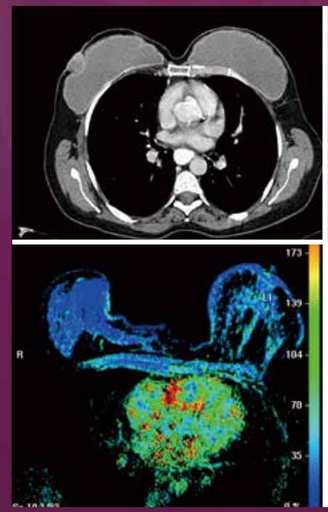
BREAST CANCER



www.amegroups.com

1A021

ISBN 978-988-77841-2-8
amegroups.com

ISBN 978-7-5487-3015-6
￥685.00 CNY

中南大學出版社
www.csupress.com.cn

AME Publishing Company

BREAST CANCER
Editors: Zhimin Shao, Peter G. Cordeiro, Charles M. Balch
中南大學出版社
www.csupress.com.cn

BREAST CANCER

Honorary Editors: Zhenzhou Shen, Bernard Fisher, Gabriel Hortobagyi
Editors: Zhimin Shao, Peter G. Cordeiro, Charles M. Balch
Associate Editors: Keda Yu, Ning Liao, V. Suzanne Klimberg, William J. Gradishar

中南大學出版社
www.csupress.com.cn

AME Publishing Company

AME Publishing Company

Room C 16F, Kings Wing Plaza 1, NO. 3 on Kwan Street, Shatin, NT, Hong Kong

Information on this title: www.amegroups.com
For more information, contact books@amegroups.com

First published in 2017
Printed in China by AME Publishing Company and Central South University Press

Zhimin Shao, Peter G. Cordeiro, Charels M. Balch
Breast Cancer (Hardcover)
ISBN: 978-988-77841-2-8
AME Publishing Company, Hong Kong
ISBN: 978-7-5487-3015-6
Central South University Press, Chang Sha

BREAST CANCER (FIRST EDITION)

Max Buttarelli
Department of Surgical Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Giuseppe Cancello
Division of Medical Senology, European Institute of Oncology, Milan, Italy

Monica Castiglione-Gertsch
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Federica Cattani
Division of Radiation Oncology, European Institute of Oncology, Milan, Italy

Emmanuelle Charafe-Jauffret
Department of Biopathology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Elisabeth Chereau
Department of Surgical Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Prakasit Chirappapha
Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Jaehee Choi
Molecular Imaging & Therapy Branch, Division of Convergence Technology, National Cancer Center, Goyang, Gyeonggi-do, Korea

Yongdoo Choi
Molecular Imaging & Therapy Branch, Division of Convergence Technology, National Cancer Center, Goyang, Gyeonggi-do, Korea

Alexandre Christinat
Institute of Oncology of Southern Switzerland (IOSI) and Breast Unit of Southern Switzerland (CSSI), Bellinzona, Switzerland

Monique Cohen
Department of Surgical Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Peter G. Cordeiro
Plastic and Reconstructive Surgery Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, USA

Anneleen Daemen
Department of Bioinformatics & Computational Biology, Genentech Inc., 1 DNA Way, South San Francisco, California 94080, USA

Haifa Dbouk
Breast Center of Excellence, Naef K. Basile Cancer Institute and Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Lucia Del Mastro
S.S. Sviluppo Terapie Innovative, IRCCS AOU San Martino- IST, Genova, Italy

Veronica Dell'Acqua
Division of Radiation Oncology, European Institute of Oncology, Milan, Italy

Serena Di Cosimo
Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Simona Di Lascio
Institute of Oncology of Southern Switzerland (IOSI) and Breast Unit of Southern Switzerland (CSSI), Bellinzona, Switzerland

Constantine Dimitrakakis
Department of Obstetrics and Gynaecology, Medical School, University of Athens, Athens, Greece

Meletios-Athanassios Dimopoulos
Department of Clinical Therapeutics, Alexandra Hospital, Medical School, University of Athens, Athens, Greece

Sophie Doisneau-Sixou
Breast Center, Department of Obstetrics & Gynecology, and Comprehensive Cancer Center, University of Munich, Munich, Germany; Faculty of Pharmacy, University Paul Sabatier Toulouse III, Toulouse, France

Angele Dubouloz
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Pierre-François Dupré
Department of Gynecology and Obstetrics, Regional University Hospital Morvan, 29 200 Brest, France

Nagi S. El Saghir
Breast Center of Excellence, Naef K. Basile Cancer Institute and Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Matthew J. Ellis
Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA

Pilar Eroles
Institute of Health Research INCLIVA, Av. Blasco Ibáñez 17.46010 Valencia, Spain

Odile Fargier-Bochaton
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Elisa Fiorito
Breast Cancer Research group, Nordic EMBL Partnership, Centre for Molecular Medicine Norway (NCMM), University of Oslo, P.O. 1137 Blindern, 0318, Oslo, Norway

Giusi Irma Forte
IBFM CNR - LATO, Contrada Pietrapollastra Pisciotto, 90015, Cefalù (PA), Italy

Simon G. Frank
Plastic and Reconstructive Surgery Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, USA

Viviana Galimberti
Division of Breast Surgery, European Institute of Oncology, Milan, Italy

Oreste Gentilini
Division of Breast Surgery, European Institute of Oncology, Milan, Italy

Axel Gerhardt
Department of Gynecology and Obstetrics, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

Frank A. Giordano
Department of Radiation Oncology, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

Mehra Golshan
Department of Surgery Brigham and Women's/Faulkner Hospital and Dana Farber Cancer Institute; Harvard Medical School: Boston, MA, USA

Jonathan A. Haas
Radiation Oncologist, Chief of Radiation Oncology, Winthrop-University Hospital, Mineola, New York, USA

Nadia Harbeck
Breast Center, Department of Obstetrics & Gynecology, and Comprehensive Cancer Center, University of Munich, Munich, Germany

Carsten Herskind
Department of Radiation Oncology, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

Gilles Houvenaeghel
Department of Surgical Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Antoni Hurtado
Breast Cancer Research group, Nordic EMBL Partnership, Centre for Molecular Medicine Norway (NCMM), University of Oslo, P.O. 1137 Blindern, 0318, Oslo, Norway; Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 Oslo, Norway

Mattia Intra
Division of Breast Surgery, European Institute of Oncology, Milan, Italy

Geraldine M. Jacobson
Departments of Radiation Oncology, West Virginia University, Robert C. Byrd Health Sciences Center, Morgantown, West Virginia 26506-9000, USA

Aurélie Jalaguier
Breast imaging Unit, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Camille Jauffret
Department of Surgical Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Virginia Kaklamani
Department of Medicine, Division of Hematology/Oncology, Feinberg School of Medicine of Northwestern University and the Robert H Lurie Comprehensive Cancer Center, 676 N St Clair St, Suite 850, Chicago, IL 606011, USA

Rasha Mohamed Kamal
Women's Imaging Unit, Cairo University, Egypt

Nermine S. Kamal
The Department of Clinical Oncology, Cairo University, Giza, Egypt

Stéphane Key
Department of Radiotherapy, Regional University Hospital Morvan, 29 200 Brest, France

Lana E. Khalil
Breast Center of Excellence, Naef K. Basile Cancer Institute and Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Katia E. Khoury
Breast Center of Excellence, Naef K. Basile Cancer Institute and Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Hyunjin Kim
Molecular Imaging & Therapy Branch, Division of Convergence Technology, National Cancer Center, Goyang, Gyeonggi-do, Korea

Youwanush Kongdan
Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Linetta B. Koppert
Erasmus MC Cancer Institute, Department of Surgical Oncology, Rotterdam, the Netherlands

Eric Lambaudie
Department of Surgical Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Matteo Lambertini
S.C. Oncologia Medica A, IRCCS AOU San Martino-IST, Genova, Italy

Lorenza Landi
Department of Medical Oncology, Istituto Toscano Tumori, Civil Hospital of Livorno, Livorno, Italy

Roberta Lazzari
Division of Radiation Oncology, European Institute of Oncology, Milan, Italy

Emmanuelle Le Fur
Department of Radiotherapy, Regional University Hospital Morvan, 29 200 Brest, France

Soo-Chin Lee
Department of Hematology-Oncology, National University Cancer Institute, National University Health System, Singapore; Cancer Science Institute, National University of Singapore, Singapore

Monchai Leesombatpaiboon
Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Maria Cristina Leonardi
Division of Radiation Oncology, European Institute of Oncology, Milan, Italy

Panuwat Lertsithichai
Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Alessia Levaggi
S.S. Sviluppo Terapie Innovative, IRCCS AOU San Martino- IST, Genova, Italy

Jennifer K. Litton
Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

Ana Elisa Lohmann
Breast Fellow, Department of Medical Oncology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada

Anne- Sophie Lucia
Department of Radiotherapy, Regional University Hospital Morvan, 29 200 Brest, France

Alberto Luini
Division of Breast Surgery, European Institute of Oncology, Milan, Italy

Cynthia X. Ma
Division of Oncology, Siteman Cancer Center, Washington University School of Medicine, St Louis, MO, USA

Marike C. Maijers
VUmc, Department of Plastic and Reconstructive Surgery, Amsterdam, the Netherlands

Patrick Maisonneuve
Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy

Rafaat A. Malak
The Department of Clinical Oncology, Cairo University, Giza, Egypt

Aidan T. Manning
Department of Surgery, University Hospital Waterford, Waterford, Ireland

Sahar Mahmoud Mansour
Women's Imaging Unit, Cairo University, Egypt

Maurie Markman
Cancer Treatment Centers of America, Philadelphia, PA, USA

Dimitrios Mavroudis
Department of Medical Oncology, University Hospital of Heraklion, University of Crete, Greece

Otto Metzger-Filho
Research Fellow, Breast Oncology Center, Dana-Farber Cancer Institute, Boston, USA

Anne-Marie Meyer
University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, USA; University of North Carolina at Chapel Hill, Cecil G Sheps Center for Health Services Research, USA

Petra Miglierini
Department of Radiotherapy, Regional University Hospital Morvan, 29 200 Brest, France

Mathieu Minsat
Department of Radiation Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Gabriele Minuti
Department of Medical Oncology, Istituto Toscano Tumori, Civil Hospital of Livorno, Livorno, Italy

Raymond Miralbell
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Omar Miranda
Department of Radiotherapy, Regional University Hospital Morvan, 29 200 Brest, France

Sindy Monnier
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Emilia Montagna
Division of Medical Senology, European Institute of Oncology, Milan, Italy

Filippo Montemurro
Unit of Investigative Clinical Oncology, Division of Medical Oncology, Fondazione del Piemonte per l'Oncologia, Candiolo Cancer Center (IRCCs), Candiolo 10060, Italy

Halle C.F. Moore
The Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA

Anna Morra
Division of Radiation Oncology, European Institute of Oncology, Milan, Italy

Tarek H. Mouhieddine
Breast Center of Excellence, Naef K. Basile Cancer Institute and Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Kimberly I. Muse
Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

Faina Nakhlis
Department of Surgery Brigham and Women's/Faulkner Hospital and Dana Farber Cancer Institute; Harvard Medical School: Boston, MA, USA

Valerie Nelson
Department of Medicine, Division of Hematology/Oncology, Feinberg School of Medicine of Northwestern University and the Robert H Lurie Comprehensive Cancer Center, 676 N St Clair St, Suite 850, Chicago, IL 606011, USA

Virginie Nepote
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Christian Neumaier
Department of Radiation Oncology, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

Philippe Nouet
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Rachel L. O'Connell
Royal Marsden NHS Foundation Trust, Sutton, UK

Roberto Orecchia
Division of Radiation Oncology, European Institute of Oncology, Milan, Italy; University of Milan, Italy

Samuel Ow
Department of Hematology-Oncology, National University Cancer Institute, National University Health System, Singapore

Olivia Pagani
Institute of Oncology of Southern Switzerland (IOSI) and Breast Unit of Southern Switzerland (CSSI), Bellinzona, Switzerland

Jessica F. Partin
Departments of Surgery, West Virginia University, Robert C. Byrd Health Sciences Center, Morgantown, West Virginia 26506-9000, USA

Ann H. Partridge
Dana-Farber Cancer Institute, Boston, MA, USA; Harvard Medical School, Boston, MA, USA

Patrick Petignat
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Ana Catarina Pinto
Medicine Department, Institut Jules Bordet, Université libre de Bruxelles, Brussels, Belgium; Br.E.A.S.T. Data Centre, Institut Jules Bordet, Brussels, Belgium

Francesca Poggio
S.S. Sviluppo Terapie Innovative, IRCCS AOU San Martino- IST, Genova, Italy

Martine Poncet
Breast imaging Unit, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Youri Popowski
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Olivier Pradier
Department of Radiotherapy, Regional University Hospital Morvan, 29 200 Brest, France

Jessica L. Profato
Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

Theodora Psaltopoulou
Department of Hygiene, Epidemiology and Medical Statistics, Medical School, University of Athens, Athens, Greece

Sarah Quillevere
Department of Radiotherapy, Regional University Hospital Morvan, 29 200 Brest, France

Alfred Rademaker
Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, 680 N Lakeshore Drive, suite 1200, Chicago, IL 60611, USA

Katherine Reeder-Hayes
University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, USA; University of North Carolina at Chapel Hill, Cecil G Sheps Center for Health Services Research, USA; University of North Carolina at Chapel Hill, School of Medicine, Department of Hematology and Oncology, USA

Michel Resbeut
Department of Radiation Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Shoshana M. Rosenberg
Dana-Farber Cancer Institute, Boston, MA, USA; Harvard School of Public Health, Boston, MA, USA

Nicole Rotmensz
Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy

Michel Rouzaud
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Alexandre Rozenholc
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Sandrine Rua
Department of Surgical Oncology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Jennifer E. Rusby
Royal Marsden NHS Foundation Trust, Sutton, UK

Virgilio S. Sacchini
Breast Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Kamal S. Saini
Scientific Co-Director, Breast International Group, Jules Bordet Institute, Brussels, Belgium

Lamiaa Adel Salah
Women's Imaging Unit, Cairo University, Egypt

Dorria Saleh Salem
Women's Imaging Unit, Cairo University, Egypt

Mohamad A. Salkeni
Departments of Internal Medicine, West Virginia University, Robert C. Byrd Health Sciences Center, Morgantown, West Virginia 26506-9000, USA

Jacqueline A. Shaw
Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester Royal In rmary, Leicester, LE2 7LX, UK

Justin Stebbing
Division of Cancer, ICTEM, Imperial College, Hammersmith Campus, London W12 0NN, UK

Thongchai Sukarayothin
Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Vera Jean Suman
Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA

Chairat Supsamutchai
Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Marc Sütterlin
Department of Gynecology and Obstetrics, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

Agnès Tallet
Department of Radiation Oncology, Institute Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Davis Torrejón
Department of Medical Oncology, Vall d'Hebron University Hospital, Barcelona, Spain

Carolien H.M. van Deurzen
Erasmus MC Cancer Institute, Department of Pathology, Rotterdam, the Netherlands

Victorien M.T. van Verschuer
Erasmus MC Cancer Institute, Department of Surgical Oncology, Rotterdam, the Netherlands

Hansjoerg Vees
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland

Marlon R. Veldwijk
Department of Radiation Oncology, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

Sandra S. Vermeulen
Radiation Oncology, Executive Director - Swedish Radiosurgery Center, Swedish Medical Center, Seattle Washington, USA

Paolo Veronesi
University of Milan, Italy; Division of Breast Surgery, European Institute of Oncology, Milan, Italy

Umberto Veronesi
Scientific Directorate, European Institute of Oncology, Milan, Italy

Giuseppe Viale
University of Milan, Italy; Division of Pathology, European Institute of Oncology, Milan, Italy

Vincent Vinh-Hung
Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland; Centre Hospitalier Universitaire de Martinique, Fort-de-France, Martinique, France

Sophocles H. Voineskos
Division of Plastic and Reconstructive Surgery, Department of Surgery, Surgical Outcomes Research Centre (SOURCE), McMaster University, Hamilton, Canada

Suparna B. Wedam
Medical Oncology Branch, Center for Cancer Research; National Clinical Target Validation Laboratory, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Frederik Wenz
Department of Radiation Oncology, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

Rasha Wessam
Women's Imaging Unit, Cairo University, Egypt

Stephanie B Wheeler
University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Health Policy and Management, USA; University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, USA; University of North Carolina at Chapel Hill, Cecil G Sheps Center for Health Services Research, USA

Ashley H. Woodson
Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

Sherry X. Yang
Medical Oncology Branch, Center for Cancer Research; National Clinical Target Validation Laboratory, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Flora Zagouri
Department of Clinical Therapeutics, Alexandra Hospital, Medical School, University of Athens, Athens, Greece; Comprehensive Cancer Center Vienna, Department of Medicine I/Division of Oncology, Medical University of Vienna, Austria

Stefano Zurrida
University of Milan, Italy; Division of Breast Surgery, European Institute of Oncology, Milan, Italy

Breast cancer is the most common malignancy in women. According to the American Cancer Society, breast cancer accounts for 30% of all female malignancies and 14% of female cancer deaths in the United States in 2017. While the prevalence of breast cancer is relatively low in China, it has kept rising in the past two decades. For many years breast cancer has ranked first among cancers diagnosed in women in large and medium cities in China. The number of Chinese women with this life-threatening condition is expected to rise from 1.6 million in 2015 to 2.5 million in 2021.

For nearly half a century, it has been gradually recognized that breast cancer is a systemic disease; as a result, clinical treatment of breast cancer has evolved from surgery alone to multidisciplinary management including surgery, chemotherapy, endocrine therapy, radiotherapy and targeted therapy; in particular, the surgical treatment has also transformed from complete radical resection of local malignancy to breast-conserving surgeries focusing on both treatment effectiveness and surgical trauma as well as other procedures (e.g. sentinel lymph node biopsy). During the same period, new developments in medical imaging, pathology, and molecular pathology also contribute to the earlier and more accurate diagnosis of breast cancer. Advances in diagnosis and treatment of breast cancer have dramatically improved the prognosis and quality of life of patients with this malignancy. New insights on the biological features of breast cancer in the 21st century, thanks to the development of molecular typing and other novel techniques, have brought the management of breast cancer from the era of evidence-based medicine to a more individualized and targeted practice - precision medicine.

Better management of breast cancer is based on the continuous improvements in clinical practices and translational research. Every year nearly 20,000 scientific articles on breast cancer are included in the PubMed database, constantly refreshing our understanding of this disease. In an era of Big Data, however, it is challenging for most clinicians to comprehensively and quickly grasp the forefront knowledge of breast cancer. In this book, therefore, we carefully collected a series of excellent review articles on breast cancer that cover the epidemiology, basic research, and clinical diagnosis and treatment of this malignancy by focusing on the hottest real-world clinical issues and the most promising concepts and theories that may substantially change the clinical practices in the coming decades.

Hopefully this book will provide the oncologists, researchers, and other interested readers a quick and reliable way to learn the cutting-edge information on the clinical practice and translational research of breast cancer. Also, we hope more similar works will be available to benefit the clinicians, researchers, and patients.

Keda Yu, Zhimin Shao
Department of Breast Surgery, Shanghai Cancer Center, Fudan University, China

Any book on breast cancer can become outdated in a very short period of time and the most up-to-date information therefore needs to compiled and rapidly disseminated in order for a work to be useful to the many diverse subspecialists in medicine and surgery who participate in the care of the breast cancer patient. This book is an amalgamation of chapters from experts around the world covering topics that range from the latest advances in the basic science of breast cancer, to every significant aspect of diagnosis and treatment of this disease. Within each of the sections are chapters that are broad and might cover general approaches and algorithms for therapy, while others might be highly focused on some of the most controversial and cutting-edge therapies. Within surgical treatment, my own specific area of expertise, for example, experts discuss the latest approaches to oncologic extirpation of breast cancer- anatomical approaches, highly aggressive versus minimal resection, breast reconstruction as well as one of the newest areas of surgical oncology that blends oncologic surgical principles with plastic surgery techniques—oncoplastic surgery. We hope that this very comprehensive compiliation of highly focused chapters will provide the practitioner with fresh new ideas and insights into the multidisciplinary approach to breast cancer.

Peter G. Cordeiro
Plastic and Reconstructive Service,
Memorial Sloan-Kettering Cancer Center,
1275 York Ave. New York,
N.Y. 10021, USA

Breast cancer is a very complex disease to understand and to treat. For physicians treating breast cancer around the world, the dramatic and rapid advances in breast cancer management, among all oncology specialties, has created an urgent need for up-to-date educational information that synthesizes the plethora of scientific publications in the world literature. Breast cancer has become a truly multimodality treatment, and the majority of breast cancer patients benefit from treatments from multiple specialties in various combinations and sequences. In addition, there are rapid advances in molecular and genetic diagnostics that are requiring physicians to adapt a multimodality treatment plan as "personalized therapy". Finally, there are chapters addressing important issues on breast cancer prevention interventions and "quality of life" issues such as breast reconstruction, premature menopause, and sexuality, fertility, and sleep disturbances. Written by global experts from North America, Europe, Asia, and the Middle East, this textbook is thus a valuable resource for all physicians and health care workers who manage the myriad of issues confronting the breast cancer patient.

This comprehensive textbook addresses the entire range of breast cancer from in situ disease to advanced metastatic disease. Chapter subjects on breast cancer include: epidemiology, Imaging, Surgical treatment, endocrine treatment, chemotherapy, targeted therapies, radiotherapy, genetic/molecular testing and prognostic factors. It includes excellent and practical chapters on molecular and genetic markers, reconstructive breast surgery, intraoperative radiation therapy, symptom management, survivorship issues, and challenging patient scenarios, such as breast cancer in young patients and those who are also pregnant.

Management of breast cancer is a global problem and the solutions are from global collaborations, not only in research (both clinical and translational) but also in educational collaborations where the "collective wisdom" of breast cancer experts from different specialties and from different nations can better present the practical "real world" application to the many presentations of breast cancer around the world.

Breast cancer is a major threat to health throughout the world. The Global Cancer Statistics (GLOBLCAN) published in February 2015 showed that there were around 1.67 million women worldwide suffering from breast cancer, more than 500,000 patients died of breast cancer, and the incidence and mortality all ranked first among cancers in women.

In the United States, breast cancer is the most common cancer among American women, except for skin cancers. About 1 in 8 (12%) women in the US will develop invasive breast cancer during their lifetime. Breast cancer is the second leading cause of cancer death in women, exceeded only by lung cancer. The American Cancer Society estimates that in the United States (for 2016), 246,660 new cases of invasive breast cancer will be diagnosed in women, 61,000 new cases of carcinoma in situ will be diagnosed, and 40,450 women will die from breast cancer. Death rates from breast cancer have been declining since about 1989, with larger decreases in women younger than 50. These decreases are believed to be the result of earlier detection through screening and increased awareness, as well as improved treatment. At this time there are more than 2.8 million breast cancer survivors in the United States.

In China, it has recently been estimated that 268,600 Chinese women developed breast cancer and 69,500 died of breast cancer in the year 2015 (Chen W. 2016). Breast cancer is the most commonly diagnosed cancer in China for women at ages 30 to 59 years, and breast cancer is the leading cause of cancer death in women younger than 45 years (Chen w. 2016).

We are grateful for this opportunity to publish these book chapters and thank the editors and publishers of AME Publishing Company for their outstanding job in bringing this comprehensive textbook to completion. We hope those who read these chapters will gain new insights about the similarities and differences in how we deliver breast cancer care. As we share more information and collaborate together on joint projects, the cancer patients will benefit wherever they live as they seek contemporary treatment in the breast cancer at all stages.

Charles M. Balch, MD, FACS
Professor of Surgery, Department of Surgical Oncology,
University of Texas MD Anderson Cancer Center, Houston, Texas, USA

The editors, Drs. Shao, Cordiero and Balch, have put together a comprehensive compendium ranging from basic research in breast cancer to translational science to innovations in clinical care. The genesis of this tome by the editors of AME publishing company has brought together an eclectic group of international scientists and clinicians with the intention of improving education and clinical care in the rapidly changing landscape of breast cancer research and treatment.

The book brings out the fundamental underpinning to breast cancer care today; that is the reliance on multidisciplinary care. The authors begin with the epidemiology of breast cancer focusing on incorporating genetics in prevention and treatment as well as ongoing work toward improving the plight and prognosis of younger patients and those who carry a genetic mutation. The authors take the opportunity to emphasize the importance of using randomized clinical trial patient outcomes to make decisions. Advances in breast tumor genomics and molecular markers for early detection of breast cancer and its recurrence as well as treatment stress the goal of personalized treatment for the breast cancer patient.

Breast imaging is covered only in terms of the role of MRI for screening in the very young high-risk patient and in those patients who are candidates for intraoperative irradiation. One chapter is devoted to the use of nanoparticles not only in imaging tumor in triple negative patients but in a treatment combination with photodynamic/chemotherapy.

The rest of the book is devoted principally to the multidisciplinary care of breast cancer. The latest techniques in oncoplastic and reconstructive surgery including in combination with radiotherapy are covered in detail with emphasis on their oncological safety. The latest adjuncts to hormonal therapy including m-tor inhibitors and bisphosphonates are covered. The newer techniques in intraoperative and partial breast irradiation (PBI) are covered in detail especially in light at how to handle the sentinel lymph node positive biopsy patient when using PBI. Unique chemotherapies especially in combination with targeted therapy are covered in detail with an emphasis on the young and HER-2-neu positive patients and those with metastatic brain metastases.

The book concludes with a section devoted to survivorship including fertility counseling and quality of life issues including premature menopause, sexual dysfunction, sleep disturbance, follow-up and rehabilitation.

The associate editors are grateful to the editors and colleagues that provided the substance of this book and patients that contributed to the research on which the data is based. Thanks to the publishers, production editors and staff for their careful attention to detail in the assembly of this book.

V. Suzanne Klimberg, MD, PhD
Courtney M. Townsend, Jr. MD. Distinguished Professor of Surgical Oncology,
The University of Texas Medical Branch, USA

One of the great challenges clinicians face is remaining up to date with new data that influences optimal care of our patients with breast cancer. The era of the Internet has allowed for the dissemination of information making it easily accessible, but the downside has been the posting of information that frequently undergoes no, or very little, vetting or editorial input. On the other hand, publishing of the classical textbook on a particular subject has been threatened by issues of timeliness and the competition provided by search engines on the Internet.

With those challenges in mind, AME Publishing Company commissioned the textbook "*Breast Cancer*" to capture all that is current in topics that span everything from epidemiology, basic research to clinical management and recent pivotal trial results. Rather than take the approach of a historically exhaustive review of every subject, the world-class investigators assembled to discuss each topic were charged with reviewing that which is new and placing it in context with current knowledge and standard of care practice. As a result, each chapter is short, providing only the most up to date new data that clinicians need to understand a rapidly changing field. Each chapter is supported by high quality figures and tables as appropriate and the key references guide readers to the original data.

"*Breast Cancer*" will be of value to any member of the multi-disciplinary team that cares for patients with breast cancer. As a clinician (surgeon, medical oncologist, radiation oncologist or plastic surgeon) cross discipline knowledge of advances is critical to optimizing a team approach to care of the breast cancer patient. Similarly, laboratory investigators will benefit from an understanding of the challenges that remain in improving outcome of patients regardless of stage of disease. Understanding some of the outstanding questions that face both clinicians and laboratory investigators can be mutually beneficial and inform the design of research questions that remain to be answered.

The faculty recruited to contribute to "*Breast Cancer*" have done an outstanding job of providing readers with the information that will enhance their knowledge and by extension improve the care of patients diagnosed with breast cancer.

William J. Gradishar, MD, FASCO, FACP
Betsy Bramsen Professor of Breast Oncology,
Interim Chief, Hematology/Oncology,
Robert H. Lurie Comprehensive Cancer Center,
Northwestern University Feinberg School of Medicine, Chicago, USA

Table of Contents

Surgical Treatment

Endocrine Therapy

Chemotherapy

Radiotherapy

Targeted Therapy

Nanoparticle Therapy

Prognosis of Breast Cancer

Epidemiology and prognosis of breast cancer in young women

Hussein A. Assi, Katia E. Khoury, Haifa Dbouk, Lana E. Khalil, Tarek H. Mouhieddine, Nagi S. El Saghir

Breast Center of Excellence, Naef K. Basile Cancer Institute and Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Correspondence to: Nagi S. El Saghir, MD, FACP, Professor of Clinical Medicine/Hematology-Oncology, Director. Breast Center of Excellence, Naef K. Basile Cancer Institute, American University of Beirut Medical Center, P.O. Box: 11-0236, Riad El Solh 1107 2020, Beirut, Lebanon. Email: nagi.saghir@aub.edu.lb.

Abstract: Breast cancer is the most common malignancy in women with 6.6% of cases diagnosed in young women below the age of 40. Despite variances in risk factors, Age Standardized Incidence Rates of breast cancer in young women vary little between different countries. Review of modifiable risk factors shows that long-term use of oral contraceptives, low body mass index (BMI) and high animal fat diet consumption are associated with increased risk of premenopausal breast cancer. Decreased physical activity and obesity increase risks of breast cancer in postmenopausal women, but data on premenopausal women rather shows that high BMI is associated with decreased risk of breast cancer. Non-modifiable risk factors such as family history and genetic mutations do account for increased risks of breast cancer in premenopausal women. Breast cancer in young women is associated with adverse pathological factors, including high grade tumors, hormone receptor negativity, and HER2 overexpression. This has a significant negative impact on the rate of local recurrence and overall survival. Moreover, younger women often tend to present with breast cancer at a later stage than their older counterparts, which further explains worse outcome. Despite these factors, age per se is still being advocated as an independent role player in the prognosis. This entails more aggressive treatment modalities and the need for closer monitoring and follow-up.

Keywords: Breast cancer; young age; epidemiology; prognosis

Submitted May 09, 2013. Accepted for publication May 25, 2013.
doi: 10.3978/j.issn.2072-1439.2013.05.24
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.05.24

Introduction

Breast cancer is the most common non-cutaneous malignancy, accounting for nearly one in three cancers diagnosed among women in the United States, and the second leading cause of cancer death around the world (1,2). Around 6.6% of all breast cancer cases are diagnosed in women less than 40 of age, 2.4% in women less than 35, and 0.65% in women less than 30 (3,4); if plotted on a curve, the cumulative incidence of breast cancer seems to follows an exponential function below the age of 40 after which it seems to rise linearly (3). The overall worldwide burden of breast cancer has doubled between 1975 and 2000, and this is thought to be attributable to the increasing life expectancy and widespread adoption of westernized lifestyle with all its risk factors (5). However, these trends are not seen in early onset breast cancer, as the rates have been more or less stable in most countries in the past 20 years (6). As for death rates, they have been steadily decreasing, especially in younger women, owing to the improved treatment and early detection (7); however, breast cancer in young women remains a great challenge to patients, families and health care providers. Although the diagnosis of breast cancer is much less common in women under the age of 40 years, it can have a greater impact than in older counterparts, as it tends to present at a later stage, be more aggressive and have a poorer prognosis (8,9).

Many studies have suggested that age is an independent prognostic factor; however, this issue is now considered controversial. Breast cancer in young women is more likely to be of a more aggressive subtype, such as triple negative

Table 1 ASR (Age Standardized Incidence Rates per 100,000 women per year) and Cumulative Risk (Cum Risk) of Breast Cancer in selected countries [Adapted from GLOBOCAN 2008 Reference (10)].

Country	Age <40		All ages	
	ASR	Cum Risk (%)	ASR	Cum Risk (%)
Italy	13.2	0.6	86.3	9.19
France	12.8	0.59	99.7	10.74
UK	11	0.5	89.1	9.49
Lebanon	9.9	0.45	55.4	5.81
US	9.8	0.45	76	8.26
Argentina	9	0.41	74	7.76
Nigeria	8.8	0.4	38.7	4.05
Japan	7.4	0.34	42.7	4.38
Egypt	7	0.32	37.3	3.65
Brazil	6.3	0.29	42.3	4.51
Turkey	5.8	0.26	28.3	2.94
Russia	5.3	0.24	43.2	4.78
China	4.4	0.2	21.6	2.24

or HER2-positive breast cancer, and is more likely to present at an advanced stage, either because of its biological aggressive subtype or because of a low index of suspicion and delayed diagnosis. This may translate into more loco-regional recurrences and distant metastases, which contributes to the poorer outcome of young women with breast cancer.

In this article, we will review epidemiology and differences between various populations and regions of the world, as well as prognosis and outcome of young women with breast cancer.

Epidemiology

According to GLOBOCAN-generated data of 2008, more than 146,660 new cases of breast cancer have been diagnosed in women less than 40 years of age worldwide, with an age-standardized rate per 100,000 (ASR) of 6 (10). Early onset breast cancer trends vary among populations and areas of the world. Although 77% of the cases occurred in developing countries, the ASR for women below the age of 40 was marginally higher in developed countries (8.8 *vs.* 5.4) (10). Overall, GLOBOCAN-generated rates of breast cancer in women less than 40 years in different countries have shown relatively stable annual rates around the world, ranging from an ASR of 1.1 to 17. This is in contrast to the overall breast cancer population rates, which vary from 8 to 109 (10). The lowest rates come from countries in Eastern and Southern Africa, while the highest rates are recorded in Europe and North America. Rates of breast cancer below and above 40 years from selected countries are presented in *Table 1*. These differences are less likely related to screening practices, since screening recommendation is not offered before the age of 40, nor to the use of HRT, since patients are premenopausal (6). It is important to note that not all countries have sufficient data and statistics on cancer rates. Most data come from high-income industrialized countries and tend to be the most accurate, precise, and up-to-date. In the USA, the Surveillance, Epidemiology and End Results (SEER) program is a principal source for cancer statistics in the country, and extensive analysis of these data are periodically published in the literature. Pertaining to our topic, SEER data between 1988 and 2003 showed an incidence of breast cancer below the age of 40 of 6.4% (15,548 patients) out of the total breast cancer population in that period (243,012 patients) (11). In addition to published data from many countries, GLOBOCAN includes other countries with lacking data by making extrapolations of old statistics or nearby population statistics (1).

Risk factors

Differences exist between populations that are not predictive of early onset breast cancer, such as fertility rates, which vary from 1.4 in Japan, to 2.1 in USA, to 5.3 in Nigeria, to 2.9 in Egypt (12). These countries have close cumulative risk rates of early onset breast cancer (0.34 in Japan, 0.45 in USA, 0.4 in Nigeria, and 0.32 in Egypt) despite varying fertility rates (10). Early onset breast cancer does not seem to be directly related to westernization or standard of living, where a weak correlation is found between country income level and early onset breast cancer (6). Genetic factors may play a role in affecting rates of early onset breast cancer in different areas, though their role cannot by itself account for international variation in risk. In the UK, approximately 3% of all breast cancers are attributable to mutations in *BRCA1* or *BRCA2* (13), whereas this number increases in Ashkenazi Jews to up to 40% (14). *TP53* mutation, although vary rare, is the causative agent of breast cancer in Li-Fraumeni syndrome, which tends to affect women between 20 and 40 years of age (15). Some populations such as in Southern Brazil have relatively high mutation frequency of *TP53* mutation, reaching one in 300 women

(16,17). Hormonal factors also vary in different populations, races, and ethnicities. In a study by Lund *et al.* (18), in Atlanta, USA, incidence rates of triple-negative tumors differed by race, with an incidence of 36.3 per 100,000 for black women, and 19.4 per 100,000 for white women.

Environmental factors

Nevertheless, most of the variation in risk is believed to be due to differential environmental exposure to certain risk factors. Studies of migrants further emphasize this hypothesis; incidence of cancers tend to rise following migration from low to high incidence countries, especially if it occurs early in life (19). Many risk factors for breast cancer have been well-established by case-control and cohort studies. However, there have been few efforts to quantify the magnitude of risk disparities between countries that might be explained by such factors.

The role of risk factors in early-onset breast cancer is even less clear. Studies involving this category of breast cancer patients are usually hindered by small sample sizes (6). Moreover, factors such as intrauterine exposures would be of utmost difficulty to follow in cohort studies. Nevertheless, case-control studies have shown that birth weight, growth rate in childhood, and attained height are all risk factors for premenopausal breast cancer (20,21). It has been postulated that prenatal influences, including hormones and growth factors, may alter the risk of breast cancer, but such correlations would be very difficult to measure (22).

Exercise, diet and obesity

Although many studies showed a favorable outcome of exercise regarding breast cancer risk (23), some studies failed to show it (24,25). In a prospective study of 64,777 premenopausal women, physical activity was associated with a 23% breast cancer risk reduction. However, in a prospective study involving 218,169 subjects in 9 European countries published in 2007, exercise was not found to be associated with early breast cancer risk (25). Overall, a systematic review of 76 studies on this topic reported that 53% of studies confirmed a significant protective effect, 37% reported a non-significant risk reduction, and only 10% failed to show a correlation (26). With regards to diet, results of observational studies were inconsistent. While several observational studies of fruit and vegetable consumption did not show any benefit in reducing breast cancer risk in both premenopausal and postmenopausal women (27), the Nurses' Health study showed nearly a 50% greater risk of breast cancer in premenopausal women who consume a high animal fat diet, but not with women on high vegetable fat diet (28). Moreover, experimental and human data shows that Mediterranean diet rich in extra virgin oil is associated with reduced risks of breast cancer (29). As for the effect of high BMI, it seems to have opposite effects in pre- and postmenopausal women. Obesity is known to increase the risk of breast cancer in postmenopausal women, probably due to the increase in estrogen exposure caused by aromatization in fatty tissues (30). In contrast, a high BMI seems to be protective in the premenopausal group (30), for reasons which are still unknown.

Female hormone exposure

In an analysis done by the Collaborative Group on Hormonal Factors in Breast Cancer (31) on 53,297 women with breast cancer and 100,239 women without breast cancer from 54 studies conducted in 25 countries, the risk of breast cancer was increased in current oral contraceptive users as well as women within 10 years of OCP stoppage. However, the increased risk is very small, being the greatest at 1.24 (95% CI, 1.15-1.33) in current OCP users (31). Regarding other reproductive risk factors, a review of 26 articles showed various degrees of effect on breast cancer risks (32). For each additional year at menarche, a decrease of about 9% of breast cancer risk was found in premenopausal women, and of about 4% when diagnosed at older age (32). Breast cancer risk increased with increasing age at first full term pregnancy (FFTP) by 5% per year for breast cancer diagnosed before menopause versus 3% for breast cancers diagnosed after menopause (32). There was a 3% reduction in premenopausal breast cancer risk for each full term pregnancy, whereas the reduction reached 12% in postmenopausal women (32). Every 12 months of breast-feeding decreased the risk by 4.3% in both premenopausal and postmenopausal women (33).

Genetic mutations

In addition to the above risk factors, it has been postulated that the set of molecular and genetic characteristics of breast carcinomas that arise in younger women, such as BRCA mutations, may be different from that of older women, much like the variance between different populations described above. In Britain, the proportion of breast cancer patients with BRCA1 or BRCA2

mutations is higher in women less than 36 years of age compared to the whole breast cancer population group (6% *vs.* 3%) (13,34).

Prognosis

Based on various prospective and retrospective studies performed in the last two decades, it has been generally accepted that young age at diagnosis correlates with a worse clinical outcome compared to their older counterparts (3,35-40). This holds true irrespective of menopausal status, as age is still a risk factor among premenopausal women (41). In addition, breast cancer survival rates are comparatively lower for women less than 40 years of age than for older women across all histological subtypes and stages (3). However, the controversy lies in the question of whether age per se is an independent risk factor for worse prognosis. Many studies have refuted this hypothesis; they rather propose that the effect of young age on outcome is merely a reflection of over-representation of other known prognostic pathological factors, such as higher grade of differentiation, presence of lymphovascular invasion, higher mitotic rate, lower ER/PR expression, and higher HER2 expression (42-45). Yet other studies have attributed the inferior outcome of young age to the more advanced presentation at diagnosis, including higher rates of axillary lymph node positivity and larger tumor size (39,46-48). Others have postulated that the effect of differential gene expression between different age groups might play a role (49,50). In any case, knowing the true impact of age on prognosis may have an effect on our management. If it is indeed an independent factor, then young women might benefit from more aggressive treatment than their older counterparts with the same clinical and pathological scenario.

Breast cancer subtypes

It is well established that there are at least 4 main subtypes of breast cancer based on different patterns of gene expression, and that they have a considerable impact on prognosis (51,52). Luminal A includes ER+ and/or PR+ and HER2-, grade 1 or 2 tumors, and they tend to have the best prognosis (52). Luminal B comprises ER+ and/or PR+ and HER2+, or ER+ and/or PR+ and HER2-, grade 3 tumors. The other 2 subtypes are the HER2 overexpressing tumors (ER-, PR-, HER2+) and the triple negative tumors (ER-, PR-, HER-), both of which confer bad prognosis (51). Many studies have confirmed the increased proportion of ER/PR-negativity, HER2 overexpression, and high grade in young women with breast cancer (50,53). Therefore, this could partly account for the worse outcome of young age. A study of 399 breast cancer patients below 40 years by Collins *et al.* (53) revealed a lower proportion of luminal A disease (33% *vs.* 60-70%) and higher proportion of luminal B disease (35% *vs.* 6-22%) compared to numbers from population studies of breast cancer (53-57). Fifty-five percent of patients had high grade tumors, and 31% of all tumors overexpressed HER2 (53), which is high compared to the 12.6% presented in a study of 1,842 breast cancer patients in Atlanta by Lund *et al.* (18). Triple negative tumors have also been found to be over-represented in young women with breast cancer, with rates close to 26% (58). To further confirm the above clinical findings, Anders *et al.* (50) studied the mRNA expression of ERα, ERβ, PR, and HER2 in 200 patients ≤45 years and 211 patients ≥65 years. Young patients had a lower expression of ERα (7.2 *vs.* 9.8, respectively; P=0.0001), ERβ (5.6 *vs.* 5.9, respectively; P=0.02), and PR (4.1 *vs.* 5.0, respectively; P=0.001) compared to their older counterparts. As for HER2 expression, it was statistically higher in the age group ≤45 years compared to the age group ≥65 years (11.1 *vs.* 9.4, respectively; P=0.0001).

Advanced stage at presentation

Several studies raised the notion that young breast cancer patients tend to present with more advanced stages than older women (39,46-48). A retrospective cohort from Denmark of 10,356 women diagnosed before 50 years reported that patients aged ≤35 years at diagnosis were at higher risk of being node positive (51% *vs.* 46%; P=0.02) compared with patients between 35 and 50 years (47). A study of 732 non-metastatic breast cancer patients from Mount Sinai Medical Center, New York showed that patients younger than 36 years had larger tumors (median 2.0 *vs.* 1.5 cm, P<0.001), more nodal involvement (50% *vs.* 37%, P=0.022), and were more likely to be diagnosed with stage II or III cancer (60% *vs.* 43%, P<0.001) than patients above 36 years (48).

Genetic mutations, gene microarrays

Another reason for young patients presenting with more aggressive tumors is the higher proportion of BRCA1 and BRCA2 mutations (34,59), which are known to be associated with higher histological grade, higher proliferation rate and ER negativity (60). Other genetic variances have also been

studied. According to Dubsky *et al.* (41), p53 mutation, c-erbB-2 over expression and tumor proliferation markers are associated with a young age and an increase in local recurrence probability, and thus more aggressive tumors. In 2008, Andres *et al.* (50) identified 367 gene sets that may make a distinction between breast tumors in young women from those in older women, which may have an impact on prognosis. Moreover, a recent study by Azim *et al.* (49) assessed the differential role of proliferation, stroma, and immune-related gene signatures in providing prognostic information in different breast cancer subtypes. They further confirmed the age-dependent differential expression of genes associated with immature mammary cell populations (RANKL, c-kit, BRCA1-mutated phenotype, mammary stem cells, and luminal progenitors cells), and growth factor signaling [mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-related].

Based on the aforementioned adverse pathological and possible genetic characteristics present in young breast cancer patients, one could explain the poor outcome in this patient population. However, many studies showed that even after accounting for these factors, young age per se seems to act independently in affecting prognosis.

Risks of local recurrence after primary therapy

Risk of local recurrence of breast cancer has been shown to be increased in young patients in two separate analyses of clinical trials, namely the EORTC group trials and NSABP group trials (61,62). The former showed a hazard ratio of 2.8 (95% CI, 1.4-5.6) for local recurrence in patients less than 35 years compared to those above 50 years. A study by Bharat *et al.* (36) estimated the risk of breast cancer recurrence for women diagnosed below the age of 40 to be 1.53 (95% CI, 1.37-1.74) times higher than in those diagnosed above 40 years. Voogd *et al.* (63) combined data on stage I and II breast cancer patients from 2 large clinical trials (EORTC and DBCG). They reported a 9.2-fold (95% CI, 3.7-23) higher risk of local recurrence in women aged 35 who underwent breast conserving surgery compared to women 65 years and above. As for distant recurrence, the risk was doubled (95% CI, 1.26-3.96) in the young patient group compared to the older patients (63). If we look at studies examining rates of contralateral breast cancer (CBC) risks, we can deduce that young age is a quite a strong risk factor (64,65). Although the absolute risk of CBC is similar between different age groups, the relative risk increase in younger populations is quite substantial, since the initial risk of breast cancer in young women is low compared their older counterparts (6,64).

Survival

Young age has also been shown to negatively affect survival. A large prospective cohort of 2,956 breast cancer patients less than 40 years diagnosed between 2000-2008 in 126 UK hospitals reported a 5-year overall survival of 82% (66). This is relatively low considering the fact that only 2.5% of patients had metastasis at presentation. Many studies compared data from young women to their older counterparts. Data from 1,398 patients analyzed by Nixon *et al.* (67) showed that young age (<35 *vs.* >35) remained an important predictor of mortality after adjusting for confounding variables, with a relative risk of 1.50. At the Institut Curie in France, it was shown that even after adjusting for clinical tumor size, node status, histological grade, hormone receptor, locoregional treatment procedure and adjuvant systemic therapy, both overall survival and disease-free survival continued to be lower in the younger age group (38). Another study by Gnerlich *et al.* (11) reviewed 243,012 breast cancer patients in the SEER database from 1988-2003. Young women less than 40 years had a higher breast cancer mortality rate (18.3% *vs.* 12.1%, P=0.001) than those older than 40 years. If adjusted for other prognostic factors and stratify by stage, younger women were more likely to die from breast cancer compared with older women if diagnosed with stage I (HR=1.44; 95% CI, 1.27 to 1.64) or stage II (HR=1.09; 95% CI, 1.03 to 1.15) disease; however, age lost its prognostic value in more advanced disease (11). A similar trend was also seen in a study of 185 patients less than 30 years in MD Anderson Cancer Center (68).

Conclusions

Age at diagnosis remains an important factor for prognostication and treatment decisions in patients with breast cancer. Although, breast cancer in women below 40 years of age constitutes a small proportion of the total incidence, it has a significant burden on women and society. Incidence rates and cumulative risk rates in women below 40 years vary little between populations, but generally remain low and do not justify screening in average risk women. Risk factors in breast cancer do not necessarily have the same effect in young and older patients. While a high BMI seems to have a protective effect against

development of breast cancer in premenopausal women, controversy still surrounds the influence of diet and physical activity in this population. Breast cancer in young women is associated with a poorer outcome, partly because of over-representation of more aggressive subtypes, such as triple negative or HER2-positive breast cancer. In addition, they are more likely to present at an advanced stage or have a delayed diagnosis because of a low index of suspicion by the patient and the primary physician. These factors predispose to more loco-regional recurrences and distant metastases which contribute to the poorer outcome of young women with breast cancer. Many studies have shown a worse prognosis even after controlling for pathological factors and staging. However, the discovery of more prognostic markers and factors might weaken the correlation between age and outcome. Management of young women with breast cancer still requires particular attention to surgical negative margins, long term follow-up after breast-conserving therapy, more aggressive adjuvant therapy because of poorly differentiated histologies, receptor negative and/or HER2-positive tumors, or poor gene signatures, and to improvement of access to care worldwide.

Review criteria

The articles that were reviewed for this manuscript were based on a keyword search using PubMed 1975 to present (April 2013), searching the title containing the word string "breast cancer" AND ("young" OR "age"). Only articles published in English were considered. All titles were reviewed but only those articles that were considered to be the most relevant to our topic were included in the review. Additional pertinent articles were included if these were deemed to be relevant by the author.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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. DeSantis C, Siegel R, Bandi P, et al. Breast cancer statistics, 2011. CA Cancer J Clin 2011;61:409-18.
3. Anders CK, Johnson R, Litton J, et al. Breast cancer before age 40 years. Semin Oncol 2009;36:237-49.
4. Fredholm H, Eaker S, Frisell J, et al. Breast cancer in young women: poor survival despite intensive treatment. PLoS One 2009;4:e7695.
5. Boyle P, Howell A. The globalisation of breast cancer. Breast Cancer Res 2010;12 Suppl 4:S7.
6. Narod SA. Breast cancer in young women. Nat Rev Clin Oncol 2012;9:460-70.
7. Ferguson NL, Bell J, Heidel R, et al. Prognostic value of breast cancer subtypes, Ki-67 proliferation index, age, and pathologic tumor characteristics on breast cancer survival in Caucasian women. Breast J 2013;19:22-30.
8. Han W, Kim SW, Park IA, et al. Young age: an independent risk factor for disease-free survival in women with operable breast cancer. BMC Cancer 2004;4:82.
9. Brennan M, French J, Houssami N, et al. Breast cancer in young women. Aust Fam Physician 2005;34:851-5.
10. Ferlay J, Shin H, 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 April 5, 2013.
11. Gnerlich JL, Deshpande AD, Jeffe DB, et al. Elevated breast cancer mortality in women younger than age 40 years compared with older women is attributed to poorer survival in early-stage disease. J Am Coll Surg 2009;208:341-7.
12. The World Factbook 2013-14. Washington, DC: Central Intelligence Agency, 2013. Available online: https://www.cia.gov/library/publications/the-world-factbook/index.html, Accessed March 18, 2013.
13. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. Br J Cancer 2000;83:1301-8.
14. Warner E, Foulkes W, Goodwin P, et al. Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. J Natl Cancer Inst 1999;91:1241-7.
15. Nichols KE, Malkin D, Garber JE, et al. Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. Cancer Epidemiol Biomarkers Prev 2001;10:83-7.
16. Palmero EI, Schüler-Faccini L, Caleffi M, et al. Detection of R337H, a germline TP53 mutation predisposing to

multiple cancers, in asymptomatic women participating in a breast cancer screening program in Southern Brazil. Cancer Lett 2008;261:21-5.
17. Gomes MC, Kotsopoulos J, de Almeida GL, et al. The R337H mutation in TP53 and breast cancer in Brazil. Hered Cancer Clin Pract 2012;10:3.
18. Lund MJ, Butler EN, Hair BY, et al. Age/race differences in HER2 testing and in incidence rates for breast cancer triple subtypes: a population-based study and first report. Cancer 2010;116:2549-59.
19. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
20. Ahlgren M, Sørensen T, Wohlfahrt J, et al. Birth weight and risk of breast cancer in a cohort of 106,504 women. Int J Cancer 2003;107:997-1000.
21. Ahlgren M, Melbye M, Wohlfahrt J, et al. Growth patterns and the risk of breast cancer in women. N Engl J Med 2004;351:1619-26.
22. Trichopoulos D, Adami HO, Ekbom A, et al. Early life events and conditions and breast cancer risk: from epidemiology to etiology. Int J Cancer 2008;122:481-5.
23. Maruti SS, Willett WC, Feskanich D, et al. A prospective study of age-specific physical activity and premenopausal breast cancer. J Natl Cancer Inst 2008;100:728-37.
24. Lynch BM, Courneya KS, Friedenreich CM. A case-control study of lifetime occupational sitting and likelihood of breast cancer. Cancer Causes Control 2013;24:1257-62.
25. Lahmann PH, Friedenreich C, Schuit AJ, et al. Physical activity and breast cancer risk: the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev 2007;16:36-42.
26. Loprinzi PD, Cardinal BJ, Winters-Stone K, et al. Physical activity and the risk of breast cancer recurrence: a literature review. Oncol Nurs Forum 2012;39:269-74.
27. van Gils CH, Peeters PH, Bueno-de-Mesquita HB, et al. Consumption of vegetables and fruits and risk of breast cancer. JAMA 2005;293:183-93.
28. Cho E, Spiegelman D, Hunter DJ, et al. Premenopausal fat intake and risk of breast cancer. J Natl Cancer Inst 2003;95:1079-85.
29. Escrich E, Moral R, Solanas M. Olive oil, an essential component of the Mediterranean diet, and breast cancer. Public Health Nutr 2011;14:2323-32.
30. Carmichael AR, Bates T. Obesity and breast cancer: a review of the literature. Breast 2004;13:85-92.
31. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Collaborative Group on Hormonal Factors in Breast Cancer. Lancet 1996;347:1713-27.
32. Clavel-Chapelon F, Gerber M. Reproductive factors and breast cancer risk. Do they differ according to age at diagnosis? Breast Cancer Res Treat 2002;72:107-15.
33. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet 2002;360:187-95.
34. Peto J, Collins N, Barfoot R, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst 1999;91:943-9.
35. El Saghir NS, Seoud M, Khalil MK, et al. Effects of young age at presentation on survival in breast cancer. BMC Cancer 2006;6:194.
36. Bharat A, Aft RL, Gao F, et al. Patient and tumor characteristics associated with increased mortality in young women (< or =40 years) with breast cancer. J Surg Oncol 2009;100:248-51.
37. Arvold ND, Taghian AG, Niemierko A, et al. Age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy. J Clin Oncol 2011;29:3885-91.
38. de la Rochefordiere A, Asselain B, Campana F, et al. Age as prognostic factor in premenopausal breast carcinoma. Lancet 1993;341:1039-43.
39. Winchester DP, Osteen RT, Menck HR. The National Cancer Data Base report on breast carcinoma characteristics and outcome in relation to age. Cancer 1996;78:1838-43.
40. Adami HO, Malker B, Holmberg L, et al. The relation between survival and age at diagnosis in breast cancer. N Engl J Med 1986;315:559-63.
41. Dubsky PC, Gnant MF, Taucher S, et al. Young age as an independent adverse prognostic factor in premenopausal patients with breast cancer. Clin Breast Cancer 2002;3:65-72.
42. Anders CK, Fan C, Parker JS, et al. Breast carcinomas arising at a young age: unique biology or a surrogate for aggressive intrinsic subtypes? J Clin Oncol 2011;29:e18-20.
43. Crowe JP Jr, Gordon NH, Shenk RR, et al. Age does not predict breast cancer outcome. Arch Surg 1994;129:483-7; discussion 487-8.
44. Ezzat A, Raja MA, Zwaan F, et al. The lack of age as a significant prognostic factor in non-metastatic breast cancer. Eur J Surg Oncol 1998;24:23-7.
45. Figueiredo JC, Ennis M, Knight JA, et al. Influence

of young age at diagnosis and family history of breast or ovarian cancer on breast cancer outcomes in a population-based cohort study. Breast Cancer Res Treat 2007;105:69-80.
46. Albain KS, Allred DC, Clark GM. Breast cancer outcome and predictors of outcome: are there age differentials? J Natl Cancer Inst Monogr 1994;:35-42.
47. Kroman N, Jensen MB, Wohlfahrt J, et al. Factors influencing the effect of age on prognosis in breast cancer: population based study. BMJ 2000;320:474-8.
48. Gajdos C, Tartter PI, Bleiweiss IJ, et al. Stage 0 to stage III breast cancer in young women. J Am Coll Surg 2000;190:523-9.
49. Azim HA Jr, Michiels S, Bedard PL, et al. Elucidating prognosis and biology of breast cancer arising in young women using gene expression profiling. Clin Cancer Res 2012;18:1341-51.
50. Anders CK, Hsu DS, Broadwater G, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. J Clin Oncol 2008;26:3324-30.
51. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-74.
52. Sotiriou C, Neo SY, McShane LM, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci U S A 2003;100:10393-8.
53. Collins LC, Marotti JD, Gelber S, et al. Pathologic features and molecular phenotype by patient age in a large cohort of young women with breast cancer. Breast Cancer Res Treat 2012;131:1061-6.
54. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006;295:2492-502.
55. Tamimi RM, Baer HJ, Marotti J, et al. Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. Breast Cancer Res 2008;10:R67.
56. Yang XR, Sherman ME, Rimm DL, et al. Differences in risk factors for breast cancer molecular subtypes in a population-based study. Cancer Epidemiol Biomarkers Prev 2007;16:439-43.
57. Caldarella A, Crocetti E, Bianchi S, et al. Female breast cancer status according to ER, PR and HER2 expression: a population based analysis. Pathol Oncol Res 2011;17:753-8.
58. Carvalho FM, Bacchi LM, Santos PP, et al. Triple-negative breast carcinomas are a heterogeneous entity that differs between young and old patients. Clinics (Sao Paulo) 2010;65:1033-6.
59. Malone KE, Daling JR, Neal C, et al. Frequency of BRCA1/BRCA2 mutations in a population-based sample of young breast carcinoma cases. Cancer 2000;88:1393-402.
60. Colleoni M, Rotmensz N, Robertson C, et al. Very young women (<35 years) with operable breast cancer: features of disease at presentation. Ann Oncol 2002;13:273-9.
61. de Bock GH, van der Hage JA, Putter H, et al. Isolated loco-regional recurrence of breast cancer is more common in young patients and following breast conserving therapy: long-term results of European Organisation for Research and Treatment of Cancer studies. Eur J Cancer 2006;42:351-6.
62. Wapnir IL, Anderson SJ, Mamounas EP, et al. Prognosis after ipsilateral breast tumor recurrence and locoregional recurrences in five National Surgical Adjuvant Breast and Bowel Project node-positive adjuvant breast cancer trials. J Clin Oncol 2006;24:2028-37.
63. Voogd AC, Nielsen M, Peterse JL, et al. Differences in risk factors for local and distant recurrence after breast-conserving therapy or mastectomy for stage I and II breast cancer: pooled results of two large European randomized trials. J Clin Oncol 2001;19:1688-97.
64. Hartman M, Czene K, Reilly M, et al. Genetic implications of bilateral breast cancer: a population based cohort study. Lancet Oncol 2005;6:377-82.
65. Kollias J, Ellis IO, Elston CW, et al. Clinical and histological predictors of contralateral breast cancer. Eur J Surg Oncol 1999;25:584-9.
66. Eccles B, Copson E, Maishman T, et al. Breast cancer diagnosis and treatment in women aged 18-40 years in the UK: Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH). Liverpool, UK: In: 8th NCRI Cancer Conference, 2012.
67. Nixon AJ, Neuberg D, Hayes DF, et al. Relationship of patient age to pathologic features of the tumor and prognosis for patients with stage I or II breast cancer. J Clin Oncol 1994;12:888-94.
68. Xiong Q, Valero V, Kau V, et al. Female patients with breast carcinoma age 30 years and younger have a poor prognosis: the M.D. Anderson Cancer Center experience. Cancer 2001;92:2523-8.

Cite this article as: Assi HA, Khoury KE, Dbouk H, Khalil LE, Mouhieddine TH, El Saghir NS. Epidemiology and prognosis of breast cancer in young women. J Thorac Dis 2013;5(S1):S2-S8. doi: 10.3978/j.issn.2072-1439.2013.05.24

Lack of patient-reported outcomes assessment in phase III breast cancer studies: a missed opportunity for informed decision making

Victoria S. Blinder

Health Outcomes Research Group, Center for Health Policy and Outcomes, Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

Correspondence to: Victoria Blinder, M.D., M.Sc., Assistant Attending Physician. Health Outcomes Research Group, Center for Health Policy and Outcomes, Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, 300 East 66th Street, 14th Floor, New York, NY 10065, USA. Email: blinderv@mskcc.org.

Abstract: A phase III study comparing capecitabine monotherapy to combination treatment with capecitabine and sunitinib in patients with metastatic breast cancer failed to demonstrate a benefit in terms of progression-free or overall survival. Both regimens were reasonably well tolerated with some differences noted in the specific toxicity profiles. However, the study failed to incorporate an assessment of patient-reported outcomes (PROs) such as self-reported pain, quality of life, or employment outcomes. This is a missed opportunity. If more clinical trials included such measures, they would provide valuable information to patients and clinicians choosing from a wide array of available and otherwise similarly effective systemic therapies for metastatic breast cancer.

Keywords: Breast cancer; oral chemotherapy; patient reported outcomes (PROs); employment; palliative treatment

Submitted Nov 11, 2013. Accepted for publication Nov 13, 2013.
doi: 10.3978/j.issn.2224-5820.2013.11.01
View this article at: http://www.amepc.org/apm/article/view/3252/4130

Therapy for metastatic breast cancer is, by definition, given with palliative intent. As oncologists, our goal is to prolong life while also minimizing cancer- and treatment-related symptoms. While some recent therapeutic advances have led to longer survival times for our patients, many unfortunately have not. In this setting, survivorship issues, such as quality of life and employment outcomes, can be determinant.

Although adherence can be a concern, an orally administered regimen is associated with obvious advantages in terms of convenience and ease of administration, particularly for patients with triple-negative breast cancer for whom chemotherapy is the only treatment option. However, few such oral regimens exist. The most widely used oral chemotherapy agent in the U.S. is capecitabine. It is active and reasonably well tolerated as demonstrated in trials administering it as a single agent or paired with parenteral chemotherapy or other targeted agents. Combining it with another oral therapy to attempt to increase the associated survival benefit could also result in significant improvements in quality of life and related outcomes for patients with metastatic breast cancer.

Crown *et al.* recently published a phase III study of patients with pretreated metastatic breast cancer in the *Journal of Clinical Oncology*, comparing treatment with single-agent capecitabine to treatment with combination capecitabine and sunitinib, an oral tyrosine kinase inhibitor with broad targeting including angiogenesis that is approved for treatment of other malignancies, such as renal cell carcinoma (1). The primary outcome of the study was progression-free survival (PFS). Study participants had previously been treated with both an anthracycline and a taxane and had received one or two prior chemotherapy regimens in the metastatic setting. Of 442 participants, 27%

in each arm had triple-negative breast cancer.

Unfortunately, the study failed to demonstrate a benefit associated with the combination regimen. PFS was 5.5 months in the combination therapy arm and 5.9 months in the monotherapy arm (P=0.9). Overall survival was likewise not significantly different between the two arms (16.4 months for the combination arm and 16.5 months for capecitabine alone, P=0.5). Subgroup analyses did not suggest a significant benefit associated with combination therapy for any specific group of patients. Although both regimens were well tolerated, patients in the single agent arm, who received a higher dose of capecitabine, experienced higher rates of hand-foot syndrome, while those in the combination arm had higher rates of neutropenia and thrombocytopenia.

These results are disappointing in that they did not lead to improved overall outcomes for patients with metastatic breast cancer. In this study even a small statistically significant difference in favor of the combination arm could have been celebrated as a step forward scientifically. The authors elegantly described the biological rationale for choosing this combination. They noted that sunitinib has several targets that have previously been shown to be important in breast cancer, and preclinical data have demonstrated a synergistic effect of the combination of sunitinib with fluorouracil, of which capecitabine is the prodrug (2-6). A positive trial result would have contributed to the scientific evidence in support of simultaneously targeting neoangiogenesis and neoplastic cellular proliferation.

