.
90. Allwein SP, Roemmele RC, Haley JJ, et al. Development and Scale-Up of an Optimized Route to the ALK Inhibitor CEP-28122. Org Process Res Dev 2012;16:148-55.
91. Fancelli D, Moll J, Varasi M, et al. 1,4,5,6-Tetrahydropyrrolo[3,4-c]pyrazoles:Identification of a Potent Aurora Kinase Inhibitor with a Favorable Antitumor Kinase Inhibition Profile. J Med Chem 2006;49:7247-51.
92. Pulford K, Morris SW, Turturro F. Anaplastic lymphoma kinase proteins in growth control and cancer. J Cell Physiol 2004;199:330-58.
93. Ardini E, Menichincheri M, Banfi P, et al. In vitro and in vivo activity of NMS-E628 against ALK mutations resistant to Xalkori. Mol Cancer Ther 2011;10:abstr A232.
94. Ardini E, Menichincheri M, De Ponti C, et al. Characterization of NMS-E628, a small molecule inhibitor of anaplastic lymphoma kinase with antitumor efficacy in ALK-dependent lymphoma and non-small cell lung cancer models. Mol Cancer Ther 2009;8:abstr A243.
95. Webb TR, Slavish J, George RE, et al. Anaplastic lymphoma kinase:role in cancer pathogenesis and small-molecule inhibitor development for therapy. Expert Rev Anticancer Ther 2009;9:331-56.
96. Mehra R, Camidge DR, Sharma S, et al. First-in-human phase I study of the ALK inhibitor LDK378 in advanced solid tumors. J Clin Oncol 2012;30:abstr 3007.
97. Garcia-echeverria C, Kanazawa T, Kawahara E, et al. Inventors. Novartis A.-G. S, Novartis Pharma G.m.b.H. and IRM LLC, assignees. Preparation of 2,4-pyrimidinediamines useful in the treatment of neoplastic diseases, inflammatory and immune system disorders. WO2005016894. Patent Application Date: August 13, 2004.
98. Kruczynski A, Mayer P, Marchand A, et al. Antitumor

activity of pyridoisoquinoline derivatives F91873 and F91874, novel multikinase inhibitors with activity against the anaplastic lymphoma kinase. Anti-Cancer Drugs 2009;20:364-72.
99. Tesaro. Pipeline. Available online: http://tesarobio.com/pipeline/ (accessed Mar 13, 2013).
100. Sequist LV, Gettinger S, Senzer NN, et al. Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. J Clin Oncol 2010;28:4953-60.
101. Chen Z, Sasaki T, Tan X, et al. Inhibition of ALK, PI3K/MEK, and HSP90 in Murine Lung Adenocarcinoma Induced by EML4-ALK Fusion Oncogene. Cancer Res 2010;70:9827-36.
102. El-Hariry I, Proia D, Vukovic V. inventors. Synta Pharmaceuticals Corp. U, assignee. Treating cancer with heat-shock protein-90 (HSP90) inhibitory compounds such as ganetespib. WO2013006864. Patent Application Date:July 9, 2012.
103. El-Deeb IM, Yoo KH, Lee SH. ROS receptor tyrosine kinase:a new potential target for anticancer drugs. Med Res Rev 2010;31:794-818.
104. araman MW, Herrgard S, Treiber DK, et al. A quantitative analysis of kinase inhibitor selectivity. Nat Biotechnol 2008;26:127-32.
105. Banerjee P. Recognition of c-ROS oncogene inhibition with a FRET-based receptor construct. MURJ 2004;10:52-7.
106. Park BS, El-Deeb IM, Yoo KH, et al. Design, synthesis and biological evaluation of new potent and highly selective ROS1-tyrosine kinase inhibitor. Bioorg Med Chem Lett 2009;19:4720-3.
107. Park BS, El-deeb IM, Yoo KH, et al. Synthesis and biological activity of new 4-(pyridin-4-yl)-(3-methoxy-5-methylphenyl)-1H-pyrazoles derivatives as ROS receptor tyrosine kinase inhibitors. Bull Korean Chem Soc 2012;33:3629-34.

Cite this article as: Puig de la Bellacasa R, Karachaliou N, Estrada-Tejedor R, Teixidó J, Costa C, Borrell JI. ALK and ROS1 as a joint target for the treatment of lung cancer: a review. Transl Lung Cancer Res 2013;2(2):72-86. doi: 10.3978/j.issn.2218-6751.2013.03.11

Activated *RET* and *ROS*: two new driver mutations in lung adenocarcinoma

Marc Bos[1,2*], Masyar Gardizi[2,3*], Hans-Ulrich Schildhaus[2,3], Reinhard Buettner[2,3], Juergen Wolf[1,2]

[1]Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany; [2]Center for Integrated Oncology Cologne/Bonn, Germany; [3]Institute of Pathology, University Hospital Cologne, Cologne, Germany

[*]These authors contributed equally in this study.

Correspondence to: Professor Jürgen Wolf, M.D. Dep. I of Internal Medicine, University Hospital Cologne, Kerpener Straße 62, D-50937 Köln, Germany. Email: juergen.wolf@uk-koeln.de.

Abstract: Rearrangements of ROS1 and RET have been recently described as new driver mutations in lung adenocarcinoma with a frequency of about 1% each. RET and ROS1 rearrangements both represent unique molecular subsets of lung adenocarcinoma with virtually no overlap with other known driver mutations described so far in lung adenocarcinoma. Specific clinicopathologic characteristics have been described and several multitargeted receptor kinase inhibitors have shown *in vitro* activity against NSCLC cells harbouring these genetic alterations. In addition, the MET/ALK/ROS inhibitor crizotinib has already shown impressive clinical activity in patients with advanced ROS1-positive lung cancer. Currently, several early proof of concept clinical trials are testing various kinase inhibitors in both molecular subsets of lung adenocarcinoma patients. Most probably, personalized treatment of these genetically defined new subsets of lung adenocarcinoma will be implemented in routine clinical care of lung cancer patients in the near future.

Keywords: Lung cancer; adenocarcinoma; RET; ROS

Submitted Feb 10, 2013. Accepted for publication Mar 12, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.08
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.08

Introduction

Recent genomic characterization of lung adenocarcinoma led to the discovery of several key genetic alterations involved in the induction of proliferation and metastatic spread as well as in prevention of apoptosis in lung adenocarcinoma cells. Nearly all of these *driver mutations* are mutually exclusive thus accounting for the classification of lung adenocarcinoma in several genetically defined subgroups. The dependency of the tumors on these driver mutations in the distinct subgroups is underlying their pharmacological vulnerability for specific inhibitors. The identification of specific activating mutations in the *EGFR* gene and specific rearrangements of the *ALK* gene have already been successfully translated into clinical routine with the use of in the meantime approved targeted therapeutic agents (erlotinib, gefitinib and crizotinib). Treatment with these targeted therapeutics results in a remarkably increased response rate, progression free survival time and overall survival compared to standard chemotherapy in these molecularly defined subgoups (1-4) thus overcoming for the first time the therapeutic nihilism in advanced adenocarcinoma based on median overall survival times of less than 1 year with chemotherapy unchanged for decades.

Unfortunately, although driver mutations can be identified in over 50% of lung adenocarcinoma (5,6) by now, only 15% of patients with lung adenocarcinoma, i.e. those with EGFR mutations or ALK aberrations, benefit from personalized treatment in clinical routine, while in the other patients either the driver mutations so far have not been fully clinically validated or no driver mutations are known at all.

Recently, two new receptor tyrosine kinase gene rearrangements affecting together up to 3% of lung adenocarcinoma patients were discovered and, based on the observations described in this review, may soon extend the spectrum of effective personalized treatment options in lung cancer.

The *rearranged during transfection (RET)* gene was found to be rearranged in lung adenocarcinoma patients (1%) for the first time in 2012 by four independent groups (7-10) and preliminary studies demonstrated sensitivity of lung cancer cell lines harboring a *RET* rearrangement to RET-kinase inhibitors like vandetanib (9).

The ROS1 rearrangement was first discovered in lung adenocarcinoma in 2007 (11). In 2012 a study determined a frequency of ROS1 rearrangements in a large lung adenocarcinoma cohort (n=1,073) of 2% (12). In addition, first results of a phase I trial investigating the use of crizotinib in patients harboring ROS1 rearrangements showed promising results (13).

Both kinases are involved in rearrangements resulting in fusion of their kinase domains to different partners. The fusion partners are responsible for the homo-dimerization underlying the oncogenic potency of the gene fusion products.

This review will focus on RET- and ROS1 kinases, their physiological role in the cell and their function as an oncogenic driver especially in lung adenocarcinoma. Furthermore, we will give an overview on current RET- and ROS1 kinase inhibitors and current clinical trials evaluating specific RET- and ROS1 inhibitors.

RET discovery and mechanism of action

The *RET* proto-oncogene was first described as an oncogene activated through DNA rearrangement in the NIH-3T3 cell model in 1985 (14). *RET* is located on chromosome 10q11.2 and spans 21 exons. It encodes for a receptor tyrosine kinase with an extracellular domain (containing four cadherin-like repeats, a calcium binding site, and a cystein rich region), a transmembrane region and an intracellular kinase domain (15). There are three common isoforms of *RET*, the long (RET51), intermediate (RET43), and short (RET9) form, which arise through alternative splicing of the mRNA at the carboxyterminal cytoplasmic tail. They are named after the number of amino acids that follow the point of divergence. RET51 and RET9 are the best characterized isoforms (16). The main ligands of the RET protein belong to the glial-derived neurotrophic factor (GDNF) family, which include GDNF, artemin, neurturin and persephin. The RET-Receptor is part of a cell surface complex, it binds a member of the GDNF family in conjunction with GDNF-family receptor alpha (GFR) co-receptors. After a ligand has bound to the RET-Receptor, it is activated through the formation of a RET-homodimer with subsequent activation of the kinase domain leading to autophosphorylation of intracellular domains (17). Multiple downstream signaling pathways are activated through the activation of the RET protein, these include the RAS/RAF/ERK, the PI3K/AKT and the JNK pathways (18). RET is expressed in neuronal subsets of the central and peripheral nervous system, the Wolffian duct, the budding ureter, the nephric duct and spermatogonia. RET kinase null mice are born alive, but die within one day because of renal aplasia or dysplasia. They also do not develop enteric nervous plexuses, which is in line with the development of Hirschprung's disease caused by loss-of-function mutations in *RET* (19).

The first link between *RET* and human cancer was established by the discovery of somatic rearrangements of *RET* in papillary thyroid carcinoma (*RET/PTC*). These rearrangements lead to a constitutive activation of the tyrosine kinase (18). Up to 30% of sporadic and up to 70% of radiation induced papillary thyroid carcinomas (PTC) show a somatic rearrangement of the *RET* gene (20). So far 12 different 5'-fusion partner genes of *RET* have been described. Germline activating point mutations of *RET* are associated with the multiple endocrine neoplasia type 2 (MEN 2) syndrome. The MEN 2 syndrome is divided into three distinct phenotypes: MEN 2A [medullary thyroid carcinoma (MTC), pheochromacytoma (PC) and hyperparathyroidism], MEN 2B (MTC and PC) and Familial Medullary Thyroid Cancer (FMTC). Each phenotype has a strong association with specific mutation sites within the *RET* gene. Somatic mutations in *RET* are also associated with 50% of sporadic medullary thyroid cancer (21).

RET in NSCLC

In 2012 four independent groups identified independently the presence of a new rearrangement involving the RET-kinase domain in NSCLC. These groups screened a total of 2,650 lung cancer patients (22) and described a frequency of 1% RET rearrangements (7-9). RET expression in the lung is under normal conditions very low, but is significantly increased with the presence of the *RET* fusion gene (7). The transforming ability of the *KIF5B-RET* fusion gene could be shown in preclinical models using Ba/F3 cells, which were shown to grow interleukin-3 independent after expression of the fusion protein and in NIH 3T3 cells, which showed anchorage-independent cell proliferation after the expression of the RET fusion protein (7). The artificial cell systems showed sensitivity to the treatment with the multi-kinase inhibitors vandetanib,

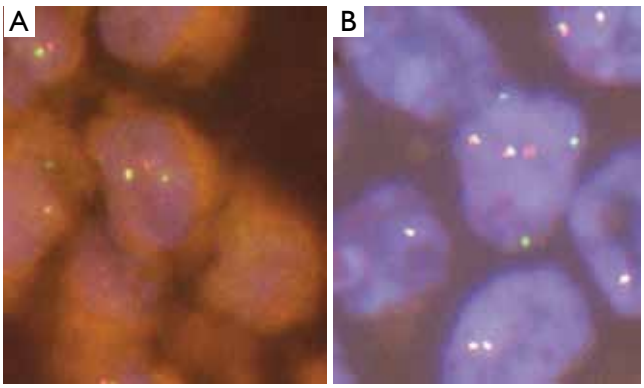


Figure 1 ret (A) and ROS1 (B) FISH assays. Rearrangements in both genes are indicated by split-off of orange and green signals.

sunitinib and sorafenib, which are able to inhibit the kinase activity of RET (9). Current data suggests, that *RET* rearrangements occur mutually exclusive with other known driver alterations in NSCLC, which further supports its role as a driving oncogene in NSCLC (9,22). The most common RET fusion protein is comprised of the first 15 exons of the *KIF5B* gene and the exons 12-20 of the *RET* gene. Exon 1-15 of *KIF5B* contains a kinesin motor and coiled-coil domains that mediate homodimerization of the fusion proteins (9). The *KIF5B* exon 15 fusion site was also shown to be present in the KIF5B-ALK fusion protein in NSCLC (23). Exons 12-20 of the *RET* gene contain the *RET* kinase domain allowing downstream kinase signaling and activation of the PI3K/Akt and/or the RAS/MAPK pathway (24). Up till now 7 variants of the *KIF5B-RET* fusion gene have been described and besides *KIF5B* two other fusion partner have been detected, i.e. the *CCDC6* gene and the *NCOA4* (nuclear receptor coactivator 4) gene (8,25). *CCDC6* and *NCOA4* have been described before in PTC as RET/PTC1 and RET/PTC3, respectively. They both also contain coiled-coil domains, which are able to mediate dimerization of the oncoproteins (26). Concerning the correlation between NSCLC harboring *RET* fusion and clinical characteristics, Wang and colleagues screened 936 patients with NSCLC and could identify 13 RET fusion positive patients (1.4%) in their population. They suggested specific clinicopathologic characteristics for RET fusion positive patients, including younger age (≤60 years), never-smoker status, early lymph-node metastases, poor differentiation of the tumor and a solid predominant subtype (25). The detection of fusion genes can be conducted using RT-PCR, FISH and IHC. Interesting data have been published showing a sensitivity of 90% and specifity of 97.8% for IHC testing for ALK translocations, when compared to FISH in NSCLC (27). So far, however, IHC has not been established for RET detection and thus was not used in trials screening for RET fusions in NSCLC (9,25). Therefore FISH, although cost and labor intensive, still seems to be the gold standard for the detection of RET fusions (*Figure 1*) in NSCLC. In addition, the use of RT-PCR might miss new fusion partners (25). However, given the necessity of simultaneous pretherapeutic assessment of numerous driver mutations in lung cancer, it seems reasonable that next generation based multiplex sequencing will substitute distinct single gene assays involving RET in the near future (28).

RET inhibitors

So far no clinical trials using RET inhibitors in NSLCL harbouring RET fusion genes have been published. Since *RET* fusions and activating mutations are present in differentiated thyroid carcinomas (DTC) and medullary thyroid carcinoma (MTC) (20) we will thus start to summarize data from clinical trials studying tyrosine kinase inhibitors with anti-RET activity in patients with DTC and MTC. However, these results may only be of limited value for the understanding of RET inhibition in NSCLC, since the spectrum of *RET* mutations and *RET* fusions in thyroid carcinoma differs from what so far is known for RET in NSCLC.

Specific aberrations in *RET* present in thyroid carcinoma can be assigned to specific subgroups of the disease. *RET* fusions are mostly present in PTC, which closely resemble the fusions present in NSCLC (8,26). Preclinical studies have shown that different tyrosine kinase inhibitors with anti-RET activity show different activity against the various aberrant *RET* forms present in thyroid carcinomas (29). For instance, cabozantinib showed greater activity compared to vandetanib in cells harboring the *RET/PTC1* fusion gene (29), a fusion gene which is also present in NSCLC. These observations should be considered in planning trials with anti-RET tyrosine kinase inhibitors also in lung cancer.

Anti-RET tyrosine kinase inhibitors have already been evaluated in NSCLC, however, not focusing of patients harbouring *RET* fusion genes in their tumors (30-32). Thus, these trials do not add valuable information concerning the use of these drugs in RET positive lung cancer patients only. Furthermore, the drugs used in these trials (vandetanib,

sunitinib, sorafenib) are no specific RET inhibitors, but rather multi kinase inhibitors. This fact further complicates the interpretation of RET-inhibition in thyroid carcinoma (24). For a list of multi-kinase inhibitors with anti-RET activity refer to *Table 1*.

Vandetanib

In April 2011 the FDA approved vandetanib, a RET, VEGF 2, VEGF 3 and EGF receptor tyrosine kinase inhibitor for the treatment of patients with metastatic MTC who were ineligible for surgery and had progressive or symptomatic disease. The approval followed the results from an open label single arm phase II study testing vandetanib in patients with hereditary MTC. This phase II study conducted by Wells *et al.* showed that 83% of the patients treated with vandetanib had a reduction in tumor size at their first assessment and 11 out of 30 patients responded with an initial decrease in tumor size ≥30% of which 6 (20%) had confirmed partial responses (PR) according to RECIST. Disease control rate at 24 weeks was 78% and the duration of response in patients with confirmed PR was durable with a median of 10.2 months (33). Following the phase II data a large phase III trial was initiated showing a significantly improved efficacy and prolongation of PFS for vandetanib compared to placebo in patients with sporadic and hereditary MTC with a hazard ratio of 0.46 (95% CI, 0.31-0.69; P<0.001) (34). Preclinical studies suggest that vandetanib has superior activity in MEN2B cell lines compared to cabozantinib (29). The predominant mutation in MEN2B is the activating M918T point mutation in the RET kinase domain, which is also the most frequent mutation in sporadic MTC (35). Vandetanib also showed activity against RET/PCT *in vitro* and *in vivo* (36).

Cabozantinib

Cabozantinib, a potent inhibitor of RET, VEGFR2 and MET tyrosine kinases, received FDA approval for its use in MTC in November 2012. Early signals of activity in MTC were seen in a phase I dose escalation trial, which led to the testing of cabozantinib in patients with MTC in an expansion cohort of the phase I study. Of the 35 patients with MTC and measurable disease included into the study 17 patients (49%) experienced a 30% or greater reduction in the sum of tumor diameters at first assessment. Disease control of at least 6 months was present in 68% of the patients (37). Following the positive data from the phase II study a large phase III study was started, which tests cabozantinib *vs.* placebo in patients with progressive, unresectable, locally advanced or metastatic MTC. First data were presented at ASCO 2012, which showed that the primary objective of significant PFS prolongation was met (HR 0.28 95% CI, 0.19-0.40; P<0.0001) (38).

In July 2012 a phase II study testing cabozantinib in KIF5B/RET positive NSCLC patients has been initiated at Memorial Sloan-Kettering Cancer Center (NCT01639508) and is thus to our knowledge the first study investigating a personalized treatment approach for this newly defined subgroup of NSCLC. Interestingly, *in vitro* studies showed a greater activity of cabozantinib compared to vandetanib in cell lines harboring the RET/PTC1 fusion gene, which also has been found in NSCLC (29).

Sorafenib

Sorafenib is a multi-tyrosine kinase inhibitor targeting VEGFR1, VEGFR2, KIT, RET, BRAF and CRAF (39). *In vitro* sorafenib was shown to inhibit RET in the low nanomolar range and exerted anti-tumor activity in RET-driven xenografts (40). Sorafenib has been tested in several phase II studies in patients with DTC, anaplastic thyroid carcinoma and MTC (41-43). In an open-label phase II study of 41 patients with PTC, 6 patients (15%) showed a PR and 23 (56%) patients had a stable disease for longer than 6 months. The PRs seen in the patients were durable with a median duration of 7.5 months. The authors concluded that sorafenib is an active drug in metastatic PTC. Genetic testing was included into the trial and the great majority of PTCs harbored an activating BRAF mutation whereas none was positive for RET/PTC1 or RET/PTC3. These observations render translation into the RET driven NSCLC setting difficult (43). In another phase II study sorafenib was tested in locally advanced or metastatic MTC. Of 15 evaluable patients with sporadic MTC, one patient had a PR and more than 50% of the patients had SD ≥15 months. The majority of tumors in the tested population had activating mutations in the *RET* gene (42) The phase II study from Gupta-Abramson *et al.* demonstrated in 30 (27 out of 30 being DTC) patients with metastatic, iodine-refractory thyroid carcinoma a PR rate of 23% (7 patients). The median PFS was stated with 19.75 months. Data of specific genetic testing were not presented in this paper. Given the PR rate of 23% and the PFS of 19.25 months the sorafenib treatment may be considered superior to chemotherapy in these patients (41).

Table 1 Multikinase inhibitors with anti-RET and anti-ROS1 activity

Drug	Targets	Provider
anti-RET acitivity		
Vandetanib	VEGF2	AstraZeneca
	VEGF3	
	EGFR	
	RET	
Sunitinib	VEGFR	Pfizer
	Flt-3	
	c-Kit	
	RET	
Sorafenib	VEGFR1	Bayer
	VEGFR2	Onyx pharmaceuticals
	c-Kit	
	RET	
	BRAF	
	CRAF	
Motesanib	VEGFR	Amgen
	PDGFR	Takeda
	c-Kit	
	RET	
Cabozantinib	RET	Exelixis
	VEGFR2	
	MET	
anti-ROS1 activity		
Crizotinib	ALK	Pfizer
	MET	
	ROS1	
AP26113	ALK	Ariad
	EGFR	
	ROS1	
ASP3026	ALK	Astellas
	MET	
	ROS1*	

*trial including ROS1 patients running, no preclinical data found.

Sunitinib

Sunitinib is a multi-tyrosine kinase inhibitor targeting VEGFR, Flt-3, c-Kit and RET (40) and has proven to be a potent inhibitor of RET/PTC oncoproteins *in vitro* and *in vivo* (36). In a phase II study in iodine refractory DTC and MTC from 33 evaluable patients one patient showed a complete response (3%), ten patients had a PR (28%) and 16 patients demonstrated stable disease (46%). There was also a significant association seen between decreased ^{18}FDG-PET uptake and RECIST response (44). Intermediate results of two studies testing sunitinib in patients with thyroid carcinoma were presented at ASCO 2008 (45,46). The study of Cohen *et al.* presented data of 31 evaluable patients with DTC treated for at least two cycles with sunitinib. Of these patients 13% showed a PR and 65% of patients a SD. In MTC there have been no PRs reported, but a SD rate of 85% (45) In a mixed patient cohort with MTC, DTC and anaplastic thyroid carcinoma Ravaud *et al.* demonstrated in 15 evaluable patients a PR rate of 7% (n=1) and a SD rate of 80% (n=12) (46). In addition two case reports have been published, one reporting a PR in a patient with MTC and one in a patient with PTC treated with sunitinib (47,48).

Motesanib

The multi-tyrosine kinase inhibitor motesanib inhibits VEGFR, PDGFR, Kit and RET and demonstrated activity in TT tumor cell xenografts expressing the RET C634W protein (49). But there have also been reports indicating the ineffectiveness of motesanib in inhibiting the C634W mutant form of RET and being only active in wild type RET (50). Motesanib was tested in two phase II studies involving patients with thyroid cancer. One study which included 93 patients with confirmed locally advanced metastatic DTC or MTC yielded a 14% PR rate and a 68% SD rate. However none of the patients genetically analyzed showed a RET mutation or RET rearrangement in their tumor (51). Another phase II trial studying motesanib in MTC included 91 patients. In this trial only 2% of the patients were reported to have achieved a PR and 81% of the patients had a SD. The objective response rate for RET-mutation negative (n=10) and for RET-mutation positive (n=28) was 10% and 0%, respectively (50).

ROS1 discovery and mechanism of action

ROS was first described as an oncogene product of the avian sarcoma RNA tumor virus UR2 (University of Rochester tumor virus 2) in 1982 and *v-ROS1* was identified in the same year as the distinctive oncogenic sequence in UR2 (52,53). In the UR 2 virus *v-ROS1* is fused to the *gag*-gene and the product of the fusion gene *gag-ros* was identified to have tyrosine kinase activity (54). In 1984 the *mf3* gene, which later was discovered to be similar to *c-ROS1*, was reported

to induce malignant transformation of NIH3T3 cells (55). *ROS1* (v-ros avian UR2 sarcoma virus oncogene homolog 1) has been mapped to chromosome 6q16-6q22 (56). The region is involved in nonrandom chromosomal rearrangements in different malignancies including, glioblastoma, cholangiocarcinoma and lung adenocarcinoma (8,57,58). The ROS1 receptor tyrosine kinase consists of an extracellular domain, a hydrophobic transmembrane region and an intracellular kinase domain. ROS is a unique receptor which is remotely related to the ALK and Insulin receptor family (59,60). The extracellular domain of ROS1 contains a YWTD -propeller domain that folds into three -propeller domains and nine fibronectin type III domains. Although ROS1 has a large extracellular domain no ligand has been found so far. The presence of fibronectin III domains is a common feature of cell adhesion molecules (CAMs), therefore the combination of fibronectin III domains in the extracellular domain of ROS1 coupled with the intracellular kinase activity might be a way of direct translating adhesion engagements to intracellular signaling pathways (60). Multiple downstream signaling pathways are activated through the activation of ROS1, these include the STAT3, PI3K/AKT and RAS/MAPK/MEK pathway, although it is important to notice that the transforming ability of the chimeric EGFR-ROS on chicken embryo fibroblasts or NIH3T3 cells was not hindered through the application of the MEK inhibitor PD98059 (61). *ROS1* was shown to be expressed in mouse, rat and chicken kidneys and intestine (60). In mice the expression of c-ROS seems to play a role in the development of the kidneys, especially in stages which involve epithelial-mesenchymal interactions. In adult mice with mature kidneys c-ROS expression is low (62). In the testicles expression of c-ROS was detected in mice and is limited to epithelial cells of the caput epididymis. The importance of ROS for the maturation of the epithelial cells of the epididymis was seen in ROS-null mice. These mice lost the ability of reproduction due to deficient sperm function, which was most likely due to improper capacitation supporting the significance of a functional epididymis for maturation of the spermatocytes. Besides from infertility c-ROS knockout mice were healthy (63). In humans c-ROS has also been detected in the epididymis, although the spatial distribution was different from what was seen in mice (64). Expression of c-ROS was also detected in other human tissues, such as lung, placenta and skeletal muscle tissue (60).

The first link between human cancer and ROS1 was established in 1987 by the discovery of somatic rearrangements involving ROS1 in glioblastoma cell lines, although the partner of ROS1 was not identified (65). The fusion of the *FIG* (Fused in Glioblastoma)-gene with *ROS1* was elucidated by Charest *et al.* in 2003. It was shown that the FIG-ROS1 fusion protein was created through a small intra-chromosomal deletion and was therefore the first example of a receptor tyrosine kinase fusion protein, which did not occur from a translocation or inversion (57). Furthermore it was shown that the FIG-ROS fusion protein was able to transform NIH3T3 cells *in vitro* and to enable tumor formation in immunocompromised nude mice (58). The expression of the FIG-ROS fusion protein in the CNS *in vivo* was able to induce the formation of glioblastomas (66). FIG-ROS fusion proteins were also discovered in cholangiocarcinoma cell lines and patient derived tumors (58).

ROS1 in NSCLC

Rearrangements in NSCLC involving ROS1 (*Figure 1*) were first described by Rikova *et al.* in 2007.

In a phosphoproteomic screen of 41 NSCLC cell lines and 150 NSCLC tumors 2 ROS1 fusions (*SLC34A2-ROS1* and *CD74-ROS1*) were detected (11). *SLC34A2-ROS1* was discovered in the HCC78 cell line. *SLC34A2* is part of the solute carrier family and is expressed in many different organs such as lung, mammary glands, testis and liver. It is believed that the gene product of *SLC34A2* the protein NaPi-IIb is involved in the reabsorbtion of Pi in the surfactant of lung alveolars. The protein is supposed to span the cell membrane in 8 loops (67). Mutations of *SLC34A2*, which abrogate the normal protein function, are associated with pulmonary alveolar microlithiasis (68). In the fusion gene 2 variants exist, either a fusion between exon 4 of SLC34A2 and exon 32 of ROS or exon 4 of SLC34A2 and exon 34 of ROS. In both cases the fusion gene expresses a protein with two transmembrane domains (11). The CD74-ROS1 fusion was discovered in a tumor from a female never-smoker with adenocarcinoma. In this tumor exon 6 of CD74 was found to be fused with exon 34 of ROS1. CD74 codes for a type II membrane protein. The protein functions as a receptor for the macrophage migration inhibitory factor and as a chaperon for MHC class II proteins (69). The transforming ability of SLC34A2-ROS was shown in the ability of the fusion gene to cause anchorage-independent growth and tumor formation in nude mice of 3T3 cells transduced with a retrovirus

containing SLC34A2-ROS (58). The oncogenic ability of the CD74-ROS1 fusion gene has also been validated in fibroblasts and NSCLC cells were the ectopic expression of the CD74-ROS1 fusion gene induced high invasiveness *in vitro* (Matrigel Boyden chamber invasion assay) and the formation of metastases *in vivo* (70).

Recently, two large studies screening together more than 2000 patients with NSCLC for the presence of ROS1 rearrangements found the frequency of ROS1 rearrangements in NSCLC to be approximately 2% (8,12). The clinical characteristics of patients with a ROS1 fusion were very similar to patients with an ALK translocation. It was found that patients with a ROS1 fusion positive tumor were more commonly light smokers (<10 pack years) or never-smokers and ROS1 fusions were associated with younger age and adenocarcinoma histology (12). The study conducted by Takeuchi *et al.* and Govindan *et al.* discovered additional fusion partners of ROS1 in NSCLC: tropomyosin 3 (TPM3), syndecan 4 (SDC4), leucine-rich repeats and immunoglobulin-like domains (LRIG3), ezrin (EZR) and endoplasmic reticulum protein retention receptor 2 (KDELR 2) (8,71). The kinase activity of ROS1 is retained in the known fusion proteins (72) and ROS1 rearrangements were not overlapping with other known oncogenic events in NSCLC, like KRAS mutations, EGFR mutations or ALK fusions (12). A transgenic mouse model expressing the EZR-ROS1 fusion protein in lung alveolar epithelial cells has been developed and could demonstrate the formation of adenocarcinoma in both lungs at an early age (73).

ROS1 inhibitors

Although ROS1 has been known to play a role as an oncogene in glioblastoma for a long time (65), selective ROS1 inhibitors have not yet been clinically tested. Given that the ROS1 kinase shares high sequence homology with ALK, which is reflected in an amino acid sequence homology of 77% at the adenosine triphosphate (ATP)-binding site (74), the activity of ALK-kinase inhibitors were tested in cell lines and tumors harboring ROS1 fusion proteins (58). The ALK-inhibitor TAE684 showed activity in the lung cancer cell line HCC78, which harbors the *SLC34A2-ROS1* fusion gene and in BaF3 cells expressing the FIG-ROS fusion protein (58,75). Crizotinib, the approved ALK/MET inhibitor for NSCLC patients harboring an ALK-translocation also showed activity in the HCC78 cell line (12,76). Following these signals the phase I trial of crizotinib (NCT00585195) was amended for the inclusion of patients with solid tumors harboring a ROS1 rearrangement. Preliminary results presented at ASCO and ESMO 2012 demonstrated promising results of crizotinib in ROS1 rearranged NSCLC with an objective response rate of 57% and a disease control rate of 80% after 2 months (13). Our group also recently published a case of a heavily pretreated NSCLC patient whose tumor harbored a *ROS1* rearrangement and showed a compete metabolic response in ^{18}FDG-PET/CT after 4 weeks of treatment with crizotinib which is maintained now for more than 4 months (77). Currently there are also two trials ongoing testing second-generation ALK inhibitors in ROS1 fusion positive tumors. These trials are evaluating the safety and activity of AP26113 (NCT01449461) and ASP3026 (NCT01284192). For a list of multi-kinase inhibitors with anti-ROS1 activity refer to *Table 1*.

Conclusions

The identification of *RET*- and *ROS1* rearrangements in NSCLC is a consequence of our increasing knowledge of the genomic basis of malignant transformation in lung cancer resulting in the identification of an increasing number of distinct and therapeutically tractable molecular subgroups. Tyrosine kinase inhibitors with anti-RET activity have shown promising preclinical and clinical activity in thyroid carcinomas. However, up till now most of the trials conducted were entity driven and did not distinguish between the molecular subtypes which are present in thyroid carcinomas and NSCLC. Thus, although these trials provide some evidence that aberrant RET may serve as a target for kinase inhibitor therapy, the translation of these observations to NSCLC seems to be problematic. Therefore, prospective trials RET translocated NSCLC are needed, although their realization may be a challenge given the low incidence of RET rearrangements in NSCLC. The ongoing trial of cabozantinib in KIF5B-RET fusion positive NSCLC (NCT01639508) is a first step in the right direction. In the case of ROS1 an impressive activity of the ALK/MET/ROS inhibitor crizotinib has already been reported as result of a still ongoing phase I trial in heavily pretreated NSCLC patients with ROS rearrangements in their tumors. Based on these results approval of crizotinib for the treatment of ROS positive NSCLC in the near future seems probable. Other clinical trials evaluating the safety and activity of second generation small molecule inhibitors with anti-ROS1 activity are also currently tested in ROS1 fusion positive patients, but no results have been

presented so far. Furthermore, it remains to be elucidated how new selective RET- and ROS-inhibitors will perform clinically (78). Given the low frequency of these two new driver mutations the execution of clinical trials addressing the efficacy of RET- and ROS-inhibitors is in particular challenging and requires the establishment of large and effective molecular screening networks providing real time molecular diagnostics of high quality (28).

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46
2. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
3. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
4. Kim D, Ahn M, Yang P, et al. Results of a Global Phase II Study with Crizotinib in Advanced ALK-positive Non-small Cell Lung Cancer (NSCLC). ASCO Annual Meeting 2012;2012:ix400-46.
5. Sequist LV, Heist RS, Shaw AT, et al. Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. Ann Oncol 2011;22:2616-24.
6. Zander T, Heukamp LC, Bos MA, et al. Regional screening network for characterization of the molecular epidemiology of non-small cell lung cancer (NSCLC) and implementation of personalized treatment. J Clin Oncol 2012;30:abstr CRA10529.
7. Kohno T, Ichikawa H, Totoki Y, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med 2012;18:375-7.
8. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med 2012;18:378-81.
9. Lipson D, Capelletti M, Yelensky R, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18:382-4.
10. Ju YS, Lee WC, Shin JY, et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. Genome Res 2012;22:436-45.
11. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190-203.
12. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-70.
13. Shaw AT, Camidge DR, Engelman J, et al. Clinical activity of crizotinib in patients with advanced non-small cell lungcancer (NSCLC) harboring ROS1 gene rearrangement. Ann Oncol 2012;30:abstr 7508.
14. Takahashi M. The GDNF/RET signaling pathway and human diseases. Cytokine Growth Factor Rev 2001;12:361-73.
15. Ishizaka Y, Itoh F, Tahira T, et al. Human ret proto-oncogene mapped to chromosome 10q11.2. Oncogene 1989;4:1519-21.
16. Myers SM, Eng C, Ponder BA, et al. Characterization of RET proto-oncogene 3' splicing variants and polyadenylation sites: a novel C-terminus for RET. Oncogene 1995;11:2039-45.
17. Airaksinen MS, Saarma M. The GDNF family: signalling, biological functions and therapeutic value. Nat Rev Neurosci 2002;3:383-94.
18. Phay JE, Shah MH. Targeting RET receptor tyrosine kinase activation in cancer. Clin Cancer Res 2010;16:5936-41.
19. Manié S, Santoro M, Fusco A, et al. The RET receptor: function in development and dysfunction in congenital malformation. Trends Genet 2001;17:580-9.
20. Jhiang SM. The RET proto-oncogene in human cancers. Oncogene 2000;19:5590-7.
21. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol 2008;21:S37-43.
22. Pao W, Hutchinson KE. Chipping away at the lung cancer genome. Nat Med 2012;18:349-51.
23. Wong DW, Leung EL, Wong SK, et al. A novel KIF5B-ALK variant in nonsmall cell lung cancer. Cancer 2011;117:2709-18.
24. Chau NG, Haddad RI. Vandetanib for the treatment of medullary thyroid cancer. Clin Cancer Res 2013;19:524-9.
25. Wang R, Hu H, Pan Y, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. J Clin Oncol 2012;30:4352-9.
26. Santoro M, Melillo RM, Fusco A. RET/PTC activation in papillary thyroid carcinoma: European Journal of Endocrinology Prize Lecture. Eur J Endocrinol 2006;155:645-53.

27. Yi ES, Boland JM, Maleszewski JJ, et al. Correlation of IHC and FISH for ALK gene rearrangement in non-small cell lung carcinoma: IHC score algorithm for FISH. J Thorac Oncol 2011;6:459-65.
28. Buettner R, Wolf J, Thomas RK. Lessons to learn from lung cancer genomics: The emerging concept of individualized diagnostics and treatment. J Clin Oncol 2013;31:1858-65.
29. Verbeek HH, Alves MM, de Groot JW, et al. The effects of four different tyrosine kinase inhibitors on medullary and papillary thyroid cancer cells. J Clin Endocrinol Metab 2011;96:E991-5.
30. Novello S, Camps C, Grossi F, et al. Phase II study of sunitinib in patients with non-small cell lung cancer and irradiated brain metastases. J Thorac Oncol 2011;6:1260-6.
31. Dy GK, Hillman SL, Rowland KM, et al. A front-line window of opportunity phase 2 study of sorafenib in patients with advanced nonsmall cell lung cancer: North Central Cancer Treatment Group Study N0326. Cancer 2010;116:5686-93.
32. Lee JS, Hirsh V, Park K, et al. Vandetanib Versus placebo in patients with advanced non-small-cell lung cancer after prior therapy with an epidermal growth factor receptor tyrosine kinase inhibitor: a randomized, double-blind phase III trial (ZEPHYR). J Clin Oncol 2012;30:1114-21.
33. Wells SA Jr, Gosnell JE, Gagel RF, et al. Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer. J Clin Oncol 2010;28:767-72.
34. Wells SA Jr, Robinson BG, Gagel RF, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol 2012;30:134-41.
35. Frank-Raue K, Rondot S, Raue F. Molecular genetics and phenomics of RET mutations: Impact on prognosis of MTC. Mol Cell Endocrinol 2010;322:2-7.
36. Kim DW, Jo YS, Jung HS, et al. An orally administered multitarget tyrosine kinase inhibitor, SU11248, is a novel potent inhibitor of thyroid oncogenic RET/papillary thyroid cancer kinases. J Clin Endocrinol Metab 2006;91:4070-6.
37. Kurzrock R, Sherman SI, Ball DW, et al. Activity of XL184 (Cabozantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer. J Clin Oncol 2011;29:2660-6.
38. Schoffski P, Elisei R, Muller S. An international, double-blind, randomized, placebo-controlled phase III trial (EXAM) of cabozantinib (XL184) in medullary thyroid carcinoma (MTC) patients (pts) with documented RECIST progression at baseline. ASCO Meeting 2012;34:5508.
39. Morgillo F, Martinelli E, Troiani T, et al. Antitumor activity of sorafenib in human cancer cell lines with acquired resistance to EGFR and VEGFR tyrosine kinase inhibitors. PLoS One 2011;6:e28841.
40. Mologni L. Development of RET kinase inhibitors for targeted cancer therapy. Curr Med Chem 2011;18:162-75.
41. Gupta-Abramson V, Troxel AB, Nellore A, et al. Phase II trial of sorafenib in advanced thyroid cancer. J Clin Oncol 2008;26:4714-9.
42. Lam ET, Ringel MD, Kloos RT, et al. Phase II clinical trial of sorafenib in metastatic medullary thyroid cancer. J Clin Oncol 2010;28:2323-30.
43. Kloos RT, Ringel MD, Knopp MV, et al. Phase II trial of sorafenib in metastatic thyroid cancer. J Clin Oncol 2009;27:1675-84.
44. Carr LL, Mankoff DA, Goulart BH, et al. Phase II study of daily sunitinib in FDG-PET-positive, iodine-refractory differentiated thyroid cancer and metastatic medullary carcinoma of the thyroid with functional imaging correlation. Clin Cancer Res 2010;16:5260-8.
45. Cohen E, Needles B, Cullen K. Phase 2 study of sunitinib in refractory thyroid cancer. J Clin Oncol. 2008;26:abstr 6025.
46. Ravaud A, De la Fouchardière C, Courbon F. Sunitinib in patients with refractory advanced thyroid cancer. J Clin Oncol 2008;26:abstr 6058.
47. Kelleher FC, McDermott R. Response to sunitinib in medullary thyroid cancer. Ann Intern Med 2008;148:567.
48. Dawson SJ, Conus NM, Toner GC, et al. Sustained clinical responses to tyrosine kinase inhibitor sunitinib in thyroid carcinoma. Anticancer Drugs 2008;19:547-52.
49. Coxon A, Bready J, Kaufman S, et al. Anti-tumor activity of motesanib in a medullary thyroid cancer model. J Endocrinol Invest 2012;35:181-90.
50. Schlumberger MJ, Elisei R, Bastholt L, et al. Phase II Study of Safety and Efficacy of Motesanib in Patients With Progressive or Symptomatic, Advanced or Metastatic Medullary Thyroid Cancer. J Clin Oncol 2009;27:3794-801.
51. Sherman SI, Wirth LJ, Droz JP, et al. Motesanib diphosphate in progressive differentiated thyroid cancer. N Engl J Med 2008;359:31-42.
52. Wang LH, Hanafusa H, Notter MF, et al. Genetic structure and transforming sequence of avian sarcoma virus UR2. J Virol 1982;41:833-41.
53. Shibuya M, Hanafusa H, Balduzzi PC. Cellular sequences related to three new onc genes of avian sarcoma virus

(fps, yes, and ros) and their expression in normal and transformed cells. J Virol 1982;42:143-52.
54. Feldman RA, Wang LH, Hanafusa H, et al. Avian sarcoma virus UR2 encodes a transforming protein which is associated with a unique protein kinase activity. J Virol 1982;42:228-36.
55. Fasano O, Birnbaum D, Edlund L, et al. New human transforming genes detected by a tumorigenicity assay. Mol Cell Biol 1984;4:1695-705.
56. Nagarajan L, Louie E, Tsujimoto Y, et al. The human c-ros gene (ROS) is located at chromosome region 6q16---- 6q22. Proc Natl Acad Sci U S A 1986;83:6568-72.
57. Charest A, Lane K, McMahon K, et al. Fusion of FIG to the receptor tyrosine kinase ROS in a glioblastoma with an interstitial del(6)(q21q21). Genes Chromosomes Cancer 2003;37:58-71.
58. Gu TL, Deng X, Huang F, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. PLoS One 2011;6:e15640.
59. Birchmeier C, O'Neill K, Riggs M, et al. Characterization of ROS1 cDNA from a human glioblastoma cell line. Proc Natl Acad Sci USA 1990;87:4799-803.
60. Acquaviva J, Wong R, Charest A. The multifaceted roles of the receptor tyrosine kinase ROS in development and cancer. Biochim Biophys Acta 2009;1795:37-52.
61. Nguyen KT, Zong CS, Uttamsingh S, et al. The role of phosphatidylinositol 3-kinase, rho family GTPases, and STAT3 in Ros-induced cell transformation. J Biol Chem 2002;277:11107-15.
62. Tessarollo L, Nagarajan L, Parada LF. c-ros: the vertebrate homolog of the sevenless tyrosine kinase receptor is tightly regulated during organogenesis in mouse embryonic development. Development 1992;115:11-20.
63. Sonnenberg-Riethmacher E, Walter B, Riethmacher D, et al. The c-ros tyrosine kinase receptor controls regionalization and differentiation of epithelial cells in the epididymis. Genes Dev 1996;10:1184-93.
64. Légaré C, Sullivan R. Expression and localization of c-ros oncogene along the human excurrent duct. Mol Hum Reprod 2004;10:697-703.
65. Birchmeier C, Sharma S, Wigler M. Expression and rearrangement of the ROS1 gene in human glioblastoma cells. Proc Natl Acad Sci USA 1987;84:9270-4.
66. Charest A, Wilker EW, McLaughlin ME, et al. ROS fusion tyrosine kinase activates a SH2 domain-containing phosphatase-2/phosphatidylinositol 3-kinase/mammalian target of rapamycin signaling axis to form glioblastoma in mice. Cancer Res 2006;66:7473-81.
67. Murer H, Forster I, Biber J. The sodium phosphate cotransporter family SLC34. Pflugers Arch 2004;447:763-7.
68. Tachibana T, Hagiwara K, Johkoh T. Pulmonary alveolar microlithiasis: review and management. Curr Opin Pulm Med 2009;15:486-90.
69. Borghese F, Clanchy FIL. CD74: an emerging opportunity as a therapeutic target in cancer and autoimmune disease. Expert Opin Ther Targets 2011;15:237-51.
70. Jun HJ, Johnson H, Bronson RT, et al. The oncogenic lung cancer fusion kinase CD74-ROS activates a novel invasiveness pathway through E-Syt1 phosphorylation. Cancer Res 2012;72:3764-74.
71. Govindan R, Ding L, Griffith M, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150:1121-34.
72. Stumpfova M, Jänne PA. Zeroing in on ROS1 rearrangements in non-small cell lung cancer. Clin Cancer Res 2012;18:4222-4.
73. Arai Y, Totoki Y, Takahashi H, et al. Mouse Model for ROS1-Rearranged Lung Cancer. PLoS One 2013;8:e56010.
74. Chin LP, Soo RA, Soong R, et al. Targeting ROS1 with anaplastic lymphoma kinase inhibitors: a promising therapeutic strategy for a newly defined molecular subset of non-small-cell lung cancer. J Thorac Oncol 2012;7:1625-30.
75. McDermott U, Iafrate AJ, Gray NS, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res 2008;68:3389-95.
76. Yasuda H, de Figueiredo-Pontes LL, Kobayashi S, et al. Preclinical rationale for use of the clinically available multitargeted tyrosine kinase inhibitor crizotinib in ROS1-translocated lung cancer. J Thorac Oncol 2012;7:1086-90.
77. Bos M, Gardizi M, Schildhaus HU. Complete metabolic response in a patient with repeatedly relapsed non-small cell lung cancer harboring ROS1 gene rearrangement after treatment with crizotinib. Lung Cancer in press.
78. El-Deeb IM, Yoo KH, Lee SH. ROS receptor tyrosine kinase: a new potential target for anticancer drugs. Med Res Rev 2011;31:794-818.