However, apart from the specifics in this case, there is also an obvious practical rationale for the use of two oral agents rather than two parenteral therapies or a combination of an oral and a parenteral therapy: such a regimen is an attractive option for patients who wish to minimize trips to the oncologist. In general, an entirely oral chemotherapy regimen is likely to be more convenient for the patient and to allow him or her to continue to live a life that is as close to "normal" as possible during treatment. The availability of oral treatment options has clear implications for patient reported outcomes (PROs) such as quality of life and employment.

Patient reported symptoms have gained prominence in clinical trials; PROs such as pain have been used as study endpoints and incorporated into drug labeling (7-9). However, other PROs, such as employment outcomes, have been virtually ignored in the clinical trials arena. My colleagues in health services research and I have been studying return to work in the adjuvant setting for several years. The Institute of Medicine cited employment concerns as paramount in their 2006 report, *From Cancer Patient to Cancer Survivor: Lost in Transition*, and recommended that "employers, legal advocates, health care providers, sponsors of support services, and government agencies should act to eliminate discrimination and minimize adverse effects of cancer on employment, while supporting cancer survivors with short-term and long-term limitations in ability to work" (10). To achieve this goal, however, we need to better understand the adverse effects of cancer treatment on employment. While we have generated some data on employment outcomes after adjuvant treatment for breast cancer, we know almost nothing about the work experiences of patients undergoing breast cancer treatment in the metastatic setting.

At Memorial Sloan-Kettering Cancer Center we have started to investigate the employment concerns of cancer patients undergoing palliative care. We surveyed 97 patients in our palliative care clinics and found that, although 79% were working at diagnosis, only 42% were still working at the time of the survey (11). Patients who continued to work reported a greater sense of normalcy and less financial distress, and 39% said they would have liked to work more hours than they were working. Factors significantly associated with not working included pain, side effects of analgesics, and fatigue. Based on these results, we can surmise that cancer-directed therapies that decrease pain and the need for analgesics might positively affect cancer patients' ability to work. On the other hand, treatments that are associated with high levels of fatigue might impair patients' ability to work. However, due to the lack of PRO data in the majority of clinical trials, we cannot draw any conclusions about the likelihood that an individual patient will experience decreased pain and/or be able to continue working while being treated with a specific regimen.

Despite the disappointing results of the study by Crown *et al.*, the push to include orally administered regimens in the armamentarium of therapeutics for metastatic breast cancer is encouraging and likely to continue. It would be useful going forward if investigators would include an assessment of quality of life as well as other relevant PROs among their study measures. Without such an assessment, we should ask ourselves how we would have incorporated the results of a similar study with positive results into our clinical practice and, indeed, how we might counsel patients. Crown *et al.* sought to demonstrate a 50% improvement in median PFS, from four to six months, with the combination

arm, deeming that such an improvement would be "clinically significant". Overall survival was a secondary endpoint in this study. Would we, as clinicians, feel comfortable recommending a treatment to our patients based on an improvement in progression-free but not overall survival without understanding the impact of either therapy on their quality of life? Indeed, PFS is sometimes cited as a surrogate for quality of life, but is it always? Using the data that are currently available in oncology, we are forced to make similar choices and guesses every day. Yet an alternative approach exists through which we could ultimately help our patients make more informed decisions.

PRO data are becoming increasingly standardized and easy to collect in the setting of a clinical trial. Basch *et al.* previously showed that patients undergoing chemotherapy can self-report symptoms using an online platform; more than 95% of patients and clinicians in their study were satisfied with the self-reporting system (12). Their research and that of other groups caught the attention of the Food and Drug Administration, which in 2009 issued a guidance document for use of PROs in the development of medical products and to support drug labeling (13). Basch *et al.* recently published recommendations in the *Journal of Clinical Oncology* for the incorporation of PROs into comparative effectiveness studies in adult oncology, including in randomized controlled trials (14). The inclusion of such measures could provide valuable insight into patients' experiences while undergoing treatment with commonly used and novel therapies. For example, in this study, although patients in the combination arm were more likely to experience hematologic toxicity, those in the single-agent arm had higher rates of hand-foot syndrome. From the perspective of a patient with metastatic breast cancer who is trying to continue to live a "normal" life, including maintaining commitments to family and to work, a lower rate of an uncomfortable and visibly disfiguring complication may be more important than a lower rate of thrombocytopenia without clinical sequelae. The inclusion of a quality-of-life measure in the study assessments would have given us a better understanding of the experiences of the patients in the two study arms. Had the trial been positive, this information would have been useful to oncologists and patients making decisions in the clinic.

Clinical trials are, by definition, patient-centered research, yet the patient experience during treatment remains incompletely understood. Our ignorance is an especially serious problem in the setting of treatment for metastatic disease. Until we demonstrate that we are able to cure patients with metastatic breast cancer, all treatment in this setting will remain palliative. As we strive to prolong our patients' lives, we cannot lose sight of the fact that one key goal should be to increase their comfort and the quality of their lives for whatever time they have remaining. The balance between these two goals is at the crux of how we practice every day in our clinics, and this same balance should be the focus of our clinical trials. The therapies we prescribe to our patients have toxicities that extend beyond what we as clinicians can see when we assess our patients, and it is our responsibility to advise patients of these toxicities when we discuss different treatment options and make clinical recommendations. However, we cannot hope to truly inform our patients if we do not have access to reliable toxicity information from clinical trials.

In their guidance document for use of PROs in drug labeling, the FDA asserted, "*Use of a PRO instrument is advised when measuring a concept best known by the patient or best measured from the patient perspective*". Based on this recommendation, many of the side effects currently reported by clinicians in the study setting, such as pain, nausea, and fatigue, should be reported by patients using accepted PRO measures. I would argue that such PROs should be incorporated into clinical trials regardless of whether or not one of these outcomes is the intended indication for drug labeling. We cannot expect this change to come from within the pharmaceutical industry, where the incentives may be different (unless we demonstrate the value of this approach to them). As clinicians, it is our responsibility to advocate for our patients by demanding that studies provide us with the information we need to make better and more informed recommendations. As researchers, it is our responsibility to ensure that clinical trials will yield the best information to advance the science of oncology, including not only our knowledge of biological targets but also our understanding of the real impact the treatments we study have on our patients' lives.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Crown JP, Diéras V, Staroslawska E, et al. Phase III trial of sunitinib in combination with capecitabine versus capecitabine monotherapy for the treatment of patients with pretreated metastatic breast cancer. J Clin Oncol 2013;31:2870-8.
2. Abrams TJ, Murray LJ, Pesenti E, et al. Preclinical evaluation of the tyrosine kinase inhibitor SU11248 as a single agent and in combination with "standard of care" therapeutic agents for the treatment of breast cancer. Mol Cancer Ther 2003;2:1011-21.
3. Banerjee S, Dowsett M, Ashworth A, et al. Mechanisms of disease: angiogenesis and the management of breast cancer. Nat Clin Pract Oncol 2007;4:536-50.
4. Goswami S, Sahai E, Wyckoff JB, et al. Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. Cancer Res 2005;65:5278-83.
5. Paulsson J, Sjöblom T, Micke P, et al. Prognostic significance of stromal platelet-derived growth factor beta-receptor expression in human breast cancer. Am J Pathol 2009;175:334-41.
6. Tsuda H, Morita D, Kimura M, et al. Correlation of KIT and EGFR overexpression with invasive ductal breast carcinoma of the solid-tubular subtype, nuclear grade 3, and mesenchymal or myoepithelial differentiation. Cancer Sci 2005;96:48-53.
7. Novantrone: mitoxantrone for injection concentrate. Silver Spring, MD, Food and Drug Administration, 2008.
8. Kantoff PW, Halabi S, Conaway M, et al. Hydrocortisone with or without mitoxantrone in men with hormone-refractory prostate cancer: results of the cancer and leukemia group B 9182 study. J Clin Oncol 1999;17:2506-13.
9. Tannock IF, Osoba D, Stockler MR, et al. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. J Clin Oncol 1996;14:1756-64.
10. Hewitt M, Greenfield S, Stovall E, eds. From Cancer Patient to Cancer Survivor: Lost in Transition. Washington, D.C. The National Academies Press, 2006.
11. Glare PA, Alickaj A, Blinder VS, et al. Survey of palliative care patients WHO work. J Clin Oncol 2013;31:abstr e20677.
12. Basch E, Artz D, Iasonos A, et al. Evaluation of an online platform for cancer patient self-reporting of chemotherapy toxicities. J Am Med Inform Assoc 2007;14:264-8.
13. Guidance for industry—patient-reported outcomes measures: use in medical product development to support labeling claims. Silver Spring, MD, Food and Drug Administration, 2009.
14. Basch E, Abernethy AP, Mullins CD, et al. Recommendations for incorporating patient-reported outcomes into clinical comparative effectiveness research in adult oncology. J Clin Oncol 2012;30:4249-55.

Cite this article as: Blinder VS. Lack of patient-reported outcomes assessment in phase III breast cancer studies: a missed opportunity for informed decision making. Ann Palliat Med 2014;3(1):12-15. doi: 10.3978/j.issn.2224-5820.2013.11.01

Breast cancer in the young: role of the geneticist

Ashley H. Woodson, Jessica L. Profato, Kimberly I. Muse, Jennifer K. Litton

Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA
Correspondence to: Jennifer K. Litton, M.D. Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1354, Houston, Texas 77030, USA. Email: jlitton@mdanderson.org.

Abstract: The genetics professional plays an important role in the care of young women with breast cancer by providing counseling on issues specific to these young women. The issues addressed in counseling include hereditary predisposition to cancer, fertility and reproductive options in the context of hereditary cancer, and the impact and implications of their history of early breast cancer on close family members. A thorough risk assessment and counseling session address the patient's personal and family history, with particular attention paid to benign and malignant findings that suggest the need for genetic testing. Genetics professionals, especially genetic counselors, also address the physical and emotional implications of an increased risk of cancer with patients and family members. This review highlights the unique aspects of care provided by these specialized healthcare providers.

Keywords: Breast cancer; genetic counseling; risk assessment

Submitted Mar 29, 2013. Accepted for publication Apr 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.04.13
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.04.13

The role of the genetics professional in the care of young women with breast cancer is growing in recognition and importance. Genetics professionals are defined here as a geneticist, genetic counselor, or any health care provider specifically trained in clinical cancer genetics. This subset of health care providers offers patients a service that extends beyond the treatment of their breast cancer and guides management and screening to prevent the development of future cancers in the patient or her family members.

Approximately 1 in 200 women under the age of 40 were diagnosed with breast cancer in 2012 (1). In the context of breast cancer, "young women" is often defined as those under the age of 40. This population has unique considerations and challenges related to their cancer management. These include treatment-related infertility, pregnancy during or after treatment, and a higher likelihood of hereditary breast cancer compared to post-menopausal women (2,3). However, guidelines set forth by the National Comprehensive Cancer Network (NCCN) recommend further genetic risk evaluation among women age 50 or younger at the time of diagnosis (4). This genetic risk assessment includes an evaluation of personal and family history of several hereditary cancer syndromes. After obtaining a detailed personal and family history of breast cancer, the genetics professional counsels the patient about her potential health risks and provides reduction strategies for the patient and her family members. Here we review the genetic syndromes associated with breast cancer at a young age, describe the application of breast cancer risk models, and discuss recommendations and potential interventions for young breast cancer patients and their families.

Hereditary genetic syndromes associated with breast cancer

Given the complexity of hereditary breast cancer syndromes, young women with breast cancer may benefit from a formal risk assessment by a trained geneticist or genetic counselor. The risk assessment includes an evaluation of family and personal history to determine whether genetic testing is indicated. In the absence of an identifiable genetic cause for the family history of breast cancer, genetics professionals use the family history to guide management and to recommend breast cancer screening for patients and their family members (5).

This process involves gathering medical information

from the patient for all family members, whether affected or unaffected. This information is then used to construct a detailed pedigree of three to four generations and includes first-degree relatives (i.e., parents, siblings, and children) and second-degree relatives (i.e., aunts, uncles, nieces, nephews, and grandparents). When collecting a cancer-focused medical and family history, the genetics professional identifies the type of cancer, age at diagnosis, number of primary tumors, primary *vs.* metastatic cancer sites, pathology, and prior treatment regimens (6,7). Other syndrome-specific details that may be included in the risk assessment include benign tumors, a history of non-cancer medical conditions, such as hypothyroidism, thyroid goiter, and fibrocystic breasts, and dermatologic findings, such as trichilemmomas, acrokeratosis, and fibrofolliculomas, among others (8). The genetics professional then uses this information to determine whether genetic testing is appropriate for the patient or for any of her family members. Based on pedigree analysis, the genetics professional estimates the likelihood of identifying a specific gene mutation by genetic testing (9). Using patient reports in genetic risk assessments has acknowledged limitations, such as inaccuracies in reported cancer histories, particularly in distant family members, as well as incomplete information, as is seen in small or adoptive families (10-13).

The majority of hereditary breast cancer is attributed to Hereditary Breast and Ovarian Cancer (HBOC) caused by mutations in *BRCA*1 and *BRCA*2 genes (14). *BRCA* mutations occur in approximately 1 in 400 to 1 in 800 individuals and are more common among those of Ashkenazi Jewish ancestry (1 in 40) (14-18). Among women with *BRCA* mutations, the lifetime risk of developing breast cancer is as much as 80%, and the increased risk of ovarian cancer in these women is as high as 45% (19,20). Increased risks of other malignancies, including male breast cancer, prostate cancer, and pancreatic cancer, also have been described in this population (21). Although the majority of hereditary breast cancer is associated with HBOC, a negative *BRCA*1 and *BRCA*2 test result may be uninformative in young women with breast cancer, because a negative result does not rule out the potential for a hereditary form of breast cancer in these patients (22).

Less common hereditary breast cancer syndromes also should be considered. Evaluation of personal and family history may prompt further investigation of more rare conditions. Li-Fraumeni syndrome, which is caused by mutations in the *TP53* gene, may account for 1% of all breast cancer (23). Mutations in the *TP53* gene also are associated with increased risks of several other types of cancer, including sarcomas (soft tissue and bone), brain, leukemia, and adrenocortical tumors (24). Although difficult to determine, the lifetime risk of cancer may be as high as 85% among individuals with *TP53* gene mutations (25,26). Often times the age of onset of these cancers, particularly breast cancer, are significantly younger than the ages of onset observed in the general population (23,24). Recent studies and an update to NCCN Guidelines suggest *TP53* genetic testing for women who are diagnosed at age 35 or younger and have negative *BRCA1* and *BRCA2* test results (27,28).

Further genetic evaluations of early-onset breast cancer may lead to testing for Cowden syndrome (associated with the *PTEN* gene), Peutz-Jeghers syndrome (*STK11* gene), or Hereditary Diffuse Gastric Cancer (*CDH1* gene) (29-31). Although these syndromes occur in fewer than 1 in 150,000 people, each syndrome is associated with an increased risk of breast cancer and other malignancies, some of which may have available screening for the patient and at-risk relatives.

Despite the identification of highly penetrant genes that are associated with hereditary breast cancer, a large proportion of breast cancer remains unexplained (32,33). Recent studies that have reviewed moderate- or low-penetrance breast cancer-susceptibility genes, such as *ATM*, *CHEK2*, *BRIP1*, *PALB2*, and RAD50, suggest that these genes account for some familial breast cancer cases (34,35). The risk of developing breast cancer among carriers of a low-penetrance gene is not well defined. As a result, standardized clinical management for these individuals and their family members is lacking and complicates the usefulness of ordering such testing (32,36).

Genetic risk assessment and genetic testing

The genetics risk evaluation of young women with breast cancer formally assesses the indication for genetic testing of one, or possibly several, hereditary cancer syndromes. The NCCN guidelines specify that women under age 45 are appropriate candidates for *BRCA* testing, with or without a family history of breast cancer. The guidelines also specify that women with breast cancer diagnosed under age 35 who have negative *BRCA*1/2 genetic test results should undergo *TP53* testing to rule out Li-Fraumeni syndrome (28). Although age of diagnosis may be an indication for genetic testing regardless of family history, the genetics professional will also review the woman's family history to ensure that the most appropriate genetic tests are ordered. The genetics professional must also ensure that all appropriate testing

was performed and that reports of all previously performed genetic tests have been reviewed. Many young women who have had *BRCA* testing in the past may not have undergone large genomic rearrangement analysis because it was not clinically available at that time. In these situations, genetics professionals are likely to order additional testing to more completely rule out the possibility of hereditary cancer (37,38). Genetics professionals also may discuss DNA banking and or research opportunities with both the patient and her family members if a genetic explanation for the young woman's cancer cannot be found through currently available genetic testing (9). Patients are often encouraged to remain in contact with genetics professionals given that new genetic tests often become available, and newer tests may provide additional information to the patient and/or her family members (39).

During discussions of genetic testing, the genetics professional will review the risks, benefits, and limitations of testing, informed consent, and implications of test results. A review of the potential results of a genetic test includes positive, true negative, uninformative negative, and variant of uncertain significance (VUS). A *positive* result indicates that a deleterious or pathogenic mutation has been identified in a cancer-causing gene, indicating an increased risk of cancer. A *true negative* result occurs when an individual undergoes site-specific genetic analysis for a known familial pathogenic mutation and is found not to be a carrier (8,9). An *uninformative negative* result describes the absence of an identified genetic mutation in the context of a personal or family history that remains concerning for a hereditary cause of cancer (8,9). An uninformative result also may be the consequence of testing an individual who was not the most appropriate family member to undergo testing. Genetic testing is most informative when performed on an individual whose personal history of cancer is most suggestive of the suspected hereditary cancer syndrome. This individual may be the one who was diagnosed with breast cancer at the youngest age in the family or the one most closely aligned with the concerning family history. Despite a diagnosis of early-onset breast cancer and possibly a family history of breast and ovarian cancer, many young women will not have a *BRCA* gene mutation (40). With an uninformative negative result, the genetics professional must re-evaluate the personal and family history in the context of this test result. For example, the genetics professional can consider whether the patient is a phenocopy, meaning a sporadic case of breast cancer in a family with hereditary breast cancer, or the family history may represent a familial clustering of cancer (41,42). Educating patients during the pre-test and post-test counseling is an important role of genetics professionals. The information enables patients to fully understand the implications and possible explanations for an uninformative negative genetic test result (43).

Providing an accurate interpretation and explanation of complicated test results is essential. This is particularly important when a VUS is found. A VUS is an alteration in the gene that may be either pathogenic or a benign polymorphism (8). Oftentimes, not enough data are available about the specific gene alternation to determine whether it is associated with an increased risk of cancer. As a result, when an individual is found to have a VUS, the clinical significance is not known and the medical management recommendations may not be clearly defined (8). Genetics professionals help patients understand the complexity of a VUS result and assist them in making decisions about medical care (44).

Risk assessment models

Several risk models have been developed to help determine the probability that a person will have a deleterious germline mutation that increases his or her risk of developing cancer. These are applied to support the genetic risk assessment achieved through pedigree analysis. These models also can estimate an individual's risk of developing breast cancer based on family history alone after common genetic syndromes have been ruled out. For example, models designed to calculate the likelihood of a *BRCA*1 or *BRCA*2 mutation include mutation prevalence tables reported by Myriad Genetic Laboratories, Inc.® in Salt Lake City, Utah, *BRCA*PRO, BOADICEA, and Penn II Risk Model, which are often used to estimate a probability range for the patient (45-49). Different models may be useful, depending on the available information for a particular family. *BRCA*PRO, for example, is a commonly used mathematical model that utilizes Bayesian analysis to calculate the probability of a *BRCA* gene mutation for the patient based on the family history of breast and ovarian cancer in first- and second-degree relatives (46). The BOADICEA model was developed in the United Kingdom by using population-based studies that evaluated patients with breast and ovarian cancer. This model integrates the possibility of genetic modifiers and accounts for other *BRCA* -associated cancers (i.e., prostate, pancreatic, and male breast cancer) in the risk assessment (41,47,48,50). Each model has a unique set of strengths and limitations that can support the clinical

judgment of the genetics professional (51). Not every hereditary cancer syndrome has available risk models; therefore, primary literature also may be used to assist in the risk assessment. For example, a scoring system and an online tool can be used to estimate the risk of a *PTEN* mutation based on the presence or absence of associated Cowden syndrome features (52).

Other empiric risk models also are available to estimate the lifetime risk of developing breast cancer among unaffected women with particular personal and family history risk factors. Among these risk estimation models are the Gail model, which is available through the National Cancer Institute, Claus model, and Tyrer-Cuzick models (5,53,54). These tools are designed to help guide management recommendations for unaffected family members of young breast cancer patients; however, each model is dependent upon different sets of criteria. The Gail model may identify a woman at increased risk of breast cancer according to personal risk factors, such as current age and history of breast biopsies, and may guide recommendations for tamoxifen use. In contrast, the Claus model takes into consideration the breast cancer history of first- and second-degree relatives, and the results of the risk estimate may lead to a recommendation of increased breast cancer screening, such as breast MRI (55,56). The younger the age at breast cancer diagnosis among first- and second-degree relatives, the greater the likelihood that an individual will be advised to receive increased breast surveillance (5). The Tyrer-Cuzick model uses both personal risk factors and family history to calculate the likelihood of a *BRCA* mutation as well as the lifetime risk of developing breast cancer in the absence of a *BRCA* mutation. This statistical model also incorporates the chance of a low penetrance gene mutation, unlike the other models available for unaffected women (54).

Potential recommendations and interventions

Identifying women with a hereditary cancer can alter treatment plans, surgical options, and/or future screening and management. Genetics professionals make management recommendations for the entire family based on genetic test results and/or cancer risk assessment models (8). Young women who test positive for a specific gene mutation (i.e., *BRCA*1/2, *TP53*, *PTEN*) are informed about management options specific to the associated hereditary cancer syndrome. Sources of consensus for management guidelines in the U.S. include organizations such as the NCCN and the American Cancer Society (8). Unfortunately, specific management guidelines are not available for many hereditary cancer syndromes, often because of their rarity and the lack of screening modalities. Recommendations are often extrapolated from the guidelines for HBOC; however, the efficacy of these recommended interventions will vary by syndrome and/or by family because of the variation in cancer risks (8).

Although a small subset of young women with breast cancer will be found to have a hereditary cancer syndrome, the majority will receive negative results from their genetic testing. For families with an uninformative negative test result, genetics professionals base screening and management recommendations on empiric risk data that account for the family history, often using such models as Claus or Tyrer-Cuzick (8). Screening recommendations for unaffected close female relatives of the young patient may include regular breast self-exams, clinical breast exams, earlier mammography, and/or increased screening that includes breast MRI, as these are the management recommendations for women at an increased risk for hereditary breast cancer (57). The age of initiation of screening is often based on the earliest age of diagnosis in the family (i.e., begin mammography 10 years earlier than the youngest age of breast cancer diagnosis in the family) (58,59).

Management of young women with hereditary or familial breast cancer also may entail several potential interventions. These include enhanced breast screening, surgical prevention strategies, and chemoprevention (57). Women with a diagnosis of a hereditary cancer syndrome may also be at an increased risk for several other types of malignancies. These include, but are not limited to, ovarian cancer, endometrial cancer, colon cancer, thyroid cancer, melanoma, and pancreatic cancer. Depending upon the clinical genetic syndrome, patients may require more individualized screening recommendations (60). These recommendations are often guided by consensus guidelines (such as those from NCCN) or, in the absence of consensus guidelines, clinical judgment and pertinent research from the medical literature. The recommendations may include annual dermatological evaluations, colonoscopy, thyroid cancer screening, or other appropriate tests (4,8).

Beyond the immediate management of young women with breast cancer, there are additional considerations regarding fertility preservation and future pregnancies. The American Society of Clinical Oncology states that early in treatment planning these young women should

be engaged in a discussion of their options for fertility preservation (61). As part of a multidisciplinary team, genetics professionals may be one of the first points of contact in a young woman's treatment plan, and referral to a reproductive endocrinologist may be expedited (62). A genetics consultation regarding a young woman's potential hereditary cancer syndrome may also address fertility preservation, possible genetic risks for future offspring, and reproductive options, such as pre-implantation genetic diagnosis (PGD) (63). PGD is one form of early prenatal diagnosis that is performed on embryos obtained through *in vitro* fertilization. An embryo can be tested for a specific genetic condition. If the embryo is found to have the gene profile for the condition in question, an unaffected embryo can be chosen for implantation (64,65).

Inadequate knowledge of PGD is common among individuals at high risk for hereditary cancer and among healthcare providers (66,67). Julian-Reynier and colleagues surveyed nearly 400 unaffected *BRCA*-positive carriers and found that 85% of respondents expected information about prenatal diagnosis. The women also expected to have PGD information provided by a cancer geneticist at the time the genetic test results were reported (68). This clearly highlights the need for patient education, but no standard clinical practice or professional guidelines are available for consumer education about PGD (69).

The implications of hereditary breast cancer among young women are complex and require attention from many different, specialized providers. Genetics professionals are responsible for educating these patients about the clinical implications of their conditions (8). Oftentimes, the genetics professional will refer patients to the appropriate healthcare providers so that screening and/or preventative surgery specific to that particular genetic syndrome can be discussed (8).

The identification of a hereditary cancer syndrome in a family has implications not only for the physical health of the individuals involved, but also for their emotional health and well being. Genetics professionals, especially genetic counselors, are responsible for addressing the possible medical and psychological implications of genetic test results (8). Genetic counselors address psychological issues such as worry about cancer, anxiety, intrusive thoughts, depression, anger, fear, guilt, family experiences with cancer, risk perception, social stressors, support and family networks, family communication, and readiness for genetic testing (8). Pre-test psychosocial assessment prior to genetic testing has been recommended to gauge a patient's anxiety level, as studies have shown that high pre-test anxiety is associated with higher post-test anxiety (70). The genetic counselor often assesses the psychosocial needs of the patient and provides assistance in managing the psychosocial responses that often occur in families that have an increased risk of developing cancer (71). Providing emotional support, reducing isolation, bolstering existing support networks, and designing innovative support interventions (i.e., multi-family support networks) have been suggested as methods for addressing the psychosocial needs of young patients with hereditary breast cancer (72). It has been suggested that formal support services for this patient population are unavailable or underutilized (72). If such support is available, genetic counselors educate patients about support groups and other resources, including peer support, Internet-based support organizations, and patient-focused gatherings on hereditary cancer (73,74). Web-based support groups also have been shown to be effective in reducing psychological distress (75). Some individuals, particularly young women, may require additional long-term support services. In these situations, genetics professionals often refer patients to long-term psychotherapy, marriage and family therapists, social workers, sexual rehabilitation counselors, depending upon the patient's needs (72). Unique psychosocial considerations for young women with hereditary breast cancer include an urgency to find a life partner and begin a family. This urgency arises from the possibility of surgical implications, the impact of genetic test results on relationship building (family and social relationships), risks to future offspring, sexuality challenges, limited availability of peers in similar circumstances, impact on career building, and other concerns related to their situation (76-78). Because of these unique and complex challenges, young women may be more likely to require long-term support and referrals to psychotherapy services.

Another unique role of genetics professionals is identifying at risk-relatives and facilitating family communication regarding genetic test results and increased cancer risks. Genetics professionals first analyze the family history (pedigree) and discuss the most likely origin of inheritance in the family (i.e., maternal, paternal, or de novo). If the origin of the mutation is unclear based on the family history, the genetics professional will identify relatives who should be tested to determine which lineage is at-risk for the particular hereditary cancer syndrome.

Genetics professionals have a duty to inform their patients about the implications of their test results for

their family members, while also protecting the patient's confidentiality (79,80). Patients with a positive test result are urged to notify at-risk relatives who may benefit from genetic testing (8). Genetics professionals also inform patients that negative genetic test results (uninformative negative or true negative) may still impact relatives' decision-making regarding genetic testing and/or medical management (81). Family members of patients with negative genetic test results need to be informed about the potential for an increased risk of breast cancer. As previously mentioned, risk models, such as the Claus model, can be used to determine whether close female relatives (i.e., daughters, sisters, mothers) may need to be followed with increased breast cancer screening (5).

Communication of information regarding hereditary cancer and the potential increased cancer risks to family members may be an emotionally overwhelming process for patients (82). Genetics professionals, particularly genetic counselors, play an important role in facilitating the communication process between the patient and his or her at-risk relatives (71). Follow-up genetic consultations after the disclosure of test results increase the proportion of relatives who are informed of their genetic risk (83).

This follow-up support often includes resources and written materials that can be shared with family members, such as individualized summary letters for the family and educational materials (81). Both male and female relatives need to be informed; however, patients are more likely to disclose their genetic results to female relatives than to male relatives (84). This may be attributable to the focus on the increased risk for breast cancer in females. Genetics professionals are responsible for educating their patients about cancer risks for males that are associated with the various hereditary cancer syndromes and for encouraging patients to inform both male and female relatives (81).

In addition, young breast cancer patients often have young children; therefore, the disclosure of genetic test results to children is another challenge that is addressed. The age at which test results are disclosed to children is often dependent on the hereditary cancer syndrome. For example, parents may wish to disclose their results and test their children for a *TP53* mutation, which confers an increased risk of certain childhood cancers. In contrast, individuals with a *BRCA* mutation may choose to delay disclosure until children are older, given that testing is not typically recommended for minors because the results lack clinical significance for children. Genetics professionals, most often genetic counselors, raise the issue of communication of genetic test results between parents and offspring and provide anticipatory guidance regarding the potential implications of sharing or not sharing the information with their children (85).

Conclusions

Genetics professionals play an important role in helping young women with breast cancer, who have a higher likelihood of having an underlying hereditary cancer syndrome. To provide the best care for these women, genetics professionals should offer a cancer genetics risk assessment that will provide a thorough evaluation of personal and family history features that may indicate the need for genetic testing. Education and counseling are essential for these young women, who need to understand the possible implications of test results for them personally and for their family members.

Genetics professionals can offer recommendations to guide cancer screening and management based on the outcome of genetic tests. The goal is to prevent future malignancies and to ensure that any malignancies that do develop are diagnosed early. The role of the genetics professional is unique in that it extends beyond the current cancer diagnosis and focuses on the future health and well-being of the young women and their family members.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10-29.
2. Cardoso F, Loibl S, Pagani O, et al. The European Society of Breast Cancer Specialists recommendations for the management of young women with breast cancer. Eur J Cancer 2012;48:3355-77.
3. Samphao S, Wheeler AJ, Rafferty E, et al. Diagnosis of breast cancer in women age 40 and younger: delays in diagnosis result from underuse of genetic testing and breast imaging. Am J Surg 2009;198:538-43.

4. National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Breast and Ovarian (Version 1.2013). Available online: http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf, last accessed 4/5/2013
5. Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. Cancer 1994;73:643-51.
6. Bennett RL. eds. The practical guide to the genetic family history (2nd ed.). Hoboken N.J.: Wiley-Blackwell, 2010.
7. Schneider KA. eds. Counseling about cancer: Strategies for genetic counseling (2nd ed). New York: Wiley-Liss, 2002.
8. Riley BD, Culver JO, Skrzynia C, et al. Essential elements of genetic cancer risk assessment, counseling, and testing: updated recommendations of the National Society of Genetic Counselors. J Genet Couns 2012;21:151-61.
9. Brown KL, Moglia DM, Grumet S. Genetic counseling for breast cancer risk: general concepts, challenging themes and future directions. Breast Dis 2006-2007;27:69-96.
10. Wood ME, Stockdale A, Flynn BS. Interviews with primary care physicians regarding taking and interpreting the cancer family history. Fam Pract 2008;25:334-40.
11. Love RR, Evans AM, Josten DM. The accuracy of patient reports of a family history of cancer. J Chronic Dis 1985;38:289-93.
12. Murff HJ, Spigel DR, Syngal S. Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. JAMA 2004;292:1480-9.
13. Douglas FS, O'Dair LC, Robinson M, et al. The accuracy of diagnoses as reported in families with cancer: a retrospective study. J Med Genet 1999;36:309-12.
14. Kauff ND, Perez-Segura P, Robson ME, et al. Incidence of non-founder BRCA1 and BRCA2 mutations in high risk Ashkenazi breast and ovarian cancer families. J Med Genet 2002;39:611-4.
15. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997;336:1401-8.
16. Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994;343:692-5.
17. Claus EB, Schildkraut JM, Thompson WD, et al. The genetic attributable risk of breast and ovarian cancer. Cancer 1996;77:2318-24.
18. Whittemore AS, Gong G, Itnyre J. Prevalence and contribution of BRCA1 mutations in breast cancer and ovarian cancer: results from three U.S. population-based case-control studies of ovarian cancer. Am J Hum Genet 1997;60:496-504.
19. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117-30.
20. Easton DF, Bishop DT, Ford D, et al. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1993;52:678-701.
21. Liede A, Karlan BY, Narod SA. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. J Clin Oncol 2004;22:735-42.
22. Bish A, Sutton S, Jacobs C, et al. No news is (not necessarily) good news: impact of preliminary results for BRCA1 mutation searches. Genet Med 2002;4:353-8.
23. Sidransky D, Tokino T, Helzlsouer K, et al. Inherited p53 gene mutations in breast cancer. Cancer Res 1992;52:2984-6.
24. Li FP, Fraumeni JF Jr, Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988;48:5358-62.
25. Le Bihan C, Moutou C, Brugières L, et al. ARCAD: a method for estimating age-dependent disease risk associated with mutation carrier status from family data. Genet Epidemiol 1995;12:13-25.
26. Kleihues P, Schäuble B, zur Hausen A, et al. Tumors associated with p53 germline mutations: a synopsis of 91 families. Am J Pathol 1997;150:1-13.
27. Masciari S, Dillon DA, Rath M, et al. Breast cancer phenotype in women with TP53 germline mutations: a Li-Fraumeni syndrome consortium effort. Breast Cancer Res Treat 2012;133:1125-30.
28. NCCN. Genetic/Familial High Risk Assessment: Breast and Ovarian V 1.2013. NCCN Clinical Practice Guidelines in Oncology, 2013.
29. Nelen MR, Kremer H, Konings IB, et al. Novel PTEN mutations in patients with Cowden disease: absence of clear genotype-phenotype correlations. Eur J Hum Genet 1999;7:267-73.
30. Kutscher AH, Zegarelli EV, Rankow RM, et al. Incidence of Peutz-Jeghers syndrome. Am J Dig Dis 1960;5:576-7.
31. Kluijt I, Sijmons RH, Hoogerbrugge N, et al. Familial gastric cancer: guidelines for diagnosis, treatment and periodic surveillance. Fam Cancer 2012;11:363-9.
32. Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med 2008;359:2143-53.

33. Pharoah PD, Antoniou A, Bobrow M, et al. Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 2002;31:33-6.
34. Nusbaum R, Vogel KJ, Ready K. Susceptibility to breast cancer: hereditary syndromes and low penetrance genes. Breast Dis 2006-2007;27:21-50.
35. Ripperger T, Gadzicki D, Meindl A, et al. Breast cancer susceptibility: current knowledge and implications for genetic counselling. Eur J Hum Genet 2009;17:722-31.
36. Njiaju UO, Olopade OI. Genetic determinants of breast cancer risk: a review of current literature and issues pertaining to clinical application. Breast J 2012;18:436-42.
37. Mazoyer S. Genomic rearrangements in the BRCA1 and BRCA2 genes. Hum Mutat 2005;25:415-22.
38. Judkins T, Rosenthal E, Arnell C, et al. Clinical significance of large rearrangements in BRCA1 and BRCA2. Cancer 2012;118:5210-6.
39. Fitzpatrick J, Hahn C, Costa T, et al. The duty to recontact: attitudes of genetics service providers. Am J Hum Genet 1997;61:A57.
40. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998;62:676-89.
41. Antoniou AC, Pharoah PD, McMullan G, et al. A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. Br J Cancer 2002;86:76-83.
42. Smith A, Moran A, Boyd MC, et al. Phenocopies in BRCA1 and BRCA2 families: evidence for modifier genes and implications for screening. J Med Genet 2007;44:10-5.
43. Hallowell N, Statham H, Murton F, et al. "Talking About Chance": The Presentation of Risk Information During Genetic Counseling for Breast and Ovarian Cancer. J Genet Counsel 1997;6:269-86.
44. Culver J, Brinkerhoff C, Clague J, et al. Variants of uncertain significance in BRCA testing: evaluation of surgical decisions, risk perception, and cancer distress. Clin Genet 2013. [Epub ahead of print].
45. Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. J Clin Oncol 2002;20:1480-90.
46. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet 1998;62:145-58.
47. Antoniou AC, Cunningham AP, Peto J, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. Br J Cancer 2008;98:1457-66.
48. Antoniou AC, Pharoah PP, Smith P, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancer. Br J Cancer 2004;91:1580-90.
49. Lindor NM, Johnson KJ, Harvey H, et al. Predicting BRCA1 and BRCA2 gene mutation carriers: comparison of PENN II model to previous study. Fam Cancer 2010;9:495-502.
50. Antoniou AC, Durocher F, Smith P, et al. BRCA1 and BRCA2 mutation predictions using the BOADICEA and BRCAPRO models and penetrance estimation in high-risk French-Canadian families. Breast Cancer Res 2006;8:R3.
51. Ready K, Litton JK, Arun BK. Clinical application of breast cancer risk assessment models. Future Oncol 2010;6:355-65.
52. Tan MH, Mester J, Peterson C, et al. A clinical scoring system for selection of patients for PTEN mutation testing is proposed on the basis of a prospective study of 3042 probands. Am J Hum Genet 2011;88:42-56.
53. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989;81:1879-86.
54. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med 2004;23:1111-30.
55. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90:1371-88.
56. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007;57:75-89.
57. Smith KL, Isaacs C. Management of women at increased risk for hereditary breast cancer. Breast Dis 2006-2007;27:51-67.
58. Dershaw DD. Are there any indications for routine breast cancer screening of asymptomatic women who are less than 40 years old? AJR Am J Roentgenol 1999;172:1136-7.
59. Dershaw DD. Mammographic screening of the high-risk woman. Am J Surg 2000;180:288-9.
60. Shannon KM, Chittenden A. Genetic testing by cancer site: breast. Cancer J 2012;18:310-9.
61. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol

2006;24:2917-31.
62. Lee S, Ozkavukcu S, Heytens E, et al. Value of early referral to fertility preservation in young women with breast cancer. J Clin Oncol 2010;28:4683-6.
63. Hurley K, Rubin LR, Werner-Lin A, et al. Incorporating information regarding preimplantation genetic diagnosis into discussions concerning testing and risk management for BRCA1/2 mutations: a qualitative study of patient preferences. Cancer 2012;118:6270-7.
64. Sermon K. Current concepts in preimplantation genetic diagnosis (PGD): a molecular biologist's view. Hum Reprod Update 2002;8:11-20.
65. Verlinsky Y, Kuliev A. Current status of preimplantation diagnosis for single gene disorders. Reprod Biomed Online 2003;7:145-50.
66. Brandt AC, Tschirgi ML, Ready KJ, et al. Knowledge, attitudes, and clinical experience of physicians regarding preimplantation genetic diagnosis for hereditary cancer predisposition syndromes. Fam Cancer 2010;9:479-87.
67. Quinn GP, Pal T, Murphy D, et al. High-risk consumers' perceptions of preimplantation genetic diagnosis for hereditary cancers: a systematic review and meta-analysis. Genet Med 2012;14:191-200.
68. Julian-Reynier C, Fabre R, Coupier I, et al. BRCA1/2 carriers: their childbearing plans and theoretical intentions about having preimplantation genetic diagnosis and prenatal diagnosis. Genet Med 2012;14:527-34.
69. Hudson KL. Preimplantation genetic diagnosis: public policy and public attitudes. Fertil Steril 2006;85:1638-45.
70. Ertmański S, Metcalfe K, Trempała J, et al. Identification of patients at high risk of psychological distress after BRCA1 genetic testing. Genet Test Mol Biomarkers 2009;13:325-30.
71. Trepanier A, Ahrens M, McKinnon W, et al. Genetic cancer risk assessment and counseling: recommendations of the national society of genetic counselors. J Genet Couns 2004;13:83-114.
72. Werner-Lin A. Formal and informal support needs of young women with BRCA mutations. J Psychosoc Oncol 2008;26:111-33.
73. Kenen RH, Shapiro PJ, Friedman S, et al. Peer-support in coping with medical uncertainty: discussion of oophorectomy and hormone replacement therapy on a web-based message board. Psychooncology 2007;16:763-71.
74. McKinnon W, Naud S, Ashikaga T, et al. Results of an intervention for individuals and families with BRCA mutations: a model for providing medical updates and psychosocial support following genetic testing. J Genet Couns 2007;16:433-56.
75. Winzelberg AJ, Classen C, Alpers GW, et al. Evaluation of an internet support group for women with primary breast cancer. Cancer 2003;97:1164-73.
76. Werner-Lin A. Beating the biological clock: the compressed family life cycle of young women with BRCA gene alterations. Soc Work Health Care 2008;47:416-37.
77. Hoskins LM, Roy KM, Greene MH. Toward a new understanding of risk perception among young female BRCA1/2 "previvors". Fam Syst Health 2012;30:32-46.
78. Hoskins LM, Greene MH. Anticipatory loss and early mastectomy for young female BRCA1/2 mutation carriers. Qual Health Res 2012;22:1633-46.
79. American Society of Clinical Oncology. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. J Clin Oncol 2003;21:2397-406.
80. ASHG statement. Professional disclosure of familial genetic information. The American Society of Human Genetics Social Issues Subcommittee on Familial Disclosure. Am J Hum Genet 1998;62:474-83.
81. DeMarco TA, McKinnon WC. Life after BRCA1/2 testing: family communication and support issues. Breast Dis 2006-2007;27:127-36.
82. Forrest K, Simpson SA, Wilson BJ, et al. To tell or not to tell: barriers and facilitators in family communication about genetic risk. Clin Genet 2003;64:317-26.
83. Forrest LE, Burke J, Bacic S, et al. Increased genetic counseling support improves communication of genetic information in families. Genet Med 2008;10:167-72.
84. Claes E, Evers-Kiebooms G, Boogaerts A, et al. Communication with close and distant relatives in the context of genetic testing for hereditary breast and ovarian cancer in cancer patients. Am J Med Genet A 2003;116A:11-9.
85. Tercyak KP, Peshkin BN, DeMarco TA, et al. Parent-child factors and their effect on communicating BRCA1/2 test results to children. Patient Educ Couns 2002;47:145-53.

Cite this article as: Woodson AH, Profato JL, Muse KI, Litton JK. Breast cancer in the young: role of the geneticist. J Thorac Dis 2013;5(S1):S19-S26. doi: 10.3978/j.issn.2072-1439.2013.04.13

An update on the genomic landscape of breast cancer: new opportunity for personalized therapy?

Anneleen Daemen

Department of Bioinformatics & Computational Biology, Genentech Inc., 1 DNA Way, South San Francisco, California 94080, USA
Correspondence to: Anneleen Daemen. Department of Bioinformatics & Computational Biology, Genentech Inc., 1 DNA Way, South San Francisco, California 94080, USA. Email: daemena@gene.com.

Submitted Oct 12, 2012. Accepted for publication Oct 22, 2012.
doi: 10.3978/j.issn.2218-676X.2012.10.05
View this article at: http://www.thetcr.org/article/view/588/html

Breast cancer is a clinically and genomically heterogeneous disease, and may therefore benefit from personalized therapeutic strategies. Incorporating genomic information obtained by molecular analysis of an individual patient's tumor into treatment decisions is a promising area of focus. This approach requires knowledge of the genomic landscape and its possible therapeutic implications, but will ultimately allow employing investigational therapeutic compounds in a more intelligent way. Many institutes worldwide have contributed to the genomic landscaping of breast cancer, of which the results of six studies were recently published in Nature. These studies, summarized in *Table 1*, differ in breast cancer spectrum covered, primary focus, and technology platforms used. Integrating the genomic data from these studies, which is the focus of this review, provides an unprecedented opportunity to better understand the substantial variation in drug response and clinical outcome and to rethink treatment strategy.

Mutational profiling for the identification of driver mutations was assessed in five studies, with the number of somatic point mutations summarized in *Table 1*. Somatic putative driver substitutions and small insertions and deletions were identified in cancer genes previously implicated in breast cancer and in cancer genes involved in other cancer types (*Figure 1*). Of particular interest for cancer treatment are new genes that may be causally implicated in oncogenesis. Cancer genes not previously associated with breast cancer but confirmed through mutation recurrence screening are potential tumor suppressors *ARID1B*, *CASP8*, *CBFB*, *CDKN1B*, *MAP3K13*, *NCOR1*, *RUNX1*, *SMARCD1*, and *TBX3*. These genes with their mutation frequency per clinical and transcriptional subtype are shown in *Figure 1*. This heatmap confirms subtype-specific mutation patterns for genes such as *AKT1*, *BRCA1*, *GATA3*, *PIK3CA* and *TP53*, with the majority of basal-like and HER2-enriched samples carrying a *TP53* mutation, whilst luminal samples are more likely to carry a *PIK3CA* mutation. It also emerges from *Figure 1* that the mutation spectrum in luminal breast cancers is heterogeneous with mutations in the majority of highly recurrent genes. HER2-enriched and particularly basal-like breast cancers harbor less recurrently mutated genes. Notably, both Banerji *et al.* and the TCGA group observed mutation rates to be lowest in luminal A and highest in the basal-like and HER2-enriched subtypes (2,4).

A new recurrent observation across the studies is the inactivation of the RUNX1/CBFB complex reported to be essential for normal hematopoietic cell differentiation (7). *CBFB* encodes the non-DNA-binding component of the transcription factor complex, whilst *RUNX1* is a transcription factor encoding the DNA-binding RUNX protein. Isolated cases of breast cancer with a somatic mutation in *CBFB* had been noted before, but not as frequently as in these recent studies with 15 tumor samples carrying a *CBFB* somatic mutation (of which 87% luminal/ER+). Interestingly, *RUNX1* was mutated in a mutually exclusive fashion in an additional 21 samples (90% luminal/ER+), and showed homozygous deletion in 2 basal-like samples. With *RUNX1* a tethering interaction partner of estrogen receptor α (8), loss of transcriptional regulation by this complex is suggested to promote breast cancer progression.

Structural variation was focused on in three studies by sequencing at the whole-genome level (*Table 1*). Consistent with mutation rates, luminal A samples showed on average 30 rearrangements, basal-like samples 237 and HER2-enriched samples 246 rearrangements (2). Within luminal

Table 1 Overview of reviewed studies with patient and omics information. The number of somatic point mutations are shown for 5 out of 6 studies with sequencing data

Study	Patient characteristics	Number of patients	Collected data	Subtype information	# somatic substitutions	# somatic indels
Sanger (1)	Primary carcinoma	100	WES [100]	54% ER+/HER2- 30% HER2+ 16% TN	6,964	277
MIT (2)	Primary, treatment-naïve carcinoma	108	WES [103] WGS [22]	35% luminal A 20% luminal B 20% Her2-enriched 12% basal-like 13% normal-like or unknown	4,668	317
WashU (3)	Pretreatment biopsies from 2 neoadj. aromatase inhibitor trials	77	WES [31] WGS [46]	100% ER+	3,221	133
TCGA (4)	Invasive carcinoma	825	WES [507] Expr [547] Meth [802] SNP [773] miRNA Seq [697] RPPA [403]	44% luminal A 25% luminal B 11% HER2-enr 18% basal-like 2% normal-like	28,319	2,302
UBC (5)	Primary, treatment-naïve carcinoma	104	WES [54] WGS [15] RNA-seq [80] SNP [104]	100% TN	2,386	107
Cambridge (6)	Primary carcinoma	1,992	Expr [1,992] SNP [1,992]	36% luminal A 25% luminal B 12% HER2-enr 17% basal-like 10% normal-like	N/A	N/A

Collected data: WES = whole exome sequencing; WGS = whole genome sequencing; Expr = expression microarray; Meth = methylation; SNP = single nucleotide polymorphism; RPPA = reverse-phase protein. Subtype information: ER = estrogen receptor; PR = progesterone receptor; TN = triple negative; N/A = not applicable

samples, the number of rearrangements was associated with treatment response, with fewer rearrangements observed in ER+ samples sensitive to aromatase inhibition (3). Although no new structural rearrangements were discovered, in-frame fusion events were revalidated, including the MAGI3-AKT3 fusion (2). It is anticipated that the latter fusion results in both loss of function of *PTEN* and activation of oncogene *AKT3*, making it targetable with an ATP-competitive AKT inhibitor. Screening for this fusion event in an additional 235 breast cancers revealed a rearrangement rate of 3.4%, among which 62% were triple negative (2). This reveals a new therapeutic option to investigate for fusion-positive triple negative breast cancer.

In the TCGA study, significant clusters obtained from a variety of data types such as methylation, copy number and miRNA expression, showed moderate concordance with the transcriptional subtypes (4). Curtis *et al.* also revealed a steady increase in number of distinct subtypes, with substantial heterogeneity within the transcriptional subtypes (6). Ten clusters were obtained by joint clustering

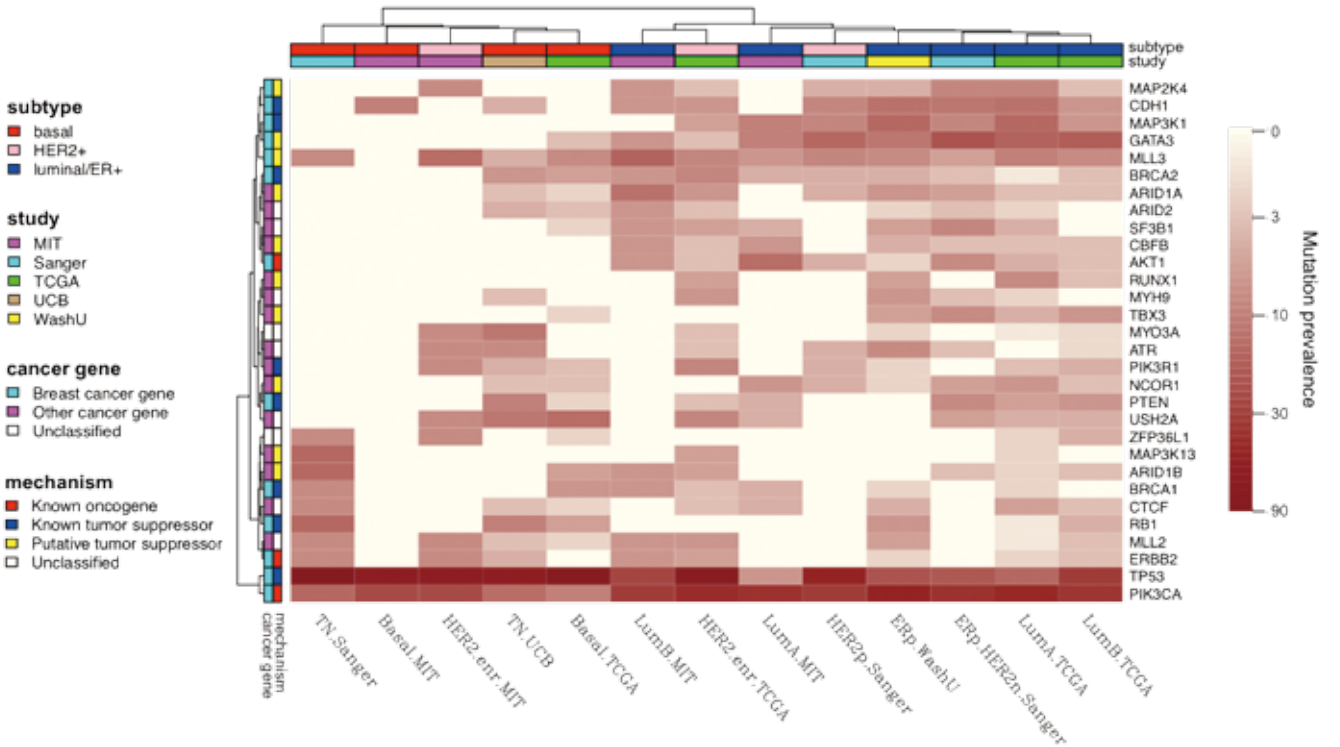


Figure 1 Heatmap of mutation prevalence per subtype for the top 30 non-silent, recurrently mutated genes. Genes were selected based on a percentage per subtype ≥5% for at least 1 study, and sum of subtype-specific percentages across all subtypes and studies ≥15%

of copy number and gene expression data. These clusters showed distinct disease-specific survival, and might influence treatment strategies. For example, the HER2-enriched cluster contained luminal cases that might benefit from HER2-specific targeted treatment. These associations with patient outcome, however, are not yet strong enough for clinical utility.

Finally, many infrequently mutated genes about which little is known together may substantially contribute to cancer, which significantly challenges therapy development. In the 100 sample cohort of Stephens *et al.*, a small portion (17%) of cancer genes (*TP53*, *PIK3CA*, *ERBB2*, *MYC*, *FGFR1/ZNF703*, *GATA3*, and *CCND1*, each frequently mutated in over 10% of cases) contributed over half (58%) of all observed driver mutations. The remaining 42% of driver mutations occurred in 83% of cancer genes, each contributing infrequently (1). Similar observations were made in two of the other studies, with somatic mutations in 21% and 12% of cases restricted to genes mutated at an insignificant frequency, many of which were part of key regulatory pathways characterized by highly recurrent cancer genes (3,5). In order for patients without recurrent abnormalities to benefit from targeted treatment, a pathway-driven approach that takes rare and tumor-specific changes into account will become a necessity. Shah *et al.* analyzed sparse mutation patterns in functionally connected genes (5). This approach revealed significantly mutated pathways for triple negative breast cancer such as *p53*-related pathways, ERBB signaling, and WNT/cadherin signaling. Targeting dysregulated pathways regardless of the mechanism by which they are dysregulated will potentially yield more and better treatment options for patients without somatic aberrations in the frequent drivers. The TCGA group and Ellis *et al.* made suggestions of drug targets based on mutation and genomic aberration spectra, with a target defined as a gene or protein with an approved drug or investigational drug in late stage development targeting the molecular pathway. These suggestions include PIK3CA, AKT1 and KIT inhibitors for luminal/ER+ cancers, PARP and DDR2 inhibitors for basal-like cancers, and combined inhibitors of *HER2*, *HER3* and/or *EGFR* for HER2-enriched cancers (3,4). It is therefore plausible that patients with chromosomal aberrations or carrying mutations in the targeted pathways might benefit from those respective inhibitors.

To move this information into routine clinical practice, it will be important to further exploit the clinical heterogeneity and new drug targets for improved treatment of breast cancer patients. Genomics can direct towards dysregulated signaling pathways or molecular functions instead of individual mutations, and therapeutic combinations can potentially reduce acquired resistance. To accelerate clinical investigation, *in vitro* cell line systems on one hand will be key to associate mutation patterns and pathway activity with response to investigational compounds (Daemen A *et al.*, submitted). On the other hand, a model for rapid assessment of phase II drugs is required. The neoadjuvant I-SPY2 trial for locally advanced breast cancer was set up according to an adaptive trial scheme to allow for concurrent drug testing using fewer patients and resources: compounds with a high probability of being more effective than standard therapy in a certain subpopulation graduate from the trial towards smaller, population-targeted phase III trials, whilst compounds with a low probability of improved efficacy are dropped (9). This design allows the simultaneous selection of biomarkers to guide patient selection, avoiding drug failure in an all-comers trial due to low aberrant pathway prevalence.

As revealed by the studies in *Table 1*, breast cancer heterogeneity cannot be fully addressed with current knowledge and standard technologies. Additional means of profiling such as epigenetics and metabolomics could aid in better defining key pathways and drivers, and add to future therapeutic selection (10). Sequencing, expression, copy number and other new technologies in combination with a pathway-targeted therapeutic approach poise the field to identify new prognostic and predictive biomarkers, and discover additional new targets and pathways for therapeutic intervention.

Acknowledgements

The author would like to thank Zemin Zhang, Gerard Manning and Zhaoshi Jiang for the fruitful discussions.

Cite this article as: Daemen A. An update on the genomic landscape of breast cancer: new opportunity for personalized therapy? Transl Cancer Res 2012;1(4):279-282. doi: 10.3978/j.issn.2218-676X.2012.10.05

Footnote

Conflicts of Interest: The author is an employee of Genentech, a member of the Roche group.

References

1. Stephens PJ, Tarpey PS, Davies H, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012;486:400-4.
2. Banerji S, Cibulskis K, Rangel-Escareno C, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 2012;486:405-9.
3. Ellis MJ, Ding L, Shen D, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature 2012;486:353-60.
4. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61-70.
5. Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 2012;486:395-9.
6. Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012;486:346-52.
7. Hart SM, Foroni L. Core binding factor genes and human leukemia. Haematologica 2002;87:1307-23.
8. Stender JD, Kim K, Charn TH, et al. Genome-wide analysis of estrogen receptor alpha DNA binding and tethering mechanisms identifies Runx1 as a novel tethering factor in receptor-mediated transcriptional activation. Mol Cell Biol 2010;30:3943-55.
9. Barker AD, Sigman CC, Kelloff GJ, et al. I-SPY 2: an adaptive breast cancer trial design in the setting of neoadjuvant chemotherapy. Clin Pharmacol Ther 2009;86:97-100.
10. Connolly R, Stearns V. Epigenetics as a Therapeutic Target in Breast Cancer. J Mammary Gland Biol Neoplasia 2012;17:191-204.

Towards the elucidation of the mechanisms underlying breast cancer mutations

Elisa Fiorito[1], Antoni Hurtado[1,2]

[1]Breast Cancer Research group, Nordic EMBL Partnership, Centre for Molecular Medicine Norway (NCMM), University of Oslo, P.O. 1137 Blindern, 0318, Oslo, Norway; [2]Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 Oslo, Norway
Correspondence to: Elisa Fiorito. Breast Cancer Research group, Nordic EMBL Partnership, Centre for Molecular Medicine Norway (NCMM), University of Oslo, P.O. 1137 Blindern, 0318, Oslo, Norway. Email: elisa.fiorito@ncmm.uio.no; Antoni Hurtado. Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 Oslo, Norway. Email: toni.hurtado@ncmm.uio.no.

Submitted Apr 14, 2013. Accepted for publication Apr 27, 2013.
doi: 10.3978/j.issn.2218-676X.2013.04.14
View this article at: http://www.thetcr.org/article/view/1121/html

The genome of cancer cells is characterized by the presence of somatic mutations acquired during the processes of neoplastic transformation and clonal expansion. A small fraction of these mutations, called *drivers*, causally affect oncogenesis by conferring growth advantage. The remaining mutations, also called *passengers*, do not contribute to the advantage of tumor growth (1,2). Somatic mutations are the result of a balance between the DNA damage and repair events occurring during tumorigenesis. With the analysis of both driver and passenger mutations in the cancer genome it is possible to follow the processes active during the lifetime of cancers. Yet, the mechanisms by which these events specifically affect somatic mutations are poorly understood. Furthermore, most of the published studies have focused only on limited number of cancer genes.

In the last decade, the development of high-throughput sequencing technologies has permitted the completion of whole cancer genome sequences (3-9) and the generation of comprehensive catalogs of somatic mutations (10,11). The investigation of the full repertoire of cancer-specific mutations can importantly contribute to our understanding of the processes modeling the genomic landscape of tumors. Recent studies have demonstrated the potential of this approach in revealing mutational signatures in melanoma and lung cancer (10,11). Very importantly, these studies have also elucidated the molecular mechanisms underlying the mutations detected in these tumors. Yet, it is unknown how the mutational processes alter the genome of breast tumors. The study published by Nik-Zainal *et al.* in *Cell* (12) aimed to identify the mutational mechanisms remodeling the genome of human breast cancers.

Nik-Zainal *et al.* sequenced the complete genome of 21 breast cancers typed for the expression of Estrogen Receptor (ER), Progesterone Receptor (PR) and Epidermal Growth Factor Receptor 2 (HER-2/ERBB2) and for the presence of BRCA1 and BRCA2 germ line mutations. The authors aimed at the identification of all the cancer-specific mutations by comparing tumor DNA and normal DNA obtained from the same patient. By performing this analysis, a comprehensive catalog of somatic mutations from the 21 breast cancer genomes was defined. In agreement with previous studies (13-15), substitutions were identified in cancer genes such as GATA3 and PIK3CA. Furthermore, the amplification of genes implicated in breast cancer development was also reported (e.g., *ERBB2*, *CCND1*, *MYC* and *ZNF703*).

The authors also focused to investigate the active mutational processes, by considering each base substitution and the bases immediately 5' and 3' to it. The analysis of base substitution evidenced that various mutational signatures and processes were present in the majority of the tumors. To define the signatures featuring the mutational processes and to evaluate the contribution of these events in each breast tumor sample, Nik-Zainal *et al.* applied a nonnegative matrix factorization (NMF) model. The evaluation of NMF decompositions revealed five mutational signatures (A, B, C, D and E) characterized by different profiles of trinucleotide mutations. In particular, signature B is mainly represented by C>T and C>G mutations at TpCpX trinucleotides. Moreover, various combinations of

each signature defined the mutational spectra in each breast cancer genome, demonstrating that multiple mutational processes may have been arranged either at the same time or in different phases of tumor growth.

In order to evaluate the possibility of regional clustering of substitutions, the authors analyzed the distance between somatic mutations. Very importantly, they showed a remarkable phenomenon of localized hypermutation, termed *kataegis*. Various extents of *kataegis* were observed in the diverse cases, with examples of hypermutation spanning both large and short regions. Moreover, these clusters showed a typical mutational pattern, similar to the one defined in signature B. Interestingly, the regions showing *kataegis* were also associated with somatic genomic rearrangements. All these findings suggested that mutational processes inducing specific localized hypermutation patterns might promote chromosomal rearrangements, which are, indeed, very relevant features of cancer genomes. In addition, the authors hypothesize that the AID/APOBEC deaminase protein family members might be involved both in kataegis as in the molecular mechanisms underlying signature B. Indeed, these proteins are involved in somatic hypermutation and class-switch recombination at immunoglobulin loci. This suggests that AID/APOBEC proteins might also play a critical role in tumors carrying signature B.

Previous studies in other cancer types have shown that transcription-coupled DNA repair processes are able to influence the mutational genomic spectrum (10,11). The work by Nik-Zainal *et al.* has revealed a mutation transcription strand bias for G>T and T>G transitions, suggesting a possible role for the transcription-coupled repair mechanisms in the removal of guanine or thymine bulky adducts. Moreover, an inverse correlation between mutation prevalence and gene expression levels was reported, confirming a similar observation in melanoma cancer (11). Interestingly, in Nik-Zainal *et al.* study, the prevalence of mutations was superior at increased distance from the transcription start site. Altogether, these data suggested that transcription processes might act as suppressors of mutagenic forces.