Cite this article as: Bos M, Gardizi M, Schildhaus HU, Buettner R, Wolf J. Activated *RET* and *ROS*: two new driver mutations in lung adenocarcinoma. Transl Lung Cancer Res 2013;2(2):112-121. doi: 10.3978/j.issn.2218-6751.2013.03.08

HER2 driven non-small cell lung cancer (NSCLC): potential therapeutic approaches

Ana Christina Garrido-Castro, Enriqueta Felip

Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain

Correspondence to: Enriqueta Felip. Medical Oncology Department, Vall d'Hebron University Hospital, P. Vall d'Hebron 119-129, 08035 Barcelona, Spain. Email: efelip@vhebron.net.

Abstract: Oncogenic driver mutations identified in non-small cell lung cancer (NSCLC) have triggered the development of drugs capable of interfering in intracellular signaling pathways involved in tumorigenesis. Tyrosine kinase inhibitors, such as erlotinib or gefitinib, have demonstrated promising results in patients with advanced NSCLC that harbor EGFR mutations. Human epidermal growth factor 2 (HER2/ERBB2/neu) is a member of the ERBB family of tyrosine kinase receptors, and is activated by homodimerization or heterodimerization with other ERBB receptors. Deregulation of HER2 gene, by overexpression and/or gene amplification has been proved important in breast and gastric cancer, in which overexpression of HER2 confers greater response to specific anti-HER2 treatment, including trastuzumab. In lung carcinogenesis, HER2 mutations are thought to be more clinically relevant than overexpression or gene amplification. HER2 mutations in NSCLC, described exclusively in adenocarcinoma histology, are present in approximately 4% of this subset of lung cancer patients, suggesting that thousands of patients per year may possibly benefit from targeted therapy. Therefore, we conclude that systematic genotypic testing in this subgroup of NSCLC patients should include detection of HER2 mutations. In addition, clinical trials with standard antiHER2 agents and new investigational therapies are ongoing, with promising preliminary results, as illustrated in this review, although further research is warranted in this field.

Keywords: HER2; lung adenocarcinoma; mutation; targeted therapy

Submitted Jan 18, 2013. Accepted for publication Feb 19, 2013.
doi: 10.3978/j.issn.2218-6751.2013.02.02
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.02.02

Introduction

Lung cancer continues to be the leading cause of cancer-related death, as estimated by the American Cancer Society, responsible for 26% of all female cancer deaths and 29% of all male cancer deaths in the U.S. in 2012 (1). Considering that non-small cell lung cancer (NSCLC) accounts for 80-85% of cases of lung cancer (2) and that significant improvement in survival rates, approximately 17% at 5 years for recently diagnosed NSCLC and less than 4% if presenting with distant metastasis (3), has not been achieved in the last decade with conventional chemotherapy, novel therapeutic approaches are warranted in this field. As a result of these advances, systematic genomic testing for patients with NSCLC is becoming the new standard of care in clinical decision-making, due to the identification of driver mutations that have triggered the development of new molecules targeting these specific alterations in cancer cells. For example, somatic mutations in epidermal growth factor receptor (EGFR) confer greater response rates to tyrosine kinase inhibitors (TKIs) that target the catalytic domain of EGFR, such as erlotinib and gefitinib, compared to standard therapy in advanced NSCLC, 70% *vs.* 33.2% in first-line trials (4,5). In a similar manner, crizotinib, the anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitor, has demonstrated response rates of approximately 60% with progression-free survival greater than 10 months in those NSCLC characterized by ALK rearrangements (6).

These studies have enabled to conclude that both EGFR-mutant and ALK-positive NSCLC constitute two defined subgroups of oncogene-driven tumors with potentially effective targeted therapy. Furthermore, approximately 15-20% of NSCLC diagnosed in Europe and North America bear EGFR mutations or ALK rearrangements (7), enhancing the significance of the development of drugs capable of interfering with their intracellular effects.

Based on these results, the identification of other activating mutations has been pursued in hopes of improving survival in NSCLC by specifically treating these genomic alterations. These potential therapeutic targets include KRAS, BRAF, HER2 and PIK3CA, in addition to ROS1 fusions. KRAS mutations, in codons 12, 13 and 61, reported in approximately 20% of cases of lung adenocarcinomas, predict negative outcome in terms of response to EGFR TKIs. No targeted therapies have demonstrated an increase in overall survival in KRAS-mutant NSCLC, although selumetinib, an inhibitor of MAPK extracellular signal-regulated kinase (MEK) 1/2 (downstream of KRAS), in combination with docetaxel in previously treated advanced NSCLC has shown promising results in a recent phase 2 trial (8).

Regarding activating mutations in BRAF, HER2 and PIK3CA, incidence reported for each group ranges from 1-4%, a lower although significant frequency that is encouraging further investigation of these genetic alterations and consequent therapeutic implications. HER2 mutations in NSCLC constitute a clear molecular target, particularly in a subset of patients with distinct clinical features, including female non-smokers with adenocarcinomas, similar to those patients with EGFR-mutant lung cancer. Here, we seek to review the characteristics of HER2 mutations that enable interaction with molecules that specifically target these receptors in lung adenocarcinomas, as well as the results of preliminary studies that assess the efficacy of anti-HER2 therapy applied to NSCLC.

Tumorigenesis induced by HER2 mutations

HER2 [also known as epidermal growth factor receptor-2 (*EGFR2*), *ERBB2* or *NEU*] is a member of the *ERBB* receptor tyrosine kinase family, which includes 3 additional members; *EGFR* (HER1/*ERBB1*), HER3 (*ERBB3*) and HER4 (*ERBB4*). The binding of ligands to the extracellular domain of EGFR, HER3 and HER4 induces homo- and heterodimerization of these receptors, catalytically activating a cascade of intracellular pathways involved in cellular proliferation, differentiation and migration. These reactions are induced by cytoplasmic signal transducers such as PLC-γ1, Ras-Raf-MEK-MAPKs, phosphatidylinositol-3 kinase (PI3K), Src or the signal transducers and activators of transcription (STATs). However, no ligand has been described for HER2, regardless of structural resemblance between *ERBB* receptors. In fact, HER2 has been identified as the preferred binding partner of the other *ERBB* receptors, in particular, of EGFR with formation of HER2/EGFR heterodimers with increased potential for signaling than EGFR homodimers (9). This unique characteristic of HER2 has been partially attributed to its increased flexibility due to a glycine-rich region following the alpha-helix C of HER2, which explains its low intrinsic catalytic activity and less stable conformation when activated (10). Consequently, HER2 overexpression potentiates EGFR signaling which relates to the increased response in EGFR-positive NSCLC with HER2 overexpression to erlotinib or gefitinib (11), specific inhibitors of active EGFR, but not of HER2 or inactive EGFR.

HER2 gene, regulated by overexpression and/or gene amplification, has been proven important in many cancers, including breast and gastric cancer, in which overexpression of HER2 confers poor prognosis although it relates to possible benefit from specific anti-HER2 therapy. With the arrival of trastuzumab, a humanized monoclonal IgG1 that targets the extracellular domain of HER2, and its effect in combination with cytotoxic chemotherapy on survival rates of breast and gastric cancer with overexpression of HER2, a new door in molecular-targeted therapy was opened. However, although HER2 overexpression and amplification has been described in 6-35% and in 10-20%, respectively, of NSCLC patients, the first clinical trials including patients treated with trastuzumab in addition to gemcitabine-cisplatin or to docetaxel, failed to demonstrate benefit in survival in HER2 IHC-positive patients (12,13).

These findings triggered investigation of activating mutations in the tyrosine kinase domain of HER2 gene, first described in 2004. HER2 mutations have been reported to exist in up to 4% of NSCLC and are more common in Asians, never smokers, women and adenocarcinomas (14), characteristically similar to patients with EGFR mutations. These mutations occur in the first four exons of the tyrosine kinase domain (exons 18-21), including the most frequently observed alteration, a 12-bp duplication/insertion of the amino acid sequence YVMA in exon 20 at codon 776 (HER^{YVMA}). The mutated region of exon 20 in the HER2

Table 1 Frequency of HER2 mutations among lung adenocarcinoma samples in recently published studies

Study group	Total (No.)	HER2 mutation (No.)	%
Tomizawa K *et al.* (*Lung Cancer* 2011)	504	13	2.58
Li C *et al.* (*J Thor Oncol* 2012)	224	8	3.57
Sun Y *et al.* (*J Clin Oncol* 2010)	52[‡]	2	3.85
Arcila M *et al.* (*Clin Cancer Res* 2012)	560	25	4.46
Zhang Y *et al.* (*Clin Cancer Res* 2012)	349[ǂ]	16	4.58
Cardarella S *et al.* (*J Thor Oncol* 2012)	276	13	4.71
Li C *et al.* (*PLos One* 2011)	202[‡]	12	5.94

[‡]Inclusion of adenocarcinoma samples of never-smokers only; [ǂ]Inclusion of adenocarcinoma samples of female never-smokers only.

gene corresponds to the nine codon region in exon 20 of the EGFR gene, where duplications and insertions have also been described, resulting in conformational changes of the tyrosine kinase domain that lead to narrowing of the ATP binding cleft and, consequently, increased kinase activity compared to wild-type receptors (HER^{WT}). *In vitro* studies have demonstrated that HER^{YVMA} induces ligand-independent transphosphorylation and stronger association with signal transducers that mediate cell proliferation, motility and survival processes than HER^{WT} (15). In fact, HER^{YVMA} activates EGFR in absence of ERBB ligands and EGFR kinase activity, which explains that EGFR TKIs erlotinib and gefitinib have no effect on EGFR and HER2 phosphorylation in HER^{YVMA} cells. However, when the effect of trastuzumab in cell proliferation was tested in these *in vitro* studies, inhibition was achieved in presence of HER^{YVMA} but not cells overexpressing HER^{WT}, findings consistent with the reported inability of the IgG1 to bind with EGF and or EGFR/HER2 heterodimers (16). Therefore, authors concluded that tumor cells harboring HER2 mutations are resistant to EGFR inhibitors although remain sensitive to HER2 inhibitors and dual EGFR/HER2 inhibitors.

Epidemiology of HER2 mutations in lung cancer

Up to date, few studies regarding HER2 mutations in NSCLC have been published, primarily in Asian patient populations in which never smokers constitute a greater percentage of lung cancer patients (approximately 30%) compared to North American and European populations (10%). Incidence of HER2 mutations has been reported in 2-5% of NSCLC adenocarcinomas (*Table 1*). In a retrospective study of pulmonary resection samples obtained at the Fudan University Shanghai Cancer Centre (17), a total of 202 patients, never smokers, with lung adenocarcinoma that had not received neoadjuvant chemotherapy, were included. The median age at diagnosis was 57.3 years and no significant differences were observed in age, stage or degree of tumor differentiation between males and females. Of these samples, 89.1% harbored known oncogenic driver mutations in EGFR (75.25%), HER2 (5.94%), ALK fusion (4.95%), KRAS (1.98%), ROS1 fusion (0.99%). Patients with no identified driver mutation were diagnosed at a younger age. 12 samples with HER2 kinase domain mutations were detected, including 11 exon 20 insertions and 1 L775P point mutation.

Recently, the Memorial Sloan Kettering Cancer Centre (MSKCC) group published the largest assessment to date of HER2 mutations in predominantly Caucasian population (18). Of 560 lung adenocarcinoma samples that resulted negative for EGFR and KRAS major mutations tested previously, 26 HER2 mutations in 25 cases were identified (5%), all mutually exclusive with point mutations in EGFR, KRAS, BRAF, NRAS, PI3KCA, MEK1 and AKT mutations as well as ALK rearrangements. No HER2 mutations were detected among 104 squamous cell carcinomas and 6 small-cell carcinomas tested. 92% (24/26) of these HER2 mutations were in-frame insertions in exon 20 (from 3 to 12 bp) between codons 775 and 881, of which the most common (83%) was the 12-bp duplication/insertion of YVMA at codon 775. The other two cases were point mutations, L775S and G776C. Median follow-up after diagnosis of advanced disease was 19 months for all patients. No significant differences in overall survival were described between HER2 and other molecular subsets. Morphologically, 92% were moderately or poorly differentiated adenocarcinomas. An additional analysis was performed to assess for HER2 gene copy number alterations by FISH in 11 HER2 mutated and 39

WT cases. None of HER2-mutant specimens were positive for HER2 amplification; 18% presented high polysomy (>4 copies of HER2 in >40% of cells) and 73% low polysomy. Amplification of HER2 was detected in one case, in the WT group, and interestingly this case was also found to harbor an EGFR exon 19 deletion. Therefore, HER2 mutation was not associated with concurrent HER2 amplification.

In this study, the overall prevalence of HER2 mutations was estimated to be approximately 2%, similar to statistics obtained in smaller European studies (19). In addition, HER2 mutations were most frequent among never-smokers (P<0.0001) although there were no associations with gender, race or stage of disease.

Therapeutic implications: HER2-targeted therapy in NSCLC

HER2 overexpression and gene amplification has been observed in breast, gastric and ovarian malignancies, inducing sensitivity to HER2-targeted drugs including trastuzumab, pertuzumab, lapatinib and T-DM1. Both amplification and high copy number gains have also been identified in NSCLC, although first clinical trials with anti-HER2 therapies in unselected patients failed to demonstrate survival benefit in HER2 positive NSCLC (defined by immunohistochemistry) (12,20). However, there is new hope that HER2 mutations may be more relevant in lung carcinogenesis than HER2 amplification or overexpression. Based on previous *in vitro* and *in vivo* studies, Cappuzzo *et al.* showed that lung cancer harboring the HER2 Gly776Leu mutation responded to treatment with trastuzumab and paclitaxel in a patient with chemotherapy-refractory lung adenocarcinoma (21).

Considering that HER2-mutant NSCLC may benefit from HER2 inhibition or dual EGFR/HER2 inhibition, but not single blockage of EGFR, novel TKIs simultaneously targeting EGFR/HER2 have been investigated. Transgenic mice models with induced expression in lung epithelium of the most common HER2 mutant, $HER2^{YVMA}$, developed lung adenosquamous carcinomas in distal and proximal bronchioles (22). In these models, treatment with erlotinib, trastuzumab, BIBW2992 and/or rapamycin revealed that the combination of BIBW2992 (afatinib), an irreversible dual TKI targeting both EGFR and HER2, and rapamycin, an inhibitor of the downstream effector protein mTOR, produced the most significant shrinkage (50.1±27.4% tumor regression measured by MRI) of tumor specimens. In addition, immunohistochemical analysis of these tumors treated with BIBW2992 and rapamycin proved this combination to be the most effective regimen for inhibition of upstream and downstream signaling of both the ERBB/PI3K/mTOR and the MAPK signaling pathways. Surprisingly, a relatively low effect was observed in $HER2^{YVMA}$ models treated with trastuzumab, with an average tumor regression of 13.59% (±10.89%), which was theoretically explained by postulating that trastuzumab is capable of inhibiting phosphorylation of membranous HER2 but unable to inhibit intracellular HER2 signaling associated with Golgi, endoplasmic reticulum, and other transport vesicles. Interestingly, continuous expression of $HER2^{YVMA}$ was proven necessary for tumor maintenance, indicating that HER2 is of great importance in lung adenosquamous tumorigenesis.

Case reports of afatinib in patients with HER2 mutant NSCLC have revealed promising results (23). Of patients who were included in an exploratory Phase II study of afatinib, five patients with non-smoking history and metastatic lung adenocarcinomas were identified to harbor HER2 mutations in cancer specimens. Three of these were evaluated, observing objective response to afatinib in all cases.

Neratinib, an irreversible pan *ERBB*-receptor family inhibitor, has been studied in a phase II trial in patients with advanced NSCLC who progressed following erlotinib or gefitinib (24). Three subgroups, EGFR mutant, wild-type EGFR and EGFR TKI naive- adenocarcinoma with light smoking history, were compared obtaining objective response rates of 3.4%, 0% and 0%, respectively. Only a small subgroup of patients with G719X mutation at exon 18 of EGFR-positive tumors, refractory to reversible TKIs, benefited from neratinib. Based on these results, neratinib is no longer in development for NSCLC although investigation in HER2-positive breast cancer continues.

PF00299804 (dacomitinib), another irreversible TKI targeting *ERBB* family members EGFR, HER2 and HER4, is being evaluated in patients with NSCLC. Preliminary data of dacomitinib in the HER2-mutant cohort reveal a 14% (3 of 22) partial response rate and 27% of these patients (6 of 22) have maintained stable disease to date (25).

In addition to TKIs, other molecules targeting EGFR and HER2 receptors have been developed. Considering that the heat shock protein 90 (Hsp90) chaperone stabilizes various oncogenic kinases necessarily involved in signal transduction and proliferation of lung carcinoma cells, when Hsp90 was demonstrated to interact with mutant EGFR, inhibition of these chaperones became a new potential therapeutic approach (26). NSCLC with

activating EGFR mutations that develop acquired resistance to EGFR TKI after treatment with erlotinib or gefitinib, have been proven sensitive to Hsp90 inhibitors both in NSCLC cell lines *in vitro* and *in vivo* (27). Other targets of Hsp90 include mutant HER2, mutant BRAF or mutant or overexpressed MET; therefore, adenocarcinomas harboring HER2 mutations may benefit from disruption of chaperone function. In fact, ganetespib, a novel non-geldanamycin potent Hsp90 inhibitor that impedes binding of Hsp90 to its co-chaperone, p23, has been proven effective in NSCLC cell lines in mice models driven by mutations in both EGFR and HER2^{YVMA} (28). These promising data support further investigation in clinical trials.

Conclusions

The discovery of oncogenic driver mutations in NSCLC is leading to the development of new therapies targeting specific molecular alterations. Detection of EGFR mutations and ALK rearrangements in tumor specimens of recently diagnosed NSCLC is currently standard of care, in order to identify subsets of patients that may respond to TKIs, such as erlotinib or gefitinib and crizotinib, respectively. Considering the prevalence of lung adenocarcinoma and clinical relevance of other mutations in NSCLC, including HER2, at diagnosis of this subgroup of lung cancer patients, we suggest expanding systematic genotype testing to include detection of these molecular alterations. In comparison with other types of cancer (i.e. breast, gastric) in which HER2 overexpression and gene amplification is associated to greater response to anti-HER2 drugs such as trastuzumab, first clinical trials in HER2 IHC-positive NSCLC failed to demonstrate benefit in the addition of trastuzumab to chemotherapy. However, HER2 mutations are thought to play a more significant role in lung cancerogenesis than overexpression or gene amplification, achieving promising results with trastuzumab in advanced HER2-mutant NSCLC. Therefore, identification of HER2 mutations, rather than HER2 IHQ-positive cancer specimens, should be studied in recently diagnosed stage IV NSCLC patients.

In addition, considering that cancer cells harboring HER2 mutations may respond to both HER2 inhibitors and dual EGFR/HER2 inhibitors, newer agents, including dacomitinib and afatinib, are currently under investigation in clinical trials specifically for this indication. Phase II studies have demonstrated promising initial results, although further investigation is necessary. Inhibition of chaperones to oncogenic kinases has revealed favorable results in preclinical models, constituting a new therapeutic strategy to be explored in both EGFR- and HER2-mutant NSCLC.

In summary, mutations in the tyrosine kinase domain of HER2 identify a subset of NSCLC adenocarcinomas, with a greater prevalence among never-smokers, which may respond to novel agents that specifically target this alteration. HER2 mutations are mutually exclusive with other driver mutations and are independent of HER2 gene amplification. Considering the prevalence of lung adenocarcinomas and given the availability of standard and investigational therapies targeting HER2, clinical genotyping of these tumors should include HER2.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10-29.
2. Peters S, Adjei AA, Gridelli C, et al. Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23:vii56-64.
3. Cetin K, Ettinger DS, Hei YJ, et al. Survival by histologic subtype in stage IV nonsmall cell lung cancer based on data from the Surveillance, Epidemiology and End Results Program. Clin Epidemiol 2011;3:139-48.
4. Petrelli F, Borgonovo K, Cabiddu M, et al. Efficacy of EGFR tyrosine kinase inhibitors in patients with EGFR-mutated non-small-cell lung cancer: a meta-analysis of 13 randomized trials. Clin Lung Cancer 2012;13:107-14.
5. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
6. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
7. Cardarella S, Ortiz TM, Joshi VA, et al. The introduction of systematic genomic testing for patients with non-small-cell lung cancer. J Thorac Oncol 2012;7:1767-74.
8. Jänne PA, Shaw AT, Pereira JR, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung

cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol 2013;14:38-47.
9. Lenferink AE, Pinkas-Kramarski R, van de Poll ML, et al. Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. EMBO J 1998;17:3385-97.
10. Aertgeerts K, Skene R, Yano J, et al. Structural analysis of the mechanism of inhibition and allosteric activation of the kinase domain of HER2 protein. J Biol Chem 2011;286:18756-65.
11. Cappuzzo F, Varella-Garcia M, Shigematsu H, et al. Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. J Clin Oncol 2005;23:5007-18.
12. Gatzemeier U, Groth G, Butts C, et al. Randomized phase II trial of gemcitabine-cisplatin with or without trastuzumab in HER2-positive non-small-cell lung cancer. Ann Oncol 2004;15:19-27.
13. Krug LM, Miller VA, Patel J, et al. Randomized phase II study of weekly docetaxel plus trastuzumab versus weekly paclitaxel plus trastuzumab in patients with previously untreated advanced nonsmall cell lung carcinoma. Cancer 2005;104:2149-55.
14. Li C, Sun Y, Fang R, et al. Lung adenocarcinomas with HER2-activating mutations are associated with distinct clinical features and HER2/EGFR copy number gains. J Thorac Oncol 2012;7:85-9.
15. Wang SE, Narasanna A, Perez-Torres M, et al. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. Cancer Cell 2006;10:25-38.
16. Agus DB, Akita RW, Fox WD, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell 2002;2:127-37.
17. Li C, Fang R, Sun Y, et al. Spectrum of oncogenic driver mutations in lung adenocarcinomas from East Asian never smokers. PLoS One 2011;6:e28204.
18. Arcila ME, Chaft JE, Nafa K, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Cancer Res 2012;18:4910-8.
19. Buttitta F, Barassi F, Fresu G, et al. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. Int J Cancer 2006;119:2586-91.
20. Langer CJ, Stephenson P, Thor A, et al. Trastuzumab in the treatment of advanced non-small-cell lung cancer: is there a role? Focus on Eastern Cooperative Oncology Group study 2598. J Clin Oncol 2004;22:1180-7.
21. Cappuzzo F, Bemis L, Varella-Garcia M. HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer. N Engl J Med 2006;354:2619-21.
22. Perera SA, Li D, Shimamura T, et al. HER2YVMA drives rapid development of adenosquamous lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy. Proc Natl Acad Sci U S A 2009;106:474-9.
23. De Grève J, Teugels E, Geers C, et al. Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. Lung Cancer 2012;76:123-7.
24. Sequist LV, Besse B, Lynch TJ, et al. Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:3076-83.
25. Kris M, Goldberg Z, Jänne P, et al. Dacomitinib (Pf-00299804), an Irreversible Pan-HER Tyrosine Kinase Inhibitor, for First-Line Treatment of EGFR-Mutant or HER2-Mutant or -Amplified Lung Cancers. Ann Oncol 2012;23:ix401-ix402.
26. Shimamura T, Shapiro GI. Heat shock protein 90 inhibition in lung cancer. J Thorac Oncol 2008;3:S152-9.
27. Kobayashi N, Toyooka S, Soh J, et al. The anti-proliferative effect of heat shock protein 90 inhibitor, 17-DMAG, on non-small-cell lung cancers being resistant to EGFR tyrosine kinase inhibitor. Lung Cancer 2012;75:161-6.
28. Shimamura T, Perera SA, Foley KP, et al. Ganetespib (STA-9090), a nongeldanamycin HSP90 inhibitor, has potent antitumor activity in in vitro and in vivo models of non-small cell lung cancer. Clin Cancer Res 2012;18:4973-85.

Cite this article as: Garrido-Castro AC, Felip E. HER2 driven non-small cell lung cancer (NSCLC): potential therapeutic approaches. Transl Lung Cancer Res 2013;2(2):122-127. doi: 10.3978/j.issn.2218-6751.2013.02.02

Targeting EML4-ALK driven non-small cell lung cancer (NSCLC)

Teresa Morán[1], Vanesa Quiroga[1], María de los Llanos Gil[1], Laia Vilà[1], Nuria Pardo[1], Enric Carcereny[1], Laia Capdevila[1], Ana M. Muñoz-Mármol[2], Rafael Rosell[1]

[1]Medical Oncology Department, Hospital Universitari Germans Trias i Pujol, Institut Català d'Oncologia-Badalona, Universitat Autònoma de Barcelona, Carretera de Canyet s/n, 08916 Badalona, Barcelona, Spain; [2]Pathology Department, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Carretera de Canyet s/n, 08916 Badalona, Barcelona, Spain

Correspondence to: Teresa Morán. Medical Oncology Department, Hospital Universitari Germans Trias i Pujol, Institut Català d'Oncologia-Badalona, Universitat Autònoma de Barcelona, Carretera de Canyet s/n, 08916 Badalona, Barcelona, Spain. Email: mmoran@iconcologia.net.

Submitted Feb 12, 2013. Accepted for publication Mar 07, 2013.
doi: 10.3978/j.issn.2218-6751.2013.03.04
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.03.04

Introduction

Recently, due to key discoveries relating to the molecular biology of many cancers and the development of effective and specific targeted treatments, the ability to personalize cancer therapy based on individual patient genotypes has become a reality in clinical practice (1). Some examples of this genotype-specific approach to anti-cancer therapeutics are BCR-ABL targeted therapy in chronic myelogenous leukemia, C-KIT inhibition in gastrointestinal stromal tumors, the use of Kristen rat sarcoma (KRAS) to negatively select EGFR inhibitors in colon cancer, HER2-directed therapy in breast cancer, and BRAF inhibitors in melanoma (2-13). Several other therapies are currently under investigation in clinical trials and will likely soon broaden this list further.

We have learned that there are different subsets of lung cancers that can be molecularly defined, targeted-treated and which exhibit differential outcomes in terms of response and survival when compared with tumors not harboring any specific mutations. The discovery of *EGFR* mutations in lung cancer represented the first event that marked this tremendous change in our understanding and management of lung cancer. Moreover, the discovery of the implications of *Anaplastic Lymphoma Kinase* (*ALK*) rearrangements in lung cancer has changed the paradigm of how we treat different subgroups of non-small cell lung cancer (NSCLC) patients (11,14).

ALK inhibitors are able to disrupt the signaling cascade related to cell survival, producing an apoptotic response (15,16). Crizotinib, an oral ALK inhibitor, has demonstrated a clinical benefit in this subset of patients that exceeds the usual expectations for this disease (13). Therefore, the inclusion of ALK screening in the molecular diagnosis of lung cancer is mandatory, considering that the frequency of ALK alterations has been reported to range from 2% to 25% of lung cancer patients between different series (1,2,17-24).

Some questions still remain a matter of debate. Firstly, which technique is most suitable to detect ALK alterations? Secondly, which patients should be included in screening programs? Thirdly, how should the sequence of available therapies be administered to these patients and, lastly, how can we understand the mechanisms of resistance that all patients invariably ultimately develop to ALK inhibitors?

ALK in lung cancer

Although *ALK* mutations do occur, the majority of ALK-positive tumors induce the aberrant signal through the formation of fusion genes. *ALK* rearrangements were initially identified in anaplastic large cell lymphoma. Since then, this alteration has been described in other tumors such as inflammatory myofibroblatic tumors, neuroblastoma and NSCLC, among others (11,25-29). These rearrangements induce a chimeric protein with ligand-independent tyrosine kinase activity that acts through different signaling pathways, such as RAS/MEK/ERK which are related to the proliferative effect, and PI3K/AKT y JAK3/STAT3 which are involved in cell survival (16,30,31).

Up to eleven different variants of *ALK* chromosomic rearrangement have been described. *Echinoderm microtubule associated protein like-4* (*EML4*) represents the most frequent partner for ALK in lung cancer. *Figure 1* shows the general

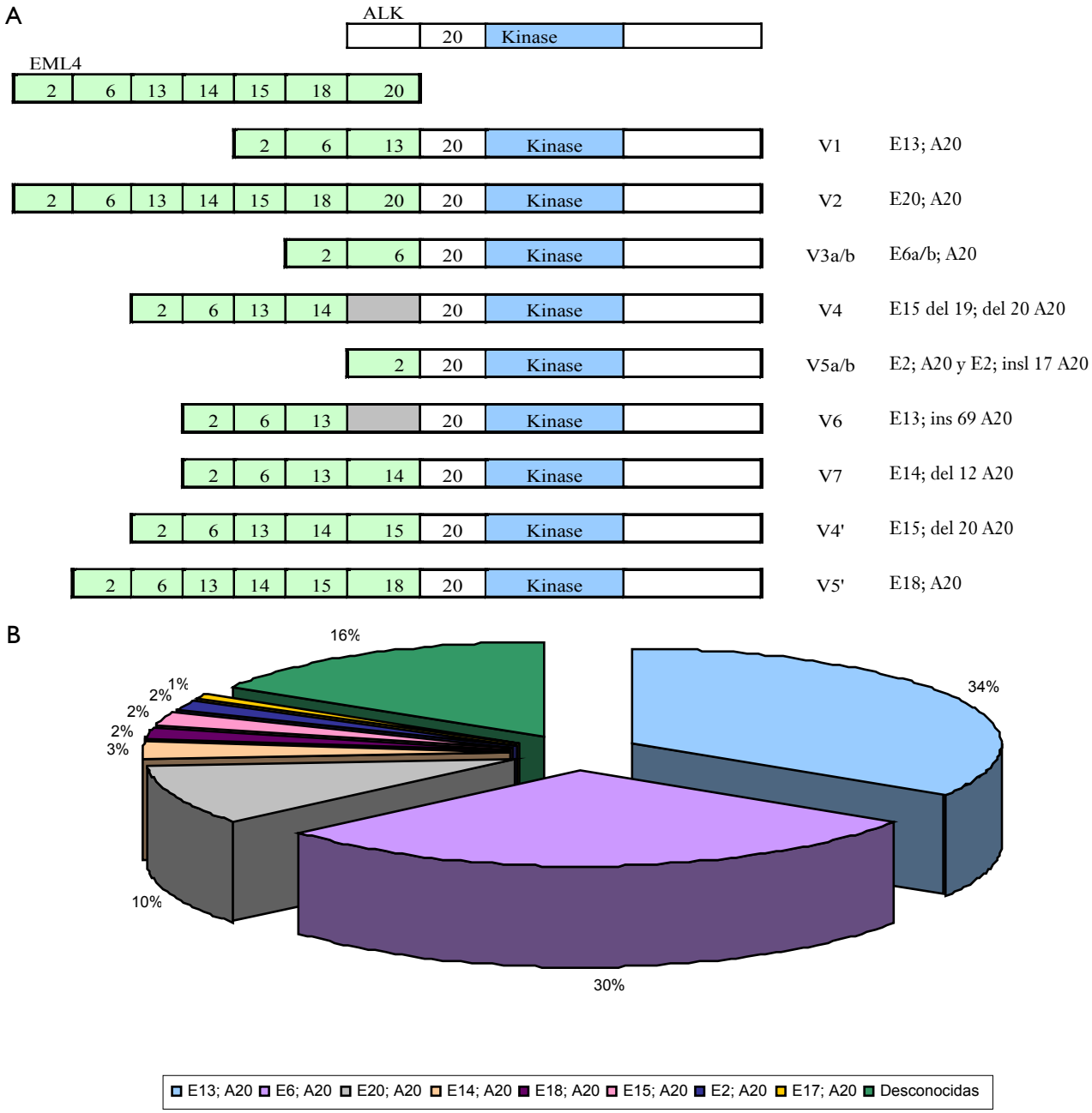


Figure 1 A. Distribution of different fusion gene variants of EML4-ALK described up to date. ALK fusion emerges on exon 20 of the kinase. Alternative variants depend on different EML4 cut points; B. Frequency of different EML4-ALK variants (11,15,17-21,32). Ins, insertion; V, variant.

distribution of *EML4-ALK* rearrangement depending on different exons of *EML4* present in the fusion forms. Other partners for *ALK* are *TFG* and *KIF5B* (30,32,33).

The presence of *ALK* rearrangements has more frequently been associated with certain clinical and pathological features, including adenocarcinoma histology (especially cribiform, signet-ring cells and solid patterns), never or light smoking history and male gender (*Table 1*). More importantly, wild type (WT) status for *EGFR* and *KRAS* mutations represents a more suitable criteria for ALK screening since simultaneous overlapping with other oncogenic driver mutations is uncommon (37,38). When considering these features,

Table 1 Summary of different studies reporting ALK positive results: results considering clinical, pathological and molecular criteria

	Clinical and pathological features	General frequencies	ALK + results by subgroups
Soda 2007 (11)	Never smokers *vs.* smokers	27.3% *vs.* 72.7%	11.1% *vs.* 8.3%
n=33	Adenocarcinoma *vs.* other	54.5% *vs.* 45.4%	5.5% *vs.* 13.3%
Japanese population	Male *vs.* female	66% *vs.* 33%	9.15% in both groups
	Age	NR	NR
Inamura 2008 (17)	Never smokers *vs.* smokers	43.6% *vs.* 56.4%	4.6% *vs.* 2.4%
n=149	Adenocarcinoma *vs.* other	67.4% *vs.* 32.6%	3.4% *vs.* 0%
Japanese population	Male *vs.* female	54% *vs.* 46%	2.5% *vs.* 4.3%
	Age	63.4	59.4
Shinmura 2008 (18)	Never smokers *vs.* smokers	35% *vs.* 65%	0% *vs.* 4.8%
n=77	Adenocarcinoma *vs.* other	65% *vs.* 35%	2% *vs.* 0%
Japanese population	Male *vs.* female	50.6% *vs.* 49.4%	2.9% *vs.* 2.6 %
	Age	64.3	54
Inamura 2009 (20)	Never smokers *vs.* smokers	41.5% *vs.* 58.1%	5.7% *vs.* 3.4%
n=363	Adenocarcinoma *vs.* other	69.7% *vs.* 30.3%	4.3% *vs.* 0%
Japanese population	Male *vs.* female	53% *vs.* 47%	3.7% *vs.* 5.1%
	Age	64	56
Shaw 2009 (12)	Never smokers *vs.* smokers	60% *vs.* 40%	23.7% *vs.* 6.1%
n=141	Adenocarcinoma *vs.* other	63% *vs.* 37%	17.9% *vs.* 5.8%
Clinical selection	Male *vs.* female	66% *vs.* 34%	22.9% *vs.* 8.6%
	Age	63	52
Wong 2009 (19)	Never smokers *vs.* smokers	53% *vs.* 47%	8.5% *vs.* 0.8%
n=266	Adenocarcinoma *vs.* other	78.6% *vs.* 21.4%	6.2% *vs.* 0%
Chinese population	Male *vs.* female	50.4% *vs.* 49.6%	1.9% *vs.* 3%
	Age	64	59
Rodig 2009 (34)	Never smokers *vs.* smokers	25.4% *vs.* 74.6%	15.4% *vs.* 6%
n=358	Adenocarcinoma *vs.* other	100% *vs.* 0%	5.6% *vs.* 0%
US	Male *vs.* female	25.9% *vs.* 74.1%	11.8% *vs.* 8.4%
	Age	66	51
Martelli 2009 (21)	Never smokers *vs.* smokers	13.3% *vs.* 86.7%	6.25% *vs.* 7.9%
n=120	Adenocarcinoma *vs.* other	52.5% *vs.* 47.5%	4.76% *vs.* 10.5%
Italy, Spain	Male *vs.* female	80% *vs.* 20%	8.3% *vs.* 4.1%
	Age	67	64
Camidge 2010 (23)	Never smokers *vs.* smokers	60% *vs.* 40%	39.4% *vs.* 0%
n=66	Adenocarcinoma *vs.* other	92.4% *vs.* 7.5%	21.3% *vs.* 0%
Caucasian, Hispanic	Male *vs.* female	NR	5M, 9F
	Age	NR	53
Salido 2011 (24)	Never smokers *vs.* smokers	15% *vs.* 85%	0% *vs.* 3.2%
n=107	Adenocarcinoma *vs.* other	65% *vs.* 35%	2.8% *vs.* 2.6%
Spain and US	Male *vs.* female	77% *vs.* 23 %	2.43% *vs.* 4%
	Age	66	73
Paik 2011 (35)	Never smokers *vs.* smokers	37.7% *vs.* 62.3%	5.8 % *vs.* 3.2%
n=465	Adenocarcinoma *vs.* other	58.1% *vs.* 41.9%	6.8% *vs.* 0.8%
Chinese population	Male *vs.* female	68.2% *vs.* 31.8%	3.6% *vs.* 5.5%
	Age	NR	48.7
Yi 2011 (36)	Never smokers *vs.* smokers	NR	NR
n=101	Adenocarcinoma *vs.* other	NR	100%
Japanese population	Male *vs.* female	NR	5M, 5F
	Age	NR	56
Kwak 2010 (13)	Never smokers *vs.* smokers	NR	76% *vs.* 24%
n=82	Adenocarcinoma *vs.* other	NR	96% *vs.* 4%
Molecular selection	Male *vs.* female	NR	52% *vs.* 48%
	Age	NR	43
Shaw 2011 (30)	Never smokers *vs.* smokers	42.5% *vs.* 54.5%	40% *vs.* 9.2%
n= 412	Adenocarcinoma *vs.* other	91.5% *vs.* 8.5%	23.3% *vs.* 11.42%
Molecular selection	Male *vs.* female	41.5% *vs.* 58.5%	27% *vs.* 19.6%
	Age	59.3	51

n, number of patients included; NR, not reported; *vs.*, versus.

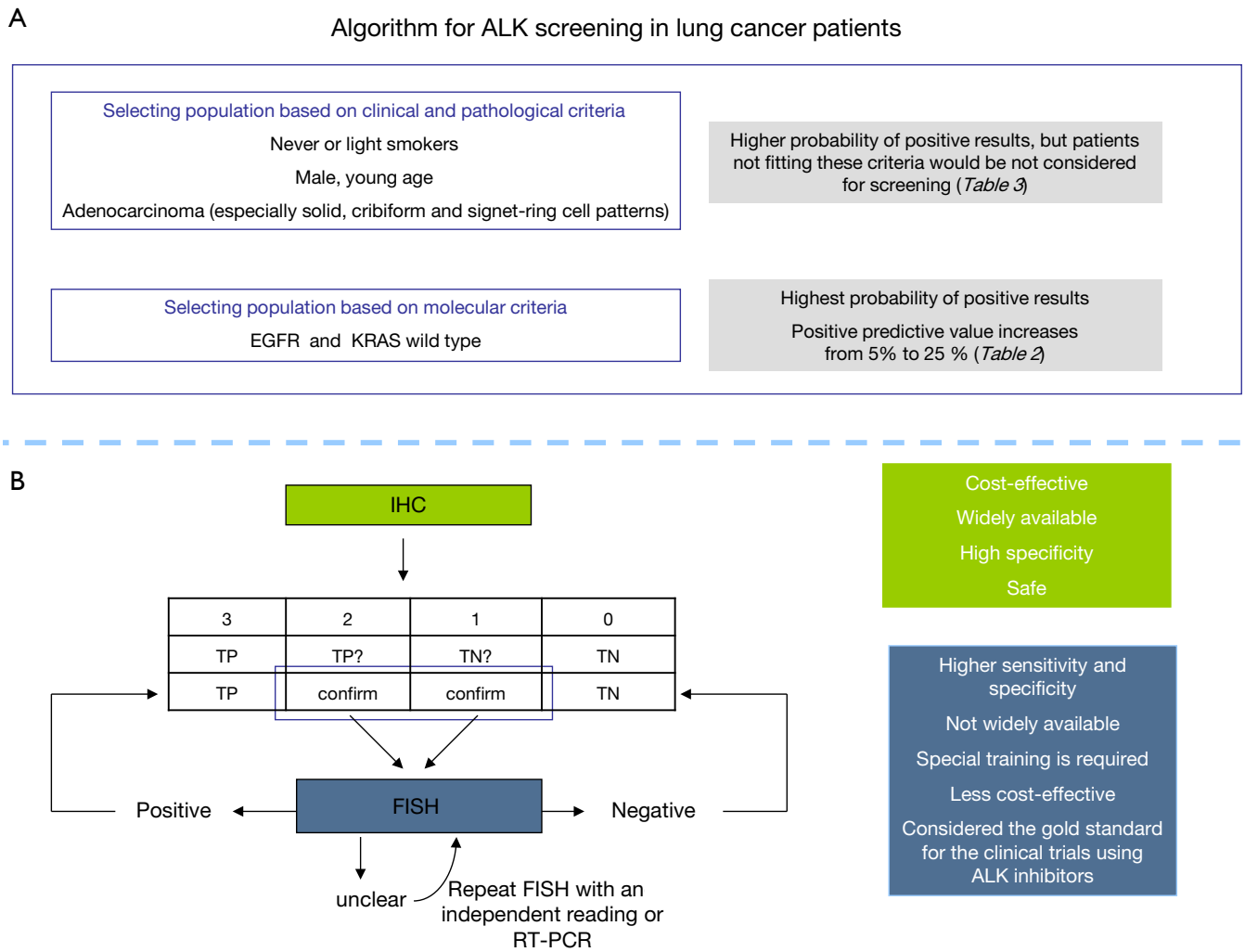


Figure 2 Algorithm for ALK screening in lung cancer patients. (A) Selection of patients to be included in the screening, based on clinical-pathological and molecular criteria. (B) Proposal for different techniques to be used in a large screening program. EGFR, Epidermal Growth Factor Receptor; PPV, positive predictive value; RT-PCR, Reverse transcription polymerase chain reaction; TN, true negative; TP, true positive.

especially molecular selection, the likelihood of detecting an *ALK* rearrangement increases from 2-10% in the general population to 24-40% in this molecularly selected population, according to different series (see References and data in *Table 1*). Thus, the criteria for ALK screening should include the prior negative result of screening for *EGFR* and *KRAS* mutations, primarily avoiding the use of clinical and pathological characteristics (*Figure 2A*). Importantly, we should consider that frequencies of *ALK* rearrangements in other subgroup of patients, such as heavy smokers and other histology subtypes different to adenocarcinoma, are still only anecdotic.

Currently, three different techniques are available for detecting *ALK* rearrangement, though which of these is the most convenient is still a matter of debate. Consideration needs to be given to the characteristics required for a diagnostic tool to become the technique of choice for large scale screening programs, such as high sensitivity and especially high specificity to detect real true positive cases and thus avoid the need for additional procedures. Moreover, this technique needs to be cost-effective and widely available (*Table 2*). However, when considering the specific use of the ALK inhibitor crizotinib in ALK-positive patients, fluorescence in situ hybridization (FISH) has been considered to be the gold standard for detecting ALK rearrangements, using the ALK Vysis LSI ALK Dual Color

Table 2 Advantages and disadvantages of different techniques used to detect ALK rearrangements

	RT-PCR	FISH	IHC
Advantages	High sensitivity	High specificity	Easy reading
	Quick method	PETT is suitable for this technique	Quick method
		Possibility of detection of new promoters	Lower cost
		Gold standard technique for the clinical trials using ALK inhibitors	Possibility of detection of new variants
			Detection of all rearrangements, no specific promoter is required
			Widely available
			Commercialized antibodies
Disadvantages	High quality and enough RNA quantity is required	Lower sensitivity	The fusion gene is indirectly detected by the protein expression
	Difficult to obtain RNA from small biopsies	Expertise in interpreting the results	Risk of false negative results
	Potential degradation of RNA in PETT	Risk of false negative results	Results can vary according to type and dilution of the antibody and reading method
	No new promoters are detected	No widely available	Compared to other tumors, the protein expression can be weaker in lung cancer (risk of false negative)
	No widely available	More time consuming	Reading method has been adapted from EGFR and HER2 score systems
		Higher cost	

PETT, paraffin embedded tumor tissue.

Break Apart Rearrangement Probe (Abbott Molecular, Abbott Park, IL). Other regulatory agencies admit the use of other diagnostic techniques, as in Japan and Europe.

FISH confers higher sensitivity and specificity when compared to real time-PCR (RT-PCR) and immunohistochemistry (IHC). However, FISH is not widely available and is less cost-effective than other techniques. The algorithm these authors propose would include the use of IHC for the first analysis; results scored as 0 and 3 could be considered as true negative and true positive, respectively. However, for results scored as 2 and 1, a confirmatory test should be performed since these two groups accumulate the highest rates of false negative and false positive results (*Table 3*). This algorithm includes confirmation by FISH and RT-PCR (*Figure 2B*).

Current status of ALK inhibition in lung cancer: crizotinib trials (*Table 4*)

Since clinical practice currently differs from country to country, it is necessary to review data from different clinical trials to understand these differences, in particular how access to different drugs depends on patients' regional backgrounds.

Crizotinib (PF-2341066; XALKori, Pfizer, New York, NY) is an oral small-molecule with tyrosine kinase inhibitor (TKI) properties of both *MET* and ALK (46). The fast approval of crizotinib in the US was based on the results of a phase I trial expansion cohort which included ALK-positive NSCLC patients (13) in which a total of 82 patients were treated. This trial demonstrated that crizotinib was an effective agent in this subset of patients with an overall response rate of 57% (56% confirmed partial responses and 33% stable disease). The estimated probability of 6 months progression-free survival (PFS) was 72%. Additionally, crizotinib was confirmed as a safe drug. The majority of adverse events were grade 1 and 2 gastrointestinal disorders (13). Based on these results, the FDA approved the use of crizotinib in NSCLC patients harboring *ALK* rearrangements independently of any prior treatment the patient had received. A more recent analysis of patients included in this expansion cohort (n=119) confirmed the previous findings: response rate was 61% and response occurred independently of clinical features such as age, gender, number of previous therapies and performance status. The median PFS was 10 months, and the estimated overall survival rates at 6 and 12 months were

Table 3 Summary of trials reporting the results of different techniques used for detecting ALK rearrangements

	Number of samples	Population	Technique	Positive results for ALK	Confirmation	Other interesting data
Soda 2007 (11)	33	Japanese, no other criteria	RT-PCR	9.10%	No	Detection of other variants, utility of cytology samples
	42	Japanese, no other criteria	RT-PCR	4.80%	No	Detection of other variants, utility of cytology samples
Inamura 2008 (17)	149 adeno (221 NSCLC)	Japanese, no other criteria	RT-PCR	3.4% in adeno; 2.3% in NSCLC	IHC, DAKO ALK1 1:20	100% of concordance with IHC; 2 variant 1 y 3 variant 2 Variant 1 in a mixed adeno (papillary and BAC) Variant 2 in acinar adenocarcinoma Exclusion of *EGFR* and *KRAS* mutations
Shinmura 2008 (18)	77	Japanese, no other criteria	RT-PCR	2.60%	No	No other variants Variant 1 y variant 2 (2 cases) Both positive results in adeno and smoking history Exclusion of *EGFR* and *KRAS* mutations, one case associated with *p53* mutation
Inamura 2009 (20)	253 adeno (363 NSCLC)	Japanese, no other criteria	IHC, DAKO ALK1 1:20	4.3% in adeno: 3.1% in NSCLC	RT-PCR	5 cases in adeno and 0 cases in other histologies Predominance in acinar adeno (54.5%) Predominance in never smokers (63.6%) Exclusion of *EGFR* and *KRAS* mutations, one case associated with *p53* mutation IHC SE 100%, SP N/R
Wong 2009 (19)	266	Chinese, no other criteria	RT-PCR	6.2% adeno, 4.9% in NSCLC	IHC, DAKO ALK1 1:1000	All cases adeno, 90,9% never smokers Exclusion of *EGFR* and *KRAS* mutations, one case associated with *p53* mutation *EGFR* and *KRAS* mutations are negative, the proportion of ALK positive results is 1.8% in never smoker males and 6.5% in never smoker females
Shaw 2009 (12)	141	Clinical selection	FISH Vysis	11.1%	IHC, DAKO ALK1, RT-PCR	At least 2 clinical criteria for selection: Asian population, adenocarcinoma, female, never smoking history. More frequent in male, adenocarcinoma (predominance in signet-ring cells), younger patients and never smoking history. Similar response to chemotherapy and lower response to TKI compared to *EGFR* and *KRAS*-mutant patients. 89% of ALK positive results in stage IV NSCLC Exclusion of *EGFR* and *KRAS* mutations
Rodig 2009 (34)	358	Clinical and pathological selection	DAKO ALK1 ALK1 1:2	5.6%	FISH	ALK positive results more frequent in younger patients, solid and signet-ring adenocarcinoma and more advanced stages. IHC SE 80 an 40% with and without tiramin amplification *vs.* FISH S 95% Exclusive with *EGFR* mutations
Martelli 2009 (21)	120	Italy, Spain	DAKO ALK1 ALK1, ALKc (SP8) y 5A4	7.5%	FISH, RT-PCR	IHC SE 0% and SP 0% (ALK detection in areas distant to the tumor)
Boland 2009 (39)	35	Clinical and pathological selection	DAKO ALK1, ALK1 1:100	2%	FISH, RT-PCR	SE100% and SP100% (validated in an independent cohort of 335 NSCLC cases)

Table 3 (*continued*)

Table 3 (*continued*)

	Number of samples	Population	Technique	Positive results for ALK	Confirmation	Other interesting data
Takeuchi 2009 (32)	130	Japanese, no other criteria	ALK1, 5A4	6.15%	RT-PCR	IHC SE 100% and SP 100% for both techniques iAEP method used for interpreting the IHC results iAEP and PCR improve the detection rates for new ALK variants.
Mino-Kenudson 2010 (40)	153	US Clinical and pathological selection	DAKO ALK1 ALK1 1:50, 1:2 D5F3	14.4%	FISH, RT-PCR	ALK-protein expression is lower in lung adenocarcinoma, risk of FN results. Use of new Ab at a higher concentrations improves SE with no effect in SP. ALK1 SE 67% y SP 97% *vs.* D5F3 SE 100% y SP 99%
Ros-Camidge 2010 (23)	61 adeno (66 NSCLC)	Caucasian, Hispanic	FISH Vysis	21.3% (19.7%)	No	Positive results in 100% adeno and 60% never smokers 1 case with concomitant *EGFR* mutation (exon 20) 0% concomitant *KRAS* mutations No concomitant MET amplification. FISH SE and SP improve to100% when at least 4 tumor areas are analyzed ALK positive result in 54% of cases when sampling tumor area *vs.* 6.8 % in areas adjacent to the tumor area, in ALK positive tumors. ALK positive result in 6% of cases when sampling tumor area *vs.* 6 % in areas adjacent to the tumor area, in ALK negative tumors.
Kwak 2010 (13)	82 de 1500	Molecular selection	FISH Vysis	5.4%	RT-PCR, IHQ (retrospective)	Clinical benefit of crizotinib: RR 57%, SD 33%, PFS rate at 6 m72%
Salido 2011 (24)	107	Spain, US, no other criteria	FISH Vysis	3%	IHQ, DAKO ALK1	2 cases EML4-ALK, 1 case ?-ALK IHC positive in 2 cases EML4-ALK and negative in ?-ALK case FISH: 63% increase GCN y 17% ALK amplification. Unknown predictive value
Paik 2011 (35)	465	Korean	IHQ, 5A4 1:30	8.6%	FISH Vysis	FISH positive in 19/453 (4.2%) FISH is concordant with IHC when score 3, 1 and 0. FISH is variable with score 2. SE and SP of IHC 100% and 95.8%, respectively. FP IHC 1.5% Exclusion of *EGFR* and *KRAS* mutations
Yi 2011 (36)	101	Japanese, clinical selection	DAKO ALK11 1:100	9.9%	FISH Vysis	IHC SE 90% and SP 97. 8% FN rate 10% and FP rate 2.2% using IHC IHC is a good initial screening technique but intermediate scores need to be confirmed

Table 3 (*continued*)

Table 3 (*continued*)

	Number of samples	Population	Technique	Positive results for ALK	Confirmation	Other interesting data
Shaw 2011 (41)	92 ALK+ *vs.* 320 ALK-	Molecular selection	FISH Vysis	22.3%	RT-PCR, IHQ (retrospective)	ALK predictive but not prognostic value ALK positive results are more frequent in male, adenecarcinoma, younger patients, never smokers and Caucasian population

Adeno, adenocarcinoma; ALK+, presence of ALK rearrangement; BAC, bronquioloalveolar carcinoma; FN, false negative; FP, false positive; GCN, gene copy number; IHC, inmunohistochemistry; m, months; N/R, no reported; RT-PCR, reverse transcription polymerase chain reaction; PFS, progression-free survival; RR, response rate; SE, sensitivity; SD, stable disease; SP, specificity; TKI, tyrosin kinase inhibitors. Brand names for different antibodies and probes: DAKO Mouse Monoclonal Anti-Human CD246, ALK Protein Clone ALK1 (Dako, Dermank and CA); D5F3 Rabbit monoclonal anti-human CD246, clones D5F3 and D9E4, Cell Signaling Technology, Danvers, MA; 5A4 Mouse monoclonal anti CD246, clone 5A4, Novocastra, Newcastle, UK; LSI ALK (Abbott) ALK Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular, Abbott Park, IL.

Table 4 Summary of the clinical trials reporting the efficacy results with crizotinib in ALK positive patients

	pI (12,42)	pII (43,44)	pIII (45)		
	crizotinib	crizotinib	crizotinib	Chemotherapy (PEM+DOC)	
n	82 [119]	135 [261]	173	174	
Overall RR (%)	61%	51%	65%	20% (PEM29%; DOC6.9%)	P<0.001
Duration of response (median, weeks)	48	42.9			
Duration of treatmente (median, weeks or cycles)	32 w	22 w	11 cycles	4 cycles	
6 months PFS	72%	NR	NR	NR	
mPFS (median, months)	NR	8.1 (6.8-9.7)	7.7	3 (PEM4.2; DOC2.6)	HR 0.49 (0.37-0.64), P<0.0001
mOS	NR	NR	20.3	22.8	HR 1.02 (0.68-1.5), P=0.5394
OS rates 6 m, 12 m	NR	90%, 81%	NR	NR	

DOC, docetaxel; m, months; m-PFS, median progression-free survival; mOS, median overall survival; n, number of patients included; PEM, pemetrexed; NR, no reported; RR, response rate; w, weeks.

90% and 81%, respectively (42).

Similar results were obtained from patients included in the PROFILE 1005, a phase II single-arm study to evaluate the efficacy and safety of crizotinib in pretreated NSCLC patients harboring *ALK* rearrangements. A total of 136 patients received crizotinib in second line (9.6%), third line (27.2%) and forth line (27.2%). Thirty six percent of patients had received more than 4 previous lines of treatment. This study demonstrated an overall response rate of 50% for a heavily pretreated population. Except for Asian patients, no other clinical characteristics influenced response, with similar benefit regardless of smoking history, performance status and previous treatment exposure (43).

Notably, standard, second line, single-agent treatments for unselected patients with advanced NSCLC achieve an overall response rate of less than 10% and PFS of less than 3 months (47,48).

An up-to-date analysis for patients included in the PROFILE 1005 trial, in which more than 900 patients were treated, has been reported (44). The first 261 patients had received treatment with a median duration of 48 weeks and had been considered as mature population. The results were consistent with those previously reported. The overall response rate was 60% (54-66%) with median duration of response of 46 weeks (35-54 weeks) and PFS was 8.1 months (6.8-9.7 months). Fifteen percent of patients discontinued crizotinib and 10% had a dose reduction due to an adverse event. The most frequent adverse events were vision disorders (54%), nausea (51%), diarrhea (44%), vomiting (44%), and constipation (37%), which were mostly grade 1 and 2 (44).

Since most of ALK-positive patients currently receive crizotinib at some point during treatment, in the absence of data from a randomized controlled trial, the effect

of this drug on overall survival remains unclear. Thus, a retrospective comparison to evaluate the impact of crizotinib on overall survival has been reported. Patients with advanced NSCLC from 3 patient cohorts were included in this analysis: 82 ALK-positive patients treated with crizotinib from the expansion cohort of a phase I trial of crizotinib, 36 ALK-positive controls who did not receive crizotinib and 253 ALK-negative/*EGFR*-negative patients. Among the ALK-positive patients treated with crizotinib, median overall survival from initiation of crizotinib was not reached and overall survival did not differ with age, gender, smoking exposure, or ethnic background. Overall survival in the ALK-positive crizotinib-naïve controls was similar to that in the entire cohort. However, overall survival was significantly improved in patients receiving crizotinib as second or third line therapy, compared with crizotinib-naïve patients receiving any other second line therapy (49).

Patient-reported outcomes of disease- and treatment-related symptoms, quality of life (QoL), and health status have been reported in the PROFILE 1005 trial (50). Data for symptom scores and QoL from the first 136 patients for whom efficacy and safety data are available have been presented (43,50,51). The results indicate that patients receiving crizotinib presented clinically meaningful and statistical (≥10-point change and P≤0.05, respectively) improvements in some symptoms from baseline. There were clinically meaningful improvements in pain, dyspnea, and cough from cycle 2, and in fatigue from cycle 5, and these improvements were maintained through subsequent cycles (49). Moreover, global QoL was maintained throughout treatment with crizotinib with clinically meaningful improvement at cycle 7 (51). Significant reductions in pain (50), dyspnea, cough, fatigue, insomnia, and alopecia symptom scales were maintained with therapy (51). Improvement in mean QoL was also reported but changes were not clinically significant, indicating that QoL was stable with more cycles of treatment (50). Clinical meaningful improvements were observed for physical, role and social functioning and for global QoL (51,52).

Recently, results for the PROFILE 1007 study have been reported (45). This large phase III trial (n=347) compared crizotinib *vs.* chemotherapy in ALK-positive patients previously treated with a prior chemotherapy regimen including a platinum-doublet. Patients were randomized to receive crizotinib or chemotherapy (pemetrexed or docetaxel, depending on the previous therapy). Those patients assigned to the chemotherapy arm were allowed to receive crizotinib when progression occurred. This crossover occurred in 62% of patients initially assigned to receive chemotherapy. The study met its primary endpoint, with a difference in PFS in favor of crizotinib [7.7 *vs.* 3 m, HR (95% CI), 0.49 (0.37-0.64), P<0.0001]. Response rate significantly favored crizotinib, with 65% of responses in the crizotinib arm *vs.* 20% in the chemotherapy arm (pemetrexed 29% and docetaxel 6.9%, P<0.0001). Interim analysis of overall survival (when 28% of survival events had occurred) showed no statistically significant difference between crizotinib and chemotherapy with a preliminary estimated median OS of 20.3 *vs.* 22.8 months; HR 3.02; 95% CI 0.68-1.5, P=0.5394), but not adjusted for crossover. The most frequent adverse events related to crizotinib were visual disturbances (59%), diarrhea (53%), nausea (52%), vomiting (44%), and elevated transaminases (36%). Frequent adverse events with chemotherapy were nausea (35%), fatigue (29%), decreased appetite (21%), and alopecia (20%). The incidence of grade 3-4 adverse events was similar in both arms (31%). Duration of treatment was longer for crizotinib *vs.* chemotherapy with a median number of administered cycles of 11 *vs.* 4, respectively (45). Crizotinib offered clinically meaningful and statistical (P<0.001) improvements in some symptoms from baseline. There were improvements in cough, dyspnea, fatigue, alopecia, insomnia, and pain. Moreover, global QoL as well as physical, role, emotional, cognitive and social functioning favored crizotinib over chemotherapy (P<0.001) (45).

This data clearly establish that crizotinib is superior to standard second line chemotherapy, usually with docetaxel and pemetrexed which were the comparators in this trial. This superiority was confirmed in terms of prolonging PFS and improving response rate, as well as improving patient symptoms and QoL.

Results from the currently ongoing PROFILE 1014 study (Clinicaltrials.gov identifier NCT01154140) comparing first line crizotinib *vs.* chemotherapy are expected to elucidate whether, mirroring the experience with EGFR-TKIs in *EGFR*-mutant lung cancer, the ALK inhibitor is a better strategy when administered upfront (53-57).

Beyond crizotinib

Despite the good activity and tolerability profile of crizotinib for treating ALK-positive patients, several molecules have been being tested to evaluate newer regimens with a more desirable toxicity profile and more convenient administration schedules for patients, though

without jeopardizing clinical activity. Moreover, patients with initial good responses to crizotinib invariably develop resistance. Therefore, further therapies are required when resistance occurs.

Based on the previous experience with *EGFR*-mutant NSCLC, mutations affecting the kinase domain of ALK were expected to mediate resistance to crizotinib. In fact, the first report of the presence of such mutations was published along with the first results of crizotinib activity in ALK-positive NSCLC (13,58). The presence of two different kinase domain mutations, L1196M and C1156Y, occurred in different clones from the same patient. Other resistant mutations have been reported to date (L1152R, G1269A, S1206Y, G1202R and 1151 Tins) with further mutations already identified. Collectively these mutations can mediate crizotinib resistance in ALK-positive tumors (59-61). These findings are in contrast with the experience in EGFR, in which resistance is mainly mediated by the emergence of a predominant mutation, T790M, and other secondary mutations are rare (62,63). Furthermore, different *ALK* mutations identified so far have shown a differential spectrum of sensitivity to crizotinib and other ALK inhibitors, suggesting that not all the newer ALK inhibitors may be equally effective in treating ALK-positive patients who develop resistance to crizotinib (60,64,65).

Other mechanisms implicated in ALK resistance have been described. These include, firstly, the copy number gain of the ALK gene fusion, which occurs simultaneously with resistant mutations (61,66). Secondly, the presence of other oncogenes that may become active via mutation or other mechanism and coexist with ALK, such as EGFR, HER2 or KIT (59-61,63). Thirdly the emergence of a separate clone that harbors other oncogenes different to ALK, such as *EGFR* or *KRAS* (61). Additionally, the underexposure of the Central Nervous System (CNS) to crizotinib may partly underlie this resistance and warrants consideration for the development of newer ALK inhibitors that can attain optimal concentration in the cerebrospinal fluid (67).

LDK378 is a next generation ALK inhibitor able to inhibit both ALK and the C1156Y variant. Results of the first in-human phase I trial have been recently reported (68). Fifty-six ALK-positive patients were included (50 patients with ALK-positive lung cancers). LDK378 was administered orally once-daily, starting at 50 mg/day. Of 47 patients evaluable for response, 24 (51%) responded and all responses were in ALK-positive NSCLC patients. Twenty one (81%) of 26 patients who had progressed to crizotinib and were treated at a dose level of ≥400 mg/day responded. The maximum tolerated dose was 750 mg/day. Dose limiting toxicities included diarrhea, vomiting, nausea, dehydration, and ALT elevation. The most frequent grade 3 side effect was diarrhea, which occurred in 5 (9%) patients. However, the most common side effects (all grades) were nausea (59%), vomiting (54%) and diarrhea (48%). Some activity has been reported in CNS metastases, which suggests good penetration in the cerebrospinal fluid.

CH5424804 is a next generation ALK inhibitor able to inhibit ALK as well as the C1156Y and L1196M variants. Recently communicated results of a phase I/II trial demonstrated very promising activity in crizotinib-naïve ALK-positive NSCLC with a response rate of 85% and range of duration of treatment from 2-46 weeks. Thirty four patients were enrolled in the trial and CH5424804 was administered at 300 mg twice-daily. The majority of patients remain on treatment at the time of this communication. The main treatment-related adverse events were ALT, AST and bilirrubin elevation (7, 6 and 3 patients, respectively), neutropenia (5 patients, 2 grade 3), rash (4 patients), nausea (4 patients), and myalgia (3 patients) which were mostly grade 1 except for neutropenia (2 cases were grade 3). Only one patient presented a treatment-related eye disorder and was grade 1. No dose reductions were necessary due to side effects. Activity in CNS metastases was shown (69).

AP26113 is a novel, synthetic, orally-active TKI that inhibits mutant forms of ALK and EGFR, as well as TKI-resistant forms such as L1196M (ALK) and T790M (EGFR) (66). This drug does not inhibit the native form of EGFR. Results of the first in-human phase 1/2 trial have been recently reported (70). A total of 34 patients were included in the dose-finding phase, starting at a dose of 30 mg/day. Twenty-seven patients had lung cancer (11 ALK-positive patients, 11 *EGFR*-mutant patients and 5 WT for ALK and EGFR). Nine ALK-positive patients were crizotinib-resistant, while 2 were crizotinib-naïve. Among the ALK patients, 8 partial responses were recorded, 6 among the crizotinib-resistant patients and 2 among crizotinib-naïve patients. The initial doses of 60 and 90 mg/day were sufficient to achieve some of these partial responses. The more frequent side effects were nausea (32%), diarrhea (18%, 3% of grade 3), loss of appetite (12%), fatigue (26%, 3% of grade 3), and vomiting (12%). Four (12%) patients presented pneumonia, in all cases grade 3. Notably, no rash or visual disturbances were reported. Similarly to previous next generation ALK inhibitors, activity in CNS disease has been reported. The phase 2 expansion will include 4 cohorts: ALK-positive lung cancers

Table 5 Current clinical investigation in ALK positive patients

clinicaltrials.gov identifier	Status	Phase	Drug(s)		Target population (ALK+)
NCT 01228435	terminated	II	IPI 504	HSP90i	both
NCT01562015	recruiting	II	STA-9090	HSP90i	C-N
NCT01752400	not yet recruitinig	II	ST-9090	HSP90i	C-R
NCT01772797	recruiting	I	LDK 378 plus AUY922	HSP90i	both
NCT 0157994	recruiting	I/II	STA-9090 plus crizotinib	HSP90i plus ALKi	C-N
NCT01801111	not yet recruitinig	I/II	RO5452802	HSP90i	C-R
NCT01712217	recruiting	I/II	AT13387+ crizotinib	HSP90i plus ALKi	C-R
			criztinib *vs.* crizo plus AT13387	HSP90i plus ALKi	C-R
			AT13387 *vs.* AT13387 plus crizotinib	HSP90i plus ALKi	C-R
NCT01288430	recruiting	I	DS-2248	HSP90i	C-R
NCT01625234	recruiting	I	X-396	ALK i	C-R

ALKi, ALK inhibitor; C-N, crizotinib-naïve; C-R, crizotinib-resistant; HSP90i, Heat Shock Protein 90 inhibitor.

naïve to crizotinib, crizotinib-resistant ALK-positive lung cancers, *EGFR* mutant lung cancers resistant to reversible TKIs, and other cancers harboring ALK abnormalities.

Another strategy to try to overcome ALK resistance consists of targeting the chaperone pathway. Results of Heat-Shock-Protein 90 (HSP90) inhibition in a cohort of ALK-positive patients have been reported (71). AUY992 is a potent, non-geldanamycin, HSP90 inhibitor. Its activity as a once-weekly, 1-hour infusion has been tested in a specific cohort of 22 ALK-positive lung cancer patients. The overall response rate was 32%, with a disease control rate of 59% and an estimated PFS at 18 weeks of 35.8%. The overall response rate in ALK-positive crizotinib-naïve patients (8) was 50%, with a disease control rate of 100% and an estimated PFS of 62.5% at 18 weeks. The most frequent treatment related side effects were eye disorders (74%), diarrhea (68%), nausea (39%), vomiting (26%), and fatigue (21%). Grade 3-4 side effects included eye disorders (7%), diarrhea (6%), and fatigue (4%). AUY922 had an acceptable safety profile. Activity was demonstrated both in crizotinib-naïve and crizotinib-resistant patients.

Other ALK inhibitors, as well as HSP90 inhibitors and different combinations are being currently tested in clinical trials to evaluate the safety profile and the activity in patients harboring *ALK* rearrangement (*Table 5*).

Conclusions

Lung cancer harboring *ALK* rearrangements has emerged as a relevant subtype of this disease, based both on its particular natural history and on the success of crizotinib in efficaciously treating this specific population. However, some challenges remain, such as a how to better manage adverse events related to treatment, more convenient therapeutic schedules for our patients, how to effectively treat CNS disease and overcome or delay the emergence of resistance. Newer strategies including next generation ALK inhibitors or novel drugs may help to address some of these questions.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Hayden EC. Personalized cancer therapy gets closer. Nature 2009;458:131-2.
2. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCRABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 2001;344:1038-42.
3. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347:472-80.
4. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230-7.
5. Lièvre A, Bachet JB, Boige V, et al. KRAS mutations as an

independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 2008;26:374-9.
6. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783-92.
7. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127-37.
8. Sequist LV, Bell DW, Lynch TJ, et al. Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. J Clin Oncol 2007;25:587-95.
9. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
10. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-500.
11. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
12. Shaw AT, Yeap BY, Kenudson MM, et al. Clinical Features and Outcome of Patients With Non Small-Cell Lung Cancer Who Harbor EML4-ALK. J Clin Oncol 2009;27:4247-53.
13. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
14. Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci U S A 2008;105:19893-7.
15. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008;14:4275-83.
16. McDermott U, Iafrate AJ, Gray NS, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res 2008;68:3389-95.
17. Inamura K, Takeuchi K, Togashi Y, et al. EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. J Thorac Oncol 2008;3:13-7.
18. Shinmura K, Kageyama S, Tao H, et al. EML4-ALK fusion transcripts, but no NPM-, TPM3-, CLTC-, ATIC-, or TFG-ALK fusion transcripts, in non-small cell lung carcinomas. Lung Cancer 2008;61:163-9.
19. Wong DW, Leung EL, So KK, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. Cancer 2009;115:1723-33.
20. Inamura K, Takeuchi K, Togashi Y, et al. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Mod Pathol 2009;22:508-15.
21. Martelli MP, Sozzi G, Hernandez L, et al. EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. Am J Pathol 2009;174:661-70.
22. Paik JH, Choe G, Kim H, et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. J Thorac Oncol 2011;6:466-72.
23. Camidge DR, Kono SA, Flacco A, et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. Clin Cancer Res 2010;16:5581-90.
24. Salido M, Pijuan L, Martínez-Avilés L, et al. Increased ALK gene copy number and amplification are frequent in non-small cell lung cancer. J Thorac Oncol 2011;6:21-7.
25. Le Beau MM, Bitter MA, Larson RA, et al. The t(2;5)(p23;q35): a recurring chromosomal abnormality in Ki-1-positive anaplastic large cell lymphoma. Leukemia 1989;3:866-70.
26. Debelenko LV, Arthur DC, Pack SD, et al. Identification of CARS-ALK fusion in primary and metastatic lesions of an inflammatory myofibroblastic tumor. Lab Invest 2003;83:1255-65.
27. Chen Y, Takita J, Choi YL, et al. Oncogenic mutations of ALK kinase in neuroblastoma. Nature 2008;455:971-4.
28. Murugan AK, Xing M. Anaplastic thyroid cancers harbor novel oncogenic mutations of the ALK gene. Cancer Res 2011;71:4403-11.
29. van Gaal JC, Flucke UE, Roeffen MH, et al. Anaplastic lymphoma kinase aberrations in rhabdomyosarcoma: clinical and prognostic implications. J Clin Oncol 2012;30:308-15.
30. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190-203.
31. Souttou B, Carvalho NB, Raulais D, et al. Activation of anaplastic lymphoma kinase receptor tyrosine kinase induces neuronal differentiation through the mitogen-activated protein kinase pathway. J Biol Chem 2001;276:9526-31.

32. Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 2009;15:3143-9.
33. Webb TR, Slavish J, George RE, et al. Anaplastic lymphoma kinase: role in cancer pathogenesis and small-molecule inhibitor development for therapy. Expert Rev Anticancer Ther 2009;9:331-56.
34. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res 2009;15:5216-23.
35. Paik JH, Choe G, Kim H, et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. J Thorac Oncol 2011;6:466-72.
36. Yi ES, Boland JM, Maleszewski JJ, et al. Correlation of IHC and FISH for ALK gene rearrangement in non-small cell lung carcinoma: IHC score algorithm for FISH. J Thorac Oncol 2011;6:459-65.
37. Wecikhardt AJ, Camide R. The therapeutic potential of anaplastic lymphoma kinase inhibitors in lung cancer: rationale and clinical evidence. Clin Invest 2011;1:1119-26.
38. Kris MG, Jonson BE, Kwiatkowski DJ, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: The NCI's Lung Cancer Mutation Consortium (LCMC). J Clin Oncol 2011;29:abstr CRA7506.
39. Boland JM, Erdogan S, Vasmatzis G, et al. Anaplastic lymphoma kinase immunoreactivity correlates with ALK gene rearrangement and transcriptional up-regulation in non-small cell lung carcinomas. Hum Pathol 2009;40:1152-8.
40. Mino-Kenudson M, Chirieac LR, Law K, et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. Clin Cancer Res 2010;16:1561-71.
41. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol 2011;12:1004-12.
42. Camidge DR, Bang Y, Kwak EL, et al. Progression-free survival (PFS) from a phase I study of crizotinib (PF-02341066) in patients with ALK-positive non-small cell lung cancer (NSCLC). J Clin Oncol 2011;29:abstr 2501.
43. CrinòL, Kim D, Riely GJ, et al. Initial phase II results with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC): PROFILE 1005. J Clin Oncol 2011;29:abstr 7514.
44. KimD, Ahn M, Yang P, et al. Updated results of a global phase II study with crizotinin in advanced ALK-positive Non-Small Cell Lung Cancer (NSCLC). Ann Oncol 2012; 23:Abstr 1230 PD.
45. Shaw AT, Kim DW, Nakagawa K, et al. Phase III Randomized Study of Crizotinib Versus Pemetrexed or Docetaxel Chemotherapy in Advanced, ALK-Positive Non-Small Cell Lung Cancer (NSCLC). Ann Oncol 2012;23:LBA1.
46. Cui JJ, Tran-Dubé M, Shen H, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem 2011;54:6342-63.
47. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22:1589-97.
48. Shepherd FA, Rodrigues PJ, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123-32.
49. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in advanced NSCLC harboring anaplastic lymphoma kinase gene rearrangement: a retrospective analysis. Lancet Oncol 2011;12:1004-12.
50. Blackhall FH, Petersen JA, Wilner K, et al. PROFILE 1005: preliminary patient-reported outcomes (PROs) from an ongoing phase 2 study of crizotinib (PF-02341066) in anaplastic lymphoma kinase (ALK)-positive advanced non-small cell lung cancer (NSCLC). Oral presentation at: 14th World Conference on Lung Cancer (WCLC); July 3-7, 2011; Amsterdam, The Netherlands.
51. Kim DW, Blackhall F, Soria JC, et al. A global phase 2 study including efficacy, safety and patient-reported outcomes (PROs) with crizotinib in patients (Pts) with ALK-positive non-small cell lung cancer (NSCLC). Eur J Cancer 2011;47:S617.
52. Blackhall FH, Evans TL, Han J, et al. Impact of Crizotinib Treatment on Patient-Reported Symptoms and Quality of Life in Advanced ALK-Positive Non-Small Cell Lung Cancer (NSCLC). Ann Oncol 2012;23:Abstr1231 PD.
53. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
54. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for Non-Small-Cell Lung Cancer with

mutated EGFR. N Engl J Med 2010;362:2380-8.
55. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-28.
56. Zhou C, Wu Y-L, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802). Lancet Oncol 2011;12:735-42.
57. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
58. Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734-9.
59. Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res 2011;71:6051-60.
60. Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. Sci Transl Med 2012;4:120ra17.
61. Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 2012;18:1472-82.
62. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
63. Balak MN, Gong Y, Riely GJ, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 2006;12:6494-501.
64. Zhang S, Wang F, Keats J, et al. Crizotinib-resistant mutants of EML4-ALK identified through an accelerated mutagenesis screen. Chem Biol Drug Des 2011;78:999-1005.
65. Heuckmann JM, Hölzel M, Sos ML, et al. ALK mutations conferring differential resistance to structurally diverse ALK inhibitors. Clin Cancer Res 2011;17:7394-401.
66. Katayama R, Khan TM, Benes C, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proc Natl Acad Sci U S A 2011;108:7535-40.
67. Costa DB, Kobayashi S, Pandya SS, et al. CSF concentration of the anaplastic lymphoma kinase inhibitor crizotinib. J Clin Oncol 2011;29:e443-5.
68. Shaw AT, Camidge DR, Felip E, et al. Results of a first-in-human phase I study of the ALK inhibitor LDK 378 in advanced solid tumors. Ann Oncol 2012;23:Abstr 440O.
69. Nishio M, Kiura K, Nakagawa K, et al. A phase I/II study of ALK inhibitor CH5424802 in patients with ALK-positive NSCLC; safety and efficacy interim results of the phase II portion. Ann Oncol 2012; 23:Abstr 441O.
70. Gettinger S, Weiss GJ, Salgia R, et al. A first-in-human dose-finding study of the ALK/EGFR inhibitir AP26113 in patients with advanced malignances. Ann Oncol 2012;23:Abstr 439O.
71. Felip E, Carcereny E, Barlesi F, et al. Phase II activity of the HSP90 inhibitor AUY992 i patients with ALK – rearranged (ALK+) or EGFR- mutated Non-Small Cell lung cancer (NSCLC). Ann Oncol 2012;23:Abstr 438O.

Cite this article as: Morán T, Quiroga V, Gil M, Vila L, Pardo N, Carcereny E, Capdevila L, Muñoz-Mármol A, Rosell R. Targeting EML4-ALK driven non-small cell lung cancer (NSCLC). Transl Lung Cancer Res 2013;2(2):128-141. doi: 10.3978/j.issn.2218-6751.2013.03.04

KRAS mutant NSCLC, a new opportunity for the synthetic lethality therapeutic approach

Javier de Castro Carpeño[1], Cristóbal Belda-Iniesta[2]

[1]Medical Oncology Unit, Department of Translational Oncology, Hospital Universitario La Paz, idiPAZ, Madrid, Spain; [2]Thoracic, H&N and Neuro-oncology Unit, CIOCC, GHM, Madrid, Spain

Correspondence to: Dr. Javier de Castro Carpeño. Medical Oncology Unit - Department of Translational Oncology, Hospital Universitario La Paz, Paseo de la Castellana, 261, 28046 Madrid (Spain). Email: javier.decastro@salud.madrid.org.

Abstract: K-RAS accounts for 90% of RAS mutations in lung adenocarcinomas, the most commonly mutated oncogene in NSCLC, with mutations detected in about 25% of all tumors. Direct inhibition of KRAS has proven clinically challenging. So far, no successful targeted therapy has been developed and remains an elusive target for cancer therapy. Despite significant efforts, currently there are no drugs directly targeting mutated KRAS. Thus, new strategies have emerged for targeting RAS including the use of synthetic lethality. A specific knowledge of individual tumor molecular abnormalities that result in oncogene-specific "synthetic lethal" interactions will allow the rationale to combine promising targeted therapies for KRAS-mutated NSCLC. In this article, we review the new approach based on testing drugs or combinations of agents that work downstream of activated K-RAS.

Keywords: RAS oncogene family; KRAS mutant; NSCLC; selumetinib; synthetic lethality

Submitted Jan 18, 2013. Accepted for publication Feb 27, 2013.
doi: 10.3978/j.issn.2218-6751.2013.02.07
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.02.07

Introduction

Lung cancer is the leading cause of cancer deaths worldwide and non–small cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases (1). The standard first-line therapy for patients with advanced NSCLC was a platinum-based doublet combination chemotherapy but modest progress has been made with the use of chemotherapy, and additional treatment strategies are needed. So cancer drug development has shifted from cytotoxic, nonspecific chemotherapies to molecularly targeted, rationally designed drugs with greater efficacy and lower toxicities. For this challenge, the best knowledge of cancer biology is required. Nowadays, we are able to identify different genetic changes that allow us to consider NSCLC as a major disease which can be molecularly reclassified into several subsets of diseases (2). RAS gene family members encode small GTPases that activate various signaling pathways involved in proliferation, differentiation and cell survival (*Figure 1*). RAS proteins function as molecular switches that cycle between a GDP-bound inactive state and GTP-bound active state. Ras proto-oncogenes are the most frequent mutated genes in NSCLC, with mutations detected in about 25% of all tumors, mainly adenocarcinoma subtype (3).

v-Ki-ras2 Kirsten rat sarcoma viral oncogene (K-RAS) accounts for 90% of RAS mutations in lung adenocarcinomas. Most oncogenic forms of RAS impair their intrinsic GTPase activity, preventing GTP hydrolysis.