The study conducted by Nik-Zainal *et al.* is the first example of analysis of the complete mutational spectra of breast cancer samples. Furthermore, this work emphasizes the importance of the whole-genome sequencing studies and the generation of comprehensive catalogs of somatic mutations accumulated in tumors. Human cells are subjected to factors that induce DNA damage, which might be repaired or transmitted to the daughter cell. Importantly, these processes may leave imprint on the genome depending on their strength and duration. The analysis of whole-genome catalogs of somatic mutations can provide a great help in our understanding of the mutational events to which every cell is subjected during the whole lifetime. It can become an important approach to shed light not only on the mechanism of neoplastic transformation and progression, but also of cell aging, and can therefore give hints on the origins of genetic instability in cancer. The extraction of mutational patterns can improve our understanding of the molecular mechanisms underlying DNA damage and repair. Moreover, they can provide information about the history of tumors and how somatic mutations are occurred, as it has been previously reported (16).

Finally, this study opens important perspectives on clinical applications. The extraction of signatures linked to specific breast cancer subtypes may improve the commonly applied histological typing. Further research may highlight signatures linked with breast cancer prognosis and efficacy of specific therapies.

The study considers mutations derived from only 21 genomes but mutational pattern analyses will be performed in thousands of cancers (17). Future studies should compare mutational signatures identified in different cancer types and correlate these with both genetic and environmental factor exposure. These studies would allow getting insight in the tumor growth mechanisms and it would give some hints about the mutational pathways to target in diverse tumor types.

All in all, signature analysis may not only be a great tool to discover DNA damage and repair mechanisms operative in cancer lifetime, but also provide remarkable insight in diagnosis and in therapy personalization.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature 2009;458:719-24.

2. Stratton MR. Exploring the genomes of cancer cells: progress and promise. Science 2011;331:1553-8.
3. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. Nature 2011;471:467-72.
4. Ding L, Ellis MJ, Li S, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature 2010;464:999-1005.
5. Mardis ER, Wilson RK. Cancer genome sequencing: a review. Hum Mol Genet 2009;18:R163-8.
6. Shah SP, Morin RD, Khattra J, et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 2009;461:809-13.
7. Tao Y, Ruan J, Yeh SH, et al. Rapid growth of a hepatocellular carcinoma and the driving mutations revealed by cell-population genetic analysis of whole-genome data. Proc Natl Acad Sci U S A 2011;108:12042-7.
8. Berger MF, Lawrence MS, Demichelis F, et al. The genomic complexity of primary human prostate cancer. Nature 2011;470:214-20.
9. Ley TJ, Mardis ER, Ding L, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature 2008;456:66-72.
10. 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.
11. Pleasance ED, Cheetham RK, Stephens PJ, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 2010;463:191-6.
12. Nik-Zainal S, Alexandrov LB, Wedge DC, et al. Mutational processes molding the genomes of 21 breast cancers. Cell 2012;149:979-93.
13. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61-70.
14. Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012;486:346-52.
15. Holland DG, Burleigh A, Git A, et al. ZNF703 is a common Luminal B breast cancer oncogene that differentially regulates luminal and basal progenitors in human mammary epithelium. EMBO Mol Med 2011;3:167-80.
16. Nik-Zainal S, Van Loo P, Wedge DC, et al. The life history of 21 breast cancers. Cell 2012;149:994-1007.
17. International Cancer Genome Consortium, Hudson TJ, Anderson W, et al. International network of cancer genome projects. Nature 2010;464:993-8.

Cite this article as: Fiorito E, Hurtado A. Towards the elucidation of the mechanisms underlying breast cancer mutations. Transl Cancer Res 2013;2(2):100-102. doi: 10.3978/j.issn.2218-676X.2013.04.14

Members of the BRCA1 complexes as new susceptibility genes for breast cancer

Pilar Eroles

Institute of Health Research INCLIVA, Av. Blasco Ibáñez 17.46010 Valencia, Spain

Correspondence to: Pilar Eroles. Institute of Health Research INCLIVA, Av. Blasco Ibáñez 17.46010 Valencia, Spain. Email: pilar.eroles@uv.es.

Submitted Mar 25, 2013. Accepted for publication Apr 22, 2013.
doi: 10.3978/j.issn.2218-676X.2013.04.06
View this article at: http://www.thetcr.org/article/view/1103/html

Breast cancer was considered a multifactorial disease resulting from the interplay of molecular, genetic and environmental factors. Germline mutations, including mutations in the tumor suppressor genes *BRCA1* and *BRCA2*, have been identified as a cause of familiar breast cancer.

BRCA1 plays an important role in both DNA damage repair mechanisms, as well as in the cell cycle checkpoint controls that maintain genome stability. BRCA1 acts as a substrate of the ATM/ATR DNA damage response kinases and is required for the homology-directed repair that facilitates the error-free repair of double-strand breaks (DSBs). Moreover, it maintains heterochromatin integrity via H2A ubiquitination (1). BRCA1 interacts with several proteins organizing into complexes. The interaction takes place through a phosphopeptide binding domain (BCRT domain) recognizing a phospho-SPxF motif (S, serine; P, proline; x, varies; F, phenylalanine). The Abraxas (also known as Abra1, CCDC98), Bach1 (also known as Brip1, FancJ) and CTIP (also known asRBBP8) proteins bind directly to the BCRT domain in a phosphorylation dependent manner, forming at least three mutually exclusive complexes.

Abraxas was the last of these three proteins identified. Wang and collaborators in 2007, using phosphopeptide affinity proteomic analysis described Abraxas as a novel protein that binds directly to the BRCT repeats of BRCA1 through a phospho-SPxF motif. Additionally, Abraxas contains a MPN domain that interacts with ubiquitin (Ub), a coiled-coil domain that binds BRCC36 and an ATM/ATR phosphorylation site (T368). Abraxas recruits Rap80 protein to form the third BRCA1 complex. Both Abraxas and Rap80 are essential factors in DNA damage resistance, repair and cell cycle checkpoint. Moreover, at least three additional components form this complex: NBA1 (also known as MERIT40), BRE (also known as BRCC45) and BRCC36 (2-9). Abraxas is the central organizer that mediates the interaction with BRCA1 and bridges the interaction of each member of the complex. Rap80 binds specifically to k63-linked polyUb chains that are mainly implicated in protein-protein interaction, protein function and subcellular localization. Furthermore, the deubiquitinating enzyme BRCC36 has activity specifically toward these chains (7,10). Even though the exact role of this complex is still not clear, it is believed that it may play an important role in the recruitment of BRCA1 to DNA damage sites through the recognition of ubiquitinated proteins (9).

Cells lacking Abraxas or Rap80 show defects in the G2/M control checkpoint, reduction homologous recombination induced by DSBs and sensitivity to the killing effect of ionizing radiation (IR), although less sensitive than BRCA1-depleted cells. These fates suggest that Abraxas and Rap80 mediate only a subset of BRCA1 functions and that additional BRCA1 complexes playing part of the roles of BRCA1 in maintaining genome stability and tumor suppression exist.

BRCA1 appears as the central mediation mechanism that maintains genome stability in response to DNA damage. Mutations in this gene have been described as clinically relevant. These mutations, however, account for no more than 20% of familiar breast cancer cases (11). This suggests that additional germline mutations are still unknown. Among these, the different members of the BRCA1 complexes are promising candidates because of their essential role in the maintenance of the BRCA1 functions.

Many of the BRCA1 mutations take place in the BRCT repeats, the domain of phosphopeptide recognition with

the capability to bind phosphorylated proteins which are essential to the functions of BRCA1. Frequently, these mutations have clinical relevance. The M1775R BRCA1 mutation disrupts the integrity of the BRCT repeat motif and avoids the interaction with Abraxas (9).

In addition to the described BRCA1 mutations, others have been found for members of the complexes. An alteration in the Rap80 UIM domain impairs the DNA damage response function (12). Common genetic variants in MERIT40 have been related to a predisposition for ovarian and hormone negative breast cancer (13,14). Germline mutations that disrupt Bach1 activity or impair the association with BRCA1 have been identified in breast cancer, indicating its role as a tumor suppressor (15). Other mutations relate this gene to ovarian and breast cancer risk (16). Furthermore, mutations of CtIP that generate a truncated form of the protein cause genome instability disorders and are associated with cancer predisposition (17).

The work of Solyom and collaborators (18) presents a novel germline mutation in Abraxas exclusively associated with familial breast cancer which disrupts the DNA damage repair functions of BRCA1. The authors screened 125 northern Finnish breast cancer families for mutations in Abraxas. Only one of the changes found has been identified by computer simulation to result in functional changes in the protein. The c.1082G>A alteration results in Arg361Gln (R361Q) changes on a putative nuclear localization signal (4). This mutation was detected in three of the families studied, but was absent in healthy controls and in the cohort of breast cancer without familiar cancer background. These data suggest that this Abraxas variant specifically correlates with familiar cancer and segregates with disease within families. The R361Q Abraxas mutant reduces the biological function in the DNA damage response in part by decreasing the efficacy of homology-directed DSB repair as a result of the defective G2 checkpoint in response to IR. R361Q maintains the interaction with BRCA1 and other components of the complex as probed by coimnunoprecipitation. Unlike wild-type, however, its location is primarily cytoplasmic and not nuclear. It suggests a deficiency in DNA repair because of an impaired nuclear location. Abraxas emerges as a new cancer susceptibility gene in breast cancer and other malignancy types in a manner similar to that of BRCA1 and BRCA2.

The identification of mutations in the components of the BCRA1 complexes is a promising strategy in the clinical setting. It has been described that PARP inhibitors (19) induce increased cellular apoptosis in patients with BCRA1 or BCRA2 mutations (20). Presumably, these drugs would act optimally on carriers of other mutations in components of the complexes. Complementary studies are necessary to assess the significance of mutations in Abraxas and other members of BRCA1 complexes in breast cancer diagnosis and in their possible use in treatment.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Zhu Q, Pao GM, Huynh AM, et al. BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. Nature 2011;477:179-84.
2. Feng L, Huang J, Chen J. MERIT40 facilitates BRCA1 localization and DNA damage repair. Genes Dev 2009;23:719-28.
3. Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science 2007;316:1202-5.
4. Kim H, Huang J, Chen J. CCDC98 is a BRCA1-BRCT domain-binding protein involved in the DNA damage response. Nat Struct Mol Biol 2007;14:710-5.
5. Liu Z, Wu J, Yu X. CCDC98 targets BRCA1 to DNA damage sites. Nat Struct Mol Biol 2007;14:716-20.
6. Patterson-Fortin J, Shao G, Bretscher H, et al. Differential regulation of JAMM domain deubiquitinating enzyme activity within the RAP80 complex. J Biol Chem 2010;285:30971-81.
7. Sobhian B, Shao G, Lilli DR, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science 2007;316:1198-202.
8. Wang B, Hurov K, Hofmann K, et al. NBA1, a new player in the Brca1 A complex, is required for DNA damage resistance and checkpoint control. Genes Dev 2009;23:729-39.
9. 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.
10. Cooper EM, Cutcliffe C, Kristiansen TZ, et al. K63-

specific deubiquitination by two JAMM/MPN+ complexes: BRISC-associated Brcc36 and proteasomal Poh1. Embo J 2009;28:621-31.
11. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. Br J Cancer 2000;83:1301-8.
12. Nikkilä J, Coleman KA, Morrissey D, et al. Familial breast cancer screening reveals an alteration in the RAP80 UIM domain that impairs DNA damage response function. Oncogene 2009;28:1843-52.
13. Antoniou AC, Wang X, Fredericksen ZS, et al. A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. Nat Genet 2010;42:885-92.
14. Bolton KL, Tyrer J, Song H, et al. Common variants at 19p13 are associated with susceptibility to ovarian cancer. Nat Genet 2010;42:880-4.
15. Cantor SB, Guillemette S. Hereditary breast cancer and the BRCA1-associated FANCJ/BACH1/BRIP1. Future Oncol 2011;7:253-61.
16. Rafnar T, Gudbjartsson DF, Sulem P, et al. Mutations in BRIP1 confer high risk of ovarian cancer. Nat Genet 2011;43:1104-7.
17. Qvist P, Huertas P, Jimeno S, et al. CtIP Mutations Cause Seckel and Jawad Syndromes. PLoS Genet 2011;7:e1002310.
18. Solyom S, Aressy B, Pylkäs K, et al. Breast cancer-associated Abraxas mutation disrupts nuclear localization and DNA damage response functions. Sci Transl Med 2012;4:122ra23.
19. Rojo F, García-Parra J, Zazo S, et al. Nuclear PARP-1 protein overexpression is associated with poor overall survival in early breast cancer. Ann Oncol 2012;23:1156-64.
20. Pessetto ZY, Yan Y, Bessho T, et al. Inhibition of BRCT(BRCA1)-phosphoprotein interaction enhances the cytotoxic effect of olaparib in breast cancer cells: a proof of concept study for synthetic lethal therapeutic option. Breast Cancer Res Treat 2012;134:511-7.

Cite this article as: Eroles P. Members of the BRCA1 complexes as new susceptibility genes for breast cancer. Transl Cancer Res 2013;2(2):103-105. doi: 10.3978/j.issn.2218-676X.2013.04.06

APOBEC3B spreads somatic mutations in breast cancer genome

Giusi Irma Forte

IBFM CNR - LATO, Contrada Pietrapollastra Pisciotto, 90015, Cefalù (PA), Italy

Correspondence to: Giusi Irma Forte, PhD. IBFM CNR - LATO, Contrada Pietrapollastra Pisciotto, 90015, Cefalù (PA), Italy. Email: giusi.forte@ibfm.cnr.it.

Submitted Mar 11, 2013. Accepted for publication Apr 10, 2013.
doi: 10.3978/j.issn.2218-676X.2013.04.03
View this article at: http://www.thetcr.org/article/view/1079/html

Cancer progression is characterized by a well-known step-wise somatic mutation accumulation mechanism, by which tumorigenic cells acquire a gradually more malign phenotype and override cell death signals.

During our genomic era, the landscape of somatic mutations has been largely studied in several types of carcinomas, producing evidences that the mutation spectra can be different at individual and clonal level, though some of these modifications, as well as, some chromosomal alterations could be repeatedly observed in similar types of tumours (1-4). Moreover, flow-sorted tumour cell clones, separated according DNA index and analysed for their genome-wide allelic state, show a gain in somatic mutations and chromosomal alterations in those cell fractions having a higher H-index, even if the clones share common alterations previously inserted (5). Then, the mutation accumulation on focused targets, such as oncogenes or cyclins, as well as, the unfocused genome allelic state changes are signs of disease progression and correlate with a gradually worse prognosis.

However, despite the particular importance of this topic to highlight tumour-progression mechanisms, few information have been collected till now on key factors implicated in cancer somatic mutation spreading.

A decade ago, a protein family composed of the 11 polynucleotide cytosine deaminases: APOBEC1, activation-induced deaminase (AID), APOBEC2, APOBEC3 proteins (A3A, A3B, A3C, A3D, A3F, A3G and A3H) and APOBEC4, having functional capacity to induce mutations in DNA and RNA of living cells, have been discovered (6). These genes are derived from a complex series of duplication and fusion across evolution (6), each one having different and roughly specific targets from cytoplasm to nucleus. Deaminating cytosine to uridine, the APOBEC family members exert a series of fundamental physiological positive activities into cells; some of which are well characterized: APOBEC1 is known to edit the ApoB pre-mRNA (7), whereas the AID conducts an antigen-driven diversification of already rearranged immunoglobulin by somatic hypermutation, gene conversion and Ig class-switch recombination (8). Less is known about APOBEC2 and APOBEC4, which do not seem to display functional activity, instead the APOBEC3 proteins exert a more pronounced retroviral replication inhibition capacity (9), noteworthy is the APOBEC3G role in HIV restriction (10). Such important deaminase family tasks, protecting cells against retroviral attack and enhancing the Ig-molecules ability to recognize a higher number of epitopes, have a dark side of the medal, concerning their role in cancer initiation and evolution, due to a non-controlled C-to-U modification spreading across the genome (11).

In breast cancer, the APOBEC3B (A3B) protein has been recently characterized as a certain enzymatic source of C-to-T mutation dissemination into the genome (12).

Among the seven APOBEC3 members analysed, the A3B mRNA was the only one highly expressed with a fold change ≥3 in 28 out 38 breast cancer cell lines and a fold change ≥10 in 12 out 38 cell lines assayed. Among these, MDA-MB-453, MDA-MB-468 and HCC1569 showed the higher up-regulation, corresponding to fold changes of 20, 21 and 61, respectively. The increased expression levels are hypothesized to be due to such transduction event, as no CpG island modifications or copy number variations have been reported for this gene.

Importantly, during cell cycle the APOBEC3 proteins have different subcellular localizations: cell-wide, cytoplasmic or nuclear, implying that only a subset of APOBEC3s contacts nuclear DNA (13). In the case of MDA-MB-453, MDA-MB-468 and HCC1569 cell lines, a nuclear localization has been observed for an A3B

fluorescent fusion protein. Furthermore, by using a DNA C-to-U fluorescent based assay, a consistent DNA editing has been demonstrated, predominantly regarding cytosine of TC dinucleotides, similarly to a retroviral hypermutation signature caused by A3B overexpression (14).

To address the dimension of the endogenous A3B contribute in DNA C-to-U modification, the genomic uracil load of MDA-MB-453 and HCC1569 has been quantified in comparison to that observed after transfection with an A3B knockdown system, finding an uracil load reduction of 70% and 30%, respectively, in transfected with respect to non-transfected cells. From this observation it has been calculated that, approximately, 30,000 to 60,000 uracils are inserted by A3B per haploid genome.

A corroboration has been obtained measuring the mutation accumulation in engineered MDA-MB-453 and HCC1569 cells expressing the herpes simplex virus type 1 TK gene, which makes the cells sensitive to ganciclovir, then transfecting them with an A3B knockdown system or a control construct. Expanded sub-clones were subjected to ganciclovir selection and resistant cells were grown to form visible colonies, showing that cells with upregulated A3B accumulate 3-5-fold more mutations.

In addition, it has been studied if the mutation accumulation can be considered a targeted or a genome-wide mechanism. In cells highly expressing A3B, TP53 and c-MYC appeared more mutated than CDKN2B, suggesting that such genomic regions are preferentially susceptible to the enzymatic deamination. Other base substitution mutations were rare.

Other effects of the A3B expression have been characterized in engineered Human Embryonic Kidney (HEK) 293 cells, stably expressing A3B. In these cells, besides C-to-T mutations, delayed cell-cycle arrest, abnormal anucleate and multinucleate cell formation, c-H2AX focus formation, DNA fragmentation and eventual cell death have been revealed.

Finally, similar results to those described in cell lines have been searched in primary breast tumours.

A confirmation of the A3B exclusive role in breast cancer cells derive from the observation that only the A3B protein, among the other APOBEC3 members tested, is found upregulated by ≥3 fold in 20 out of 52 primary tumours compared to matched normal tissue and in 44 out of 52 tumours compared to the reduction mammoplasty tissue.

Then, the availability of RNA sequencing (RNA-seq) and somatic mutation databases allowed making a comparison of *in-vivo* and *in-vitro* A3B signatures. This analysis revealed that, whereas C-to-T frequency is low (20%) and random in liver tumour, it is high (80%) in melanoma, focused at dipyrimidines, as expected due to ultraviolet load. Instead, breast cancer is featured by an intermediate (40%) C-to-T frequency, preferentially focused at trinucleotide sites, miming the *in-vitro* A3B signature. Also, the same approach allowed to observe that the A3B upregulation is strongly correlated to mutation accumulation and TP53 inactivation in breast primary tumours and cancer cell lines, being perhaps the TP53 inactivation crucial to override DNA damage stop signals triggered by A3B.

According to the literature, the cytosine deaminase involvement in cancer mutation accumulation and genomic instability is a still not deeply explored argument, as a relative limited number of studies have been dedicated to this topic (14-17), nonetheless their involvement in cancer development is more certain than just supposed.

In this respect, the APOBEC members could represent key factors for targeted therapies, to block mechanisms producing genome-wide alterations, which drive tumour development.

The study described herein makes evident the necessity of future scientific efforts to deeply highlight the cytosine deaminase family contribution and that one of similar molecules in specific tumour types, the causes of their activation and the correlation with grading and prognosis.

Acknowledgements

Thanks to the researchers of IBFM CNR - LATO for helpful discussions. Special thanks to Dott. Alexandros Xynos for English editing.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Middeldorp A, van Eijk R, Oosting J, et al. Increased frequency of 20q gain and copy-neutral loss of heterozygosity in mismatch repair proficient familial colorectal carcinomas. Int J Cancer 2012;130:837-46.
2. Gray J, Druker B. Genomics: the breast cancer landscape. Nature 2012;486:328-9.
3. Bravatà V, Cammarata FP, Forte GI, et al. "Omics" of HER2-Positive Breast Cancer. OMICS 2013;17:119-29.

4. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.
5. Corver WE, Middeldorp A, ter Haar NT, et al. Genome wide allelic state analysis on flow-sorted tumor fractions provides an accurate measure of chromosomal aberrations. Cancer Res 2008;68:10333-40.
6. Conticello SG. The AID/APOBEC family of nucleic acid mutators. Genome Biol 2008;9:229.
7. Blanc V, Davidson NO. APOBEC-1-mediated RNA editing. Wiley Interdiscip Rev Syst Biol Med 2010;2:594-602.
8. Pavri R, Nussenzweig MC. AID targeting in antibody diversity. Adv Immunol 2011;110:1-26.
9. Izumi T, Shirakawa K, Takaori-Kondo A. Cytidine deaminases as a weapon against retroviruses and a new target for antiviral therapy. Mini Rev Med Chem 2008;8:231-8.
10. Monajemi M, Woodworth CF, Benkaroun J, et al. Emerging complexities of APOBEC3G action on immunity and viral fitness during HIV infection and treatment. Retrovirology 2012;9:35.
11. Schmitz KM, Petersen-Mahrt SK. AIDing the immune system-DIAbolic in cancer. Semin Immunol 2012;24:241-5.
12. Burns MB, Lackey L, Carpenter MA, et al. APOBEC3B is an enzymatic source of mutation in breast cancer. Nature 2013;494:366-70.
13. Lackey L, Law EK, Brown WL, et al. Subcellular localization of the APOBEC3 proteins during mitosis and implications for genomic DNA deamination. Cell Cycle 2013;12:762-72.
14. Barlow JH, Faryabi RB, Callén E, et al. Identification of early replicating fragile sites that contribute to genome instability. Cell 2013;152:620-32.
15. Ebrahem Q, Mahfouz RZ, Ng KP, et al. High cytidine deaminase expression in the liver provides sanctuary for cancer cells from decitabine treatment effects. Oncotarget 2012;3:1137-45.
16. Andersen A, Jones DA. APC and DNA demethylation in cell fate specification and intestinal cancer. Adv Exp Med Biol 2013;754:167-77.
17. Nik-Zainal S, Alexandrov LB, Wedge DC, et al. Mutational processes molding the genomes of 21 breast cancers. Cell 2012;149:979-93.

Cite this article as: Forte GI. APOBEC3B spreads somatic mutations in breast cancer genome. Transl Cancer Res 2013;2(2):97-99. doi: 10.3978/j.issn.2218-676X.2013.04.03

From genomic data analysis to drug development: a new generation of trials using molecular marker assessment in breast cancer

Sophie Doisneau-Sixou[1,2], Nadia Harbeck[1]

[1]Breast Center, Department of Obstetrics & Gynecology, and Comprehensive Cancer Center, University of Munich, Munich, Germany; [2]Faculty of Pharmacy, University Paul Sabatier Toulouse III, Toulouse, France

Correspondence to: Prof. Sophie Doisneau-Sixou. Breast Center and Comprehensive Cancer Center, University of Munich, Campus Innenstadt, Maistraße 11, 80337 Munich, Germany. Email: Sophie.DoisneauSixou@med.uni-muenchen.de; Prof. Nadia Harbeck. Breast Center, Department of Obstetrics & Gynecology, and Comprehensive Cancer Center, University of Munich, Campus Großhadern, Marchioninistr. 15, 81337 Munich, Germany. Email: nadia.harbeck@med.uni-muenchen.de.

Abstract: With the advent of molecular subtypes in breast cancer, the landscape of clinical trials and drug development is rapidly changing. Molecular screening identifies numerous mutations but actually druggable targets are few and far between. Thus, new clinical trial concepts are needed which are feasible in clinical practice and successful from the point of view of drug development. This article highlights the evolution of the sequencing technologies, the current molecular screening efforts and their impact on drug development as well as novel successful trial designs, focusing on the hormone receptor (HR) positive breast cancer patients. The range of mutations to identify in order to adapt the treatment to each patient and limit the resistance mechanisms is quite wide already, but theoretical or practical restriction may have to be considered to optimize the development of such adaptive combinations of targeted therapies.

Keywords: Breast cancer; clinical trial; druggable gene; driver mutation; high-throughput technology

Submitted May 24, 2014. Accepted for publication May 26, 2014.
doi: 10.3978/j.issn.2304-3865.2014.05.15
View this article at: http://dx.doi.org/10.3978/j.issn.2304-3865.2014.05.15

Introduction

Breast cancer is the leading cause of cancer death for women worldwide (1,2) and breast cancer diversity is a crucial point to consider accurate categorization and treatment of patient subpopulations (3,4). Current breast cancer classification and assessment remain strongly based on clinicopathological criteria (patient age, tumor size, lymph node invasion, histological type, and grade) (5). Besides, histological characterization is driven by development of targeted therapies, including endocrine and anti-HER2 therapies [estrogen receptors (ER) and progesterone receptors (PR) and HER2 expressions respectively], and by proliferation index evaluation (Ki67).

In parallel to chemotherapy, personalized treatments were initiated with the first targeted therapy, tamoxifen in the 1970's. This antiestrogenic treatment was followed later by fulvestrant and aromatase inhibitor commercialization. About 25 years later, the antiHER2-antibody trastuzumab was the next step in sub-dividing large cohorts of patient populations for personalized treatment. The efficiency of trastuzumab was based on the specific amplification of the *ERBB2* gene in some breast cancers. Since then, no other targeted drug could add such benefits, using gene mutations or rearrangements specific for breast cancers.

Besides individual mutation analysis, gene expression microarrays have allowed simultaneous expression analysis of thousands of genes in a single experiment in order to create molecular tumor profiles. In 2000, Perou and colleagues published the first classification of breast cancers into four intrinsic subtypes, based on the gene expression profiling from unsupervised hierarchical analysis of complementary DNA (cDNA) microarray data (6,7): luminal A, luminal

B, HER2-enriched (HER2-E), and basal-like. New classifications have emerged defining now two additional subtypes: normal breast-like and more recently, the claudin-low or mesenchymal-like subtype (8,9). Although the so-called triple negative tumors are mainly represented among the basal-like subtype, the two subtypes do not completely overlap (10-12). These new intrinsic classifications are in permanent evolution and new signaling pathway identification for each subtype has proven to be useful for drug discovery and for identification of new molecular markers, such as luminal androgen receptor (AR) subtypes (4,12,13).

At primary diagnosis, approximately 60% of patients with invasive breast cancer are node-negative, with 94% of these expected to have no distant metastasis at 10 years if treated by locoregional and adjuvant systemic therapy (www.tumoregister-muenchen.de). The HER2-negative, hormone receptor (HR) positive population can be intrinsically divided in subpopulations with low, average, and high risks of recurrence. Adjuvant therapy, either endocrine or chemotherapy, should be decided accordingly but risk stratification based on only clinicopathological parameters may cause under- or overtreatment. Since 2007, international guidelines [e.g., St Gallen, American Society of Clinical Oncology (ASCO), Arbeitsgemeinschaft Gynäkologische Onkologie (AGO)] have recommended to combine validated protein or gene expression tests reflecting the intrinsic tumor characteristics to improve the clinical risk stratification (14,15). Genomic information is now combined with the clinicopathological characteristics to estimate recurrence risk (prognostic value) and to predict therapy efficacy (predictive value). Identifying high-risk patients for recurrence and administering optimal therapies but avoiding overtreatment are major issues in breast cancer management as therapy resistance and metastasis processes need to be effectively targeted for improvement of survival (16).

Endocrine therapy resistance is a constant threat during patient treatment, as tumor cells may escape the antiestrogenic drugs by adapting the ER-pathway and/or by switching from their usual signaling pathway to an alternate one (17-21). Moreover, the intrinsic phenotypic and genotypic heterogeneities of the primary tumors and of the micrometastases are omitted whenever a single biopsy from the primary tumor is performed to establish the treatment (22-24). Other resistance mechanisms clearly involve miRNA and epigenetic regulations [for review (25-28)]. Recent advances in next-generation sequencing technologies provide the opportunity for new prospective clinical trials that should ideally be affordable as routine tests, using limited amounts of material. This may not be an utopia any longer to allow for each patient, the identification of druggable genes, i.e., targets of efficient drugs or drug combination against resistance mechanisms specifically hormone therapy ones (13,29,30).

We will present in this review a brief history of the genome analysis evolution over the last 15 years, followed by their main applications in breast cancer sub-typing and clinical trials for HR positive breast cancer, and their implications for drug development.

Genomic characterization of breast cancers

The human genome project [for review (31)]

The human genome project was initiated in 1986, with the hypothesis, among others, that some of the cellular genes could drive cancer apparition and progression. The impressive amount of data collected over 13 years, after the project completion in 2003, was only the basement for the elucidation of the numerous genes involved in each individual cancer (32). Within the last 10 years, another revolution happened with the development of the large-scale or high-throughput studies of mutations and other gene alterations, leading far beyond the traditional Sanger sequencing technique.

Next generation sequencing (NGS) and actual high-throughput genomic assays

From 2005, NGS or massive parallel sequencing (MPS) technologies allowed time and money affordable analyses as they are applicable on tumor material to generate information on the whole genome within few days and thousands euros (33-35). They both rely on the sequencing of a sequence library of the targeted nucleic acid, followed by synthesis sequencing. The huge amount of data has then to be processed through powerful and rapid data analysis tools. After the current marker leader, Illumina HiSeq sequencer, three major new sequencing platforms have been released in 2011, Ion Torrent' PGM, Pacific Biosciences' RS and Illumina MiSeq (36). The applications of NGS are extending every day not only to DNA, but to RNA and epigenome sequences as presented below, competing with the microarray techniques.

The Whole Genome Sequencing (WGS) developed enough in the recent years to generate data that considerably

improve the diagnostic, prognostic and treatments of cancer patients (37). It allows the analysis of genes and regulatory elements with all types of mutations and aberrations from single base point mutations to copy number aberrations, DNA rearrangements or chromosome-scale amplification.

More recently, the amplification and sequencing of each coding exon [Whole Exome Sequencing (WES)] of 18,000 genes clearly evidenced and confirmed some "cancer" genes (e.g., TP53 for breast cancers) and recurrent "driver" mutations to distinguish from "one-off" mutations (38). Although the WES only identifies point mutations and small insertion/deletion in the coding regions of the genes, but not larger scale DNA rearrangements, it may become a routine analysis due to its lower cost and number of data to process and store (just over 1% of the whole genome), compared to WGS. The routine feasibility of WES allowed its integration in numerous large scale analyses of tumors from up to 500 patients, for rational decision making.

The cDNA sequencing or RNA-seq. was then the next approach to explore any alteration from the transcriptome, by sequencing mRNA, mi-RNA and other RNAs. RNA-seq provides first of all gene expression levels, but information on the aberrant splicings, chimeric gene fusion transcripts features, too (39-41). Nonetheless, the reverse transcription step is not reliable enough to allow the characterization of point mutations.

The targeted sequencing of specific genes or regions, known to be highly involved in the disease development or progression may be a more affordable and rapid option than the WGS and WES (42). The development of such disease-targeted sequencing is a promising approach to develop.

The technology for a systematic analysis of chromatin and epigenetic modifications in cancer cells is still in development but encouraging data arose from the ENCODE Project Consortium's genome-wide (43).

Other "omics" technologies

In parallel to the DNA and RNAs analysis, high-throughput sequencing technologies have been developed for protein comprehensive analysis, such as the Reverse-Phase Protein Array (RPPA) (44). The proteome and the phosphoproteome analysis generate very sensible data for the functional interpretation of the alterations observed in the signaling pathways of tumor cells.

Using these powerful tools, The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov), the Cancer Genome Project (http://www.sanger.ac.uk/genetics/CGP), the International Cancer Genome Consortium [(ICGC), (45)] and others initiated the complex comprehensive analysis of the mutations present in various cancers (46,47). By using multiplexed screens, i.e., multiple high throughput genotyping platforms, the data of combined powerful technologies are pooled for an integrated analysis. The open questions for many cancer researchers today may be the criteria to select NGS rather than microarray technology for each definite project or trial to set up. There is no clear answer as both have advantages and limitations. The main advantages of microarrays are still the much lower cost of the analyses, the quicker preparation of the samples and analysis of the data, and the 20-year expertise of many laboratories worldwide. Regarding the NGS, each application has to be considered independently: for RNA-Seq, the main advantage over microarrays may be that it covers all aspects the transcriptome (non-coding RNAs, splice junctions, novel transcripts, etc) without previous definition of specific probes. Similarly, for the methylation projects, the NGS is much more complete but much more expensive too at the moment. Consequently, a good balance may be to prefer the NGS approach for wide screenings and then the microarrays for rapid profiling. Any progression of the NGS technologies towards diagnostic application will need strong validation through clinical trials. We will now present the TCGA breast cancer project analysis that cleverly combined both technologies.

TCGA breast cancer project

The TCGA breast cancer project was conducted to analyze 510 breast cancers on six platforms for DNA sequencing (WES by MPS technology), DNA copy numbers [Affymetrix 6.0 Single Nucleotide Polymorphism (SNP) arrays], DNA methylation (Illumina Infinium DNA methylation chips), mRNA expression (Agilent mRNA expression microarrays), miRNA sequencing, and protein and phosphoprotein expression (RPPA) (30,32,48).

In the initial four breast cancer subtypes defined by Perou in 2000 (6), the luminal breast cancers have been first described as having a gene expression signature that includes estrogen receptor 1 (*ESR1*), GATA-binding protein 3 (*GATA-3*), forkhead bow protein A1 (*FOXA1*), B-cell chronic lymphocytic leukemia (CLL)/lymphoma 2 (*BCL2*), X-Box binding protein 1 (*XPB1*) and the myeloblastosis gene (*MYB*), which are highly characteristic of the luminal epithelial cells in the inner layer of a normal breast duct. Luminal B cells differ from luminal A in their lower levels of these luminal gene expressions, higher level of

proliferation genes and a worse clinical outcome (7). The HER2-E breast cancers expressed higher levels of HER2 and of growth factor-bound protein 7 (GRB7), but not all HER2-E cells are HER2 amplified and they can belong to the luminal or basal-like subtypes in some cases.

The TCGA innovative large scale screening demonstrated 30,626 mutations in the 510 analyzed tumors. The four main subtypes were confirmed by presenting striking differences in their mutation spectra. The luminal breast cancers exhibited a lower mutation rate than the two other subtypes but the most diverse and recurrent Significantly Mutated Genes (SMG), i.e., genes mutated more frequently than the background mutation rate, suggesting their causative role in the specific initiation and development of the luminal cancers. Luminal A breast cancers were characterized by a high frequency of mutations in the phosphatidylinositol-4,5-biphosphonate 3-kinase, catalytic subunit alpha (*PIK3CA*) gene (45%), a low frequency of mutation of TP53 (12%) and multiple SMGs such as mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*), GATA3, cadherin 1 (*CDH1*), and mitogen-activated protein kinase kinase 4 (*MAP2K4*). *MAP2K4* mutation was observed almost exclusively in this subtype.

Compared to the luminal A subtype, luminal B breast cancers exhibited a higher rate of *TP53* mutation (29%) and a lower rate of *PIK3CA* mutation (29%). The TP53 antagonist Murine Double Minute 2, MDM2, exhibits a gene amplification associated to the luminal B status (30%).

Regarding both the luminal A and B subtypes, *Cyclin D1* is amplified in 40% of them (29% in A and 58% in B), the fibroblastic growth factor receptor 1 (*FGFR1*) gene is frequently amplified and the histone trimethyltransferase, mixed lineage leukemia gene 3 (*MML3*), is found mutated too (8% in A and 6% in B). Unique to luminal subtypes are the mutations of *GATA3* (14% in A and 15% in B) and to a lower extent the forkhead bOX protein A1 (*FOXA1*) and of the RUNt-related transcription factor 1 (*RUNX1*), the three of them being important for the genomic activity of ER (48). Similarly, it was confirmed that the serine threonine kinase MAP3K1 that regulates the extracellular signal-regulated kinase (ERK) and c-JuN amino-terminal kinase (JNK) kinase pathways and the nuclear-factor-kappa-B (NFκB) signaling, is mutated almost exclusively in the luminal subtypes (13% in A and 5% in B), with a mutual exclusivity with *MAP2K4* mutations, the serine threonine kinase downstream of MAP3K1 that activates JNK. Both kinases appear then as driver events of this subtype, although MAP3K1 mutations are associated to a lower tumor grade and proliferation index.

As expected, the HER2-E subtype was characterized by 80% of *HER2* amplification, plus highly mutated *TP53* (72%) and *PIK3CA* (39%) genes, and a much lower frequency of the other SMGs.

In comparison, the basal-like subtype carried the highest rate of *TP53* mutation (80%), without SMGs except *PIK3CA* (9%).

It is essential to distinguish the driver mutations from passenger one (29,48); the driver mutations being recurrent mutations observed at higher frequency than expected from background mutation across tumors. They may be the first one to target in drug development. To date, we know that breast cancer genes make up to 25 to 30% of the heritability, including BRCA1, BRCA2, CHEK2, ATM, PALB2, BRIP1, TP53, PTEN, CDH1 and STK11 (49). Genome-Wide Association Studies (GWAS) and international consortia confirmed common polymorphisms individually associated with breast cancer risk, adding a further 14% (49-51). SNPs complete this percentage to 50%, leaving around 50% of the breast cancer without any heritability (48).

The druggable genes against endocrine resistance

As cancer genome sequencing consortia are providing thousands of somatic mutations from the analysis of hundreds of patients, new specific powerful tools are developed in parallel to integrate all the data and rank the priorities of the various candidate druggable genes (52-54). For example, dGene is an annotation tool specific for cancer genome sequencing data, designed to allow any cancer researcher to rapidly identify genes belonging to 1 of 10 druggable classes frequently targeted in drug development, without any biostatistician support (55). The seven druggable classes that have been extracted from the analysis of 77 breast cancer tumors from the TCGA breast cancer project (56) are the G-protein coupled receptors, PI3K receptors, protease inhibitors, proteases, phosphotyrosine phosphatases, serine/threonine kinases and tyrosine kinases.

Introduction of molecular subtypes opened new ways for clinicians to classify, diagnose and treat breast cancers (8,13,57) and endocrine therapy resistance is now clearly defined through guidelines (15). Nonetheless, the diversity and complexity of the biological pathways involved in the endocrine resistance mechanisms are very high limitations. Moreover, besides ERα signaling pathway, other nuclear receptor, essentially PR, ERβ and the AR may be involved in ERα activity modulation (58-63).

Concentrating on the HR positive/luminal tumors, the TCGA data demonstrated that the *PIK3CA* gene is the most mutated gene in these tumors. The PI3K/AKT/mTOR pathway is involved in essential cell functions including cell growth, proliferation, survival, migration and angiogenesis. As the *PIK3CA* mutations trigger a gain of function, *PIK3CA* is a druggable gene. Indeed, the most recent new drug available to overcome endocrine resistance is the rapamycin analog everolimus, a mTOR inhibitor (64), mTOR being a pathway component downstream of PIK3CA. Nonetheless, when everolimus was combined with the aromatase inhibitor exemestane in endocrine resistant advanced breast cancers, *PIK3CA* mutation status failed to predict benefit outlining the complexity of the interpretation and validation of the genomic data generated (30,65).

Many clinical trials are evaluating PI3K pathway inhibitors, because the direct targeting of PIK3CA seems to be the most promising approach and may lead to molecules with a better tolerability than the rapamycin analogues [for review (30)].

Apart from the PI3K pathway mutations, most of the other gene mutations trigger a loss of function, with predominantly tumor suppressor genes such as the most frequent apoptotic *TP53*. Although mutations of *TP53* cannot be targeted, because they are significantly enriched in the luminal B subtypes (29% compared to 19% in the luminal A) and in tumors with a higher histological grade, a potential role as prognostic marker is suggested (30,32,56,66-69). Moreover, the TP53 antagonist MDM2 exhibits a gene amplification associated to the luminal B status and to endocrine therapy resistance. This was the basis for MDM2 inhibitor development, now in phase I [NCT01462175 (52)].

GATA3 mutations are then the third most common mutations in luminal tumors, following *PIK3CA* and *TP53*. These inactivating mutations do not appear to change the proliferation level except in response to neoadjuvant aromatase inhibitor therapy, suggesting that GATA3 mutation could serve as predictor of aromatase inhibitor sensitive disease (56).

The cyclin dependant kinases 4 and 6 (CDK4/6) normally bind to Cyclin D1 to allow pRb phosphorylation and G1/S cell cycle progression. As Cyclin D1 is amplified in 40% of the luminal cancers, especially in luminal B, inhibitors of CDK4/6 have successfully been tested in combination with the aromatase inhibitor letrozole in a completed phase II clinical trial with advanced or metastatic breast cancers patients (30,32,56,66-69).

Clinical trials combining entinostat (a histone desacetylase inhibitor and epigenetic modulator) with the aromatase inhibitor exemestane proved to be active on HR positive advanced breast cancer patients too (30). This outlines the importance of target genes involved in epigenetic regulation, such as the histone trimethyltransferase *MML3* mutated in luminal cancers (30,32,56,66-69).

Amplification of the *FGFR1* gene is another relatively frequent event in luminal cancers and both antibodies and small inhibitors are developed at the moment (52).

Because multiple inhibitors of specific pathways are proposed (e.g., PI3K/PKB/mTOR), a clear ranking of the specific class and inhibitor needed for each tumor is needed (25,29), remembering that low frequency mutations may be very relevant to target. Other necessary approaches are the functional validation of the relevance of the candidate gene, but cell lines, primary or xenograft cell lines are useful but still limited models. The only ultimate demonstration of the relevance of a definite targeted treatment, in agreement with the related genotype of a subpopulation of patients, will be prospective clinical trials that allow the adaptation of the treatment according to the tumor genotype evolution.

The new generation of prospective clinical trials

In parallel to the breast cancer sub-typing by gene expression profiling, multigene assays have been developed based on a specific prognosis and/or predictive signature [for review (70)]. Among the most widely used platforms are MammaPrint® (microarray) and Oncotype DX® (qRT-PCR), but several other assays are either marketed or in development. Based on numerous retrospective studies, the most advanced tests are now in prospective clinical trials in order to reach a high level of evidence (3,4,71). The development of comprehensive affordable high-throughput genomic platforms was the prerequisite to conduct prospective clinical marker trials in molecularly defined patient populations, and these technologies are now ready to follow the same path as the multigene assays to eventually become decisive treatment decision tools.

Few prospective clinical therapy trials using molecular markers for patient stratification have already been completed (MINDACT for validation of the Mammaprint® assay and TAILORx and WSG Plan B trials for further validation of Oncotype DX®) and results are still expected (72-74). Besides ongoing trials are the RxPONDER/SWOG S1007 (Oncotype DX® in 9,400 node-positive disease) and WSG-Adjuvant Dynamic marker-Adjusted Personalized

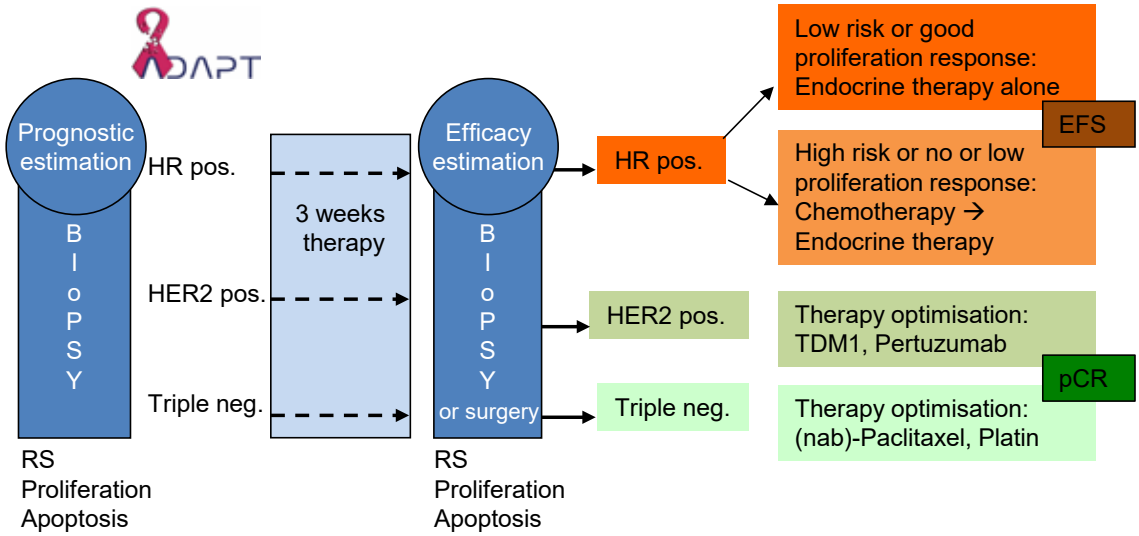


Figure 1 Design of the ongoing ADAPT umbrella trial. Patients are allocated to one of the distinct ADAPT sub-trials, depending on the HR and HER2 status of the tumor. Patients are treated subtype-specific according to their individual disease, starting with subtype-specific induction therapy for 3 weeks. Central pathological assessment includes HR, HER2 and Ki-67. For HR positive tumors, an initial RS is determined by Oncotype DX®. After induction therapy, efficacy estimation is performed using repeat core biopsy or surgical specimen. Either EFS or pCR are assessed according to the sub-trials. ADAPT, Adjuvant Dynamic marker-Adjusted Personalized Therapy; HR, hormone receptor; RS, recurrence score; EFS, event-free survival; pCR, pathological complete response; T-DM1, Trastuzumab-Emtansine.

Therapy (ADAPT) trials (Oncotype DX® in 4,936 invasive early breast cancers from pre-/post-menopausal women with node-negative and -positive disease). The WSG-ADAPT trial (ADAPT trial optimizing risk assessment and therapy response prediction in early breast cancers) is set up as a prospective, multi-center, controlled, non-blinded, randomized phase II/III trial, launched in May 2012 by the WSG, (www.wsg-online.com) (75) (*Figure 1*). ADAPT is one of the first new generation adjuvant trials expected to establish early predictive molecular surrogate markers for outcome by assessing response to a short 3-week induction treatment, using a baseline diagnostic core biopsy and a second biopsy or surgical tissue sample after induction treatment. ADAPT combines static assessment of prognosis of patients by Oncotype DX® recurrence score in HR positive HER2-negative disease and conventional prognostic markers (nodal status) with dynamic measurement of proliferation/apoptosis changes during the short course of preoperative therapy.

Investigation of Serial studies to Predict Your Therapeutic Response with Imaging And moLecular analysis (I-SPY 1 TRIAL) was a multicenter breast cancer study integrating clinical, imaging and genomic data to evaluate pathological response, recurrence free survival (RFS), and their relationship and predictability based on tumor biomarkers (76-78). The 221 included patients with tumors >3 cm received neoadjuvant anthracycline-based chemotherapy, followed by taxanes. The various molecular classifiers tested were highly correlated. In multivariate analysis, the molecular signatures that added to the ability of HR and HER2 receptors, clinical stage, and pathological complete response (pCR) in predicting RFS included 70-gene signature, wound healing signature, p53 mutation signature, and PAM50 risk of recurrence (78). The I-SPY 1 TRIAL demonstrated the increase of the ability of pCR to predict outcome when analyzed within tumor subtypes. Indeed, pCR is the primary endpoint of the next generation study, the I-SPY 2 TRIAL, which is designed to identify agents early in the drug development cycle that improve the rates of pCR (www.ispy2.org). Patients that are included in the I-SPY 2 TRIAL have newly diagnosed locally advanced breast cancers, with high risk of recurrence. Since the first approval in 2009, five investigational drug combinations have been already tested and should be extended to 12 when the trial will be completed (with more than 1,000 patients) (79,80). This trial is based on adaptive designs that rely on information, including from patients who have not achieved the trial's primary endpoint.

Such innovant trial designs should allow parallel

translational research, by performing adequate NGSs on the tumor samples, before and after the endocrine induction treatment. Moreover, they represent a therapy concept for studying drugs which are supposed to overcome endocrine resistance under routine conditions in early breast cancer.

Such MPS has already been performed on 77 samples from HR-positive tumors from breast cancer patients previously included in two independent trials (56). In both trials, patients received neoadjuvant aromatase inhibitor and tumor materials were collected before treatment. WGS was performed on 46 samples and WES on the 31 others, followed by extensive analysis of somatic alterations and their association with aromatase inhibitors. A total of 18 SMGs were identified among them genes previously identified as mutated in breast cancers (*PIK3CA*, *TP53*, *GATA3*, *CDH1*, *RB1*, *MLL3*, *MAP3K1* and *CDKN1B*) as well as genes not previously observed in clinical breast cancer samples (*RUNX1*, *TBX3*, *LDLRAP1*, *STNM2*, *MYH9*, *AGTR2*, *STMN2*, *SF3B1* and *CBFB*). Mutated *MAP3K1* was associated with the luminal A subtype, low grade histology and low proliferation rate whereas *p53* was associated to the opposite pattern. As already cited above, mutant *GATA3* was correlated with suppression of proliferation upon aromatase inhibitor treatment. Pathway analysis demonstrated mutations in the MAP3K1 substrate, MAP2K4, inducing similar alteration as direct MAP3K1 loss. Besides, many SMGs appear with low frequencies, making the correlative data analysis more complex. Nonetheless, the authors suggest that for patients with *MAP3K1* mutated tumors, luminal A subtype, neoadjuvant aromatase inhibitors are a favorable option. On the contrary, for patients with *TP53* mutated tumors, resistance to aromatase inhibitor is expected and an alternate treatment could be selected. PIK3CA was the most common mutation in the luminal subtypes (41.3%) but was neither associated to clinical nor KI67 response. Nonetheless, a positive association was observed between MAP3K1/MAP2K4 mutations and PIK3CA mutation, suggesting an *in vivo* crosstalk. In any cases, the initial tumor heterogeneity, its evolution along the disease progression and after the pressure of the successive treatments is a key element to consider when analyzing such huge amounts of data.

Another innovative study was the prospective trial SAFIRO/UNICANCER (NCT01414933), conducted with 423 metastatic breast cancer patients enrolled within 11 months in 18 centres in France (81). The aim was to perform molecular screenings to identify abnormalities in individual patients, with the aim of providing targeted therapy matched to individual genomic alterations. Comparative genomic hybridization (CGH) array and Sanger sequencing on *PIK3CA* (exon 10 and 21) and on *AKT1* (exon 4) were used to assess the biopsy samples. Therapy target was then decided accordingly. A targetable genomic alteration was observed in 46% of the patients, and the most frequent one were *PIK3CA* (25%), *CCND1* (19%) and *FGFR1* (13%). Mutations and amplifications described above and others (*AKT1*, *EGFR*, *MDM2*, *FGFR2*, *AKT2*, *IGFR1* and *MET amplification*), were observed but with a low frequency (<5%). Therapy could be personalized and assessed in 43 patients, with four objective responses (9%) and nine stable diseases for more than 16 weeks (21%). Antitumor activity was observed in patients with *PIK3CA*, *FGFR1*, *IGF1R*, *FGF3*, *AKT1*, *AKT2* and *EGFR* gene alterations, although the mutations of the last three genes were located in two rare gene segments that are not conventional driver mutations to focus on.

This challenging clinical trial definitely proved the relevance of such large scale genomic analysis, where ideally, no theoretical or practical restriction should limit the range of mutations to identify in order to adapt the treatment to each patient and limit the resistance mechanisms.

Expected clinical impact

As technologies for DNA/RNA sequencing are improving so quickly to the accuracy, acceptable cost and technical feasibility needed, they reach the standards to be implemented in clinic for the identification of driver mutations. Then, a strict definition of the targeted drug combination to use will be possible, to block the tumor cells of using new signaling pathways that drive endocrine therapy resistances.

All the large-scale genomic analysis already introduced a wide spectrum of candidate genes but reality will force us to focus on a small definite number. Of course, the SMGs and driver mutations are the favorite candidates to target, but we are aware that the low frequency mutated genes and passenger mutations may be relevant, too. Because breast cancers are so common, even a low percentage of patients that would benefit from a targeted therapy could represent a significant number of patients worldwide. As not each mutated gene possesses a related drug, then, extensive/exhaustive genomic analysis may not be the most adequate strategy for rational decision-making that could be directed according to available targeted drugs.

So far, clinical trials using NGS for clinical identification of druggable targets in advanced breast cancer have shown

that this concept is feasible in clinical routine but requires large patient numbers and sufficient downstream trials with specific inhibitors to make this effort worthwhile for patients, investigators, and industry sponsors. Such a concept for rapid *in vivo* testing of novel drugs will need a tailored approach by all stakeholders in drug development to be successful in the future. Yet, another alternative to move new compounds rapidly into the early breast cancer setting provided that sufficient safety information is available, is the umbrella trial concept as already successfully established by the I-SPY consortium (77,78,82) or WSG ADAPT (83).

Conclusions

As more genomic data and information on mutations are added today, prospective trials should open wide screening tests. Then, patients can expect to benefit from the identification of the specific driver mutations of their tumor and the related drug combination. No general consensus for targeted drug prescription will be then possible as individual tumor molecular specificities and the tumor heterogeneity will drive the therapeutic choice. Aside from clinical practice, large scale molecular testing will also aid drug development but requires coherent concepts for testing and downstream trials, which can only be performed by close national and international collaboration between all stakeholders. Moreover, novel trial concepts such trials including several tumor entities or umbrella trials with several biology-based sub-protocols supported by national and international research consortia will also speed up progress in this area.

Acknowledgements

None.

Footnote

Conflicts of Interest: NH has received honoraria for lectures from Genomic Health and obtained research funding from Genomic Health and NanoString. SDS declares that she has no interest to disclose related to the publication of this manuscript.

References

1. Levi F, Bosetti C, Lucchini F, et al. Monitoring the decrease in breast cancer mortality in Europe. Eur J Cancer Prev 2005;14:497-502.
2. 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.
3. Eroles P, Bosch A, Perez-Fidalgo JA, et al. Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. Cancer Treat Rev 2012;38:698-707.
4. Dawson SJ, Rueda OM, Aparicio S, et al. A new genome-driven integrated classification of breast cancer and its implications. EMBO J 2013;32:617-28.
5. Ellis IO, Galea M, Broughton N, et al. Pathological prognostic factors in breast cancer. II. Histological type. Relationship with survival in a large study with long-term follow-up. Histopathology 1992;20:479-89.
6. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
7. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418-23.
8. Prat A, Parker JS, Karginova O, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010;12:R68.
9. Perou CM, Parker JS, Prat A, et al. Clinical implementation of the intrinsic subtypes of breast cancer. Lancet Oncol 2010;11:718-9; author reply 20-1.
10. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5:5-23.
11. Prat A, Adamo B, Cheang MC, et al. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist 2013;18:123-33.
12. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750-67.
13. Harbeck N, Rody A. Lost in translation? Estrogen receptor status and endocrine responsiveness in breast cancer. J Clin Oncol 2012;30:686-9.
14. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287-312.
15. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011;22:1736-47.

16. Rodenhiser DI, Andrews JD, Vandenberg TA, et al. Gene signatures of breast cancer progression and metastasis. Breast Cancer Res 2011;13:201.
17. Hamilton-Burke W, Coleman L, Cummings M, et al. Phosphorylation of estrogen receptor beta at serine 105 is associated with good prognosis in breast cancer. Am J Pathol 2010;177:1079-86.
18. O'Hara J, Vareslija D, McBryan J, et al. AIB1:ERalpha transcriptional activity is selectively enhanced in aromatase inhibitor-resistant breast cancer cells. Clin Cancer Res 2012;18:3305-15.
19. Santen RJ, Fan P, Zhang Z, et al. Estrogen signals via an extra-nuclear pathway involving IGF-1R and EGFR in tamoxifen-sensitive and -resistant breast cancer cells. Steroids 2009;74:586-94.
20. Ross-Innes CS, Stark R, Teschendorff AE, et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature 2012;481:389-93.
21. Martin HL, Smith L, Tomlinson DC. Multidrug-resistant breast cancer: current perspectives. Breast Cancer (Dove Med Press) 2014;6:1-13.
22. Geyer FC, Weigelt B, Natrajan R, et al. Molecular analysis reveals a genetic basis for the phenotypic diversity of metaplastic breast carcinomas. J Pathol 2010;220:562-73.
23. Navin N, Krasnitz A, Rodgers L, et al. Inferring tumor progression from genomic heterogeneity. Genome Res 2010;20:68-80.
24. Navin N, Kendall J, Troge J, et al. Tumour evolution inferred by single-cell sequencing. Nature 2011;472:90-4.
25. Eccles SA, Aboagye EO, Ali S, et al. Critical research gaps and translational priorities for the successful prevention and treatment of breast cancer. Breast Cancer Res 2013;15:R92.
26. Viedma-Rodríguez R, Baiza-Gutman L, Salamanca-Gómez F, et al. Mechanisms associated with resistance to tamoxifen in estrogen receptor-positive breast cancer (Review). Oncol Rep 2014. [Epub ahead of print].
27. Shah NR, Chen H. MicroRNAs in pathogenesis of breast cancer: Implications in diagnosis and treatment. World J Clin Oncol 2014;5:48-60.
28. Byler S, Goldgar S, Heerboth S, et al. Genetic and epigenetic aspects of breast cancer progression and therapy. Anticancer Res 2014;34:1071-7.
29. Ellis MJ. Mutational analysis of breast cancer: guiding personalized treatments. Breast 2013;22 Suppl 2:S19-21.
30. Ellis MJ, Perou CM. The genomic landscape of breast cancer as a therapeutic roadmap. Cancer Discov 2013;3:27-34.
31. Wheeler DA, Wang L. From human genome to cancer genome: the first decade. Genome Res 2013;23:1054-62.
32. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61-70.
33. Stephens PJ, McBride DJ, Lin ML, et al. Complex landscapes of somatic rearrangement in human breast cancer genomes. Nature 2009;462:1005-10.
34. Pleasance ED, Cheetham RK, Stephens PJ, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 2010;463:191-6.
35. Chin L, Hahn WC, Getz G, et al. Making sense of cancer genomic data. Genes Dev 2011;25:534-55.
36. Quail MA, Smith M, Coupland P, et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC Genomics 2012;13:341.
37. Khurana E, Fu Y, Colonna V, et al. Integrative annotation of variants from 1092 humans: application to cancer genomics. Science 2013;342:1235587.
38. Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108-13.
39. Maher CA, Kumar-Sinha C, Cao X, et al. Transcriptome sequencing to detect gene fusions in cancer. Nature 2009;458:97-101.
40. Shah SP, Morin RD, Khattra J, et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 2009;461:809-13.
41. Tuch BB, Laborde RR, Xu X, et al. Tumor transcriptome sequencing reveals allelic expression imbalances associated with copy number alterations. PLoS One 2010;5:e9317.
42. Rehm HL. Disease-targeted sequencing: a cornerstone in the clinic. Nat Rev Genet 2013;14:295-300.
43. Bernstein BE, Birney E, Dunham I, et al. An integrated encyclopedia of DNA elements in the human genome. Nature 2012;489:57-74.
44. Malinowsky K, Wolff C, Ergin B, et al. Deciphering signaling pathways in clinical tissues for personalized medicine using protein microarrays. J Cell Physiol 2010;225:364-70.
45. International Cancer Genome Consortium, Hudson TJ, Anderson W, et al. International network of cancer genome projects. Nature 2010;464:993-8.
46. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499:214-8.

47. Garraway LA, Lander ES. Lessons from the cancer genome. Cell 2013;153:17-37.
48. Ma CX, Ellis MJ. The Cancer Genome Atlas: clinical applications for breast cancer. Oncology (Williston Park) 2013;27:1263-9, 1274-9.
49. Melchor L, Benitez J. The complex genetic landscape of familial breast cancer. Hum Genet 2013;132:845-63.
50. Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 2013;45:353-61, 361e1-2.
51. Sakoda LC, Jorgenson E, Witte JS. Turning of COGS moves forward findings for hormonally mediated cancers. Nat Genet 2013;45:345-8.
52. Tabchy A, Ma CX, Bose R, et al. Incorporating genomics into breast cancer clinical trials and care. Clin Cancer Res 2013;19:6371-9.
53. Ciruelos Gil EM. Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. Cancer Treat Rev 2014;40:862-71.
54. Ferrario C, Batist G. Advances in the approach to novel drug clinical development for breast cancer. Expert Opin Drug Discov 2014;9:647-68.
55. Kumar RD, Chang LW, Ellis MJ, et al. Prioritizing Potentially Druggable Mutations with dGene: An Annotation Tool for Cancer Genome Sequencing Data. PLoS One 2013;8:e67980.
56. Ellis MJ, Ding L, Shen D, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature 2012;486:353-60.
57. Herschkowitz JI, Simin K, Weigman VJ, et al. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol 2007;8:R76.
58. Bartlett JM, Brookes CL, Robson T, et al. Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial. J Clin Oncol 2011;29:1531-8.
59. Honma N, Horii R, Iwase T, et al. Clinical importance of estrogen receptor-beta evaluation in breast cancer patients treated with adjuvant tamoxifen therapy. J Clin Oncol 2008;26:3727-34.
60. Yan Y, Li X, Blanchard A, et al. Expression of both estrogen receptor-beta 1 (ER-beta1) and its co-regulator steroid receptor RNA activator protein (SRAP) are predictive for benefit from tamoxifen therapy in patients with estrogen receptor-alpha (ER-alpha)-negative early breast cancer (EBC). Ann Oncol 2013;24:1986-93.
61. De Amicis F, Thirugnansampanthan J, Cui Y, et al. Androgen receptor overexpression induces tamoxifen resistance in human breast cancer cells. Breast Cancer Res Treat 2010;121:1-11.
62. Garay JP, Park BH. Androgen receptor as a targeted therapy for breast cancer. Am J Cancer Res 2012;2:434-45.
63. Hickey TE, Robinson JL, Carroll JS, et al. Minireview: The androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? Mol Endocrinol 2012;26:1252-67.
64. Gnant M. Overcoming endocrine resistance in breast cancer: importance of mTOR inhibition. Expert Rev Anticancer Ther 2012;12:1579-89.
65. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 2012;366:520-9.
66. Banerji S, Cibulskis K, Rangel-Escareno C, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 2012;486:405-9.
67. Stephens PJ, Tarpey PS, Davies H, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012;486:400-4.
68. Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012;486:346-52.
69. Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 2012;486:395-9.
70. Harbeck N, Sotlar K, Wuerstlein R, et al. Molecular and protein markers for clinical decision making in breast cancer: today and tomorrow. Cancer Treat Rev 2014;40:434-44.
71. Gökmen-Polar Y, Badve S. Molecular profiling assays in breast cancer: are we ready for prime time? Oncology (Williston Park) 2012;26:350-7, 361.
72. Zujewski JA, Kamin L. Trial assessing individualized options for treatment for breast cancer: the TAILORx trial. Future Oncol 2008;4:603-10.
73. Sparano JA. TAILORx: trial assigning individualized options for treatment (Rx). Clin Breast Cancer 2006;7:347-50.
74. Gluz, Kreipe H, Dehenhardt T, et al. Prospective Comparison of Risk Assessment Tools in Early Breast Cancer (Recurrence Score®, uPA/PAI-1, Central Grade, and Luminal Subtypes): Final Correlation Analysis from the Phase III WSG planB Trial. San Antonio Breast Cancer symposium 2011:Abstract S4-3.

75. Gluz O, Hofmann D, Kates RE, et al. ADAPT - Adjuvant Dynamic marker-Adjusted Personalized Therapy trial optimizing risk assessment and therapy response prediction in early breast cancer. Cancer Res 2012;72:Abstract nr OT3-3-02.
76. Berry DA. Adaptive clinical trials in oncology. Nat Rev Clin Oncol 2011;9:199-207.
77. Esserman LJ, Berry DA, DeMichele A, et al. Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL--CALGB 150007/150012, ACRIN 6657. J Clin Oncol 2012;30:3242-9.
78. Esserman LJ, Berry DA, Cheang MC, et al. Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). Breast Cancer Res Treat 2012;132:1049-62.
79. Barker AD, Sigman CC, Kelloff GJ, et al. I-SPY 2: an adaptive breast cancer trial design in the setting of neoadjuvant chemotherapy. Clin Pharmacol Ther 2009;86:97-100.
80. Printz C. I-SPY 2 may change how clinical trials are conducted: researchers aim to accelerate approvals of cancer drugs. Cancer 2013;119:1925-7.
81. André F, Bachelot T, Commo F, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). Lancet Oncol 2014;15:267-74.
82. Lin C, Buxton MB, Moore D, et al. Locally advanced breast cancers are more likely to present as Interval Cancers: results from the I-SPY 1 TRIAL (CALGB 150007/150012, ACRIN 6657, InterSPORE Trial). Breast Cancer Res Treat 2012;132:871-9.
83. Hofmann D, Nitz U, Gluz O, et al. WSG ADAPT - adjuvant dynamic marker-adjusted personalized therapy trial optimizing risk assessment and therapy response prediction in early breast cancer: study protocol for a prospective, multi-center, controlled, non-blinded, randomized, investigator initiated phase II/III trial. Trials 2013;14:261.

Cite this article as: Doisneau-Sixou S, Harbeck N. From genomic data analysis to drug development: a new generation of trials using molecular marker assessment in breast cancer. Chin Clin Oncol 2014;3(2):16. doi: 10.3978/j.issn.2304-3865.2014.05.15

MET deregulation in breast cancer

Gabriele Minuti, Lorenza Landi

Department of Medical Oncology, Istituto Toscano Tumori, Civil Hospital of Livorno, Livorno, Italy

Contributions: (I) Conception and design: G Minuti; (II) Administrative support: All authors; (III) Provision of Study material: PubMed; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretetion: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Gabriele Minuti, MD. Department of Medical Oncology, Istituto Toscano Tumori, Civil Hospital of Livorno, Viale Alfieri 36, Livorno 57100, Italy. Email: gabriele.minuti@gmail.com.

Background: Mesenchymal-epithelial transition (*MET*) is an oncogene encoding for a trans-membrane tyrosine kinase receptor activated by the hepatocyte growth factor (HGF). *MET* has a normal function in organ development during embryogenesis and in tissue homeostasis during adult life. Deregulation of HGF/MET signaling pathway is frequently observed in many cancer types, conferring invasive growth and tendency to progression. MET deregulation is due to gene amplification or increased copy number, gene mutation, receptor over-expression or ligand autocrine loops activation. These events lead to migration, invasion, proliferation, metastatic spread and neo-angiogenesis of cancer cells, suggesting that anti-HGF/MET agents may represent a potential antitumor strategy. In breast cancer (BC), preclinical and clinical data demonstrated the role of HGF/MET signalling pathway in carcinogenesis, disease progression and resistance features.

Methods: For this review article, all published data on HGF/MET in BC were collected and analyzed.

Results: Several evidences underline that, in early BC, MET over-expression has an independent negative prognostic significance, regardless of method used for evaluation and BC subtypes. Available data suggest that MET is a relevant target particularly in basal-like (BL) and in triple negative BC. Moreover, preclinical and retrospective data support the critical role of MET deregulation in the development of resistance to target-agents, such as anti-HER2 strategies.

Conclusions: MET is a promising new target in BC. Several anti-MET agents are under investigation and ongoing clinical trials will clarify its relevance in BC treatment.

Keywords: Mesenchymal-epithelial transition (MET); hepatocyte growth factor (HGF); breast cancer (BC); triple negative; basal-like (BL)

Submitted Jun 23, 2015. Accepted for publication Jun 24, 2015.
doi: 10.3978/j.issn.2305-5839.2015.06.22
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.06.22

Introduction

Mesenchymal-epithelial transition (*MET*) factor oncogene is located on chromosome 7q31 and encodes for the dimeric tyrosine kinase receptor of the hepatocyte growth factor (HGF), also known as scatter factor (SF). The ligand binding to MET induces dimerization and cytoplasmic auto-phosphorylation of the receptor kinase domain, favouring a cascade of intracellular signalling involved in invasive cell programs (1) as illustrated in *Figure 1*. In normal tissue, MET-regulated pathways have a key role for critical physiologic functions, including embryogenesis, angiogenesis, cell growth and wound healing (2,3). The activation of HGF/MET axis has been described as a relevant process for cancer initiation and progression, leading to invasiveness, cell survival, neo-angiogenesis, cell migration and metastatic spread (4). MET is frequently deregulated in cancer and the main mechanisms include gene amplification or increased copy number (GCN), germinal or somatic mutation, receptor over-expression. These molecular events have been described in a wide spectrum of human malignancies, such as non-small cell lung cancer (NSCLC) (5,6), gastric cancer (7), oesophageal

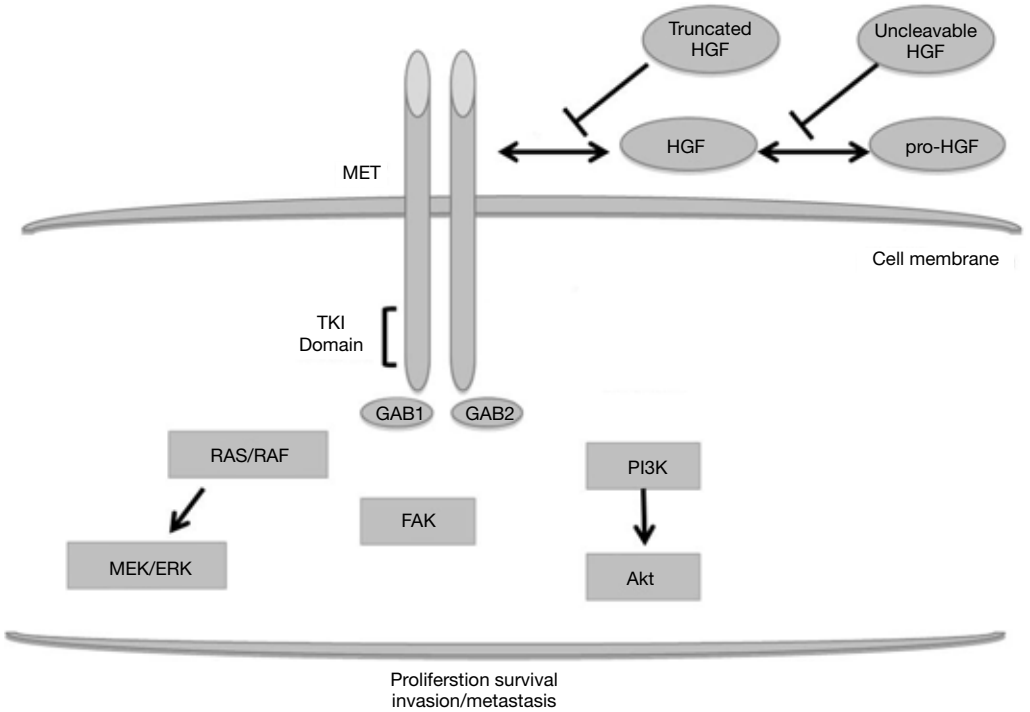


Figure 1 HGF/MET signaling pathway. MET, mesenchymal-epithelial transition factor; HGF, hepatocyte growth factor.

cancer (8), endometrial cancer (9), hepatocarcinoma (10), head and neck cancer (11), colorectal cancer (12) and kidney cancer (13). In these cases, aberrant HGF/MET signalling pathway confers aggressive phenotype characterized with high risk of progression and poor outcome. In addition, MET deregulation is often involved in acquired resistance to targeted agents (12,14,15).

In breast cancer (BC), preclinical and clinical studies highlighted the role of MET deregulation on carcinogenesis and development of aggressive phenotypes, as suggested by the higher incidence of mammary invasive carcinomas in mouse models harbouring *MET* mutations (16). Moreover, an imbalance in MET expression between neoplastic lesion and adjacent normal tissue is associated with aggressive behaviour of *in situ* ductal carcinoma (DCIS) (17). Across all BCs, MET is over-expressed in at least 20-30% of cases, with difference related to methods and scoring system used for biomarker testing (18). Several investigations showed a preferential, but not exclusive, expression in basal-like (BL) subgroup, mostly represented by triple negative breast cancer (TNBC). However, up-regulation of HGF/MET axis represents a strong and independent predictor of poor outcome and aggressiveness, irrespective of histological subtype (19,20). Available data suggest that molecular events, such as *MET* mutation or amplification, are rare in primary BC tumours (21,22). Finally, in advanced stage disease, MET deregulation plays a critical role in cancer progression and development of acquired resistance to target agents, including trastuzumab (23).

Aim of this review is to discuss available data on MET deregulation and its therapeutic implications in BC.

MET/HGF expression as a prognostic factor in BC

During the 90s, several studies showed a potential prognostic relevance of HGF/MET expression in BC. In 1994, Yamashita and colleagues measured the intra-tumour immunoreactive (ir)-HGF concentration of 258 primary human BCs. Patients with high ir-HGF concentration had a significantly shorter relapse-free survival (RFS) and overall survival (OS) when compared to those with low ir-HGF concentration. In multivariate analysis, ir-HGF level was the most important independent factor in predicting RFS and OS, greater than lymph node involvement (24). Furthermore, in a retrospective series of 113 node-negative BC cases, Camp *et al.* demonstrated that high-MET *vs.* low-MET expression in the primary lesion significantly impact on 5-year survival. Interestingly, the authors described,

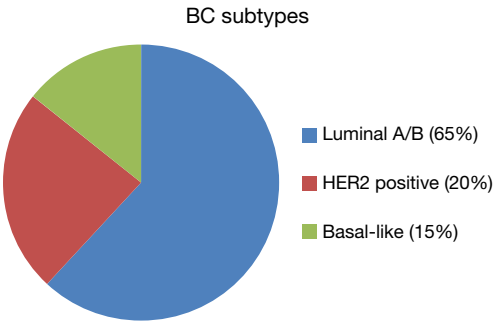


Figure 2 Breast cancer (BC) subtypes.

at the time of relapse, an increased rate of MET over-expression also in patients with previously negligible value, suggesting a possible selection of MET-positive clones in the process of progression (25). Subsequently, using a tissue microarray, Kang and co-workers confirmed the strong and negative prognostic relevance of MET expression in a cohort of 330 node-negative breast carcinomas. In the study, also matriptase and HAI-I, an HGF-activator expressed on mammary epithelium and its cognate inhibitor respectively, were associated with poor patient outcome. The prognostic impact of MET expression was independent by the traditional BC biomarkers, as confirmed in the multivariate analysis (26). Recently, using a reverse phase protein array, Raghav *et al*. assessed, once more, the negative role of MET expression on patient outcome among 257 BCs cases. The level of MET and phoshpo-MET, its activated form, had a strong prognostic impact in BC patients, while no significant differences in mean expression of both biomarkers were seen among the different subtypes (19). In addition, in the 2014, Baccelli and colleagues investigated the role of MET and CD47, a ligand involved in cancer cell evasion from macrophage scavenging, on BC patient outcome. The expression of both biomarkers was assessed with immunohistochemistry (IHC) in a series of 255 hormone receptor-positive early BCs. The authors described a 10.3-year mean OS difference between MET/CD47 double-positive and double-negative cases, demonstrating a novel and independent couple of prognostic factors and underling again the relevance of MET over-expression in term of patients' outcome (20).

Recently, a comprehensive meta-analysis including 6,010 cases showed that MET over-expression is significantly associated with poor survival in BC patients, especially in the TNBC patients. The results of subgroup analysis suggest that, in Asian and HER2 positive BC individuals, MET expression might not be associated with prognosis (27).

In summary, collecting all these clinical data together, MET over-expression results as a robust negative prognostic factor for BC patients, regardless of evaluation method used or cancer subtypes.

Role of MET in BLBC and TNBC

BLBC accounts for 10% to 20% (*Figure 2*) of all BCs and represents an aggressive subgroup of mammary carcinoma, with worse prognosis and limited therapeutic options. BLBCs are characterized by high histological grade and mitotic indexes, pushing borders and large areas of necrosis. IHC features of BL tumours usually include lack of hormonal and human epidermal receptor-2 (HER2) expression, positivity of one or more basal cytokeratins (e.g., CK5/6, CK14 and CK17) and/or expression of HER1 [also named epidermal growth factor receptor (EGFR)]. Among cancers with BL features, TNBCs are the main prominent with a global incidence of approximately 15% (28). Currently, chemotherapy is the only modality of systemic therapy available for patients with BL and triple-negative disease, although, in the small group of *BRCA1* and *BRCA2* mutated BC patients, poly (ADP-ribose) polymerase (PARP) inhibitors may have a therapeutic role (29). For such reasons, the identification of targetable biomarkers is an urgent clinical need and a major theme of discussion (28).

In preclinical models, genomic and proteomic analyses led to dichotomize BC into two main groups: the luminal and the BL subtype (30,31). In gene expression profile of BC cell lines, comparison between BL and luminal cluster showed differential expression of *MET* gene (30,31). Clinical studies confirmed these findings. In 2007, Garcia and colleagues analyzed 930 BC specimens by using IHC, demonstrating a strong association between high MET levels and expression of basal-cluster features, such as CK5, CK6, caveolin 1, c-KIT and p63 (32). On these bases, MET over-expression could be considered as an additional constituent of BL phenotype. Similar results were obtained in some other studies conducted in both early and metastatic diseases, thus confirming that MET is preferentially expressed in BLBC (31,33). In addition, these studies suggested that high MET expression levels correlated with worse prognosis. Recently, Ho-Yen and colleagues analyzed 1,274 primary tumour samples of early BC aiming to evaluate the relationship between MET IHC expression and BC subtypes (34). Authors found

that MET was independently associated with BL feature (odds ratio =6.44; P=0.005). More interestingly, MET over-expression negatively affected RFS in all subtypes [hazard ratio (HR) =1.85; P=0.027], with a trend toward reduced OS in BL tumors (33). Similarly, a strong correlation between BL features and MET over-expression (57.5%, P<0.001) has been found by Kim and co-workers, which analyzed 924 BCs specimens using IHC (35). The study also confirmed the prognostic impact of high-MET expression levels in terms of recurrence (DFS, P=0.010) and survival (OS, P=0.001). More interestingly, the same authors demonstrated that, in TNBC cell lines, MET levels were high and MET inhibition by RNA-interference reduced cell proliferation and migration, suggesting the potential therapeutic role of MET inhibition in TNBC patients (35).

Finally, Zagouri *et al*. retrospectively evaluated MET expression in a series of 170 TN tumours, showing high expression levels in approximately half of cases (52%) (36). As previously described in BLBCs (34), MET over-expression significantly predicted shorter survival (adjusted HR for death 3.74; P=0.002) and also associated with poorly differentiated carcinomas (P=0.02).

Recently, as previously described, a large meta-analysis including more than 6,000 BC cases confirmed that, especially in TNBC patients, MET over-expression is significantly associated with worst survival (27).

MET and acquired resistance to targeted agents

Several evidences suggest that MET deregulation plays a critical role in the development of acquired resistance to targeted agents through a functional interaction, the so called "cross-talk", with other TK receptors, particularly with EGFR family. In a preclinical model, Engelman and colleagues demonstrated that *MET* amplification was responsible for acquired resistance to first-generation *EGFR* TK inhibitors in up to 20% of *EGFR*-mutant NSCLCs (37). Similarly, Bardelli *et al*. showed that *MET* amplification driven *de novo* and acquired resistance to anti-EGFR monoclonal antibodies cetuximab and panitumumab in metastatic colorectal cancers, both *in vitro* and *in vivo* models (12).

As HER2 belongs to EGFR family and represents a therapeutic target in BC (38), several studies have investigated the potential impact of MET status in modulating efficacy of anti-HER2 strategies. In 2007, Lindeman and colleagues firstly demonstrated that MET over-expression occurred in approximately a quarter of HER2-positive BC cases, suggesting that HER2 and MET could have a synergistic effect in promoting tumour growth and aggressiveness (17). Therefore, in a preclinical model, Shattuck *et al*. reported that a significant proportion of HER2-positive tumours also displayed high levels of MET expression by Western blot analysis (23). In addition, authors demonstrated that MET could contribute to resistance to the anti-HER2 monoclonal antibody trastuzumab. Indeed, in cell lines MET inhibition, either through RNA interference-mediated depletion or small molecule-mediated inhibition, increased sensitivity to trastuzumab while MET activation protected cells from the anti-apoptotic effects of trastuzumab by preventing drug-mediated p27 induction. More recently, a preclinical study evaluating MET/HER2 "cross-talk" in human BC, confirmed that a relevant percentage of HER2 positive cases co-expressed MET and HER2, despite an intratumoral heterogeneity (39). In a double positive (MET+/HER2+) BC cell-line the authors showed that MET depletion resulted in increased HER2 activation and, conversely, HER2 depletion resulted in MET activation. Moreover, functional analysis of TK receptors activation during HER2 knockdown indicated that MET signaling was a compensatory pathway of resistance.

In 2013, we retrospectively analyzed a cohort of 130 HER2-positive metastatic BC patients, aiming to evaluate the impact of *MET* and *HGF* GCN in predicting trastuzumab-sensitivity (40). Increased *MET* or *HGF* GCNs were detected in approximately one-fourth of cases and were significantly associated with higher risk of treatment failure. Indeed, *MET* FISH-positive cases (n=36) had both significantly higher trastuzumab failure rate (P=0.001) and shorter time to progression (HR =1.74; P=0.006) than *MET* FISH-negative cases. Also *HGF* FISH-positive status (n=33) significantly associated with higher risk of failure (P=0.007) when compared with *HGF* FISH-negative cases.

In summary, our data suggested that in HER2 positive BCs, *MET/HGF* GCNs increased the risk of trastuzumab failure, thus supporting the investigation of dual HER2 and MET inhibitors in such population. Notably, our experience confirmed the absence of *MET* amplification in HER2-positive cases, as reported in previous other investigations (22,40).

Anti-MET agents under investigation in BC

There are at least four possible strategies that are useful for blockade of the HGF/MET pathway, including

Table 1 Anti-MET compounds under investigation in BC

Agent	Category	Targets	Clinical trial
Tivantinib (ARQ197)	TK inhibitor	MET (non-ATP kinase), GSK3α and GSK3β	NCT01575522 (TNBC)
Cabozantinib (XL184)	TK inhibitor	MET, VEGFR-2, RET, c-KIT, AXL, TIE-2 and FLT1/3/4	NCT01738438 (TNBC); NCT02260531 (TNBC/HER2+/HR+)
Foretinib (XL880)	TK inhibitor	MET, RON, AXL, VEGFR-2, FGFR2, PDGFR, c-KIT, TIE-2 and FLT3	NCT01147484 (TNBC); NCT01138384 (HER2+)
Onartuzumab MetMab	Monoclonal antibody	MET	NCT01186991 (TNBC)

MET, mesenchymal-epithelial transition factor; VEGFR-2, vascular endothelial growth factor 2; RET, rearranged during transfection; c-KIT, mast/stem cell growth factor receptor (SCFR), also known as proto-oncogene c-KIT; AXL, encoding for tyrosine-protein kinase receptor UFO; TIE-2, tyrosine kinase with Ig and EGF (epidermal growth factor) homology domains; FLT 1/2/3, encoding for vascular endothelial growth factor receptor 1/2/3; RON, a tyrosine kinase receptor of MET family, also known as macrophage stimulating 1 receptor (MST1R); FGFR2, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; HR+, hormonal receptor positive breast cancer.

agents interfering with HGF binding to MET, anti-MET monoclonal antibodies, small molecule MET TK inhibitors, and small molecule inhibitors of the downstream pathways. Data from preclinical studies suggest that the modality of HGF/MET activation (i.e., autocrine/paracrine stimulation, gene amplification or gene mutation), could predict the class of agents more likely to interrupt the signalling pathway. A list of anti-MET agents currently under investigation in metastatic BCs patients is shown in *Table 1*. Notably, the majority of these agents are TK inhibitors and restricted to BL/TN BC patients.

Preliminary results from an ongoing 2-stage single arm trial with foretinib demonstrated a potential activity in metastatic TNBC (NCT01147484). In the first cohort of patients evaluable for response (n=15) the MET TK inhibitor showed a disease control rate (DCR) of 47% (n=7), including one partial response and six stable disease (SD). Interestingly, 6/8 MET IHC positive cases obtained SD. Stage 2 of accrual is currently enrolling (41).

Recently, also cabozantinib monotherapy showed evidence of antitumor activity in TNBC (42). In a single-arm phase II trial (NCT02260531) including 35 patients with advanced TNBC, the multiple receptor TK inhibitor demonstrated an overall response rate (ORR) and a DCR of 11% and 34%, respectively. Preliminary results on exploratory biomarker analysis suggest a possible correlation between baseline plasma MET levels and PFS.

Conclusions

In the last years, many progresses have been made in the understanding of HGF/MET signaling pathway in cancer development and progression. Literature data also supported its critical role in mammary tumours and several studies have clearly demonstrated that high level of MET expression correlated with worse prognosis, both in early and advanced stage.

Treatment of metastatic BC is now based on hormonal and HER2 status. Today, endocrine treatments as well as anti-HER2 agents, alone or in combination with chemotherapy, offer to metastatic BC patients the concrete possibility to prolong survival and preserve their quality of life. Unfortunately, for patients defined as TN, therapeutic options remain limited and largely unsatisfactory, with long-lasting disease control representing a major challenge. As MET over-expression often correlates with poorly differentiated and aggressive disease including BL/TN BCs, MET inhibition could be beneficial in this particular group of patients and results of ongoing trials exploring the activity of anti-MET agents are urgently awaited.

Finally, "cross-talk" of MET with other TK receptors seems in part explain the failure of target agents, particularly trastuzumab. As a consequence, combination of anti-HER2 and anti-MET strategies could represent a suitable option to delay or possibly overcome acquired resistance, at least in those cases displaying *MET* alteration, such as amplification or high GCN. At this proposal, it is important to remember that only a proper selection of patients, through prognostic/predictive models and validated biomarker, can lead to identify those individuals who may maximally benefit from tailored treatments, such as anti-MET agents.

Acknowledgements

Funding: This work was supported by the Italian Association for Cancer Research (AIRC) (IG 2012-13157), Fondazione Ricerca Traslazionale (FoRT) and Istituto Toscano Tumori (ITT), (Project F13/16).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: AIRC, FoRT, ITT.

References

1. 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.
2. Bussolino F, Di Renzo MF, Ziche M,et al. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol 1992;119:629-41.
3. Uehara Y, Minowa O, Mori C, et al. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. Nature 1995;373:702-5.
4. Boccaccio C, Comoglio PM. Invasive growth: a MET-driven genetic programme for cancer and stem cells. Nat Rev Cancer 2006;6:637-45.
5. Tsao MS, Liu N, Chen JR, et al. Differential expression of Met/hepatocyte growth factor receptor in subtypes of non-small cell lungcancers. Lung Cancer 1998;20:1-16.
6. 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.
7. Hara T, Ooi A, Kobayashi M, et al. Amplification of c-myc, K-sam, and c-met in gastric cancers: detection by fluorescence in situ hybridization. Lab Invest 1998;78:1143-53.
8. Miller CT, Lin L, Casper AM, et al. Genomic amplification of MET with boundaries within fragile site FRA7G and upregulation of MET pathways in esophageal adenocarcinoma. Oncogene 2006;25:409-18.
9. Samuelson E, Nordlander C, Levan G, et al. Amplification studies of MET and Cdk6 in a rat endometrial tumor model and their correlation to human type I endometrial carcinoma tumors. Adv Exp Med Biol 2008;617:511-7.
10. Lee SJ, Lee J, Sohn I, et al. A survey of c-MET expression and amplification in 287 patients with hepatocellular carcinoma. Anticancer Res 2013;33:5179-86.
11. Nisa L, Aebersold DM, Giger R, et al. Biological, diagnostic and therapeutic relevance of the MET receptor signaling in head and neck cancer. Pharmacol Ther 2014;143:337-49.
12. Bardelli A, Corso S, Bertotti A, et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 2013;3:658-73.
13. Fay AP, Signoretti S, Choueiri TK. MET as a target in papillary renal cell carcinoma. Clin Cancer Res 2014;20:3361-3.
14. Graziano F, Galluccio N, Lorenzini P, et al. Genetic activation of the MET pathway and prognosis of patients with high-risk, radically resected gastric cancer. J Clin Oncol 2011;29:4789-95.
15. Lo Muzio L, Farina A, Rubini C, et al. Effect of c-Met expression on survival in head and neck squamous cell carcinoma. Tumour Biol 2006;27:115-21.
16. Gastaldi S, Comoglio PM, Trusolino L. The Met oncogene and basal-like breast cancer: another culprit to watch out for? Breast Cancer Res 2010;12:208.
17. Lindemann K, Resau J, Nährig J, et al. Differential expression of c-Met, its ligand HGF/SF and HER2/neu in DCIS and adjacent normal breast tissue. Histopathology 2007;51:54-62.
18. Lengyel E, Prechtel D, Resau JH, et al. C-Met overexpression in node-positive breast cancer identifies patients with poor clinical outcome independent of Her2/neu. Int J Cancer 2005;113:678-82.
19. Raghav KP, Wang W, Liu S, et al. cMET and phospho-cMET protein levels in breast cancers and survival outcomes. Clin Cancer Res 2012;18:2269-77.
20. Baccelli I, Stenzinger A, Vogel V, et al. Co-expression of MET and CD47 is a novel prognosticator for survival of luminal-type breast cancer patients. Oncotarget 2014;5:8147-60.
21. Bièche I, Champème MH, Lidereau R. Infrequent mutations of the MET gene in sporadic breast tumours. Int J Cancer 1999;82:908-10.
22. Carracedo A, Egervari K, Salido M, et al. FISH and immunohistochemical status of the hepatocyte growth factor receptor (c-Met) in 184 invasive breast tumors. Breast Cancer Res 2009;11:402.
23. 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.

24. Yamashita J, Ogawa M, Yamashita S, et al. Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. Cancer Res 1994;54:1630-3.
25. Camp RL, Rimm EB, Rimm DL, et al. Met expression is associated with poor outcome in patients with axillary lymph node negative breast carcinoma. Cancer 1999;86:2259-65.
26. Kang JY, Dolled-Filhart M, Ocal IT, et al. Tissue microarray of hepatocyte growth factor/Met pathway components reveals a role for Met, matriptase, and hepatocyte growth factor activator inhibitor 1 in the progression of node-negative breast cancer. Cancer Res 2003;63:1101-5.
27. Yan S , Jiao X , Zou H, et al. Prognostic significance of c-Met in breast cancer: a meta-analysis of 6010 cases. Diagn Pathol 2015;10:62.
28. Badve S, Dabbs DJ, Schnitt SJ, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Mod Pathol 2011;24:157-67.
29. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol 2015;33:244-50.
30. Charafe-Jauffret E, Ginestier C, Monville F, et al. Gene expression profiling of breast cell linesidentifies potential new basal markers. Oncogene 2006;25:2273-84.
31. Gonçalves A, Charafe-Jauffret E, Bertucci F, et al. Protein profiling of human breast tumor cells identifies novel biomarkers associated with molecular subtypes. Mol Cell Proteomics 2008;7:1420-33.
32. Garcia S, Dalès JP, Charafe-Jauffret E, et al. Poor prognosis in breast carcinomas correlates with increased expression of targetable CD146 and c-Met and with proteomic basal-like phenotype. Hum Pathol 2007;38:830-41.
33. Wu JM, Fackler MJ, Halushka MK, et al. Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res 2008;14:1938-46.
34. Ho-Yen CM, Green AR, Rakha EA, et al. C-Met in invasive breast cancer: is there a relationship with the basal-like subtype? Cancer 2014;120:163-71.
35. Kim YJ, Choi JS, Seo J, et al. MET is a potential target for use in combination therapy with EGFR inhibition in triple-negative/basal-like breast cancer. Int J Cancer 2014;134:2424-36.
36. Zagouri F, Bago-Horvath Z, Rössler F, et al. High MET expression is an adverse prognostic factor in patients with triple-negative breast cancer. Br J Cancer 2013;108:1100-5.
37. 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.
38. 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.
39. Paulson AK, Linklater ES, Berghuis BD, et al. MET and ERBB2 are co-expressed in ERBB2 breast cancer and contribute to innate resistance. Mol Cancer Res 2013;11:1112-21.
40. Minuti G, Cappuzzo F, Duchnowska R, et al. Increased MET and HGF gene copy numbers are associated with trastuzumab failure in HER2-positive metastatic breast cancer. Br J Cancer 2012;107:793-9.
41. Rayson D, Lupichuk SM, Chia SKL, et al. A phase II study of foretinib in triple-negative, recurrent/metastatic breast cancer: NCIC CTG trial IND.197 (NCT01147484). 2012 ASCO Annual Meeting. J Clin Oncol 2012;30:abstr 1036.
42. Tolaney SM, Ziehr DR, Guo H, et al. A phase II study of cabozantinib for metastatic triple-negative breast cancer (TNBC). J Clin Oncol 2015;33:abstr 1080.

Cite this article as: Minuti G, Landi L. MET deregulation in breast cancer. Ann Transl Med 2015;3(13):181. doi: 10.3978/j.issn.2305-5839.2015.06.22

Circulating free DNA in the management of breast cancer

Jacqueline A. Shaw[1], Justin Stebbing[2]

[1]Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester Royal Infirmary, Leicester, LE2 7LX, UK; [2]Division of Cancer, ICTEM, Imperial College, Hammersmith Campus, London W12 0NN, UK

Correspondence to: Professor Jacqueline A. Shaw. Department of Cancer Studies and Molecular Medicine, University of Leicester, Level 3 Robert Kilpatrick Building, Leicester Royal Infirmary, Leicester, LE2 7LX, UK. Email: js39@le.ac.uk.

Abstract: Circulating cell-free DNA (cfDNA) is now under investigation as a "liquid biopsy" in the real time management of cancer. In *The New England Journal of Medicine* Dawson *et al.* reported a proof of concept investigation of tumour specific alterations in cfDNA and demonstrate that this fraction termed "circulating tumour DNA" (ctDNA) shows greater correlation with changes in tumour burden than two other circulating biomarkers (CA 15-3 and circulating tumor cell counts) in individuals with metastatic breast cancer receiving therapy.

Keywords: Breast cancer; biomarkers; circulating tumour DNA; circulating tumour cells (CTCs)

Submitted May 24, 2013. Accepted for publication Jun 24, 2013.
doi: 10.3978/j.issn.2305-5839.2013.06.06
View this article at: http://www.atmjournal.org/article/view/2930/3850

Breast cancer is the most common cancer in women worldwide. Although metastatic breast cancer is currently incurable, there are a number of endocrine, cytotoxic and biological therapies that benefit some patients though determination of tumor burden remains problematic. Treatment response can be assessed by imaging, the serum biomarker cancer antigen 15-3 (CA 15-3) and the FDA approved CellSearch system, which enumerates circulating tumour cells (CTCs). An increase in CA 15-3 levels or a CTC count of ≥5 cells per 7.5 mL blood is associated with poorer prognosis; however, both methods have a sensitivity of only 60% to 70% (1-3) and imaging often fails to rapidly detect changes in tumour burden. There is a need for improved biomarkers with greater sensitivity and specificity to monitor treatment response, help determine the benefit of new and emerging therapies and provide more accurate means for determining prognosis.

Circulating free DNA (cfDNA) first described over 60 years ago (4), has potential as a "liquid biopsy" to monitor cancer in real time. Elevated levels of cfDNA are observed in cancer, particularly in advanced disease, but have also been suggested for the diagnosis of breast (5) and other cancers (6). However, detection of tumour specific alterations in cfDNA [e.g., mutations, loss of heterozygosity (LOH), hypermethylation] has the potential to provide tumour specific markers and has been more widely investigated [reviewed in (7)]. Some studies have suggested that the proportion of cfDNA, which carries tumour specific alterations termed ctDNA, is variable and represents only a small fraction of total cfDNA (8,9). However, there are currently no consensus protocols either for isolation of cfDNA, or for enrichment of this ctDNA, suggested to be largely associated with low molecular weight fractions (10,11), making comparison between different studies and data difficult.

Our group was the first to report emergence of HER2 amplification in cfDNA in patients who were HER2 negative at diagnosis through analysis of cfDNA (12). We also demonstrated whole genome wide analysis of cfDNA using an SNP 6.0 array and reported common tumour-associated copy number variation in cfDNA of 65 breast cancer patients (13). Rapid developments in next generation sequencing have enabled similar genome-wide analysis of cfDNA (14,15) and other recent studies have shown the emergence of acquired resistance to targeted therapies (16,17) through analysis of cfDNA.

The data presented by Dawson *et al.* (18) extend this developing field through the combined use of digital PCR

and targeted deep sequencing (TDS) to assess serial blood samples in 52 patients with metastatic breast cancer while undergoing treatment. Using a combination of a candidate gene approach to screen for somatic mutations in *PIK3CA* and *TP53* and whole genome paired-end sequencing of primary tumour tissue, they identified point mutations and patient specific somatic structural variants, i.e., ctDNA, in 30 of the 52 patients. Results demonstrated a sensitivity of mutant allele detection of 0.1% for digital PCR and 0.14% for TDS. In some patients ctDNA results were discordant with the primary tumour, suggesting tumour evolution and/or emergence of an original minor clone. One critical question to emerge from these data, which will require follow up in new clinical trials, is what level of alteration/mutation in a key driver is sufficient to initiate a switch in disease management? For those patients in whom mutations could not be observed, probably as a result of lack of analysis of all genes, results were inconclusive; the test appears to work well on finding mutations in both the solid tumor and pairing this with the blood sample. Overall, using a modified bootstrap approach Dawson *et al.* demonstrated improved sensitivity of ctDNA over both CA 15-3 (85% *vs.* 59%) and CTCs (90% *vs.* 67%). In 20 of the patients, for whom blood data was available for three or more time points they showed that fluctuations in ctDNA correlated with treatment response observed by imaging. Similar results were also seen for CTC counts and CA 15-3 but again with less sensitivity. Finally, using a Cox proportional hazards model increasing ctDNA levels and CTC counts were both correlated with poorer overall survival ($P<0.0001$ and $P<0.03$, respectively).

Much work is still to be done before sensitive mutation analysis of ctDNA, whether by TDS or another method, becomes routine in the clinic, but rapidly accumulating data presented by Dawson *et al.* and others (12-14,16-18) show the potential of this approach for sensitive and specific serial sampling to provide a "liquid biopsy" of cancer in real time. As our understanding of the genetic heterogeneity of breast and other cancers develops, this will allow for intelligent design of custom assays to survey ctDNA in cfDNA. Alongside this rapidly advancing technology will likely improve in terms of throughput, sensitivity, cost and ease of use. In the not too distant future, ctDNA/cfDNA analysis has the potential to revolutionise the management of common cancers and as we move into the era of personalised medicine but these tests require standardization so they are reliable and reproducible in the same manner as CTC tests. Despite low numbers of patients in this study, in aggregate it appears that ctDNA is reliable in detecting tumor burden but their role in the clinic will take years to establish, so incorporation into prospective clinical trials would be optimal. Whether such tests can replace or adjunct traditional imaging is unclear, and ctDNA/cfDNA is likely to be as heterogenous as the original tumors themselves.

Acknowledgements

With thanks to funders Cancer Research UK, The National Institute for Health Research, The Roy Castle Lung Cancer Foundation. Pancreatic Cancer UK and Hope Against Cancer.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287-312.
2. Duffy MJ, Evoy D, McDermott EW. CA 15-3: uses and limitation as a biomarker for breast cancer. Clin Chim Acta 2010;411:1869-74.
3. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781-91.
4. Mandel P, Metais P. Les acides nucleiques du plasma sanguin chez l'homme. C R Seances Soc Biol Fil 1948;142:241-3.
5. Huang ZH, Li LH, Hua D. Quantitative analysis of plasma circulating DNA at diagnosis and during follow-up of breast cancer patients. Cancer Lett 2006;243:64-70.
6. Mead R, Duku M, Bhandari P, et al. Circulating tumour markers can define patients with normal colons, benign polyps, and cancers. Br J Cancer 2011;105:239-45.
7. Jung K, Fleischhacker M, Rabien A. Cell-free DNA in the blood as a solid tumor biomarker--a critical appraisal of the literature. Clin Chim Acta 2010;411:1611-24.
8. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011;11:426-37.
9. Gormally E, Caboux E, Vineis P, et al. Circulating free

DNA in plasma or serum as biomarker of carcinogenesis: practical aspects and biological significance. Mutat Res 2007;635:105-17.
10. Kuhlmann JD, Schwarzenbach H, Wimberger P, et al. LOH at 6q and 10q in fractionated circulating DNA of ovarian cancer patients is predictive for tumor cell spread and overall survival. BMC Cancer 2012;12:325.
11. Schwarzenbach H, Eichelser C, Kropidlowski J, et al. Loss of heterozygosity at tumor suppressor genes detectable on fractionated circulating cell-free tumor DNA as indicator of breast cancer progression. Clin Cancer Res 2012;18:5719-30.
12. Page K, Hava N, Ward B, et al. Detection of HER2 amplification in circulating free DNA in patients with breast cancer. Br J Cancer 2011;104:1342-8.
13. Shaw JA, Page K, Blighe K, et al. Genomic analysis of circulating cell-free DNA infers breast cancer dormancy. Genome Res 2012;22:220-31.
14. Beck J, Urnovitz HB, Mitchell WM, et al. Next generation sequencing of serum circulating nucleic acids from patients with invasive ductal breast cancer reveals differences to healthy and nonmalignant controls. Mol Cancer Res 2010;8:335-42.
15. Leary RJ, Sausen M, Kinde I, et al. Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. Sci Transl Med 2012;4:162ra154.
16. Diaz LA Jr, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012;486:537-40.
17. Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012;486:532-6.
18. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199-209.

Cite this article as: Shaw JA, Stebbing J. Circulating free DNA in the management of breast cancer. Ann Transl Med 2014;2(1):3. doi: 10.3978/j.issn.2305-5839.2013.06.06

Breast imaging in the young: the role of magnetic resonance imaging in breast cancer screening, diagnosis and follow-up

Dorria Saleh Salem, Rasha Mohamed Kamal, Sahar Mahmoud Mansour, Lamiaa Adel Salah, Rasha Wessam

Women's Imaging Unit, Cairo University, Egypt

Correspondence to: Rasha Mohamed Kamal. Professor of Radiology, Director of the "Women's Health Outreach program", Cairo, Egypt. Email: rashaakamal@hotmail.com.

Abstract: Diagnosis of breast cancer in young individuals (younger than 40 years old) poses a real challenge to breast radiologists because their breast tissue is often denser than the breast tissue of older women. Magnetic Resonance Imaging (MRI) may be particularly helpful in such situations. The American Cancer Society (ACS) recommended breast MRI screening as an adjunct to mammography for: BRCA mutation carriers and their first-degree relatives; women with a lifetime breast cancer risk ≥20% to 25%; women with a history of chest radiation between ages of 10 and 30 years; and women with predisposing genetic syndromes. Currently, breast MRI demonstrates a high sensitivity in the range of 93-100%. As many benign lesions also show enhancement or other atypical features on MRI, the primary weakness of contrast enhanced MRI remains in its low specificity, reported to be in the range of 37-97%. Breast MRI is helpful in demonstrating the true tumor size initially, as well as identifying residual tumor following the completion of neo-adjuvant therapy. In general, sensitivities ranging from 61% to 86% for detecting residual disease have been reported. The absence of enhancement virtually excludes a recurrence and the presence of enhancement is very specific for tumor even in the radiated breast. MRI is also the preferred modality for assessment of the breast after re- constructive surgery. The role of Magnetic Resonance Imaging (MRI) in breast diagnosis will continue to evolve as technology improves and clinical experience with new techniques expands.

Keywords: Breast cancer; young females; magnetic resonance imaging (MRI)

Submitted Apr 18, 2013. Accepted for publication May 06, 2013.
doi: 10.3978/j.issn.2072-1439.2013.05.02
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.05.02

Introduction

Breast cancer is a disease that knows no boundaries. It can strike women at any age. Doctors may not take young women seriously when they express concerns about breast cancer (1). The wrong perception that young women do not get breast cancer often leads to an initial misdiagnosis. Many breast symptoms and signs in young individuals are dismissed by clinicians and radiologists as cysts or benign breast lesions and they usually adopt a 'follow up' protocol.

By the time a lump can be diagnosed in a young woman, it is often large enough and advanced enough to lower the chances of survival. In addition, the cancer may be more aggressive and less responsive to hormone therapy. Breast carcinoma in young patients has been reported to present with more aggressive biologic characteristics and to behave poorly compared with the disease in older patients (2).

Five-year relative survival is lower among women with a more advanced stage at diagnosis. Considering all races, 5-year relative survival is 99% for localized disease, 84% for regional disease, and 23% for distant-stage disease. Larger tumor size at diagnosis is also associated with decreased survival (1). Thus the early detection and diagnosis of breast cancer is thus an emotive issue and a test is required that is both sensitive and specific. In general, regular mammograms are not recommended for women under 40 years of age, in part because breast tissue tends to be denser in young women, making mammograms less effective as a

screening tool. In addition, most experts believe the low risk of developing breast cancer at a young age does not justify the radiation exposure or the cost of mammography. Ultrasound (US) although an excellent alternative method for assessing palpable abnormalities in young individuals, yet, it has limitations as a screening modality with a false negative rate ranging from 0.3% to 47% in some series (3).

Breast MRI is no longer an experimental modality, but has attained a solid position in the diagnosis and workup of breast lesions (4). MRI may be particularly helpful in certain situations. This includes high risk patients especially those who have dense breast tissue. Dense breast tissue in young women may obscure signs of malignancy on mammography and limit the evaluation of the true extent of disease (5).

In this review article we will discuss the role of MRI in the screening, diagnosis and follow up of breast cancer in young individuals.

Technique of MRI

MRI utilizes magnetic fields to produce detailed cross-sectional images of tissue structures, providing very good soft tissue contrast. MRI creates images of the breast by measuring changes in the movement of protons in fat and water with the application of changing magnetic fields and by utilizing the differences in tissue relaxation characteristics. Contrast between tissues in the breast (fat, glandular tissue, lesions, etc.) depends on the mobility and magnetic environment of the hydrogen atoms in water and fat that contribute to the measured signal that determines the brightness of tissues in the image. In the breast, this results in images showing predominantly parenchyma and fat, and lesions, if they are present. The use of MRI for breast cancer detection is based on the concept of tumor angiogenesis or neo-vascularity. Tumor associated blood vessels have increased permeability, which leads to prompt take up and release of gadolinium within the first one to two minutes after administration, leading to a pattern of rapid enhancement and washout on MRI. This dynamic rapid enhancement pattern helps to distinguish breast cancers from benign lesions. Thus, contrast enhanced MRI has been shown to have a high sensitivity for detecting breast cancer in high-risk asymptomatic and symptomatic women, although reports of specificity have been more variable (6). This high signal from enhancing lesions can be difficult to separate from fat, leading to the use of subtraction images or fat suppression, or both, to assess disease. Because parenchymal tissue also enhances, but generally more slowly than malignant lesions, and also because contrast can wash out rapidly from some tumors, it is important to look at images at an early time point after contrast injection (typically 1 to 3 minutes). MRI examinations may involve examining images at one time point or, more often, will collect a pre-injection image with sequential sets of images after contrast injection [dynamic contrast-enhanced (DCE)-MRI]. Both the appearance of lesions and, where available, the uptake and washout pattern can be used to identify malignant disease and discriminate it from benign conditions. These techniques, which have been widely employed for assessing symptomatic disease, have recently been shown to provide good sensitivity as a screening tool for breast cancer in women at increased risk based on family history (7-9).

MRI in screening for breast cancer in young females

Breast cancer is diagnosed in over one million women worldwide every year. Until breast cancer can be prevented, early detection offers the best chance for cure (10).

In generic terms, for a screening procedure to be considered useful, it should not only find lesions at an earlier stage, but also it should demonstrate that earlier diagnosis results in some clinical benefit, preferably a reduction in breast cancer mortality (11). Although mammography screening is frequently offered to women with a genetic predisposition to breast cancer at a younger age, the efficacy of this approach is unproven. Preliminary results in such women showed that mammographic screening has a low sensitivity for detecting tumors, especially in carriers of BRCA mutation. These women have a cumulative lifetime risk of developing breast cancer of 21-65%. Women genetically predisposed to breast cancer often develop the disease at young age when dense breast tissue reduces the sensitivity of mammography. Other possible reasons include changes seen on mammography in carriers of BRCA mutation as compared with non carriers of the same age (12-14) (*Figure 1*).

Owing to the debate regarding the role of MRI as a screening test, the American Cancer Society has outlined recommendations for the use of breast MRI for breast cancer screening. It should be stressed that if MRI is used, it should be in addition to, not instead of, a screening mammogram. This is because although an MRI is a more sensitive test (it's more likely to detect cancer than a mammogram), it may still miss some cancers that a

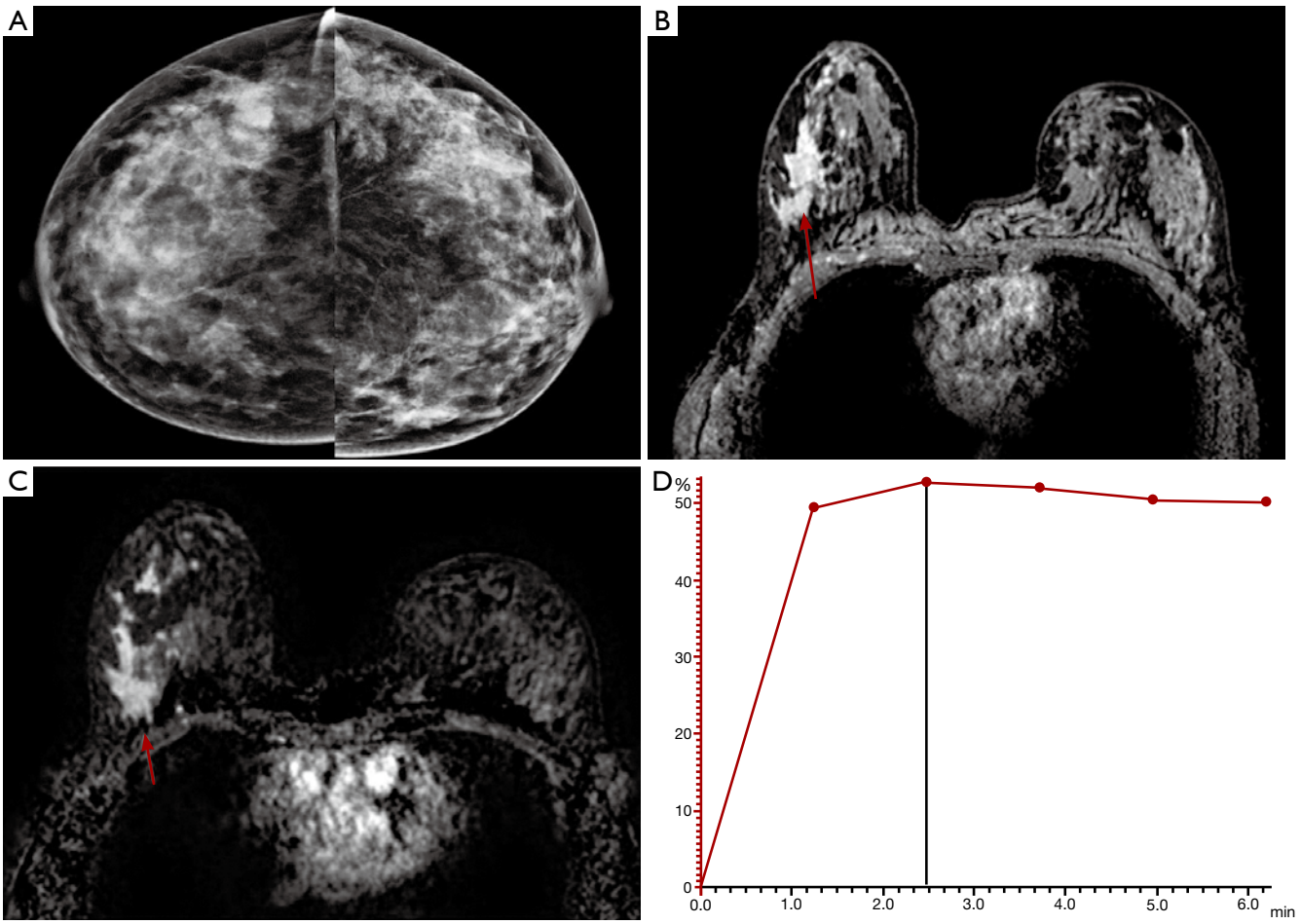


Figure 1 39 year-old female complaining of cyclic mastalgia. Craino-caudal view of the mammogram (A) shows a heterogeneous dense parenchyma. Intense right UOQ contrast uptake (red arrow) was seen in the dynamic post contrast (B) and corresponding subtraction (C) MR images. Post processing kinetics elicits early enhancement reaching 60 % and plateau curve pattern (D). Pathology revealed invasive duct carcinoma (grade II).

mammogram would detect. For most women at high risk the ACS recommended screening with MRI and mammograms should begin at age 30 years and continue for as long as a woman is in good health. But because the evidence is limited about the best age at which to start screening, this decision should be based on shared decision-making between patients and their health care providers, taking into account personal circumstances and preferences. The American Cancer Society (ACS) recommended breast MRI screening as an adjunct to mammography for: *BRCA* mutation carriers and their first-degree relatives; women with a lifetime breast cancer risk ≥20% to 25%; women with a history of chest radiation between ages of 10 and 30 years; and women with predisposing genetic syndromes (e.g., Li-Fraumeni, Cowden). The group felt there was insufficient evidence to recommend for or against MRI screening among women with a personal history of invasive breast cancer or duct carcinoma in situ (15).

In 2010, the European Society of Breast Cancer Specialists (EUSOMA) published a paper evaluating the available evidence regarding clinical value of and indications for breast MRI. This paper reported the results of all the cohort studies investigating the diagnostic performance of different imaging modalities in the surveillance of high-risk women. They recommended that women with a family history suggesting an inherited predisposition to breast cancer should have their risk assessed by an appropriately trained professional group (e.g., genetic

counseling). If found to be at high risk (20-30% lifetime risk or greater), these women should be given oral and written information regarding their risk and the risks and benefits of mammography and MRI screening or alternative risk-reducing interventions. If these women accept to be screened by MRI, they should be informed about screening intervals and logistics. This should be determined on the basis of regional or national considerations reflecting an area-specific cumulative risk in the general population, resource availability and practical feasibility. They recommended that annual MRI screening should be available starting at age 30 (16).

Based on several observational studies that have yielded consistent results, the combination of annual magnetic resonance imaging (MRI) plus mammography is now the standard of care for screening women with BRCA mutations who decline risk-reducing mastectomy. Because of its high sensitivity, multiple investigators have studied the potential role of MRI in screening women at high risk. In the past few years, results from eight major clinical trials exploring breast MRI as a screening tool have been published. Combined, the studies included 4,271 patients and found 144 breast cancers detected by MRI, for an overall cancer yield of 3%. The sensitivity of MRI ranged from 71% to 100% across the studies. Although its reported specificity was variable, the call-back rates and risk of benign biopsies were within acceptable limits. In general, patients who underwent breast MRI screening had a 10% risk of being called back, and a 5% risk of having a benign biopsy (17).

A study was conducted to summarize the sensitivity, specificity, likelihood ratios, and posttest probability associated with adding MRI to annual mammography screening of women at very high risk for breast cancer in eleven relevant, prospective, nonrandomized studies that ranged from small single-center studies with only one round of patient screening to large multicenter studies with repeated rounds of annual screening were identified. Characteristics of women that varied across study samples included age range, history of breast cancer, and BRCA1 or BRCA2 mutation status. Studies used dynamic contrast-enhanced MRI with axial or coronal plane images (European studies) or sagittal images (North American studies) that were usually interpreted without knowledge of mammography results. The summary negative likelihood ratio and the probability of a BI-RADS-suspicious lesion (given negative test findings and assuming a 2% pretest probability of disease) were 0.70 (95% CI, 0.59 to 0.82) and 1.4% (CI, 1.2% to 1.6%) for mammography alone and 0.14 (CI, 0.05 to 0.42) and 0.3% (CI, 0.1% to 0.8%) for the combination of MRI plus mammography, using a BI-RADS score of 4 or higher as the definition of positive. The authors concluded that screening with both MRI and mammography might rule out cancerous lesions better than mammography alone in women who are known or likely to have an inherited predisposition to breast cancer (18).

Should we perform MRI of the breast to screen all women?

At this time, MRI is used mostly in breast cancer diagnosis and staging, rather than in screening. Given this impressive ability to detect tumors not found on mammograms, MRI might seem to be a logical choice for breast cancer screening. Yet none of the nationally recognized advisory groups is recommending it for women at average risk. There are several important reasons for this:

(I) MRI screening is time consuming, requires the injection of intravenous contrast, generates more false-positive results, and has not been shown to impact breast cancer mortality (19);

(II) High-quality breast MRI is still unavailable everywhere;

(III) Although screening with MRI may improve survival for women with familial risk of breast cancer, but is expensive. It has been found to be cost effective for women with a *BRCA1/2* mutation, it remains unclear whether this is the case for women with a family history of breast cancer without a proven genetic predisposition (20). The projected cost-effectiveness of annual combined screening with MR imaging and screen-film mammography is strongly dependent on the cost of an MR imaging examination and on the underlying breast cancer risk in the women being screened (21);

(IV) Moreover, breast MRI can't be performed to women who have certain devices in place such as pacemakers or implantable cardioverter-defibrillators;

(V) The ability of MRI to detect tiny calcifications of early pre-invasive breast cancer (duct carcinoma in situ, or DCIS) is limited;

(VI) Because MRI is so good at picking up any abnormal tissue, whether cancerous or not, it leads to too many negative biopsies;

(VII) False negatives after MRI screening can be attributed to inherent technological limitations of MRI, patient characteristics, quality assurance

failures and human error (19);

(VIII) False positives can be attributed to the same factors, as well as heightened medical concerns over the consequences of missed cancers. A screening exam is considered to be false positive when its results recommend further testing or a biopsy of a suspicious finding, but no cancer is found. While MRI is more sensitive than mammograms, it also has a higher false-positive rate (it is more likely to find something that turns out not to be cancer). False-positive results during breast MRI screening may have adverse psychological effects. They would lead to unneeded biopsies and other tests in many of the women screened, which can lead to a lot of worry and anxiety (19,22).

MRI in the diagnosis of breast cancer in young individuals

Diagnosis means characterization of detected lesions whether benign or malignant. Staging should pursue this step when malignant pathology is identified.

Sensitivity and specificity contribute to the accuracy of any diagnostic tool. In the case of contrast-enhanced breast MRI, there is strong evidence that the sensitivity is greater than the sensitivity of other techniques of imaging the breast. Currently, breast MR demonstrates a high sensitivity in the range of 93-100%. As many benign lesions also show enhancement or other atypical features on MRI, the primary weakness of contrast enhanced MRI remains in its low specificity, reported to be in the range of 37-97%. However, for the further implementation of diagnostic breast MRI in clinical practice, a reduced overall number of false-positive findings remain a major aim. For sufficient reliable and standardized differential diagnosis of malignant and benign lesions, the characterization of specific features of the lesions is vital (23).

Typically, the first step in evaluating lesion morphology on breast MRI is to classify the lesion as a mass, a focal lesion, or a non-mass-like enhancement. The BI-RADS breast MRI lexicon gives the following clear definitions for mass and non-mass-like enhancement: “Mass—A mass is a three-dimensional space-occupying lesion that comprises one process, usually round, oval, lobular, or irregular in shape”; “Non-mass-like enhancement—Enhancement of an area that is not a mass” (24).

In the case of mass-type lesions there are several parameters that can be used for constructing the differential diagnosis. For example, dark T2 signal, spiculation (morphology), rim or heterogeneous enhancement (texture) and the wash-out kinetic pattern are typical features of malignant lesions; whereas smooth margin (morphology), low and homogeneous enhancement (texture) and a persistent kinetic pattern typically indicate a benign mass (25) (*Figure 2*).

On the other hand, diagnosis of non-mass-like enhancement lesions is much more challenging. Malignant lesions such as duct carcinoma in situ (DCIS) and invasive lobular cancer (ILC) are likely to present as non-mass-like enhancement (*Figure 3*). Benign fibrocystic changes, which also appear as non-mass-like enhancement, are a frequent finding on DCE-MRI. Unlike mass lesions, non-mass-like enhancement lesions exhibit poorly defined boundaries, leading to difficulty in the analysis of morphology. Furthermore, the malignant non-mass lesions often do not show the typical wash-out pattern in enhancement kinetics, so this very useful diagnosis criterion for mass lesions has a limited diagnostic value for non-mass lesions. Diagnosis of these lesions is challenging because the enhancement of normal tissues and some benign processes, such as fibrocystic change, might show similar appearances (25).

When breast carcinoma is diagnosed, the extent of disease may not be apparent either by palpation or by mammography. Because of its very high sensitivity, MRI is particularly well suited for staging women diagnosed with breast cancer (26).

Traditionally, breast cancer was treated with mastectomy, although equivalent long-term survival is obtained with breast-conserving surgery and radiotherapy. Whether a patient is suitable for breast-conserving surgery depends on the size of the mass, particularly in relation to the size of the breast, the presence of multifocal or multi-centric disease, and involvement of the nipple (*Figure 4*). Multifocal or multi-centric disease has been demonstrated in 31% of patients with known breast cancer. MRI is quite sensitive to multi-focality, provided the scan has been performed to cover the entire breast, or both breasts. Residual breast cancer at the lumpectomy site can result in recurrence. Therefore, successful treatment depends on accurate pre-surgical knowledge of the extent of the disease. Tumor size is under estimated by mammography and ultrasound. Contra lateral occult synchronous tumors could be also identified (27).

Tumors located posterior in the breast are difficult to evaluate fully with mammography and muscle invasion is often difficult to detect by ultrasound due to acoustic shadowing from the tumor. Breast MRI can also identify

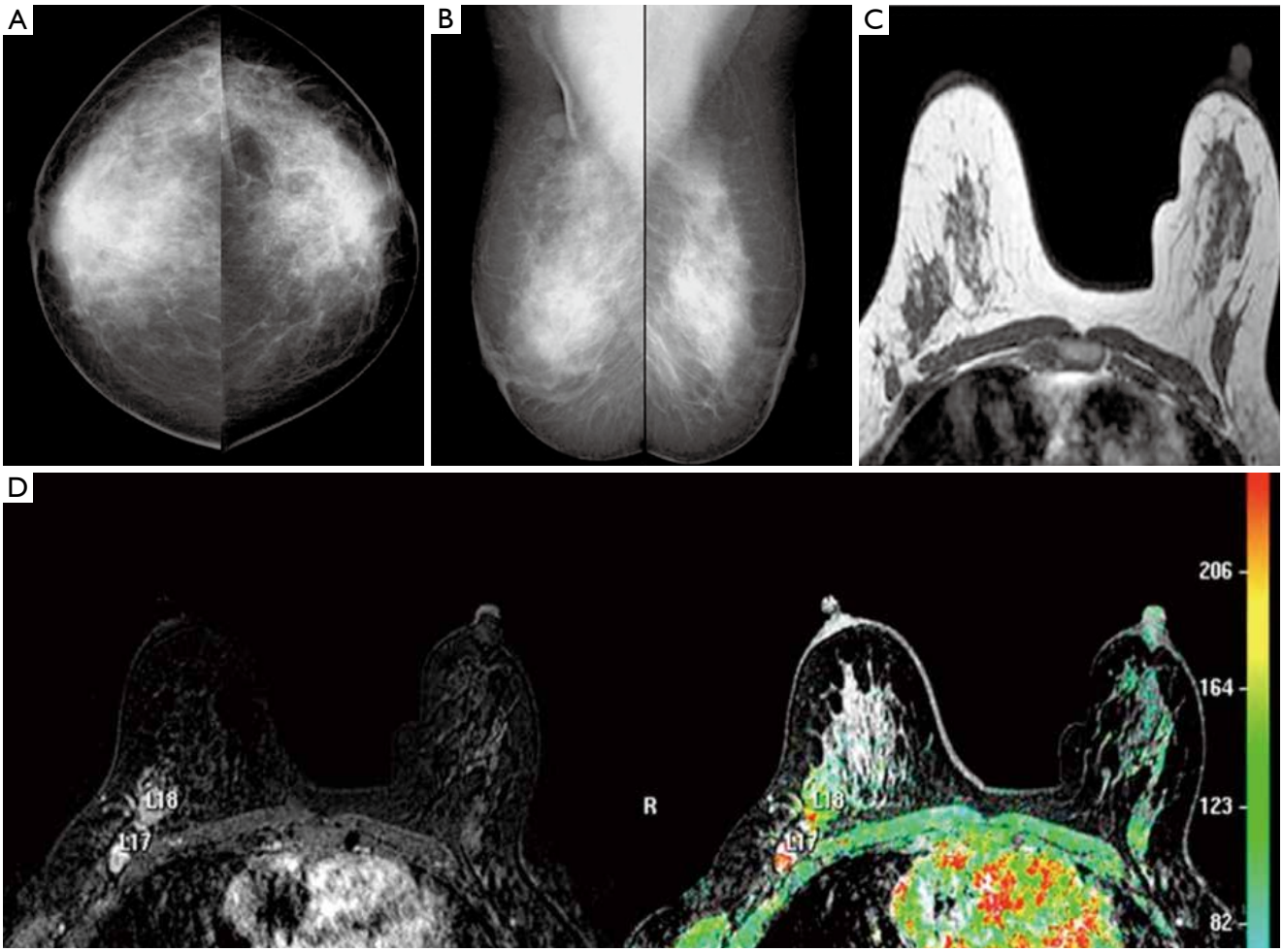


Figure 2 37 year-old female complaining of right breast inflammatory changes first diagnosed as mastitis resistant to antibiotic therapy. Her mammogram (A and B) showed subtle diffuse increased right breast density. MRI showed a spiculated outlined mass lesion in the right UOQ with ipsilateral enlarged axillary nodes. The mass showed a dark T2 signal (C) and on the dynamic post contrast sequence (D) showed intense heterogeneous contrast uptake as seen in the subtraction (right) and color coded (left) images. Biopsy revealed IDC.

chest wall invasion which changes the disease stage to IIIB regardless of primary tumor size. Tumor invasion is identified as muscle enhancement, which may have an infiltrative or mass-like appearance. Muscle enhancement is the only finding which has been shown to reliably indicate tumor invasion. Loss of fat planes between the tumor mass and muscle, and vascular structures extending from the tumor through the muscle, do not indicate tumor invasion (28,29).