RAS proteins acquire the potential to transform the cells when an amino acid at position 12, 13, or 61 is replaced as a result of a point mutation in the gene but 97% of K-RAS mutations in NSCLC involve codons 12 or 13 at P-Loop also known as Walker A motif. This domain interacts with the phosphate group of GTP helped by GAP protein. In this regard, mutations at codon 12 avoid K-Ras to be stimulated by GAP protein. As GAP acts as a catalyst to

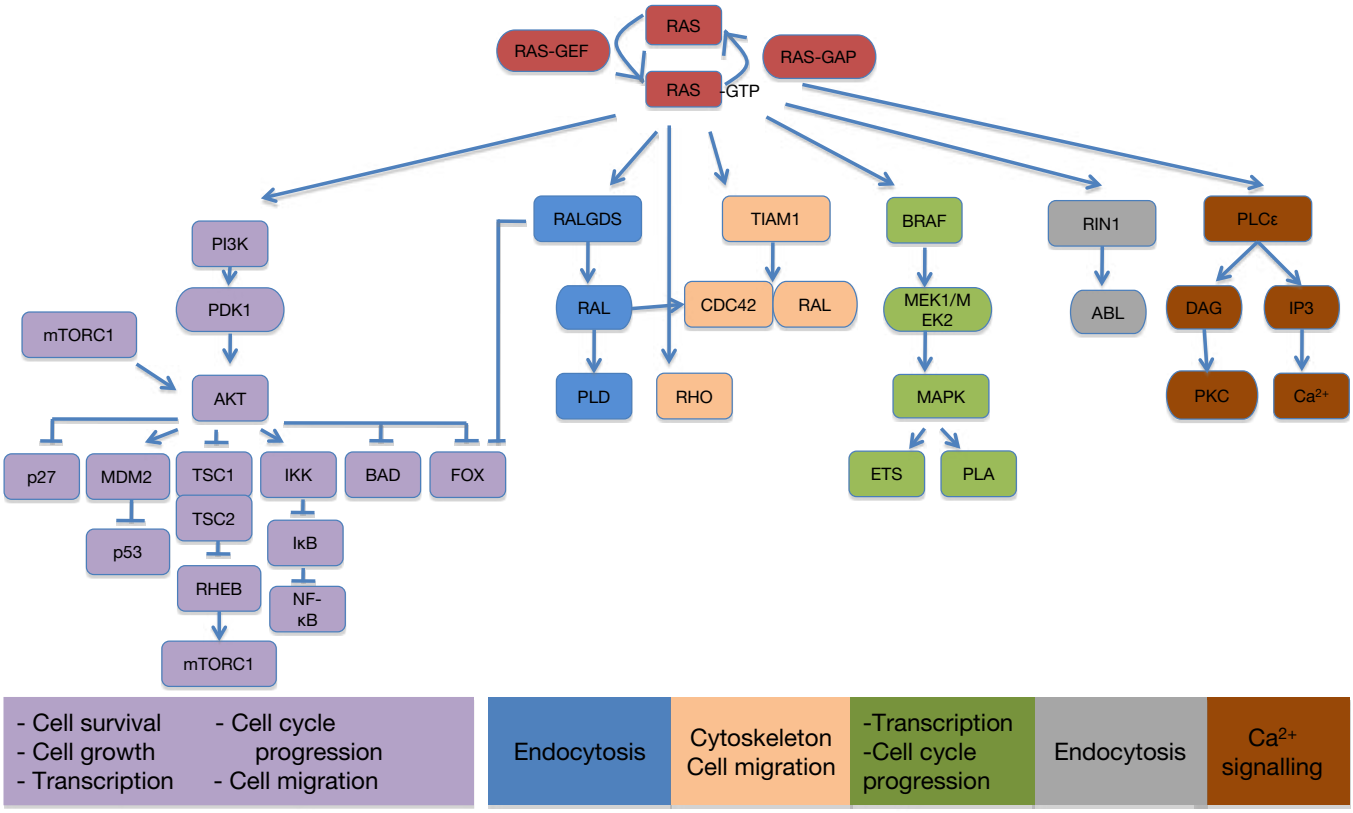


Figure 1 The major RAS effector pathways. CDC42, cell division cycle 42; DAG, diacylglycerol; FOX, forkhead transcription factor; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; IKK, IκB kinase; IP3, inositol-1,4,5-trisphosphate; mTORC, mTOR complex; NF-κB, nuclear factor-κB; PDK1, phosphoinositide-dependent kinase 1; PKC, protein kinase C; PLA, phospholipase A; PLCε, phospholipase Cε; PLD, phospholipase D; RALGDS, RAL guanine nucleotide dissociation stimulator; RHEB, RAS homologue enriched in brain; RIN1, RAS and RAB interactor 1; TIAM1, cell lymphoma invasion and metastasis 1.

speed up GTPase activity, mutations at that position slow GTP transition to GDP increasing GTP levels. Mutations at codon 61 affect the energy gradient needed to transform substrate (GTP) into product (GDP) because wild-type residue at that position stabilizes the transition state for GTP hydrolysis. So, it is critical to know specific site and biochemical effects when a K-Ras mutation is diagnosed because pharmacological modulation is completely different.

Although KRAS mutations have been widely hypothesized to be related to direct tobacco exposure, they do occur in approximately 15% of lung adenocarcinomas from never-smokers (4). Thus, KRAS tumor status cannot be easily predicted on the basis of smoking history alone. KRAS transversion mutations (G/TorG/C) are more common in former or current smokers and transition mutations (G/A) are more common in patients who never smoked cigarettes.

KRAS mutations have been associated with a poor prognosis such as a lower expectancy for survival (5), reduced benefit from adjuvant chemotherapy, they predict resistance towards EGFR tyrosine kinase inhibitors (6), and obtain less clinical benefits from chemotherapy compared with the general NSCLC population (7).

Treatment of KRAS mutated NSCLC: an unresolved issue

Direct inhibition of KRAS has proven clinically challenging. Although KRAS mutations were identified in lung cancer nearly 30 years ago (8), no successful targeted therapy has been developed and remains an elusive target for cancer therapy (9). So far, there is no yet effective treatment for patients with these types of tumors although we consider that K-RAS is not a unique target but a myriad of targets that combine absence of affinity for a catalyst (GAP) or

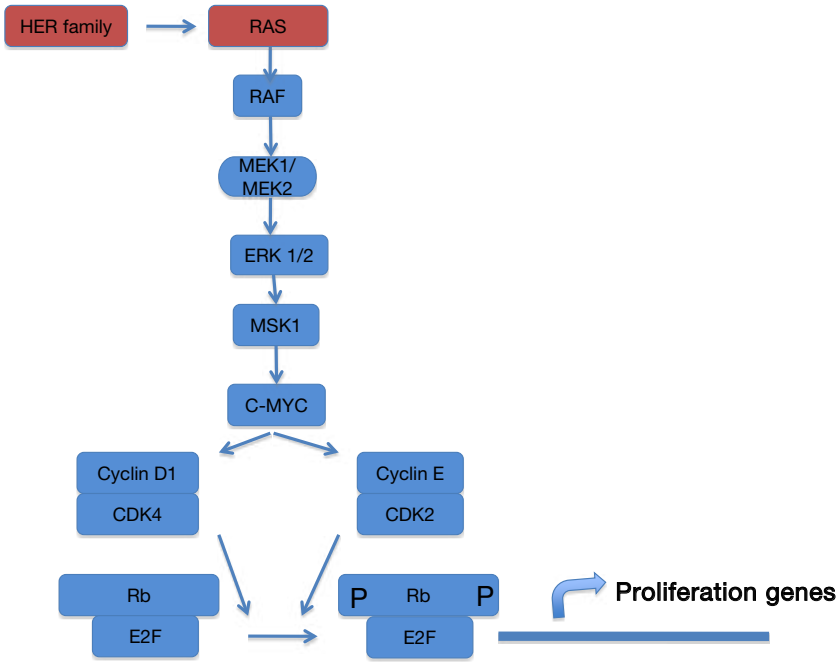


Figure 2 The relationship between HER family, KRAS and cyclin-dependent kinases (Cdk).

decreasing affinity for GTP (P-Loop impairing) as well as other biochemical complexities.

Until now, all efforts to inhibit mutant KRAS in NSCLC have failed and few compounds have been assessed by clinical trial. One of the reasons to explain this point is because RAS enzyme kinetics is hard to inhibit due to affinity to substrates, catalyst proteins and sequential conformational changes after first signal that occurs inside this multi-target protein. In fact, the lack of specificity of KAS inhibitors could be related to this biochemical complexity that could be targeted at different levels: membrane attachment, P-Loop and thermodynamic requirements.

Various potent and selective inhibitors of RAS function were developed in the 1990s, with the aim to prevent association of RAS with the inner face of cell membrane (10). First, farnesyl transferase inhibitors avoid a critical post-translational modification in pre-RAS protein blocking isoprenylation. As farnesyl residues are needed to attach K-RAS to membrane it was hypothesized that this sort of inhibitors could inhibit RAS proteins (11). In fact, these inhibitors blocked RAS-dependent oncogenic activity "*in vitro*" and in preclinical animal models, but unfortunately failed in the clinical practice and showed little clinical efficacy because of a sequential post-translational modification at pre-Ras that compensates first steps of K-RAS maturation (12).

Although effective KRAS inhibitors are not currently available, genetic approaches have identified novel drug targets that are essential for RAS cellular localization and function, raising hope that new inhibitors of specific biochemical functionality of K-RAS will soon be developed.

Rationale for a new treatment strategy for K-RAS mutated NSCLC

A different approach has been based on testing drugs or combinations of agents that work downstream of activated K-RAS. If you take into account that different KRAS-mutant tumors can activate several signalling pathways, a new treatment strategy for KRAS-mutant NSCLC should be based on the combination of targeted agents that inhibit downstream effectors of K-RAS dependent-tumors according to the "RAS-ome" (*Figures 2,3*). In this way, a specific knowledge of individual tumor molecular abnormalities that result in oncogene-specific "synthetic lethal" interactions will allow the rationale to combine promising targeted therapies for KRAS-mutated NSCLC.

Targeting HER pathway

Epiregulin (EREG) is ligand of the EGF receptor/EGFR and ERBB4 and is a putative transcriptional target of

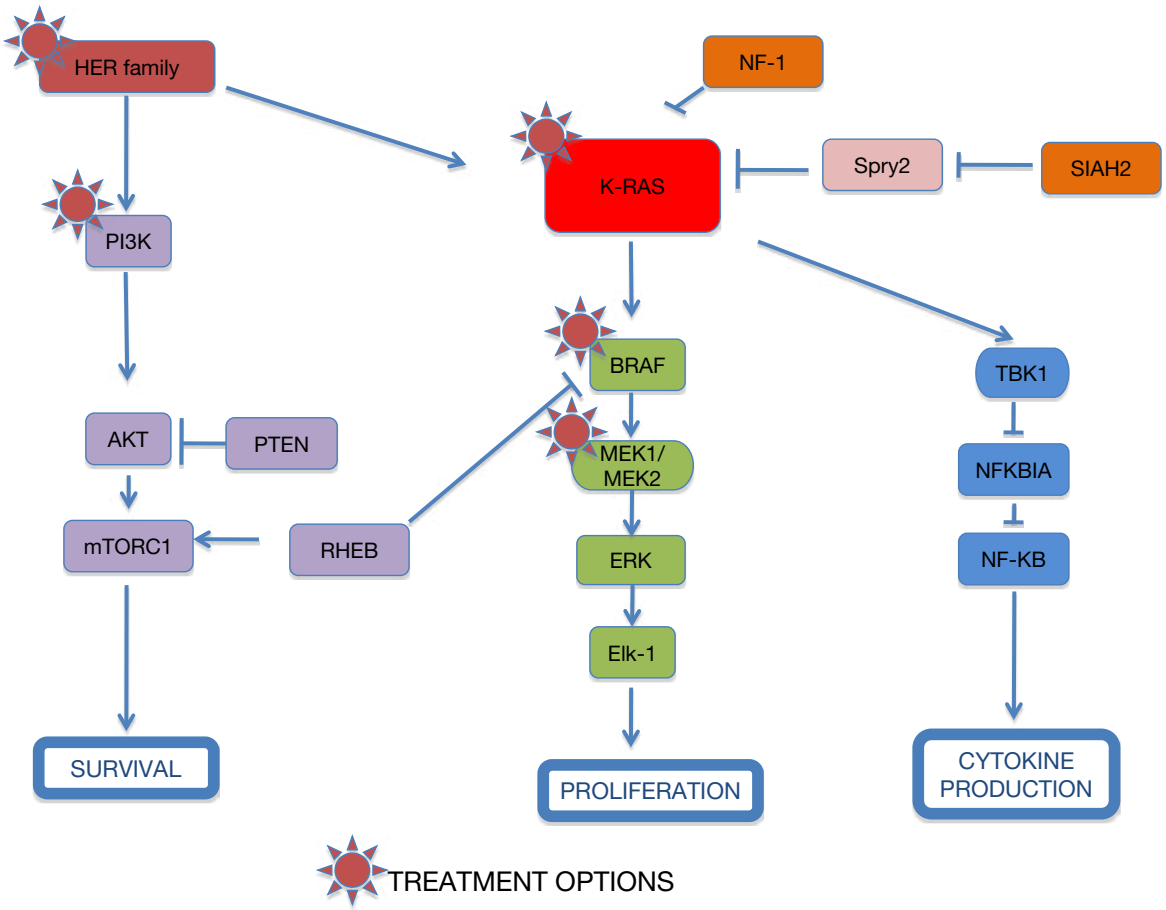


Figure 3 Major interactions in the KRAS pathway.

mutated KRAS dependent signaling that contributes to an aggressive phenotype and could be a promising therapeutic target in oncogenic KRAS-driven NSCLC (13) (*Figure 2*).

Targeting MEK pathway

Initial efforts focused on proteins downstream K-Ras at the RAS/RAF/mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway. The MAPK pathway converges at the MEK1/MEK2 kinases, for which the only known substrates are the ERK1/ERK2 kinases (*Figures 2,3*). In fact, MEK inhibition would block ERK signalling irrespective of the upstream stimulus.

MEK1 and MEK2 are dual specificity kinases, RAF-phosphorylated, that phosphorylate the tyrosine and threonine residues on ERK1 and 2, leading to proliferation and migration activation. Mutations in RAS or RAF lead to a sustained oncogenic signal and predict response to MEK inhibition in laboratory models.

Selumetinib (AZD6244, ARRY-142886; AstraZeneca, Alderley Park, Cheshire, UK) is an orally available, potent, selective, non-ATP competitive inhibitor of MEK1/MEK2 kinases (IC50 14 nM for MEK1). Preclinical data from KRAS-mutant NSCLC tumor xenografts showed that selumetinib significantly suppressed tumor growth (14), especially in tumors harboring RAS mutations (15). Initial clinical studies of selumetinib showed target inhibition and tumor responses (16). A phase I trial demonstrating tolerability and preliminary efficacy of selumetinib at 100 mg twice daily (17), identified an acneiform rash as the main dose-limiting toxicity (DLT). However, treatment with selumetinib alone, showed little clinical efficacy in a phase II clinical trial in unselected pre-treated patients with NSCLC when selumetinib was compared with pemetrexed (18).

Results of additional preclinical *in-vivo* studies have shown that the combination of selumetinib and docetaxel leads to greater tumor-growth inhibition or regression, and

apoptosis (19,20). This combination showed a manageable tolerability profile in advanced solid tumors (21) in phase I. With this rationale, a randomised, double-blind, phase II clinical trial combining docetaxel (75 mg/m^2 on day 1 of a 21-day cycle) with or without oral selumetinib (75 mg twice daily in a 21-day cycle) in KRAS-mutant NSCLC patients after first-line progression (22). Mature data evidenced a promising trend in overall survival for patients treated at experimental arm (median OS 9.4 *vs.* 5.2 mo; HR 0.80; 80% CI, 0.56-1.14; one-sided P=0.21). Additionally, median progression-free survival was statistically significant (5.3 *vs.* 2.1 mo, HR 0.58; 80% CI, 0.42-0.79; one-sided P=0.014), and an impressive response rate around 37% in the combination group compared with 0% in the docetaxel alone group (P<0.0001). In post-hoc analyses, there were also improvements in lung cancer symptoms and all these benefits might be attributable to the cytoreductive effects of the treatment. However, a higher rate of febrile neutropenia (18% *vs.* 0%), diarrhea, vomiting, stomatitis, and dry skin with selumetinib plus docetaxel were communicated.

Obviously, this is a phase II study and requires further validation in a large phase III clinical trial. Furthermore, the study has potential limitations such as the small sample size and the absence of independent confirmation of progression-free survival and tumor response. Moreover, the control group of the study who received docetaxel alone clearly had poor evolution, lower than expected in previous clinical trials in unselected patients receiving docetaxel at second line setting (23). Furthermore, a new question emerges because poor efficacy of docetaxel in K-RAS mutant NSCLC patients should be investigated. Conversely, the potential synergy of docetaxel and selumetinib remains unclear and additional studies are needed. *In-vivo* mechanistic drug sequencing studies have shown that administration of selumetinib after docetaxel, rather than before, induced more apoptosis. This finding could have important clinical implications for any dosing schedule of this combination. This contrasts with the majority of previous studies in NSCLC, in which addition of a targeted agent to chemotherapy has not resulted in improved efficacy.

Another important issue is the therapeutic effect of specific KRAS mutations, to define a subpopulation of KRAS-mutant NSCLC in which the combination of selumetinib and docetaxel leads to improved efficacy. Previous studies showed that KRAS mutation subtype seems to be an important predictor of treatment outcome (24).

Wide genomic approaches have evidenced that it is usual for many mutations to co-exist. In this regard, K-RAS mutations in NSCLC patients could be co-expressed with additional sequence alterations. Thus, a recent study done in mice showed that overlapping mutations at p53 or LKB1 affect efficacy of selumetinib plus docetaxel (25) as well as docetaxel alone in tumors that harbors a mutated Kras sequence. For example, combination of selumetinib plus docetaxel provides substantial benefit in K-Ras^{mt}/p53^{mt} lung cancer models. Conversely, mice harboring Kras^{mt}/LKB1^{mt} tumors show primary resistance to this schedule. LKB1 (liver kinase B1) also known as serine threonine kinase 11 (STK11) the defective sequence of which is a cause of Peutz-Jeghers syndrome. Its role is critical in p53-dependent apoptosis, mainly involved at mitochondrion steps. When LKN1 is unable to exert its activity, p53-dependent death is impaired. LKB1 is somatically inactivated in about 30% of NSCLC (26), and the combination of LKB1 loss and KRAS mutation results in a more aggressive phenotype than tumors only harboring KRAS mutations (27). In fact, the decreased activation of ERK phosphorylation in KRAS/LKB1 tumors suggests that the proliferation of these tumors may be driven through other signaling pathways. KRAS/LKB1-mutant tumors have heightened activation of both AKT and SRC. This type of tumors with KRAS mutated and LKB1 inactivated show sensitivity to rapamycin or the MEK inhibitor CI-1040.

Several selumetinib trials are currently enrolling patients, including a phase II study (NCT01229150) in previously treated NSCLC stratified by KRAS status. Mutated KRAS and wild-type KRAS patients are randomized to receive selumetinib and erlotinib or selumetinib alone (28). In addition, the drug is being evaluated with thoracic radiation in one trial (NCT01146756) and in two multi-arm trials (NCT01306045 and NCT01248247) that assign treatment by molecular tumor characteristics.

Other MEK inhibitors have been already tested. Trametinib (GSK 1120212 or JTP-74057) is a reversible, allosteric MEK1/MEK2 inhibitor with an IC50 of 0.7 nM for MEK1, and a high specificity as demonstrated by limited activity against a panel of 180 other kinases. A multi-arm phase I/Ib trial (NCT01192165) is assessing many treatment combinations, specifically with a goal of identifying appropriate regimens for lung and pancreatic cancer treatment. An open-label, randomized phase II trial (NCT01362296) in second-line NSCLC that harbors muta¬tion in KRAS, NRAS, BRAF, or MEK1 is currently recruiting patients.

Dual targeting of MEK with inhibition of other kinases in the same pathway, such as EGFR, or with inhibition

of a parallel pathway are also promising directions for ongoing trials.

Targeting PI3K pathway

PI3K is a site of convergence and stem for multiple pathways resulting in complex regulation of signaling and the potential for significant off-target effects, including activation of alternative networks to promote oncogenesis (*Figure 3*).

NSCLC harbors several molecular alterations involving the PI3K pathway, including PIK3CA amplification and mutation, decrease or loss of phosphate, and tensin homologue (PTEN), AKT mutations, LKB1 loss and KRAS mutation. For all of these features, PI3K pathway is one of the promising approaches to target RAS downstream signaling proteins. Conversely, K-RAS mutations have been predicted to mediate resistance to PI3K inhibitors (29). For this reason, a potential strategy of treatment of KRAS mutant tumors will be focused on dual inhibition of PI3K and MEK/ERK signaling.

MK-2206 is an oral pan-Akt inhibitor that binds Akt in its inactive configuration. MK-2206 has shown preclinical activity in a panel of NSCLC lines, with the greatest activity in a PIK3CA-mutated model (30). Combination therapy with selumetinib demonstrated synergy (31) and is being evaluated clinically (NCT01021748) (32).

Targeting nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) pathway

KRAS mutated tumors can activate nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) pathway and produce anti-apoptotic signals, essential for NSCLC survival through cREL and Bcl-xL (33) (*Figures 1,3*). So, NF-κB signaling and the non-canonical IκB kinase, TBK1, may represent an alternative strategy for targeting $KRAS^{mt}$-driven tumors. These observations suggest a pharmacological alternative for potential treatment of cancers harboring RAS mutations (34).

Neurofibromatosis type 1 pathway

Neurofibromatosis type 1 (NF1) gene regulates cell motility and invasion, and displays high homology with RAS GTPase activating protein (*Figure 3*). Loss of NF1 produces hyper-activation of RAS signaling in 40% of NSCLC (35). NF1-deficient malignancies and KRas/p53-mutant lung cancer exhibit an aggressive phenotype in murine models. However, agents that enhance proteotoxic stress, including the HSP90 inhibitor IPI-504 showed relevant responses when combined with rapamycin (36). Other HSP90 inhibitors are under evaluation (37). Since the mTOR inhibitor rapamycin has shown potential activity against NF1-associated tumors, it could be a new option of treatment (38).

Wilms tumor gene pathway

The Wilms Tumor gene (WT1) is a tumor suppressor gene that recognizes and binds to the DNA sequence 5'-CGCCCCCGC-3'. Curiously, function may be isoform-specific as isoforms lacking the KTS motif may act as transcription factors and isoforms containing the KTS motif may bind mRNA and play a role in mRNA metabolism or splicing. This biological complexity offers many possibilities for drug development, including those that affect $KRAS^{mt}$ driven biology. Recently, a study in both mouse and human cells has shown that the loss of WT1 could activate a senescence program in $KRAS^{mt}$ cells (39). If this observation is confirmed, a new approach of treatment will be opened.

GATA2 pathway

The development of RNA interference technology has enabled the possibility of testing biological roles of putative genes in wide-genome scale. In this regard, several screenings assays have been carried out in cell libraries aimed to identify genes the inhibition of which is selectively deleterious to $K\text{-}RAS^{mt}$ cells (40). Candidate genes were then tested in larger panel of KRAS mutant and wild-type cancer cells. Finally, $K\text{-}RAS^{mt}$ cancer cell lines were found to be dependent on some genes such as the transcription factor GATA2 (41).

GATA-binding Factor 2 or erythroid transcription factor (GATA2) can be involved in regulation of the proteasome activity, IL-1 and Rho-signaling pathways. Recently, it has been observed that loss of GATA2 reduced the viability of NSCLC cells harboring RAS mutations, whereas wild-type cells were unaffected (42). Although GATA2 itself is likely undruggable, combined suppression of GATA2-regulated pathways with clinically approved inhibitors caused marked tumor clearance. Pharmacological inhibition of GATA2-mediated pathways with bortezomib and fasudil results in dramatic tumor inhibition (43). These observations present a new treatment option to KRAS mutant NSCLC.

Seven in absentia homolog 2 pathway

The human homolog of Drosophila seven-in-absentia--SIAH-1 and SIAH-2 are ubiquitin E3 ligases and driving ubiquitin-mediated degradation of conserved downstream components of the RAS pathway that are required for mammalian RAS signal transduction (*Figure 3*). In this regard, SIAH-2 regulates the tumor growth by degradation of SPRY2 and subsequent activation of the RAS-ERK pathway. Since SIAH-2 can be involved in different NSCLC, SIAH-2 may be a viable target for novel anti-RAS and anticancer agents aimed at inhibiting EGFR and/or RAS-mediated tumorigenesis (44).

RNA-binding motif 5 pathway

RBM5 (RNA-binding motif protein 5, also named H37/LUCA-15) gene is a component of the spliceosome. A complex (also known as the prespliceosome) that regulates the alternative splicing of a number of mRNAs. It has demonstrated tumor suppressor activity (45). RBM5 can inhibit the growth of lung cancer cells and induce apoptosis both *in vitro* and *in vivo* (46). RBM5 is downregulated by the constitutively activated RAS mutant protein, RAS (G12V), in rat embryonic fibroblast cells, which indicates a correlation between the RAS pathways and RBM5 activity (47). Further evaluation of interrelationships between RBM5 expression and KRAS gene must be carried out to open a novel therapeutic approach.

IL-8 pathway

Interleukin-8 (IL-8; CXCL8) is a cytokine of the CXC chemokine family that is involved in neutrophil recruitment and activation. In addition, IL-8 is an angiogenic growth factor that is overexpressed in different cancers, including NSCLC (48). Lung adenocarcinoma and muco-epidermoid carcinoma cells produce substantial amounts of IL-8, and express both CXCR1 and CXCR2 IL-8 receptors. Activating mutations of KRAS upregulate IL-8 expression in NSCLC and IL-8 can play a role in cell growth and migration in oncogenic KRAS-driven NSCLC (49).

Twist-related protein 1 pathway

Twist1 acts as a transcriptional regulation as a heterodimer with E proteins. Interestingly, Twist1 regulates gene expression differentially, depending on dimer composition: homodimers induce expression of FGFR2 and POSTN while heterodimers repress FGFR2 and POSTN expression and induce THBS1 expression. Additionally, it has been suggested to play an important role during tumor progression. For example, transgenic mouse models have shown that Twist1 cooperates with KRAS (G12D) to markedly accelerate lung tumorigenesis by abrogating cellular senescence programs and promoting the progression from benign adenomas to adenocarcinomas. Moreover, the suppression of Twist1 to physiological levels is enough to cause KRAS mutant lung tumors to undergo senescence losing their neoplastic features (50). The suppression of TWIST1 in human tumors may be an effective example of pro-senescence therapy.

Conclusions

Traditionally, treatment decisions for patients with lung cancer have historically been based on tumor histology and TNM stage. One promising treatment strategy involves the further subdivision of NSCLC into clinically relevant molecular subsets, according to a classification schema based on specific so-called driver mutations.

Although mutational activation of the KRAS pathway is the most frequent genetic event in NSCLC, it remains an elusive target for cancer therapy. In fact, it has been considered an "undruggable" genetic alteration.

A key goal in cancer research is the discovery of new drug targets that will selectively impair the viability of tumoral cells such as KRAS mutant NSCLC. Therefore, a specific knowledge of individual tumor molecular abnormalities that result in oncogene-specific "synthetic lethal" interactions will allow the rationale to combine promising targeted therapies for KRAS-mutated NSCLC. Recently, a MEK inhibitor, selumetinib, has shown interesting efficacy when combined with docetaxel in patients with KRAS-mutant tumors. Several pathways may provide attractive approaches to develop new treatments in KRAS-mutated NSCLC.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.

2. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med 2008;359:1367-80.
3. Mitsudomi T, Viallet J, Mulshine JL, et al. Mutations of ras genes distinguish a subset of non-small-cell lung cancer cell lines from small-cell lung cancer cell lines. Oncogene 1991;6:1353-62.
4. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res 2008;14:5731-4.
5. Mascaux C, Iannino N, Martin B, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 2005;92:131-9.
6. Mitsudomi T, Yatabe Y. Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer. Cancer Sci 2007;98:1817-24.
7. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005;23:5900-9.
8. Santos E, Martin-Zanca D, Reddy EP, et al. Malignant activation of a K-ras oncogene in lung carcinoma but not in normal tissue of the same patient. Science 1984;223:661-4.
9. Ahearn IM, Haigis K, Bar-Sagi D, et al. Regulating the regulator: post-translational modification of RAS. Nat Rev Mol Cell Biol 2011;13:39-51.
10. Sousa SF, Fernandes PA, Ramos MJ. Farnesyltransferase inhibitors: a detailed chemical view on an elusive biological problem. Curr Med Chem 2008;15:1478-92.
11. Blumenschein G, Ludwig C, Thomas G, et al. O-082 A randomized phase III trial comparing ionafarnib/carboplatin/paclitaxel versus carboplatin/paclitaxel (CP) in chemotherapy-naive patients with advanced or metastatic non-small cell lung cancer (NSCLC). Lung Cancer 2005;49:S30.
12. Whyte DB, Kirschmeier P, Hockenberry TN, et al.K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. J Biol Chem 1997;272:14459-64.
13. Sunaga N, Kaira K, Imai H, et al. Oncogenic KRAS-induced epiregulin overexpression contributes to aggressive phenotype and is a promising therapeutic target in non-small-cell lung cancer. Oncogene 2013;32:4034-42.
14. Dry JR, Pavey S, Pratilas CA, et al. Transcriptional pathway signatures predict MEK addiction and response to selumetinib (AZD6244). Cancer Res 2010;70:2264-73.
15. Garon EB, Finn RS, Hosmer W, et al. Identification of common predictive markers of in vitro response to the Mek inhibitor selumetinib (AZD6244; ARRY-142886) in human breast cancer and non-small cell lung cancer cell lines. Mol Cancer Ther 2010;9:1985-94.
16. Banerji U, Camidge DR, Verheul HM, et al. The first-in-human study of the hydrogen sulfate (Hyd-sulfate) capsule of the MEK1/2 inhibitor AZD6244 (ARRY-142886): a phase I open-label multicenter trial in patients with advanced cancer. Clin Cancer Res 2010;16:1613-23.
17. Adjei AA, Cohen RB, Franklin W, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol 2008;26:2139-46.
18. Hainsworth JD, Cebotaru CL, Kanarev V, et al. A phase II, open-label, randomized study to assess the efficacy and safety of AZD6244 (ARRY-142886) versus pemetrexed in patients with non-small cell lung cancer who have failed one or two prior chemotherapeutic regimens. J Thorac Oncol 2010;5:1630-6.
19. Holt SV, Logié A, Odedra R, et al. The MEK1/2 inhibitor, selumetinib (AZD6244; ARRY-142886), enhances anti-tumour efficacy when combined with conventional chemotherapeutic agents in human tumour xenograft models. Br J Cancer 2012;106:858-66.
20. Davies BR, Logie A, McKay JS, et al. AZD6244 (ARRY-142886), a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 kinases: mechanism of action in vivo, pharmacokinetic/pharmacodynamic relationship, and potential for combination in preclinical models. Mol Cancer Ther 2007;6:2209-19.
21. Kim K, Infante J, Cohen R, et al. A phase I dose-escalation study of selumetinib in combination with docetaxel in patients with advanced solid tumors. Mol Cancer Ther 2011;10:B225.
22. Jänne PA, Shaw AT, Pereira JR, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol 2013;14:38-47.
23. Douillard JY, Shepherd FA, Hirsh V, et al. Molecular

predictors of outcome with gefitinib and docetaxel in previously treated non-small-cell lung cancer: data from the randomized phase III INTEREST trial. J Clin Oncol 2010;28:744-52.
24. Ihle NT, Byers LA, Kim ES, et al. Effect of KRAS oncogene substitutions on protein behavior: implications for signaling and clinical outcome. J Natl Cancer Inst 2012;104:228-39.
25. Chen Z, Cheng K, Walton Z, et al. A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response. Nature 2012;483:613-7.
26. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455:1069-75.
27. Mahoney CL, Choudhury B, Davies H, et al. LKB1/KRAS mutant lung cancers constitute a genetic subset of NSCLC with increased sensitivity to MAPK and mTOR signalling inhibition. Br J Cancer 2009;100:370-5.
28. Goldman JW, Garon EB. Targeting MEK for the treatment of non-small-cell lung cancer. J Thorac Oncol 2012;7:S377-8.
29. Ihle NT, Lemos R Jr, Wipf P, et al. Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. Cancer Res 2009;69:143-50.
30. Hirai H, Sootome H, Nakatsuru Y, et al. MK-2206, an allosteric Akt inhibitor, enhances antitumor efficacy by standard chemotherapeutic agents or molecular targeted drugs in vitro and in vivo. Mol Cancer Ther 2010;9:1956-67.
31. Engelman JA, Chen L, Tan X, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 2008;14:1351-6.
32. Meng J, Dai B, Fang B, et al. Combination treatment with MEK and AKT inhibitors is more effective than each drug alone in human non-small cell lung cancer in vitro and in vivo. PLoS One 2010;5:e14124.
33. Meylan E, Dooley AL, Feldser DM, et al. Requirement for NF-kappaB signalling in a mouse model of lung adenocarcinoma. Nature 2009;462:104-7.
34. Barbie DA, Tamayo P, Boehm JS, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature 2009;462:108-12.
35. Roberts PJ, Stinchcombe TE, Der CJ, et al. Personalized medicine in non-small-cell lung cancer: is KRAS a useful marker in selecting patients for epidermal growth factor receptor-targeted therapy? J Clin Oncol 2010;28:4769-77.
36. De Raedt T, Walton Z, Yecies JL, et al. Exploiting cancer cell vulnerabilities to develop a combination therapy for ras-driven tumors. Cancer Cell 2011;20:400-13.
37. Acquaviva J, Smith DL, Sang J, et al. Targeting KRAS-mutant non-small cell lung cancer with the Hsp90 inhibitor ganetespib. Mol Cancer Ther 2012;11:2633-43.
38. Johannessen CM, Johnson BW, Williams SM, et al. TORC1 is essential for NF1-associated malignancies. Curr Biol 2008;18:56-62.
39. Vicent S, Chen R, Sayles LC, et al. Wilms tumor 1 (WT1) regulates KRAS-driven oncogenesis and senescence in mouse and human models. J Clin Invest 2010;120:3940-52.
40. Luo J, Emanuele MJ, Li D, et al. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell 2009;137:835-48.
41. Steckel M, Molina-Arcas M, Weigelt B, et al. Determination of synthetic lethal interactions in KRAS oncogene-dependent cancer cells reveals novel therapeutic targeting strategies. Cell Res 2012;22:1227-45.
42. Kumar MS, Hancock DC, Molina-Arcas M, et al. The GATA2 transcriptional network is requisite for RAS oncogene-driven non-small cell lung cancer. Cell 2012;149:642-55.
43. Barbacid M. Opening a new GATAway for treating KRAS-driven lung tumors. Cancer Cell 2012;21:598-600.
44. Ahmed AU, Schmidt RL, Park CH, et al. Effect of disrupting seven-in-absentia homolog 2 function on lung cancer cell growth. J Natl Cancer Inst 2008;100:1606-29.
45. Oh JJ, Razfar A, Delgado I, et al. 3p21.3 tumor suppressor gene H37/Luca15/RBM5 inhibits growth of human lung cancer cells through cell cycle arrest and apoptosis. Cancer Res 2006;66:3419-27.
46. Shao C, Zhao L, Wang K, et al. The tumor suppressor gene RBM5 inhibits lung adenocarcinoma cell growth and induces apoptosis. World J Surg Oncol 2012;10:160.
47. Edamatsu H, Kaziro Y, Itoh H. LUCA15, a putative tumour suppressor gene encoding an RNA-binding nuclear protein, is down-regulated in ras-transformed Rat-1 cells. Genes Cells 2000;5:849-58.
48. Luppi F, Longo AM, de Boer WI, et al. Interleukin-8 stimulates cell proliferation in non-small cell lung cancer through epidermal growth factor receptor transactivation.

Lung Cancer 2007;56:25-33.
49. Sunaga N, Imai H, Shimizu K, et al. Oncogenic KRAS-induced interleukin-8 overexpression promotes cell growth and migration and contributes to aggressive phenotypes of non-small cell lung cancer. Int J Cancer 2012;130:1733-44.
50. Tran PT, Shroff EH, Burns TF, et al. Twist1 suppresses senescence programs and thereby accelerates and maintains mutant Kras-induced lung tumorigenesis. PLoS Genet 2012;8:e1002650.

Cite this article as: de Castro Carpeño J, Belda-Iniesta C. KRAS mutant NSCLC, a new opportunity for the synthetic lethality therapeutic approach. Transl Lung Cancer Res 2013;2(2):142-151. doi: 10.3978/j.issn.2218-6751.2013.02.07

Translational Medicine

The role of SOX2 in small cell lung cancer, lung adenocarcinoma and squamous cell carcinoma of the lung

Niki Karachaliou[1], Rafael Rosell[1,2], Santiago Viteri[1]

[1]Pangaea Biotech, Dexeus University Institute, Barcelona, Spain; [2]Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Badalona, Spain
Correspondence to: Rafael Rosell. Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Badalona, Spain. Email: rrosell@iconcologia.net.

Abstract: SOX2 is a stem cell transcription factor that plays a crucial role in the regulation of embryonic development. It is one of the genes in a set of factors (Oct4, SOX2, Nanog) that are able to reprogram human somatic cells to pluripotent stem cells. Overexpression of SOX2 has been described in all types of lung cancer tissues, including small cell and squamous cell carcinoma but also adenocarcinoma. An in-depth view of the spectrum of genomic alterations in small cell lung cancer (SCLC) has identified SOX2 as a potential target for therapeutic intervention. Amplification of 3q, the most common genomic aberration in squamous lung cancer, has been demonstrated in the evolution of preinvasive squamous lung cancer and implicates SOX2 as a key target of this dynamic process, making SOX2 and its downstream effector components potential targets for biological therapeutics of squamous carcinomas. SOX2 is expressed in nearly 20% of lung adenocarcinoma and is associated with poor prognosis. SOX2 activity was found to promote squamous identity instead of a loss of cellular differentiation consistent with the role of SOX2 as a "lineage-survival oncogene." Interestingly, SOX2 transcription factor is the predominant downstream target of EGFR signaling and plays a major role in self-renewal growth and expansion of side population cells. In light of the complex actions of SOX2 in regulating normal and tumor development, the elucidation of SOX2-dependent pathways may identify new therapeutic vulnerabilities in lung cancer and uncover additional common pathways between cancer, normal development, and the maintenance of pluripotency.

Keywords: SOX2; transcription factor; non-small cell lung cancer; small-cell lung cancer; cancer stem cells

Submitted Dec 05, 2012. Accepted for publication Jan 04, 2013.
doi: 10.3978/j.issn.2218-6751.2013.01.01
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2013.01.01

Sex-determining region Y (Sry)-related high mobility group (HMG)-box (SOX) genes are indispensable for multiple aspects of development (1). Members of the SOX family are expressed in a wide variety of tissues and have important roles in the regulation of organ development and cell-type specification (1). SOX2 was initially reported to be strongly associated with the inhibition of neuronal differentiation (2). More recent studies indicate that SOX2 exists in the nuclei of embryonic stem (ES) cells and acts as a transcriptional factor to maintain their unique characters such as clonogenicity, pluripotency, and self-renewal (3). Some cancer stem cells (CSCs) have, on their cell surface, ATP-binding cassette transporters (ABCG) that pump out the DNA-binding dye Hoechst 33342 and are characterized as side population (SP) cells (4). Because these malignant SP cells proliferate in a sustained fashion and readily export many cytotoxic drugs, they may be resistant to therapy and contribute to disease relapse (5). It was found that isolated SP cells show higher expression levels of stem cell genes, such as SOX2 and Oct4 and tumorigenesis properties than non SP cells (6).

SOX2 is transcriptionally regulated by an enhancer containing a composite SOX-OCT element that the octamer-binding transcription factor 4 (Oct4) and SOX2 bind in a combinatorial interaction (7). It appears that the SOX2-Oct4 regulatory complex upregulates a large number of genes important for the maintenance of the pluripotency of ES cells and downregulates genes responsible for the

initiation of differentiation (8). There is abundant evidence that SOX proteins might also affect the Wnt/β-catenin pathway. They can either antagonize or facilitate β-catenin/TCF-mediated transcription in the context of different SOX species (9). In parallel, G1/S-specific cyclin-D1 (CCND1) has been identified as the downstream target of SOX2 which agrees well with the cellular behavior of SOX2 in promoting the G0/G1 to S transition (10). Above all, SOX2 has been recently recognized as a novel target of EGFR-Src-AKT signaling in NSCLCs that modulates self-renewal and expansion of stem-like cells, making the relative SOX2 expression and functions within the tumor-CSCs a major determinant in EGFR-targeted therapy (11).

A number of links have been found between SOX transcription factors and human cancers. For instance, SOX2 promoter silencing by DNA methylation has been reported in some human gastric carcinomas (12). In contrast, several publications report overexpression of SOX2 in glioblastomas (13), non-small cell lung cancer (NSCLC) (14,15), SCLC (16), prostate cancer (17), hepatocellular carcinomas (18) osteosarcomas (19), and breast carcinomas (20), supporting a role of SOX2 as an oncogene in these tissues. These reports suggest that SOX2 could activate important gene cascades involved in tumor initiation and progression and in the maintenance of a poorly differentiated state. In this review, we will attempt to deepen our knowledge on the underlying molecular mechanism of SOX2's function in lung tumorigenesis, which may emerge as a novel promising strategy for lung cancer therapy.

The role of SOX2 in small cell lung cancer

SCLC is a distinct clinical and histological entity within the range of lung cancer (21). The incidence and mortality of SCLC worldwide make this disease a notable health-care issue. SCLC represents 13% of all newly diagnosed cases of lung cancer worldwide, and its prognosis remains poor, with an overall median survival following treatment of 10 months and a 5-year survival of 5% (22). Its management has followed the major developments of modern cancer treatment through the integration of biology, imaging, chemotherapy, and radiotherapy.

The immunohistochemical analysis of SOX2 expression in various types of lung cancer found that SCLC tissues revealed a higher expression level of SOX2 than NSCLC tissues (23). In parallel, SOX2 was found to cooperate with important oncogenes like Wnt1, Wnt2, c-Myc and Notch to promote lung tumor occurrence, while downregulation of SOX2 inhibited proliferation and induced apoptosis in tumor cells (23). Another study of more than 50 tumour samples and SCLC cell lines (H446 and H720) has shown that SOX2 is amplified in approximately 27% of cancers (16).

SOX2 plays a pivotal role in the maintenance of ES cell pluripotency by regulating lineage commitment factors and later in development, is involved in specification and maintenance of neural stem cells during neurogenesis. Notably, conditional induction of SOX2 in lung epithelial cells is also known to increase the number of neural progenitor cells (24). SCLCs are tumors with neuroendocrine features. SOX2 protein overexpression has previously been noted in high-grade SCLC, and immunoreactive antibodies against SOX2 have been detected in sera from SCLC patients (25,26).

Two possibilities may account for the increased expression of SOX2 in SCLC. One is that the normal progenitor cell of SCLC, generally presumed to be the neuroendocrine Kulchitsky cell, expresses SOX genes; thus, the expression of this antigen in SCLC represents the persistence of these differentiation characteristics during neoplastic clonal expansion. The other possibility is that SOX genes are not expressed in normal adult Kulchitsky cells or bronchial epithelium and that the expression of these genes in SCLC represents a reactivation of lineage-specific embryonic markers, reflecting the developmental stage at which SOX2 is coexpressed. Rudin *et al*., found that cell proliferation can be suppressed in vitro by silencing SOX2 (using short hairpin RNAs) which implicates this gene in driving SCLC and suggests a plausible novel therapeutic strategy (16).

The role of SOX2 in squamous cell lung cancer

It has been found that SOX2 amplification and consequent SOX2 protein overexpression represent important mechanisms of tumor initiation and progression in a considerable subset of squamous cell carcinomas (SCCs) (27). The reported frequency of SOX2 amplification in lung SCCs varies from 20% to 60% with these variations in frequency being most likely due to methodological discrepancies applied by different laboratories but also differences between the cohorts and tumor heterogeneity (28-31). Lung SCCs are known to commonly harbor an amplification of the genomic region 3q. The amplification locus of 3q comprises additional genes, such as the defective in cullin neddylation 1, domain containing 1 (DCUN1D1) and the

phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA) that previously have been proposed to be target oncogenes of the 3q amplicon. Recent studies have provided strong evidence that SOX2 is the primary amplification target within the common 3q amplicon and functional studies using shRNA against SOX2 showed an impact on tumor biology, thus making SOX2 the most promising candidate 3q oncogene (28,29,32).

A peak of genomic amplification on chromosome 3q26.33 that contains SOX2 gene, in SCCs of the lung and esophagus has been recently reported (28). However SOX2 alone cannot transform immortalized tracheobronchial epithelial cells and Forkhead box E1(FOXE1) or Fibroblast growth factor receptor 2 (FGFR2) are required as transforming cooperative genes (28). Toschi *et al*., evaluated SOX2 and FGFR1 gene copy number by fluorescence in situ hybridization (FISH) in tissue microarray cores in 447 surgically resected NSCLCs, to investigate their prognostic relevance and their association with clinico-pathological characteristics. They reported that increased SOX2 and FGFR1 gene copy number is a common event in lung cancer patients with squamous cell histology and that SOX2 gene gain is a favorable prognostic factor in early stage resected patients (33).

It seems that SOX2 correlates with markers of squamous differentiation in lung SCCs. For instance, TP63 and Keratin 6A (KRT6A), which encode for the squamous markers p63 and cytokeratin 6A, respectively, were among the transcripts most correlated with SOX2 expression in lung SCCs (28). When SOX2 was ectopically expressed in the NCI-H2009 lung adenocarcinoma cell line, both TP63 and KRT6A were induced, demonstrating actions of SOX2 that promote squamous identity rather than de-differentiation to a pluripotent state, thus consistent with its role as a lineage survival oncogene (28). It has also been demonstrated that SOX2 overexpression in epithelial cells of the adult lung drives development of histologically well differentiated adenocarcinoma with significant squamous cell features including widespread expression of p63, FoxE1 and Desmoglein-3, a phenotype not unique to tumors induced by SOX2 (14). Inducible deletion of the tumor suppressor liver kinase B1 (LKB1) along with inducible expression of oncogenic K-Ras leads to adenocarcinoma with squamous features as well (34). It is possible that SOX2 alone can drive expression of some squamous tumor markers, but an additional oncogenic stimulus is required to drive complete squamous differentiation.

Within the primitive foregut there is reciprocal expression of NKX2.1 (*also known as thyroid transcription factor 1; TITF1*) and SOX2 in compartments that form the trachea and esophagus, respectively (35). As the developmental transcription factor *NKX2-1* is an amplified lineage survival oncogene in lung adenocarcinoma, SOX2 may similarly represent a lineage survival oncogene in lung SCCs (35). Bass *et al*., found that SOX2 amplification was enriched in the lung SCC tumor population, while *NKX2-1* amplification was enriched in lung adenocarcinoma (28). The complementary roles of SOX2 and *NKX2-1* in distinct cancer lineages thus parallel their actions in development.

Functional studies underscore the oncogenic role of SOX2 in squamous cell carcinomas. In squamous cell carcinoma cell lines harboring SOX2 amplification, suppression of SOX2 had an anti-proliferative effect (28). Furthermore, cell lines overexpressing SOX2 exhibited increased migratory activity and enhanced colony formation (29). In preinvasive lesions of the lung, SOX2 expression has been reported to occur in normal bronchial epithelium, alveolar bronchiolization, squamous dysplasia, as well as carcinoma in situ (30). Furthermore, SOX2 amplification was reported in none of a series of low-grade bronchial lesions, but in all high-grade lesions, suggesting upregulation during preinvasive disease progression (36). Consistently, it has been shown that conditional homozygous SOX2 overexpression in Clara cells induces bronchial epithelial hyperplasia with 50% of cases showing a progression to lung cancer in mice (14). Taken together, these results strongly indicate that SOX2 harbors oncogenic potential and has a role during tumorigenesis.

An association between elevated SOX2 expression and indicators of better patient outcome, most importantly prolonged overall survival, was recently demonstrated (37). Increased levels of SOX2 amplification indicated a better histological differentiation grade and a trend to improved patient survival (37). In a cohort of early stage lung SCCs, patients with SOX2 expression above the median showed prolonged overall survival (14). The molecular mechanisms accounting for SOX2 being associated with favorable prognosis in lung SCCs is still unknown and further studies are needed to clarify the functional aspects of SOX2. SOX2 overexpression might recapitulate transcription networks active in normal squamous precursor cells and thus counteract the chaos of malignant dedifferentiation or alternatively, SOX2 overexpression might occur early during lung SCC carcinogenesis and might be lost during disease progression, due to genetic inactivation. Furthermore tumors arising from an upregulation of SOX2 exhibit a clear squamous cell differentiation and thus can

be associated with better prognostic features, similar to NKX2-1 in lung adenocarcinomas.

Besides the lung, SOX2 has been found to be amplified and expressed in squamous cell carcinomas originating from other organ sites, predominantly derived from the embryonic foregut, but also from non-foregut tissues, such as the skin, the cervix, and the penis (27,28,38,39). Squamous carcinogenesis from diverse body sites may thus share similar underlying mechanisms and SOX2 might be a general marker for SCC differentiation regardless the tissue of origin. All the above studies used a similar strategy of chromosomal aberrations screening to identify the SOX2 locus as one of the most frequently amplified sites over the SCC genome and further highlighted the recurrent SOX2 activation and its indispensable role for squamous cell survival. However, it remains to assess the impact of the recurrent activation of SOX2 in advanced primary tumors and how SOX2 may mechanistically be involved in tumor progression and aggressiveness.

The role of SOX2 in lung adenocarcinomas

Previous findings revealed that SOX2 is expressed in bronchial epithelial cells of the lung, whereas it is absent in alveolar cells (30,38). Likewise, adenocarcinoma precursor lesions, such as atypical adenomatous hyperplasia, proved to be negative for SOX2 expression (30). In the study of Cai *et al.*, the amplification of SOX2 in SCCs and adenocarcinomas was 31.6% and 20%, respectively (40). No SOX2 amplification was found among smokers with adenocarcinoma (40). In contrast, 10 of 38 (26.3%) cases involving patients with no history of smoking and with adenocarcinoma presented SOX2 amplification, indicating that SOX2 amplification may be an activating pathway to adenocarcinoma (40).

Another recent study showed that SOX2 is strongly and diffusely expressed in approximately 90% of pulmonary SCC and 20% of adenocarcinoma (41). When SOX2 expression was examined in stage I lung adenocarcinoma patients was detected in 50% of cases and it was more frequent in tumors from older and male patients (41). Compared to SOX2-negative tumors, SOX2 expression predicted a shorter time to tumor progression and shorter overall survival and appeared to be an independent predictor of poor outcome in stage I lung adenocarcinomas which may help stratify patients at increased risk for recurrence (41). Taken together, these results might suggest a prognostic role for SOX2 in lung adenocarcinomas. However, given the overall low frequency of SOX2 amplification and overexpression, the significance of this finding needs further evaluation.

As previously described, SOX2 gene amplification is more common in the SCCs of smokers while the incidence of SOX2 amplification is in the early stage of tumorigenesis in NSCLC. However SOX2 is also activated in more advanced SCC tumors (26,29). Therefore the SOX2 gene is not only activated by amplification but is also affected by other regulators that promote its transcription, affecting its downstream genes. SP cells isolated from established human NSCLC cell lines and tumors are highly enriched in NSCLC-CSCs and EGFR-Src-AKT signaling axis contributes significantly to the self-renewal of SP cells (11). Interestingly, SOX2 transcription factor is the predominant downstream target of EGFR signaling in these cells and plays a major role in self-renewal growth and expansion of SP cells, independent of Oct4 and Nanog (11).

EGFR tyrosine kinase inhibitors are able to downregulate self-renewal and SP phenotype. Singh *et al.*, have reported that blocking EGF-receptors results in a significant decrease in SP frequency in both A549 and H1650 cells along with decreased EGFR phosphorylation as well as ABCG2 expression in both cell lines (11). Depletion of EGFR expression by erlotinib or gefitinib inhibits the self-renewal of SP cells and so does the combination of gefitinib with the irreversible EGFR-tyrosine kinase inhibitor BIBW2992 in the H1975 cell line with acquired resistance to gefitinib or erlotinib due to the secondary point mutation in exon 20 of EGFR (T790M) (11). When the downstream signaling events of EGFR expression were examined, it was found that c-Src, ERK and AKT signaling impinge transcription factors associated with stemness (*Figure 1A*) (11). EGFR inhibition by gefitinib or BIBW as well as inhibition of Src activity by dasatinib markedly decreased phosphorylation of EGFR, Src, ERK and AKT and reduced SOX2 expression; Oct4 and Nanog levels were not affected (*Figure 1B,C*) (11). The contribution of ERK and AKT pathways to EGFR mediated induction of SOX2 has also been examined. Phosphorylation of ERK is suppressed by the MEK inhibitor PD98059 and AKT phosphorylation is suppressed by the PI3-kinase inhibitor, LY294002. PI3-kinase inhibitors can also slightly inhibit ERK phosphorylation (*Figure 1D*) (11). However, as shown in *Figure 1E*, inhibition of MEK activity does not affect the levels of SOX2 while the PI3-kinase inhibition, markedly reduces its levels with corresponding reduction in SP frequency (11). Therefore, relative SOX2 expression and functions within the tumor-

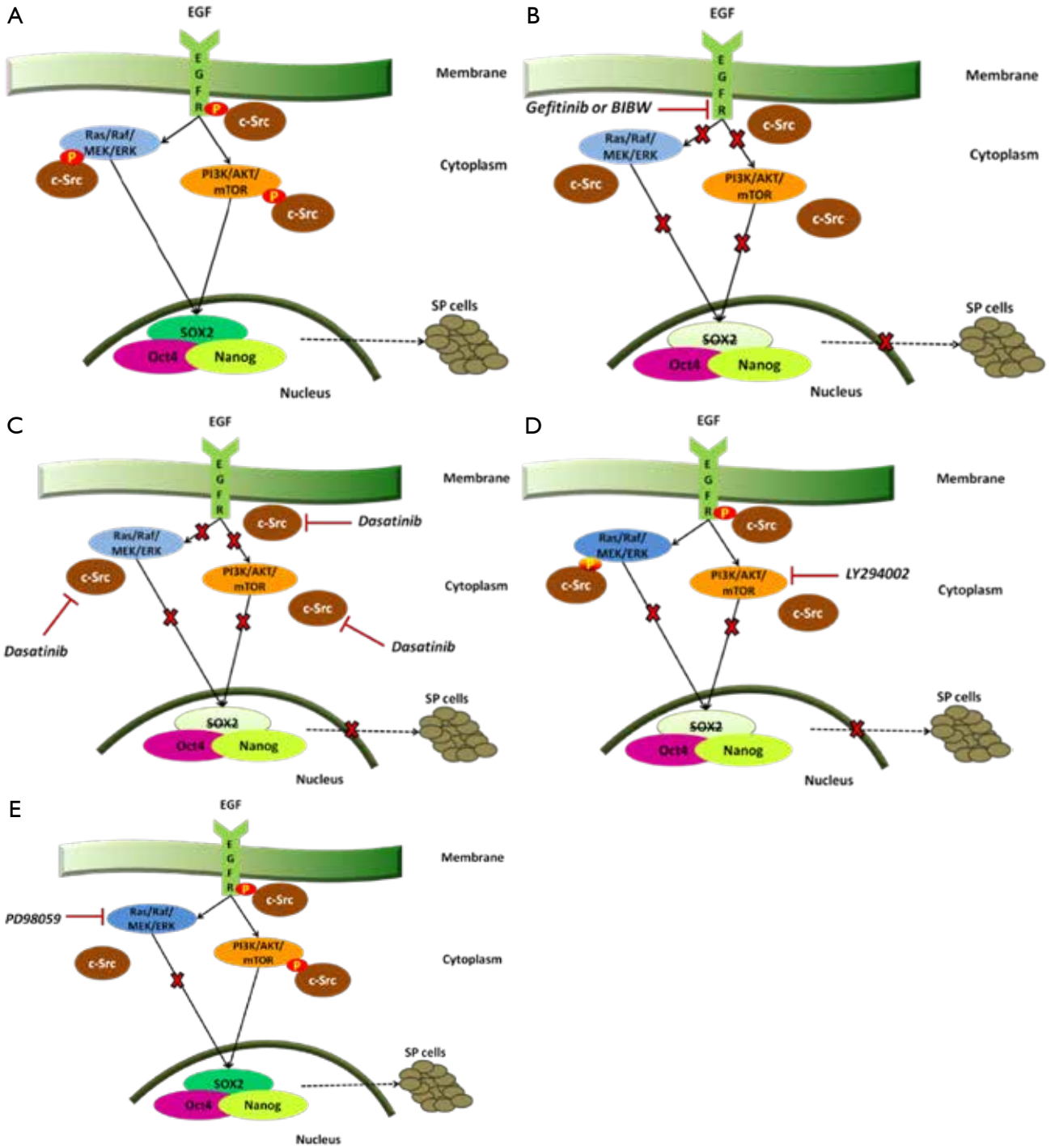


Figure 1 (A) EGFR activation as well as c-Src signaling result in phosphorylation of EGFR, ERK and AKT and mediate induction of SOX2 that modulates self-renewal of SP cells together with other transcription factors like Oct4 and Nanog; (B) inhibition of EGFR with gefitinib or BIBW results in decreased phosphorylation of EGFR, ERK and AKT and reduces SOX2 levels with corresponding reduction in SP frequency. The expression of Oct4 and Nanog is not affected; (C) inhibition of c-Src with dasatinib results in decreased phosphorylation of EGFR, ERK and AKT and reduces SOX2 levels with corresponding reduction in SP frequency. The expression of Oct4 and Nanog is not affected; (D) the PI3K inhibitor LY294002 supresses AKT phosphorylation, slightly inhibits ERK phosphorylation and reduces the levels of SOX2; E. Inhibition of MEK activity does not affect the levels of SOX2.

CSCs may be a major determinant in EGFR-targeted therapy against NSCLCs. This information might also be potentially useful to overcome the acquired resistance to EGFR therapies, by targeting downstream targets of EGFR signaling, including SOX2.

In summary, SOX2 might be a molecular target of lung adenocarcinomas. Transient transfection of SOX2 siRNA completely abrogated the tumorigenicity of SP cells in a lung adenocarcinoma cell line (LHK2) (42). SOX2 has a role in maintenance of stemness and tumorigenicity of human lung adenocarcinoma CSCs but further molecular analysis especially upstream and downstream of SOX2 should reveal the mechanisms of its tumorigenicity, making SOX2 a potential target for treatment.

Conclusions

SOX2 has been shown as hall mark of lung cancer but its role in lung cancer formation or progression has been partially elucidated. Amplification and overexpression of SOX2 are strongly associated with SCC morphology and favorable clinicopathological features in SCCs, including longer overall survival. In contrast, both events are less frequent in SCLC and rare in adenocarcinoma and of uncertain prognostic significance. The finding of SOX2 amplification/upregulation being frequent in lung SCCs, but rare in lung adenocarcinomas might reflect a fundamental molecular difference in carcinogenesis between these tumor entities.

The elucidation of SOX2-dependent pathways may identify novel therapeutic vulnerabilities in lung cancer and may uncover additional common pathways between cancer, normal development and the maintenance of pluripotency. The appearance of compensatory mechanisms favoring survival of cancer cells after therapy represents a limitation in therapies targeting EGFR and understanding but also overcoming EGFR-TKI resistance mechanisms in NSCLC patients has become a burning issue lately. Molecular pathways are interconnected, and thus, combination therapy is emerging as an appropriate strategy to treat those patients. Unfortunately very few patients undergo repeated tumor biopsies at the time when resistance develops to help guide appropriate therapeutic choices and the need to develop noninvasive methods to identify resistance mechanisms becomes more evident. For instance we should consider the possibility of using quantitative reverse transcriptase-PCR in measuring plasma SOX2 mRNA in lung cancer patients with gained-resistance to EGFR tyrosine kinase inhibitors and confirm whether high circulating plasma mRNA levels of SOX2 could be undocumented as a mechanism of resistance to EGFR-targeted therapy. In parallel the plasma mRNA measurement of the druggable downstream targets of EGFR signaling that regulate SOX2, can be also of great significance. Moreover SOX2 amplification may be more effectively identified by examining copy number changes by FISH specifically on individual circulating tumor cells. The recent advances in isolating circulating tumor cells suggest that this may be possible and can be combined with genotyping studies to examine mechanisms of resistance to EGFR tyrosine kinase inhibitors.

Except from targeting downstream targets of EGFR signaling that regulate SOX2 as mentioned above, being able to target SOX2 itself and other transcription factors involved in tumor initiation and maintenance can provide a unique opportunity for anti-cancer intervention. However, because of their lack of small molecule binding pockets, transcription factors are currently an example of 'undruggable targets'. Thus, novel strategies to effectively down-regulate these targets are required. Recently Zinc-finger-based artificial transcription factors (ATFs) were able to reactivate the expression of the tumor-suppressor genes and repress potential oncogenes including SOX2 in breast cancer cell lines (43). These data suggest that the targeted down-regulation of highly expressed oncogenes using ATF-based technologies can be used as a powerful tool for the long-term targeting of oncogenic TFs with potential application in cancer biology. In summary, the above data elucidate and offer novel perspectives on the multiple roles that the transcription factor SOX2 exerts on carcinogenesis. SOX2 that is expressed in lung SCC and adenocarcinoma, but also in SCLC tissues can act as novel unite marker and ideal therapeutic target. In view of the fact that the transcriptional activity of SOX2 is critical in mediating tumorigenesis, we believe that further studies investigating how SOX2 activity is regulated will be highly worthwhile.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Kiefer JC. Back to basics: Sox genes. Dev Dyn 2007;236:2356-66.
2. Bylund M, Andersson E, Novitch BG, et al. Vertebrate neurogenesis is counteracted by Sox1-3 activity. Nat

Neurosci 2003;6:1162-8.
3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-76.
4. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 2001;7:1028-34.
5. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci U S A 2004;101:14228-33.
6. Kruger JA, Kaplan CD, Luo Y, et al. Characterization of stem cell-like cancer cells in immune-competent mice. Blood 2006;108:3906-12.
7. Seo E, Basu-Roy U, Zavadil J, et al. Distinct functions of Sox2 control self-renewal and differentiation in the osteoblast lineage. Mol Cell Biol 2011;31:4593-608.
8. Okumura-Nakanishi S, Saito M, Niwa H, et al. Oct-3/4 and Sox2 regulate Oct-3/4 gene in embryonic stem cells. J Biol Chem 2005;280:5307-17.
9. Sinner D, Rankin S, Lee M, et al. Sox17 and beta-catenin cooperate to regulate the transcription of endodermal genes. Development 2004;131:3069-80.
10. Chen Y, Shi L, Zhang L, et al. The molecular mechanism governing the oncogenic potential of SOX2 in breast cancer. J Biol Chem 2008;283:17969-78.
11. Singh S, Trevino J, Bora-Singhal N, et al. EGFR/Src/Akt signaling modulates Sox2 expression and self-renewal of stem-like side-population cells in non-small cell lung cancer. Mol Cancer 2012;11:73.
12. Li XL, Eishi Y, Bai YQ, et al. Expression of the SRY-related HMG box protein SOX2 in human gastric carcinoma. Int J Oncol 2004;24:257-63.
13. Gangemi RM, Griffero F, Marubbi D, et al. SOX2 silencing in glioblastoma tumor-initiating cells causes stop of proliferation and loss of tumorigenicity. Stem Cells 2009;27:40-8.
14. Lu Y, Futtner C, Rock JR, et al. Evidence that SOX2 overexpression is oncogenic in the lung. PLoS One 2010;5:e11022.
15. Leung EL, Fiscus RR, Tung JW, et al. Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PLoS One 2010;5:e14062.
16. Rudin CM, Durinck S, Stawiski EW, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 2012;44:1111-6.
17. Bae KM, Su Z, Frye C, et al. Expression of pluripotent stem cell reprogramming factors by prostate tumor initiating cells. J Urol 2010;183:2045-53.
18. Xu XL, Xing BC, Han HB, et al. The properties of tumor-initiating cells from a hepatocellular carcinoma patient's primary and recurrent tumor. Carcinogenesis 2010;31:167-74.
19. Basu-Roy U, Seo E, Ramanathapuram L, et al. Sox2 maintains self renewal of tumor-initiating cells in osteosarcomas. Oncogene 2012;31:2270-82.
20. Lengerke C, Fehm T, Kurth R, et al. Expression of the embryonic stem cell marker SOX2 in early-stage breast carcinoma. BMC Cancer 2011;11:42.
21. van Meerbeeck JP, Fennell DA, De Ruysscher DK. Small-cell lung cancer. Lancet 2011;378:1741-55.
22. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
23. Chen S, Xu Y, Chen Y, et al. SOX2 gene regulates the transcriptional network of oncogenes and affects tumorigenesis of human lung cancer cells. PLoS One 2012;7:e36326.
24. Gontan C, de Munck A, Vermeij M, et al. Sox2 is important for two crucial processes in lung development: branching morphogenesis and epithelial cell differentiation. Dev Biol 2008;317:296-309.
25. Güre AO, Stockert E, Scanlan MJ, et al. Serological identification of embryonic neural proteins as highly immunogenic tumor antigens in small cell lung cancer. Proc Natl Acad Sci U S A 2000;97:4198-203.
26. Sholl LM, Long KB, Hornick JL. Sox2 expression in pulmonary non-small cell and neuroendocrine carcinomas. Appl Immunohistochem Mol Morphol 2010;18:55-61.
27. Maier S, Wilbertz T, Braun M, et al. SOX2 amplification is a common event in squamous cell carcinomas of different organ sites. Hum Pathol 2011;42:1078-88.
28. Bass AJ, Watanabe H, Mermel CH, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41:1238-42.
29. Hussenet T, Dali S, Exinger J, et al. SOX2 is an oncogene activated by recurrent 3q26.3 amplifications in human lung squamous cell carcinomas. PLoS One 2010;5:e8960.
30. Yuan P, Kadara H, Behrens C, et al. Sex determining region Y-Box 2 (SOX2) is a potential cell-lineage gene highly expressed in the pathogenesis of squamous cell carcinomas of the lung. PLoS One 2010;5:e9112.
31. Sasaki H, Yokota K, Hikosaka Y, et al. Increased Sox2 copy number in lung squamous cell carcinomas. Exp Ther Med 2012;3:44-48.
32. Hussenet T, du Manoir S. SOX2 in squamous cell

carcinoma: amplifying a pleiotropic oncogene along carcinogenesis. Cell Cycle 2010;9:1480-6.
33. Toschi L, Finocchiaro G, Teresa T. SOX2 and FGFR1 gene copy number in surgically resected non-small cell lung cancer (NSCLC). J Clin Oncol 2012;30:abstr 7061.
34. Ji H, Ramsey MR, Hayes DN, et al. LKB1 modulates lung cancer differentiation and metastasis. Nature 2007;448:807-10.
35. Que J, Okubo T, Goldenring JR, et al. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 2007;134:2521-31.
36. McCaughan F, Pole JC, Bankier AT, et al. Progressive 3q amplification consistently targets SOX2 in preinvasive squamous lung cancer. Am J Respir Crit Care Med 2010;182:83-91.
37. Wilbertz T, Wagner P, Petersen K, et al. SOX2 gene amplification and protein overexpression are associated with better outcome in squamous cell lung cancer. Mod Pathol 2011;24:944-53.
38. Long KB, Hornick JL. SOX2 is highly expressed in squamous cell carcinomas of the gastrointestinal tract. Hum Pathol 2009;40:1768-73.
39. Freier K, Knoepfle K, Flechtenmacher C, et al. Recurrent copy number gain of transcription factor SOX2 and corresponding high protein expression in oral squamous cell carcinoma. Genes Chromosomes Cancer 2010;49:9-16.
40. Cai YR, Zhang HQ, Zhang ZD, et al. Detection of MET and SOX2 amplification by quantitative real-time PCR in non-small cell lung carcinoma. Oncol Lett 2011;2:257-264.
41. Sholl LM, Barletta JA, Yeap BY, et al. Sox2 protein expression is an independent poor prognostic indicator in stage I lung adenocarcinoma. Am J Surg Pathol 2010;34:1193-8.
42. Nakatsugawa M, Takahashi A, Hirohashi Y, et al. SOX2 is overexpressed in stem-like cells of human lung adenocarcinoma and augments the tumorigenicity. Lab Invest 2011;91:1796-804.
43. Stolzenburg S, Rots MG, Beltran AS, et al. Targeted silencing of the oncogenic transcription factor SOX2 in breast cancer. Nucleic Acids Res 2012;40:6725-40.

Cite this article as: Karachaliou N, Rosell R, Viteri S. The role of SOX2 in small cell lung cancer, lung adenocarcinoma and squamous cell carcinoma of the lung. Transl Lung Cancer Res 2013;2(3):172-179. doi: 10.3978/j.issn.2218-6751.2013.01.01

Inhibition of insulin-like growth factor receptor: end of a targeted therapy?

Rathi N. Pillai, Suresh S. Ramalingam

Winship Cancer Institute, Emory University, GA, USA

Correspondence to: Suresh S. Ramalingam, MD, Professor, Director of Medical Oncology. 1365 Clifton Road NE, Rm C-3090, Atlanta, GA 30322, USA. Email: suresh.ramalingam@emory.edu.

Abstract: The Insulin-like Growth Factor 1 (IGF-1) signaling pathway activates several downstream signals important to lung cancer development and survival. IGF-1R activation has been linked to cancer risk in epidemiological studies and tumorigenesis in preclinical models. Several inhibitors of the insulin-like growth factor 1 receptor (IGF-1R) have been tested in clinical trials. Despite promising data in early phase studies, most studies of IGF-1R antagonists in combination with chemotherapy or with epidermal growth factor receptor (EGFR) inhibitors in non-small cell lung cancer (NSCLC) yielded disappointing results. Biomarker studies of clinical trials have identified IGF-1 levels as a potential marker of sensitivity to IGF-1R inhibition. Further study will need to focus on selection of NSCLC patients most likely to benefit from the addition of IGF-1R antagonists to standard therapy and the development of rational strategies for combination therapy in NSCLC.

Keywords: Insulin-like Growth Factor 1 (IGF-1) ; insulin-like growth factor 1 receptor (IGF-1R); non-small cell lung cancer (NSCLC); epidermal growth factor receptor (EGFR)

Submitted Oct 06, 2012. Accepted for publication Nov 08, 2012.
doi: 10.3978/j.issn.2218-6751.2012.11.05
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2012.11.05

Introduction

Lung cancer remains the most lethal form of cancer worldwide, with 1.37 million deaths in 2008 (1). Due to lack of effective screening programs until recently, most patients present with advanced disease, where the mainstay of therapy is chemotherapy. Frontline treatment for patients with lung cancer consists of platinum doublet therapy, based on seminal publications that established improvement in survival over best supportive care (2). Recent advances in the genetic characterization of lung cancers have resulted in use of targeted agents for specific subsets of NSCLC. However, the majority of patients still receive systemic chemotherapy since targetable molecular abnormality is detected only in approximately 20%. There is a great need to find new targets to improve the efficacy of treatment for lung cancer patients.

The insulin-like growth factor (IGF) pathway has been extensively studied as an important signaling pathway in cancer. IGF-1 and its receptor, the insulin growth factor 1 receptor (IGF-1R), have been implicated in carcinogenesis and to cancer risk in the population. IGF-1, produced in the liver, mediates the effects of growth hormone. IGF-1R is a member of the insulin receptor subclass of tyrosine kinase membrane receptors and shares structural homology with the insulin receptor (3). IGF-1R consists of a tetramer of two alpha subunits that bind IGF-1, and insulin less avidly, and two intracellular beta subunits, which have tyrosine kinase domains with the ATP binding site. Once IGF-1R binds to IGF-1, this causes a conformational change in the receptor, leading to activation of the kinase domain. IGF-1R can then signal through adapter proteins insulin receptor substrate (IRS) 1 and 2 to activate downstream targets including the PI3K/AKT/mTOR and Ras/Raf/MAPK pathways, leading to cell cycle progression, cell proliferation, and cell survival (*Figure 1*). Serum IGF-binding proteins (IGFBPs) bind 90% of circulating IGF-1 and prevent receptor binding and prolong half-life of IGF-1. IGF signaling has been

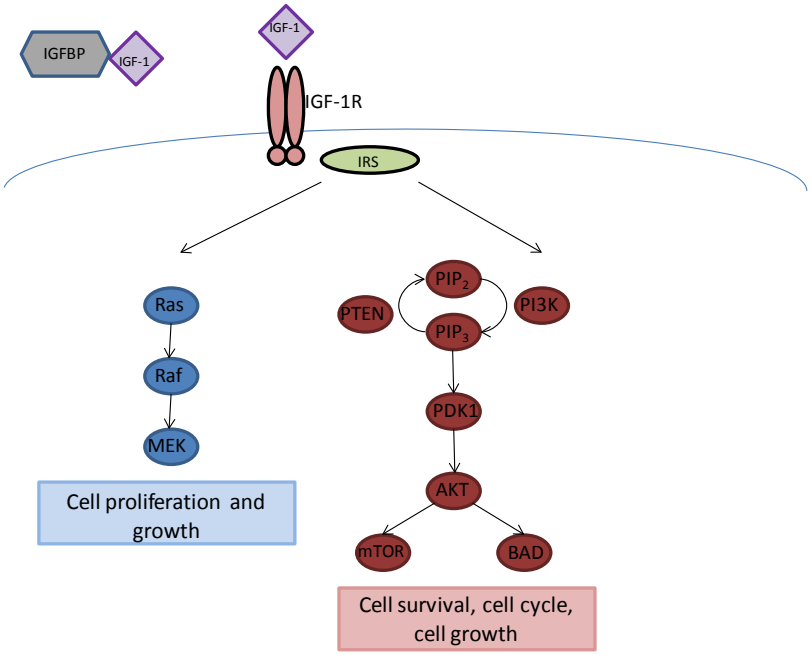


Figure 1 IGF-1R Signaling Pathway. IGF-1 is regulated by binding to insulin growth factor binding protein (IGFBP). Free IGF-1 can bind to IGF-1R, and activate downstream signaling through the insulin receptor substrate (IRS) to promote cell growth and proliferation (Ras/Raf/MEK pathway) and cell survival and cell cycle progression (PI3K/AKT/mTOR pathway).

implicated in regulation of angiogenesis, as this pathway can activate HIF-1α expression and VEGF secretion in lung cancer cell lines (3). There also appears to be crosstalk between IGF pathway and EGFR signaling; inhibition of the EGFR pathway is less effective in the presence of IGF-1R overexpression, suggesting a potential mechanism behind development of resistance to EGFR inhibitors.

The IGF signaling pathway has been implicated in carcinogenesis and cancer risk in many studies. In a landmark paper, lack of IGF-1R expression in mouse fibroblasts resulted in loss of oncogenic transformation by simian virus 40 (SV40) large T antigen expression (4). Wild-type fibroblasts were transformed by SV40 large T antigen expression and able to form foci in both culture and soft agar, demonstrating hallmarks of cancer cells with loss of contact inhibition and anchorage-independent growth. These findings were further verified by the loss of transformation seen in fibroblasts treated with antisense RNA against IGF-1R. This was the first demonstration of the importance of IGF-1R in carcinogenesis.

IGF-1 was also shown to be important for tumor growth in a breast cancer xenograft model in mice; mice that were homozygous for *lit* mutation, resulting in loss of IGF-1/growth hormone axis, had significantly smaller tumors than in control mice (5). With regards to lung cancer, IGF overexpression in transgenic mice led to the development of adenomatous hyperplasia (6). IGF-1R is overexpressed in cancer cell lines and in human cancers (3,7). IGF-1 and IGF-1R overexpression has been studied in multiple epidemiological studies and linked to increased risk of lung, ovarian, pancreatic, breast, and colorectal cancers (7).

Clinical trials with IGF-1R inhibitors in lung cancer

Based on the sound pre-clinical rationale supporting IGF-1R as a therapeutic target, agents that inhibit IGF-1R were evaluated in clinical trials (*Table 1*). The majority of the studies conducted to date have evaluated monoclonal antibodies that bind to, and inhibit the IGF-1R. Presently, small molecule tyrosine kinase inhibitors of IGF-1R are also under development.

Figitumumab

The first specific IGF-1R antagonist which was developed in the clinic is figitumumab (*Table 2*), previously known as CP-751,871, a fully humanized monoclonal antibody against IGF-1R (Pfizer, New London, CT). Phase II data

Table 1 Selected IGF-1R inhibitors evaluated in the clinic

Drug	Company	Phase of study
Figitumumab (CP-751, 871)	Pfizer	III
R1507	Roche	II
Cixutumumab (IMC-A12)	Imclone	II
Dalotuzumab (MK-0646)	Merck	II
OSI-906	OSI pharmaceuticals	I, II

Table 2 Clinical data with figitumumab

	Primary endpoint	P value
Phase II (8)		
Carboplatin/paclitaxel/figitumumab	ORR 54%	P<0.0001 (95% CI, 0.44 to 0.64)
Carboplatin/paclitaxel	ORR 42%	
Phase III (9)		
Carboplatin/paclitaxel/figitumumab	OS 10.3 months	HR 1.23 (95% CI, 1.0-1.5), P=0.051
Carboplatin/paclitaxel	OS 8.5 months	
Phase III (not published)		
Figitumumab/erlotinib	OS: terminated for futility	n/a

were published in 2009, which showed very promising activity in combination with platinum doublet chemotherapy (8). Patients with chemotherapy-naïve advanced and metastatic non-small cell lung cancer (NSCLC) were randomized 2:1 to carboplatin/paclitaxel/figitumumab (PCF) or carboplatin/paclitaxel (PC). Carboplatin was dosed to achieve an area under the plasma concentration-time curve (AUC) of 6 and paclitaxel at 200 mg/m^2 every 3 weeks; figitumumab was given to the first cohort of patients at 10 mg/kg IV and second cohort at 20 mg/kg IV, every 3 weeks. Patients in the control arm could receive figitumumab with or without PC per investigator's discretion at progression (n=20 patients). Patients in the treatment arm could continue on single agent figitumumab after discontinuation of chemotherapy (n=47 patients). The study demonstrated an objective response rate (ORR) of 54% with PCF compared to 42% in PC arm. The most remarkable findings were from a subgroup analysis: patients with squamous cell carcinoma were found to have an ORR of 78% and 12-week progression-free survival (PFS) rate of 89% with figitumumab 20 mg/kg. An additional 30 patients were enrolled to single arm expansion cohort to confirm these results. There was no difference in PFS between the PCF and PC groups, but for patients in the control arm that received figitumumab at progression, this translated into improved PFS with HR 0.56 (P=0.0153, 95% CI, 0.28 to 0.87). The combination therapy was associated with a higher incidence of grade 3 and 4 hyperglycemia seen in PCF arm (15%) compared to PC arm (8%). There were eight deaths during study treatment, with 5 reported in the PCF arm compared to 3 deaths in PC. Based on these phase II data, there was great excitement in proceeding with a phase III, particularly in patients with squamous cell histology, for whom there have been few successful therapies.

The results of the phase III study were presented at the 2010 American Society for Clinical Oncology (ASCO) meeting (9). The planned enrollment was 820 patients with non-adenocarcinoma NSCLC to be randomized to PCF with 20 mg/kg or PC in the first line setting. The primary endpoint was overall survival (OS). The study was terminated after 681 patients (86% squamous and 88% stage IV) were enrolled based on a planned interim analysis that demonstrated a hazard ratio that crossed futility boundary favoring PC. The OS in PCF was 10.3 months compared to 8.5 months in PC arm (HR 1.23, 95% CI, 1.0-1.5, P=0.051). In patients with serum IGF-1 level greater than or equal to 1 ng/mL, OS was 10.2 months in PCF group compared to 7 months in PC group (HR 0.97, 95% CI, 0.6-1.7). The OS hazard ratio favored PC in patients with low baseline IGF-1 (HR 1.6 PCF/PC ratio for patients with IGF1 <120 ng/mL, P=0.006) and PCF in patients with high baseline IGF1 levels (over 145 ng/

mL, HR=0.62, P=0.13). The disappointing results of the phase III study were likely a consequence of the lack of predictive biomarkers for patient selection, particularly in light of the absence of survival benefit in the preceding phase II study.

Based on the correlation between efficacy of figitumumab and the serum IGF-1 levels in the Jassem study, biomarker analysis was done in the serum specimens collected from patients that participated in the phase II study described earlier (10). The study team evaluated IGF-1, IGF-2, IGFBP1-3, insulin, and cotinine in plasma samples obtained from patients. Of all these, high pre-treatment levels of free IGF-1 defined as at least 0.54 ng/mL were found to correlate with improved PFS (P=0.007) in patients who received 20 mg/kg dose of figitumumab. Conversely, low levels of free IGF-1 was associated with a median PFS of <3 months (P=0.026). For patients treated with chemotherapy alone, IGF-1 levels were not predictive of PFS improvement, suggesting that the marker is specific for therapy with figitumumab. The authors found that a threshold of free IGF-1 level above 0.7 ng/mL predicted different median PFS by treatment group: 2.63 months with chemotherapy alone, 3.97 with PCF using 10 mg/kg figitumumab dose, and 6.53 months in PCI group who received 20 mg/kg figitumumab. In patients with free IGF-1 levels below 0.7 ng/mL, there was no difference in median PFS between the three treatment groups. There was a correlation between high free IGF-1 levels and expression of vimentin, a mesenchymal marker that is linked with epithelial to mesenchymal transition (EMT). The results from these two studies suggest that baseline serum IGF-1 level could help select patients that might benefit from figitumumab combination. These results may be useful to the clinical development of other IGF-1R inhibitors.

Figitumumab was also studied in combination with erlotinib in the phase III ADVIGO 1018 study. This trial enrolled patients with advanced NSCLC with non-adenocarcinoma histology in the second or third line setting. The study was halted due to an interim analysis which demonstrated the futility of this combination in March 2010 (11). As the data have never been presented, it is unclear what factors may have impacted the disappointing results.

R1507

R1507 is a humanized recombinant monoclonal antibody against IGF-1R developed by Roche (Basel, Switzerland). Based on preclinical observations that IGF-1R signaling interacts with EGFR signaling and may mediate resistance to EGFR inhibitors, R1507 was studied in a phase II trial in combination with erlotinib in patients with advanced NSCLC (12). Patients with metastatic NSCLC that had progressed on one or two prior chemotherapy regimens were randomized to erlotinib 150 mg orally daily in combination with either R1507 9 mg/kg IV weekly, 16 mg/kg IV every 3 weeks or placebo. The primary endpoint was PFS rate at 12 weeks. Patient tumor samples were tested for activating *EGFR* mutations and *KRAS* mutations. In this patient population of predominantly male patients (65-68%) with a minority of never smokers (9-16%), there was no difference in 12-week PFS rate between the treatment groups: 41% with erlotinib alone, 42% erlotinib and weekly R1507, and 45% erlotinib and every 3 weekly R1507. The OS was 8.1 months in erlotinib alone, 8.1 months erlotinib and weekly R1507, and 12.1 months with erlotinib and 3 week R1507, which was not statistically significant: the hazard ratio was 0.84 with weekly R1507 (0.58 to 1.21, 90% CI, P=0.43) and 0.72 with three weekly R1507 (0.53 to 0.99, 90% CI, P=0.09). However, in the 27% of patients with mutated *KRAS*, the 12-week PFS rate was improved to 36% in patients who received R1507 compared to 0 in patients treated with placebo (P=0.039). It is interesting that the *KRAS* mutated patients seem to benefit from the addition of IGF-1 inhibitor R1507 to EGFR inhibition, as these patients are typically resistant to EGFR inhibition. The results suggest that patients with *KRAS* mutations may benefit from combined inhibition of the IGF-1R and EGFR pathways.

In order to determine other predictive biomarkers for treatment with R1507 and erlotinib, archived tumor tissue and plasma were assessed for free and total IGF-1, IGFBP-3, IGF-1R, pAKT, PTEN, EGFR, and *KRAS* and correlated with the primary endpoint of the study, 12-week PFS rate (13). Free IGF-1 level was significantly correlated with improved 12-week PFS rate in the patients treated in the 16 mg/kg dose of R1507: 46% patients with elevated free IGF-1 level treated with R1507 achieved 12 week PFS compared to 18% patients in placebo arm, HR=3.94 (95% CI, 1.2-13.6). None of the other biomarkers had a significant impact on treatment response with R1507. These results further substantiate the observations that high levels of serum IGF-1 might be a useful selection parameter for treatment with IGF-1R inhibitors.

Cixutumumab

Cixutumumab (IMC-A12) is a fully humanized monoclonal IgG1 antibody against IGF-1R developed by Imclone

(Bridgewater, NJ). Preclinical studies demonstrated high affinity binding of IMC-A12 to IGF-1R and inhibition of the IGF-1R signaling pathway; in addition, both single agent activity as well as additive and synergistic effects with cytotoxic agents and targeted therapies like cetuximab and mTOR inhibitors were observed (14). Two phase II studies have been performed by ECOG investigating IMC-A12 activity in lung cancer patients. The ECOG 4508 study (15) randomized patients with metastatic NSCLC that were ineligible for bevacizumab to treatment with carboplatin and paclitaxel combined with either cetuximab weekly, IMC-A12 every two weeks, or both. The trial was terminated early for safety concerns related to excessive 30-day mortality with the four-drug regimen, after only 129 patients of planned 180 patients were enrolled. The median PFS was similar between the arms: 3.4 months in cetuximab arm, 4.3 months in IMC-A12 arm, and 4.1 months in combination arm. OS was also comparable in all treatment groups: 11.7 months cetuximab arm, 9.6 months IMC-A12 arm, and 8.4 months in combination arm. There were 13 deaths on treatment, including 9 patients who died within 1 month of initiation of study drug. There were higher rates of neutropenia, hyperglycemia, and thromboembolic events in the treatment arms that included IMC-A12. However, 6 of the 13 deaths occurred in patients who did not receive IMC-A12 so the high mortality seen was not solely due to IGF-1R inhibition, and could have resulted from pre-existing medical conditions. Studies utilizing IMC-A12 with other agents in lung cancer have proceeded without excessive toxicities.

IMC-A12 is also being studied in patients with small cell lung cancer. The ECOG 1508 study enrolled patients with extensive stage small lung cancer and randomized them to treatment in one of three arms: standard cisplatin and etoposide for 4 cycles, cisplatin and etoposide in combination with GDC-0449, an oral Hedgehog inhibitor, for 4 cycles with continuation of GDC-0449 as maintenance therapy, or cisplatin and etoposide with IMC-A12 on days 1, 8, 15 with IMC-A12 maintenance therapy until disease progression. The primary endpoint of the study is PFS; the study has completed accrual and the results are anticipated to be reported soon.