Should we perform MRI of the breast to diagnose all cases?

Prudence should be used with the application of Breast MRI in evaluating breast cancer patients due to:

(I) There is significant overlap of contrast enhancement in benign and malignant breast lesions on MRI;
(II) The large number of false positive results in additional biopsy in about 4-21% of patients;
(III) There is significant overlap between normal tissues and malignant tissues;
(IV) Suspicious uptake has been recorded with a variety of benign and benign precancerous conditions;
(V) Many enhancing features on MRI, particularly those with diffuse or regional distribution that show moderate, progressive-to-stabilized enhancement, do not turn out to be cancer. This pattern can

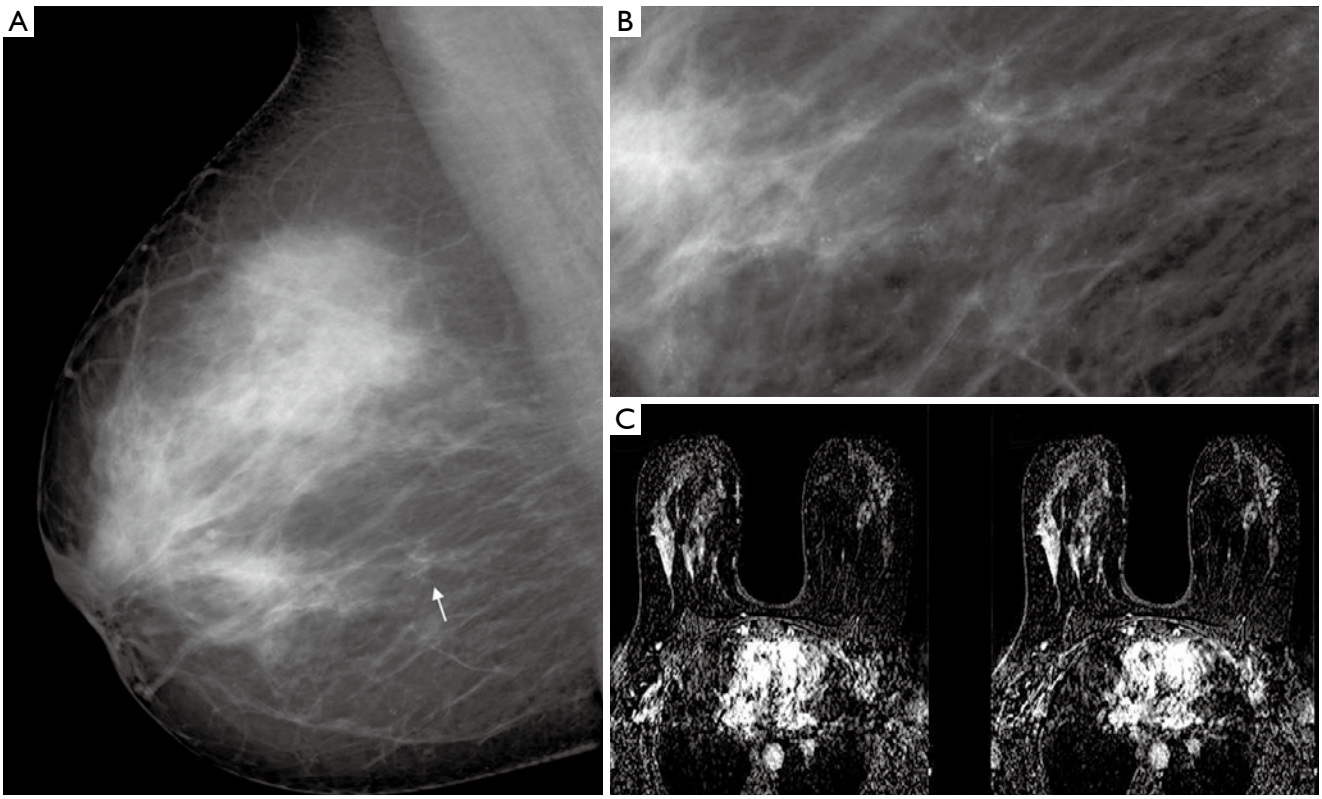


Figure 3 34 year-old female complaining of mastalgia. Her mammogram (A) showed clustered, segmental pleomorphic calcifications (arrow) seen on the magnified view (B). Post contrast MRI study showed linear segmental non mass enhancement (subtraction image, C). Biopsy revealed DCIS.

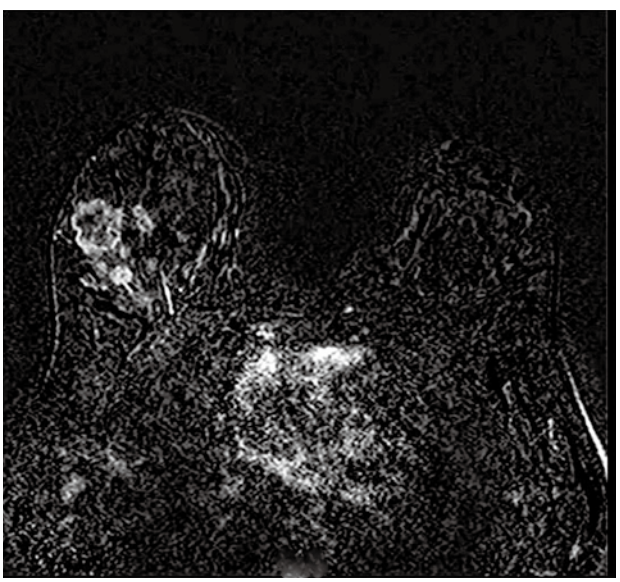


Figure 4 Subtraction contrast MR image of 27 year-old female with a multifocal right breast carcinoma.

also be associated however, with DCIS, lobular carcinoma, or low grade invasive duct carcinoma. Such findings present a diagnostic dilemma and MR-guided biopsy capabilities are not yet readily available;

(VI) While MRI can demonstrate enhancing lesions on the order of 1-2 mm in size, it is virtually impossible to obtain histo pathologic validation of these small imaging occurrences, making it difficult to determine the true sensitivity of breast MRI (26,27).

MRI in the follow up of breast cancer cases in young individuals

Follow-up post-neoadjuvant chemotherapy

It is becoming increasingly common to treat women with locally advanced disease with neo-adjuvant therapy.

Clinical response alone is not a very accurate measure of response to therapy however, and many investigators have pursued imaging to track response. Traditionally, palpation, mammography, and sonography have been used, but edema and necrosis at the tumor site may hinder measurement of the tumor's true size. Clinical breast exam has been found to underestimate residual disease. MRI is emerging as a very important modality, not only because it can delineate the extent of disease and accurately assess response to therapy, but also because it enables us to look at the morphology of tumors and identify tumor patterns that are distinct at initial presentation (26).

Neo-adjuvant chemotherapy is given to patients after the diagnosis of malignancy has been made but prior to definitive surgical treatment, to decrease the size of the tumor. The change in appearance on post chemotherapy may be decrease in tumor size, change in tumor cellularity, or change in tumor vascularity. The extent of response to chemotherapy and amount of residual tumor determines the treatment options in this setting. Delineating the response poses a clinical challenge. Breast MRI is helpful in demonstrating the true tumor size initially, as well as identifying residual tumor following the completion of neo-adjuvant therapy. Although, MRI is limited by both over- and under estimation of residual disease, it has been shown to correlate more accurately with pathologic specimens pathological complete response (pCR), in the range of 71% to 90%, *vs.* clinical exam (19% to 60% accuracy), ultrasound (35% to 75% accuracy) and mammography (26% to 70% accuracy). It is important to recognize that even though no residual disease maybe evident by MRI, surgical resection is still required due to the potential under estimation of residual disease. For this reason, it is important to place a tissue marker prior to treatment. MRI provides not only an anatomic evaluation of the tumor but also a physiologic one. As MRI findings are based on the vascularity of the tumor, the effect of chemotherapy agents that inhibit tumor angiogenesis can be seen. Diminished contrast enhancement following chemotherapy would support reduction in tumor vascularity. Decrease in peak contrast uptake and flattening of the contrast uptake curve have been seen in tumors following chemotherapy (30-33) (*Figure 5*).

A systematic literature research including forty four studies (2,050 patients) was conducted to examine MRI accuracy in detecting residual tumor, investigates variables potentially affecting MRI performance, and compares MRI with other tests. MRI had higher accuracy than mammography (P=0.02); there was only weak evidence that MRI had higher accuracy than clinical examination (P=0.10). No difference in MRI and ultrasound accuracy was found (P=0.15). The authors concluded that MRI accurately detects residual tumor after neo adjuvant chemotherapy. Accuracy was lower when pCR was more rigorously defined, and specificity was lower when test negativity thresholds were more stringent; these definitions require standardization. They stated that although MRI is more accurate than mammography; however, they recommended further studies comparing MRI and ultrasound (34).

Follow up after breast operations

Evaluation of patients who have undergone mastectomy and breast reconstruction is another very difficult issue. As most of the breast tissue has been removed, the site of recurrence is beneath the skin or near the chest wall. These areas are difficult to image with mammography, and post surgical changes can be easily interpreted as malignant. Patients who have been treated with breast conservation therapy with resultant positive surgical margins are typically offered one additional attempt at excision. Mastectomy is usually performed if negative margins are not achieved. Breast MRI in these patients is helpful to identify the extent of residual disease, which may aid in surgical planning for re-excision and may prospectively identify those patients who would ultimately require mastectomy. The purpose of MRI is to detect the presence of multifocal and multi-centric disease as well as to detect bulky residual disease at the lumpectomy site in order to allow directed re-excision. Microscopic residual disease at the surgical margins is known to be present and surgical excision is still required, even if the MRI findings are negative (30).

The evaluation of the tumor bed with MRI is limited, as granulation tissue may enhance in the early postoperative period. Frei *et al.* determined that the least number of false-positive results were found when MRI was performed between 35 to 42 days following surgery (35). In general, sensitivities ranging from 61% to 86% for detecting residual disease have been reported (36). Studies have shown that the absence of enhancement virtually excludes a recurrence and the presence of enhancement is very specific for tumor even in the radiated breast (30) (*Figure 6*).

Follow up after breast reconstructive surgery

Breast surgery to rebuild the normal contour of the affected

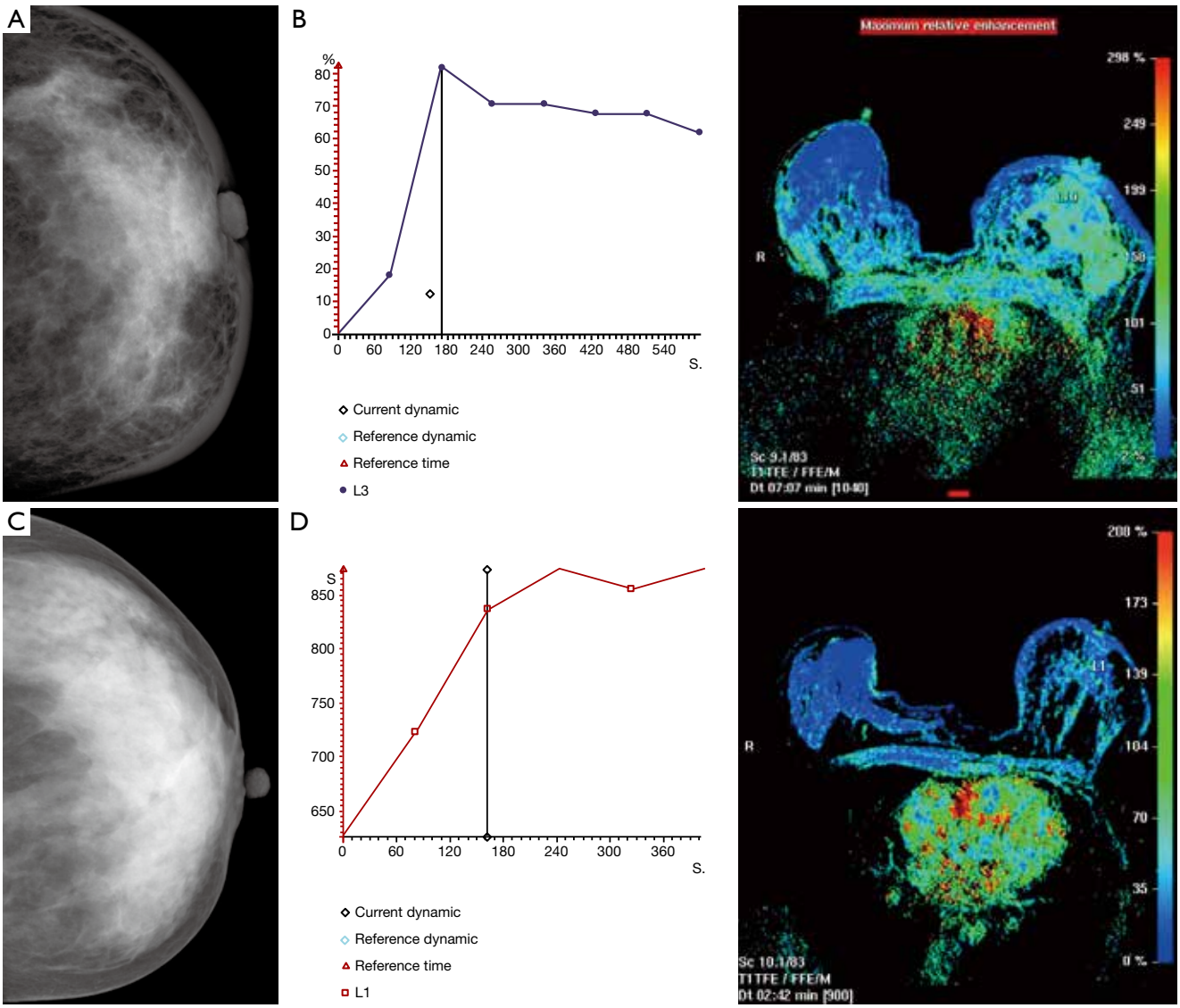


Figure 5 23 year-old lactating female diagnosed as left breast lactation mastitis. Her mammogram (A) showed a diffuse edema pattern of the left breast with coarsened trabeculae and marked skin thickening. A post contrast MRI study (B) was performed and showed an extensive locally advanced carcinoma of the left breast with a type 3 kinematic curve (peak enhancement 80% after 3 minutes). Biopsy revealed IDC grade II. She received 3 cycles of chemotherapy and came for a follow up study to assess response. Her mammogram (C) showed resolution of the edema. MRI study showed faint parenchymal enhancement with a type 2 curve showing delayed peak enhancement of 45% in 6 minutes.

and the contra-lateral unaffected breast to produce a more normal appearance, is considered reconstructive surgery. It is performed following a mastectomy, lumpectomy, or other breast surgery to treat breast cancer. The number of procedures and timing of these procedures varies, depending on the individualized treatment plan devised by the treating physician(s) and the individual and may be impacted by the overall treatment plan for the breast cancer itself (37,38). There are two ways to recreate the breast after a mastectomy: using saline breast implants or the patient's own tissue (muscle flap reconstruction).

Although imaging with ultrasound and mammography have both been used successfully to evaluate the integrity of implants and detect possible problems over time, MRI is the preferred modality to detect implant rupture (39). Advantages of using MRI to detect implant rupture include

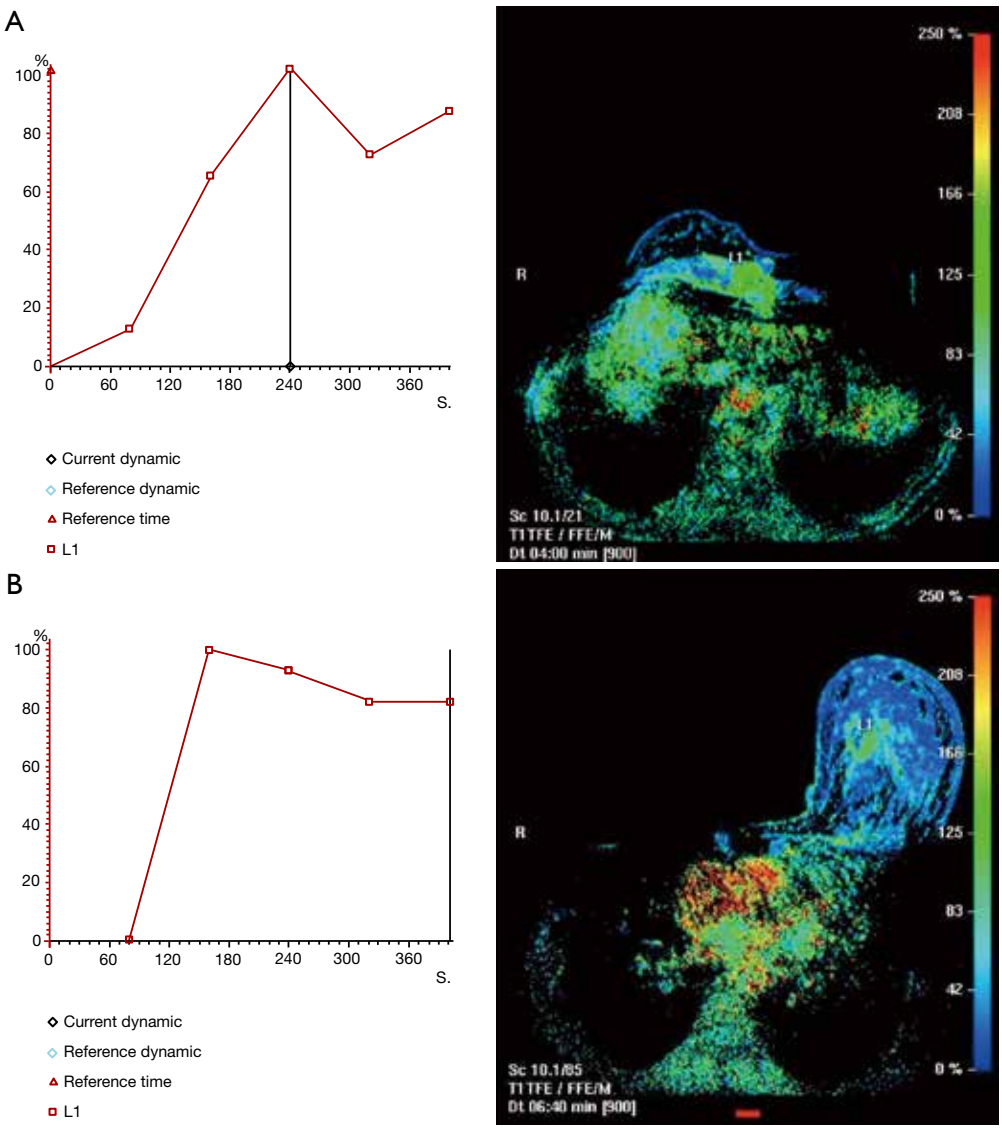


Figure 6 High risk 35 year-old female with history of right mastectomy. An MRI examination was requested as part of her annual check-up. In (A) there is local recurrence involved the chest wall. (B) Contra lateral upper inner quadrant newly developed malignant mass were identified.

imaging with a high sensitivity and specificity, the ability to image the entire implant, and the avoidance of ionizing radiation exposure (40,41).

Conclusions

Contrast-enhanced breast MR imaging is a powerful tool in the breast imaging diagnostic workup especially in high risk young individuals. New evidence on Breast MRI screening has become available since the American Cancer Society last issued guidelines for the early detection of breast cancer in 2003. If MRI is used, it should be in addition to, not instead of, a screening mammogram. The role of Magnetic Resonance Imaging in breast diagnosis will continue to evolve as technology improves and clinical experience with new techniques expands.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Alteri R, Bandi P, Brinton L, et al. Breast Cancer Facts and Figures 2011-2012 a publication of the American Cancer Society, Atlanta, Georgia.
2. Gonzalez-Angulo AM, Broglio K, Kau SW, et al. Women age < or = 35 years with primary breast carcinoma: disease features at presentation. Cancer 2005;103:2466-72.
3. Rankin SC. MRI of the breast. Br J Radiol 2000;73:806-18.
4. Mann RM, Kuhl CK, Kinkel K, et al. Breast MRI: guidelines from the European Society of Breast Imaging. Eur Radiol 2008;18:1307-18.
5. Berg WA. Tailored supplemental screening for breast cancer: what now and what next? AJR Am J Roentgenol 2009;192:390-9.
6. Liu PF, Debatin JF, Caduff RF, et al. Improved diagnostic accuracy in dynamic contrast enhanced MRI of the breast by combined quantitative and qualitative analysis. Br J Radiol 1998;71:501-9.
7. Kuhl CK, Schrading S, Leutner CC, et al. Mammography, breast ultrasound, and magnetic resonance imaging for surveillance of women at high familial risk for breast cancer. J Clin Oncol 2005;23:8469-76.
8. Leach MO, Boggis CR, Dixon AK, et al. Screening with magnetic resonance imaging and mammography of a UK population at high familial risk of breast cancer: a prospective multicentre cohort study (MARIBS). Lancet 2005;365:1769-78.
9. Lehman CD, Blume JD, Weatherall P, et al. Screening women at high risk for breast cancer with mammography and magnetic resonance imaging. Cancer 2005;103:1898-905.
10. World Health Organization. Cancer Statistics worldwide. Cancer Fact sheet No. 297. Globocan, IARC, 2008.
11. Wollins DS, Somerfield MR. Q and a: magnetic resonance imaging in the detection and evaluation of breast cancer. J Oncol Pract 2008;4:18-23.
12. Kriege M, Brekelmans CT, Boetes C, et al. Efficacy of MRI and mammography for breast-cancer screening in women with a familial or genetic predisposition. N Engl J Med 2004;351:427-37.
13. Lord SJ, Lei W, Craft P, et al. A systematic review of the effectiveness of magnetic resonance imaging (MRI) as an addition to mammography and ultrasound in screening young women at high risk of breast cancer. Eur J Cancer 2007;43:1905-17.
14. Antoniou AC, Cunningham AP, Peto J, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. Br J Cancer 2008;98:1457-66.
15. American Cancer Society recommendations for early breast cancer detection in women without breast symptoms. Available online: http://www.cancer.org/cancer/breastcancer/moreinformation/breastcancerearlydetection. Accessed on 30/08/2012.
16. Sardanelli F, Boetes C, Borisch B, et al. Magnetic resonance imaging of the breast: recommendations from the EUSOMA working group. Eur J Cancer 2010;46:1296-316.
17. Lehman CD. Role of MRI in screening women at high risk for breast cancer. J Magn Reson Imaging 2006;24:964-70.
18. Warner E, Messersmith H, Causer P, et al. Systematic review: using magnetic resonance imaging to screen women at high risk for breast cancer. Ann Intern Med 2008;148:671-9.
19. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007;57:75-89.
20. Saadatmand S, Heijnsdijk EA, Rutgers EJ, et al. Cost-effectiveness of screening with additional MRI for women with familial risk for breast cancer without a genetic predisposition. Cancer Res 2012;72:P3-02-09.
21. Lee JM, McMahon PM, Kong CY, et al. Cost-effectiveness of breast MR imaging and screen-film mammography for screening BRCA1 gene mutation carriers. Radiology 2010;254:793-800.
22. O'Neill SM, Rubinstein WS, Sener SF, et al. Psychological impact of recall in high-risk breast MRI screening. Breast Cancer Res Treat 2009;115:365-71.
23. Baltzer PA, Benndorf M, Dietzel M, et al. False-positive findings at contrast-enhanced breast MRI: a BI-RADS descriptor study. AJR Am J Roentgenol 2010;194:1658-63.
24. Agrawal G, Su MY, Nalcioglu O, et al. Significance of breast lesion descriptors in the ACR BI-RADS MRI lexicon. Cancer 2009;115:1363-80.
25. Newell D, Nie K, Chen JH, et al. Selection of diagnostic features on breast MRI to differentiate between malignant and benign lesions using computer-aided diagnosis: differences in lesions presenting as mass and non-mass-like enhancement. Eur Radiol 2010;20:771-81.
26. Esserman L, Wolverton D, Hylton N. Magnetic resonance imaging for primary breast cancer management: current role and new applications. Endocr Relat Cancer 2002;9:141-53.

27. Gundry KR. The Application of Breast MRI in Staging and Screening for Breast Cancer. Available online: http://www.cancernetwork.com/breast-cancer/content/article/10165/105413
28. Argus A, Mahoney MC. Indications for breast MRI: case-based review. AJR Am J Roentgenol 2011;196:WS1-14.
29. Morris EA, Schwartz LH, Drotman MB, et al. Evaluation of pectoralis major muscle in patients with posterior breast tumors on breast MR images: early experience. Radiology 2000;214:67-72.
30. Argus A, Mahoney MC. Clinical indications for breast MRI. Applied Radiol 2010;39:32-5.
31. Belli P, Costantini M, Malaspina C, et al. MRI accuracy in residual disease evaluation in breast cancer patients treated with neoadjuvant chemotherapy. Clin Radiol 2006;61:946-53.
32. Chagpar AB, Middleton LP, Sahin AA, et al. Accuracy of physical examination, ultrasonography, and mammography in predicting residual pathologic tumor size in patients treated with neoadjuvant chemotherapy. Ann Surg 2006;243:257-64.
33. Yeh E, Slanetz P, Kopans DB, et al. Prospective comparison of mammography, sonography, and MRI in patients undergoing neoadjuvant chemotherapy for palpable breast cancer. AJR Am J Roentgenol 2005;184:868-77.
34. Marinovich ML, Houssami N, Macaskill P, et al. Meta-analysis of magnetic resonance imaging in detecting residual breast cancer after neoadjuvant therapy. J Natl Cancer Inst 2013;105:321-33.
35. Frei KA, Kinkel K, Bonel HM, et al. MR imaging of the breast in patients with positive margins after lumpectomy: influence of the time interval between lumpectomy and MR imaging. AJR Am J Roentgenol 2000;175:1577-84.
36. Lee JM, Orel SG, Czerniecki BJ, et al. MRI before reexcision surgery in patients with breast cancer. AJR Am J Roentgenol 2004;182:473-80.
37. Diana M, Robb GL, Talavera F, et al. Breast Reconstruction: Nipple-Areola Reconstruction. Updated July 15, 2011. Available online: http://www.emedicine.com/plastic/topic144.htm. Accessed on July 12, 2012.
38. Zion SM, Slezak JM, Sellers TA, et al. Reoperations after prophylactic mastectomy with or without implant reconstruction. Cancer 2003;98:2152-60.
39. O'Toole M, Caskey CI. Imaging spectrum of breast implant complications: mammography, ultrasound, and magnetic resonance imaging. Semin Ultrasound CT MR 2000;21:351-61.
40. Di Benedetto G, Cecchini S, Grassetti L, et al. Comparative study of breast implant rupture using mammography, sonography, and magnetic resonance imaging: correlation with surgical findings. Breast J 2008;14:532-7.
41. Gorczyca DP, Gorczyca SM, Gorczyca KL. The diagnosis of silicone breast implant rupture. Plast Reconstr Surg 2007;120:49S-61S.

Cite this article as: Salem DS, Kamal RM, Mansour SM, Salah LA, Wessam R. Breast imaging in the young: the role of magnetic resonance imaging in breast cancer screening, diagnosis and follow-up. J Thorac Dis 2013;5(S1):S9-S18. doi: 10.3978/j.issn.2072-1439.2013.05.02

Challenges in managing breast cancer during pregnancy

Flora Zagouri[1,2], Theodora Psaltopoulou[3], Constantine Dimitrakakis[4], Rupert Bartsch[2], Meletios-Athanassios Dimopoulos[1]

[1]Department of Clinical Therapeutics, Alexandra Hospital, Medical School, University of Athens, Athens, Greece; [2]Comprehensive Cancer Center Vienna, Department of Medicine I/Division of Oncology, Medical University of Vienna, Austria; [3]Department of Hygiene, Epidemiology and Medical Statistics, Medical School, University of Athens, Athens, Greece; [4]Department of Obstetrics and Gynaecology, Medical School, University of Athens, Athens, Greece

Correspondence to: Flora Zagouri, MD, PhD. Dept of Clinical Therapeutics, Alexandra Hospital, University of Athens, Greece; Vas Sofias Ave & Lourou str, Athens 11521, Greece. Email: florazagouri@yahoo.co.uk.

Abstract: Pregnancy-associated breast cancer (PABC) is defined as breast cancer occurring anytime during gestation, lactation or within one year after delivery. The optimal management of pregnant women with breast cancer is challenging and not well established; the main concern is the effect of the drugs on the developing fetus and long-term complications after in utero exposure to anti-cancer drugs. Surgical resection is the mainstay of treatment for early breast cancer diagnosed during pregnancy. Modified radical mastectomy is standard of care in first trimester, whereas breast-conserving surgery (lumpectomy with lymph node dissection) can be performed preferably in the second and third trimester. Of note, breast-conserving surgery is not contraindicated per se during the first trimester, but owing to the potential impact of delaying radiotherapy. Radiation therapy is not favored during pregnancy. Moreover, tamoxifen is contraindicated during pregnancy; the agent has been associated with birth defects in up to 20% of exposures. Chemotherapy is generally contraindicated during the first trimester because of the possible damage to organogenesis. Anthracyclines-based regimens are the most widely used is breast cancer treatment and were been shown to be associated with favourable safety profile when administered during pregnancy. As for taxanes, more limited data is available. The use of trastuzumab is contraindicated during pregnancy, given the apparent risk of oligo- and/or anhydramnios as well as the unknown long-term sequelae on the fetus. It is obvious that, diagnosis of breast cancer during pregnancy adds complexity to cancer treatment recommendations. In all cases, a multidisciplinary therapeutic approach among obstetricians, gynaecologists, surgical oncologists, radiation oncologists, medical oncologists, pediatricians and hematologists is clearly warranted.

Keywords: Breast cancer; pregnancy; controversies; chemotherapy

Submitted Apr 18, 2013. Accepted for publication May 28, 2013.
doi: 10.3978/j.issn.2072-1439.2013.05.21
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.05.21

Introduction

Pregnancy-associated breast cancer (PABC) is defined as breast cancer occurring anytime during gestation, lactation or within one year after delivery (1,2). Breast cancer is one of the most common tumor during pregnancy along with melanoma and cervical cancers and occurs in approximately one out of 3,000-10,000 pregnancies (3). Diagnosis of PABC is expected to become more frequent in the forthcoming years since there is an increasing trend for women to delay childbearing (4,5).

Significant controversy exists in the literature regarding the influence of pregnancy upon breast cancer prognosis. Some studies did not demonstrate any aggravating role (6-8), whereas other studies have reported that pregnancy itself may not represent a veritable poor prognostic factor for breast cancer, attributing any detrimental effects to the delayed diagnosis of tumours in pregnancy (9-13). On the other hand, some studies point to the opposite direction, indicating an independent, aggravating role of

pregnancy (14-16). Of note, a recently published meta-analysis, including 30 studies, have shown that PABC is independently associated with poor survival particularly when diagnosed shortly post-partum (17).

In this context, it should be noted that diagnosis of breast cancer during pregnancy adds complexity to cancer treatment recommendations, taking into consideration that treatment strategies offered for pregnant women with breast cancer should not differ from their non-pregnant counterparts. Hence, the optimal management of pregnant women with breast cancer is challenging and not well established; the main concern is the effect of the drugs on the developing fetus and long-term complications after in utero exposure to anti-cancer drugs. This review, taking into consideration all available data, focuses on critical issues regarding the management of breast cancer during pregnancy, such as consultation of pregnant patients, surgical procedure, administration of chemotherapy regimens during pregnancy and lactation, radiation therapy, targeted treatment administration during pregnancy, etc.

Surgical procedure

Surgical resection is the mainstay of treatment for early breast cancer diagnosed during pregnancy (3,18). Modified radical mastectomy is standard of care in first trimester of pregnancy. Of note, breast-conserving surgery is not contraindicated per se during the first trimester, but owing to the potential impact of delaying radiotherapy; hence, mastectomy is considered in these cases. Breast-conserving surgery (lumpectomy with lymph node dissection) can be performed preferably in the second and third trimester because of the necessary ensuing radiotherapy that in any case must be delayed up until after delivery (3,18). The decision to proceed with breast-conservation or mastectomy should be based upon the clinical situation of each patient. In this context, it should be noted that surgery is a safe procedure and can be performed in all trimesters with minimal risk for the fetus; after the 12th week of gestation, in particular, the risk of abortion is minimal (19-23). Radical mastectomy may be followed by immediate breast reconstruction; however, there are no data on reconstructive breast surgery during pregnancy. Hence, reconstruction-if needed-should be better restricted to a prosthetic implant or preferably should be carried out post partum (24).

As far as sentinel lymph node biopsy (SLNB) in pregnant women is concerned, there are insufficient safety data to support this procedure during pregnancy owing to radiation concerns. However, a dosimetry study followed by a prospective trial on 12 pregnant breast cancer patients (25,26) supported the safety of SLNB, when performed with low-dose lymphoscintigraphy using 99m-Tc human serum albumin nanocolloids. In this study, eleven healthy babies were born with no malformation and with normal weight, whereas one newborn had a ventricular septal defect suspected before lymphoscintigraphy. Moreover, there was no evidence of axillary recurrence at a median follow-up of 32 months. By contrast, blue dye is associated with a risk of an anaphylactic maternal reaction, which would probably distress the fetus (27). Therefore, the use of blue dye should be avoided during pregnancy. Hence, SLNB with low-dose lymphoscintigraphy using 99m-Tc human serum albumin nanocolloids may be considered in selected cases and within centers with experience in carrying out this technique (28).

Radiation therapy

Radiation therapy is not favored during pregnancy owing to its teratogenic effects on the fetus; hence, there is a general agreement to postpone radiotherapy up until after delivery (3,18,24,29). In the first trimester (before the completion of organogenesis), radiotherapy may be related to fetal death, malformations, microcephaly, intrauterine growth retardation, mental retardation, and induction of childhood neoplasms and hematologic disorders (30,31). Adjuvant radiotherapy to the breast is never an "urgent" procedure; hence, postponing it could be better given the potential hazards of the fetus. Of note, the latter remains very low anyways during the first and second trimester with adequate shielding given that the uterus is far from the radiation field (31,32). However, in patients with brain metastases, radiotherapy to the brain is certainly given during pregnancy because there is an urgent clinical need with very low potential fetal adverse effects (31,32).

Hormonal treatment

According to current clinical recommendations, tamoxifen is contraindicated during pregnancy; the agent has been associated with birth defects in up to 20% of exposures, including Goldenhar's syndrome (33), ambiguous genitalia, vaginal bleeding, and spontaneous abortion (34-36). During pregnancy, tamoxifen and its metabolites interact with rapidly growing and developing embryonic or fetal tissues (37). Although several case reports describe tamoxifen exposure and healthy neonatal outcomes (38), there is a general

agreement to postpone tamoxifen up until after delivery (29). In this context, it should be noted that aromatase inhibitors are not indicated in premenopausal women.

Chemotherapy administration

Chemotherapy plays a key role in improving the survival of patients with early stage breast cancer. The decision to administer chemotherapy in pregnant women with breast cancer should follow the same guidelines as applied to non-pregnant patients. Chemotherapy is generally contraindicated during the first trimester because of the possible damage to organogenesis, whereas several recent studies have shown that certain chemotherapy regimens can be relatively safely administered during the second and third trimester (39-41). Worthy of note, in the first trimester, the risk of congenital malformations ranges from 10-20%, whilst it drops to 1.3% in the third trimester of pregnancy (39).

In this context, it should be noted that although pregnancy will alter the pharmacokinetics of cytotoxic drugs, there are currently no studies justifying a change in dosage. Hence, during pregnancy, dosages should not differ from those used outside pregnancy, even if few pharmacokinetic and pharmacodynamic data are available during pregnancy (24).

Anthracyclines-based regimens are the most widely used is breast cancer treatment and were been shown to be associated with favourable safety profile when administered during pregnancy (42). More specifically, the most commonly used regimens, in the adjuvant setting, include 5-fluorouracil combined with doxorubicin (5-FU-A) and epirubicin or doxorubicin in combination with cyclophosphamide (E or A-C). Of note, no clear differences could be attributed to the aforementioned different regimens regarding maternal toxicities, short or long term fetal outcome and pregnancy outcome. Moreover, in the neo-adjuvant and in the advanced/metastatic setting, anthracyclines and anthracycline-based regimens remain the best choice (42,43).

More limited data is available on taxanes. More specifically, they have recently been incorporated in the ESMO and NCCN guidelines (3,18), as being considered relatively safe to administer beyond the first gestational trimester; the risk of abortion or congenital anomalies increases when they are administered during the first trimester. Moreover, the Food and Drug Administration classify docetaxel and paclitaxel as a category D drug (i.e., able to be administered in pregnancy if necessary).

According to a recent systematic review, a completely healthy neonate was born with a normal Apgar score, appropriate fetal growth and acceptable weight in the majority of breast cancer patients with taxanes administration during pregnancy (44). Moreover, 27 out of 30 children (90%) were completely healthy at a median follow-up of 16 months; among the remaining cases, one child with recurrent otitis media, one child with IgA deficiency and mild constipation and another child with delayed speech were reported (44,45). However, it should be underlined that there is limited information concerning the long-term consequences for the offspring. Moreover, only *ex vivo* data are available on the transplacental transfer of taxanes in humans, whilst in a human placental perfusion model, the transplacental transfer rate of paclitaxel was found to be low (<5%) (46).

Hence, as for taxanes, if required in the adjuvant setting, limited data is available in pregnancy (44). Still, acknowledging the limited amount of evidence, taxanes could be offered in sequence to anthracyclines following delivery (29). Regarding the metastatic setting, it seems that single agent taxane (paclitaxel or docetaxel) may represent an appealing option, especially for patients who are not suitable candidates for anthracycline-based regimens (44,47).

Targeted therapy during pregnancy

Trastuzumab

According to ESMO and NCCN guidelines (3,18), the use of trastuzumab is contraindicated during pregnancy, given the apparent risk of oligo- and/or anhydramnios as well as the unknown long-term sequelae on the fetus (48). Notably, the Food and Drug Administration classify trastuzumab as a pregnancy category B drug. While studies in cynomolgus monkeys reported no harm to the fetus, they failed to reveal placental transfer of trastuzumab in monkeys [reviewed in (48)].

A recent meta-analysis has shown that trastuzumab administration emerges as relatively safe during the first trimester of pregnancy, whereas a high incidence of oligohydramnios and/or anhydramnios is observed when this agent is used beyond the first trimester (49). An intriguing observation of this meta-analysis is that all children exposed to trastuzumab exclusively during the first trimester of pregnancy were completely healthy and showed no evidence of congenital malformations (50-52). Indeed, the occurrence of oligohydramnios/or anhydramnios was

confined to pregnancies exposed during the second or third trimesters (49). A study by Pentsuk *et al.* concurred with this meta-analysis (53), showing that fetal exposure to trastuzumab is very low during the first trimester, and increases during the second half of gestation, to reach a drug concentration at birth similar to that of the mother.

Hence, as concerns trastuzumab administration in the adjuvant setting during pregnancy, it should be noted that there is no cause for exposing the pregnant HER2-positive woman and the fetus to the potential hazard of the agent. Mounting evidence outside pregnancy confirm the efficacy of trastuzumab even after 6 months of adjuvant chemotherapy (54), suggesting that a monoclonal antibody could be safely administered after delivery. On the other hand, as far as metastatic HER2- positive breast cancer is concerned, trastuzumab should be avoided and chemotherapy could start from the second trimester. However, in selected cases, where the agent may be urgently needed, its administration is recommended for a short period with careful control of the amniotic fluid, fetal growth and kidney function; should signs of oligohydramnios be observed, the agent should immediately be discontinued (49).

Moreover, unlike chemotherapy, trastuzumab does not induce amenorrhea (55), thus, an accidental pregnancy during its administration cannot be precluded if no adequate contraception is used. Of note, according to Azim *et al.* (56), patients who became pregnant after a trastuzumab-free interval of more than 3 months appeared to have normal pregnancy courses and outcomes. These data may be of particular significance to women who accidentally fall pregnant during trastuzumab administration but do not wish to terminate the pregnancy; in this setting, trastuzumab should be discontinued and pregnancy be allowed to continue without urging an abortion. However, it should be stressed that no definite conclusion can be drawn given the limited number of observations; clinicians should always advise women to use active contraception while on trastuzumab therapy and to continue doing so for up to 6 months following completion of treatment (48,49,52).

Other biologics

There are insufficient data on lapatinib administration during pregnancy, but its pharmacological characteristics (massive transplacental transfer) would strongly caution against its use during pregnancy; hence, lapatinib cannot be recommended during pregnancy (3,18,24). Of note, there is only one report on lapatinib exposure in a woman during the first and second trimesters; the agent was discontinued and the delivery was uncomplicated with a healthy newborn (57).

Moreover, the use of bevacizumab during pregnancy cannot be recommended, given its mode of action and the lack of available data (3,18,24).

Supportive treatment

Antiemetics such as 5HT antagonists, steroids, or antihistamines are not contraindicated during pregnancy. Granulocyte-stimulating factors are considered as pregnancy category C; hence, they should be used during pregnancy by the clinical necessity (1). Concerning bisphosphonates, limited data is available for their use during pregnancy. More specifically, data on 51 pregnant women for different indications did not reveal any increase in maternal and/or fetal morbidity (58). However, given that bisphosphonates remain in mineralised bone for several years and that available data on pregnant patients are limited, it should be clearly stated that bisphosphonates should be used with caution and on personalized basis; if used, hypocalcaemia affecting the contractility of the uterus should be avoided (58,59).

Fetal and pregnancy monitoring

A multidisciplinary approach involving medical and surgical oncologists, high-risk obstetric care, genetic counsellors, pharmacists, radiation oncologists, and neonatologists is mandatory for the successful management of women with breast cancer during pregnancy (24). It is without doubt that strict fetal monitoring with morphometric ultrasound and umbilical artery Doppler should be performed at regular intervals during gestational chemotherapy (3,18,24).

The timing of delivery should be balanced according to the oncological treatment schedule and the maturation of the fetus; as in non-cancer patients, the aim of a full term delivery (>37 weeks' gestation) is important since prematurity affects the cognitive and emotional development of children (60-62). Moreover, it is recommended that patients should not receive any chemotherapeutic agents for at least 3 weeks prior to delivery so as to avoid problems associated with haematopoeitic suppression (bleeding, infection, anaemia) in the mother and baby, and to prevent drug accumulation in the fetus (24,43,63). The mode of delivery is determined based upon the obstetrical indication (24). Although metastases to the placenta is a rare event in breast cancer

patients, the placenta should always be evaluated after delivery (64,65).

In the absence of safety data, breastfeeding in the first weeks after chemotherapy is not recommended (3,18,24). Of note, primary inhibition of milk production is needed because especially lipophylic agents such as taxanes can accumulate in the milk.

Conclusions

In this context, it should be noted that treating cancer during pregnancy represents a relatively uncommon situation. The available data are limited and consist mainly of case reports, case series, and retrospective registries; hence, in order to provide further information for this challenging clinical situation, improved collaboration between registries and cancer centers is more than warranted given the long-term implications for both the breast cancer patient and neonate.

Moreover, it should be stressed that in all cases, a multidisciplinary therapeutic approach among obstetricians, gynaecologists, surgical oncologists, radiation oncologists, medical oncologists and hematologists is clearly warranted; the optimal therapeutic strategy in a pregnant patient with breast cancer diagnosis should take into consideration the gestational age, stage of breast cancer, treatment options, the wishes of the patient, and a host of psychological, ethical, religious, and even legal considerations.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Viswanathan S, Ramaswamy B. Pregnancy-associated breast cancer. Clin Obstet Gynecol 2011;54:546-55.
2. Asgeirsson KS. Pregnancy-associated breast cancer. Acta Obstet Gynecol Scand 2011;90:158-66.
3. Pentheroudakis G, Orecchia R, Hoekstra HJ, et al. Cancer, fertility and pregnancy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21:v266-73.
4. Mir O, Berveiller P, Ropert S, et al. Emerging therapeutic options for breast cancer chemotherapy during pregnancy. Ann Oncol 2008;19:607-13.
5. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2008, National Cancer Institute. Bethesda, MD. Available online: http://seer.cancer.gov/csr/1975_2008/, based on November 2010 SEER data submission, posted to the SEER web site, 2011.
6. Ezzat A, Raja MA, Berry J, et al. Impact of pregnancy on non-metastatic breast cancer: a case control study. Clin Oncol (R Coll Radiol) 1996;8:367-70.
7. Beadle BM, Woodward WA, Middleton LP, et al. The impact of pregnancy on breast cancer outcomes in women < or =35 years. Cancer 2009;115:1174-84.
8. Zemlickis D, Lishner M, Degendorfer P, et al. Maternal and fetal outcome after breast cancer in pregnancy. Am J Obstet Gynecol 1992;166:781-7.
9. Ishida T, Yokoe T, Kasumi F, et al. Clinicopathologic characteristics and prognosis of breast cancer patients associated with pregnancy and lactation: analysis of case-control study in Japan. Jpn J Cancer Res 1992;83:1143-9.
10. Zhang J, Liu G, Wu J, et al. Pregnancy-associated breast cancer: a case control and long-term follow-up study in China. J Exp Clin Cancer Res 2003;22:23-7.
11. Murphy C, Mallam D, Stein S, et al. Pathologic features and outcomes of pregnancy-associated breast cancer (PABC): A case control study. J Clin Oncol 2010;28:1589.
12. Lethaby AE, O'Neill MA, Mason BH, et al. Overall survival from breast cancer in women pregnant or lactating at or after diagnosis. Auckland Breast Cancer Study Group. Int J Cancer 1996;67:751-5.
13. Azim HA Jr, Botteri E, Renne G, et al. The biological features and prognosis of breast cancer diagnosed during pregnancy: a case-control study. Acta Oncol 2012;51:653-61.
14. Rodriguez AO, Chew H, Cress R, et al. Evidence of poorer survival in pregnancy-associated breast cancer. Obstet Gynecol 2008;112:71-8.
15. Bonnier P, Romain S, Dilhuydy JM, et al. Influence of pregnancy on the outcome of breast cancer: a case-control study. Societe Francaise de Senologie et de Pathologie Mammaire Study Group. Int J Cancer 1997;72:720-7.
16. Moreira WB, Brandão EC, Soares AN, et al. Prognosis for patients diagnosed with pregnancy-associated breast cancer: a paired case-control study. Sao Paulo Med J 2010;128:119-24.
17. Azim HA Jr, Santoro L, Russell-Edu W, et al. Prognosis of pregnancy-associated breast cancer: a meta-analysis of 30 studies. Cancer Treat Rev 2012;38:834-42.

18. NCCN Clinical Practice Guidelines in Oncology. Breast Cancer. Version 2.2012. Available online: http://www.nccn.com
19. Rovera F, Frattini F, Coglitore A, et al. Breast cancer in pregnancy. Breast J 2010;16:S22-5.
20. Molckovsky A, Madarnas Y. Breast cancer in pregnancy: a literature review. Breast Cancer Res Treat 2008;108:333-8.
21. Navrozoglou I, Vrekoussis T, Kontostolis E, et al. Breast cancer during pregnancy: a mini-review. Eur J Surg Oncol 2008;34:837-43.
22. Vinatier E, Merlot B, Poncelet E, et al. Breast cancer during pregnancy. Eur J Obstet Gynecol Reprod Biol 2009;147:9-14.
23. Amant F, Loibl S, Neven P, et al. Breast cancer in pregnancy. Lancet 2012;379:570-9.
24. Amant F, Deckers S, Van Calsteren K, et al. Breast cancer in pregnancy: recommendations of an international consensus meeting. Eur J Cancer 2010;46:3158-68.
25. Gentilini O, Cremonesi M, Trifirò G, et al. Safety of sentinel node biopsy in pregnant patients with breast cancer. Ann Oncol 2004;15:1348-51.
26. Gentilini O, Cremonesi M, Toesca A, et al. Sentinel lymph node biopsy in pregnant patients with breast cancer. Eur J Nucl Med Mol Imaging 2010;37:78-83.
27. Khera SY, Kiluk JV, Hasson DM, et al. Pregnancy-associated breast cancer patients can safely undergo lymphatic mapping. Breast J 2008;14:250-4.
28. Azim H Jr, Gentilini O, Locatelli M, et al. Managing pregnant women with cancer: personal considerations and a review of the literature. Ecancermedicalscience 2011;5:204.
29. Azim HA Jr, Del Mastro L, Scarfone G, et al. Treatment of breast cancer during pregnancy: regimen selection, pregnancy monitoring and more... Breast 2011;20:1-6.
30. Behrman RH, Homer MJ, Yang WT, et al. Mammography and fetal dose. Radiology 2007;243:605; author reply 605-6.
31. Martin DD. Review of radiation therapy in the pregnant cancer patient. Clin Obstet Gynecol 2011;54:591-601.
32. Guidroz JA, Scott-Conner CE, Weigel RJ. Management of pregnant women with breast cancer. J Surg Oncol 2011;103:337-40.
33. Cullins SL, Pridjian G, Sutherland CM. Goldenhar's syndrome associated with tamoxifen given to the mother during gestation. JAMA 1994;271:1905-6.
34. Cunha GR, Taguchi O, Namikawa R, et al. Teratogenic effects of clomiphene, tamoxifen, and diethylstilbestrol on the developing human female genital tract. Hum Pathol 1987;18:1132-43.
35. Isaacs RJ, Hunter W, Clark K. Tamoxifen as systemic treatment of advanced breast cancer during pregnancy--case report and literature review. Gynecol Oncol 2001;80:405-8.
36. Tewari K, Bonebrake RG, Asrat T, et al. Ambiguous genitalia in infant exposed to tamoxifen in utero. Lancet 1997;350:183.
37. Braems G, Denys H, De Wever O, et al. Use of tamoxifen before and during pregnancy. Oncologist 2011;16:1547-51.
38. Oksüzoglu B, Güler N. An infertile patient with breast cancer who delivered a healthy child under adjuvant tamoxifen therapy. Eur J Obstet Gynecol Reprod Biol 2002;104:79.
39. Ring AE, Smith IE, Jones A, et al. Chemotherapy for breast cancer during pregnancy: an 18-year experience from five London teaching hospitals. J Clin Oncol 2005;23:4192-7.
40. Berry DL, Theriault RL, Holmes FA, et al. Management of breast cancer during pregnancy using a standardized protocol. J Clin Oncol 1999;17:855-61.
41. Hahn KM, Johnson PH, Gordon N, et al. Treatment of pregnant breast cancer patients and outcomes of children exposed to chemotherapy in utero. Cancer 2006;107:1219-26.
42. Azim HA Jr, Peccatori FA, Pavlidis N. Treatment of the pregnant mother with cancer: a systematic review on the use of cytotoxic, endocrine, targeted agents and immunotherapy during pregnancy. Part I: Solid tumors. Cancer Treat Rev 2010;36:101-9.
43. Loibl S, von Minckwitz G, Gwyn K, et al. Breast carcinoma during pregnancy. International recommendations from an expert meeting. Cancer 2006;106:237-46.
44. Zagouri F, Sergentanis TN, Chrysikos D, et al. Taxanes for breast cancer during pregnancy: a systematic review. Clin Breast Cancer 2013;13:16-23.
45. Cardonick E, Bhat A, Gilmandyar D, et al. Maternal and fetal outcomes of taxane chemotherapy in breast and ovarian cancer during pregnancy: case series and review of the literature. Ann Oncol 2012;23:3016-23.
46. Berveiller P, Mir O. Taxanes during pregnancy: probably safe, but still to be optimized. Oncology 2012;83:239-40.
47. Schackmuth EM, Harlow CL, Norton LW. Milk fistula: a complication after core breast biopsy. AJR Am J Roentgenol 1993;161:961-2.
48. Azim HA Jr, Azim H, Peccatori FA. Treatment of cancer during pregnancy with monoclonal antibodies: a real challenge. Expert Rev Clin Immunol 2010;6:821-6.
49. Zagouri F, Sergentanis TN, Chrysikos D, et al.

Trastuzumab administration during pregnancy: a systematic review and meta-analysis. Breast Cancer Res Treat 2013;137:349-57.
50. Goodyer MJ, Ismail JR, O'Reilly SP, et al. Safety of trastuzumab (Herceptin) during pregnancy: two case reports. Cases J 2009;2:9329.
51. Waterston AM, Graham J. Effect of adjuvant trastuzumab on pregnancy. J Clin Oncol 2006;24:321-2.
52. Azim HA Jr, Peccatori FA, Liptrott SJ, et al. Breast cancer and pregnancy: how safe is trastuzumab? Nat Rev Clin Oncol 2009;6:367-70.
53. Pentsuk N, van der Laan JW. An interspecies comparison of placental antibody transfer: new insights into developmental toxicity testing of monoclonal antibodies. Birth Defects Res B Dev Reprod Toxicol 2009;86:328-44.
54. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005;353:1659-72.
55. Abusief ME, Missmer SA, Ginsburg ES, et al. The effects of paclitaxel, dose density, and trastuzumab on treatment-related amenorrhea in premenopausal women with breast cancer. Cancer 2010;116:791-8.
56. Azim HA Jr, Metzger-Filho O, de Azambuja E, et al. Pregnancy occurring during or following adjuvant trastuzumab in patients enrolled in the HERA trial (BIG 01-01). Breast Cancer Res Treat 2012;133:387-91.
57. Kelly H, Graham M, Humes E, et al. Delivery of a healthy baby after first-trimester maternal exposure to lapatinib. Clin Breast Cancer 2006;7:339-41.
58. Djokanovic N, Garcia-Bournissen F, Koren G. Medications for restless legs syndrome in pregnancy. J Obstet Gynaecol Can 2008;30:505-7.
59. Levy S, Fayez I, Taguchi N, et al. Pregnancy outcome following in utero exposure to bisphosphonates. Bone 2009;44:428-30.
60. Amant F, Van Calsteren K, Halaska MJ, et al. Long-term cognitive and cardiac outcomes after prenatal exposure to chemotherapy in children aged 18 months or older: an observational study. Lancet Oncol 2012;13:256-64.
61. Tamaru S, Kikuchi A, Takagi K, et al. Neurodevelopmental outcomes of very low birth weight and extremely low birth weight infants at 18 months of corrected age associated with prenatal risk factors. Early Hum Dev 2011;87:55-9.
62. Løhaugen GC, Gramstad A, Evensen KA, et al. Cognitive profile in young adults born preterm at very low birthweight. Dev Med Child Neurol 2010;52:1133-8.
63. Sorosky JI, Sood AK, Buekers TE. The use of chemotherapeutic agents during pregnancy. Obstet Gynecol Clin North Am 1997;24:591-9.
64. Alexander A, Samlowski WE, Grossman D, et al. Metastatic melanoma in pregnancy: risk of transplacental metastases in the infant. J Clin Oncol 2003;21:2179-86.
65. Dunn JS Jr, Anderson CD, Brost BC. Breast carcinoma metastatic to the placenta. Obstet Gynecol 1999;94:846.

Cite this article as: Zagouri F, Psaltopoulou T, Dimitrakakis C, Bartsch R, Dimopoulos MA. Challenges in managing breast cancer during pregnancy. J Thorac Dis 2013;5(S1):S62-S67. doi: 10.3978/j.issn.2072-1439.2013.05.21

Premature menopause in young breast cancer: effects on quality of life and treatment interventions

Shoshana M. Rosenberg[1,2], **Ann H. Partridge**[1,3]

[1]Dana-Farber Cancer Institute, Boston, MA, USA; [2]Harvard School of Public Health, Boston, MA, USA; [3]Harvard Medical School, Boston, MA, USA
Correspondence to: Shoshana M. Rosenberg, ScD, MPH. Dana-Farber Cancer Institute, 450 Brookline Ave, LW601, Boston, MA 02215, USA. Email: shoshana_rosenberg@dfci.harvard.edu.

Abstract: Many young women are at increased risk for premature menopause following adjuvant treatment for breast cancer. These women must deal with consequences of menopause, including loss of fertility and physiologic symptoms such as night sweats, hot flashes, vaginal dryness, and weight gain. These symptoms can be particularly distressing for young women and can adversely affect both health-related and psychosocial quality of life (QOL). While there are a wide range of pharmacologic and non-pharmacologic interventions available to help with these symptoms and in turn, improve QOL, there is little data available about the use and efficacy of these interventions in younger women who become menopausal as a result of their breast cancer treatment. Future studies should focus on this vulnerable population, with the goal of identifying effective strategies to relieve symptoms and improve quality of life in young breast cancer survivors.

Keywords: Premature menopause; young women; breast cancer; quality of life (QOL)

Submitted May 29, 2013. Accepted for publication Jun 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.06.20
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.06.20

Introduction

It is well established that the diagnosis and treatment of breast cancer can have a profound impact on a patient's short and long term quality of life (QOL). Young women with breast cancer have unique health and psychosocial concerns and are also more likely to experience poorer QOL outcomes following diagnosis compared to older women (1-3). In particular, fertility concerns and outcomes as well as associated menopausal issues may greatly impact on a young women's survivorship (4).

Amenorrhea is a common consequence of systemic breast cancer therapy among young women, the vast majority of whom are premenopausal at diagnosis. In many cases, it is temporary, with menses resuming in the months following the end of treatment. For some women, amenorrhea is permanent, and heralds the onset of early menopause. Even if menses do resume, many breast cancer survivors are at an increased risk of premature ovarian failure (POF) (5). Most women who remain amenorrheic for at least a year will not resume menstruation and will be considered to have POF (6).

POF resulting from chemotherapy is largely dependent on both age and treatment type (7-9). For example, in women under 40, a regimen consisting of cyclophosphomide, methotrexate, and 5-fluorouracil (CMF) for 6 months is associated with a risk of premature menopause approximated to be between 30-40%; in women aged 40 and older this risk is greater than 80% and has been reported to be as high as 96% (10). Treatment with doxorubicin and cyclophosphomide (AC) is associated with a lower risk of menopause: 13% for women younger than 40 and 57-63% in women aged 40 and older (10). Adjuvant tamoxifen therapy has also been identified as a risk factor for premature menopause in young breast cancer survivors. Additionally, choices like bilateral prophylactic oophorectomy as a means of ovarian suppression or as a risk reduction strategy in women at high risk for ovarian cancer (i.e., BRCA 1 or 2 mutation carriers) may also cause early menopause among young women with breast cancer. Regardless of the reason, these women face the physiological changes that frequently accompany menopause, as well as the emotional

reality of becoming prematurely potentially infertile. While these experiences might be "normal" for women who are in their 50s, for women in their 20s, 30s and early 40s, these changes can be extremely distressing and have the potential to negatively affect QOL.

Poorer QOL outcomes among younger women are strongly related to the physiologic changes experienced during the menopausal transition. Declining estrogen levels due to ovarian failure is associated with symptoms such as hot flashes, night sweats, vaginal dryness, dyspareunia, and weight changes. Ovarian damage from chemotherapy treatment can also negatively affect sex hormone levels, including androgens (11). Menopausal symptoms have been reported to be more severe among women who become menopausal from treatment, as well as among women who undergo a surgical menopause, as compared to the symptom burden in women experiencing a normal menopausal transition (12-16).

While many symptoms of premature menopause are the consequence of changes in the hormonal milieu and manifest themselves in the domain of physical health, the potential for compromised mental health is also of concern. In their Cancer and Menopause Study (CAMS), Ganz *et al.* (17) reported lower scores the Mental Component Summary Scale (MCS) of the SF-36 among women who entered menopause following treatment compared to those who did not, indicating that young women are more likely to experience compromised psycho-social QOL following the onset of early menopause.

In this review, we summarize the current body of literature regarding QOL, including that related to both physical and psychosocial functioning, in young breast cancer survivors who experience premature menopause. We also review medical and psychosocial interventions that have been tested and that are available to help improve those areas of QOL most often affected in younger women with early menopause.

Fatigue and sleep problems

Fatigue is frequently reported both during and after breast cancer treatment, and can negatively impact many aspects of QOL (18). The presence of menopausal symptoms has been found to be positively associated with more problems with fatigue following the end of chemotherapy treatment (15,19,20). Furthermore, fatigue is also linked to increased depressive symptoms (18). However the directionality of the relationship between fatigue and menopausal symptoms is unclear. Alternatively, fatigue might be an independent symptom related to the onset of menopause, i.e., a consequence of changing hormonal levels.

Sleep disturbance can be a direct consequence of hot flashes and/or night sweats, in turn leading to increased levels of fatigue. In one study inclusive of both pre- and post- menopausal women, hot flashes and night sweats were not predictive of changes in sleep quality, however women with a history of chemotherapy treatment were more likely to experience altered sleeping patterns in the months and years after treatment (21).

It is unclear if fatigue or compromised sleep quality is worse among women with POF *vs.* women who are menopausal before diagnosis, as most studies do not distinguish between these types of women. However given the severity of menopausal symptoms is often worse among women with POF, because of the strong relationship between vasomotor symptoms and fatigue, it is conceivable that fatigue and sleep problems can be significant issues for women experiencing an early menopause following breast cancer treatment.

Weight gain

Weight gain is commonly seen in both women of all ages both during and after breast cancer treatment (22). Among women who are pre-menopausal at diagnosis, those who become menopausal as a result of treatment seem to experience more weight gain relative to those who do not (23,24). A recent study exploring weight gain in a small cohort of young women found that those with POF due to chemotherapy gained more truncal fat compared to women who did not experience treatment-associated ovarian failure, though this difference was not statistically significant (25). Additionally, the women who became menopausal also lost truncal lean mass, in contrast to the women who remained premenopausal, who did not experience any significant lean mass loss (25).

Weight gain is a distressing issue for many young women. Nearly two-thirds of women diagnosed with breast cancer at age 40 or younger who responded to a web-based survey said that weight gain was at least moderately bothersome (12) and another study inclusive of women up to 50 years old at diagnosis found that approximately 40% of women reported weight gain to be at least somewhat bothersome (26). Other studies have also identified weight gain as an important determinant of body image concerns, an important psycho-social QOL domain, among young women (27,28).

Bone health

Women with POF are at increased risk for reduced bone mineral density (BMD) following adjuvant chemotherapy compared to women who remain premenopausal (29,30). Bone loss can lead to osteopenia and osteoporosis if not intervened upon, putting women at increased risk for fractures at an earlier age. Of concern, it appears many women are not following recommended bone health guidelines, including BMD screening, physical activity, and taking supplemental calcium and vitamin D when warranted (31,32).

Tamoxifen appears to have a protective effect on BMD specifically among women with POF, similar to what is seen in women who are post-menopausal. In one study, women who remained premenopausal following chemotherapy who were also treated with tamoxifen experienced significant BMD loss relative to their baseline levels; in contrast, women with POF on tamoxifen had reduced bone loss (33).

Cognitive function

Most studies have not detected measurable differences in cognitive function between women who become menopausal from treatment and those who remain premenopausal or who were post-menopausal at diagnosis (34-36). However, inherent in many of the studies that have examined whether POF is a risk factor for adverse cognitive outcomes are methodological limitations that preclude any conclusions about this outcome in younger women (37). Additional studies, including those that evaluate longer-term outcomes are needed to better evaluate the effect of POF on cognition.

Cardiovascular health

In the general population, women with POF are at increased risk for developing cardiovascular disease (CVD) (38). There is little data about CVD risk in young breast cancer survivors with POF. Additional long-term studies are needed of young survivors to determine the burden of excess CVD risk among women with POF as a consequence of breast cancer treatment.

Depression and anxiety

There is significant psychological morbidity related to symptoms of depression among younger women with breast cancer (1). Few studies, however, have described specifically how POF is related to depression in younger women. While Gorman *et al.* did not find an association between treatment-associated amenorrhea and depressive symptoms among women who were diagnosed at age 40 or younger, they did find that women with more reproductive concerns, less social support, and worse physical functioning had more symptoms of depression (39). Bloom *et al.* reported a relationship between depression and several health related and psycho-social QOL domains, including symptom severity, pain, and body image, suggesting these variables primarily affected depression through illness intrusiveness (40).

Anxiety surrounding a cancer diagnosis—both early in the treatment period and many years after treatment has ended—can have a measurable impact on QOL. One study examining the potential role of oophorectomy and abrupt onset of menopause on QOL in young survivors found anxious symptoms to be prevalent, however no differences were detected between women who were treated with chemotherapy and those who did not, and between women who had an oophorectomy and those who did not (41).

Sexuality

Premature menopause has a major impact on sexuality and intimacy, with sexual problems more prevalent among young women who become amenorrheic from treatment compared to women who continue to menstruate (17,42-44). Vaginal dryness and dyspareunia are the main drivers of sexual dysfunction, and are strongly related to the onset of menopause. In addition to physiologic symptoms, decreased interest in sex is another significant factor. It is important to consider that sexual problems are complex, often interrelated, and if ignored or untreated, can lead to long term sexual health issues. When intercourse is painful, women are less likely to be interested in sex, sex can become less frequent, and vaginal atrophy may result. Adding to the complexity of sexual dysfunction is the potential of other QOL issues to affect intimacy. Anxiety, depression, body image concerns, fatigue, and side effects from medication (i.e., anti-depressants) can influence both sexual interest and functioning.

Interventions for symptom management

Interventions aimed at ameliorating menopausal symptoms in breast cancer survivors, with the goal of improving QOL, are varied and include pharmacologic, psycho-educational,

Table 1 Interventions to consider for menopausal symptom management in young breast cancer survivors.

Hormonal medication (often contraindicated in this setting)
Non-hormonal medication
• Antidepressants, gabapentin, and clonidine for hot flashes
• Topical lubricants and moisturizers for vaginal health
Behavioral strategies and interventions
• Exercise
• Yoga
• Acupuncture
• Cold packs, dressing in layers, etc.
Psychoeducation
• Cognitive behavioral therapy (CBT)
• Individual or couples counseling

and behavioral interventions (See *Table 1*). It is important to consider that because many aspects of QOL are inter-related, intervening in one often leads to improvement of others. For example, managing symptoms of fatigue can potentially help improve other related QOL domains, such as sexual dysfunction and depression.

An important limitation of most trials assessing the efficacy of interventions aimed to improve QOL in cancer survivors is that they have generally been conducted in older women who were post-menopausal at diagnosis, and it is unclear how generalizable findings are to young women with POF.

Pharmacologic

Hormonal

In the general population, hormone replacement therapy (HRT) is very efficacious in managing menopausal symptoms. However, systemic treatment with exogenous hormones is generally contraindicated in women who have a history of breast cancer, particularly when they have had hormone sensitive disease. Several trials have evaluated localized estrogen therapeutic options, such as vaginal rings, tablets and creams, to help ameliorate vaginal dryness in breast cancer survivors. While these improve symptoms and rely on a low, localized dose of estrogen, some estrogen is released systemically into the blood stream, leading to a consensus among experts that is a highly individualized decision, with women advised to speak with their physicians (42,45,46). Testosterone-based therapies have been considered as well but have not been shown to be effective without HRT (45).

Non-hormonal

A wide range of non-hormonal pharmacological agents have been tested in an effort to diminish both the occurrence and intensity of hot flashes. Anti-depressants, including venlafaxine and paroxetine, as well as other pharmacologic, most notably gabapentin and clonidine, have been found to be effective in improving hot flash burden in randomized controlled trials (RCTs) (47-50). In contrast, there has not been strong evidence that natural supplements, including Vitamin E, phyto-estrogens (e.g., soy), and black cohosh are better compared to placebos (51-53).

Several non-hormonal options also exist to reduce vaginal dryness and help with dyspareunia. Vaginal lubricants can help improve symptoms of vaginal dryness and make sexual intercourse more comfortable. Vaginal moisturizers are designed to hydrate the vaginal mucosa and are effectual for approximately 2-3 days (54). Most importantly, it is crucial to advise women to maintain sexual activity. The potential for long-term detrimental impact—e.g., vaginal atrophy-argues for early intervention to recognize and treat symptoms implicated in poor sexual functioning.

There are several pharmacologic options that have been tested in an effort to prevent or treat bone loss among young women with treatment induced POF, thereby decreasing the risk of osteoporosis. Results from one trial (55) indicate that administering zoledronic acid concomitantly with chemotherapy treatment can effectively reduce BMD loss among young women who develop POF.

Findings from other studies of another bisphosphonate, clodronate, in women with POF have been mixed, with one study finding oral clodronate effective at lessening BMD loss (56) while another study (57) found no significant difference in BMD loss between the group randomized to intravenous clodronate and the control group, although the authors acknowledge that this might be due to power limitations due to the small study size, or alternatively, the relatively limited duration women received the drug during this trial. The optimal timing of and indications for treatment with bisphosphonates in this setting are unclear at this time.

Psycho-educational

A wide range of interventions grounded in psycho-social theory have been developed and tested to help achieve better menopausal symptom management, ultimately improving QOL in the both the short and long term. A recent trial demonstrated that cognitive behavioral therapy (CBT), physical activity, and a combination of the two can helping improve menopausal symptoms as well as select QOL outcomes, including sexuality, urinary problems, and physical functioning in women with premature menopause (58). In addition to decreasing the severity of vasomotor symptoms, other trials, inclusive of both pre- and post menopausal breast cancer survivors, have also found CBT to be helpful with sleep, emotional, and cognitive issues (59,60). Interventions teaching relaxation strategies have also demonstrated some effectiveness in improving vasomotor symptoms in breast cancer survivors (61).

Several psycho-educational interventions have been shown to help breast cancer survivors with problems related to sexuality and intimacy. Effective strategies have included both individual and couple–based therapies which focus on improving communication, facilitating coping, and helping couples deal with potential intimacy issues (62).

Exercise

Greater levels of physical activity after breast cancer have been associated with improved survival outcomes (63-65). Exercise has also been associated with better QOL outcomes in breast cancer survivors of all ages, largely the result of improving symptoms of anxiety, depression, and fatigue (66), as well as having a beneficial effect on bone health. There have been promising results from yoga interventions, with reported improvements in fatigue, depressive symptoms, and vasomotor symptoms following the intervention period (67-69), however many of these studies are small, and follow-up data is needed as to whether the initial benefit can be sustained over the longer term. Specifically among prematurely menopausal survivors, a recent RCT demonstrated that a regimen incorporating both a strength and resistance component was effective in both reducing bone loss and maintaining body fat (70).

Additional strategies

There are some additional behavioral modifications that should be considered in an effort to reduce distressing side effects and improve overall QOL in breast cancer survivors. Simple changes, such as dressing in layers, using cold packs, or temperature adjustments, can often help women suffering from hot flashes and night sweats. Acupuncture is another alternative medical approach that has been investigated in breast cancer patients, with mixed findings as to the effectiveness in relieving menopausal symptoms. While one randomized found acupuncture was just as effective as venlafaxine in reducing hot flashes and depressive symptoms, as well as improving other QOL domains in women with hormone positive breast cancer (71), other studies have not confirmed that acupuncture is any better sham interventions (72).

Concerning sexual dysfunction, strategies to address vaginal atrophy and stenosis include pelvic floor strengthening and vaginal dilation. It is vital to maintain blood flow to the vagina to prevent atrophy, and as noted above, it is therefore important to maintain regular sexual activity.

Conclusions

Life following a cancer diagnosis will invariably be different for every breast cancer survivor, however many young women will be faced with the additional physical and emotional challenges of menopause years earlier than would otherwise be expected. There is a clear need for additional studies of interventions among women in younger age groups in order to identify useful and effective therapies for these young survivors, with the goal of improving the QOL in young women through treatment and into long-term survivorship.

Acknowledgements

Dr. Rosenberg is funded by NIH 5 R25 CA057711.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Howard-Anderson J, Ganz PA, Bower JE, et al. Quality of life, fertility concerns, and behavioral health outcomes in younger breast cancer survivors: a systematic review. J Natl Cancer Inst 2012;104:386-405.
2. Wenzel LB, Fairclough DL, Brady MJ, et al. Age-related differences in the quality of life of breast carcinoma patients after treatment. Cancer 1999;86:1768-74.
3. Kroenke CH, Rosner B, Chen WY, et al. Functional impact of breast cancer by age at diagnosis. J Clin Oncol 2004;22:1849-56.
4. Ruddy KJ, Partridge AH. Fertility (male and female) and menopause. J Clin Oncol 2012;30:3705-11.
5. Partridge A, Gelber S, Gelber RD, et al. Age of menopause among women who remain premenopausal following treatment for early breast cancer: long-term results from International Breast Cancer Study Group Trials V and VI. Eur J Cancer 2007;43:1646-53.
6. Partridge AH, Ruddy KJ. Fertility and adjuvant treatment in young women with breast cancer. Breast 2007;16 Suppl 2:S175-81.
7. Goodwin PJ, Ennis M, Pritchard KI, et al. Risk of menopause during the first year after breast cancer diagnosis. J Clin Oncol 1999;17:2365-70.
8. Petrek JA, Naughton MJ, Case LD, et al. Incidence, time course, and determinants of menstrual bleeding after breast cancer treatment: a prospective study. J Clin Oncol 2006;24:1045-51.
9. Abusief ME, Missmer SA, Ginsburg ES, et al. The effects of paclitaxel, dose density, and trastuzumab on treatment-related amenorrhea in premenopausal women with breast cancer. Cancer 2010;116:791-8.
10. Partridge AH, Burstein HJ, Winer EP. Side effects of chemotherapy and combined chemohormonal therapy in women with early-stage breast cancer. J Natl Cancer Inst Monogr 2001;(30):135-42.
11. Alder J, Zanetti R, Wight E, et al. Sexual dysfunction after premenopausal stage I and II breast cancer: do androgens play a role? J Sex Med 2008;5:1898-906.
12. Leining MG, Gelber S, Rosenberg R, et al. Menopausal-type symptoms in young breast cancer survivors. Ann Oncol 2006;17:1777-82.
13. Burstein HJ, Winer EP. Primary care for survivors of breast cancer. N Engl J Med 2000;343:1086-94.
14. Crandall C, Petersen L, Ganz PA, et al. Association of breast cancer and its therapy with menopause-related symptoms. Menopause 2004;11:519-30.
15. Mar Fan HG, Houédé-Tchen N, Chemerynsky I, et al. Menopausal symptoms in women undergoing chemotherapy-induced and natural menopause: a prospective controlled study. Ann Oncol 2010;21:983-7.
16. Benshushan A, Rojansky N, Chaviv M, et al. Climacteric symptoms in women undergoing risk-reducing bilateral salpingo-oophorectomy. Climacteric 2009;12:404-9.
17. Ganz PA, Greendale GA, Petersen L, et al. Breast cancer in younger women: reproductive and late health effects of treatment. J Clin Oncol 2003;21:4184-93.
18. Ganz PA, Bower JE. Cancer related fatigue: a focus on breast cancer and Hodgkin's disease survivors. Acta Oncol 2007;46:474-9.
19. Broeckel JA, Jacobsen PB, Horton J, et al. Characteristics and correlates of fatigue after adjuvant chemotherapy for breast cancer. J Clin Oncol 1998;16:1689-96.
20. Glaus A, Boehme Ch, Thürlimann B, et al. Fatigue and menopausal symptoms in women with breast cancer undergoing hormonal cancer treatment. Ann Oncol 2006;17:801-6.
21. Alfano CM, Lichstein KL, Vander Wal GS, et al. Sleep duration change across breast cancer survivorship: associations with symptoms and health-related quality of life. Breast Cancer Res Treat 2011;130:243-54.
22. Helms RL, O'Hea EL, Corso M. Body image issues in women with breast cancer. Psychol Health Med 2008;13:313-25.
23. Goodwin PJ, Ennis M, Pritchard KI, et al. Adjuvant treatment and onset of menopause predict weight gain after breast cancer diagnosis. J Clin Oncol 1999;17:120-9.
24. McInnes JA, Knobf MT. Weight gain and quality of life in women treated with adjuvant chemotherapy for early-stage breast cancer. Oncol Nurs Forum 2001;28:675-84.
25. Gordon AM, Hurwitz S, Shapiro CL, et al. Premature ovarian failure and body composition changes with adjuvant chemotherapy for breast cancer. Menopause 2011;18:1244-8.
26. Avis NE, Crawford S, Manuel J. Psychosocial problems among younger women with breast cancer. Psychooncology 2004;13:295-308.
27. Rosenberg SM, Tamimi RM, Gelber S, et al. Body image in recently diagnosed young women with early breast cancer. Psychooncology 2012. [Epub ahead of print].

28. Fobair P, Stewart SL, Chang S, et al. Body image and sexual problems in young women with breast cancer. Psychooncology 2006;15:579-94.
29. Shapiro CL, Manola J, Leboff M. Ovarian failure after adjuvant chemotherapy is associated with rapid bone loss in women with early-stage breast cancer. J Clin Oncol 2001;19:3306-11.
30. Bruning PF, Pit MJ, de Jong-Bakker M, et al. Bone mineral density after adjuvant chemotherapy for premenopausal breast cancer. Br J Cancer 1990;61:308-10.
31. Tham YL, Sexton K, Weiss HL, et al. The adherence to practice guidelines in the assessment of bone health in women with chemotherapy-induced menopause. J Support Oncol 2006;4:295-8, 304.
32. McCune JS, Games DM, Espirito JL. Assessment of ovarian failure and osteoporosis in premenopausal breast cancer survivors. J Oncol Pharm Pract 2005;11:37-43.
33. Vehmanen L, Elomaa I, Blomqvist C, et al. Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status. J Clin Oncol 2006;24:675-80.
34. Hermelink K, Henschel V, Untch M, et al. Short-term effects of treatment-induced hormonal changes on cognitive function in breast cancer patients: results of a multicenter, prospective, longitudinal study. Cancer 2008;113:2431-9.
35. Vearncombe KJ, Rolfe M, Andrew B, et al. Cognitive effects of chemotherapy-induced menopause in breast cancer. Clin Neuropsychol 2011;25:1295-313.
36. Jenkins V, Shilling V, Deutsch G, et al. A 3-year prospective study of the effects of adjuvant treatments on cognition in women with early stage breast cancer. Br J Cancer 2006;94:828-34.
37. Vearncombe KJ, Pachana NA. Is cognitive functioning detrimentally affected after early, induced menopause? Menopause 2009;16:188-98.
38. Archer DF. Premature menopause increases cardiovascular risk. Climacteric 2009;12 Suppl 1:26-31.
39. Gorman JR, Malcarne VL, Roesch SC, et al. Depressive symptoms among young breast cancer survivors: the importance of reproductive concerns. Breast Cancer Res Treat 2010;123:477-85.
40. Bloom JR, Stewart SL, Johnston M, et al. Intrusiveness of illness and quality of life in young women with breast cancer. Psychooncology 1998;7:89-100.
41. Sayakhot P, Vincent A, Deeks A, Potential adverse impact of ovariectomy on physical and psychological function of younger women with breast cancer. Menopause 2011;18:786-93.
42. Schover LR. Premature ovarian failure and its consequences: vasomotor symptoms, sexuality, and fertility. J Clin Oncol 2008;26:753-8.
43. Burwell SR, Case LD, Kaelin C, et al. Sexual problems in younger women after breast cancer surgery. J Clin Oncol 2006;24:2815-21.
44. King J, Wynne CH, Assersohn L, et al. Hormone replacement therapy and women with premature menopause--a cancer survivorship issue. Eur J Cancer 2011;47:1623-32.
45. Melisko ME, Goldman M, Rugo HS. Amelioration of sexual adverse effects in the early breast cancer patient. J Cancer Surviv 2010;4:247-55.
46. Wills S, Ravipati A, Venuturumilli P, et al. Effects of vaginal estrogens on serum estradiol levels in postmenopausal breast cancer survivors and women at risk of breast cancer taking an aromatase inhibitor or a selective estrogen receptor modulator. J Oncol Pract 2012;8:144-8.
47. Boekhout AH, Vincent AD, Dalesio OB, et al. Management of hot flashes in patients who have breast cancer with venlafaxine and clonidine: a randomized, double-blind, placebo-controlled trial. J Clin Oncol 2011;29:3862-8.
48. Loprinzi CL, Kugler JW, Sloan JA, et al. Venlafaxine in management of hot flashes in survivors of breast cancer: a randomised controlled trial. Lancet 2000;356:2059-63.
49. Bordeleau L, Pritchard K, Goodwin P, et al. Therapeutic options for the management of hot flashes in breast cancer survivors: an evidence-based review. Clin Ther 2007;29:230-41.
50. Nelson HD, Vesco KK, Haney E, et al. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. JAMA 2006;295:2057-71.
51. Jacobson JS, Troxel AB, Evans J, et al. Randomized trial of black cohosh for the treatment of hot flashes among women with a history of breast cancer. J Clin Oncol 2001;19:2739-45.
52. Pockaj BA, Gallagher JG, Loprinzi CL, et al. Phase III double-blind, randomized, placebo-controlled crossover trial of black cohosh in the management of hot flashes: NCCTG Trial N01CC1. J Clin Oncol 2006;24:2836-41.
53. Rada G, Capurro D, Pantoja T, et al. Non-hormonal interventions for hot flushes in women with a history of breast cancer. Cochrane Database Syst Rev 2010;(9):CD004923.
54. Carter J, Goldfrank D, Schover LR. Simple strategies for vaginal health promotion in cancer survivors. J Sex Med

2011;8:549-59.
55. Shapiro CL, Halabi S, Hars V, et al. Zoledronic acid preserves bone mineral density in premenopausal women who develop ovarian failure due to adjuvant chemotherapy: final results from CALGB trial 79809. Eur J Cancer 2011;47:683-9.
56. Saarto T, Blomqvist C, Välimäki M, et al. Chemical castration induced by adjuvant cyclophosphamide, methotrexate, and fluorouracil chemotherapy causes rapid bone loss that is reduced by clodronate: a randomized study in premenopausal breast cancer patients. J Clin Oncol 1997;15:1341-7.
57. Vehmanen L, Saarto T, Risteli J, et al. Short-term intermittent intravenous clodronate in the prevention of bone loss related to chemotherapy-induced ovarian failure. Breast Cancer Res Treat 2004;87:181-8.
58. Duijts SF, van Beurden M, Oldenburg HS, et al. Efficacy of cognitive behavioral therapy and physical exercise in alleviating treatment-induced menopausal symptoms in patients with breast cancer: results of a randomized, controlled, multicenter trial. J Clin Oncol 2012;30:4124-33.
59. Mann E, Smith MJ, Hellier J, et al. Cognitive behavioural treatment for women who have menopausal symptoms after breast cancer treatment (MENOS 1): a randomised controlled trial. Lancet Oncol 2012;13:309-18.
60. Savard J, Simard S, Ivers H, et al. Randomized study on the efficacy of cognitive-behavioral therapy for insomnia secondary to breast cancer, part I: Sleep and psychological effects. J Clin Oncol 2005;23:6083-96.
61. Tremblay A, Sheeran L, Aranda SK. Psychoeducational interventions to alleviate hot flashes: a systematic review. Menopause 2008;15:193-202.
62. Taylor S, Harley C, Ziegler L, et al. Interventions for sexual problems following treatment for breast cancer: a systematic review. Breast Cancer Res Treat 2011;130:711-24.
63. Holmes MD, Chen WY, Feskanich D, et al. Physical activity and survival after breast cancer diagnosis. JAMA 2005;293:2479-86.
64. Holick CN, Newcomb PA, Trentham-Dietz A, et al. Physical activity and survival after diagnosis of invasive breast cancer. Cancer Epidemiol Biomarkers Prev 2008;17:379-86.
65. Irwin ML, Smith AW, McTiernan A, et al. Influence of pre- and postdiagnosis physical activity on mortality in breast cancer survivors: the health, eating, activity, and lifestyle study. J Clin Oncol 2008;26:3958-64.
66. Alfano CM, Smith AW, Irwin ML, et al. Physical activity, long-term symptoms, and physical health-related quality of life among breast cancer survivors: a prospective analysis. J Cancer Surviv 2007;1:116-28.
67. Bower JE, Garet D, Sternlieb B, et al. Yoga for persistent fatigue in breast cancer survivors: a randomized controlled trial. Cancer 2012;118:3766-75.
68. Carson JW, Carson KM, Porter LS, et al. Yoga of Awareness program for menopausal symptoms in breast cancer survivors: results from a randomized trial. Support Care Cancer 2009;17:1301-9.
69. Harder H, Parlour L, Jenkins V. Randomised controlled trials of yoga interventions for women with breast cancer: a systematic literature review. Support Care Cancer 2012;20:3055-64.
70. Winters-Stone KM, Dobek J, Nail LM, et al. Impact + resistance training improves bone health and body composition in prematurely menopausal breast cancer survivors: a randomized controlled trial. Osteoporos Int 2013;24:1637-46.
71. Walker EM, Rodriguez AI, Kohn B, et al. Acupuncture versus venlafaxine for the management of vasomotor symptoms in patients with hormone receptor-positive breast cancer: a randomized controlled trial. J Clin Oncol 2010;28:634-40.
72. Loibl S, Lintermans A, Dieudonné AS, et al. Management of menopausal symptoms in breast cancer patients. Maturitas 2011;68:148-54.

Cite this article as: Rosenberg SM, Partridge AH. Premature menopause in young breast cancer: effects on quality of life and treatment interventions. J Thorac Dis 2013;5(S1):S55-S61. doi: 10.3978/j.issn.2072-1439.2013.06.20

Conservative mastectomies for breast cancer and risk-reducing surgery: the Memorial Sloan Kettering Cancer Center experience

Aidan T. Manning[1], Virgilio S. Sacchini[2]

[1]Department of Surgery, University Hospital Waterford, Waterford, Ireland; [2]Breast Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Contributions: (I) Conception and design: All authors; (II) Administrative support: All authors; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Virgilio S. Sacchini, MD. Breast Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, 300 East 66th Street, New York, NY 10065, USA. Email: sacchinv@mskcc.org.

Abstract: Demand for conservative mastectomies continues to increase as more patients choose to undergo breast reconstruction, often with simultaneous contralateral prophylactic mastectomy (CPM). In addition, the increasing use of risk-reducing surgery in high-risk groups has contributed to the increased use of these techniques. We have reviewed the indications and outcomes of a large group of patients undergoing nipple-sparing mastectomy (NSM) at this institution. In total, 728 nipple-sparing mastectomies (NSMs) were performed in 413 patients between 2000 and 2013, for treatment of breast cancer (n=269) or risk reduction (n=459). Of 728 NSMs performed, 177 (24.3%) were in patients known to have a BRCA1 or BRCA2 germline mutation, or a genetic variant of uncertain significance. There was an incidental finding of ductal carcinoma in situ (DCIS) or invasive carcinoma in 22 (4.8%) and 8 (1.7%) of 459 prophylactic NSMs, respectively. In addition, unexpected invasive carcinoma was found in 17 of 98 therapeutic NSMs (17.3%) performed for DCIS. At median follow-up of 49 months, there were no known cases of local recurrence and only one case of regional recurrence. Immediate breast reconstruction was performed in 409 patients, most of whom underwent tissue expander/implant based procedures (n=401). Although 273 breasts (37.5%) had some evidence of skin desquamation at follow-up, most resolved spontaneously with 47 breasts (6.5%) requiring debridement. Other complications included hematoma in seven breasts (1%) and wound infection in 31 breasts (4.3%). Expander/implant removal was required in 20 cases (2.8%). The nipple-areola complex (NAC) was subsequently excised in 10 of 728 breasts (1.4%) due to oncologic concerns following assessment of retroareolar tissue. NSM was successful in most patients with an acceptable complication rate and in few patients subsequently undergoing removal of the NAC. Patients requiring mastectomy for breast cancer or risk reduction may now benefit from conservative mastectomy techniques such as NSM, resulting in improved cosmesis and, possibly, a reduced psychological impact.

Keywords: Breast cancer; conservative mastectomy; risk-reducing surgery

Submitted Aug 11, 2015. Accepted for publication Sep 24, 2015.
doi: 10.3978/j.issn.2227-684X.2015.10.02
View this article at: http://dx.doi.org/10.3978/j.issn.2227-684X.2015.10.02

Introduction

The surgical management of breast cancer has changed significantly in recent decades, from the disfiguring radical mastectomy, commonly performed until the mid 1970s, to breast-conserving surgery and sentinel lymph node biopsy, in which minimal breast tissue is removed and the morbidity associated with more extensive axillary surgery is avoided. There are, however, several circumstances in which mastectomy is still indicated, such as multicentric disease and inflammatory breast cancer (IBC), and circumstances in

which breast radiation is contraindicated (such as pregnancy, previous breast-conserving therapy, and connective tissue diseases). In addition, some women diagnosed with breast cancer will choose mastectomy as their treatment of choice, even when breast conservation is possible, often undergoing a contralateral prophylactic mastectomy (CPM) at the same time (1). Risk-reducing surgery is also commonly performed in high-risk patients, such as those with a genetic predisposition to breast cancer and—in particular—those carrying a germline BRCA1 or BRCA2 mutation (2).

Oncoplastic surgery techniques enable surgeons to perform mastectomy with immediate or delayed reconstruction, preserving much of the skin envelope and sometimes the nipple-areola complex (NAC). Importantly, these dramatic changes toward more-conservative surgery have evolved without evidence of compromise to oncologic safety (3-17).

Increasing evidence that breast cancer is a systemic heterogenous disease requiring targeted systemic therapies has allowed for a change from the aggressive local therapies of the Halsted era to more conservative surgical approaches. The concept of performing mastectomy with preservation of skin and, if possible, the NAC, has developed gradually with the evolution of breast reconstruction, using either autologous tissue or implant-based techniques. "Subcutaneous mastectomy" followed by reconstruction was first reported in 1962 by Freeman, who described this procedure for patients with benign disease (18). The decline of the Halsted radical mastectomy in favor of the modified technique, which preserved muscle and some of the native breast skin, allowed for development of reconstructive surgery in breast cancer patients as well. The need for muscle cover to avoid implant exposure led to the use of tissue expanders to stretch the subpectoral pocket in preparation for a permanent implant.

The concept of a "skin-sparing mastectomy" was introduced by Toth and Lappert in 1991, with preservation of more of the native breast skin compared to the traditional modified radical mastectomy (19), resulting in improved cosmesis and a decrease in the need for contralateral symmetrization procedures (20). Initially, there was concern that preservation of more skin would result in increased rates of local recurrence; however, several studies have shown similar local recurrence rates to the modified radical mastectomy (3-9). It is now generally accepted that skin-sparing mastectomy is the standard mastectomy procedure, without an increased risk of disease recurrence and allowing for immediate breast reconstruction if desired.

Skin-sparing mastectomy with immediate reconstruction is now commonly performed; however, this procedure always involves removal of the NAC. Although several techniques exist for immediate or delayed nipple reconstruction, results are often unsatisfactory (21), and this has led to an increase in demand for the "nipple-sparing mastectomy (NSM)" procedure for both therapeutic and prophylactic surgery. The oncologic safety of NSM has been reported in many retrospective studies, with encouraging results (10-17), and as length of follow-up increases, this procedure is being accepted more and more as an option when specific criteria are met. In particular, it is often performed in the prophylactic setting, when such concerns regarding local recurrence do not apply.

The Memorial Sloan Kettering Cancer Center (MSKCC) experience

Indications for NSM

There are no widely accepted criteria that need to be fulfilled in order for NSM to be performed; however, several factors are considered for each potential candidate. Relative contraindications to NSM include smoking history, larger breast size, and ptosis. In breast cancer patients, those with skin or nipple involvement, larger (T3) tumors, central tumors close to the NAC, or blood stained nipple discharge are generally considered unsuitable candidates for NSM (22). All patients undergoing NSM meet with a breast surgeon and a plastic surgeon with a special interest in breast reconstruction preoperatively, to discuss the risks and benefits of NSM compared to skin-sparing mastectomy. Risks of NSM are discussed and documented, including inadequate blood supply leading to skin desquamation or necrosis, with the possible need for debridement or excision of the NAC. Concerns regarding oncologic safety, including lack of long-term data on local recurrence rates, are discussed with all patients, including those undergoing risk-reducing surgery.

Technical considerations

The most common incision performed for NSM at MSKCC is circumareolar, with lateral extension if required; however, the actual incision is decided on an individual case basis, following discussion between patient, breast surgeon, and plastic surgeon. Chen *et al*. have previously described the frequency, advantages, and disadvantages of different

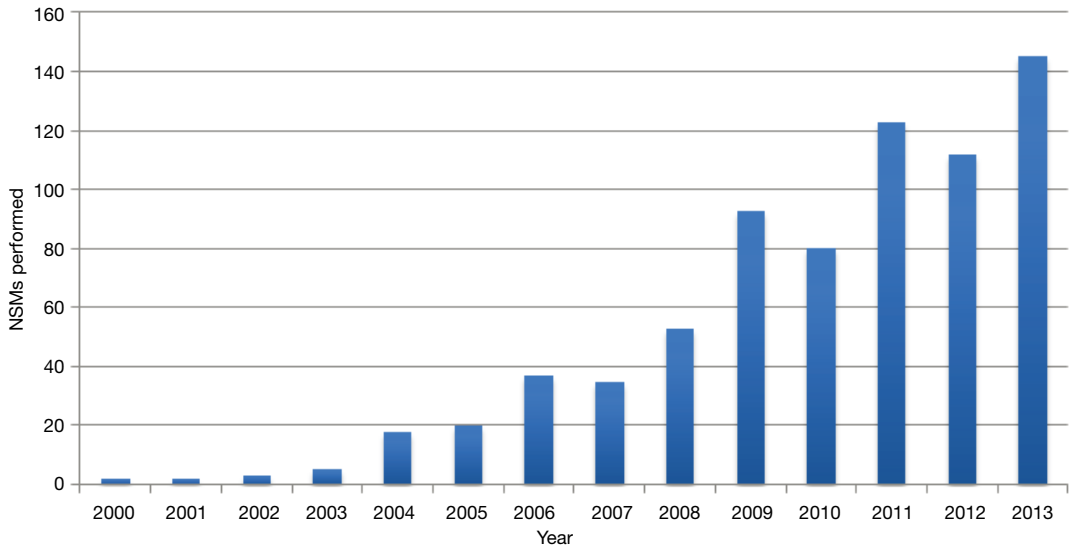


Figure 1 Number of nipple-sparing mastectomies (NSMs) performed at Memorial Sloan Kettering Cancer Center (MSKCC).

incisions used for NSM at our institution (23). The plastic surgeon will usually recommend a specific incision and discuss it with the breast surgeon. Agreement is reached between both surgeons and confirmed again on the day of surgery. We find that the circumareolar approach, with lateral extension as required, allows adequate access to the axilla for sentinel lymph node biopsy and axillary lymph node dissection when required. Although the lateral inframammary fold incision may have advantages in terms of cosmesis, most will agree that axillary surgery is more challenging using this approach, particularly in a large breast and when axillary lymph node dissection is required. Several factors are considered in each case when planning incision, including breast size, scars from previous surgery (if any), need for axillary dissection, and patient preference.

As with skin-sparing mastectomy, the aim is to remove all glandular breast tissue and retaining only a thin layer of subdermal tissue behind the NAC (<3 mm). The retroareolar space is typically infiltrated with 10 mL of saline at the beginning of the case to help develop the tissue plane between the breast tissue and the NAC. In all therapeutic cases, a section of retroareolar tissue ("nipple-margin") is removed and examined either by intraoperative frozen section or routine permanent section as appropriate.

Review of NSMs performed at MSKCC

We recently reviewed our experience with NSM at this institution. Although skin-sparing mastectomy is by far the most common type of mastectomy performed, the number of patients undergoing NSM has significantly increased, as shown in *Figure 1*. During the period between 2000 and 2013, 728 NSMs were performed in 413 patients, 315 of whom underwent bilateral procedures (630 NSMs) and 98 of whom underwent a unilateral procedure. There were 269 therapeutic NSMs performed in 261 patients (eight patients had bilateral therapeutic NSMs). The remaining 152 patients underwent NSM for risk reduction. In addition, 176 patients undergoing therapeutic NSM also underwent a simultaneous CPM; therefore, the total number of patients undergoing a risk-reducing procedure was 328 (459 NSMs). The indications for NSM are shown in *Tables 1,2*.

Of 728 NSMs performed during this period, 177 were performed for risk reduction or treatment of breast cancer in 89 patients with a confirmed BRCA1 or BRCA2 mutation or a genetic variant of uncertain significance. We have recently reported our experience of NSM in this group of patients (24), and although follow-up is short at 27 months, there were no cases of local or regional recurrence, supporting the view that NSM is an acceptable option in patients with a BRCA1 or BRCA2 mutation.

Disease stage for patients undergoing therapeutic NSM

Following 269 therapeutic NSMs in 261 patients, the majority were confirmed to have pre-invasive or early-stage breast cancer (stage 0, n=81; stage 1, n=114; stage 2, n=51; stage 3, n=9; stage 4, n=0). The remaining patients had

Table 1 Indications for nipple-sparing mastectomy (NSM)

Indication	n
Therapeutic	
Current breast cancer	223
Completion mastectomy*	41
Phyllodes	5
Prophylactic	
BRCA1 or BRCA2 mutation	125
Contralateral prophylactic mastectomy	198
Family history	72
Lobular carcinoma in situ	42
Other**	8
Previous breast cancer	14
Total	728

*, it includes four women who had lumpectomies for atypical ductal hyperplasia; **, four patients underwent bilateral prophylactic NSMs for "other" indications. These were bilateral papillomatosis, previous mantle radiation, bilateral calcifications not amenable to biopsy, and previous bilateral intramammary biogel injections.

Table 2 Indications for therapeutic nipple-sparing mastectomy (NSM)

Indication	n
Invasive ductal carcinoma	135
Invasive lobular carcinoma	13
Mixed ductal and lobular carcinoma	11
Ductal carcinoma in situ (DCIS)	98
Phyllodes (malignant)	4
Phyllodes (benign)	1
Malignant myoepithelial carcinoma	1
Metaplastic carcinoma	1
Occult breast cancer (presented with lymph node metastases)	1
Atypical ductal hyperplasia	4
Total	269

either phyllodes tumour (n=4) or had NSM for recurrent breast cancer (n=2) and were not assigned a disease stage.

Incidental diagnosis of ductal carcinoma in situ (DCIS) or invasive breast cancer following NSM

Invasive breast cancer was found unexpectedly in 26 patients. In eight cases, the patient was undergoing NSM for prophylaxis, and pathology revealed invasive ductal carcinoma (four cases) or invasive lobular carcinoma (four cases). The other 18 cases of unexpected invasive cancer were in patients undergoing therapeutic NSM for non-invasive disease who were subsequently upstaged; 17 of 98 women undergoing NSM for DCIS and one of four women undergoing NSM for atypical ductal hyperplasia. The type of invasive cancer was invasive ductal carcinoma in 17 cases, and mixed ductal and lobular in one case.

In addition, DCIS was found unexpectedly in 21 of 328 patients (22 breasts) undergoing prophylactic NSM (6.4%). This included a BRCA2 mutation carrier undergoing bilateral NSMs who was subsequently diagnosed with bilateral DCIS. One patient undergoing NSM for a benign phyllodes tumor was found to have DCIS. In total, 29 of 328 patients undergoing prophylactic NSM (8.8%) were diagnosed unexpectedly with either DCIS or invasive breast cancer.

Assessment of the "nipple margin"

Pathological assessment of the retroareolar "nipple margin" was positive for atypia or DCIS in 11 of 269 therapeutic cases. In 7 of these 11 cases, the NAC was excised, revealing residual DCIS in one case, atypia in another case, and five cases with no further disease in the excised specimen. Repeat nipple margin biopsies were performed in the remaining four cases, sometimes during the expander/implant exchange procedure. All of these were benign, allowing the NAC to be preserved. Of 459 prophylactic NSMs performed, four had a positive finding of DCIS in the retroareolar specimen. Three of these patients returned for excision of the NAC, and one patient, who was found to have only a very small focus of DCIS, decided against further surgery.

Follow-up

At median follow-up of 49 months (range, 0-149 months), 402 of 413 patients were alive with no evidence of disease. Four patients had died, one having developed regional and distant metastases 15 months after NSM for stage IIA disease, and another who had undergone nipple-sparing CPM developed metastatic disease from her initial stage IIIB breast cancer. One patient died of unknown cause, and another died of metastatic ovarian cancer. There was therefore one death attributed to metastatic breast cancer

for which NSM had been performed.

Seven patients were alive with metastatic disease, two of whom had undergone nipple-sparing CPMs and developed metastases from the initial breast cancer. Five patients who had undergone therapeutic NSMs developed distant metastatic disease (all were initially diagnosed with either stage II or stage III disease). One of these patients was diagnosed simultaneously with both regional and bone metastases. No patient was diagnosed with local recurrence.

Breast reconstruction and complications

Immediate breast reconstruction was performed in almost all cases, with only 4 of 413 patients not undergoing reconstruction due to small breast size. The procedures performed were tissue expander/implant based (n=370), implant only (n=31), autologous flap (n=7), and fat injection only (n=1). Frequent use of ADM to cover the lower pole of the expander allows for greater initial fill volume, reducing the number of outpatient expansions. It also facilitates subsequent overexpansion, allowing for increased implant size if desired (23). The mean length of time between tissue expander insertion and exchange procedure was 169 days (median 143 days). Although 273 of 728 breasts (37.5%) had some degree of skin desquamation at follow-up, most of these were mild and fully resolved without intervention. Only 47 breasts (6.5%) developed skin necrosis requiring debridement. There were seven hematomas requiring evacuation (1%) and 31 wound infections (4.3%). Removal of expander/implant was required in 20 of 711 cases in which an expander/implant reconstruction was performed (2.8%).

Discussion

The evolution of conservative mastectomy techniques enables patients requiring mastectomy and patients undergoing risk-reducing surgery to benefit from advances in oncoplastic surgery, with improved cosmetic outcomes and reduced psychological impact. There are several forms of breast reconstruction available, using either the patient's own tissue or prosthetic implants, or both. These procedures are made possible by skin-sparing and NSM techniques, which may allow the entire skin envelope and NAC to be preserved.

Data continue to show equivalence of these conservative techniques to the more traditional modified radical mastectomy in terms of local and regional recurrence rates (3-17). A recent database study from the United States of more than 20,000 women undergoing mastectomy for breast cancer from 1998 to 2007 showed a dramatic rise in the use of breast reconstruction (46% in 1998 to 63% in 2007). This change was predominantly due to a rise in the number of patients undergoing implant-based reconstruction, which is generally performed following a conservative mastectomy approach, with a decrease in the number of patients undergoing autologous tissue techniques during the same period (56% in 1998 to 25% in 2007) (25). Skin-sparing mastectomy has been the standard type performed at our institution for many years, and most patients undergo immediate implant-based reconstruction (26). The increased demand for nipple-sparing techniques, as discussed above, is further evidence of an expanding role for conservative mastectomies, in keeping with the increased demand for breast reconstruction in the United States (25).

Another significant change in breast surgery has been the increasing demand for CPM in breast cancer patients (27,28). We performed 198 NSMs in this context at our institution, including some patients with a previous history of mastectomy for breast cancer now presenting for delayed CPM. Patients will often choose bilateral mastectomy, even in the context of unilateral breast cancer amenable to breast-conserving surgery. Our surgeons spend many hours each week discussing the risks associated with bilateral surgery and the lack of survival benefit according to available evidence (29). Despite this, patients are often determined to pursue this course, and will sometimes change surgeons or hospitals in order to achieve this. It is essential that patients are making a fully informed decision regarding bilateral mastectomy and are aware of the risks, benefits, lack of impact on survival, and alternatives. Our cohort also includes patients with BRCA1 and BRCA2 mutations, and it is of course easier to justify CPM in these circumstances, particularly with recent evidence showing improved overall survival (30).

Suitability for NSM depends on several patient factors, including tumor size, skin involvement, and tumor proximity to the NAC. Although the number of patients requesting and being offered these procedures is increasing, it is certain that a group remains for whom conservative techniques are contraindicated. Patients with IBC require modified radical mastectomy following neoadjuvant chemotherapy, with subsequent post-mastectomy radiation therapy (PMRT) (31). By definition, these patients have skin involvement involving at least one-third of the breast, and skin biopsy may reveal tumor cells within dermal lymphatics. Immediate breast reconstruction in these patients is controversial and should

generally be avoided (32). Although there have been reports of immediate reconstruction in IBC patients, local recurrence rates were high, particularly in the presence of positive mastectomy margins (33,34).

The requirement for PMRT, and the detrimental effect of this treatment to breast reconstruction of any form, is an important issue when considering the possibility of immediate breast reconstruction (35). Recent data from meta-analysis show that patients are likely to benefit from PMRT in the setting of limited axillary lymph node metastases (36), and it is likely that the use of radiation therapy in these circumstances will increase for this reason. In addition to the negative cosmetic effects of radiation therapy to a reconstructed breast, morbidities associated with reconstructive procedures can result in delays to commencement of PMRT, thereby possibly compromising oncologic treatment. Many patients will therefore forego immediate breast reconstruction in preference of a delayed procedure, and, in these circumstances, the benefit of the conservative mastectomy approach is less.

Almost all patients in this study underwent tissue expander/implant based reconstruction, and we have reported an acceptable complication rate, with 6.5% of patients developing skin necrosis requiring debridement. Only 31 patients (7.6%) underwent a single-stage implant-based reconstruction. Our preference is for a two-stage procedure for several reasons, as outlined in an earlier paper from our institution (23). Issues relating to nipple position asymmetry and implant asymmetry can be managed at the time of the replacement of the tissue expander with a permanent implant. Secondly, by limiting the volume of the tissue expander at the initial operation such that the skin envelope is expanded but not under tension, the risk of mastectomy skin flap and nipple-areola ischaemia is reduced. Finally, patients not infrequently request a size change, particularly if they are small breasted initially. Beginning the reconstructive process with a tissue expander allows the surgeon to customize the results to patient preference (23).

Although our experience with NSM is predominantly in patients with early-stage breast cancer (only 9 of 261 patients had stage III disease), there is emerging evidence that this technique is being used in patients with more advanced breast disease and with acceptable results. A recent report by Peled showed that of 753 patients undergoing NSM, 139 (18%) had locally advanced disease at diagnosis. The local recurrence rate was 5% at mean follow-up of 41 months (range, 4-111 months), and there were no recurrences in the preserved NAC (37). Although such reports are encouraging, it is important for patients to be informed that long-term data are lacking and that most existing data are based on patients with favorable disease characteristics. However, it is likely that we will continue to see an increase in the number of patients availing themselves of the NSM technique, particularly in the setting of risk-reduction surgery. The increasing use of conservative mastectomies represents further progress in the evolution of breast cancer surgery, lessening the psychological burden on those diagnosed with the disease and those undergoing risk-reducing surgery.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Albornoz CR, Matros E, Lee CN, et al. Bilateral Mastectomy versus Breast-Conserving Surgery for Early-Stage Breast Cancer: The Role of Breast Reconstruction. Plast Reconstr Surg 2015;135:1518-26.
2. Domchek SM, Friebel TM, Singer CF, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA 2010;304:967-75.
3. Carlson GW, Styblo TM, Lyles RH, et al. The use of skin sparing mastectomy in the treatment of breast cancer: The Emory experience. Surg Oncol 2003;12:265-9.
4. Medina-Franco H, Vasconez LO, Fix RJ, et al. Factors associated with local recurrence after skin-sparing mastectomy and immediate breast reconstruction for invasive breast cancer. Ann Surg 2002;235:814-9.
5. Meretoja TJ, Rasia S, von Smitten KA, et al. Late results of skin-sparing mastectomy followed by immediate breast reconstruction. Br J Surg 2007;94:1220-5.
6. Kroll SS, Schusterman MA, Tadjalli HE, et al. Risk of recurrence after treatment of early breast cancer with skin-sparing mastectomy. Ann Surg Oncol 1997;4:193-7.
7. Newman LA, Kuerer HM, Hunt KK, et al. Presentation, treatment, and outcome of local recurrence afterskin-sparing mastectomy and immediate breast reconstruction.

Ann Surg Oncol 1998;5:620-6.
8. Kroll SS, Khoo A, Singletary SE, et al. Local recurrence risk after skin-sparing and conventional mastectomy: a 6-year follow-up. Plast Reconstr Surg 1999;104:421-5.
9. Simmons RM, Fish SK, Gayle L, et al. Local and distant recurrence rates in skin-sparing mastectomies compared with non-skin-sparing mastectomies. Ann Surg Oncol 1999;6:676-81.
10. Benediktsson KP, Perbeck L. Survival in breast cancer after nipple-sparing subcutaneous mastectomy and immediate reconstruction with implants: a prospective trial with 13 years median follow-up in 216 patients. Eur J Surg Oncol 2008;34:143-8.
11. Gerber B, Krause A, Dieterich M, et al. The oncological safety of skin sparing mastectomy with conservation of the nipple-areola complex and autologous reconstruction: an extended follow-up study. Ann Surg 2009;249:461-8.
12. Petit JY, Veronesi U, Rey P, et al. Nipple-sparing mastectomy: risk of nipple-areolar recurrences in a series of 579 cases. Breast Cancer Res Treat 2009;114:97-101.
13. de Alcantara Filho P, Capko D, Barry JM, et al. Nipple-sparing mastectomy for breast cancer and risk-reducing surgery: the Memorial Sloan-Kettering Cancer Center experience. Ann Surg Oncol 2011;18:3117-22.
14. Sakurai T, Zhang N, Suzuma T, et al. Long-term follow-up of nipple-sparing mastectomy without radiotherapy: a single center study at a Japanese institution. Med Oncol 2013;30:481.
15. Coopey SB, Tang R, Lei L, et al. Increasing eligibility for nipple-sparing mastectomy. Ann Surg Oncol 2013;20:3218-22.
16. Eisenberg RE, Chan JS, Swistel AJ, et al. Pathological evaluation of nipple-sparing mastectomies with emphasis on occult nipple involvement: the Weill-Cornell experience with 325 cases. Breast J 2014;20:15-21.
17. Adam H, Bygdeson M, de Boniface J. The oncological safety of nipple-sparing mastectomy - a Swedish matched cohort study. Eur J Surg Oncol 2014;40:1209-15.
18. Freeman BS. Subcutaneous mastectomy for benign breast lesions with immediate or delayed prosthetic replacement. Plast Reconstr Surg Transplant Bull 1962;30:676-82.
19. Toth BA, Lappert P. Modified skin incisions for mastectomy: the need for plastic surgical input in preoperative planning. Plast Reconstr Surg 1991;87:1048-53.
20. Carlson GW, Bostwick J 3rd, Styblo TM, et al. Skin-sparing mastectomy. Oncologic and reconstructive considerations. Ann Surg 1997;225:570-5; discussion 575-8.
21. Jabor MA, Shayani P, Collins DR Jr, et al. Nipple-areola reconstruction: satisfaction and clinical determinants. Plast Reconstr Surg 2002;110:457-63; discussion 464-5.
22. Garcia-Etienne CA, Cody Iii HS 3rd, Disa JJ, et al. Nipple-sparing mastectomy: initial experience at the Memorial Sloan-Kettering Cancer Center and a comprehensive review of literature. Breast J 2009;15:440-9.
23. Chen CM, Disa JJ, Sacchini V, et al. Nipple-sparing mastectomy and immediate tissue expander/implant breast reconstruction. Plast Reconstr Surg 2009;124:1772-80.
24. Manning AT, Wood C, Eaton A, et al. Nipple-sparing mastectomy in patients with BRCA1/2 mutations and variants of uncertain significance. Br J Surg 2015;102:1354-9.
25. Jagsi R, Jiang J, Momoh AO, et al. Trends and variation in use of breast reconstruction in patients with breast cancer undergoing mastectomy in the United States. J Clin Oncol 2014;32:919-26.
26. Manning AT, Cassella D, Ugras S, et al. Initial experience with an ambulatory extended recovery program for patients undergoing mastectomy. Cancer Res 2015:75:Abstr. P2-13-08.
27. Tuttle TM, Habermann EB, Grund EH, et al. Increasing use of contralateral prophylactic mastectomy for breast cancer patients: a trend toward more aggressive surgical treatment. J Clin Oncol 2007;25:5203-9.
28. Tuttle TM, Jarosek S, Habermann EB, et al. Increasing rates of contralateral prophylactic mastectomy among patients with ductal carcinoma in situ. J Clin Oncol 2009;27:1362-7.
29. Lostumbo L, Carbine NE, Wallace J. Prophylactic mastectomy for the prevention of breast cancer. Cochrane Database Syst Rev 2010;(11):CD002748.
30. Heemskerk-Gerritsen BA, Rookus MA, Aalfs CM, et al. Improved overall survival after contralateral risk-reducing mastectomy in BRCA1/2 mutation carriers with a history of unilateral breast cancer: a prospective analysis. Int J Cancer 2015;136:668-77.
31. Rueth NM, Lin HY, Bedrosian I, et al. Underuse of trimodality treatment affects survival for patients with inflammatory breast cancer: an analysis of treatment and survival trends from the National Cancer Database. J Clin Oncol 2014;32:2018-24.
32. Merajver SD, Iniesta MD, Sabel MS. Inflammatory Breast Cancer. In: Harris JR, Lippman ME, Morrow M, et al, editors. Diseases of the Breast. 4th edition. Philadelphia, Lippincott Williams & Wilkins, 2010:762-73.

33. Slavin SA, Love SM, Goldwyn RM. Recurrent breast cancer following immediate reconstruction with myocutaneous flaps. Plast Reconstr Surg 1994;93:1191-204; discussion 1205-7.
34. Chin PL, Andersen JS, Somlo G, et al. Esthetic reconstruction after mastectomy for inflammatory breast cancer: is it worthwhile? J Am Coll Surg 2000;190:304-9.
35. Barry M, Kell MR. Radiotherapy and breast reconstruction: a meta-analysis. Breast Cancer Res Treat 2011;127:15-22.
36. EBCTCG (Early Breast Cancer Trialists' Collaborative Group), McGale P, Taylor C, et al. Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: meta-analysis of individual patient data for 8135 women in 22 randomised trials. Lancet 2014;383:2127-35.
37. Peled AW, Wang F, Foster RD, et al. Expanding the Indications for Total Skin-Sparing Mastectomy: Is It Safe for Patients with Locally Advanced Disease? Ann Surg Oncol 2015. [Epub ahead of print].

Cite this article as: Manning AT, Sacchini VS. Conservative mastectomies for breast cancer and risk-reducing surgery: the Memorial Sloan Kettering Cancer Center experience. Gland Surg 2016;5(1):55-62. doi: 10.3978/j.issn.2227-684X.2015.10.02

Oncoplastic techniques in breast surgery for special therapeutic problems

Prakasit Chirappapha, Panuwat Lertsithichai, Thongchai Sukarayothin, Monchai Leesombatpaiboon, Chairat Supsamutchai, Youwanush Kongdan

Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Correspondence to: Dr. Prakasit Chirappapha. Breast & Endocrine Surgery Unit, Department of Surgery, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand. Email: onco.prakasit@gmail.com.

Abstract: Resection of large tumors can be challenging, from the view point of breast preservation. Oncoplastic techniques are a valuable component of breast surgery in patients with large breast tumors who desire breast preservation. These techniques have been shown to be oncologically safe, while maintaining acceptable breast cosmesis. For locally advanced or recurrent breast cancers, the goals of surgery include local disease control and palliation of clinical symptoms. Oncoplastic surgery is also effective and oncologically safe in these situations. The need to completely remove all foci of cancers with adequate surgical margins often requires the displacement of adjacent or distant skin and soft tissue to cover the resulting soft tissue defect. Sometimes doing so can be cosmetically pleasing as well. In this article we present three special therapeutic problems in three distinct conditions, all resolved with oncoplastic techniques: the benign breast condition, malignant breast condition, and the palliative setting.

Keywords: Breast conservative treatment (BCT); oncoplastic technique; breast reconstruction; phyllodes tumor; giant fibroadenoma; breast flap; random skin flap; salvage mastectomy; stage IV breast cancer

Submitted Mar 20, 2015. Accepted for publication Apr 29, 2015.
doi: 10.3978/j.issn.2227-684X.2015.05.04
View this article at: http://dx.doi.org/10.3978/j.issn.2227-684X.2015.05.04

Special therapeutic problems in benign breast conditions

Benign proliferative breast lesions are most frequently observed in women 30 to 40 years of age, sometime causing significant breast asymmetry because of the large size. The differential diagnoses for these lesions include pseudoangiomatous stromal hyperplasia (PASH), benign phyllodes tumors, juvenile fibroadenoma, and giant fibroadenoma with increased stromal cellularity. The principles of surgical treatment are different for each diagnostic category. The crucial steps in management consist of preoperative tissue diagnosis and surgical techniques for breast reconstruction after removal of the tumor.

Core needle biopsy (CNB) is preferable to fine needle aspiration for preoperative tissue diagnosis, because fibroadenomas and phyllodes tumors have similar cytologic features. Clinical findings that could increase the suspicion of phyllodes tumors include older patient age, larger tumor size, and history of rapid growth (1). The major pathological feature that distinguishes a phyllodes tumor from a giant fibroadenoma is the cellularity of the stromal component in the former (2). However, the histologic features of benign phyllodes tumors can be difficult to distinguish from those of fibroadenomas on CNB.

It is common for a CNB of either a phyllodes tumor or fibroadenoma to be interpreted as a “fibroepithelial lesion”, hence a phyllodes tumor cannot be ruled out in such a situation. The clinical challenge for the surgeon is to decide whether to remove the entire lesion for management, as is done for a typical fibroadenoma, or to excise the lesion with wide margins, as is therapeutically indicated for phyllodes tumors. If large benign phyllodes tumors are excised with narrow or no margin, reexcision should be performed. Several publications advocated margins of at least 1 cm as adequate (3,4).

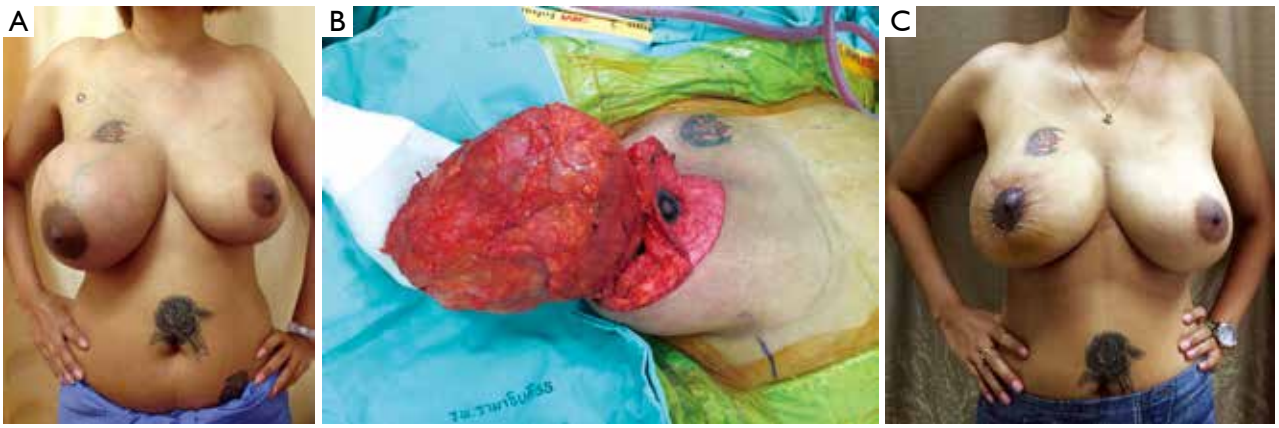


Figure 1 Presentation and management of a giant fibroadenoma. A 40-year-old woman presented with a palpable mass at the right middle inner quadrant, which had grown from 2.4 to 10 cm over 2 years. Imaging and core needle sampling at first presentation were interpreted as "fibroadenoma". The final pathology on excision was a giant fibroadenoma. (A) Preoperative presentation with bulging mass apparent on inspection; (B) intraoperative view showing the large tumor and planned skin excision (outer de-epithelized line), which is drew immediately superficial to the mass; (C) postoperative view after the "round block" mastopexy technique with 325 cc subglandular implant.

Appropriate techniques for breast reconstruction are crucial after removal of a large benign tumor. Lesions with microscopic appearance of a conventional fibroadenoma, however large, should still be classified as fibroadenomas and may be managed adequately by enucleation. Cosmetic sequelae after enucleation of large tumors are common. If an estimated 20% to 50% of breast volume has been resection, a type II breast deformity can occur (5). Reshaping the breast by using a "round block" technique such as the periareolar Benelli mastopexy is required to correct the defect after removing a large volume of the tumor (*Figure 1A-C*) (6). If total mastectomy is considered for a large benign phyllodes tumor, then a free flap or a pedicled flap such as a pedicled transverse rectus abdominis (TRAM) flap can be used to reconstruct the breast (*Figure 2A,B*).

Special therapeutic problems in malignant conditions

In patients with a CNB result interpreted as "malignant phyllodes tumor", the crucial information is whether the tumor to breast size ratio is favourable (e.g., a low ratio) or not. A pseudocapsule of dense, compressed, normal tissue, often containing microscopic malignant cells, surrounds malignant phyllodes tumors. As a result, more tissue typically needs to be removed to achieve adequate margins (7). Simple mastectomy without axillary dissection has been recommended for malignant phyllodes tumors with high tumor to breast size ratio. Margins can be typically wider than 1 cm, but a width greater than 2 cm is associated with the lowest risk of recurrence (8). After removing the tumor with negative margins, a large skin and soft tissue defect can be covered with a pedicled TRAM flap reconstruction (*Figure 3A-D*). In a patient who presented with local recurrence (LR) after performing left breast conservative treatment (BCT) for a malignant phyllodes tumor, and who also had large breasts with severe ptosis, we performed a restaging work-up to rule out distant metastases. The majority of such patients with LR after BCT are treated with mastectomy, although the use of repeat breast conservation surgery for LR has been reported (9). In the case of our patient, after a restaging work up ruled out distant metastasis, we performed a left mastectomy, and a reduction mammoplasty of the opposite breast to reduce breast weight, with a good cosmetic result (*Figure 4A,B*) (10). A reduction mammoplasty in the present setting can help relieve back pain and achieve good body balance, with only one remaining but smaller breast.

Special therapeutic problems in the palliative setting

Breast cancer patients who have concurrent distant metastases (stage IV disease) are primarily treated by palliative systemic therapy. Surgical removal of the breast tumor does not provide survival benefit. On occasion the primary tumor is removed in these patients for palliative reasons, such as for

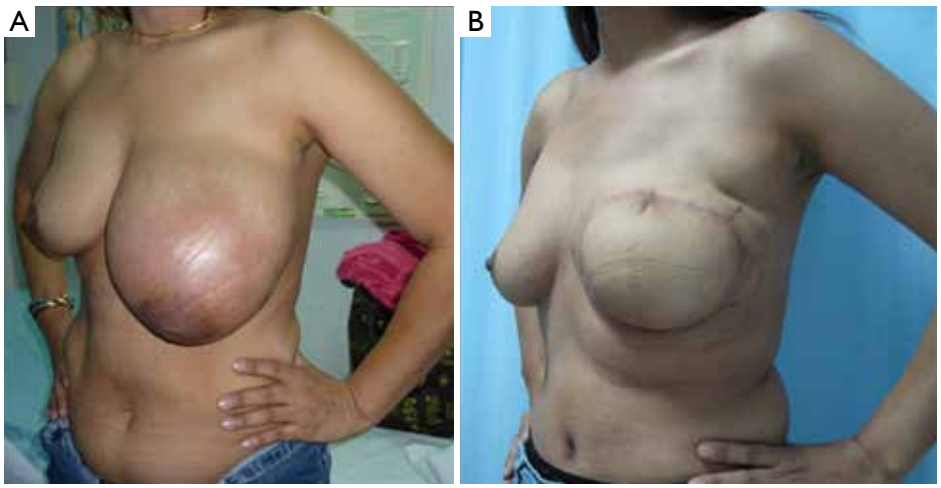


Figure 2 Presentation and resection of a large benign phyllodes tumor. A 39-year-old woman presented with a large mass in the left breast. Core needle biopsy (CNB) was reported as "benign phyllodes tumor". (A) Preoperative presentation with bulging mass apparent on inspection; (B) postoperative view after performing a pedicled transverse rectus abdominis (TRAM) flap.