IMC-A12 has also been studied in combination with EGFR inhibitors. Results from a phase I study of erlotinib in combination with IMC-A12 were recently published (16). This trial examined the safety of erlotinib 150 mg daily combined with 3 different doses of IMC-A12: 6 mg/kg weekly or 5 mg/kg weekly given on 28 day cycle or 15 mg/kg every 21 days. Eighteen patients were treated and the most frequent toxicities seen were fatigue, rash, and diarrhea. Four patients in the 6 mg/kg dose cohort had DLTs including 3 patients with grade 3 fatigue and 1 patient with grade 3 acneiform rash. The 5 mg/kg dose was declared as the maximum tolerated dose for the weekly schedule. Five patients had stabilization of disease as best response while the remaining 13 patients progressed on study. The median PFS was 39 days (range, 21-432 days), with no significant differences in efficacy seen between the three dose cohorts. Three patients with activating EGFR mutations had a median duration on study of 217 days compared to 37 days in the wild-type EGFR group. Only 13 patients had serum available for biomarker analysis. There was a non-significant trend towards benefit in patients with the highest quartile of free IGF-1. This study was terminated from a planned expansion into phase II study due to lack of robust efficacy with the combination regimen (12). The strategy of combining EGFR tyrosine kinase inhibitors with IGF-1R, though promising from preclinical studies, has failed to translate into meaningful improvement in the treatment of unselected NSCLC patients.

Two other studies utilizing IMC-A12 in NSCLC patients are currently ongoing. A phase II study by ECOG evaluates carboplatin/paclitaxel/bevacizumab with or without IMC-A12 in patients with metastatic or recurrent NSCLC in the frontline setting (ClinicalTrials.gov NCT 00955305). The primary endpoint will be PFS with secondary endpoints of OS, ORR, and toxicity. The other open study is the JAEM trial sponsored by Eli Lilly (Indianapolis, Indiana), a phase II trial randomizing patients with metastatic NSCLC with non-squamous histology to treatment with either cisplatin/pemetrexed/IMC-A12 or cisplatin/pemetrexed (ClinicalTrials.gov NCT 01232452). The primary endpoint will be PFS with secondary outcomes including ORR, OS, duration of response, time to progression, change in tumor size, and quality of life assessment in a total of 220 patients. The study will have a major emphasis on biomarker assessment.

MK-0646

MK-0646 (Dalotuzumab) is another monoclonal antibody against IGF-1R that was developed by Merck (Whitehouse Station, NJ). Phase I data were published last year examining the safety of MK-0646 in patients with tumors that expressed IGF-1R as determined by immunohistochemistry (IHC) (17). Eighty patients were treated with breast and colon cancer being the most common tumor-types. The drug was well tolerated with the most frequent toxicities of hyperglycemia,

asthenia, chills, back pain, and aspartate aminotransferase elevation. There were 3 patients with grade 3 or higher toxicity, including tumor pain, hyperglycemia, and a biopsy proven leukocytoclastic vasculitis, which resolved once study drug was discontinued. MK-0646 dose was escalated from 1.25 to 20 mg/kg in a total of 6 dose cohorts without an MTD being achieved. Pharmacokinetic and pharmacodynamics data was also collected; various protein levels were assessed by IHC and quantified by histochemical scores (H-scores). In 33 matched pairs of baseline and on-treatment tumor samples and 69 matched pairs of baseline and on-treatment skin biopsy samples, there was decrease in IGF-1R levels in both tumor (P=0.02) and skin (P=0.04) after 3 weeks of MK-0646 treatment. Reduced expression of proteins regulated by IGF-1R signaling, such as EGFR and phosphorylated MAP kinase, was also seen. In the 76 patients who had evaluable responses by RECIST criteria, 6 patients had stable disease, including 2 patients with Ewing's sarcoma. It is interesting that although all the patients were selected for the study by the presence of IGF-1R expression by IHC, this did not predict treatment response with MK-0646 monotherapy.

Subsequently, a phase I/II study of erlotinib in combination with MK-0646 in unselected patients with advanced or metastatic NSCLC that has progressed after first-line therapy was conducted (ClinicalTrials.gov NCT 00654420). Another study that has completed accrual is a phase Ib study of erlotinib and MK-0646 to determine imaging and molecular determinants of response. This study utilized positron emission tomography (PET) response at weeks 1 and 3 to guide therapy with PFS and OS as secondary endpoints. Patients with locally advanced or metastatic NSCLC that progressed on 1 or 2 prior chemotherapy regimens were treated with erlotinib for one week. If there was a decrease in FDG uptake seen on PET, the patients continued on erlotinib. If there was no PET response, the patients were continued on erlotinib in combination with MK-0646 until disease progression. Patients were allowed to crossover from erlotinib to combination arm at time of disease progression. Results for this study have not yet been reported.

IGF-1R inhibition in other malignancies

To date, the efficacy of IGF-1R inhibition in NSCLC has been disappointing. There have been multiple studies of IGF-1R blockade in other tumor types, including colon cancer, pancreatic cancer, and breast cancer that have shown limited activity. The most promising data have been seen in Ewing's sarcoma. Ewing's sarcoma is defined by the presence of the *EWS/FL-1* fusion gene, which results in malignant transformation in an IGF-1R dependent manner (18). Inhibition of IGF-1R inhibits Ewing's sarcoma growth in tumor xenograft models (19). Based on these preclinical data, the phase I study of figitumumab included expansion cohorts for patients with refractory advanced sarcomas and specifically for Ewing's sarcoma (20). There were 29 sarcoma patients with 16 with Ewing's sarcoma. Of the 28 patients with response data, 2 patients with Ewing's sarcoma had objective responses, including 1 complete response. Eight patients achieved stable disease for at least 4 months.

Further studies of IGF-1R monoclonal antibodies in Ewing's sarcoma have also failed to show impressive single agent efficacy. The phase I/II study of figitumumab enrolled 31 patients with sarcoma to 2 dose escalation cohorts (21). There were 107 patients with Ewing's sarcoma enrolled in the phase II portion of the study; 15 of 106 evaluable patients had partial response (ORR 14.2%) and 25 achieved stable disease with a median OS of 8.9 months. Again, free IGF-1 levels predicted patients that benefited most from IGF-1R inhibition: patients with baseline IGF-1 higher than 0.65 ng/mL had median OS of 10.4 months (P<0.001) compared to OS of 3.6 months in those with lower levels. A phase II study of R1507 enrolled 115 patients with refractory or recurrent Ewing's sarcoma to treatment with R1507 dosed at 9 mg/kg weekly; however, the study was amended after ongoing PK studies demonstrated that higher peak serum concentrations attained with 27 mg/kg every 3 weeks resulted in increased tumor shrinkage in a xenograft model (22). A total of 109 patients were enrolled to the weekly dose cohort and 6 patients to the higher dose cohort. The objective response rate was 10% with a median duration of response of 29 weeks and median OS of 7.6 months. Treatment was well tolerated with most common grade 3 or 4 AEs of pain, anemia, thrombocytopenia, asthenia, and hyperglycemia (3 patients). Factors found to predict survival in multivariate analysis included bone primary tumor, Karnofsky performance status of at least 90%, total IGF-1 level above 110 ng/mL, and higher percentage increase in total IGF-1 from baseline to week 6. Similarly, IMC-A12 was investigated in a phase I/II study of patients with refractory solid tumors: the recommended phase II dose was defined at 9 mg/kg (23). In 30 patients treated with Ewing's sarcoma, only 3 patients had partial response with single agent IMC-A12. Again despite promising preclinical data, IGF-1R inhibition with monoclonal antibodies has not resulted in significant single agent activity in Ewing's sarcoma.

Is there a future for IGF-1R targeting in oncology?

Despite promising preclinical data and strong rationale for targeting IGF-1R, clinical efficacy has been disappointing in multiple studies, in lung cancer and other diseases. There are multiple potential reasons behind the failure of IGF-1R inhibition to live up to its promise. First, no study has studied the effects of IGF-1R inhibitors on the receptor at the level of the tumor and subsequent downstream signals. Defining specificity of a targeted agent against its target in patients is challenging, as often tumor tissue is not easily accessible for multiple biopsies to test IGF-1R expression for baseline and post-treatment levels. Nonetheless, this is an integral determination to make to assess if a targeted therapy fails because it does not truly achieve the desired effects on its intended target or because inhibition of the target by itself was not adequate to induce disease response.

However, the presence of IGF-1R expression may not be enough to predict response to IGF-1R inhibition, as was seen in the MK-0646 phase I study (17). Most tumors are not dependent on only one aberrant signaling pathway for tumor growth. There are multiple downstream signaling pathways that are activated by IGF-1R signaling; any of these pathways can become constitutively activated to overcome loss of IGF-1R signaling. For example, mTOR signaling has been shown to be activated in breast cancer cell lines resistant to IGF-1R inhibition; this could be overcome by treatment with the mTOR inhibitor everolimus (24). This combination has been studied in phase I studies and shown to have promising activity, particularly in patients with Ewing's sarcoma and adrenocortical carcinoma (25-27). Given the genetic complexity of most tumors, particularly lung cancer, targeting of multiple signaling pathways will likely be required to result in therapeutic efficacy.

The insulin receptor (IR) also signals through the PI3K-AKT pathway. IR signaling is up-regulated when IGF-1R expression is decreased by treatment with monoclonal antibodies (28). OSI Pharmaceuticals (Melville, NY) is developing orally bioavailable dual IGF-1R and IR inhibitor, OSI-906, which has been shown to have efficacy in preclinical cell line and tumor xenograft models with activated IR and IGF-1R signaling (29). This compound binds reversibly with the ATP binding cleft of the catalytic domains of both receptors and can bind both the active and inactive forms of the receptor. This dual targeting may be another way to make IGF-1R blockade a more effective strategy.

One of the major lessons to be learnt from the development of IGF-1R inhibitors in NSCLC is the lack of efforts to develop patient selection methods as part of early phase studies. The negative results of large phase III studies quickly quelled the enthusiasm for development of these agents and halted further investment into biomarker discovery. However, as can be seen from the studies described in this article, elevated baseline serum IGF-1 levels have shown near consistent correlation with improved efficacy with IGF-1R antagonists. These observations were noted in phase I and II studies. However, the phase III studies were conducted in unselected patients even before the results of phase I and II studies could be carefully analyzed for predictive biomarkers. For example, figitumumab was immediately pushed into phase III studies with combinations of chemotherapy and with erlotinib without waiting for biomarker analysis from the earlier phases of study. Unfortunately, both strategies utilizing figitumumab have been proven futile. The pressure to bring drugs to patients early, has no doubt contributed to the conduct of phase III studies with limited supporting data. Unfortunately, too often, potentially useful drugs have been discarded because of an accelerated development path without careful biomarker discovery efforts. We believe that the biological data that link IGF-1R pathway activation to cancer are compelling and the rationale to inhibit IGF-1R is sufficiently strong. IGF-1R inhibition is likely still a viable target in lung cancer.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. World Health Organization. Cancer Fact Sheet 2012 (cited 2012 September 28). Available online: http://www.who.int/mediacentre/factsheets/fs297/en/
2. Rapp E, Pater JL, Willan A, et al. Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer--report of a Canadian multicenter randomized trial. J Clin Oncol 1988;6:633-41.
3. Tao Y, Pinzi V, Bourhis J, et al. Mechanisms of disease: signaling of the insulin-like growth factor 1 receptor pathway--therapeutic perspectives in cancer. Nat Clin Pract Oncol 2007;4:591-602.
4. Sell C, Rubini M, Rubin R, et al. Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor. Proc Natl Acad Sci U S A 1993;90:11217-21.
5. Yang XF, Beamer WG, Huynh H, et al. Reduced growth

of human breast cancer xenografts in hosts homozygous for the lit mutation. Cancer Res 1996;56:1509-11.
6. Frankel SK, Moats-Staats BM, Cool CD, et al. Human insulin-like growth factor-IA expression in transgenic mice promotes adenomatous hyperplasia but not pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2005;288:L805-12.
7. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008;8:915-28.
8. Karp DD, Paz-Ares LG, Novello S, et al. Phase II study of the anti-insulin-like growth factor type 1 receptor antibody CP-751,871 in combination with paclitaxel and carboplatin in previously untreated, locally advanced, or metastatic non-small-cell lung cancer. J Clin Oncol 2009;27:2516-22.
9. Jassem J, Langer CJ, Karp DD, et al. Randomized, open label, phase III trial of figitumumab in combination with paclitaxel and carboplatin versus paclitaxel and carboplatin in patients with non-small cell lung cancer (NSCLC). J Clin Oncol 2010;28:abstr 7500.
10. Gualberto A, Hixon ML, Karp DD, et al. Pre-treatment levels of circulating free IGF-1 identify NSCLC patients who derive clinical benefit from figitumumab. Br J Cancer 2011;104:68-74.
11. Pfizer. Pfizer Discontinues A Phase 3 Study Of Figitumumab In Previously Treated Patients With Advanced Non-Small Cell Lung Cancer. 2010 (cited 2012 November 1); Available online: http://www.pfizer.com/news/press_releases/pfizer_press_release_archive.jsp?guid=20100311006790en&source=2010&page=11
12. Ramalingam SS, Spigel DR, Chen D, et al. Randomized phase II study of erlotinib in combination with placebo or R1507, a monoclonal antibody to insulin-like growth factor-1 receptor, for advanced-stage non-small-cell lung cancer. J Clin Oncol 2011;29:4574-80.
13. Habben K, Delmar P, Brownstein CM, et al. Investigation of predictive biomarkers for R1507, an anti-IGF1R antibody, in patients with advanced non-small cell lung cancer with progression after first-line chemotherapy. J Clin Oncol 2011;29:abstr 7584.
14. Rowinsky EK, Youssoufian H, Tonra JR, et al. IMC-A12, a human IgG1 monoclonal antibody to the insulin-like growth factor I receptor. Clin Cancer Res 2007;13:5549s-5555s.
15. Hanna NH, Dahlberg SE, Kolesar J, et al. Three-arm randomized phase II study of carboplatin (C) and paclitaxel (P) in combination with cetuximab (CET), IMC-A12, or both for advanced non-small cell lung cancer (NSCLC) patients who will not receive bevacizumab-based therapy: An Eastern Cooperative Oncology Group (ECOG) study (E4508). J Clin Oncol 2012;30:abstr 7516.
16. Weickhardt A, Doebele R, Oton A, et al. A phase I/II study of erlotinib in combination with the anti-insulin-like growth factor-1 receptor monoclonal antibody IMC-A12 (cixutumumab) in patients with advanced non-small cell lung cancer. J Thorac Oncol 2012;7:419-26.
17. Atzori F, Tabernero J, Cervantes A, et al. A phase I pharmacokinetic and pharmacodynamic study of dalotuzumab (MK-0646), an anti-insulin-like growth factor-1 receptor monoclonal antibody, in patients with advanced solid tumors. Clin Cancer Res 2011;17:6304-12.
18. Toretsky JA, Kalebic T, Blakesley V, et al. The insulin-like growth factor-I receptor is required for EWS/FLI-1 transformation of fibroblasts. J Biol Chem 1997;272:30822-7.
19. Scotlandi K, Benini S, Nanni P, et al. Blockage of insulin-like growth factor-I receptor inhibits the growth of Ewing's sarcoma in athymic mice. Cancer Res 1998;58:4127-31.
20. Olmos D, Postel-Vinay S, Molife LR, et al. Safety, pharmacokinetics, and preliminary activity of the anti-IGF-1R antibody figitumumab (CP-751,871) in patients with sarcoma and Ewing's sarcoma: a phase 1 expansion cohort study. Lancet Oncol 2010;11:129-35.
21. Juergens H, Daw NC, Geoerger B, et al. Preliminary efficacy of the anti-insulin-like growth factor type 1 receptor antibody figitumumab in patients with refractory Ewing sarcoma. J Clin Oncol 2011;29:4534-40.
22. Pappo AS, Patel SR, Crowley J, et al. R1507, a monoclonal antibody to the insulin-like growth factor 1 receptor, in patients with recurrent or refractory Ewing sarcoma family of tumors: results of a phase II Sarcoma Alliance for Research through Collaboration study. J Clin Oncol 2011;29:4541-7.
23. Malempati S, Weigel B, Ingle AM, et al. Phase I/II trial and pharmacokinetic study of cixutumumab in pediatric patients with refractory solid tumors and Ewing sarcoma: a report from the Children's Oncology Group. J Clin Oncol 2012;30:256-62.
24. Ekyalongo RC, Mukohara T, Kataoka Y, et al. Mechanisms of acquired resistance to insulin-like growth factor 1 receptor inhibitor in MCF-7 breast cancer cell line. Invest New Drugs 2013;31:293-303.
25. Quek R, Wang Q, Morgan JA, et al. Combination mTOR and IGF-1R inhibition: phase I trial of everolimus and figitumumab in patients with advanced sarcomas and other solid tumors. Clin Cancer Res 2011;17:871-9.
26. Naing A, Kurzrock R, Burger A, et al. Phase I trial of

cixutumumab combined with temsirolimus in patients with advanced cancer. Clin Cancer Res 2011;17:6052-60.
27. Naing A, LoRusso P, Fu S, et al. Insulin growth factor-receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with refractory Ewing's sarcoma family tumors. Clin Cancer Res 2012;18:2625-31.
28. Buck E, Gokhale PC, Koujak S, et al. Compensatory insulin receptor (IR) activation on inhibition of insulin-like growth factor-1 receptor (IGF-1R): rationale for cotargeting IGF-1R and IR in cancer. Mol Cancer Ther 2010;9:2652-64.
29. Mulvihill MJ, Cooke A, Rosenfeld-Franklin M, et al. Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor. Future Med Chem 2009;1:1153-71.

Cite this article as: Pillai RN, Ramalingam SS. Inhibition of insulin-like growth factor receptor: end of a targeted therapy? Transl Lung Cancer Res 2013;2(1):14-22. doi: 10.3978/j.issn.2218-6751.2012.11.05

Translational Medicine

MET inhibition in lung cancer

Jessica Menis[1], Matteo Giaj Levra[2], Silvia Novello[2]

[1]Department of Oncology, A.O.U Santa Maria della Misericordia of Udine, Italy; [2]Department of Oncology, A.O.U San Luigi - Orbassano, University of Turin, Italy

Correspondence to: Jessica Menis, MD. Department of Oncology, A.O.U Santa Maria della Misericordia of Udine, Piazzale Santa Maria della Misericordia, 33100 Udine, Italy. Email: jessica.menis@libero.it.

Abstract: Targeted agents have completely changed cancer treatment strategy, leading it from a "one size fits all" approach to a customized therapy. In this scenario Met, a heterodimere receptor tyrosine kinase deeply involved into embryogenesis and organogenesis, has been introduced many years ago as a potential target for biological agents, becoming "druggable" only in this last period of time. Met can be altered through receptor overexpression, genomic amplification, mutations or alternative splicing, autocrine or paracrine secretion of hepatic growth factor (HGF): these dysregulations stimulate tumorigenesis (in terms of cell-cell detachment, proliferation, invasion, angiogenesis and survival) and metastatization. Met is overexpressed in lung cancer and Met gene amplification can drive the dependency of cell survival and proliferation upon the Met signaling. Both Met overexpression and amplification seem to correlate with poor prognosis. Met amplification is also described to be linked to EGFR acquired resistance. Several Met inhibitors have been tested both in preclinical and human trials, demonstrating activity in lung cancer treatment. This paper aims to summarize data on Met biological function, on its interaction with cell signaling and other pathways and to present data on those Met inhibitors currently under evaluation.

Keywords: Lung cancer; Met; targeted therapy

Submitted Nov 16, 2012. Accepted for publication Dec 17, 2012.
doi: 10.3978/j.issn.2218-6751.2012.12.04
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2012.12.04

Introduction

The discovery of new cancer-driver genes and the enforcement of molecules targeting them have changed the landscape of Non Small Cell Lung Cancer (NSCLC) treatment.

As a matter of fact, the previous scenario of advanced NSCLC treatment has been completely revolutionized, switching from a "one size fits all" approach to a personalized therapy.

Somatic mutations of the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase domain positively correlated with clinical responsiveness to specific inhibitors: gefitinib, erlotinib and afatinib, two reversible and one irreversible EGFR inhibitors, have consistently demonstrated significant increase of Response Rate (RR) and Progression-Free-Survival (PFS) compared to standard chemotherapy in EGFR mutated NSCLC patients with advanced disease (1-7).

The Anaplastic Lymphoma Kinase (ALK), firstly identified from a chromosomal translocation leading to the production of merged proteins in Non-Hodgkin lymphomas, was then detected as a fusion with the echinoderm microtubule-associated protein-like 4 (EML4) in 6.7% of NSCLC patients (8,9). Crizotinib (PF02341066, Xalkori) targets EML4-ALK thus gaining astonishing response rates in a phase I/II trial and more recently in a phase III trial (10,11).

Unfortunately, other biomarkers already identified in NSCLC are still "undraggable" and one clear example is KRAS. KRAS is a member of the RAS family of oncogenesis, involved in signal transduction and tumorigenesis and its mutations, frequently in codons 12 and 13, have been reported in 20-30% NSCLCS (12-15). Some sign of activity

came in the last year from a targeted agent (Selumetinib), which compared to standard chemotherapy in KRAS mutated patients gave interesting results in terms of RR and PFS (16).

Several other molecular markers' alterations have been described in NSCLC such as: phosphatidylinositol 3-kinases (PI3K) (2%), lipid kinases that regenerate a key mediator between growth-factor receptors and intracellular downstream signaling pathways; ERBB-2 (2%); B-RAF (1-3%), a Ser-Thr kinase that links RAS GTPases to downstream proteins of the MAPK family, thus controlling cell proliferation; ROS1 (about 1%), oncogene that encodes a transmembrane tyrosine kinase receptor; AKT; RET and MET (17-21).

Since the first MET pathway description, several inhibitors have been preclinically and clinically tested, both alone and in combination with chemotherapy or other targeted therapies.

This paper will focus on MET biology, its role in the cell function and tumorigenesis, specifically in lung cancer, as well as on the molecules that target it.

Met discovery and mechanism of action

Met is a heterodimer receptor tyrosine kinase composed of a α-chain and a β-chain, linked by a disulphide bond.

Met was originally isolated as the product of a human oncogene, *trp-met*, in tumor cells treated with a chemical carcinogen. Met gene encodes a 170-kD protein ($p170^{met}$) that has constitutive and ligand-independent tyrosin-kinase activity. Met has pivotal functions in embryogenesis and organogenesis of placenta, liver, kidney, neurons and muscles (22-25).

Moreover, *in vivo*, Met receptor activation determines a phenomenon called "invasive growth", which includes cell proliferation, scattering, survival, motility and invasion, epithelial-mesenchymal transition and branched morphogenesis (26,27).

The natural ligand for this receptor is the HGF, produced by stromal and mesenchymal cells, that acts primarily on Met-expressing epithelial cells in an endocrine and/or paracrine fashion (24,28). HGF-induced Met tyrosine kinase activation is regulated by paracrine ligand delivery, ligand activation at the target cell surface and ligand-activated receptor internalization and degradation (29). Going more into details, when HGF binds to the Met receptor, Met major autophosphorylation sites (located within the tyrosine kinase domain) are phosphorylated, with subsequent intrinsic catalytic activation of multiple signaling cascades involved in cell proliferation, survival, angiogenesis, morphogenesis, cell scattering, motility, migration and invasion. An activated docking site in the kinase domain further recruits intracellular adaptor molecules through the SH2 domains and other recognition motifs, such as GAB1 (a key coordinator of the cellular responses to Met). Downstream signaling of the GRB2-mitogen-activated protein kinase (MAPK) cascade, PI3K-mTOR pathway, and STAT pathway are eventually activated, mediating various cellular functions (27,30,31). Finally, in order to activate the receptor, proteolytic cleavage of proHGF is necessary (25).

HGF is mainly produced by stromal tissue like liver and bone marrow, and is expressed in a multitude of mesenchymal-derived cells. Being Met expression detected in the epithelium of most tissues, this indicates that HGF-Met signal transduction pathway contributes to mesenchymal-epithelial interactions (24,32-34).

Met downregulation occurs through rapid internalization of Met itself and subsequent degradation by the lysosome: this process is regulated by ligand-dependent ubiquitination of Met, a process also modulated by specific tyrosine phosphatases and recently identified as proteins decorin and LRIG1 (35,36).

Met can be altered through receptor overexpression, genomic amplification, mutations or alternative splicing. These alterations lead to signaling deregulation that can be mediated through ligand (HGF)-independent receptor activation or through its ligand (HGF)-dependent activation via autocrine (intratumoral HGF), paracrine (mesenchymal or microenvironmental HGF), or endocrine (circulatory HGF) loop signaling cascades (29).

HGF and Met are highly expressed in various stem and progenitors cells, but are only expressed as low levels in their mature cells (25). In preclinical animal models, whereas the overexpression of Met and/or HGF has been shown to stimulate tumorigenesis and metastasis, down-regulation of Met or HGF expression resulted in increased apoptosis and decreased tumor growth and blood vessel density (37-40). Moreover, Met interacts synergistically with VEGF to promote angiogenesis, cell proliferation and invasion (41). This occurs through the transcriptional up-regulation of the hypoxia inducible factor-1α and amplified HGF signaling, that resulted in both induction of invasion and increased expression of VEGF (41).

Met pathway is also one of the key players in the development of acquired resistance to VEGF pathway inhibitors: the inhibition of Met expression prevented hypoxia-induced invasion growth (42,43).

The increased Met expression described in case of response to ionizing radiation through the ATM-NFκB signaling pathway, could lead to radioresistance and cancer invasion (44).

Met pathway and cross-talks

The cross-talk of Met with various signaling pathways is described in literature and that one between Met and EGFR/HER family receptors is particularly important in lung cancer (45-49).

Met and EGF family receptors are often described co-expressed in tumors and transactivation of Met depends on elevated expression of EGFR in many human tumors (46,50,51). Conversely, HGF stimulation promotes transactivation of EGFR in multiple cell lines, including NSCLC (49).

Cooperation between Met and EGFR occurs also indirectly: when Met activates Src, this lead to EGFR phosphorylation and the creation of docking sites for EGFR interactors involved in downstream signaling (52).

Moreover, through receptor cross-talk, Met exerts a key role in the development of resistance to EGFR family inhibitors. One example is the stimulation of HER-3 phosphorylation and signaling to Akt (a key signaling molecule required for cell survival and proliferation) when Met is amplified and overexpressed (53,54). Inhibition of Met in EGFR inhibitors resistant cells, either *in vitro* or *in vivo*, promotes apoptosis, tumor growth reduction and significant necrosis (49,53).

Met and EGFR inhibitors combined together, cooperatively abrogate ErbB3 signaling activation (49). An alternative mechanism in this context is the Src-induced EGFR phosphorylation (52).

Preclinical data also support that Met cross-talks and cooperates with other members of the EGF receptor family, including HER2, to enhance cell invasion and this lead to the possibility to explore therapeutic activity of dual Met and HER2 therapies (55,56).

Stimulation with both HGF and EGF enhances downstream activation of several signaling pathways including Akt, Erk and STAT3 in a way that Met inhibitors abolished their baseline phosphorylation (57,58).

The already mentioned interaction between decorin and LRIG1 proteins, promotes ligand-independent receptor downregulation and degradation of EGFR family members. Decorin binds to the EGFR family, inducing receptor dimerization, internalization and eventual lysosomal degradation, whereas LRIG1 and EGFR associated via their extracellular domains, allow enhanced EGFR phosphorylation. Thus, Met promotes resistance to VEGFR and EGFR inhibitors (59,60).

Cross-talk between Met and KRAS signaling has also been described both in preclinical and clinical findings (61,62). Met activates RAS directly or via a protein-tyrosine phosphatase (63). Similarly, PI3K could be directly activated by Met or indirectly by RAS protein (30).

Moreover, Met directly binds to and sequesters the Fas receptor. This interaction prevents Fas self-aggregation and ligand binding, thus inhibiting Fas activation and apoptosis (64).

Finally, preclinical studies exploring a combination of anti-Met therapeutic agents with mTOR inhibitors have also demonstrated an increased growth suppression, compared to mTOR inhibitors alone (62).

Met plays also a functional role in signaling pathways mediated by other membrane proteins. Integrin-dependent signaling could trigger ligand-independent Met phosphorylation following cellular adhesion, and Met and integrins might have independent yet synergistic roles in cell invasion. Plexins, single-pass transmembrane receptors for semaphorins, acts cooperatively with Met for cell adhesion and migration (45).

MET and NSCLC

Met receptor is overexpressed in both Small Cell Lung Cancer (SCLC) and NSCLC, mainly in *non-squamous* histotype (65-67).

Recent tumor microarray expression analysis demonstrated a 72% Met expression in human lung cancer tissue and 40% Met receptor over-expression; such values are higher than in breast (16%) and ovarian cancer (31%), but lower than in renal (70%) and colorectal cancers (CRC; 78%) (67). Phospho-Met expression is found to be at the highest levels in lung cancer (73%), followed by ovarian (33%), breast (23%), and renal (18%) cancer (67).

Met gene amplification can guide the dependency of cell survival and proliferation upon the Met signaling, even in lung cancer cell lines. Blocking Met causes significant growth inhibition, G1-S arrest and apoptosis in cell lines harboring Met gene amplification. When Met is not amplified, its levels of activation are low and cells are unable to grow (68).

Different studies have reported primary Met amplification to be in the wide range of 2% to 21%, in

NSCLC lung adenocarcinomas, particularly in TKI-naïve cohorts (69-72).

In lung cancer, Met receptor mutations were mainly found clustered in the non-tyrosine kinase domain, in the juxtamembrane (JM) domain and in the sema domain (67). These mutations are oncogenic activating variants, that result in a deletion in the juxtamembrane domain with enhanced oncogenic signaling, tumorigenicity, cell motility, and migration (27,73). Met kinase domain mutations have been found to be somatically selected in the metastatic tissues, compared with the primary solid cancers (74).

Literature data are quite discordant on the prognostic value of Met over-expression, amplification and mutation.

The overexpression of circulating Met in patients with NSCLC has been strongly associated with early tumor recurrence and patients with adenocarcinoma and Met amplification have also demonstrated a trend for poor prognosis (69,75,76).

Concerning the correlation between Met FISH status and clinical characteristics, only Okuda and colleagues demonstrated an association with male gender and smoking status, showing also a relationship with high Met gene copy number (77). In the same trial, both FISH positive and gene amplified cases had a worse prognosis, although the difference was not statistically significant and among the Met FISH-positive NSCLCs, patients with gene amplification showed not significantly worse OS compared to those with high polysomy.

All FISH-positive cases had squamous histology, adenocarcinoma had Met amplification: high Met gene copy number tended to have shorter OS and PFS than those with low Met gene copy number, being this difference statistically significant only in the squamous histotype.

Moreover, at multivariate analysis done on squamous histology, increased Met gene copy number and Met amplification were confirmed to be independent poor prognostic factors.

No significant difference in prognosis was found in patients having adenocarcinoma regardless Met FISH status in the korean study. In contrast, Beau-Faller and colleagues found a tendency toward shorter event-free survival in adenocarcinoma patients with increased Met gene copy number, whereas Kanteti and colleagues demonstrated that the high Met gene copy number in adenocarcinoma was associated with a trend of better prognosis (69). However, the above mentioned study has some critical methodology aspects as it was conducted on a small sample size and qPCR was used as test and not FISH, done on DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) archival tumor tissues (70).

Capuzzo and colleagues found no patient with EGFR mutation was Met FISH positive, but increased Met gene copy number significantly correlated with EGFR FISH-positive status (78).

Acquired Met amplification has also been linked to approximately 22% of non-T790M mediated secondary gefitinib resistance in NSCLC patients, although it can also occur concurrently but independently (52,53,78-80).

Using *in vitro* cell line models, the Met gene amplification in gefitinib-resistant cell clones was identified (53).

Rho and colleagues tried to demonstrate that Met activation, rather than gene amplification, is sufficient to promote EGFR resistance, but the activation appear to be secondary to increase passage numbers rather than to EGFR-Tki exposure (81).

More recently, two prospective analyses have investigated the mechanism of EGFR-Tki resistance through the tissue rebiopsy: high Met gene copy number was found in 11% and 5% of the tissue samples, respectively (82,83).

Met inhibitors

Several inhibitors have been tested so far: they can be classified according to their mechanism of action in selective Met inhibitors, unselective Met inhibitors and antibodies targeting Met or HGF (*Figure 1*, *Table 1*).

Selective Met inhibitors

Tivantinib

Tivantinib (ARQ 197) is the first non-ATP-competitive small molecule that selectively targets the Met RTK, locking and stabilizing the kinase in a "closed" and "inactive" conformation, causing the disruption of Met phosphorylation and the downstream signaling.

Moreover, tivantinib enhances Met degradation through the ubiquitin/proteasome pathway *in vitro*, induces apoptosis in Met activated cell-lines and it's active in multiple human cancer xenografts (84,85).

Tivantinib acts synergically with antiangiogenentc drugs in preclinical studies on solid tumor cell lines (86).

Studies *in vitro* and *in vivo* demonstrated its activity in several types of cancer such as breast, colorectal and gastric cancer (85,87).

Met cancer expressing cell lines treated with tivantinib displayed either a dose-dependent loss of proliferative

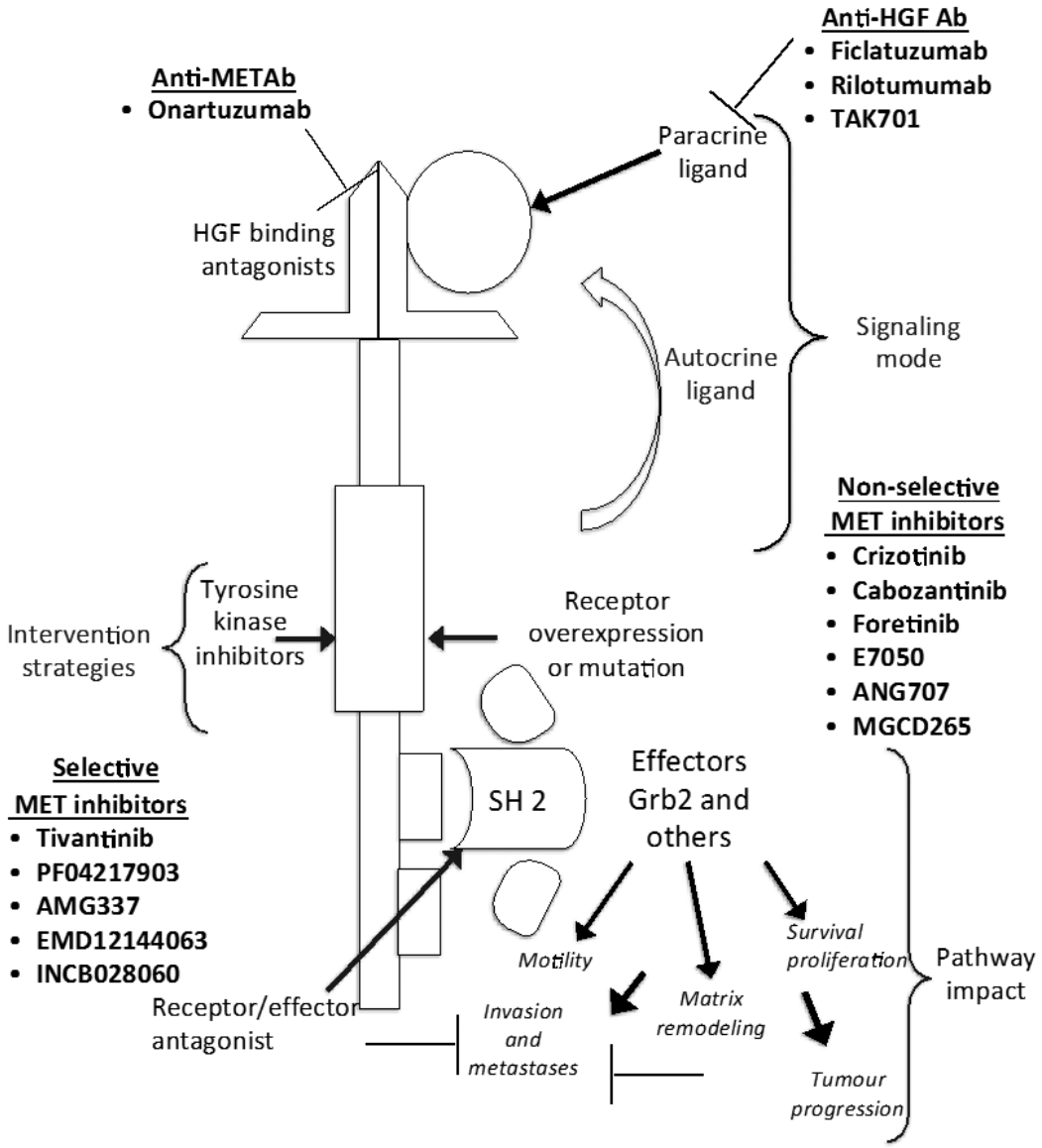


Figure 1 Met inhibitors in the clinic.

capacity or caspase-dependent Met apoptosis, which positively correlated with either ligand-dependent Met activity or constitutively active Met. Tivantinib does not exert any activity in cancer cell lines not expressing Met or phospho-Met.

Tivantinib has been investigated in three phase I trials, as single agent and in combination.

In the first dose-escalation phase I trial, tivantinib is administered as single-agent in patients with advanced solid tumors. Initially, an intermittent dosing was planned but, due to the bradycardia experienced in the other phase I trial using this schedule, the protocol was amended and the following 79 patients received a continuous dose (88).

No MTD was reached in this study and less than 33% of patients experienced DLTs at any given dose. Thus, the recommended phase II dose was confirmed at 360 mg twice a day as per a concomitant phase I study, where this MTD was identified (88).

The most commonly reported drug-related adverse events of any grade included fatigue, gastrointestinal (GI) disorders (nausea, vomiting and diarrhea) and anemia.

Pharmacokinetic was linear. There was considerable

Table 1 Ongoing trials on Met inhibitors

Molecule	Targets	Type	Phase	Monotherapy or combination	Drug associated	Patient populations
Tivantinib	c-Met	TKI	I	Combination	Topotecan	SCLC
			II	Combination	Erlotinib	EGFR pos NSLC
			II	Combination	Erlotinib	KRAS pos NSCLC
AMG 337	c-Met	TKI	I	Monotherapy	-	Solid tumors
Cabozantinib	c-Met, VEGFR2, RET, Kit, AXL, FLT3	TKI	II	Monotherapy	-	Solid tumors
			II	Monotherapy	-	KIF5B/RET NSCLC
			II	Combination	Erlotinib	EGFR neg NSCLC
Foretinib	c-Met, VEGFR, PDGFRb, Tie-2, RON, Kit, FLT3	TKI	I-II	Combination	Erlotinib	NSCLC
Golvatinib	c-Met, VEGFR	TKI	I	Monotherapy	-	Solid tumors
MGCD265	c-Met, VEGFR, RON, Tie2	TKI	I-II	Combination	Erlotinib/ Docetaxel	NSCLC
Onartuzumab	c-Met	MoAb	II rand	Combination	CBDCA-Pacl	Squamous NSCLC
			III	Combination	Erlotinib	Met positive NSCLC
			II rand	Combination	Platinum + Pem; Platinum + Pacl + Bev	Non-squamous NSCLC
Rilotumumab	HGF	MoAb	I-II	Combination	Erlotinib	NSCLC

CBDCA, carboplatin; Pacl, paclitaxel; Pem, pemetrexed; Bev, bevacizumab; rand, randomised; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; EGFR, epidermal growth factor; KIF5B/RET, kinesin family member 5B/ret proto-oncogene; Met, met proto-oncogene; TKI, tyrosine kinase inhibitor; MoAb, monoclonal antibody.

inter-patient variability, but no relationship between drug-related adverse events (AEs), dose and extent of tivantinib exposure; consequently, this inter-patient variability was not considered relevant for its clinical safety. Partial responses registered in this trial were equal to 4.8% (89).

In another phase I trial, two formulations of tivantinib were tested: the amorphous and the crystalline A formulation. The trial was lead in a single institution, the Royal Marsden Hospital (Sutton, United Kingdom) and highlighted the following DLTs: one patient had grade 3 fatigue at 200 mg, one patient presented a grade 3 febrile neutropenia, one other a grade 3 mucositis, one a grade 3 palmar-plantar erythrodysesthesia and one a grade 3 hypokalemia at 400 mg. The MTDs- recommended phase 2 doses (RP2Ds) were 300 mg bi-daily for the amorphous formulation and 360 mg bi-daily for the crystalline A formulation. The main grade 1-2 AEs, all generally self-limiting, were fatigue (15.7%), nausea (13.7%), vomiting (11.8%). Tivantinib is metabolized by CYP2C19: one patient with CYP2C19 deficiency experienced grade 4 febrile neutropenia and grade 3 mucositis as the drug's AUC was 3-fold higher (90).

The crystalline A formulation of tivantinib resulted in lower drug exposure at 300 and 360 mg twice daily, compared with the amorphous form at 300 mg twice daily (likely due to different dissolution characteristics). RECIST stable disease ≥4 months was the best response in 14 patients, together with minor tumor regressions (88).

As the ratio of the poor metabolizers of CYP2C19 in Asians is around 20% (while is very low in Caucasians), a Japanese phase I trial was designed to evaluate drug's safety profile of tivantinib in this group of patients with metastatic solid tumors and the drug was well tolerated, but CYP2C19 genotype clearly affected the exposure and the RP2Ds differed for "no poor metabolizers" (360 mg bi-daily) and for "poor metabolizers" (240 mg bi-daily). Most common AEs were similar to those mentioned above (91). A phase III trial was conducted in Asia in advanced NSCLC

patients, comparing erlotinib + tivantinib versus erlotinib + placebo at the dose calculated considering the CYP2C19 polymorphism (92). A press release in August 2012 announced a suspension in the accrual for this study, due to suspected cases of interstitial lung disease (93).

Based on the preclinical data showing a synergistic action between EGFR-TKi and Met inhibitors, an open-label sequential dose escalation phase I trial on tivantinib + erlotinib was set up. Thirty-two metastatic cancer patients were included: 59% were males, 75% PS 1 and mean age was 60 years. The MTD was not established, however, the RP2D was 360 mg bi-daily for tivantinib and 150 mg daily for erlotinib. Two DLT were experienced at 360 mg (grade 4 neutropenia, grade 3 thrombocytopenia), none at 240 or 120 mg. The most common AEs were cutaneous rash, fatigue, nausea, abdominal pain, diarrhea, bradycardia and anemia, mostly grade 1 and 2. No drug related death, but 11% grade 3-4 neutropenia and 8% grade 3-4 nausea were recorded (94).

This combination of erlotinib (150 mg daily) + tivantinib (360 mg bi-daily) every 4 weeks was further studied in a phase II, double-blind, randomized open-label study in comparison with erlotinib 150 mg daily + placebo, in previously treated locally advanced or metastatic NSCLC patients. One hundred and sixty-seven patients were enrolled and homogeneously distributed between the two arms (mainly males, never or former smoker, with stage IV disease and adenocarcinoma histology): 10% in the combination arm versus 18% in the standard arm presented an EGFR mutation, 10% versus 17% a KRAS mutation, 26% versus 26.5% had 4 or more MET gene copy number. The ORR was 10% for erlotinib + tivantinib versus 7% for the control arm.