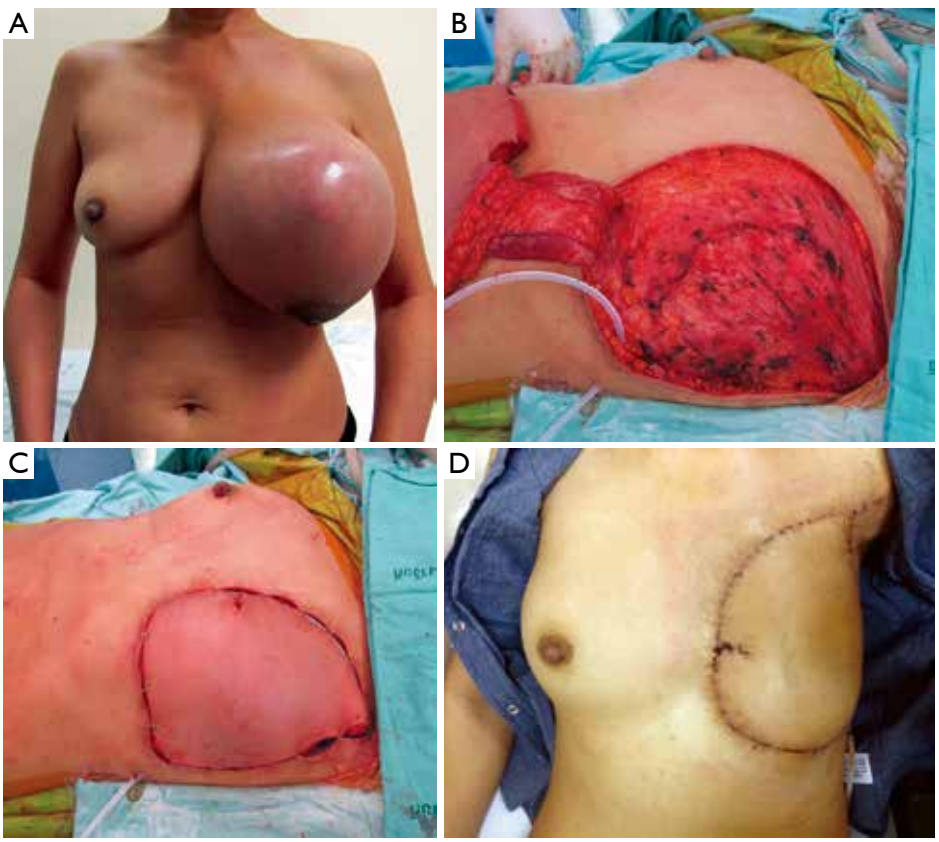


Figure 3 Presentation and management of a malignant phyllodes tumor. A 44-year-old woman presented with a large mass in the left breast. CNB was reported as "malignant phyllodes tumor". (A) Preoperative presentation with a bulging mass apparent on inspection; (B) intraoperative view after simple mastectomy with 3 cm lateral margins of surrounding soft tissue; (C) the defect was covered with a pedicle TRAM flap; (D) postoperative view 2 weeks after surgery. CNB, core needle biopsy; TRAM, transverse rectus abdominis.

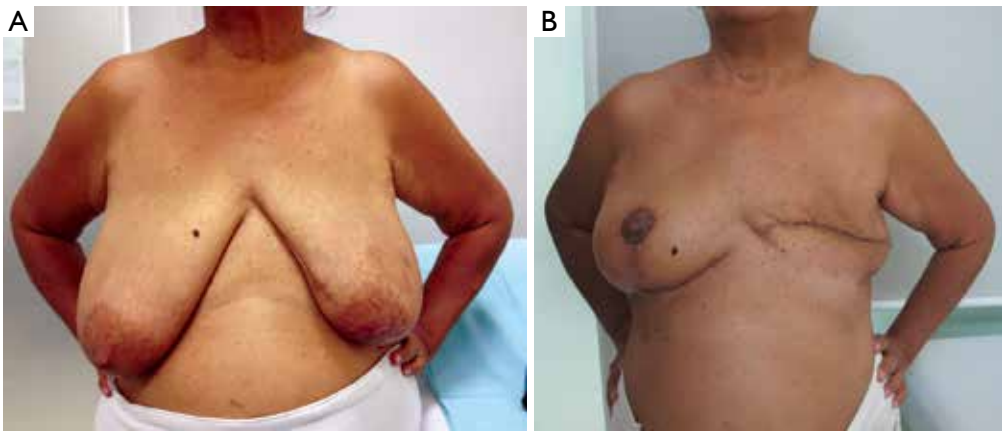


Figure 4 Presentation of local recurrence (LR) after left BCT for a malignant phyllodes tumor. Large breasts with severe ptosis can be seen. An assessment for metastatic disease showed no lesion on computed tomography of the chest and abdomen, bone scan and combined positron emission tomography-computed tomography. (A) Preoperative view in preparation for an inverted T inferior-pedicle breast reduction; (B) anterior view of the results at 6 weeks after performing left mastectomy and reduction mammoplasty of the opposite breast. BCT, breast conservative treatment.

disabling pain, infection, ulceration or bleeding. Nonetheless, these patients should be initiated on systemic therapy as the first-line treatment. Patients who respond to systemic therapy, or have persistent but non-progressive metastatic diseases, with good performance status, may be considered for palliative or salvage surgery for quality of life (QoL) reasons. The QoL benefits have been highlighted in a recent study (11). A salvage resection is defined as the resection of all visible lesions, extending to the surrounding skin with a safety margin of at least 2 cm (12). Closure or reconstruction of the soft tissue defect of the chest wall can be performed using skin grafts or different types of vascularized pedicled musculo-cutaneous flaps.

The choice of closure or reconstruction methods depend on the location and size of the defect, availability of the local and pedicled flaps, previous surgery or radiotherapy at the donor and recipient site, and the general condition of the patient. Direct simple closure is possible for small lesions. Skin grafts can be used for superficial chest wall defects involving only the soft tissue. Previous or post-operative radiation therapy may compromise the healing of skin grafts.

Local flaps

Breast flap

The breast parenchyma can be used as a flap to cover defects located predominantly in the midline (*Figure 5A-D*). This flap is suitable for elderly patients with associated comorbidities, because of the short operative time required. The blood supply of breast flap is good, but the cosmetic outcome is rather poor (13).

Random skin flap from the lateral chest wall

This flap can cover small and moderate sized defect on the anterior and lateral aspects of the chest wall, and can be used in combination with the other flaps (*Figure 6A-E*). It is also suitable for the elderly, or for patients with poor functional status, due to the short operative time. The weakness of this method is a lack of sufficient volume to cover large defect.

Pedicled flaps

The regional pedicled musculocutaneous flaps available for reconstruction include the latissimus dorsi (LD) flap or TRAM flap. We prefer the use of the LD flap when available, and it is usually large enough to cover most defects (*Figure 7A,B*). The LD flap can be rotated widely, is easy to harvest, and can be tailored to cover the anterior, lateral, and posterior regions of the chest wall. In addition, this technique can be performed within a relatively short period of time, and patients experience fewer postoperative complications afterwards.

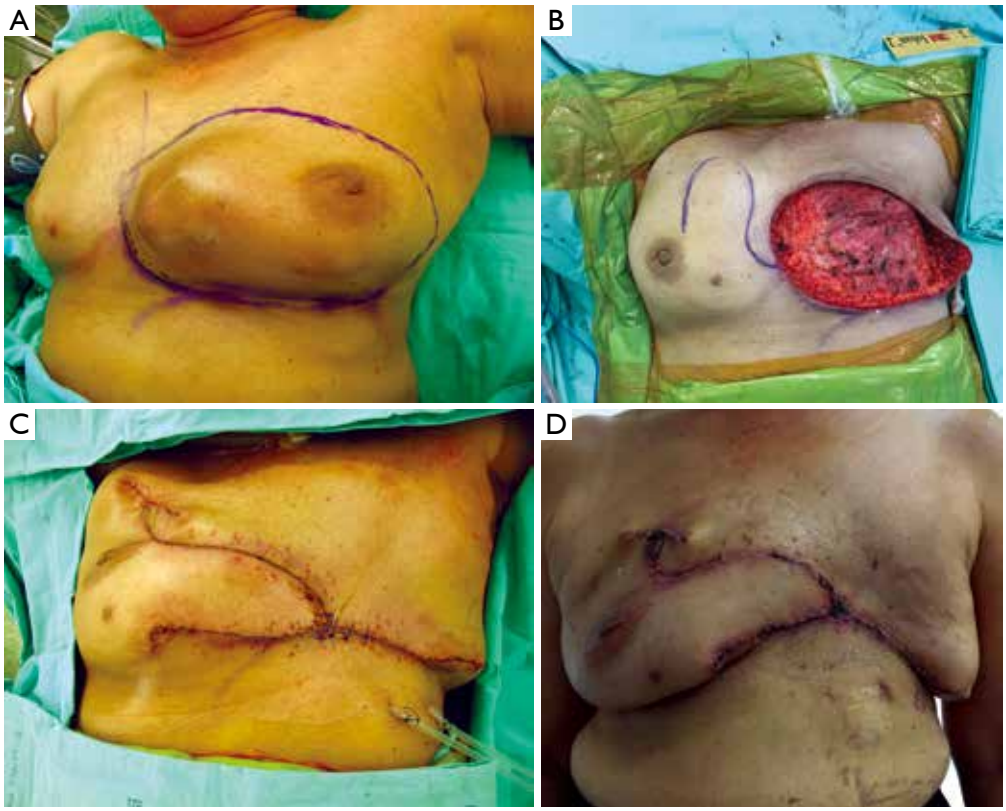


Figure 5 Presentation and management of invasive ductal carcinoma at left breast with stable bone metastasis. A 65-year-old woman presented with a large mass at the left breast. CNB was reported as "invasive ductal carcinoma". An assessment for metastatic disease showed no lesion on computed tomography of the chest and abdomen, but multiple bone metastases were found on radionuclide scintigraphy. She received systemic endocrine therapy and her bone metastases stabilized. (A) Preoperative presentation with large mass apparent on left breast; (B) intraoperative view of medial chest wall defect after salvage mastectomy; (C) the defect was covered with a right breast flap; (D) anterior view of the results at 6 weeks after performing right breast flap. CNB, core needle biopsy.

Complications of oncoplastic surgery after radiation

Previous studies suggested that the surgeon should be more cautious in performing oncoplastic surgery in patients with irradiated breasts. The study by Losken *et al*. suggested that radiation therapy might decrease compliance of the covering soft tissue (14). Our results demonstrate that oncoplastic surgery is a simple and reliable technique to correct nipple areola complex (NAC) malposition after previous breast procedures, even in those patients who previously underwent locoregional radiotherapy that could negatively affect wound healing and graft intake (15).

In previously irradiated patient, our experience showed a mastectomy skin flap necrosis occurred after performing nipple sparing mastectomy (NSM) with LD flap plus implant reconstruction (*Figure 8A-D*). This finding may due to the individual surgeon's technique. The surgeon must carefully make the dissection of the gland more precisely and the preservation of the subdermal vessel network to the cutaneous flaps. To reduce severity of necrotic complications, the reconstruction should be performed with autologous flap (LD flap, TRAM flap) with the use of an additional implant. When mastectomy skin flap or NAC necrosis occurred, we sometimes performed only skin flap debridement with or without NAC and we did not remove implant because the flap could protect and cover it.

Conclusions

Breast reconstruction techniques are of crucial importance after removal of large benign proliferative lesions with an adequate margin. For large phyllodes tumors, oncoplastic surgery can

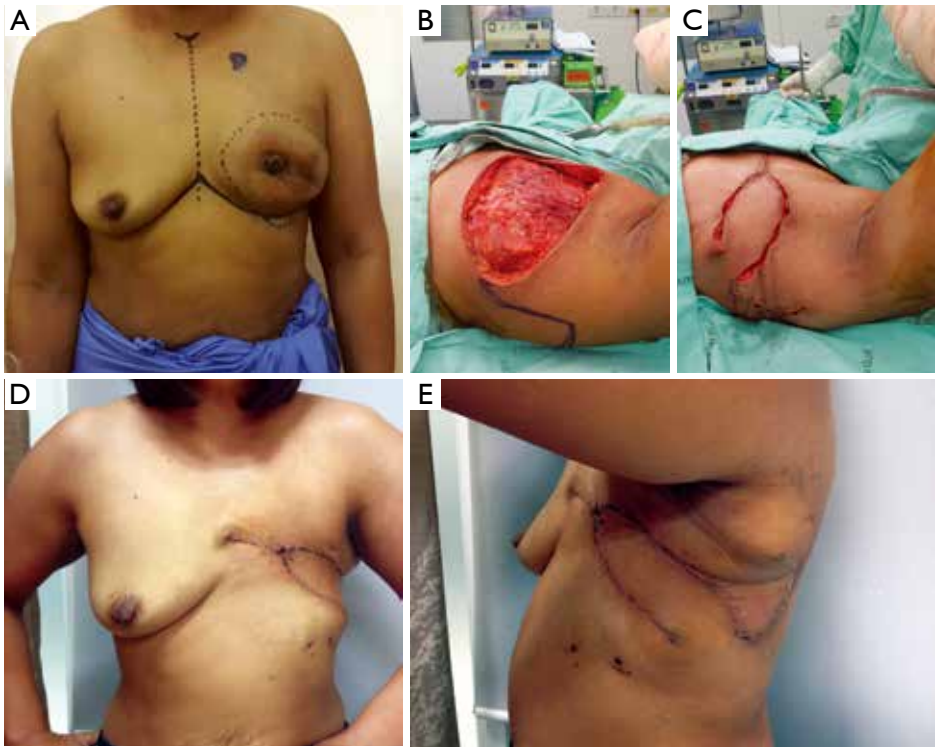


Figure 6 Presentation and management of invasive ductal carcinoma at left breast with stable bone metastasis. A 60-year-old woman presented with a large mass at the left breast. CNB was reported as "invasive ductal carcinoma". An assessment for metastatic disease showed no lesion on computed tomography of the chest and abdomen, but bone metastases were found on radionuclide scintigraphy. She received systemic therapy for stage IV disease until her bone metastases stabilized. The large tumor was partially responsiveness to systemic treatment. The patient requested tumor removal because of pain. (A) Preoperative presentation with large mass apparent at left breast; (B) intraoperative view of the chest wall defect after salvage mastectomy; (C) the defect was covered with a random skin flap from lateral chest wall; (D) anterior view of the results at 6 weeks after surgery; (E) lateral view of the results at 6 weeks after surgery. CNB, core needle biopsy.

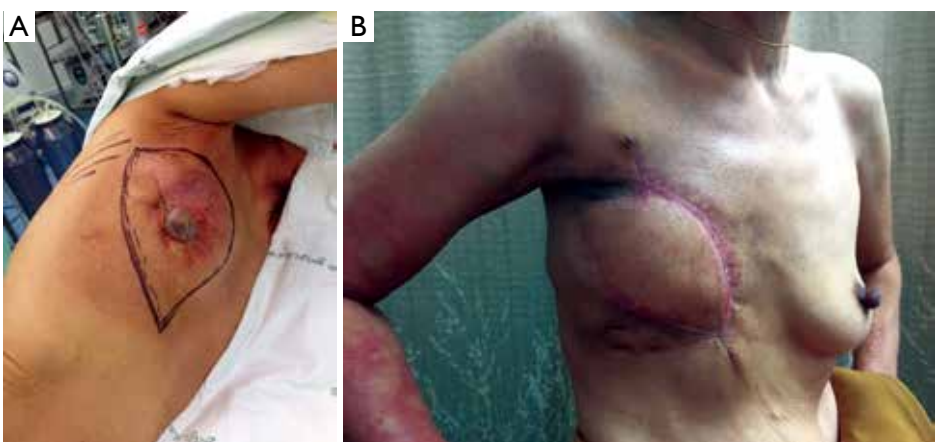


Figure 7 Presentation and management of invasive ductal carcinoma at right breast with stable bone metastasis. A 64-year-old woman presented with a tumor at the right breast. Skin involvement can be seen. CNB was reported as "invasive ductal carcinoma". An assessment for metastatic disease showed no lesion on computed tomography of the chest and abdomen, but bone metastases was found on radionuclide scintigraphy. She received systemic endocrine therapy until bone metastases were stabilized. (A) Preoperative presentation with skin involvement; (B) anterior view of the results at 6 weeks after performing right LD flap closure of defect. CNB, core needle biopsy; LD, latissimus dorsi.

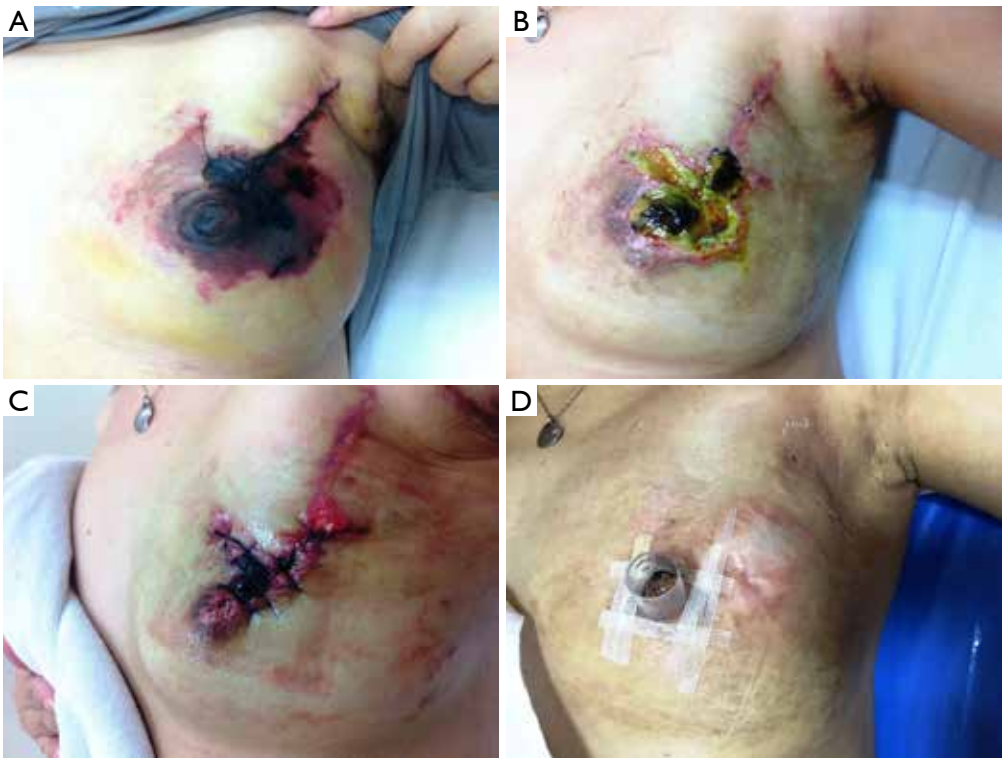


Figure 8 Presentation and management of a mastectomy skin flap necrosis occurred after performing NSM with LD flap plus implant reconstruction. A 46-year-old woman presented with recurrent tumor at the left breast. She had previously undergone a left lumpectomy with whole breast radiation. We performed NSM and immediate breast reconstruction with LD flap plus implant. (A) NAC necrosis with mastectomy skin flap necrosis around NAC. The necrosis occupied mostly in the superior outer quadrant and incision is supero-lateral radial incision; (B) we performed only skin flap debridement with NAC; (C) resuture mastectomy skin flap was performed with nylon 4-0; (D) postoperative view of the results at 6 weeks after debridement and nipple reconstruction. NSM, nipple sparing mastectomy; LD, latissimus dorsi; NAC, nipple areola complex.

prevent and correct breast deformities after adequate removal with wide margins, resulting in a good cosmetic outcome. Larger soft tissue and skin defects can be closed using oncoplastic methods. Salvage mastectomy and reconstruction for stage IV breast cancer is a feasible procedure, providing adequate local disease control and excellent palliation of very disabling symptoms in selected patients.

Acknowledgements

The authors wish to acknowledge their clinical fellows team as follows: Dr. Natthapong Saengow, Dr. Rujira Panawattanakul, Dr. Saowanee Kitudomrat, Dr. Paweena Luadthai, Dr. Pongsakorn Srichan and Dr. Piyawan Kensakoo to encourage these operations.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Gabriele R, Borghese M, Corigliano N, et al. Phyllodes tumor of the breast. Personal contribution of 21 cases. G Chir 2000;21:453-6.
2. Ashikari R, Farrow JH, O'Hara J. Fibroadenomas in the breast of juveniles. Surg Gynecol Obstet 1971;132:259-62.
3. de Roos WK, Kaye P, Dent DM. Factors leading to local recurrence or death after surgical resection of phyllodes tumours of the breast. Br J Surg 1999;86:396-9.

4. Reinfuss M, Mituś J, Duda K, et al. The treatment and prognosis of patients with phyllodes tumor of the breast: an analysis of 170 cases. Cancer 1996;77:910-6.
5. Clough KB, Cuminet J, Fitoussi A, et al. Cosmetic sequelae after conservative treatment for breast cancer: classification and results of surgical correction. Ann Plast Surg 1998;41:471-81.
6. Benelli L. A new periareolar mammaplasty: the "round block" technique. Aesthetic Plast Surg 1990;14:93-100.
7. August DA, Kearney T. Cystosarcoma phyllodes: mastectomy, lumpectomy, or lumpectomy plus irradiation. Surg Oncol 2000;9:49-52.
8. Belkacémi Y, Bousquet G, Marsiglia H, et al. Phyllodes tumor of the breast. Int J Radiat Oncol Biol Phys 2008;70:492-500.
9. Galper S, Blood E, Gelman R, et al. Prognosis after local recurrence after conservative surgery and radiation for early-stage breast cancer. Int J Radiat Oncol Biol Phys 2005;61:348-57.
10. Dennis CH. Reduction Mammaplasty and Mastopexy: General Considerations. In: Spear SL, editor. Surgery of the breast: principles and art. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2005:972-5.
11. Levy Faber D, Fadel E, Kolb F, et al. Outcome of full-thickness chest wall resection for isolated breast cancer recurrence. Eur J Cardiothorac Surg 2013;44:637-42.
12. Veronesi G, Scanagatta P, Goldhirsch A, et al. Results of chest wall resection for recurrent or locally advanced breast malignancies. Breast 2007;16:297-302.
13. Tukiainen E. Chest wall reconstruction after oncological resections. Scand J Surg 2013;102:9-13.
14. Losken A, Pinell XA, Sikoro K, et al. Autologous fat grafting in secondary breast reconstruction. Ann Plast Surg 2011;66:518-22.
15. Rietjens M, De Lorenzi F, Andrea M, et al. Free nipple graft technique to correct nipple and areola malposition after breast procedures. Plast Reconstr Surg Glob Open 2013;1:e69.

Cite this article as: Chirappapha P, Lertsithichai P, Sukarayothin T, Leesombatpaiboon M, Supsamutchai C, Kongdan Y. Oncoplastic techniques in breast surgery for special therapeutic problems. Gland Surg 2016;5(1):75-82. doi: 10.3978/j.issn.2227-684X.2015.05.04

Oncological safety of prophylactic breast surgery: skin-sparing and nipple-sparing versus total mastectomy

Victorien M.T. van Verschuer[1], Marike C. Maijers[2], Carolien H.M. van Deurzen[3], Linetta B. Koppert[1]

[1]Erasmus MC Cancer Institute, Department of Surgical Oncology, Rotterdam, the Netherlands; [2]VUmc, Department of Plastic and Reconstructive Surgery, Amsterdam, the Netherlands; [3]Erasmus MC Cancer Institute, Department of Pathology, Rotterdam, the Netherlands

Correspondence to: Victorien M.T. van Verschuer, MD. Erasmus MC Cancer Institute, Department of Surgical Oncology, Room DHD-407, P.O. Box 5201, 3008 AE Rotterdam, the Netherlands. Email: v.vanverschuer@erasmusmc.nl.

Abstract: Women with a *BRCA1/2* gene mutation and others with a high breast cancer risk may opt for bilateral prophylactic mastectomy. To allow for immediate breast reconstruction the skin envelope is left *in situ* with or without the nipple-areola complex (NAC). Although possibly leading to a more natural aesthetic outcome than the conventional total mastectomy, so-called skin-sparing mastectomies (SSM) and nipple-sparing mastectomies (NSM) may leave some breast glandular tissue *in situ*. The oncological risk associated with remaining breast glandular tissue is unclear. We present a case of primary breast cancer after prophylactic mastectomy followed by a review of the literature on remaining breast glandular tissue after various mastectomy techniques and oncological safety of prophylactic mastectomies.

Keywords: Risk-reduction; skin-sparing mastectomy (SSM); nipple-sparing mastectomy (NSM); total mastectomy; primary breast cancer; breast glandular tissue; terminal duct lobular units

Submitted Dec 17, 2014. Accepted for publication Jan 29, 2015.
doi: 10.3978/j.issn.2227-684X.2015.02.01
View this article at: http://dx.doi.org/10.3978/j.issn.2227-684X.2015.02.01

Introduction

BRCA1/2 mutation carriers have a cumulative lifetime breast cancer risk of 55-85% by the age of 70 (1-5). As an alternative to surveillance, *BRCA1* and *BRCA2* mutation carriers and other women with a high breast cancer risk may choose to undergo bilateral prophylactic mastectomy, reducing breast cancer risks by 90-100% after 3-13 years of follow-up (6-10). The prophylactic character of the bilateral mastectomy emphasizes the importance of a natural aesthetic outcome (11), which can be achieved by various immediate autologous and implant breast reconstruction techniques. Instead of the conventional total mastectomy, to allow for an immediate breast reconstruction and to achieve a natural aesthetic outcome so-called conservative mastectomies are increasingly performed for risk reduction. In conservative mastectomies, all breast glandular tissue is removed while leaving the skin envelope and, if spared, the nipple-areola complex (NAC) *in situ* [skin-sparing mastectomy (SSM) and nipple-sparing mastectomy (NSM), respectively].

Safety of conservative mastectomies in women at high breast cancer risk is subject to an ongoing debate. The presumed oncological risk of the conservative technique lies in potential remaining breast glandular tissue with the skin flap and, if spared, with the NAC. Smaller incisions that are tailored to individual reconstruction wishes, however, may result in a technically difficult surgical approach. Therefore, the oncological safety of the conservative mastectomy remains a challenge for the oncological surgeon. We present a case of primary breast cancer developed after prophylactic conservative mastectomy. Further, we provide a review of the literature on the oncological safety of prophylactic conservative mastectomies.

Case: a 43-year-old woman with primary breast cancer in the prophylactic mastectomy scar

In 2011, a 43-year-old woman presented a lesion clinically suspicious of breast cancer. In 1982, at the age of 15, she had been successfully treated for stage IIa Hodgkin's disease

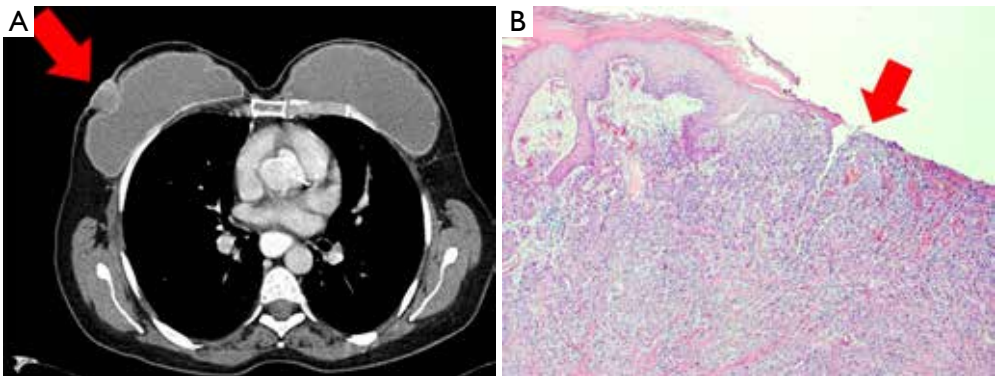


Figure 1 A 43-year-old woman presented with a primary, ulcerous breast cancer in the right prophylactic mastectomy scar. Eight years before presentation she had undergone prophylactic mastectomy and immediate breast implant reconstruction because of a history of Mantle field radiation at the age of 15. Histology of the mastectomy specimens showed no (*in situ*) malignancy. (A) Computer-assisted Tomography (CT) scan of the thorax shows the tumor of 2.1×2.7 cm^2 that invades the skin and causes dimpling of the subpectoral implant; (B) microscopic examination showed a grade 3 invasive ductal carcinoma with skin involvement, indicated by the arrowhead. Haematoxylin and eosin stained (H&E); 4× objective.

in her neck and mediastinum with 40 Gy mantle field radiation. After 10 years there were no signs of recurrence and she was discharged from follow-up.

In 1998, a mammography—performed because of a wish for breast reduction—revealed suspect microcalcifications in the left breast. The suspect lesion was excised by upper outer quadrantectomy. Pathological examination of the lumpectomy specimen showed grade 2 ductal carcinoma *in situ*. No adjuvant radiotherapy was administered due to the history of mantle field radiation. Initially, physicians and patient agreed to frequent radiological screening instead of a completing mastectomy. However, after several additional diagnostic procedures due to suspect lesions of the left breast, in 2001, the patient chose to undergo a SSM and immediate implant reconstruction. In 2003, this was followed by a prophylactic SSM of the right breast and bilateral implant reconstruction. In both cases, histologic investigation showed no (*in situ*) malignancy.

In 2011, she returned with an ulcerous lesion in the right mastectomy scar. On CT-scan a superficial tumor of 21×27 mm^2 was seen (*Figure 1A*). Ultrasonography of the axilla did not show pathological lymph nodes. A wide local excision with axillary lymph node dissection was performed and the implants were removed. Histological examination of the excised specimen showed an invasive ductal carcinoma with a diameter of 2.4 cm, Bloom Richardson grade 3, estrogen receptor (ER) positive, progesterone receptor (PR) and human epithelial growth factor-2 receptor (HER2 receptor) negative (*Figure 1B*). Adjacent to the tumor, normal glandular breast tissue was found. One out of eight dissected axillary nodes showed a metastasis. According to our national protocol, she received adjuvant chemotherapy, hormonal therapy and re-irradiation with hyperthermia of the chest wall. At the time of writing the patient is alive without breast cancer recurrence.

Surgical techniques of conservative mastectomies: SSM and NSM

Examples of conservative mastectomies include SSM and NSM. In SSM, a periareolar incision is used with caudal or lateral extension if necessary ("racquet" incision). The skin envelope is created by subcutaneously excising the breast glandular tissue while preserving a thin subcutaneous layer to support skin vascularization. Nipple-papilla and surrounding pigmented areola (NAC) are removed. In NSM, the skin envelope is created through a semicircular periareolar or an inframammary incision. The NAC is dissected as thin as possible by macroscopically removing all breast glandular tissue while preserving vascularization. The nipple-papilla is "cored" by inverting it and excising residual breast glandular tissue. The NAC is then left *in situ* adherent to the skin envelope. A breast reconstruction is performed during the same procedure. The oncological safety of SSM in the prophylactic setting is generally acknowledged, whereas safety of NSM is still subject to debate.

In the last two decades of the past century it was

common to perform a so-called subcutaneous mastectomy. Although subcutaneous mastectomy encompassed a skin- and nipple-sparing technique as well, it is likely that this was not comparable to current NSM and SSM techniques. A description of the 'state of the art' subcutaneous mastectomy in 1983 mentions that a plaque of one centimeter of breast glandular tissue should be left *in situ* with the areola (12). In contrast, current NSM and SSM techniques aim for skin flaps <5 mm and NACs of 2-3 mm thickness (13).

Breast glandular tissue or terminal duct lobular units (TDLUs): residuals after mastectomy

The hazard of remaining breast glandular tissue after mastectomy for development or recurrence of breast cancer has been a recurring subject to debate since more than half of a century. Anatomically the NAC is a continuation of the mammary gland and therefore should be removed when pursuing a complete mastectomy. Therefore, especially sparing of nipple and areola in NSM has been a controversial topic. However, the growing ability of more specifically identifying women at high breast cancer risk and the consequently increasing interest in prophylactic mastectomies has revived the discussion. Breast cancer is thought to originate in TDLUs, defined as a terminal duct combined with an associated lobule (14-16). Consequently, theoretically any remaining TDLUs may represent a lifelong potential breast cancer hazard. To estimate the remaining risk after prophylactic mastectomy, some authors have studied whether TDLUs are left *in situ*. Several others have simply examined the presence of remaining ductal or lobular structures or more non-specifically the presence of glandular tissue.

Residual breast glandular tissue after total mastectomies

The first study to investigate the amount of glandular tissue left *in situ* after a conventional total mastectomy was already in 1940 by Hicken *et al*. (17). The authors had been triggered by two cases of women who developed breast cancer and mastitis of residual axillary breast tissue 15 and 10 years, respectively, after an ipsilateral mastectomy for a benign indication. Mammographies of 385 breasts using intraductal contrast showed that mammary ducts frequently extend beyond regular mastectomy resection planes. In 95%, mammary ducts extended into the axillary fossa, in 15% downward into the epigastric region, in 2% beyond the lateral limits of the latissimus dorsi muscle and in two cases even past the midsternal line to the contralateral side (17). A histological analysis of 17 total mastectomies was performed in the same study by preoperatively injecting methylene blue dye into the ducts of the nipple-papilla. Any resection plane that colored blue during surgery meant that ducts had been cut and the resection site was defined as 'irradical' (17). Results showed that breast glandular tissue had been excised irradically underneath the skin flap in 94% of cases, in 12% the axillary tail had been removed irradically, in 23% the ducts had been cut in the sternal region and in 11% in the epigastric region (17). The authors therefore concluded that, even when it is intended to perform a total mastectomy, it is seldom accomplished (17).

In 1991, a small study was performed in ten total mastectomies in five women (18). Frozen sections of skin flaps, pectoral muscle and axillary tail were examined. Similar to the results of Hicken, residual breast glandular tissue was found in caudal skin flaps, the axillary tail and even in the pectoral fascia (18). Another small study separately resected specimens specifically of the inframammary fold (IMF) and encountered small amounts of residual breast tissue in 13/24 IMF specimens (with breast glandular tissue volume/IMF specimen volume rates of 0.04%) (19).

In 2013, Griepsma *et al*. studied the superficial dissection planes of 206–mostly total–mastectomy specimens (20). Per mastectomy 36 biopsies were obtained from standardized locations of the subcutaneously dissected part of the total mastectomy specimens. In 76% of mastectomies, one or more biopsies contained breast glandular tissue at the resection plane. Areas of predilection were the lower outer quadrant (15% positive biopsies) and halfway the subcutaneous dissection plane between the peripheral pectoral muscle margin and central skin margin (12% positive biopsies) (20).

Residual breast glandular tissue after conservative mastectomy: SSM and NSM

Three decades after the first report on total mastectomies by Hicken *et al*., Goldman and Goldwyn picked up on the issue of conservative prophylactic mastectomy by performing 12 subcutaneous (skin- and nipple-sparing) mastectomies in six cadavers through an inframammary incision (21). Biopsies of post-mastectomy skin flaps, resection planes and any fibrous or adipose tissue remaining elsewhere showed residual breast glandular tissue after 83% of mastectomies (21). In all cases even, residual breast glandular tissue was

found behind the spared NAC. However, the authors do not describe which biopsy sites were positive for breast glandular tissue, nor the surgical technique used for dissection of the NAC (21).

Aiming to investigate the potential value of NSM in the treatment of lobular carcinoma in situ (LCIS), Rosen and Tench (22) vertically sectioned 101 nipples in conventional mastectomies performed for breast cancer. In 17% of the nipples lobules were found and in 13% (*in situ*) carcinoma was encountered. The authors propose that "coring" of the nipple-papilla in NSM, which had been described before (23), is necessary to remove as much glandular tissue as possible. The NAC was further examined in 1993 (24). By inverting the projected center of the NAC—the nipple-papilla—and grossly removing all glandular tissue inside the papilla, the nipple was cored. Despite nipple-coring the authors did encounter mammary ducts in the areolar dermis (24).

In 1991, Barton *et al*. compared 27 conservative mastectomies with 28 modified radical mastectomies (25). Post-mastectomy biopsies were taken at the inframammary fold, parasternal region, infraclavicular chest wall, latissimus dorsi muscle border, anterior lower axilla and skin flaps. The NAC was not examined. No differences were found between the number of biopsies containing residual breast glandular tissue after conservative mastectomy (22%) and after total mastectomy (21%) (25). After conservative mastectomy, most positive biopsies (50%) originated in the skin flap. In contrary, after total mastectomy, most positive biopsies (38%) originated at the latissimus dorsi border (25).

The skin flap after conservative mastectomy was further examined in 1998 (26). The authors removed 114 small (0.5×2.0 cm^2) strips of skin from the remaining skin flap in 32 patients for complete histological examination. In none of the strips ductal breast tissue was encountered (26), however, regarding the size of the strips, this negative finding may be due to a sampling error. Somewhat larger skin flaps have been examined in a more recent study (27). In 66 SSMs, skin specimens that had been removed additionally to the SSM specimen to facilitate reconstruction were examined for residual glandular tissue. Skin specimens had a mean volume of 93.9 cm^3 and in specimens of only four patients (6%) residual breast tissue was found (27). However, since only a minimum of three sites per skin specimen was analyzed, again in this study a sampling error cannot be ruled out. A study of 168 SSMs for therapeutic indication analyzed the superficial margin to the dermis just above the tumor that would have been left *in situ* otherwise. In contrast with the two studies described above, in 89 (53%) of the cases benign breast ducts were present in the superficial margin specimen (28).

Residual TDLUs after conservative mastectomy: SSM and NSM

Several studies have more specifically studied whether TDLUs remain after SSM or NSM (22,29-31). The only study on SSM was by Torresan *et al*. in 2005 (32). In 42 total mastectomies, they resected the skin flap that would have been left *in situ* if it were a SSM and submitted 80 slides per skin specimen for examination. In contrary to the two studies mentioned earlier, they found TDLUs in 60% of the skin flaps (32). The risk of finding TDLUs strongly increased for skin flaps thicker than 5 mm (32).

The other five studies focus on NSM. Stolier *et al*. examined the nipple-papilla for presence of TDLUs in 2008 (29). During mastectomies, 32 nipple-papillas were transected at the junction of papilla and areola. Nipple-papilla's were sectioned, entirely embedded and examined microscopically for presence of TDLUs. Only in three out of 32 nipple-papilla TDLUs were found. Therefore, it was concluded that TDLUs are scarce in the nipple-papilla (29). Reynolds *et al*. collected 62 mastectomy specimens from 33 *BRCA1/2* mutation carriers and excised the NAC for histologic evaluation (30). In 24% of the NACs, TDLUs were found; only 8% was located in the papilla (30). Similarly, Kryvenko *et al*. studied 105 NACs from mastectomy specimens (31). Sixty-five NACs were entirely embedded for examination of presence of TDLUs; of 40 NACs only one vertical section was examined. TDLUs were found in 26% of NACs but most frequently were located in the papilla (31)—in contrast to the results of Reynolds and Stolier (29,30). It has been suggested that an areola-sparing mastectomy rather than a NAC-sparing mastectomy should be performed for risk reduction. Removing the nipple-papilla might further reduce any remaining breast cancer risk. However, this is not supported by the abovementioned studies since two of the three show a higher incidence of TDLUs in the areola versus the nipple-papilla.

Recently, our own group compared presence and numbers of TDLUs between skin flap and NAC (33). In 105 total mastectomies, the NAC and an adjacent skin-island were dissected as if an NSM was performed, and the papilla was cored. TDLUs were found in 61% of the NACs *vs*. 24% of the skin islands (33). Also after adjustment for volume of the excised specimens, density of TDLUs was significantly higher in the NACs as compared with the skin. Further, risk factors for presence of TDLUs were

younger age and parity (*vs.* nulliparity) (33). We concluded that NACs, as well as skin flaps might harbor a risk for developing breast cancer, albeit very small.

Oncological safety of prophylactic mastectomy: clinical studies

In addition to the histopathological studies, we assessed whether there are any oncological consequences of the residual glandular tissue. We performed a systematic PubMed search using the term "prophylactic mastectomy [Title/Abstract] OR skin-sparing mastectomy [Title/Abstract] OR nipple-sparing mastectomy [Title/Abstract] OR subcutaneous mastectomy [Title/Abstract] OR conservative mastectomy [Title/Abstract] OR risk-reducing mastectomy [Title/Abstract] AND breast cancer [Title/Abstract]", yielding 680 titles. Titles and abstracts were checked for relevance. Reviews and case reports were excluded, as were articles that were not in English. Also excluded were: studies that focused: (I) on merely therapeutic mastectomy and/or comprised <20 prophylactic mastectomies and/or did not report clinical follow-up outcome of prophylactic mastectomies; (II) on survival benefits of contralateral prophylactic mastectomy or oophorectomy; (III) on uptake, counseling and decision-making of prophylactic surgery.

Twenty-four studies from 1976-2014 met our criteria and are summarized in *Table S1*. All are observational studies describing prospective or retrospective cohorts or a case-control series. In 24 studies, 7,173 mastectomies are described of which 1,392 were for therapeutic indications and which were not considered in further analysis. Most prophylactic mastectomies were performed in *BRCA1/2* gene mutation carriers and other women at high breast cancer risk. Average follow-up periods range from 10.4-168 months. Most recent studies focus on NSM rather than SSM; while in older studies conservative mastectomies are defined as 'subcutaneous mastectomy', suggesting that the NAC is–partly–spared. However, as described above, it is likely that in subcutaneous mastectomy the NAC and skin are not dissected as thin as modern NSM or SSM techniques dictate.

As reported by the 24 studies in *Table S1*, grossly, 21 primary breast cancers occurred after 6,044 prophylactic mastectomies. Of these, three occurred after a total mastectomy (0.6% of all total mastectomies), 17 occurred after a conservative mastectomy (0.3% of all subcutaneous mastectomies, NSM or SSM) and for one breast cancer the prophylactic mastectomy technique was not specified. Besides, four patients presented with distant metastases with unknown primary site. Most prophylactic mastectomies included in these studies, as well as the ones in which a primary breast cancer developed, were subcutaneous mastectomies, NSM or SSM. Nonetheless, the majority of primary breast cancers did not originate near the NAC or skin flap. Of the 21 breast cancers that developed after prophylactic mastectomy, five were encountered at the chest wall, four in the axilla, (two in the axillary tail, one in an axillary lymph node, one in an unknown location), one in the outer quadrant, one in the nipple and one "above the areola" (not further specified). In nine cases the location was unclear or not reported.

The 21 loco-regional primary breast cancers correspond with an incidence of 0.7% per woman who undergoes bilateral prophylactic mastectomy (0.35% per mastectomy). Most breast cancers that developed after conservative mastectomy were found at the chest wall or in the axilla. Although the chest wall and the axilla may be at risk in total mastectomy as well, two things should be considered: First, the origin of the breast cancer may have been the skin flap, even though it was described as 'chest wall'. Most breast implants in immediate breast reconstruction are placed underneath the pectoral muscle. Consequently, skin-flap and chest wall are in direct contact. Therefore, although we have no information on the reconstruction techniques used in these studies, it is possible that the breast cancers developing at the chest wall actually did originate in the skin flap. Second, as mentioned before, the surgical technique of SSM and NSM using small peri-areolar or inframammary incisions can be challenging. A suboptimal exposure may impede thorough removal of remaining breast glandular tissue in all quadrants and in the axillary tail.

In four cases, breast cancer presented as metastatic disease and the primary tumor site was never found. Pathological findings specific for breast cancers, the high a priori breast cancer risk of the patient and elimination of other potential first sites because of negative radiological examinations may all have led to the conclusion that the metastatic disease most probably originated from breast cancer. The possibility that the primary tumor already may have been present in the prophylactic mastectomy specimen emphasizes the importance of standardized pathological examination of the excised specimen, and—even more—thorough radiological screening by MRI before prophylactic mastectomy.

In conclusion, the incidence of primary breast cancers

after prophylactic mastectomy is very low after total as well as after conservative mastectomies. However, theoretically, according to these data, approximately one out of 140 women undergoing bilateral prophylactic mastectomy for breast cancer prevention will develop a primary breast cancer over time. Oncological surgeons should be aware of this risk and may minimize it by putting extra care in dissecting all glandular tissue, especially in the axillary tail and chest wall, and by dissecting skin flaps and NAC as thin as possible. More studies are warranted that further assess long-term oncological safety. Further, it is important to more specifically study patient satisfaction after NSM and SSM and potential differences in patient expectations. Ultimately, surgeons and patients may be able to balance any remaining oncological risk against expected benefits of NSM or SSM.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. King MC, Marks JH, Mandell JB, et al. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643-6.
2. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997;336:1401-8.
3. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117-30.
4. Graeser MK, Engel C, Rhiem K, et al. Contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers. J Clin Oncol 2009;27:5887-92.
5. van der Kolk DM, de Bock GH, Leegte BK, et al. Penetrance of breast cancer, ovarian cancer and contralateral breast cancer in BRCA1 and BRCA2 families: high cancer incidence at older age. Breast Cancer Res Treat 2010;124:643-51.
6. Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. J Natl Cancer Inst 2001;93:1633-7.
7. Heemskerk-Gerritsen BA, Menke-Pluijmers MB, Jager A, et al. Substantial breast cancer risk reduction and potential survival benefit after bilateral mastectomy when compared with surveillance in healthy BRCA1 and BRCA2 mutation carriers: a prospective analysis. Ann Oncol 2013;24:2029-35.
8. Kaas R, Verhoef S, Wesseling J, et al. Prophylactic mastectomy in BRCA1 and BRCA2 mutation carriers: very low risk for subsequent breast cancer. Ann Surg 2010;251:488-92.
9. Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol 2004;22:1055-62.
10. Heemskerk-Gerritsen BA, Kriege M, Seynaeve C. Association of risk-reducing surgery with cancer risks and mortality in BRCA mutation carriers. JAMA 2010;304:2695; author reply 2695-6.
11. Bresser PJ, Seynaeve C, Van Gool AR, et al. Satisfaction with prophylactic mastectomy and breast reconstruction in genetically predisposed women. Plast Reconstr Surg 2006;117:1675-82; discussion 1683-4.
12. Woods JE. Subcutaneous mastectomy: current state of the art. Ann Plast Surg 1983;11:541-50.
13. Sacchini V, Pinotti JA, Barros AC, et al. Nipple-sparing mastectomy for breast cancer and risk reduction: oncologic or technical problem? J Am Coll Surg 2006;203:704-14.
14. Jensen HM. On the origin and progression of human breast cancer. Am J Obstet Gynecol 1986;154:1280-4.
15. Wellings SR, Jensen HM, Marcum RG. An atlas of subgross pathology of the human breast with special reference to possible precancerous lesions. J Natl Cancer Inst 1975;55:231-73.
16. Parks AG. The micro-anatomy of the breast. Ann R Coll Surg Engl 1959;25:235-51.
17. Hicken NF. Mastectomy: a clinical pathologic study demonstrating why most mastectomies result in incomplete removal of the mammary gland. Arch Surg 1940;40:6-14.
18. Temple WJ, Lindsay RL, Magi E, et al. Technical considerations for prophylactic mastectomy in patients at high risk for breast cancer. Am J Surg 1991;161:413-5.
19. Carlson GW, Grossl N, Lewis MM, et al. Preservation of the inframammary fold: what are we leaving behind? Plast Reconstr Surg 1996;98:447-50.

20. Griepsma M, de Roy van Zuidewijn DB, Grond AJ, et al. Residual breast tissue after mastectomy: how often and where is it located? Ann Surg Oncol 2014;21:1260-6.
21. Goldman LD, Goldwyn RM. Some anatomical considerations of subcutaneous mastectomy. Plast Reconstr Surg 1973;51:501-5.
22. Rosen PP, Tench W. Lobules in the nipple. Frequency and significance for breast cancer treatment. Pathol Annu 1985;20 Pt 2:317-22.
23. Randall P, Dabb R, Loc N. "Apple coring" the nipple in subcutaneous mastectomy. Plast Reconstr Surg 1979;64:800-3.
24. Schnitt SJ, Goldwyn RM, Slavin SA. Mammary ducts in the areola: implications for patients undergoing reconstructive surgery of the breast. Plast Reconstr Surg 1993;92:1290-3.
25. Barton FE Jr, English JM, Kingsley WB, et al. Glandular excision in total glandular mastectomy and modified radical mastectomy: a comparison. Plast Reconstr Surg 1991;88:389-92; discussion 393-4.
26. Slavin SA, Schnitt SJ, Duda RB, et al. Skin-sparing mastectomy and immediate reconstruction: oncologic risks and aesthetic results in patients with early-stage breast cancer. Plast Reconstr Surg 1998;102:49-62.
27. Dreadin J, Sarode V, Saint-Cyr M, et al. Risk of residual breast tissue after skin-sparing mastectomy. Breast J 2012;18:248-52.
28. Cao D, Tsangaris TN, Kouprina N, et al. The superficial margin of the skin-sparing mastectomy for breast carcinoma: factors predicting involvement and efficacy of additional margin sampling. Ann Surg Oncol 2008;15:1330-40.
29. Stolier AJ, Wang J. Terminal duct lobular units are scarce in the nipple: implications for prophylactic nipple-sparing mastectomy: terminal duct lobular units in the nipple. Ann Surg Oncol 2008;15:438-42.
30. Reynolds C, Davidson JA, Lindor NM, et al. Prophylactic and therapeutic mastectomy in BRCA mutation carriers: can the nipple be preserved? Ann Surg Oncol 2011;18:3102-9.
31. Kryvenko ON, Yoon JY, Chitale DA, et al. Prevalence of terminal duct lobular units and frequency of neoplastic involvement of the nipple in mastectomy. Arch Pathol Lab Med 2013;137:955-60.
32. Torresan RZ, dos Santos CC, Okamura H, et al. Evaluation of residual glandular tissue after skin-sparing mastectomies. Ann Surg Oncol 2005;12:1037-44.
33. van Verschuer VM, van Deurzen CH, Westenend PJ, et al. Prophylactic nipple-sparing mastectomy leaves more terminal duct lobular units in situ as compared with skin-sparing mastectomy. Am J Surg Pathol 2014;38:706-12.
34. de Alcantara Filho P, Capko D, Barry JM, et al. Nipple-sparing mastectomy for breast cancer and risk-reducing surgery: the Memorial Sloan-Kettering Cancer Center experience. Ann Surg Oncol 2011;18:3117-22.
35. Arver B, Isaksson K, Atterhem H, et al. Bilateral prophylactic mastectomy in Swedish women at high risk of breast cancer: a national survey. Ann Surg 2011;253:1147-54.
36. Colwell AS, Tessler O, Lin AM, et al. Breast reconstruction following nipple-sparing mastectomy: predictors of complications, reconstruction outcomes, and 5-year trends. Plast Reconstr Surg 2014;133:496-506.
37. Contant CM, Menke-Pluijmers MB, Seynaeve C, et al. Clinical experience of prophylactic mastectomy followed by immediate breast reconstruction in women at hereditary risk of breast cancer (HB(O)C) or a proven BRCA1 and BRCA2 germ-line mutation. Eur J Surg Oncol 2002;28:627-32.
38. Evans DG, Baildam AD, Anderson E, et al. Risk reducing mastectomy: outcomes in 10 European centres. J Med Genet 2009;46:254-8.
39. Garcia-Etienne CA, Cody Iii HS 3rd, Disa JJ, et al. Nipple-sparing mastectomy: initial experience at the Memorial Sloan-Kettering Cancer Center and a comprehensive review of literature. Breast J 2009;15:440-9.
40. Hagen AI, Mæhle L, Vedå N, et al. Risk reducing mastectomy, breast reconstruction and patient satisfaction in Norwegian BRCA1/2 mutation carriers. Breast 2014;23:38-43.
41. Harness JK, Vetter TS, Salibian AH. Areola and nipple-areola-sparing mastectomy for breast cancer treatment and risk reduction: report of an initial experience in a community hospital setting. Ann Surg Oncol 2011;18:917-22.
42. Hartmann LC, Schaid DJ, Woods JE, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med 1999;340:77-84.
43. Jensen JA, Orringer JS, Giuliano AE. Nipple-sparing mastectomy in 99 patients with a mean follow-up of 5 years. Ann Surg Oncol 2011;18:1665-70.
44. Meijers-Heijboer H, van Geel B, van Putten WL, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2001;345:159-64.
45. Munhoz AM, Aldrighi CM, Montag E, et al. Clinical

outcomes following nipple-areola-sparing mastectomy with immediate implant-based breast reconstruction: a 12-year experience with an analysis of patient and breast-related factors for complications. Breast Cancer Res Treat 2013;140:545-55.
46. Peled AW, Irwin CS, Hwang ES, et al. Total skin-sparing mastectomy in BRCA mutation carriers. Ann Surg Oncol 2014;21:37-41.
47. Pennisi VR. Subcutaneous mastectomy and fibrocystic disease of the breast. Clin Plast Surg 1976;3:205-16.
48. Skytte AB, Crüger D, Gerster M, et al. Breast cancer after bilateral risk-reducing mastectomy. Clin Genet 2011;79:431-7.
49. Spear SL, Willey SC, Feldman ED, et al. Nipple-sparing mastectomy for prophylactic and therapeutic indications. Plast Reconstr Surg 2011;128:1005-14.
50. Wagner JL, Fearmonti R, Hunt KK, et al. Prospective evaluation of the nipple-areola complex sparing mastectomy for risk reduction and for early-stage breast cancer. Ann Surg Oncol 2012;19:1137-44.
51. Warren Peled A, Foster RD, Stover AC, et al. Outcomes after total skin-sparing mastectomy and immediate reconstruction in 657 breasts. Ann Surg Oncol 2012;19:3402-9.
52. Wijayanayagam A, Kumar AS, Foster RD, et al. Optimizing the total skin-sparing mastectomy. Arch Surg 2008;143:38-45; discussion 45.

Cite this article as: van Verschuer VM, Maijers MC, van Deurzen CH, Koppert LB. Oncological safety of prophylactic breast surgery: skin-sparing and nipple-sparing versus total mastectomy. Gland Surg 2015;4(6):467-475. doi: 10.3978/j.issn.2227-684X.2015.02.01

Anatomy relevant to conservative mastectomy

Rachel L. O'Connell, Jennifer E. Rusby

Royal Marsden NHS Foundation Trust, Sutton, UK

Correspondence to: Jennifer E. Rusby. Royal Marsden NHS Foundation Trust, Sutton, UK. Email: Jennifer.Rusby@rmh.nhs.uk.

Abstract: Knowledge of the anatomy of the nipple and breast skin is fundamental to any surgeon practicing conservative mastectomies. In this paper, the relevant clinical anatomy will be described, mainly focusing on the anatomy of the "oncoplastic plane", the ducts and the vasculature. We will also cover more briefly the nerve supply and the arrangement of smooth muscle of the nipple. Finally the lymphatic drainage of the nipple and areola will be described. An appreciation of the relevant anatomy, together with meticulous surgical technique may minimise local recurrence and ischaemic complications.

Keywords: Anatomy; nipple; conservative mastectomy; nipple-sparing

Submitted Dec 17, 2014. Accepted for publication Feb 10, 2015.
doi: 10.3978/j.issn.2227-684X.2015.02.06
View this article at: http://dx.doi.org/10.3978/j.issn.2227-684X.2015.02.06

Introduction

The detailed surgical anatomy will be the breast was of almost no consequence during the Halsteadian era when the standard treatment was a radical mastectomy. The resurgence of interest in preservation of the skin and nipple with a view to optimizing aesthetic outcome, so called "conservative mastectomy", has led researchers to attempt to build upon the seminal work of Sir Astley Cooper (1).

The anatomy of the breast, in particular the nipple, is highly relevant to surgeons considering conservative mastectomy. This paper will describe the clinical anatomy of the ducts as this pertains to the margins of a conservative mastectomy, but also the vascular anatomy of the breast skin and nipple as this has implications for the risk of ischaemic complications. An understanding of the anatomy, together with careful surgical technique may minimise these. We will briefly consider the nerve supply to the nipple and the arrangement of smooth muscle in of the nipple as these are relevant to residual function of the nipple after conservative mastectomy. While the detailed lobar anatomy of the breast (2-5) is of interest in optimising breast conservation it is not relevant in the case of mastectomy so will not be covered here.

Embryological development of the nipple and ducts

Paired mammary ridges, also known as milk lines develop on the ventral surface of the embryo. These extend from the axilla to the inguinal region, however much of each line atrophies leaving only the part overlying the pectoral region (6). The ectoderm is responsible for the formation of the ducts and alveoli and the mesenchyme is responsible for the connective tissue and the vasculature of the breast. The ectodermal thickening of the mammary primordium grows downwards into the dermis (7) producing solid cords of ectodermal cells growing within the underlying mesoderm. These buds become canalized and later form the lactiferous ducts and alveoli. When the foetus is near term the nipple becomes everted and ready to accept the lactiferous ducts. Developmental abnormalities in this process in a minority of foetuses result in congenital abnormalities such as amastia (absence of one or both breasts), athelia (absence of one or both nipples) and polythelia (more than two nipples).

Anatomy for skin-sparing mastectomy

From a surgical perspective, there is a clear compromise between completeness of excision of at-risk ducts and likelihood of damaging the blood supply of the skin and nipple. Thus skin-sparing mastectomy requires careful surgical technique, as described in subsequent chapters (on skin-sparing and skin-reducing mastectomy).

The development of the breast from ectoderm and mesenchyme may explain the presence of an "oncoplastic

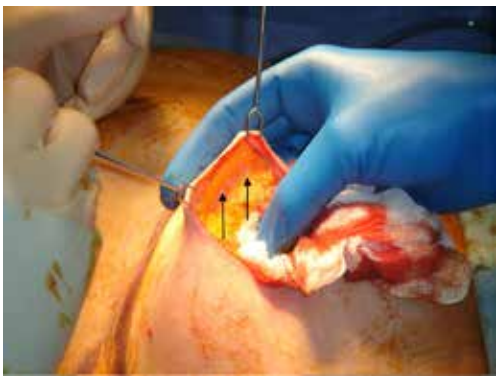


Figure 1 Operative image to show the "oncoplastic plane" with white connective tissue between subcutaneous fat and parenchymal fat shown by black arrows.

plane", seen by surgeons between the subcutaneous fat, and the fat of the breast itself (see *Figure 1*). Named, like the discipline of oncoplastic surgery, to reflect the marriage of ablative oncological surgery, with aesthetic plastic surgery, this is the key to an oncologically-sound skin-sparing mastectomy.

The breast tissue lies deep to this plane and the blood vessels, upon which the skin depends, run in the subdermal layer and are preserved with the skin, enhancing the aesthetic outcome of reconstruction. Failure to preserve the blood supply of the skin may result in necrosis of the skin flap, requiring debridement and possibly skin-grafting and risking infection and implant loss. Surgeons must, therefore, seek this plane, but in some patients it is easily found, and in others, more difficult. Anatomical (histological) studies shed some light on the reasons for this:

Beer *et al.* presented a histological study of thickness of the skin flap (i.e., depth of the oncoplastic plane) and showed great variability (8). Furthermore, they discovered that the fascial plane was not histologically distinguishable in 44% of resection specimens, and in some cases breast tissue came to within 0.4 mm of the surface of the skin. Larson *et al.* (9) also carried out histological examination of 76 breast specimens from 38 women undergoing reduction mammoplasty. The median subcutaneous tissue thickness (deep dermis to most superficial breast tissue) was 10 mm but with a wide range of 0-29 mm. The interquartile range was 6-17 mm. There was no correlation between the thickness of this subcutaneous tissue and body mass index, patient age, breast specimen weight, or dermis-to-breast thickness of the contralateral breast. Technical considerations (sampling and preservation of specimens) may partially explain these findings, but it is not uncommon, surgically, to find that the plane lies quite superficially in some patients and deeper in others, and indeed there may be variation within a patient in different quadrants. Hence no optimum mastectomy skin flap thickness can be recommended (10). Rather, the surgeon must be observant and careful when developing the plane.

Anatomy of the ducts

In addition to careful adherence to the oncoplastic plane, nipple-sparing mastectomy requires an understanding of the anatomy of ducts, their position within the nipple and their relationship to the vasculature and to the overall nipple shape. Again, surgical techniques for best managing this compromise will be discussed in later chapters. Here we present the relevant anatomy.

Number of ducts

In Sir Astley Cooper's book "On the anatomy of the Breast", he stated "The greatest number of lactiferous tubes I have been able to inject, has been twelve, and more frequently from seven to ten. But the greatest number of orifices I have been able to reckon has been twenty-two; however, some of these might be been follicles only, and not open ducts" (1). The variable results according to technique used, is reflected in the 21st century literature.

Going and Moffat (11) examined a single coronal section through the base of 72 nipples and found a median of 27 (IQR 21-30) collecting ducts. Similarly, Rusby *et al.* (12) studied 129 nipples and found the median number of ducts was 23 (IQR 19-28). Taneri *et al.* (13) sampled 226 mastectomy nipples histologically and found a mean of 17 (range, 18-30) ducts. Other techniques tend to result in smaller estimates of the number of ducts. For example, Ramsay *et al.* (14) used ultrasound to study 21 lactating women and found a mean of 9.6 ducts beneath the nipple of the left breast and 9.2 on the right. However, the equipment had insufficient resolution to identify ducts of less than 0.5 mm in diameter. Love and Barsky (15) employed several approaches to the study of ductal openings. Using serial sectioning and cytokeratin immunocytochemistry of ten nipples they identified 5-9 duct openings per nipple. They noted a mean of 5 duct openings by direct in vivo observations of lactating women and 6-8 openings by observation of passive conduction of lymphazurin from a subareolar injection to the nipple tip in mastectomy specimens. These findings are restricted to the number

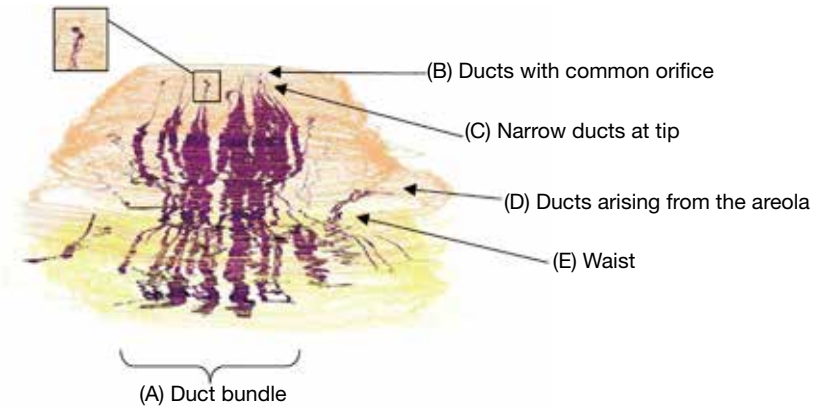


Figure 2 Three-dimensional reconstruction of a nipple. Skin in tan, cut edge in yellow and ducts in purple. Reproduced with permission from ref (12).

of ductal openings and do not establish the number of underlying ducts or their interconnections.

Relationship between ducts and openings

Four groups using histological techniques have noted the discrepancy between duct number and opening number and postulated that duct branching may be responsible (11,13,15). Going and Mohun (4) tried to elucidate the path of the 19 identifiable ducts in a 2.2 mm thick block at the tip of a nipple using episcopic fluorescence image capture (EFIC). However, they found that EFIC has insufficient resolution to discriminate reliably between keratin plugging and discontinuity between the duct and the skin surface. Using hematoxylin and eosin staining (H&E) sections from an entire nipple-tip, Rusby *et al*. showed that several ducts arose in the same cleft of the nipple (12), accounting for the discrepancy between the number of ducts in the nipple and the number of openings that can be counted externally.

Duct diameter

Estimating diameter at different levels has shown that most ducts are very narrow at the tip of the nipple with only a few ducts of a size that could be cannulated. At 1 and 1.5 mm beneath the tip the average duct diameter was 0.06 mm, and this increased to 0.7 mm at 3 mm deep (12).