Median investigator's PFS was 3.8 months for the tivantinib + erlotinib arm versus 2.3 months for the erlotinib + placebo arm (HR=0.81, P=0.24); the reviewer's PFS was 3.6 versus 2 months (HR=0.74, P=0.09). Median OS was 8.5 for the investigational arm versus 6.9 months for the control arm (HR=0.87, P=0.47). Pre-planned exploratory survival analysis in non-squamous histology showed a trend of benefit from the combination arm in both PFS (HR=0.71) and OS (HR=0.72). Even in a small number of patients, the subgroup analysis showed an advantage in terms of PFS for EGFR wild type (HR=0.70), KRAS mutated patients (HR=0.76) and for Met FISH positive patients (>5, HR=0.45).

Treatment was well tolerated both in the investigational and in the control arm: low grade rash (9.5% versus 7.2%) and diarrhea (7.1% versus 7.2%), fatigue (4.8% versus 6%), nausea (1.2% versus 4.8%), vomiting (3.6% versus 1.2%), dyspnea (7.1% versus 13.3%), anemia (6% versus 7.2%) were the most common reported toxicities (95).

On the basis of data coming from this phase II trial, the phase III MARQUEE trial was designed in non-squamous NSCLC patients with the same schema, having the overall survival (OS) as primary end-point. Unfortunately, a press release in October 2012, revealed that the primary end point in the intent to treat population was not met, but no further data are yet available (96,97).

Others selective Met inhibitors

PF-04217903 is a selective ATP-competitive small inhibitor of Met kinase. It inhibits tumor cell proliferation, survival, migration/invasion in Met-amplified cell lines *in vitro*, and shows marked antitumor activity in tumor models harbouring either Met gene amplification or a HGF/Met autocrine loop. PF-04217903 also demonstrates potent antiangiogenic properties *in vitro* and *in vivo* (98). In 2012 a phase I trial with PF-04217903 in patients with advanced solid tumors was prematurely discontinued, due to strategic development decision by Pfizer. No safety concerns were reported (99).

AMG 337 is a selective inhibitor of the proto-oncogene Met thereby disrupting Met signal transduction pathway. A phase I, open-label, sequential dose escalation and expansion study with AMG 337 in subjects with advanced solid tumors is currently ongoing (100) (*Table 1*).

INCB028060 is an oral potent and highly selective Met inhibitor, capable of suppressing tumor growth *in vivo* at doses that are extremely well tolerated (101,102).

Good tolerance was confirmed in a phase I standard 3+3 dose-escalation study once or twice daily on a continuous 28-day schedule in patients with advanced solid tumors. The MTD was not reached and no grade 3-4 AEs were noted, except grade 3 ALT increase in a patient with liver metastases and grade 2 ALT levels at baseline. Grade 1-2 AEs experienced were mild tremor, fatigue, nausea, diarrhea, indigestion and headache (103).

Non-selective Met inhibitors

Crizotinib

Crizotinib was synthesized primarily as a Met inhibitor. It was engineered based on interactions of a precursor (PHA-665752) with the ATP-binding sites of the Met kinase domain thus resulting in displacement of the kinase

activation loop, that interferes with ATP and substrate binding to the Met receptor tyrosine kinase. Crizotinib was designed in order to be less lipophilic and to have a small hinge binder with the possibility to better interact in the kinase pocket (104).

Crizotinib was proved to be active in NSCLC cell lines carrying Met amplification. However, no activity was described in Met mutated, EGFR mutated or normal cell lines. Moreover, crizotinib markedly inhibited AKT, Met and ERK phosphorylation. By doing that, it induced apoptosis even though a mediation of BIM up-regulation (pro-apoptotic member of the Bcl-2 family) and survivin down-regulation (a member of the inhibitor of apoptosis protein family) has also been reported.

Interestingly, in Met or EGFR mutated but also in normal cell lines, whit a low Met phosphorylation, the Met phosphorylation is completely inhibited, whereas the ERK and AKT are not (105).

During drug development, Ou and colleagues described a case of prolonged partial response to crizotinib in a NSCLC patient carrying Met amplification (defined as Met/CEP7 ratio >5) but no ALK translocation (106).

The first phase I trial was designed as open-label, multicenter, to evaluate safety and efficacy of crizotinib: this study was emended with an expanded cohort for patients with lung cancers carrying ALK rearrangements. The recommended crizotinib dose was 250 mg twice daily in 28-day cycles.

In the overall NSCLC population a phase I trial investigated crizotinib in association to dose escalating erlotinib: 5 DLTs were reported (at 150/100 mg grade 2 vomiting, grade 2 esophagitis and dysphagia, grade 3 diarrhea and dehydration; at 200/100 mg, grade 3 dry eye and grade 3 esophagitis). Ninety-two percent of the patients experienced treatment-related AEs, mainly grade 1 or grade 2: diarrhea (72%), rash (56%) and fatigue (44%) (107).

Another phase I trials evaluated crizotinib in combination with dacomitinib, an irreversible pan-erb inhibitor in previously treated advanced NSCLC patients (108).

Cabozantinib

Cabozantinib (XL184) is a potent Met/VEGFR2/RET/KIT/AXL/FLT3 inhibitor that targets tumor survival, metastasization and angiogenesis.

It selectively inhibits KIT, RET, AXL, TIE2 and FLT3 (all kinases implicated in tumor pathobiology) through strong, reversible, ATP-competitive binding. Moreover, cabozantinib inhibits HGF and VEGF-mediated cell migration and also Met and VEGFR phosphorylation and the tubule formation, with no evidence of cytotoxicity.

This effect described *in vitro*, turned into *in vivo* significant tumor regression, without any relevant toxicity (109).

Several phase I trials have already been published verifying the schedule, the formulation, the dose of the drug, both as single-agent and in combination with other molecules.

Kurzrock and colleagues studied single-agent cabozantinib both in suspension and capsule formulation, at intermittent (5 days on, 9 off) and continuous schedule: MTD was defined at 175 mg continuous schedule, being DLT mucositis, elevated lipase and altered liver function (110).

The continuous dose was further investigated in a Japanese only population: MTD was 60 mg, being grade 3 hypertension the DLT (111).

Regarding combination regimens, a phase I study analyzed the interaction of the combination cabozantinib and rosiglitazone, as the latter is a CYP2C8 substrate, but no interaction was found between these two compounds (112,113).

Cabozantinib was further studied in several phase II trials in different tumor types. Among them, one phase II trial investigated treatment with cabozantinib in NSCLC patients previously treated with anti-EGFR TKi (50%) and anti-VEGF therapies (32%). At week 12 the ORR was 10% and the overall DCR 40%. No difference in terms of PFS (median 4.2 months) was seen in the two populations according to the treatment response at 12 weeks. The most common grade 3-4 events were diarrhea (7%), palmar-plantar erythrodyesthesia (8%), fatigue (13%) and asthenia (7%) (114).

Likewise tivantinib, also cabozantinib was tested together with erlotinib or gefitinib *in vivo* and *in vitro* in EGFR TKi resistant NSCLC xenograft models harboring Met amplification. Gefitinib and cabozantinib were tested on gefitinib resistant cell lines either alone and in combination and the two molecules together were substantially more potent than each drug alone (>50% inhibition). The same result was obtained with the combination of erlotinib and cabozantinib on erlotinib resistant cell lines (115).

The combination of cabozantinib and erlotinib was tested on 54 NSCLC patients in a phase Ib/II study. Patients were divided into 5 cohorts in two parallel arms: arm A (75 mg cabozantinib + 100 mg erlotinib; 125 mg cabozantinib + 100 mg erlotinib; 125 mg cabozantinib + 50 mg erlotinib) and arm B (75 mg cabozantinib +150 mg erlotinib; 50 mg cabozantinib +150 mg erlotinib). Twelve patients experienced at least 1 DLT: diarrhea, increased AST levels, palmar-plantar erythrodysesthesia, mucositis,

hypertension, hypokalemia, elevated lipase and fatigue. The most common grade 3-4 adverse events were diarrhea (26%), fatigue (15%), dyspnea (12%) and hypoxia (9%) (116).

In advanced NSCLC patients two phase II trials are ongoing: the first one randomizes EGFR wild type patients to erlotinib, cabozantinib or erlotinib plus cabozantinib as second or third line therapy; the second study investigates cabozantinib in patients with KIF5B/RET positive NSCLC (117,118) (*Table 1*).

Foretinib

Foretinib (XL-880, EXEL-2880) is an oral multi-kinase inhibitor developed to target Met and several other receptor tyrosine kinases involved in tumor angiogenesis. It is an ATP-competitive inhibitor and binds the ATP pocket of both Met and VEGFR-2 tyrosine kinase domains with high affinity.

Both *in vitro* and *in vivo*, foretinib inhibits Met and VEGF receptor-2 (VEGFR-2) and have high *in vitro* affinity for PDGFRb, Tie-2, RON, Kit, and FLT3 kinases, preventing tumor growth through a direct effect on tumor cell proliferation and inhibition of invasion and angiogenesis, mediated by HGF and VEGF receptor (119).

Two phase I trials have been published: the first investigated foretinib administered for 5 consecutive days every 14 days in a 3+3 dose escalation study; in the second study foretinib was administered once daily for 28 days. Both trials were conducted in patients with metastatic or unresectable solid tumors. MDT was defined as 3.6 mg/kg for 5 consecutive days every 14 days in the first study and as 80 mg daily in the second; DLTs in the first study included grade 3 elevations in aspartate aminotransferase and lipase, whereas in the second trial hypertension, dehydration and diarrhea were described.

Additional AEs in both studies included hypertension, fatigue, diarrhea, vomiting, proteinuria, and hematuria. In these studies no responses were observed and most of patients achieved a stable disease as best response (120,121).

A phase I, open-label, randomized, 2-part crossover study assessed the safety, pharmacokinetics and relative bioavailability of single doses of foretinib free base tablet formulation compared to a bisphosphate salt capsule formulation: both were well tolerated and their pharmacokinetics and relative bioavailability were not clinically different (122).

On the basis of preclinical data, showing that combining foretinib with erlotinib or lapatinib effectively decrease the phosphorylation of Met, HER1, HER2, HER3, AKT, and ERK in cell lines, a phase I/II study of erlotinib in association or not with foretinib in previously treated NSCLC patients has been designed and is currently ongoing (123,124) (*Table 1*).

Golvatinib

Golvatinib (E7050) is a novel small molecule ATP-competitive inhibitor of Met receptor, that potently and selectively inhibits the autophosphorylation of Met and VEGF-induced phosphorylation of VEGFR (125).

Golvatinib also circumvents resistance to reversible, irreversible, and mutant-selective EGFR-TKIs induced by exogenous and/or endogenous HGF in EGFR mutant lung cancer cell lines, by blocking the Met/Gab1/PI3K/Akt pathway *in vitro* and also prevents the emergence of gefitinib-resistant cells, induced by continuous exposure to HGF (126).

A phase I study with oral daily golvatinib administered continuously once a day in patients with advanced solid tumors was performed. Three DLTs were observed: grade 3 increase in GGT and alkaline phosphatase levels and grade 3 fatigue, all at 450 mg. The MTD was determined to be 400 mg every day. Frequently occurring AEs were fatigue (68%), diarrhea (65%), nausea (62%), vomiting (53%), decreased appetite (47%), ALT increase (38%) and AST increase (23%). No grade 4 AEs were observed (127).

Other molecules

MGC D265 is an oral receptor tyrosine kinase inhibitor targeting Met, VEGF, RON and Tie2. Preclinical data have demonstrated synergism of action with erlotinib and early clinical trials are currently ongoing (128) (*Table 1*).

ANG707 is another non-selective Met inhibitor under investigation in early phase trials (129).

Antibodies

Antibodies against Met

Onartuzumab (MetMab)

MetMAb is a recombinant, fully humanized, monovalent monoclonal anti-Met antibody based on the human IgG1k framework sequence. It binds in the sema domain of Met within the extracellular domain, where it acts to inhibit HGF binding and initiation of receptor activation. The unique monovalent design of MetMAb eliminates the potential for Met activation via antibody-driven receptor dimerization (130).

The activity shown *in vitro* by MetMAb did not translate

into a full activity *in vivo*: only about 65% tumor inhibition was demonstrated, indicating that blockade of HGF by MetMAb is not sufficient for full tumor inhibition in specific tumors (130).

A phase I trial investigated sequential 3+3 dose-escalation of endovenous MetMAb in advanced solid tumors: MetMAb was three weekly intravenously administered, both as single agent and in combination with bevacizumab 15 mg/kg every three weeks, until progression.

Most frequent MetMAb AEs as single-agent were: fatigue (56%), peripheral edema (35%), decreased appetite (32%), constipation (29%), nausea (27%), vomiting (24%) and hypoalbuminemia (24%); there was no consistent relationship between AEs and dose level.

Grade 3 AEs were peripheral edema (9%), abdominal pain, AST increase, fever and hyponatremia. No Grade 4 toxicity was observed. The combination arm had similar toxicities; no grade 3 or 4 toxicity was experienced. MTD was not reached. The best response was stable disease (131).

The phase II trial was a global, randomized, double-blind trial evaluating the combination of MetMAb + erlotinib versus placebo + erlotinib in second/third line NSCLC advanced patients. One hundred and twenty-eight NSCLC patients were enrolled with a baseline immunohistochemical evaluation of Met: 54% of the patients were considered as Met positive (high protein expression at IHC). Met positive patients treated in the experimental arm had a significantly higher PFS (3.0 *vs*. 1.5 months; HR 0.47; P=0.01) and OS (12.6 *vs*. 4.6 months; HR 0.37; P=0.002) (132).

Based on phase II data, a randomized, phase III, multicenter, double-blind, placebo-controlled study evaluating the efficacy and safety of onartuzumab in combination with erlotinib in patients with Met positive NSCLC who have received standard chemotherapy for advanced disease is currently recruiting patients (133) (*Table 1*).

The positive results of the phase I trial on MetMAb in combination with bevacizumab have paved the way to the ongoing randomized phase II multicentric double-blind placebo-controlled study evaluating the efficacy and safety of MetMAb in combination with either bevacizumab + platinum + paclitaxel or pemetrexed + platinum as first-line treatment in patients with stage IIIB and IV non-squamous NSCLC (134).

Antibodies against HGF

Ficlatuzumab

Ficlatuzumab (AV-299) is a potent hepatocyte growth factor (HGF) inhibitor IgG1 monoclonal antibody, that binds to the HGF ligand with high affinity and specificity. Ficlatuzumab was studied in two phase I trials and one phase II study. In both phase I trials it was associated with gefitinib and erlotinib. In the first phase I trial ficlatuzumab was biweekly administered intravenously over 30-60 minutes both as single-agent and in combination with erlotinib at 150 mg continuously in advanced solid tumors. There were no DLT in the monotherapy arm; consequently no MTD was identified.

For the combination arm there was one DLT (grade 3 mucositis). The RP2D for both monotherapy and combination regimen was defined as 20 mg/kg every 2 weeks. Ficlatuzumab as a single-agent demonstrated a stabilisation of disease in 50% of the cases (135).

The second phase Ib trial enrolled only Asiatic patients with unresectable NSCLC: ficlatuzumab was administered intravenously every 2 weeks at two dose levels (10 and 20 mg/kg) in combination with gefitinib at 250 mg daily. No DLTs were observed in the dose-escalation cohorts; 20 mg/kg of ficlatuzumab every 2 weeks + gefitinib 250 mg daily was selected as RP2D. Among 12 patients in the 20 mg/kg cohort, 5 partial responses were achieved (136). Most frequent treatment-emergent adverse events (AEs) were fatigue (27-33%), dermatitis acneiform (53%, particularly for the combination regimens), diarrhea (33-46%) and edema (16-27%) for both single-agent and combination therapy (135,136).

The efficacy of ficlatuzumab together with gefitinib was further investigated in a multicenter, open-label, exploratory, 2-arm randomized phase 2 study in previously untreated Asian NSCLC patients with the doses defined in the phase I. One-hundred eighty-eight patients were randomized with a baseline evaluation of Met by IHC and gene copy number. In the low Met group, ORR (41 versus 22%) and median PFS (7.3 versus 2.8 m) favored the combination regimen with a manageable toxicity profile (137).

Rilotumumab

Rilotumumab (AMG 102) is a fully human monoclonal antibody that selectively targets and neutralizes hepatocyte growth factor/scatter factor (HGF/SF). It preferentially bound to the β-chain of the human, mature, active form of HGF, and had no apparent effect on proteolytic processing of the inactive HGF precursor (138).

Two phase I trials have been published so far with AMG 102 in advanced refractory solid tumors: one as single agent and one in combination with bevacizumab or motesanib (139).

In the monotherapy trial, AMG 102 was well tolerated up to the planned maximum dose of 20 mg/kg, MTD was not reached and pharmacokinetic was linear. Two patients

experienced DLTs: one grade 3 hypoxia and grade 3 dyspnea (0.5 mg/kg cohort) and one grade 3 upper GI hemorrhage (1 mg/kg cohort). Treatment-related AEs were generally mild and included fatigue (13%), constipation (8%), nausea (8%), vomiting (5%), anorexia (5%), myalgia (5%), and hypertension (5%). Seventy percent of the evaluable patients had a SD as best response (139).

The phase Ib combination study sequentially enrolled patients into four cohorts, but the number of those receiving AMG 102 plus motesanib was insufficient to adequately assess safety and the accrual was early suspended because of reports of cholecystitis in other motesanib studies. No dose-limiting toxicities were reported and the combination of AMG 102 with bevacizumab seemed to have acceptable toxicity. AEs were generally mild and included fatigue (75%), nausea (58%), constipation (42%) and peripheral edema (42%) (140).

TAK 701

TAK-701 is a humanized monoclonal antibody that binds HGF thus inhibiting its bound to Met receptor. TAK-701 in combination with gefitinib blocks the phosphorylation of Met, EGFR, extracellular signal-regulated kinase, and AKT in HGF expressing human NSCLC cell lines with an activating EGFR mutation. Combination therapy also markedly inhibited the tumor growth *in vivo* (141).

Preliminary data of a phase I study in advanced solid malignancies with TAK-701 showed that the most common AEs were cough, abdominal pain, constipation and fatigue, all grade 1-2. There were 3 grade 3 AEs (gastrointestinal ileus, pleural effusion, urinary tract infection) and 1 grade 4 AE (dyspnea). No DLT was found and the MTD has not been reached (142).

Conclusions

In patients with advanced NSCLC, a correct definition of the histotype is still the first step to design a proper therapeutic algorithm, but personalized molecular diagnosis is becoming more and more relevant.

Genetically defined subsets of cancers may share dependence on a specific signaling pathway: specific inhibitors targeting these pathways would be most effectively tested in patient populations characterized by molecular markers.

Moreover, genetic events that arise and are selected during tumor progression may become essential for tumor survival, a phenomenon generally described as "oncogene addiction": cancer cells appear to depend on a single overactive oncogene to proliferate and survive (143). Optimal case selection, diagnostic and pharmacodynamic biomarker development, the identification and testing of rationally designed anticancer drugs and combination strategies are crucial to develop the best treatment for the right patient (144).

New generations of molecularly targeted drugs will allow more personalized medicine and more efficacious and less toxic antitumor therapies in patients with defined molecular aberrations, sparing normal cells thus sparing toxicity (145,146).

Met can act as an 'oncogene expedient' even in absence of genetic alterations and might potentiate the effect of other oncogenes, promote malignant progression and participate in tumor angiogenesis (147).

Met dysregulation correlates with disease prognosis in numerous cancers and represents a possible target for personalized treatment. The clinical efficacy of Met targeting agents in lung cancer needs further details from the ongoing trials as well as more information are necessary to establish the most appropriate diagnostic test to identify Met expression or amplification.

Several molecules are currently under investigation and two of them already reached phase III trials in advanced NSCLC.

Acknowledgements

Disclosure: All the authors have no conflict of interest to declare.

References

1. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
2. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
3. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-8.
4. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.

5. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
6. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
7. Yang JC, Schuler MH, Yamamoto N, et al. LUX-Lung 3: A randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. J Clin Oncol 2012;30:abstr LBA7500.
8. Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1994;263:1281-4.
9. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
10. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
11. Shaw AT, Kim DW, Nakagawa K, et al. Phase III study of crizotinib versus pemetrexed or docetaxel chemotherapy in patients with advanced ALK-positive non-small cell lung cancer (NSCLC) (PROFILE 1007). Ann Oncology 2012;23:abstr LBA1_PR.
12. Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. Clin Cancer Res 2002;8:2286-91.
13. Tsao MS, Aviel-Ronen S, Ding K, et al. Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. J Clin Oncol 2007;25:5240-7.
14. Huncharek M, Muscat J, Geschwind JF. K-ras oncogene mutation as a prognostic marker in non-small cell lung cancer: a combined analysis of 881 cases. Carcinogenesis 1999;20:1507-10.
15. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005;23:5900-9.
16. Janne PA, Shaw AT, Pereira JR, et al. Phase II double-blind, randomized study of selumetinib (SEL) plus docetaxel (DOC) versus DOC plus placebo as second-line treatment for advanced KRAS mutant non-small cell lung cancer (NSCLC). J Clin Oncol 2012;30:abstr 7503.
17. Jimenez C, Jones DR, Rodríguez-Viciana P, et al. Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. EMBO J 1998;17:743-53.
18. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
19. Stephens P, Hunter C, Bignell G, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature 2004;431:525-6.
20. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.
21. Chin LP, Soo RA, Soong R, et al. Targeting ROS1 with anaplastic lymphoma kinase inhibitors: a promising therapeutic strategy for a newly defined molecular subset of non-small-cell lung cancer. J Thorac Oncol 2012;7:1625-30.
22. Cooper CS, Park M, Blair DG, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature 1984;311:29-33.
23. Park M, Dean M, Kaul K, et al. Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors. Proc Natl Acad Sci U S A 1987;84:6379-83.
24. Bottaro DP, Rubin JS, Faletto DL, et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science 1991;251:802-4.
25. Birchmeier C, Gherardi E. Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. Trends Cell Biol 1998;8:404-10.
26. Comoglio PM. Pathway specificity for Met signalling. Nat Cell Biol 2001;3:E161-2.
27. Ma PC, Kijima T, Maulik G, et al. c-MET mutational analysis in small cell lung cancer: novel juxtamembrane domain mutations regulating cytoskeletal functions. Cancer Res 2003;63:6272-81.
28. Jeffers M, Rong S, Vande Woude GF. Enhanced tumorigenicity and invasion-metastasis by hepatocyte growth factor/scatter factor-met signalling in human cells concomitant with induction of the urokinase proteolysis network. Mol Cell Biol 1996;16:1115-25.

29. Cecchi F, Rabe DC, Bottaro DP. Targeting the HGF/Met signalling pathway in cancer. Eur J Cancer 2010;46:1260-70.
30. Ponzetto C, Bardelli A, Zhen Z, et al. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. Cell 1994;77:261-71.
31. Birchmeier C, Birchmeier W, Gherardi E, et al. Met, metastasis, motility and more. Nat Rev Mol Cell Biol 2003;4:915-25.
32. Gohda E, Tsubouchi H, Nakayama H, et al. Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure. J Clin Invest 1988;81:414-9.
33. Montesano R, Matsumoto K, Nakamura T, et al. Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. Cell 1991;67:901-8.
34. Rosen EM, Nigam SK, Goldberg ID. Scatter factor and the c-met receptor: a paradigm for mesenchymal/epithelial interaction. J Cell Biol 1994;127:1783-7.
35. Hammond DE, Urbé S, Vande Woude GF, et al. Down-regulation of MET, the receptor for hepatocyte growth factor. Oncogene 2001;20:2761-70.
36. Goldoni S, Humphries A, Nyström A, et al. Decorin is a novel antagonistic ligand of the Met receptor. J Cell Biol 2009;185:743-54.
37. Rong S, Segal S, Anver M, et al. Invasiveness and metastasis of NIH 3T3 cells induced by Met-hepatocyte growth factor/scatter factor autocrine stimulation. Proc Natl Acad Sci U S A 1994;91:4731-5.
38. Boccaccio C, Sabatino G, Medico E, et al. The MET oncogene drives a genetic programme linking cancer to haemostasis. Nature 2005;434:396-400.
39. Takayama H, LaRochelle WJ, Sharp R, et al. Diverse tumorigenesis associated with aberrant development in mice overexpressing hepatocyte growth factor/scatter factor. Proc Natl Acad Sci U S A 1997;94:701-6.
40. Abounader R, Lal B, Luddy C, et al. In vivo targeting of SF/HGF and c-met expression via U1snRNA/ribozymes inhibits glioma growth and angiogenesis and promotes apoptosis. FASEB J 2002;16:108-10.
41. Eder JP, Vande Woude GF, Boerner SA, et al. Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. Clin Cancer Res 2009;15:2207-14.
42. Shojaei F, Lee JH, Simmons BH, et al. HGF/c-Met acts as an alternative angiogenic pathway in sunitinib-resistant tumors. Cancer Res 2010;70:10090-100.
43. Pennacchietti S, Michieli P, Galluzzo M, et al. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003;3:347-61.
44. De Bacco F, Luraghi P, Medico E, et al. Induction of MET by ionizing radiation and its role in radioresistance and invasive growth of cancer. J Natl Cancer Inst 2011;103:645-61.
45. Lai AZ, Abella JV, Park M. Crosstalk in Met receptor oncogenesis. Trends Cell Biol 2009;19:542-51.
46. Jo M, Stolz DB, Esplen JE, et al. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. J Biol Chem 2000;275:8806-11.
47. Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science 2007;318:287-90.
48. Guo A, Villén J, Kornhauser J, et al. Signaling networks assembled by oncogenic EGFR and c-Met. Proc Natl Acad Sci U S A 2008;105:692-7.
49. Tang Z, Du R, Jiang S, et al. Dual MET-EGFR combinatorial inhibition against T790M-EGFR-mediated erlotinib-resistant lung cancer. Br J Cancer 2008;99:911-22.
50. Shattuck DL, Miller JK, Carraway KL 3rd, et al. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. Cancer Res 2008;68:1471-7.
51. Nakajima M, Sawada H, Yamada Y, et al. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. Cancer 1999;85:1894-902.
52. Mueller KL, Hunter LA, Ethier SP, et al. Met and c-Src cooperate to compensate for loss of epidermal growth factor receptor kinase activity in breast cancer cells. Cancer Res 2008;68:3314-22.
53. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
54. Cappuzzo F, Jänne PA, Skokan M, et al. MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol 2009;20:298-304.
55. Swiercz JM, Worzfeld T, Offermanns S. ErbB-2 and met reciprocally regulate cellular signaling via plexin-B1. J Biol Chem 2008;283:1893-901.
56. Khoury H, Naujokas MA, Zuo D, et al. HGF converts ErbB2/Neu epithelial morphogenesis to cell invasion. Mol Biol Cell 2005;16:550-61.
57. Matsubara D, Ishikawa S, Oguni S, et al. Molecular predictors of sensitivity to the MET inhibitor PHA665752 in lung carcinoma cells. J Thorac Oncol 2010;5:1317-24.

58. Turke AB, Zejnullahu K, Wu YL, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 2010;17:77-88.
59. Goldoni S, Iozzo RV. Tumor microenvironment: Modulation by decorin and related molecules harboring leucine-rich tandem motifs. Int J Cancer 2008;123:2473-9.
60. Gur G, Rubin C, Katz M, et al. LRIG1 restricts growth factor signaling by enhancing receptor ubiquitylation and degradation. EMBO J 2004;23:3270-81.
61. Long IS, Han K, Li M, et al. Met receptor overexpression and oncogenic Ki-ras mutation cooperate to enhance tumorigenicity of colon cancer cells in vivo. Mol Cancer Res 2003;1:393-401.
62. Ma PC, Jagadeeswaran R, Jagadeesh S, et al. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. Cancer Res 2005;65:1479-88.
63. Pelicci G, Giordano S, Zhen Z, et al. The motogenic and mitogenic responses to HGF are amplified by the Shc adaptor protein. Oncogene 1995;10:1631-8.
64. Wang X, DeFrances MC, Dai Y, et al. A mechanism of cell survival: sequestration of Fas by the HGF receptor Met. Mol Cell 2002;9:411-21.
65. Olivero M, Rizzo M, Madeddu R, et al. Overexpression and activation of hepatocyte growth factor/scatter factor in human non-small-cell lung carcinomas. Br J Cancer 1996;74:1862-8.
66. Maulik G, Kijima T, Ma PC, et al. Modulation of the c-Met/hepatocyte growth factor pathway in small cell lung cancer. Clin Cancer Res 2002;8:620-7.
67. Ma PC, Tretiakova MS, MacKinnon AC, et al. Expression and mutational analysis of MET in human solid cancers. Genes Chromosomes Cancer 2008;47:1025-37.
68. Lutterbach B, Zeng Q, Davis LJ, et al. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. Cancer Res 2007;67:2081-8.
69. Beau-Faller M, Ruppert AM, Voegeli AC, et al. MET gene copy number in non-small cell lung cancer: molecular analysis in a targeted tyrosine kinase inhibitor naïve cohort. J Thorac Oncol 2008;3:331-9.
70. Kanteti R, Yala S, Ferguson MK, et al. MET, HGF, EGFR, and PXN gene copy number in lung cancer using DNA extracts from FFPE archival samples and prognostic significance. J Environ Pathol Toxicol Oncol 2009;28:89-98.
71. Onozato R, Kosaka T, Kuwano H, et al. Activation of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. J Thorac Oncol 2009;4:5-11.
72. Onitsuka T, Uramoto H, Ono K, et al. Comprehensive molecular analyses of lung adenocarcinoma with regard to the epidermal growth factor receptor, K-ras, MET, and hepatocyte growth factor status. J Thorac Oncol 2010;5:591-6.
73. Kong-Beltran M, Seshagiri S, Zha J, et al. Somatic mutations lead to an oncogenic deletion of met in lung cancer. Cancer Res 2006;66:283-9.
74. Di Renzo MF, Olivero M, Martone T, et al. Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas. Oncogene 2000;19:1547-55.
75. Cheng TL, Chang MY, Huang SY, et al. Overexpression of circulating c-met messenger RNA is significantly correlated with nodal stage and early recurrence in non-small cell lung cancer. Chest 2005;128:1453-60.
76. Go H, Jeon YK, Park HJ, et al. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol 2010;5:305-13.
77. Okuda K, Sasaki H, Yukiue H, et al. Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer. Cancer Sci 2008;99:2280-5.
78. Cappuzzo F, Jänne PA, Skokan M, et al. MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol 2009;20:298-304.
79. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932-7.
80. Cappuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol 2009;27:1667-74.
81. Rho JK, Choi YJ, Jeon BS, et al. Combined treatment with silibinin and epidermal growth factor receptor tyrosine kinase inhibitors overcomes drug resistance caused by T790M mutation. Mol Cancer Ther 2010;9:3233-43.
82. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17:1169-80.
83. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.

84. Tomoko K, Kadofuku T, Yamaoka T, et al. The novel c-MET inhibitor, ARQ 197, shows additive growth-inhibitory effect with erlotinib through enhanced degradation of c-MET protein via ubiquitin/proteasome pathway. Cancer Res 2010;70:abstr1639.
85. Munshi N, Jeay S, Li Y, et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther 2010;9:1544-53.
86. Chen CR, Szwaya J, Rajanackarin A, et al. Combination studies of tyrosine kinase inhibitors (TKIs): assessment of potential cytotoxic synergy of ARQ 197 with sorafenib or sunitinib. AACR Meeting 2009; abstr 820.
87. Jeay S, Munshi N, Hill J, et al. ARQ 197, a highly selective small molecule inhibitor of c-Met, with selective antitumor properties in a broad spectrum of human cancer cells. AACR Annual Meeting 2007; abstr 2369.
88. Yap TA, Olmos D, Brunetto AT, et al. Phase I trial of a selective c-MET inhibitor ARQ 197 incorporating proof of mechanism pharmacodynamic studies. J Clin Oncol 2011;29:1271-9.
89. Rosen LS, Senzer N, Mekhail T, et al. A phase I dose-escalation study of Tivantinib (ARQ 197) in adult patients with metastatic solid tumors. Clin Cancer Res 2011;17:7754-64.
90. Rodriguez-Antona C, Ingelman-Sundberg M. Cytochrome P450 pharmacogenetics and cancer. Oncogene 2006;25:1679-91.
91. Nishina T, Hirashima T, Sugio K, et al. The effect of CYP2C19 polymorphism on the tolerability of ARQ 197: Results from phase I trial in Japanese patients with metastatic solid tumors. J Clin Oncol 2011;29:abstr 2516.
92. NCT01377376; Available online: www.clinicaltrial.gov
93. Available online: http://propthink.com/arqule-setbacks-increase-risk-discount-rates-increased-for-forward-revenue/1766
94. Goldman JW, Laux I, Chai F, et al. Phase 1 dose-escalation trial evaluating the combination of the selective MET (mesenchymal-epithelial transition factor) inhibitor tivantinib (ARQ 197) plus erlotinib. Cancer 2012;118:5903-11.
95. Sequist LV, von Pawel J, Garmey EG, et al. Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. J Clin Oncol 2011;29:3307-15.
96. Scagliotti GV, Novello S, Schiller JH, et al. Rationale and design of MARQUEE: a phase III, randomized, double-blind study of tivantinib plus erlotinib versus placebo plus erlotinib in previously treated patients with locally advanced or metastatic, nonsquamous, non-small-cell lung cancer. Clin Lung Cancer 2012;13:391-5.
97. Available online: http://propthink.com/tivantinib-will-not-meet-primary-endpoint-phase-iii-trial-discontinued/2390
98. Zou HY, Li Q, Lee JH, et al. Sensitivity of selected human tumor models to PF-04217903, a novel selective c-Met kinase inhibitor. Mol Cancer Ther 2012;11:1036-47.
99. NCT00706355. Available online: www.clinicaltrial.gov
100. NCT01253707. Available online: www.clinicaltrial.gov
101. Koblish HK, Liu X, Hall L, et al. Preclinical in vivo characterization of INCB028060, a novel, potent and highly selective c-Met inhibitor. J Clin Oncol 2008;26:abstr 14561.
102. Liu X, Wang Q, Yang G, et al. A novel kinase inhibitor, INCB28060, blocks c-MET-dependent signaling, neoplastic activities, and cross-talk with EGFR and HER-3. Clin Cancer Res 2011;17:7127-38.
103. Donehower RC, Scardina A, Hill M, et al. A phase I dose-escalation study of INCB028060, an inhibitor of c-MET receptor tyrosine kinase, in patients with advanced solid tumors. J Clin Oncol 2011;29:abstr 3091.
104. Cui JJ, Tran-Dubé M, Shen H, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem 2011;54:6342-63.
105. Tanizaki J, Okamoto I, Okamoto K, et al. MET tyrosine kinase inhibitor crizotinib (PF-02341066) shows differential antitumor effects in non-small cell lung cancer according to MET alterations. J Thorac Oncol 2011;6:1624-31.
106. Ou SH, Kwak EL, Siwak-Tapp C, et al. Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with de novo MET amplification. J Thorac Oncol 2011;6:942-6.
107. Ou SH, Govindan R, Eaton KD, et al. Phase I/II dose-finding study of crizotinib (CRIZ) in combination with erlotinib (E) in patients (pts) with advanced non-small cell lung cancer (NSCLC). J Clin Oncol 2012;30:abstr 2610.
108. Janne PA, Shaw AT, Giaccone G, et al. Phase I trial of irreversible pan-erbb inhibitor dacomitinib (dac) in combination with ALK/MET inhibitor crizotinib (criz) in previously treated advanced non-small cell lung cancer (NSCLC). Annals of Oncology 2012;23:abstr 1290P.
109. Yakes FM, Chen J, Tan J, et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously

suppresses metastasis, angiogenesis, and tumor growth. Mol Cancer Ther 2011;10:2298-308.
110. Kurzrock R, Sherman SI, Ball DW, et al. Activity of XL184 (Cabozantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer. J Clin Oncol 2011;29:2660-6.
111. Nokihara H, Yamamoto N, Nakamichi S, et al. Molecular profile and anti-tumor activity in nonsmallcell lung cancer (NSCLC) patients (pts) in a phase 1 study of cabozantinib (XL184) in Japan. ESMO Meeting 2012; Ann Oncol 2012;23:abstr 17O8PD.
112. Choueiri TK, Pal SK, Cabanillas ME, et al. Antitumor activity observed in a phase I drug-drug interaction study of cabozantinib (XL184) and rosiglitazone in patients (pts) with renal cell carcinoma (RCC) and differentiated thyroid cancer (DTC). J Clin Oncol 2011;29:abstr e13042.
113. Choueiri TK, Pal SK, McDermott DF, et al. Efficacy of cabozantinib (XL184) in patients (pts) with metastatic, refractory renal cell carcinoma (RCC). J Clin Oncol 2012;30:abstr 4504.
114. Hellerstedt BA, Edelman G, Vogelzang NJ, et al. Activity of cabozantinib (XL184) in metastatic NSCLC: Results from a phase II randomized discontinuation trial (RDT). J Clin Oncol 2012;30:abstr 7514.
115. Jänne PA, Wax M, Leach J, et al. Targeting MET with XL 184 to reverse EGFR tyrosine kinase inhibitor (TKI) resistance in NSCLC: impact of preclinical studies on clinical trial design. 20th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, poster 552.
116. Wakelee HA, Gettinger SN, Engelman JA, et al. A phase Ib/II study of XL184 (BMS 907351) with and without erlotinib in patients (pts) with non-small cell lung cancer (NSCLC). J Clin Oncol 2010;28:abstr 3017.
117. NCT01708954. Available online: www.clinicaltrial.gov
118. NCT01639508. Available online: www.clinicaltrial.gov
119. Qian F, Engst S, Yamaguchi K, et al. Inhibition of tumor cell growth, invasion, and metastasis by EXEL-2880 (XL880, GSK1363089), a novel inhibitor of HGF and VEGF receptor tyrosine kinases. Cancer Res 2009;69:8009-16.
120. Eder JP, Shapiro GI, Appleman LJ, et al. A phase I study of foretinib, a multi-targeted inhibitor of c-Met and vascular endothelial growth factor receptor 2. Clin Cancer Res 2010;16:3507-16.
121. Shapiro GI, McCallum S, Adams LM, et al. A Phase 1 dose-escalation study of the safety and pharmacokinetics of once-daily oral foretinib, a multi-kinase inhibitor, in patients with solid tumors. Invest New Drugs 2013;31:742-50.
122. Naing A, Kurzrock R, Adams LM, et al. A comparison of the pharmacokinetics of the anticancer MET inhibitor foretinib free base tablet formulation to bisphosphate salt capsule formulation in patients with solid tumors. Invest New Drugs 2012;30:327-34.
123. Liu L, Shi H, Liu Y, Anderson A, et al. Synergistic effects of foretinib with HER-targeted agents in MET and HER1- or HER2-coactivated tumor cells. Mol Cancer Ther 2011;10:518-30.
124. NCT01068587. Available online: www.clinicaltrial.gov
125. Nakagawa T, Tohyama O, Yamaguchi A, et al. E7050: a dual c-Met and VEGFR-2 tyrosine kinase inhibitor promotes tumor regression and prolongs survival in mouse xenograft models. Cancer Sci 2010;101:210-5.
126. Wang W, Li Q, Takeuchi S, et al. Met kinase inhibitor E7050 reverses three different mechanisms of hepatocyte growth factor-induced tyrosine kinase inhibitor resistance in EGFR mutant lung cancer. Clin Cancer Res 2012;18:1663-71.
127. Daniele G, Ranson M, Blanco-Codesido M, et al. Phase I dose-finding study of golvatinib (E7050), a c-Met and Eph receptor targeted multi-kinase inhibitor, administered orally QD to patients with advanced solid tumors. J Clin Oncol 2012;30:abstr 3030.
128. Bonfils C, Beaulieu N, Fournel M, et al. The combination of MGC D265, a MET/VEGFR inhibitor in clinical development, and erlotinib potently inhibits tumour growth by altering multiple pathways including glycolysis. AACR 2012, abstr 1790.
129. Peters S, Adjei AA. MET: a promising anticancer therapeutic target. Nat Rev Clin Oncol 2012;9:314-26.
130. Prat M, Crepaldi T, Pennacchietti S, et al. Agonistic monoclonal antibodies against the Met receptor dissect the biological responses to HGF. J Cell Sci 1998;111:237-47.
131. Moss RA, Bothos J, Patel P, et al. Complete results from phase I dose escalation study of MetMAb, a monovalent antagonist antibody to the receptor met, dosed as single agent and in combination with bevacizumab in patients with advanced solid malignancies. Ann Oncol 2010;21:abstr 504P.
132. Spigel DR, Ervin TJ, Ramlau R, et al. Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. J Clin Oncol 2011;29:abstr 7505.
133. NCT01496742. Available online: www.clinicaltrial.gov
134. NCT01456325. Available online: www.clinicaltrial.gov

135. Patnaik A, Weiss GJ, Papadopoulos K, et al. Phase I study of SCH 900105 (SC), an anti-hepatocyte growth factor (HGF) monoclonal antibody (MAb), as a single agent and in combination with erlotinib (E) in patients (pts) with advanced solid tumors. J Clin Oncol 2010; 28:abstr 2525.
136. Tan E, Park K, Lim W, et al. A Phase 1b Study of Ficlatuzumab (AV-299), an anti-hepatocyte growth factor monoclonal antibody, in combination with gefitinib in asian patients with NSCLC. J Clin Oncol 2011;29:abstr 7571.
137. Mok TS, Park K, Geater SL, et al. A randomized phase 2 study with exploratory biomarker analysis of ficlatuzumab a humanized hepatocyte growth factor inhibitory MAB in combination with gefitinib versus gefitinib in Asian patients with lung adenocarcinoma. Ann Oncol 2012;23:abstr 1198P.
138. Burgess TL, Sun J, Meyer S, et al. Biochemical characterization of AMG 102: a neutralizing, fully human monoclonal antibody to human and nonhuman primate hepatocyte growth factor. Mol Cancer Ther 2010;9:400-9.
139. Gordon MS, Sweeney CS, Mendelson DS, et al. Safety, pharmacokinetics, and pharmacodynamics of AMG 102, a fully human hepatocyte growth factor-neutralizing monoclonal antibody, in a first-in-human study of patients with advanced solid tumors. Clin Cancer Res 2010;16:699-710.
140. Rosen PJ, Sweeney CJ, Park DJ, et al. A phase Ib study of AMG 102 in combination with bevacizumab or motesanib in patients with advanced solid tumors. Clin Cancer Res 2010;16:2677-87.
141. Okamoto W, Okamoto I, Tanaka K, et al. TAK-701, a humanized monoclonal antibody to hepatocyte growth factor, reverses gefitinib resistance induced by tumor-derived HGF in non-small cell lung cancer with an EGFR mutation. Mol Cancer Ther 2010;9:2785-92.
142. Jones SF, Cohen RB, Bendell JC, et al. Safety, tolerability, and pharmacokinetics of TAK-701, a humanized anti-hepatocyte growth factor (HGF) monoclonal antibody, in patients with advanced non hematologic malignancies: First-in-human phase I dose-escalation study. J Clin Oncol 2010; 28:abstr 3081.
143. Sharma SV, Settleman J. Oncogene addiction: setting the stage for molecularly targeted cancer therapy. Genes Dev 2007;21:3214-31.
144. de Bono JS, Ashworth A. Translating cancer research into targeted therapeutics. Nature 2010;467:543-9.
145. de Bono JS, Tolcher AW, Rowinsky EK. The future of cytotoxic therapy: selective cytotoxicity based on biology is the key. Breast Cancer Res 2003;5:154-9.
146. de Bono JS, Yap TA. Future directions in the evaluation of c-MET-driven malignancies. Ther Adv Med Oncol 2011;3:S51-60.
147. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Discov 2008;7:504-16.

Cite this article as: Menis J, Giaj Levra M, Novello S. MET inhibition in lung cancer. Transl Lung Cancer Res 2013;2(1):23-39. doi: 10.3978/j.issn.2218-6751.2012.12.04

Irreversible EGFR-TKIs: dreaming perfection

Lorenza Landi, Federico Cappuzzo

Istituto Toscano Tumori, Ospedale Civile, Viale Alfieri 36, 57100 Livorno, Italy

Correspondence to: Federico Cappuzzo, MD. Medical Oncology Department, Istituto Toscano Tumori, Ospedale Civile, Viale Alfieri 36, 57100 Livorno, Italy. Email: f.cappuzzo@gmail.com.

Abstract: In the last few years, the treatment of Non-Small-Cell Lung Cancer (NSCLC) has dramatically changed. Presence of activating mutations in the *Epidermal Growth Factor Receptor (EGFR)* identified a particular group of NSCLC patients with different clinical characteristics and outcome. For *EGFR* mutant patients first-generation EGFR tyrosine-kinase inhibitors (TKIs), such as gefitinib and erlotinib, represent the best therapeutic option in first, second and maintenance setting. Unfortunately, all patients develop acquired resistance and despite an initial benefit, virtually all patients progress due to the development of resistance. Several molecular mechanisms are responsible for acquired resistance and the two prominent are the up-regulation of the downstream signal by mesenchymal-epidermal transition (MET) amplification and the emergence of T790M *EGFR* gatekeeper mutation. Preclinical and early clinical trials suggested a potential efficacy of a new class of panHER inhibitor, also called irreversible or covalent inhibitor, in overcome acquired resistance related to T790M. Afatinib, dacomitinib and neratinib, are currently in development in different setting and results from these trials are awaited in order to establish the role of these new compounds in the treatment of NSCLC.

Keywords: NSCLC (non-small-cell lung cancer); EGFR (epidermal growth factor receptor); afatinib; dacomitinib; neratnib; acquired resistance

Submitted Nov 16, 2012. Accepted for publication Dec 17, 2012.
doi: 10.3978/j.issn.2218-6751.2012.12.05
View this article at: http://dx.doi.org/10.3978/j.issn.2218-6751.2012.12.05

Introduction

It is hard to believe that only a decade ago the treatment of non-small-cell lung cancer (NSCLC) was based on simple exclusion of small-cell phenotype. In the last 10 years, steps toward a better knowledge of the mechanisms underlying this lethal disease moved researchers to investigate potential molecular alterations responsible for tumor growth and, consequently, for therapeutic approach. The discovery of mutations in the epidermal growth factor receptor (EGFR) has dramatically changed the treatment of NSCLC (1-3). For patients with lung adenocarcinoma and activating EGFR mutations who received first-generation EGFR-tyrosine kinase inhibitors (TKIs) - such as erlotinib or gefitinib - median overall survival (OS) ranges between 24 and 30 months (4-6), contrasting with the historical plateau of 10 months obtained with front line platinum-based chemotherapy in molecularly unselected populations (7).

Seven large phase III randomized trials conducted in more than 1,400 patients harboring classical EGFR mutations - such as deletion in exon 19 or the L858R substitution in exon 21 - have established a new standard of care (4,5,8-12). In fact, all of these studies demonstrated the superiority of gefitinib, erlotinib or, more recently, afatinib in terms of response rate (RR) and progression free-survival (PFS) when compared to conventional platinum-doublet chemotherapy (*Table 1*). Because the vast majority of subjects enrolled in chemotherapy arm received an EGFR-TKIs at progression, no formal advantage in overall survival has emerged from the aforementioned trials. Nevertheless, in all trials median survival was up to 2-3 years, indicating that EGFR-TKIs are changing natural history of EGFR mutated NSCLC. Finally, since TKI toxicity is generally less severe than the one observed with platinum-based chemotherapy, offering an EGFR-TKIs to a sensitive

Table 1 Studies of EGFR TKIs versus chemotherapy as first-line therapy in NSCLC with typical *EGFR* mutations

Study	EGFR TKI	n	Median PFS in TKI arm (months)	P value	HR
OPTIMAL (11)	Erlotinib	154	13.1	<0.0001	0.16
First Signal (8)	Gefitinib	42	8.4	0.084	0.61
IPASS (4)	Gefitinib	261	9.5	<0.0001	0.48
WJTOG 3405 (9)	Gefitinib	177	9.2	<0.001	0.48
NEJSG 002 (10)	Gefitinib	200	10.8	<0.001	0.36
EURTAC (5)	Erlotinib	174	9.4	<0.0001	0.42
LUX-3 (12)	Afatinib	308	13.6	<0.0001	0.47

Table 2 Main criticisms reported with first-generation EGFR-TKIs

(I)	No response in near 30% of NSCLC with classical exon 19-21 mutation
(II)	No clear benefit in presence of uncommon mutations
(III)	Toxicity
(IV)	No patient is cured: median duration of response 9-12 months
(V)	Lack of efficacy in presence of "acquired" T790M mutation

patient means delay toxic effects of chemotherapy and preserve quality of life (QoL). Similarly, a significant benefit was observed in those EGFR mutant patients treated with erlotinib or gefitinib as second- or third-line treatment (13,14) as well as in maintenance setting (15,16). Taken into account, all these data reinforced the conviction that patients carrying an activating EGFR mutation should never loose the opportunity of receiving an EGFR-TKI during the course of their disease.

However, the enthusiasm generated by these findings has been modulated by the awareness that, until now, no patient can be cured and inevitably all our patients progress and die for their disease. Aim of the present article is to briefly discuss the pitfalls of the first generation EGFR TKIs and to highlight the available data on a new class of inhibitors, also called irreversible or covalent, in the treatment of NSCLC.

Unmet needs with reversible EGFR-TKIs

Main criticisms related to first-generation EGFR-TKIs are listed in *Table 2*.

First, a consistent proportion of *EGFR* mutant patients, approximately 30%, never respond to anti-EGFR TKIs due to primary resistance and the mechanism of this phenomenon is poorly understood (17). On the other hand, we know that *EGFR* mutation does not mean sensitive mutation. *EGFR* mutations exist in exon 18-21 of the tyrosine-binding domain of the EGFR (1,2,18). As previously reported, deletion in exon 19 and L858R point mutation in exon 21 account for the 90% of EGFR mutations detected in NSCLC and are clearly associated with benefit to EGFR TKIs (4,5,8-12). Beside these classical or typical mutations, there is still a small group of "uncommon" mutations, as G719, S768, L861 and others, that can occur with or without a common mutation (19) and for which the clinical impact is poorly understood. Wu *et al.*, analyzed a large series of 1,261 lung cancer cases of which 627 were *EGFR* mutant, with the aim to evaluate the outcome to erlotinib or gefitinib according to the type of mutation (20). The authors confirmed that typical mutations derived the greatest benefit in terms of RR, PFS and OS (74%, 8.5 and 19.6 months respectively) from such treatment; nevertheless the absolute difference in outcome was not so huge when considering the less frequent G719 and L861 mutations (RR 53.3% and 60.0%, PFS 8.1 and 6.0 months, OS 16.4 and 15.2 months for G719 and L861 respectively); on the other hand, some rare uncommon mutations (i.e., V769M and A871E) failed to respond to EGFR TKIs (RR 20%, PFS 1.6 months and OS 11.1 months) with a clinical trend that was very similar to that observed for EGFR wild type population (RR 16.5%, PFS 2.0 months and OS 10.4). Although, the retrospective nature of the investigation and the low sample size of uncommon mutations in large phase III trials, only 6% and 3.8% in the NEJ002 and IPASS respectively (4,10), do not permit to drawn any definitive conclusion, at the present time it is not recommended in clinical practice to treat in first-line a patient with uncommon mutation with erlotinib or gefitinib.

Second, treatment with reversible EGFR TKIs is generally defined as "overall well tolerated". Indeed in the large phase III trials comparing erlotinib and gefitinib

versus standard platinum based chemotherapy, also the toxicity profile was significantly better in the "experimental" arms; the incidences of grade >3 skin rash, diarrhea and liver dysfunction, the three most common adverse events related to EGFR TKIs treatment, did not exceed 20% and the proportion of patients that discontinued therapy due to toxic effects is less than 10% (4,5,8-10). Nevertheless, this small amount of patients, even if molecularly-favored, no longer benefited from therapy. On the other hand, unlike conventional chemotherapy, treatment with targeted agents is continued until disease progression; as a consequence also a long-lasting grade 2 toxicity could became "psicologically serious" over the time mainly because, more often, treated patients are young and able to normal activities.

Last but not least, the most relevant problem related to EGFR TKI therapy is the emergence of acquired resistance (21-23). Indeed, despite an initial dramatic tumor regression in up to 80% of cases after a median time of 9-12 months, all patients progress and the possibility of further control tumor growth inevitably decreases.

Acquired resistance to EGFR TKIs: clinical, biological and therapeutic implications

From a clinical point of view, we refer to acquired resistance according to the criteria proposed by Jackman and coworkers (24) in 2010 considering as "resistant" those patients treated with single-agent erlotinib or gefitinib (I) who progressed while on treatment and (II) who harbored a sensitive EGFR mutation or (III) if *EGFR* status is wild type or unknown, who obtained partial or complete response or a significant and durable (>6 months) clinical benefit - according to RECIST or WHO criteria - after initiation of EGFR TKI therapy. Two important issues derived from this work: first, the utility of a relative simple criteria to correctly define and select for novel clinical trials a population otherwise too heterogeneous; second, the concept that a progression that occur *while on treatment* could be interpreted as a transitory clinical condition related to the type of therapy (i.e., reversible EGFR TKIs) rather than to a true EGFR-pathway-independent tumor growth. In other words, the sensitivity to an anti-EGFR TKIs could be restore after a break period (3,22,25); for this reason many trials with sequential use of chemo- and EGFR targeted therapies are ongoing (25).

From biological point of view, prolonged exposure to erlotinib or gefitinib provides selective pressure for the development of tumor clones able to growth irrespective of the drug inhibition. The mechanisms underlying the phenomenon of secondary resistance are object of extensive evaluation and some of these are so far elucidated (22,23,26). Several preclinical studies demonstrated that the two main mechanisms responsible for acquired resistance are the up-regulation of the downstream signal by mesenchymal-epidermal transition (MET) amplification and the emergence of T790M *EGFR* gatekeeper mutation (26-30). Other mechanisms include EGFR amplifications, PI3KCA mutations or a transition from epiyhelial to mesenchymal differentiation (26). More interestingly, for a little percentage of resistant tumors occurs transformation into SCLC (26).

MET amplification is found to be associated with acquired resistance in up to 20% of cases and inhibition of MET with the use of monoclonal antibodies (31-33) or small molecule TK inhibitor (34) alone or in combination with other targeted agents are currently under investigations. Anti-MET strategies have been extensively discussed elsewhere (35-37).

The "acquired" T790M mutation - a characteristic point mutation in the exon 20 of the *EGFR* gene - is associated with lack of activity of first generation EGFR TKI and is responsible for secondary resistance in at least 50% of patients exposed to erlotinib or gefitinib (22,23,26,38). Initial data showed that this event occur in less than 3% of mutated patients before starting and EGFR TKI therapy (30). More recently, using high sensitive methods, the EGFR T790M mutation was detected in up to 40% of previously untreated NSCLC, suggesting that what we call an "acquired resistance" is a pre-existing phenomenon (39). Retrospective data from Memorial Sloan Kettering Cancer Center suggested that this molecular event is largely underestimated, when assessed by low-sensitive technique (39). Whereas the vast majority of *EGFR* mutations are sensitive to TKIs because they decrease the affinity of the receptor for its natural substrate ATP, the presence of T790M, altering the conformation of the tyrosine kinase domain of the EGFR, restore its affinity for ATP at the levels similar than reported for *EGFR* wild type thus reducing the ability of reversible TKIs to effectively compete with ATP (40-41). *In vitro* studies demonstrated that gefitinib-resistant as well T790M mutation positive clones remain sensitive to irreversible EGFR TKIs that are structurally similar to erlotinib and gefitinib (42); unlike reversible TKIs, this new class of inhibitor contain an acceptor-group that binds covalently with the Cys797 present at the ATP-binding site of mutant EGFR. As discussed above, due to their characteristics irreversible EGFR TKIs seemed to be the ideal compounds

to test in order to overcome T790M acquired resistance (42).

A fascinating way to interfere with the signaling cascade of the EGFR, in order to overcome resistance, is to simultaneously inhibit both the extracellular and intracellular receptor domains. The clinical proof of the so-called "vertical inhibition" comes from previous experience in HER2-overexpressing trastuzumab-resistant metastatic breast cancer, in which the combination of trastuzumab and lapatinib was superior to lapatinib alone in terms of RR and PFS (43).

Similarly in NSCLC, the combination of afatinib and cetuximab induced nearly complete tumor regression in T790M transgenic murine models (44). On this base, a pivotal phase Ib study has been recently conducted in NSCLC patients with clinically defined acquired resistance with the aim to explore the safety and activity of the combination (45). In the initial cohort, 22 patients were exposed to afatinib at the oral daily dose of 40 mg and cetuximab 500 mg/m^2 intravenously every 2 weeks. Adverse events were consistent with the typical class-effects previously reported (i.e., diarrhea and skin rash) and were generally mild, with only 3 patients experiencing grade 3 skin toxicity. Every patient obtained disease control with a median reduction in tumor size of 76% and a promising activity of 36% (8/22 including 4/13 T790M positive cases), leading to enrollment of an additional cohort of 80 patients. Final results have been recently presented. Main grade 3 adverse events were skin rash (12%) and diarrhea (6%); 96 patients were evaluable for efficacy and treatment resulted in 75% of disease control rate with a response rate of 30%, without significant difference between T790M positive and T790M negative patients (32% versus 28% months); median PFS was 4.7 months (46). These encouraging results deserve further validation in large phase III trials.

New generations EGFR TKIs

The second generation of EGFR inhibitors, also-defined irreversible or covalent EGFR inhibitors, afatinib, dacomitinib and neratinib, are pan-ErbB inhibitors and their activity against both EGFR activating mutations and the T790M mutation has been demonstrated in *in vivo* models (47-49).

Afatinib

Afatinib (BIBW2992) binds irreversibly to EGFR, HER2, HER4 and also to EGFR receptors carrying the T790M mutation, suggesting a potential role in overcoming resistance. Multiple phase I studies identified in 50 mg once daily the maximum tolerated dose (MTD) with main toxicities represented by diarrhea and skin rash (50). On this basis, the LUX-Lung clinical trial program has been launched for testing this molecule in different setting in advanced NSCLC patients.

In the phase 2b/3 LUX-Lung 1 trial (51), a total of 585 adenocarcinoma patients who met criteria for acquired resistance to EGFR-TKIs as proposed by Jackman *et al.* (24), were randomized in a 2:1 fashion to receive daily oral afatinib 50 mg plus best supportive care (BSC) or placebo plus BSC as third or subsequent line of therapy. The primary end-point was overall survival. Interestingly, the trial did not need archival tumor tissue and the subjects were not screened for *EGFR* status, but the prior disease control for >3 months under TKIs treatment was used as surrogate criterion to increase probability of *EGFR* mutations. The treatment with afatinib resulted in better activity (RR 7% versus 0.5%) and longer PFS (3.3 months, 95% CI, 2.79-4.40 months) than it was in placebo group (1.1 months, 95% CI, 0.95-1.68 months, HR 0.38, P<0.0001). Surprisingly, the PFS benefit did not translate in survival benefit. Median overall survival was 10 and 12 months for the afatinib and placebo arm respectively; the reason behind this unusual finding could be the confounding effect of post-study therapies; indeed, a greater proportion in the placebo arm than in the afatinib arm receive subsequent treatment, including chemotherapy and EGFR TKI.

Similar activity was preliminary reported in the LUX-Lung 4, a phase II open label trial, in which 62 Japanese patients who progressed after 1 or 2 chemotherapy lines and prior erlotinib or gefitinib underwent therapy with afatinib at the dose 50 mg (52). Response rate was 8%, with DCR of 66%, while PFS resulted of 4.4 months.

Afatinib was also evaluated as first line and second line therapy in patients who had not received a first generation TKI. The LUX-Lung 2 trial was a single-arm, multicenter phase II study evaluating the efficacy of afatinib 40-50 mg daily in advanced adenocarcinoma with *EGFR* activating mutations (53). A total of 129 subjects (first line N=61; second line, N=68) were enrolled onto the study; notably 18% of patients presented an uncommon mutation. In overall population objective RR, DCR and PFS were 59%, 83% and 14 months respectively, with a median overall survival of 24 months; no difference in outcome was noted between patients harbored L858R or deletion in exon 19 irrespective of line of therapy, while the efficacy in terms

of RR, PFS and OS was lower in those patients with uncommon mutations (RR 39%; median PFS 3.7 months; OS 16.3 months).

The LUX-lung 3, the first phase III study using the combination of pemetrexed and cisplatin as a comparator arm, randomly assigned in a 2:1 fashion *EGFR* mutant adenocarcinoma patients to receive as front line therapy afatinib 40 mg daily or six cycles of chemotherapy (12). The study, which enrolled 345 patients, met its primary end point of PFS. Patients treated with afatinib had a 42% relative reduction in risk of progression compared with those receiving standard chemotherapy (11.1 versus 6.9 months, HR 0.58; 13.1 versus 6.9 months, HR 0.47 for patients with classical EGFR mutations). Treatment with afatinib was also associated with higher response rate (56% versus 23%, ITT population) and better toxicity profile than chemotherapy, although G3 diarrhea and skin rash occurred in 14% and 16% of cases receiving the study drug.

Dacomitinib

Dacomitinib (PF0299804), covalently binds the adenosine triphosphate domain of each of three kinase active members of the HER family: EGFR/HER1, HER2 and HER4. In preclinical experiences, dacomitinib showed greater antitumor activity in gefitinib-resistant NSCLC *in vitro* and *in vivo* models (49). In NSCLC clinical trials, Dacomitinib has been evaluated in three different setting: after EGFR TKI failure (54-56), in second line in patients not previously exposed to a reversible EGFR TKI and in front line in *EGFR* mutants patients (57,58).

In a phase I study (54), a disease control rate (PR + SD) of 34% was seen in 44 patients pretreated with first-generation EGFR TKIs (94%) and chemotherapy (79%); most frequently any-grade adverse events observed at the recommended daily dose of 45 mg were diarrhea (78%) and skin rash (65%). In another phase I/II trial conducted in 36 advanced NSCLC patients who progressed after one or two prior chemotherapy regimen and erlotinib (55), DCR was observed in 67% and 40 % of patients with adenocarcinoma and squamous cell carcinoma respectively. In another Korean phase II trial (56), enrolling 42 patients with similar characteristics, preliminary results demonstrated an activity of 15% with a DCR of 25%.

Ramalingam *et al*. published the results of the first randomized trial on irreversible EGFR TKI in lung cancer patients never exposed to TKI treatment (59). Subjects enrolled onto this phase II study were randomly assigned to receive as second line treatment erlotinib (N=94) or dacomitinib (N=94). The primary end point was PFS. In the dacomitinib arm there was a higher number of patients with ECOG performance status 2, *EGFR* mutant and treated with 2 or more prior chemotherapy than in the erlotinib arm. PFS resulted in favor of the experimental arm (median PFS 2.8 versus 1.91 months; HR 0.66); the improvement in PFS was reported across most of the subgroup considered and particularly in KRAS wild type/EGFR any status (median PFS 3.71 versus 1.91 months; HR 0.55), KRAS wild type/EGFR wild type (median PFS 2.21 versus 1.84 months; HR 0.61), while for EGFR mutant patients median PFS resulted of 7.44 in both arms. The objective RR was lower in the erlotinib arm than in dacomitinib arm (5.3% versus 17%), as DCR (14.9% versus 29.8%) did. However, grade diarrhea and skin rash were more frequent with dacomitinib than with erlotinib.

More recently, Kris *et al*. reported the results of the 1017 study of dacomitinib at the dose of 30-45 mg daily in NSCLC patients with *EGFR* mutations or *HER-2* mutations (i.e., exon 20 insertions or point mutations) or *HER-2* amplification (57). Endpoints included progression-free survival rate at 4 months (PFS at 4 M), PFS, partial response (PR) rate and safety. EGFR cohort included never or light-former smoker (<10 pack year) patients with metastatic non-pretreated adenocarcinoma or treatment-naïve patients with known *EGFR* mutations, while HER2 cohort enrolled subjects with *HER2* mutations or amplification who received any number of prior therapy. In the EGFR cohort (Cohort A, N=89), 46 of patients harbored a classical mutation (exon 19, N=25; exon 21, N=21); in this subgroup, RR rate was 76% while PFS at 4M and PFS were 95.5% (95% CI, 83.2-98.9%) and 18.2 months (95% CI, 12.8-23.8 months) respectively. As expected, common side effects were diarrhea, skin toxicity and nail changes. Cohort B is still recruiting and in the first 22 enrolled patients (*HER2* amplification, N=4; *HER2* mutation, N=18) an interesting activity of 14% was observed, but limited to those patients carrying a *HER-2* mutation.

Neratinib

Neratinib (HKI-272), an irreversible HER family inhibitor targeting EGFR/HER-1, HER-2 and HER-4, was initially tested in a phase I trial of 72 patients with advanced ErbB2 or ErbB1/EGFR IHC positive tumors (58). Maximum tolerated dose (MTD) was determined to be 320 mg and the most common related adverse event at this dose was

Table 3 Comparison of best reported phase II results for EGFR TKIs in patients with *EGFR*-Mutant lung cancers (Exon 19 and Exon 21)

	Pts Enrolled, N	RR, %	mPFS, mos	mOS, mos
Dacomitinib (57)	46	74	17	NR
Afatinib (53)	129*	66	15	32-39
Erlotinib (61)	33	70	14	31
Gefitinib (62)	27	59	9.2	17.5

*51 treated first-line.

diarrhea. Strikingly, a long-lasting disease control (defined as stable disease for >24 weeks) was observed in 43% of refractory NSCLC patients.

A large non-randomized phase II tria l explored the activity of neratinib in three different cohorts of advanced pretreated NSCLC patients (60). Arm A included patients with activating EGFR mutation (N=91), arm B included *EGFR* wild-type patients (N=48) while arm C included EGFR TKI-naïve patients selected for adenocarcinoma histology and smoking history (N=28). Subjects in arms A and B had to have received at least 12 weeks of prior erlotinib/gefitinib treatment. In the overall population (N=158), the activity was lower than expected, with only 2% of responders (RR 3.4% arm A; 0% arm B; 0% arm C). Interestingly, the three responding patients harbored the rare G719X point mutation in exon 18, maybe suggesting that neratinib could be less effective in presence of classical *EGFR* mutations; on the contrary, the presence of T790M mutation did not seem guarantee any benefit from such treatment. Median PFS was 15.3 weeks in the entire cohort, without significant difference between the three arms (15.3, 16.1 and 9.3 weeks in arm A, B and C respectively). Nevertheless, in the first 39 patients receiving neratinib at the dose of 320 mg daily the occurrence of grade 3 diarrhea was unacceptably high (50%); as a consequence, a dose reduction to 240 mg was required in order to improve tolerability with the hypothetical disadvantage of negatively affect response. Anyway, this major limitation led to dissipate the interest to further explore neratinib in NSCLC.

Discussion

The ideal inhibitor might be equally effective irrespective of the type of *EGFR* mutations, highly similar to the binding site of the receptor, active even in presence of T790M clones and - from the patient point of view - at least with identical or better toxicity profile than older compounds. Have the irreversible EGFR TKIs met all this endpoints?

In front line setting, the efficacy of covalent inhibitors is comparable to the one reported for reversible TKIs. In the LUX Lung 3 trial median PFS for patients with typical *EGFR* mutations is more than 13 months, with an absolute improvement of nearly 7 months respect to chemotherapy arm (12). These results is quite similar to those reported in the OPTIMAL trial, in which an impressive HR of 0.16 for PFS in favour of erlotinib arm was observed (11); nevertheless, unlike OPTIMAL, in the LUX-3 the difference in outcome between *EGFR*-TKI therapy and chemotherapy appears to be real, considering the high performance of the comparator arm. In phase II trial, Dacomitinib showed an unexpected PFS of nearly 18 months, but this finding deserves further validation in prospective large phase III studies (57). In terms of activity, best response rate observed in phase II trials of first and second generation EGFR-TKIs seemed almost identical for both class of inhibitors (53,57,61,62) (*Table 3*). Large phase III trials comparing head-to-head irreversible versus reversible EGFR TKIs are urgently needed to define whether covalent inhibitors may improve outcomes and possibly delay the onset of resistance.

Once again, patients harboring a classical mutation gained the greatest benefit from such treatments. In the LUX Lung 2, in which 18% of patients presented uncommon mutations, the RR and PFS was lower for this population and in any case, were consistent with those reported for gefitinib and erlotinib (53). In the LUX Lung 3 study (12,63), 48 (10.6%) patients presented uncommon mutations that were were categorized into 5 groups: T790M, G719X, S768I, exon 20 insertions, L861Q; the first 3 groups included double mutant patients. Tumour response and prolonged PFS were noted in 2 double mutant patients (L858R + T790M; S768I + L858R) and in 2 with single uncommon mutation (G719X and S768I), while in the other cases SD was the best response. Nevertheless these results are inconclusive, as the effect of afatinib in doublet mutant patients could be in part referred to the presence of the L858R mutation. As previously reported (60), neratinib

Table 4 Grade >3 toxicity with EGFR-TKIs

	Gefitinib				Erlotinib		Afatinib
	NEJSG 002 (10)	IPASS (4)	First-SIGNAL (8)	WJTOG3405 (9)	OPTIMAL (11)	EURTAC (5)	LUX-3 (12)
	n=114	n=607	n=159	n=87	n=83	n=84	n=229
Rash	71.0 [5.3]	66.2 [3.1]	72.3 [1.3]	74 [2]	73.5 [2.4]	11 [13.0]	37 [16.2]
Diarrhea	34.2 [0.9]	46.6 [3.8]	NR	47[1]	25.3 [1.2]	4 [5.0]	33 [14.4]
Fatigue	10.5 [2.6]	NR	28.3 [0.6]	34 [2]	4.8 [0]	5 [6.0]	3 [1.3]
Anorexia	NR	21.9 [1.5]	44.7 [0]	NR	NR	0 [0]	7 [3.1]
Stomatitis	9.6 [0]	17.0 [0.2]	NR	19 [0]	13.3 [1.2]	NR	20 [8.7]
Paronychia	NR	13.5 [0.3]	NR	28 [1]	3.6 [0]	NR	26 [11.4]
Vomiting	6.1 [0.9]	12.9 [0.2]	NR	NR	NR	NR	7 [3.1]

seemed to be more effective in presence of the rare G719X mutation; this might simply reflect a different sensitivity of specific mutations to an EGFR TKI. Furthermore, is it not possible to exclude that this result was obtained by chance because of the very small number of patients.

Irreversible TKIs have been developed with a specific focus on patients with acquired resistance to erlotinib or gefitinib. LUX-Lung 1 (51) and LUX-Lung 4 (52) trials failed to demonstrate a clear benefit in terms of RR in patients with acquired resistance and particularly in those cancers with T790M; the activity reported in the 2 studies was only 7% and 8%, lower than expected. We recently presented a retrospective analysis of 68 advanced lung adenocarcinoma patients with acquired resistance to reversible EGFR TKIs treated with afatinib and we reported a response rate of 10.6% with a disease control rate of 65%. Four of the five responding patients harbored a classical mutation including 1 patient with T790M; in 9 patients in which tumor biopsy was repeated before starting afatinib, only 2 patients had T790M mutation, with no evidence of response (64). All these results are disappointing and suggest that the ability of covalent inhibitor in overcome acquired resistance may have limitations unpredicted in preclinical experiences; a possible explanation could be the different drug concentration achieved in humans respect to preclinical models.

Another critical issue concerns the toxicity profile of the irreversible inhibitors. In metastatic setting, the preservation of QoL still remains one of the goals of therapy, mainly when considering second and subsequent line of treatment. In the case of neratinib, an unacceptable incidence of 50% of grade diarrhea required a dose reduction in the Sequist's phase II trial (60). Grade 3 adverse events reported in LUX 1 and 2 trials (51,52), led the clinicians to consider 40 mg as the "optimal" tolerated dose, instead of 50 mg defined in phase I trial (50). Anyway, indirect comparison of phase III trials showed higher incidences of diarrhea, skin rash and stomatitis for afatinib respect to erlotinib or gefitinib (4,5,8). Main grade >3 toxicities with EGFR-TKIs are listed in *Table 4*. Taken into account, all these data suggested that toxicities of covalent inhibitors are probably higher than those observed with first-generation compounds.

Conclusions

Irreversible EGFR TKIs could represent a promising therapeutic option in the treatment of NSCLC. Although in absence of trials directly comparing reversible versus irreversible TKIs, available data failed to demonstrated a superior efficacy respect to first-generation inhibitors. Furthermore, the activity reported in patients harbouring an EGFR uncommon mutation is consistent with the one observed for gefitinib and erlotinib. Although the clinical development of covalent inhibitors focused on T790M-dependent acquired resistance, activity observed in this particular subgroup was only modest. The high affinity for ATP binding site could in part explain the prevalence of typical class-effects observed with afatinib, neratinib and dacomitinib. Results from ongoing and planned clinical trials, will help us to define the role of second generation TKIs in our clinical practice.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib

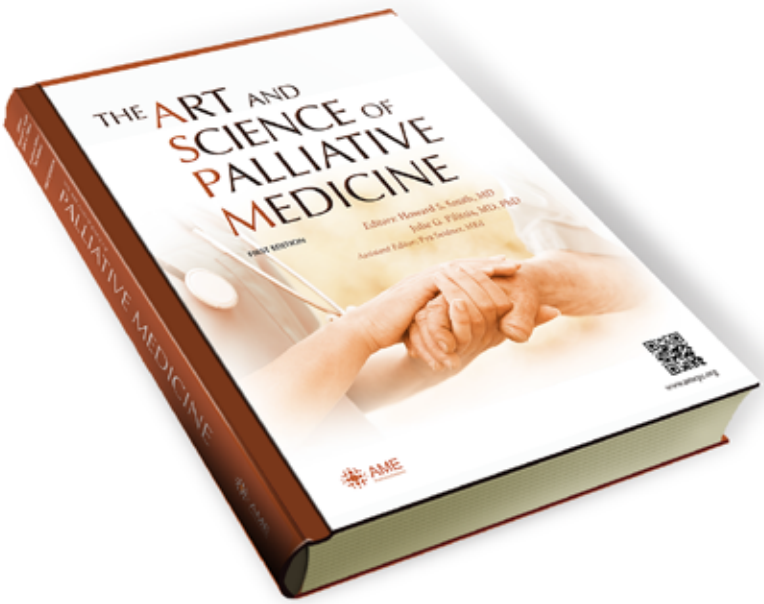
therapy. Science 2004;304:1497-500.
2. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 2004;101:13306-11.
3. Soria JC, Mok TS, Cappuzzo F, et al. EGFR-mutated oncogene-addicted non-small cell lung cancer: current trends and future prospects. Cancer Treat Rev 2012;38:416-30.
4. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
5. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
6. Mok TS, Lee JS, Zhang L, et al. Biomarkers analyses and overall survival (OS) from the randomized, placebo-controlled, phase 3, FASTACT-2 study of intercalated erlotinib with first- line chemotherapy in advanced non-small cell lung cancer (NSCLC). Ann Oncol 2012;29:ix 400;abstr 12260.
7. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92-8.
8. Han JY, Park K, Kim SW, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol 2012;30:1122-8.
9. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-8.
10. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
11. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011;12:735-42.
12. Yang JC, Schuler MH, Yamamoto N, et al. LUX-Lung 3: A randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. J Clin Oncol 2012;30:abstr LBA7500.
13. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123-32.
14. Douillard JY, Shepherd FA, Hirsh V, et al. Molecular predictors of outcome with gefitinib and docetaxel in previously treated non-small-cell lung cancer: data from the randomized phase III INTEREST trial. J Clin Oncol 2010;28:744-52.
15. Cappuzzo F, Ciuleanu T, Stelmakh L, et al. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. Lancet Oncol 2010;11:521-9.
16. Zhang L, Ma S, Song X, et al. Gefitinib versus placebo as maintenance therapy in patients with locally advanced or metastatic non-small-cell lung cancer (INFORM; C-TONG 0804): a multicentre, double-blind randomised phase 3 trial. Lancet Oncol 2012;13:466-75.
17. Cappuzzo F, Jänne PA, Skokan M, et al. MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol 2009;20:298-304.
18. Riely GJ, Politi KA, Miller VA, et al. Update on epidermal growth factor receptor mutations in non-small cell lung cancer. Clin Cancer Res 2006;12:7232-41.
19. Chen Z, Feng J, Saldivar JS, et al. EGFR somatic doublets in lung cancer are frequent and generally arise from a pair of driver mutations uncommonly seen as singlet mutations: one-third of doublets occur at five pairs of amino acids. Oncogene 2008;27:4336-43.
20. Wu JY, Yu CJ, Chang YC, et al. Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. Clin Cancer Res 2011;17:3812-21.
21. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786-92.
22. Brugger W, Thomas M. EGFR-TKI resistant non-small cell lung cancer (NSCLC): new developments and implications for future treatment. Lung Cancer 2012;77:2-8.
23. Engelman JA, Jänne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin

Cancer Res 2008;14:2895-9.
24. Jackman D, Pao W, Riely GJ, et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. J Clin Oncol 2010;28:357-60.
25. Moran T, Sequist LV. Timing of epidermal growth factor receptor tyrosine kinase inhibitor therapy in patients with lung cancer with EGFR mutations. J Clin Oncol 2012;30:3330-6.
26. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
27. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
28. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932-7.
29. Chen HJ, Mok TS, Chen ZH, et al. Clinicopathologic and molecular features of epidermal growth factor receptor T790M mutation and c-MET amplification in tyrosine kinase inhibitor-resistant Chinese non-small cell lung cancer. Pathol Oncol Res 2009;15:651-8.
30. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.
31. Tan E, Park K, Lim WT, et al. Phase Ib study of ficlatuzumab (formerly AV-299), an anti-hepatocyte growth factor (HGF) monoclonal antibody (MAb) in combination with gefitinib (G) in Asian patients (pts) with NSCLC. J Clin Oncol 2011;29:abstr 7571.
32. Mok TS, Park K, Geater SL, et al. A Randomized Phase 2 Study with Exploratory Biomarker Analysis of Ficlatuzumab, a Humanized Hepatocyte Growth Factor (HGF) Inhibitory Monoclonal Antibody, in Combination with Gefitinib versus Gefitinib Alone in Asian Patients With Lung Adenocarcinoma. Ann Oncol 2012;23:abstr.
33. Spigel DR, Ervin TJ, Ramlau R, et al. Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. J Clin Oncol 2011;29:abstr 7505.
34. Sequist LV, von Pawel J, Garmey EG, et al. Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. J Clin Oncol 2011;29:3307-15.
35. Blumenschein GR Jr, Mills GB, Gonzalez-Angulo AM. Targeting the hepatocyte growth factor-cMET axis in cancer therapy. J Clin Oncol 2012;30:3287-96.
36. Appleman LJ. MET signaling pathway: a rational target for cancer therapy. J Clin Oncol 2011;29:4837-8.
37. Toschi L, Cappuzzo F. Clinical implications of MET gene copy number in lung cancer. Future Oncol 2010;6:239-47.
38. Oxnard GR, Arcila ME, Sima CS, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res 2011;17:1616-22.
39. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17:1169-80.
40. Yun CH, Mengwasser KE, Toms AV, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 2008;105:2070-5.
41. Suda K, Onozato R, Yatabe Y, et al. EGFR T790M mutation: a double role in lung cancer cell survival? J Thorac Oncol 2009;4:1-4.
42. Kwak EL, Sordella R, Bell DW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. Proc Natl Acad Sci U S A 2005;102:7665-70.
43. Blackwell KL, Burstein HJ, Storniolo AM, et al. Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. J Clin Oncol 2010;28:1124-30.
44. Regales L, Gong Y, Shen R, et al. Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. J Clin Invest 2009;119:3000-10.
45. Janjigian YY, Groen HJ, Horn L, et al. Activity and tolerability of afatinib (BIBW2992) and cetuximab in NSCLC patients with acquired resistance to erlotinib or gefitinib. J Clin Oncol 2011; 29:abstr 7525.
46. Janjigian YY, Smith EE, Horn L, et al. Activity of afatinib/cetuximab in patients with EGFR mutant non-small cell lung cancer and acquired resistance to EGFR inhibitors. Ann Oncol 2012;23:ix 401.
47. Li D, Ambrogio L, Shimamura T, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 2008;27:4702-11.

48. Ninomiya T, Takigawa N, Kubo T, et al. Effect of afatinib on lung cancer burden induced by an exon 19 EGFR mutation in transgenic mice [abstract 3566]. Presented at the 102nd Annual Meeting of the American Association for Cancer Research; Orlando, Florida; April 2-11, 2011.
49. Engelman JA, Zejnullahu K, Gale CM, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. Cancer Res 2007;67:11924-32.
50. Yap TA, Vidal L, Adam J, et al. Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. J Clin Oncol 2010;28:3965-72.
51. Miller VA, Hirsh V, Cadranel J, et al. Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. Lancet Oncol 2012;13:528-38.
52. Atagi S, Katakami N, Hida T, et al. LUX Lung 4: a phase II of Afatinib (BIBW2992) in advanced NSCLC patients previously treated with erlotinib or gefitinib. J Thor Oncol 2011;6:abstr O19.06.
53. Yang JC, Shih JY, Su WC, et al. Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): a phase 2 trial. Lancet Oncol 2012;13:539-48.
54. Janne PA, Schellenns JH, Engelman JA, et al. Preliminary activity and safety results from a phase I clinical trial of PF-00299804, an Irreversible pan-HER inhibitor in patients with NSCLC. J Clin Oncol 2008;26:abstr 8027.
55. Janne PA, Reckamp K, Koczywas M, et al. A phase 2 trial of PF-00299804, an oral irreversible HER kinase inhibitor (TKI) in patients with advanced NSCLC after failure of prior chemotherapy: preliminary efficacy and safety results. J Thorac Oncol 2009;4:S293-94.
56. Park K, Heo DS, Cho BC, et al. PF299804 in Asian patients with non-small-cell lung cancer refractory to chemotherapy and erlotinib or gefitinib: aphase I/II study. J Clin Oncol 2010;28:abstr 7599.
57. Kris MG, Mok T, Ou SH, et al. Dacomitinib (PF-00299804), an Irreversible pan-HER Tyrosine Kinase Inhibitor, for First-Line Treatment of EGFR-Mutant or HER2-Mutant or -Amplified Lung Cancers. J Clin Oncol 2012;30:abstr 7530.
58. Wong KK, Fracasso PM, Bukowski RM, et al. A phase I study with neratinib (HKI-272), an irreversible pan ErbB receptor tyrosine kinase inhibitor, in patients with solid tumors. Clin Cancer Res 2009;15:2552-8.
59. Ramalingam SS, Blackhall F, Krzakowski M, et al. Randomized phase II study of dacomitinib (PF-00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2012;30:3337-44.
60. Sequist LV, Besse B, Lynch TJ, et al. Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. J Clin Oncol 2010;28:3076-83.
61. Jänne PA, Wang X, Socinski MA, et al. Randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 trial. J Clin Oncol 2012;30:2063-9.
62. Sequist LV, Martins RG, Spigel D, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. J Clin Oncol 2008;26:2442-9.
63. Yang JC, Schuler M, Yamamoto N, et al. Activity of afatinib in uncommon epidermal growth factor receptor (EGFR) mutations in LUX-Lung 3, a phase III trial of afatinib or cisplatin/pemetrexed in EGFR mutation-positive lung cancer. Ann Oncol 2012;23:ix410, abstr 1256p.
64. Landi L, galetta D, Bennati C, et al. Efficacy of the irreversible EGFR-HER2 dual inhibitor afatinib in pretreated lung adenocarcinoma. Ann Oncol 2012;23:ix423, abstr 1288P.

Cite this article as: Landi L, Cappuzzo F. Irreversible EGFR-TKIs: dreaming perfection. Transl Lung Cancer Res 2013;2(1):40-49. doi: 10.3978/j.issn.2218-6751.2012.12.05

ISBN: 978-988-12997-2-7 (hardback)
ISBN: 978-988-12997-3-4 (eBook)



Editors:
Howard S. Smith, MD
Julie G. Pilitsis, MD, PhD

Assistant Editor:
Pya Seidner, MEd

Page count: 598

The goal of the book was to provide a resource that is usable in all countries, providing straightforward data as well as food for thought for providers worldwide. Its design by Howard Smith, MD, was brilliant in its simplicity as well as its breadth of coverage. It is useful both for the student and resident physician being first exposed to death and ding as well as the palliative care specialist that may be an expert in one facet of the patient's disease, but not in others. After reading this book, it was Dr. Smith's goal to arm the reader with a new set of tools in their daily responsibility and to be the best provider possible for their patients. It is meant to spawn interest in further reading on topics of interest and to promote future directions of study.

Julie G. Pilitsis, MD, PhD
Albany, NY, USA

"... to cure sometimes, to relieve often, to comfort always."
Attributed to Dr. Edward Livingston Trudeau, founder of a 19th century tuberculosis sanatorium, this could easily be a defining slogan for palliative care because nearly all care models highlight the reigning importance of the individual as the central point of care.

Amy P. Abernethy, M.D., Ph.D.
Current President, American Academy of Hospice & Palliative Medicine;
Duke University School of Medicine, Durham, USA

Please scan the above code for purchase information, or contact apm@amepc.org.

AME Publishing Company

More online at www.amepc.org

Sydney Adventist Hospital… leading the way in minimally invasive cardiothoracic surgery

Sydney Adventist Hospital (SAH) was established in 1903 and has a rich history in education and training. SAH is Sydney's largest private hospital and home to Sydney Adventist Hospital Clinical School of the University of Sydney. With its comprehensive and acute care services, the hospital is renowned for being at the forefront of cardiac surgery and minimally invasive cardiothoracic surgery, offering state-of-the-art services, including:

- Minimally invasive aortic valve replacement
- MInimally invasive mitral valve repair
- Sutureless valve technology
- Minimally invasive aortic surgery (Mini-Bentall)
- Robotic thymectomy
- VATS lobectomy

If you or your patients would like more information, please contact Carolyn Austin at carolyn.austin@sah.org.au or Stephen Wang at stephen.wang@sah.org.au

SYDNEY ADVENTIST HOSPITAL

www.sah.org.au 185 Fox Valley Road Wahroonga 2076 Sydney Australia **Tel:** +61 2 9487 9111