Position of the ducts within the nipple

For conservative mastectomy, the exact number and size of the ducts is less relevant than their position and relationships to other structures in the nipple. The surgical community is divided over whether it is necessary to attempt to excise all of the ducts (potentially compromising blood supply) and it certainly might seem unnecessary to remove the duct core in prophylactic mastectomy since most tumours develop in the terminal ductal lobular units. However, it has been reported that 9-17% of nipples do contain lobular tissue (16,17), thus, potentially carrying the risk of *de novo* cancer formation within the nipple in high-risk women.

Duct arrangement is best seen in a three-dimensional image of a reconstructed nipple (12) (*Figure 2*).

This shows:

(A) The ducts are arranged in a central bundle with a peripheral duct-free rim;
(B) The bundle narrows to a "waist" just beneath the skin, possibly at the level of the superficial fascia;
(C) Some ducts originate on the areola or part way up the nipple;
(D) Most ducts are very narrow as they approach the tip of the nipple;
(E) Many of the ducts originate within a smaller number of openings on the nipple surface.

The finding that the majority of ducts form a central bundle that occupies 21-67% of the cross-sectional area of the papilla (12) suggests that near-complete surgical excision of the central duct bundle is feasible if it is deemed advisable. The changing cross-sectional area of the duct bundle forms a "waist" as shown in the three-dimensional reconstructions (12,18). This may have a developmental origin as sagittal sections illustrate that the narrowest point of the duct bundle occurs at the level of the superficial

fascia, perhaps indicating that in-growing ducts pierce this fascia together before dispersing into the developing breast. The waist may also correspond to the operative finding that the plane between breast and subcutaneous fat becomes more fibrous at the border of the nipple and this must be freed before the nipple can be inverted.

Going and Moffat (11) classified nipple ducts into three categories, ducts with a wide lumen, ducts with a minute lumen at the origin in the vicinity of the apex of the nipple and a minor duct population which arise from around the base of the papilla. Similar findings have been reproduced in other three-dimensional studies as well as identifying ducts originating in the areola (12). Going and Moffat's hypothesis that larger ducts might be connected to larger duct systems were not confirmed in the aforementioned study by Rusby *et al*. as there was no organized relationship between size of duct and whether it terminated within the nipple or passed deeper into the breast.

Vascular anatomy of the nipple

Nipple necrosis after nipple-sparing mastectomy may result in a requirement for excision of the nipple. Nipple necrosis can also occur following surgery to correct inversion, for mammary duct fistula, and after Hadfield's major duct excision. An understanding of the vascular anatomy is, therefore, clinically-relevant beyond nipple-sparing mastectomy.

Much of the available anatomical information about vascular anatomy within the breast and about supply to the nipple-areola complex is found in literature on breast reduction, where nipple viability is of key importance. Several studies have demonstrated that the blood supply of the breast is from the external and internal thoracic arteries, the intercostal, and the thoracoacromial arteries (19-22). Many of these studies were carried out in a small number of cadavers, which may account for discrepancies in comments on predominant supply to the nipple-areola complex.

Würinger (23) described two main sources of neurovascular supply to the nipple: a central and a superficial network. The central supply travels in a ligamentous septum originating from pectoralis fascia at the level of the 5th rib and inferior border of pectoralis major. Branches of the thoracoacromial, lateral thoracic and intercostal arteries and the deep branch of the 4th intercostal nerve passed within this septum. Würinger also described a medial ligament arising from the sternum and guiding blood vessels of the internal thoracic artery and anterior cutaneous intercostal nerve branches. A lateral ligament attached to the lateral border of pectoralis minor guides branches of the lateral thoracic and lateral cutaneous intercostal nerves. These ligaments merge and carry a blood supply to the superficial fascia.

O'Dey *et al*. (22) found that the lateral thoracic artery supplied up to three separate branches to the nipple-areola complex during its descending course. However, these passed through deep breast tissue before ascending towards the nipple-areola complex to reach the superolateral edge. While important in breast reduction, these branches would be divided during a mastectomy. O'Dey concluded that the internal thoracic artery, in particular, supplies the nipple-areola complex. 86% of cases studied had one or two perforating vessels usually emerging in the 2nd or 4th intercostal spaces. These vessels had a curved course with superior convexity and arrived at the supero-medial border of the nipple-areola complex. These are described as traversing the subcutaneous tissue, converging on the nipple-areola complex at a depth of 1.5±0.4 cm.

These studies all report that there is a superficial and a deep blood supply: the deep blood supply to the nipple shown in whole breast anatomical studies runs either through breast parenchyma (22) or in a ligamentous septum (24) and will be excised with the mastectomy specimen. If, according to O'Dey *et al*., the "superficial" supply runs approximately 1.5 cm deep to the skin surface it, too, is unlikely to be preserved during a good oncological mastectomy as it is unusual to leave skin flaps that are 1.5 cm thick (as described above). Furthermore, this implies that despite leaving 0.5 cm thickness of glandular tissue beneath the nipple as advocated by some surgeons, the most important vessels are likely to have been severed. Nakajima *et al*. (19) described branches of the external and internal mammary arteries travelling in the subcutaneous tissue and communicating with one another above and below the areola. Small branches derived from the communicating vessels were found running toward the nipple-areola complex. These small vessels reached the base of the nipple, giving off fine vessels to the areolar skin, and ascended in the nipple in a circular fashion. Nakajima found that these arborised in the upper and middle thirds of the nipple. The close proximity of these vessels to the ducts implies that any technique in which the nipple core is excised will result in disruption of the major neurovascular supply within the nipple. A subsidiary part of Nakajima's work involved angiograms of breast skin specimens in which mammary glands and subcutaneous tissue had been resected. These showed rather sparse dermal and subdermal plexuses around the nipple-areola complex. It appears to be these plexuses upon which the survival of the nipple-areola complex depends if complete duct excision is

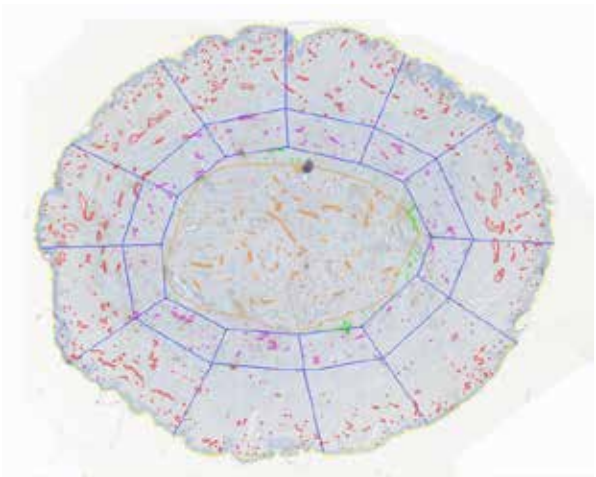


Figure 3 Coronal section of a nipple with nipple outline, duct bundle and peripheral 2 and 3 mm rims marked. Vessels stained with anti-factor VIII antibody to vascular endothelium have been highlighted and counted. Ducts are faintly visible within the central duct bundle. In this example, leaving either a 2 or 3 mm rim would have removed all ductal tissue. Reproduced with permission from ref (29).

attempted in nipple-sparing mastectomy.

Thus the two conflicting challenges of nipple preservation, ensuring oncological safety and maintaining nipple viability, are dependent on the underlying anatomy and on surgical technique and are inextricably linked through surgical judgment about the value of excising as much duct tissue as possible. Clinical series reporting necrosis rates often do not report in sufficient detail on surgical technique to allow readers to evaluate the trade-off being made.

Incision placement, however, is usually reported and many different incisions have been described for the conservative mastectomy with some high quality retrospective studies addressing this. A review of 48 studies by Munhoz *et al.* (25) demonstrated that the most common incision was the radial, followed by periareolar, inframmammary, mastopexy and transareaolar. Wijayanayagam *et al.* (18) found that the radial incision had the greatest likelihood of avoiding ischaemia of the nipple-areola complex in a series of 64 conservative mastectomies. However the scar from this incision is prominent. Colwell *et al.* (26) reviewed 500 nipple-sparing mastectomy procedures and found that a periareolar incision was an independent predictor of complications on multivariate analysis and the inferolateral inframammary fold incision was associated with a decreased risk of total and ischaemic complications. Similar results for the periareolar incision have been found in another study (27). Garwood *et al.* (28) found on logistic regression analysis that using an incision that was more than one third of the circumference of the nipple-areola complex was an independent risk factor for complete or partial nipple loss and skin flap necrosis. It can be assumed that if the sparse dermal and subdermal plexuses around the nipple-areola complex are disturbed in addition to division of the deeper vessels during the mastectomy, the risk of ischaemic complications is higher.

A study to investigate the microanatomy of the un-irradiated nipple vasculature used anti-factor VIII antibody to highlight blood vessels in sections from coronal 3 mm thick blocks of resected nipples. Within a 2 mm rim of peripheral nipple tissue 50% of the vessels were contained, and within a 3 mm rim, 66%. Only 29% of the vessels were located within the duct bundle (*Figure 3*). However, in terms of density, the mean microvascular density was 16 per mm^2 in the duct bundle and 9 per mm^2 in the peripheral tissue (29). The proportion of vessels in the duct bundle and the microvessel density was unchanged by radiation. These data are of anatomical interest, though it is difficult to apply these microscopic findings to improve surgical practice.

Anatomy of retained function

Opatt *et al.* (30) argue that sparing the nipple serves little purpose if the nipple is insensate. However, there is some evidence that nipple sensation and erection can be regained after nipple-sparing mastectomy (31-35).

The sensory innervation of the breasts comes from the lateral and anterior cutaneous branches of intercostal nerves (36,37). Controversies as to which intercostal nerves are relevant and their course are likely to be due to difficulty in dissecting thin nerves and the small number of cadavers in each study. Schlenz *et al.* (38) undertook an anatomic study of 28 female cadavers. They found that the nipple and areola were always innervated by the lateral and cutaneous branches of the 3^{rd}, 4^{th} and 5^{th} intercostal nerves with the most constant innervation pattern being from the 4^{th} lateral cutaneous branch. The anterior cutaneous branches took a superficial course within the subcutaneous tissues of the medial breast and terminated at the medial areolar border. The lateral cutaneous branches took a deep course within the pectoral fascia and reached the nipple via the breast parenchyma and pierced the nipple via its posterior surface. Montagne and Macpherson (39) demonstrated that the neural elements are concentrated at the base of the nipple with few at the side of the nipple and even fewer in the areolar. Therefore it is unsurprising that the nipple is

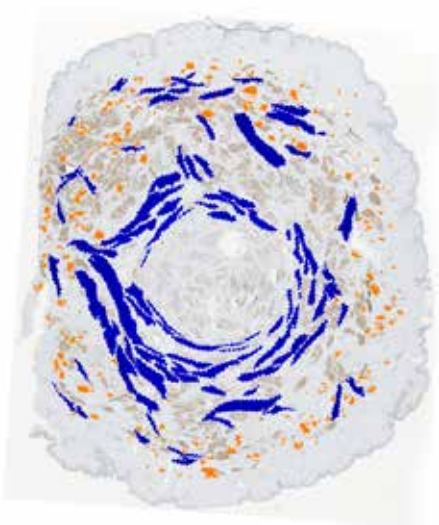


Figure 4 Section with muscle fibres. Circular fibres highlighted in blue, longitudinal fibres in orange. Reproduced with permission from ref (40).

largely insensate after nipple-sparing mastectomy due to injury of the anterior cutaneous nerves as the anatomical plane between the subcutaneous fat and breast parenchyma is developed and the lateral cutaneous nerves are divided as the breast parenchyma is separated from the pectoral fascia.

Although most authors report that sensation is lost, some preserved nipples remain erectile and therefore behave more naturally than a reconstructed nipple.

The arrangement of smooth muscle highlighted in *Figure 4* (40) is reminiscent of the concentric muscle layers of the gastrointestinal tract or of a sphincter. At the base of the papilla the circular smooth muscle is particularly prominent around the duct bundle suggesting that contraction of this muscle could lead to erection of the nipple and possibly occlusion of the ducts. Conversely, towards the tip of the nipple, the concentrations of muscle fibres surround individual ducts as they narrow and unite close to the tip of the nipple.

Anatomy of lymphatic drainage

Sappey first described the anatomical basis of the breast lymphatics in the 1870s (41). He demonstrated a subareolar plexus of lymphatics and a small number of large lymphatic vessels draining into the axillary lymph nodes. Sappey concluded that the lymphatics of the breast collected in a subareolar plexus and then drained towards the axilla. Many of his observations contributed significantly to the development of breast lymphatic mapping and sentinel lymph node biopsy. In 1959 Turner-Warwick (42) studied the lymphatics and concluded that lymphatic pathways passed directly from the tumour injection site to the axillary lymph nodes without passing though the subareolar plexus. He suggested Sappey had mistaken mammary ducts for a lymphatic vessel, therefore overemphasizing the importance of the subareolar plexus. Whether or not the subareolar plexus drains the breast tissues and then lymph then drains towards the sentinel lymph node is still controversial and calls into question the optimal location of dye or radioisotope for sentinel lymph node biopsy. Suami *et al.* (43) undertook lymphatic mapping of 14 cadavers using hydrogen peroxide and injecting with a lead oxide mixture and then imaging the specimens. Similarly to Sappey they found the lymphatics deep to the nipple and areola were a dense network of lymph capillaries, however they favoured the Turner-Warwick findings that suggested a direct pathway from the injection site to the axilla, not via the subareolar plexus.

Conclusions

Together with careful surgical technique, a good working knowledge of the blood supply of the skin and nipple of the breast contributes to the avoidance of ischaemic complications in conservative mastectomy. Similarly, an understanding of the spatial relationships of ducts and blood vessels within the nipple will help surgeons make decisions on the relative benefits of removing or preserving the nipple core, and optimising technique to do so should this be deemed necessary.

Acknowledgements

The authors acknowledge the NIHR as the Royal Marsden and Institute for Cancer Research are an NIHR funded Biomedical Research Centre.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Cooper AP. editor. On the anatomy of the breast. London:

Longman, 1840.
2. Going JJ. Ductal-lobar organisation of human breast tissue, its relevance in disease and a research objective: vector mapping of parenchyma in complete breasts (the Astley Cooper project). Breast Cancer Res 2006;8:107.
3. Tot T. The theory of the sick breast lobe and the possible consequences. Int J Surg Pathol 2007;15:369-75.
4. Going JJ, Mohun TJ. Human breast duct anatomy, the 'sick lobe' hypothesis and intraductal approaches to breast cancer. Breast Cancer Res Treat 2006;97:285-91.
5. Mannino M, Yarnold J. Effect of breast-duct anatomy and wound-healing responses on local tumour recurrence after primary surgery for early breast cancer. Lancet Oncol 2009;10:425-9.
6. Seltzer V. The breast: embryology, development, and anatomy. Clin Obstet Gynecol 1994;37:879-80.
7. Skandalakis JE, Colborn GL, Skandalakis PN, et al. Breast. In: Skandalakis JE. eds. Surgical Anatomy: The Embryologic and Anatomic Basis of Modern Surgery. Athens: Paschalidis Medical Publications 2004:155-88.
8. Beer GM, Varga Z, Budi S, et al. Incidence of the superficial fascia and its relevance in skin-sparing mastectomy. Cancer 2002;94:1619-25.
9. Larson DL, Basir Z, Bruce T. Is oncologic safety compatible with a predictably viable mastectomy skin flap? Plast Reconstr Surg 2011;127:27-33.
10. Robertson SA, Rusby JE, Cutress RI. Determinants of optimal mastectomy skin flap thickness. Br J Surg 2014;101:899-911.
11. Going JJ, Moffat DF. Escaping from Flatland: clinical and biological aspects of human mammary duct anatomy in three dimensions. J Pathol 2004;203:538-44.
12. Rusby JE, Brachtel EF, Michaelson JS, et al. Breast duct anatomy in the human nipple: three-dimensional patterns and clinical implications. Breast Cancer Res Treat 2007;106:171-9.
13. Taneri F, Kurukahvecioglu O, Akyurek N, et al. Microanatomy of milk ducts in the nipple. Eur Surg Res 2006;38:545-9.
14. Ramsay DT, Kent JC, Hartmann RA, et al. Anatomy of the lactating human breast redefined with ultrasound imaging. J Anat 2005;206:525-34.
15. Love SM, Barsky SH. Anatomy of the nipple and breast ducts revisited. Cancer 2004;101:1947-57.
16. Rosen PP, Tench W. Lobules in the nipple. Frequency and significance for breast cancer treatment. Pathol Annu 1985;20 Pt 2:317-22.
17. Stolier AJ, Wang J. Terminal duct lobular units are scarce in the nipple: implications for prophylactic nipple-sparing mastectomy: terminal duct lobular units in the nipple. Ann Surg Oncol 2008;15:438-42.
18. Wijayanayagam A, Kumar AS, Foster RD, et al. Optimizing the total skin-sparing mastectomy. Arch Surg 2008;143:38-45; discussion 45.
19. Nakajima H, Imanishi N, Aiso S. Arterial anatomy of the nipple-areola complex. Plast Reconstr Surg 1995;96:843-5.
20. Ricbourg B. Applied anatomy of the breast: blood supply and innervation. Ann Chir Plast Esthet 1992;37:603-20.
21. Wueringer E, Tschabitscher M. New aspects of the topographical anatomy of the mammary gland regarding its neurovascular supply along a regular ligamentous suspension. Eur J Morphol 2002;40:181-9.
22. O'Dey Dm, Prescher A, Pallua N. Vascular reliability of nipple-areola complex-bearing pedicles: an anatomical microdissection study. Plast Reconstr Surg 2007;119:1167-77.
23. Würinger E. Secondary reduction mammaplasty. Plast Reconstr Surg. 2002;109:812-4.
24. Würinger E, Mader N, Posch E, et al. Nerve and vessel supplying ligamentous suspension of the mammary gland. Plast Reconstr Surg 1998;101:1486-93.
25. Munhoz AM, Montag E, Filassi JR, et al. Immediate nipple-areola-sparing mastectomy reconstruction: An update on oncological and reconstruction techniques. World J Clin Oncol 2014;5:478-94.
26. Colwell AS, Tessler O, Lin AM, et al. Breast reconstruction following nipple-sparing mastectomy: predictors of complications, reconstruction outcomes, and 5-year trends. Plast Reconstr Surg 2014;133:496-506.
27. Regolo L, Ballardini B, Gallarotti E, et al. Nipple sparing mastectomy: an innovative skin incision for an alternative approach. Breast 2008;17:8-11.
28. Garwood ER, Moore D, Ewing C, et al. Total skin-sparing mastectomy: complications and local recurrence rates in 2 cohorts of patients. Ann Surg 2009;249:26-32.
29. Rusby JE, Brachtel EF, Taghian A, et al. George Peters Award. Microscopic anatomy within the nipple: implications for nipple-sparing mastectomy. Am J Surg 2007;194:433-7.
30. Opatt D, Morrow M. The dual role of nipple preservation. J Support Oncol 2006;4:233-4.
31. Petit JY, Veronesi U, Orecchia R, et al. Nipple sparing mastectomy with nipple areola intraoperative radiotherapy: one thousand and one cases of a five years experience at the European institute of oncology of Milan (EIO). Breast Cancer Res Treat 2009;117:333-8.

32. Nahabedian MY, Tsangaris TN. Breast reconstruction following subcutaneous mastectomy for cancer: a critical appraisal of the nipple-areola complex. Plast Reconstr Surg 2006;117:1083-90.
33. Denewer A, Farouk O. Can nipple-sparing mastectomy and immediate breast reconstruction with modified extended latissimus dorsi muscular flap improve the cosmetic and functional outcome among patients with breast carcinoma? World J Surg 2007;31:1169-77.
34. Yueh JH, Houlihan MJ, Slavin SA, et al. Nipple-sparing mastectomy: evaluation of patient satisfaction, aesthetic results, and sensation. Ann Plast Surg 2009;62:586-90.
35. Benediktsson KP, Perbeck L, Geigant E, et al. Touch sensibility in the breast after subcutaneous mastectomy and immediate reconstruction with a prosthesis. Br J Plast Surg 1997;50:443-9.
36. Sarhadi NS, Shaw Dunn J, Lee FD, et al. An anatomical study of the nerve supply of the breast, including the nipple and areola. Br J Plast Surg 1996;49:156-64.
37. Sarhadi NS, Shaw-Dunn J, Soutar DS. Nerve supply of the breast with special reference to the nipple and areola: Sir Astley Cooper revisited. Clin Anat 1997;10:283-8.
38. Schlenz I, Kuzbari R, Gruber H, et al. The sensitivity of the nipple-areola complex: an anatomic study. Plast Reconstr Surg 2000;105:905-9.
39. Montagna W, Macpherson EE. Proceedings: Some neglected aspects of the anatomy of human breasts. J Invest Dermatol 1974;63:10-6.
40. Rusby JE. An anatomical study of the skin, nipple and areola of the breast. Towards a scientific basis for nipple-sparing mastectomy. University of Oxford DM Thesis 2012.
41. Sappey MP. Anatomie, Physiologie, Pathologie des vaisseaux Lymphatiques consideres chez L'homme at les Vertebres. Paris: A. Delahaye and E. Lecrosnier, 1874.
42. Turner-Warwick RT. The lymphatics of the breast. Br J Surg 1959;46:574-82.
43. Suami H, Pan WR, Mann GB, et al. The lymphatic anatomy of the breast and its implications for sentinel lymph node biopsy: a human cadaver study. Ann Surg Oncol 2008;15:863-71.

Cite this article as: O'Connell RL, Rusby JE. Anatomy relevant to conservative mastectomy. Gland Surg 2015;4(6):476-483. doi: 10.3978/j.issn.2227-684X.2015.02.06

Breast reconstruction following conservative mastectomies: predictors of complications and outcomes

Sophocles H. Voineskos[1,2], Simon G. Frank[3], Peter G. Cordeiro[3]

[1]Division of Plastic and Reconstructive Surgery, Department of Surgery, [2]Surgical Outcomes Research Centre (SOURCE), McMaster University, Hamilton, Canada; [3]Plastic and Reconstructive Surgery Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, USA
Correspondence to: Peter G. Cordeiro, M.D. Plastic and Reconstructive Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave. New York, N.Y. 10021, USA. Email: cordeirp@mskcc.org.

Abstract: Breast reconstruction can be performed using a variety of techniques, most commonly categorized into an alloplastic approach or an autologous tissue method. Both strategies have certain risk factors that influence reconstructive outcomes and complication rates. In alloplastic breast reconstruction, surgical outcomes and complication rates are negatively impacted by radiation, smoking, increased body mass index (BMI), hypertension, and prior breast conserving therapy. Surgical factors such as the type of implant material, undergoing immediate breast reconstruction, and the use of fat grafting can improve patient satisfaction and aesthetic outcomes. In autologous breast reconstruction, radiation, increased BMI, certain previous abdominal surgery, smoking, and delayed reconstruction are associated with higher complication rates. Though a pedicled transverse rectus abdominis myocutaneous (TRAM) flap is the most common type of flap used for autologous breast reconstruction, pedicled TRAMs are more likely to be associated with fat necrosis than a free TRAM or deep inferior epigastric perforator (DIEP) flap. Fat grafting can also be used to improve aesthetic outcomes in autologous reconstruction. This article focuses on factors, both patient and surgical, that are predictors of complications and outcomes in breast reconstruction.

Keywords: Breast reconstruction; complications; outcomes; tissue expander/implant; autologous reconstruction

Submitted Dec 18, 2014. Accepted for publication Mar 06, 2015.
doi: 10.3978/j.issn.2227-684X.2015.04.13
View this article at: http://dx.doi.org/10.3978/j.issn.2227-684X.2015.04.13

Introduction

Breast reconstruction following mastectomy can be performed using alloplastic techniques, most commonly tissue expansion followed by implant placement, or autologous techniques in which numerous flap options exist. The goal of breast reconstruction surgery, whether autologous or alloplastic, is to create a breast mound that appears as natural as possible under clothing, and ideally without clothing as well (1). To achieve this goal, certain patient factors and surgical factors that can influence outcomes and complication rates must be taken into consideration.

Patient factors affecting complication rates and outcomes in breast reconstruction that are typically investigated include radiation, chemotherapy, smoking, obesity, age, and medical comorbidities (1-3). Surgical factors common to both alloplastic and autologous reconstruction, such as the timing of the reconstruction and the use of fat grafting, have an effect on outcomes and complications (4-6).

In alloplastic reconstructions, patients are exposed to less surgical risk, fewer scars, less donor site morbidity and fewer irreversible consequences. However, surgical factors like implant type, number of surgical stages, and use of an acellular dermal matrix (ADM) can influence outcomes (7-10). Typical complications and their frequencies in four large series of alloplastic based reconstruction are displayed in *Table 1*.

The optimal method of breast reconstruction differs from patient to patient, however reconstruction with autologous tissue can provide a long lasting, natural

feeling breast mound (11). An obvious surgical factor that influences outcomes in autologous reconstruction is the type of autologous flap used. Complication rates in seven large series of autologous reconstruction patients are presented in *Tables 2* and *3*.

This article will describe patient factors and surgical factors that are predictors of outcomes and complications in alloplastic and autologous breast reconstruction.

Tissue expander/implant based reconstruction

Patient factors influencing complications and outcomes

Radiation

Radiation adversely impacts expander/implant based breast reconstruction. Regardless of the timing of administration of radiation therapy, expander placement in a radio-treated field, radiation to temporary expanders postmastectomy, or radiation postmastectomy to implant, patients are at an increased risk of complications and reconstructive failure (2,22). Capsular contracture (23,24), infection (25) and wound-related complications are more common (1), with a wide spectrum of reported complication rates, ranging from 5% to 48% (26). Both aesthetic satisfaction and general satisfaction rates appear to be similar in expander/implant based reconstruction patients with and without radiotherapy (23,27). However, a long-term multicenter analysis demonstrated that patients receiving radiation had significantly lower satisfaction with the surgical outcome, as well as their psychosocial, sexual, and physical well-being (28). The increased complication rate does not exclude a patient requiring radiotherapy from an expander/implant based reconstruction, but the potential for the requirement of an autologous/prosthetic combination, in the form of a latissimus dorsi flap with implant, or a completely autologous reconstructive approach, should be discussed with the patient (1,2).

Chemotherapy

Both neoadjuvant and adjuvant chemotherapy regimens have been investigated in the setting of postoperative complications after mastectomy and breast reconstruction. It appears that neither neoadjuvant (2,13,29,30) nor adjuvant (12,13,30) chemotherapy increase the rate of complications or implant failure in patients undergoing postmastectomy expander/implants breast reconstruction, including in patients who undergo tissue expansion concomitantly. Bevacizumab in particular has been shown to affect surgical wound healing (31). To date, it has not been shown to increase complications in breast reconstruction, though evidence is limited (32). It is suggested to wait 6-8 weeks after completing bevacizumab therapy before performing surgery to minimize risks of complications (31).

Smoking

Smoking is universally considered to be a risk factor for surgical complications. For patients undergoing expander/implant based breast reconstruction, smoking is an independent risk factor for the development of perioperative complications and is associated with an increased risk of reconstructive failure (2,13,33). The rates of mastectomy skin flap necrosis and infectious complications are significantly higher in smokers compared to non-smokers (33). Complication rates as high as 37.9% in smokers have been reported (33), a 2-3 fold increase compared to non-smokers (13,33). Smokers are also five times more likely to experience reconstructive failure (13). The rate of complications in ex-smokers, defined as patients who have stopped smoking between 1 and 12 months preoperatively, can also be higher than non-smokers (33). The significant association between cigarette smoking and complications in the setting of tissue expander/implant reconstruction necessitates advising patients on smoking cessation and informing them of the increased risks.

Obesity/body mass index (BMI)

Obesity is defined as a BMI of 30 or greater. Obesity is an independent risk factor for the development of perioperative complications in patients undergoing expander/implant based reconstruction (13,14). Patients who are obese have nearly twice the risk of developing a perioperative complication (13). The risk of reconstructive failure is seven times greater in obese patients when compared to non-obese patients. Overweight patients, defined as a BMI of 25 or greater, are also at an increased risk of postoperative complications and reconstructive failure, though their risk is notably smaller (2,4,9).

Breast size

Some genetic factors that contribute to breast size are shared with those that influence BMI. Though the extent to which they are related is not clear, they are covariates (34). In patients undergoing expander/implant based reconstruction, large preoperative breast size, a cup size of D or larger, may be associated with an increased risk of complication and an increased risk of reconstructive failure (25). However, the

Table 1 Complication rates in tissue expander/implant reconstruction

Publication	Total patients	Total expanders	Unilateral	Bilateral	Total complications, N (%)	Failed expansion, N (%)	Expander deflation, N (%)	Mastectomy skin flap necrosis, N (%)	Hematoma, N (%)	Seroma, N (%)	Infection, N (%)
Spear *et al*. 1998 (11)	142	171	113	29	–	12 (7.0)	3 (1.8)	14 (8.1)	2 (1.0)	–	8 (4.7)
Cordeiro *et al*. 2006 (12)	1,221	1,522	920	301	(5.8)	2 (0.1)	2 (0.1)	45 (2.0)	10 (0.4)	5 (0.2)	58 (2.5)
McCarthy *et al*. 2008 (13)	884	1,170	597	287	206 (17.6)	2 (0.2)	–	102 (8.7)	–	–	57 (4.9)
Colwell *et al*. 2014 (14)	274	471	–	–	60 (12.7)	–	–	25 (5.2)	8 (1.7)	8 (1.7)	16 (3.3)

Table 2 Complication rates in autologous reconstruction

Publication	Total patients	Total flaps	Unilateral	Bilateral	Total complications, N (%)	Total flap loss, N (%)	Partial flap loss, N (%)	Arterial occlusion, N (%)	Venous occlusion, N (%)	Fat necrosis, N (%)
Blondeel *et al*. 1999 (15)	87	100	74	13	–	2 (2.0)	7 (7.0)	2 (2.0)	4 (4.0)	6 (6.0)
Chang *et al*. 2000 (16)	718	936	–	–	328 (45.7)*	8 (0.9)	13 (1.4)	–	–	55 (5.9)
Gill *et al*. 2004 (17)	609	758	460	149	229 (30.2)◆	4 (0.5)	19 (2.5)	4 (0.5)	29 (3.8)	98 (12.9)
Mehrara *et al*. 2006 (18)	952	1,195	695	250	266 (27.9)*	6 (0.5)	26 (2.3)	9 (0.8)	15 (1.3)	134 (11.2)
Selber *et al*. 2006 (19)	500	569	431	69	119 (20.9)◆	2 (0.3)	9 (1.6)	1 (0.2)	–	19 (3.3)
Damen *et al*. 2013 (20)	285	406	164	121	120 (42.1)*	2 (0.5)	10 (3.5)	2 (0.5)	7 (1.7)	14 (3.4)
Fischer *et al*. 2013 (21)	849	1,303	395	454	542 (63.9)*	18 (2.1)	7 (0.8)	–	–	49 (5.8)

*, percentage reported using number of patients as denominator; ◆, percentage reported using number of flaps as denominator.

Table 3 Complication rates in autologous reconstruction

Publication	Total patients	Total flaps	Mastectomy skin flap necrosis, N (%)	Hematoma, N (%)	Seroma, N (%)	Infection, N (%)	Donor site* complication, N (%)	Abdominal wall* laxity/hernia, N (%)	Delayed donor* site healing, N (%)
Blondeel *et al*. 1999 (15)	87	100	1 (1.0)	2 (2.0)	1 (1.0)	3 (3.0)	–	1 (1.0)	2 (2.0)
Chang *et al*. 2000 (16)	718	936	96 (10.3)	16 (1.7)	38 (4.1)	17 (1.8)	106 (14.8)	41 (5.7)	11 (1.5)
Gill *et al*. 2004 (17)	609	758	–	14 (1.8)	35 (4.6)	21 (2.8)	103 (13.6)	5 (0.7)	–
Mehrara *et al*. 2006 (18)	952	1,195	–	19 (1.6)	–	110 (9.2)	–	29 (3.0)	–
Selber *et al*. 2006 (19)	500	569	17 (3.0)	3 (0.5)	7 (1.2)	20 (3.5)	–	11 (1.9)	19 (3.3)
Damen *et al*. 2013 (20)	285	406	15 (3.7)	19 (4.7)	–	7 (2.5)	–	10 (3.5)	13 (4.6)
Fischer *et al*. 2013 (21)	849	1,303	–	–	45 (3.9)	64 (7.5)	–	29 (3.4)	154 (18.2)

*, percentage reported using number of patients as denominator.

effect of breast size has not been isolated from BMI and therefore it is not yet established whether large breast size on its own contributes to complications in these patients (2).

Age

Expander/implant reconstruction rates have been increasing in the elderly (28). Age is another factor that is universally associated with poorer outcomes following surgical procedures. Limited data exists on the relationship between age and outcomes in expander/implant based breast reconstruction. Age might be an independent risk factor for complications, though it does not appear to be a significant predictor of reconstructive failure (13). Patients older than 65 may have an increased risk of perioperative complications when compared to younger patients (13).

Medical comorbidities

Hypertension

In a review of 1,170 consecutive expander/implant reconstructions (884 patients) hypertension was found to be an independent risk factor for perioperative complications (13). In this series, a patient was classified as having hypertension if they required medical therapy. The risk was quantified as being two times greater than in a patient without hypertension. The odds of premature removal of a tissue expander and/or explantation of a permanent implant were four times higher in the hypertensive patient (13).

Diabetes mellitus

No significant associations between implant infection and diabetes have been found (13,35). Diabetes has not been shown to be an independent risk factor for the development of postoperative complications or for reconstructive failure (2,13,22). However, it is still advised that breast cancer patients attempt glycemic control in the perioperative period (2).

Prior breast conserving therapy (lumpectomy/ irradiation combination)

Expander/implant based reconstruction may be an option in carefully selected patients with cancer recurrence following lumpectomy with irradiation. Patients who have undergone breast conserving therapy are at higher risk of early complications, of higher capsular contracture grade, and slightly inferior aesthetic results (36). Patients with severe breast deformity, multiple scars on the irradiated breast, or with tight/poor soft tissue might be appropriate candidates for the use of a latissimus dorsi flap to cover the prosthesis or for autologous reconstruction (36,37).

Mastectomy type: nipple sparing, skin sparing, skin reducing

The proportion of patients undergoing nipple sparing mastectomies (NSM) is increasing due to its perceived aesthetic benefits (38). The oncologic safety of NSMs is the greatest concern associated with this procedure, as nipple areola complex (NAC) involvement is related to tumor size, distance from the NAC, multicentricity, nuclear grade and lymph node status (38). A percentage of patients undergoing this procedure will have occult disease in the NAC [reported at 9.1% in one series of 66 patients (38)]. Wound healing problems within the NAC and either partial or complete NAC loss are unique complications to this procedure. Patients with larger breasts are at greater risk of nipple necrosis (39). The overall rate of complications in NSMs appears to be similar to that in skin-sparing mastectomies (SSM) (39). NAC preservation is associated with favorable results in aesthetic outcome, nipple sensitivity, and patient satisfaction (40).

SSMs are the conventional approach where the skin ellipse surrounding the NAC is extended (41). SSM is the most common type of mastectomy surgery performed for breast cancer treatment and does not have any unique complications.

Skin reducing mastectomies (SRMs) are performed using a Wise Pattern incision when skin envelope reduction is required (41). The vertical scar approach is an alternative to the Wise pattern technique (41). SRMs are often used for large breasts which in turn are at an increased risk of complications and reconstructive failure (25).

Surgical factors influencing complications and outcomes

Implant texture, shape, and material

Saline and silicone gel implants are available as the final implant material for expander/implant based postmastectomy reconstruction. All implant models have a bladder, or outside shell, made of solid silicone. The shell can be either textured or smooth. Modern expanders are textured to help prevent migration and early capsular contracture. Both saline and silicone implants can be either round, or anatomically shaped (like a teardrop). Patient satisfaction and aesthetic outcome does not appear to be affected by the shape (round or anatomic) of the implant used in the reconstruction (42,43).

Silicone gel implants are traditionally thought to provide a softer, more natural feeling breast when compared to saline implants (3). Decreased visible wrinkling has been

thought to be an benefit of silicone implants, however this advantage is not always apparent (44). Patients receiving silicone implants have greater satisfaction with their breasts than those with saline implants (7,8). Silicone is no longer believed to be linked to immunologic (45) or other systemic diseases (3), however degradation of the silicone bladder over time will cause an implant to rupture (1). Thus, due to the possibility of silicone leakage into local tissues, some patients may choose saline implants for peace of mind.

Timing of reconstruction

Alloplastic reconstruction can be performed concomitantly with the mastectomy (immediate), or weeks, months or years later (delayed). While the timing of reconstruction can depend on many factors, immediate reconstruction is generally preferable as the mastectomy skin flaps are pliable and the native inframammary fold is present (1). The greatest benefit of immediate reconstruction could be the potential for fewer operations.

The impact of the timing of alloplastic breast reconstruction on outcomes is not clear. In a prospective, multicenter study, Alderman *et al*. found complications (both total and major) to be associated with immediate reconstructions (4). They suggested that the higher complication rate in the immediate setting might be due to any additional complications from the mastectomy procedure. In comparison, a review of a prospectively maintained database, from a single center examining only expander/implant reconstruction, did not find the timing of reconstruction to be a significant predictor of reconstructive failure (13). Satisfaction with immediate reconstruction has been reported to be greater than delayed reconstruction (5).

Single-stage breast reconstruction

Single-stage breast reconstruction is appropriate in a patient with small, non-ptotic breasts, and good quality skin and muscle (3). An implant is placed at the time of mastectomy and an ADM is used for support and implant coverage. This is also known as direct-to-implant reconstruction. The disadvantage of a direct-to-implant reconstruction is that aesthetic outcomes might not be as good as tissue expander/implant reconstructions, and often a revision procedure is required (3). Increasing breast cup size is associated with a need for early revision surgery (46). When direct-to-implant reconstruction is used in the right patient, both complication rates and revision rates appear to be comparable to two-staged tissue expander/implant based reconstruction (10). The role for this procedure in patients who will require post-mastectomy radiation is still unclear (46).

Use of an acellular dermal matrix

Traditional submuscular placement of a tissue expander requires the elevation of, and coverage with, the pectoralis major and serratus anterior (and sometimes the rectus abdominis). The use of an ADM has been increasing (47), whereby the pectoralis muscle is used to cover the prosthesis anteromedially, and the ADM is used for coverage laterally. This technique allows placement of tissue expanders with greater intraoperative fill volumes, and therefore fewer expansions are required before exchange for the permanent implant (47). In addition, it might have the potential to reduce the rate of encapsulation (48,49).

The use of ADM avoids elevation of the serratus anterior, which was once thought to decrease post-operative pain. However, a multicenter, blinded, randomized controlled trial did not demonstrate any reduction in postoperative pain when using ADM (50). In addition, an increased risk in complications has been demonstrated when using ADM, in particular, seroma (9,47,51), infection (51,52), and reconstructive failure (9,51) rates.

Use of an autologous flap

Tissue expansion/implant based reconstruction requires enough of a healthy skin envelope for a tension-free closure. The native skin and/or muscle envelope may not be adequate to undergo expansion if there are multiple scars, previous radiation injury, or if there was a large skin resection during mastectomy. In these cases, the use of an autologous flap (most commonly the latissimus dorsi myocutaneous flap) can provide coverage of the expander, and eventually implant. Patients requiring a salvage mastectomy after failed lumpectomy/irradiation can benefit from a latissimus dorsi/implant reconstruction (53).

Use of an autologous flap in previously irradiated breasts appears to reduce the incidence of implant related complications (54). The addition of an autologous flap to the implant based procedure increases the length and complexity of the operation, and adds a donor site with potential morbidity (3). In previously irradiated patients, complication rates and reconstructive failure rates in latissimus dorsi flap plus implant reconstruction are not statistically significant when compared to purely abdominal based autologous reconstruction (55). The most common complication when using a latissimus dorsi flap is a dorsal seroma (56).

Use of fat grafting

Fat grafting is an important tool to manage contour deformities in breast reconstruction. It can smooth out a "step-off" between the chest wall and implant, and help camouflage implant rippling. Fat grafting might help to achieve greater satisfaction, improve surrounding skin quality, and decrease implant exposure in patients who undergo implant based reconstruction after radiation (57,58). However, multiple procedures are often necessary, and potential complications include infection, fat necrosis, and oil cysts. Concerns have also been related to the theoretical interference with breast cancer detection (59), though the American Society of Plastic Surgeons task force did not find evidence to support this (60).

Volume of implant-based breast reconstruction practice

High volume implant-based breast reconstruction teams (surgical oncologist and plastic surgeon) tend to have lower complication rates when compared to low volume teams (where high volume teams had performed greater than 300 procedures together) (61). Low volume teams (fewer than 150 procedures performed together) were shown to have higher rates of infection (61). However other studies have failed to show this relationship between complications and surgical team volume (62).

Autologous reconstruction

Patient factors influencing complications and outcomes

Radiation

Radiation appears to negatively affect certain outcomes in autologous breast reconstruction. Radiation contributes to poor cosmesis (63,64), though does not appear to increase major complication rates (63,65). Flaps experience a higher rate of fat necrosis when irradiated. When irradiated muscle-sparing free transverse rectus abdominis myocutaneous (TRAM) flaps were compared to irradiated deep inferior epigastric perforator (DIEP) flaps, rates of fat necrosis were similar (66).

Challenges exist when radiotherapy is required after reconstruction (67). The autologous breast mound can compromise the design and delivery of radiotherapy (68), however increased tumor recurrence and worse clinical outcomes have not been demonstrated (1). Nevertheless, it has been suggested that the technique of delayed-immediate reconstruction (explained below under "Timing of Reconstruction") can be used to balance aesthetic outcomes with the ability to provide optimal radiotherapy (67).

Chemotherapy

Neoadjuvant chemotherapy does not seem to be a predictor of flap loss, microvascular complications (18), or reoperation rate (69). Similarly, fascial healing at the donor site does not appear to be adversely affected (18). However, it has been associated with an increase in overall complications (70), early complications, in the form of wound healing difficulties, and late complications, such as fat necrosis (18). The timing of chemotherapy does not seem to have a significant effect on surgical outcomes (30).

Smoking

The effect of smoking on wound healing and blood supply is known to be harmful. In autologous breast reconstruction, studies have confirmed the deleterious relationship between smoking and post-operative complications (17,19), however the specific complications demonstrated have been variable. Smoking has been associated with an increased risk of wound infection (19), mastectomy flap necrosis (19,71,72), abdominal flap necrosis (19,71,72), abdominal hernia (71), and fat necrosis (19). On the other hand, some studies have not demonstrated an association between smoking and complications (4,18). Regardless, many reconstructive surgeons insist their patients quit smoking before proceeding with an autologous reconstruction.

Obesity/BMI

Patients with a higher BMI are prone to complications (9). Risks increase with the patient's BMI, and obese patients have a greater risk of overall complications when compared to normal weight and overweight patients (73). This increased risk has partly been attributed to intraoperative technical difficulty, as obesity is associated with longer operative times in abdominally based autologous reconstruction (74). Increased health care resource consumption and greater hospital costs also appear to be consequences of the increased perioperative risk in these patients (74).

Overall, minor, early, and late complications are shown to be greater in the obese patient, with a 1.5- to 2-fold increase in flap complications (16) and a 3-fold increase in donor site complications (18). While the majority of overweight and even obese patients can complete autologous breast reconstruction successfully, they should be appropriately counselled that both the risk of failure, and complication rates are higher than normal weight

patients (16,18). On the other hand, a retrospective analysis comparing implant reconstruction versus abdominal-based free flap reconstruction concluded that obese patients, particularly morbidly obese patients, experience lower failure rate with autologous reconstruction rather than implant reconstruction (75).

Age

In general, increasing age is associated with poorer outcomes following surgical procedures. Limited data exists on the relationship between age and outcomes in autologous breast reconstruction. Older patients are more likely to stay in hospital longer than younger patients (76) after autologous breast reconstruction. Rates of post-operative complications, including flap thrombosis (77), do not appear to be significantly different in elderly patients (76). Autologous breast reconstruction can be performed safely in the elderly (76), and age by itself should probably not be viewed as a risk factor for complications. However, older patients are more likely to have other medical comorbidities, and therefore this should be taken into account.

Other medical comorbidities

Hypertension

Hypertension is a risk factor for complications in the setting of autologous breast reconstruction. Hypertension is associated with both minor and major surgical complications (21), and with both breast and abdominal (donor) complications (17). It is also an independent predictor of unplanned readmission after autologous reconstruction, with the risk of readmission quantified as being at least 2 times greater than in a patient without hypertension (78).

Diabetes mellitus

The predisposition of diabetics to infection (79) and microvascular and macrovascular disease (79) are valid reasons to expect an increased rate of complications in these patients. Diabetes has been correlated with both minor surgical complications and post-operative medical complications (21). However, in other studies, diabetes mellitus has demonstrated trends toward association with complications but no statistically significant associations (17,18). Nevertheless, it is sensible for a breast reconstruction patient to attempt glycemic control in the perioperative period.

Mastectomy type: nipple sparing, skin sparing, skin reducing

A high quality autologous reconstruction can be obtained using either a NSM or SSM technique (80). With the preservation of the original skin envelope, inframammary fold, and the NAC in a NSM, the flap can be used to recreate the volume and shape of the original breast. SSM and immediate autologous reconstruction is an oncologically safe procedure (81). For patients undergoing NSMs, aesthetic results are significantly better when compared to SSM (82). However, in NSMs, anastomosis of the pedicle to the internal mammary artery can be difficult due to limited exposure (83), and traction during the operation can increase the chance of partial or complete nipple areola necrosis. While cancer recurrence in the NAC remains a concern, autologous reconstruction after NSM is a reasonable option in the appropriate patient (84).

Prior abdominal surgery

When planning to use an abdominal flap for autologous reconstruction, the finding of an abdominal scar on physical exam could potentially alter the approach to breast reconstruction due to concerns of flap loss and/or donor site complication. Prior abdominal surgery in patients undergoing TRAM based breast reconstruction is associated with minor, major, and overall complication rates (18). Most of the major complications involve partial flap loss (18). Donor site complication rates, including hernia/laxity and wound healing, are also found to be greater. Careful patient selection is especially important in these patients, as smokers with a subcostal scar have been found to have a greater than 6-fold increase in donor site complications (85).

Surgical factors influencing complications and outcomes

Free flap choice

The pedicled TRAM is most common method for autologous breast reconstruction in the United States (1,86,87). Common free tissue transfer options for reconstruction use tissue from the abdomen in the form of either a TRAM, DIEP, or superficial inferior epigastric artery (SIEA) flap. Autologous reconstruction can also be performed using tissue from the thigh or buttock in the form of transverse upper gracilis (TUG), superior gluteal artery perforator (SGAP), inferior gluteal artery perforator (IGAP), or profunda artery perforator (PAP) flaps. The distinct advantage of an autologous reconstruction is the ability to replace "like with like", and provide the patient with a lifelong, natural feeling breast.

When comparing outcomes of pedicled TRAM reconstructions to free flap reconstructions, the incidence of complications (overall, flap-related and nonflap-related) was greater in free flaps in a review of over 2,000 flaps (88).

However, after regression modelling these differences did not appear to be significant. The pedicled TRAM tends to be associated with more fat necrosis than free abdominal flaps (89,90) and with an increased risk of partial and total flap loss in obese patients (91). To decrease these types of complications, especially in "high risk" patients, a vascular delay procedure can be used, where the inferior vascular pedicle is ligated 2 to 3 weeks before reconstruction (92).

The criticism of the free TRAM flap has been related to morbidity from sacrificing the rectus muscle at the donor site (93,94). Patients reconstructed with a free TRAM flap have decreased abdominal strength and have twice the risk of an abdominal bulge or hernia compared to DIEP reconstructions (95). The DIEP flap is thought to offer patients decreased donor site morbidity. Although many studies are able to demonstrate the advantage of the DIEP with respect to the donor site objectively, changes in the ability to perform activities of daily living do not appear to be significantly different from TRAM patients (96). In a systematic review of studies comparing DIEP and free TRAM flaps, DIEP flaps were found to have a higher rate of flap-related complications, and a 2-fold increase in the risk of fat necrosis and flap loss compared to free TRAM flaps (95). Therefore the reconstructive advantage of the DIEP flap has remained uncertain, in general seems to be less reliable than the free TRAM flap, and has gained only cautious acceptance among many reconstructive surgeons (95).

The major benefit of the SIEA flap is the ability to harvest abdominal tissue without violating the abdominal wall fascia, therefore leaving both the fascia and rectus muscle intact and minimizing donor site morbidity (97). On the other hand, the flap has a smaller pedicle length and diameter (98), and flap size is limited to only half of the abdominal skin island for reconstruction (1). When compared to free TRAM and DIEP flaps, use of the SIEA flap has also been found to be a risk factor for flap thrombosis (77), and is associated with an increased risk of fat necrosis (1). The significantly higher rate of thrombotic complications associated with the SIEA flap limits the indications for this type of reconstruction.

Autologous reconstruction using tissue from the thigh or buttock (TUG, SGAP, IGAP, PAP) is less common, typically only indicated in patients who require a small to medium size breast reconstruction, have either abdominal scarring or limited abdominal tissue, and excess tissue in the thigh/buttock region. The literature describing outcomes and complications using autologous thigh/buttock flaps is in its infancy compared to abdominal based flaps.

Timing of reconstruction

Similar to alloplastic reconstruction, autologous reconstruction can be performed either immediately or in a delayed fashion with respect to the mastectomy. Immediate reconstruction potentially exposes the patient to fewer operations, can save resource costs (99,100), and gives the patient the best chance at a good aesthetic result (101). In delayed reconstruction, mastectomy skin flaps are often scarred and less compliant (1), and a higher rate of free flap thrombosis has been found to occur (77). However similar rates of both major and minor complications have been reported between patients undergoing either immediate or delayed reconstruction with a TRAM free-flap (102).

The requirement of post-mastectomy radiotherapy has been considered to be a relative contraindication to immediate reconstruction (103). An alternative strategy, known as "delayed-immediate" autologous reconstruction, has been used (104). This is a two stage approach in which a filled tissue expander is placed after mastectomy. If radiotherapy is not required, definitive autologous reconstruction is performed. If radiotherapy is required, the expander is deflated, radiotherapy is administered, the expander is re-inflated, and autologous reconstruction performed (104). When compared to "delayed" reconstruction, "delayed-immediate" has been shown to have similar flap-related complication rates, decreased rates of revision surgery (105), and a better aesthetic outcome (106).

Fat grafting

Fat grafting can be used to address step-off deformities (between the chest wall and the flap), intrinsic deformities (e.g., from fat necrosis) and extrinsic deformities (e.g., from radiation or scar contracture) (6). Fat grafting can also be used to help augment size in a volume-deficient reconstruction, therefore allowing certain patients with barely enough soft tissue for a microvascular free flap to undergo autologous reconstruction (107). In a review of mostly autologous reconstructed patients, aesthetic outcomes were significantly improved with fat grafting, though half of the patients required more than one procedure, and complications occurred in approximately 6% of procedures (6).

Volume of autologous breast reconstruction practice

High volume autologous breast reconstruction centers tend to have lower complication rates when compared to low and medium volume centers (where high volume was "greater

than 44 procedures per year") (108). Both surgery-specific and systemic complications were inversely related to volume of reconstruction at the center (108). When examining microsurgical cases, low-volume centers had a 2-fold increase in surgery-specific complications when compared to high-volume centers (108).

Summary

Alloplastic breast reconstruction outcomes can be negatively affected by certain patient factors. Pre- or post-mastectomy radiotherapy, smoking, increased BMI, hypertension, and prior breast conserving therapy are all associated with an increase in complications and/or inferior outcomes. Silicone gel implants provide a softer, more natural feeling breast and these patients appear to have greater satisfaction than those with saline implants. Patient satisfaction and aesthetic outcomes are not different between reconstructions that use either round or anatomically shaped implants. Immediate reconstruction, and the use of fat grafting techniques are likely to improve aesthetic outcomes.

Autologous breast reconstruction outcomes are affected in a deleterious manner by radiation, increased BMI, certain previous abdominal surgery, delayed reconstruction, smoking, hypertension, and most likely diabetes. When these risk factors are present, a free microvascular reconstructive technique is preferred over a pedicled flap for patients undergoing autologous reconstruction. Reduced donor site morbidity can be seen in DIEP flap reconstruction, compared to TRAM flap, but is more obvious in bilateral reconstructions. The use of the SIEA flap in breast reconstruction is limited due to the higher rate of vessel thrombosis. Other types of free flaps, TUG, SGAP, IGAP and PAP flaps, tend to be options when abdominal tissue is not available. Fat grafting can be used to improve aesthetic outcomes, and high volume centers are associated with fewer complications, especially in free flap reconstruction.

Offering patients an opportunity for breast reconstruction is an important component of the treatment for breast cancer. There are many options for both alloplastic and autologous reconstruction. Ultimately, patient and surgical risk factors should be considered in concert with the patient's wishes when deciding upon a reconstructive strategy.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Serletti JM, Fosnot J, Nelson JA, et al. Breast reconstruction after breast cancer. Plast Reconstr Surg 2011;127:124e-35e.
2. Alderman A, Gutowski K, Ahuja A, et al. ASPS Clinical Practice Guideline Summary on Breast Reconstruction with Expanders and Implants. Plast Reconstr Surg 2014;134:648e-55e.
3. Cordeiro PG. Breast reconstruction after surgery for breast cancer. N Engl J Med 2008;359:1590-601.
4. Alderman AK, Wilkins EG, Kim HM, et al. Complications in postmastectomy breast reconstruction: two-year results of the Michigan Breast Reconstruction Outcome Study. Plast Reconstr Surg 2002;109:2265-74.
5. Fernández-Delgado J, Lopez-Pedraza MJ, Blasco JA, et al. Satisfaction with and psychological impact of immediate and deferred breast reconstruction. Ann Oncol 2008;19:1430-4.
6. de Blacam C, Momoh AO, Colakoglu S, et al. Evaluation of clinical outcomes and aesthetic results after autologous fat grafting for contour deformities of the reconstructed breast. Plast Reconstr Surg 2011;128:411e-8e.
7. McCarthy CM, Klassen AF, Cano SJ, et al. Patient satisfaction with postmastectomy breast reconstruction: a comparison of saline and silicone implants. Cancer 2010;116:5584-91.
8. Macadam SA, Ho AL, Cook EF, et al. Patient satisfaction and health-related quality of life following breast reconstruction: patient-reported outcomes among saline and silicone implant recipients. Plast Reconstr Surg 2010;125:761-71.
9. Antony AK, McCarthy CM, Cordeiro PG, et al. Acellular human dermis implantation in 153 immediate two-stage tissue expander breast reconstructions: determining the incidence and significant predictors of complications. Plast Reconstr Surg 2010;125:1606-14.
10. Salzberg CA, Ashikari AY, Koch RM, et al. An 8-year experience of direct-to-implant immediate breast reconstruction using human acellular dermal matrix (AlloDerm). Plast Reconstr Surg 2011;127:514-24.
11. Spear SL, Majidian A. Immediate breast reconstruction in two stages using textured, integrated-valve tissue

expanders and breast implants: a retrospective review of 171 consecutive breast reconstructions from 1989 to 1996. Plast Reconstr Surg 1998;101:53-63.
12. Cordeiro PG, McCarthy CM. A single surgeon's 12-year experience with tissue expander/implant breast reconstruction: part I. A prospective analysis of early complications. Plast Reconstr Surg 2006;118:825-31.
13. McCarthy CM, Mehrara BJ, Riedel E, et al. Predicting complications following expander/implant breast reconstruction: an outcomes analysis based on preoperative clinical risk. Plast Reconstr Surg 2008;121:1886-92.
14. Colwell AS, Tessler O, Lin AM, et al. Breast reconstruction following nipple-sparing mastectomy: predictors of complications, reconstruction outcomes, and 5-year trends. Plast Reconstr Surg 2014;133:496-506.
15. Blondeel PN. One hundred free DIEP flap breast reconstructions: a personal experience. Br J Plast Surg 1999;52:104-11.
16. Chang DW, Wang B, Robb GL, et al. Effect of obesity on flap and donor-site complications in free transverse rectus abdominis myocutaneous flap breast reconstruction. Plast Reconstr Surg 2000;105:1640-8.
17. Gill PS, Hunt JP, Guerra AB, et al. A 10-year retrospective review of 758 DIEP flaps for breast reconstruction. Plast Reconstr Surg 2004;113:1153-60.
18. Mehrara BJ, Santoro TD, Arcilla E, et al. Complications after microvascular breast reconstruction: experience with 1195 flaps. Plast Reconstr Surg 2006;118:1100-9; discussion 1110-1.
19. Selber JC, Kurichi JE, Vega SJ, et al. Risk factors and complications in free TRAM flap breast reconstruction. Ann Plast Surg 2006;56:492-7.
20. Damen TH, Morritt AN, Zhong T, et al. Improving outcomes in microsurgical breast reconstruction: lessons learnt from 406 consecutive DIEP/TRAM flaps performed by a single surgeon. J Plast Reconstr Aesthet Surg 2013;66:1032-8.
21. Fischer JP, Sieber B, Nelson JA, et al. Comprehensive outcome and cost analysis of free tissue transfer for breast reconstruction: an experience with 1303 flaps. Plast Reconstr Surg 2013;131:195-203.
22. Ascherman JA, Hanasono MM, Newman MI, et al. Implant reconstruction in breast cancer patients treated with radiation therapy. Plast Reconstr Surg 2006;117:359-65.
23. Cordeiro PG, Pusic AL, Disa JJ, et al. Irradiation after immediate tissue expander/implant breast reconstruction: outcomes, complications, aesthetic results, and satisfaction among 156 patients. Plast Reconstr Surg 2004;113:877-81.
24. McCarthy CM, Pusic AL, Disa JJ, et al. Unilateral postoperative chest wall radiotherapy in bilateral tissue expander/implant reconstruction patients: a prospective outcomes analysis. Plast Reconstr Surg 2005;116:1642-7.
25. Francis SH, Ruberg RL, Stevenson KB, et al. Independent risk factors for infection in tissue expander breast reconstruction. Plast Reconstr Surg 2009;124:1790-6.
26. Ho A, Cordeiro P, Disa J, et al. Long-term outcomes in breast cancer patients undergoing immediate 2-stage expander/implant reconstruction and postmastectomy radiation. Cancer 2012;118:2552-9.
27. Krueger EA, Wilkins EG, Strawderman M, et al. Complications and patient satisfaction following expander/implant breast reconstruction with and without radiotherapy. Int J Radiat Oncol Biol Phys 2001;49:713-21.
28. Albornoz CR, Matros E, McCarthy CM, et al. Implant breast reconstruction and radiation: a multicenter analysis of long-term health-related quality of life and satisfaction. Ann Surg Oncol 2014;21:2159-64.
29. Song J, Zhang X, Liu Q, et al. Impact of neoadjuvant chemotherapy on immediate breast reconstruction: a meta-analysis. PLoS One 2014;9:e98225.
30. Warren Peled A, Itakura K, Foster RD, et al. Impact of chemotherapy on postoperative complications after mastectomy and immediate breast reconstruction. Arch Surg 2010;145:880-5.
31. Gordon CR, Rojavin Y, Patel M, et al. A review on bevacizumab and surgical wound healing: an important warning to all surgeons. Ann Plast Surg 2009;62:707-9.
32. Kansal KJ, Dominici LS, Tolaney SM, et al. Neoadjuvant bevacizumab: surgical complications of mastectomy with and without reconstruction. Breast Cancer Res Treat 2013;141:255-9.
33. Goodwin SJ, McCarthy CM, Pusic AL, et al. Complications in smokers after postmastectomy tissue expander/implant breast reconstruction. Ann Plast Surg 2005;55:16-9; discussion 19-20.
34. Wade TD, Zhu G, Martin NG. Body mass index and breast size in women: same or different genes? Twin Res Hum Genet 2010;13:450-4.
35. Nahabedian MY, Tsangaris T, Momen B, et al. Infectious complications following breast reconstruction with expanders and implants. Plast Reconstr Surg 2003;112:467-76.
36. Cordeiro PG, Snell L, Heerdt A, et al. Immediate tissue expander/implast breast reconstruction after salvage

mastectomy for cancer recurrence following lumpectomy/irradiation. Plast Reconstr Surg 2012;129:341-50.
37. Butler CE, Kronowitz SJ. Discussion. Immediate tissue expander/implant breast reconstruction after salvage mastectomy for cancer recurrence following lumpectomy/irradiation. Plast Reconstr Surg 2012;129:351-3.
38. Chen CM, Disa JJ, Sacchini V, et al. Nipple-sparing mastectomy and immediate tissue expander/implant breast reconstruction. Plast Reconstr Surg 2009;124:1772-80.
39. Gould DJ, Hunt KK, Liu J, et al. Impact of surgical techniques, biomaterials, and patient variables on rate of nipple necrosis after nipple-sparing mastectomy. Plast Reconstr Surg 2013;132:330e-8e.
40. Didier F, Radice D, Gandini S, et al. Does nipple preservation in mastectomy improve satisfaction with cosmetic results, psychological adjustment, body image and sexuality? Breast Cancer Res Treat 2009;118:623-33.
41. Nava MB, Catanuto G, Pennati A, et al. Conservative mastectomies. Aesthetic Plast Surg 2009;33:681-6.
42. Macadam SA, Ho AL, Lennox PA, et al. Patient-reported satisfaction and health-related quality of life following breast reconstruction: a comparison of shaped cohesive gel and round cohesive gel implant recipients. Plast Reconstr Surg 2013;131:431-41.
43. Gahm J, Edsander-Nord A, Jurell G, et al. No differences in aesthetic outcome or patient satisfaction between anatomically shaped and round expandable implants in bilateral breast reconstructions: a randomized study. Plast Reconstr Surg 2010;126:1419-27.
44. Cordeiro PG, McCarthy CM. A single surgeon's 12-year experience with tissue expander/implant breast reconstruction: part II. An analysis of long-term complications, aesthetic outcomes, and patient satisfaction. Plast Reconstr Surg 2006;118:832-9.
45. Karlson EW, Hankinson SE, Liang MH, et al. Association of silicone breast implants with immunologic abnormalities: a prospective study. Am J Med 1999;106:11-9.
46. Gdalevitch P, Ho A, Genoway K, et al. Direct-to-implant single-stage immediate breast reconstruction with acellular dermal matrix: predictors of failure. Plast Reconstr Surg 2014;133:738e-47e.
47. Sbitany H, Serletti JM. Acellular dermis-assisted prosthetic breast reconstruction: a systematic and critical review of efficacy and associated morbidity. Plast Reconstr Surg 2011;128:1162-9.
48. Stump A, Holton LH 3rd, Connor J, et al. The use of acellular dermal matrix to prevent capsule formation around implants in a primate model. Plast Reconstr Surg 2009;124:82-91.
49. Basu CB, Leong M, Hicks MJ. Acellular cadaveric dermis decreases the inflammatory response in capsule formation in reconstructive breast surgery. Plast Reconstr Surg 2010;126:1842-7.
50. McCarthy CM, Lee CN, Halvorson EG, et al. The use of acellular dermal matrices in two-stage expander/implant reconstruction: a multicenter, blinded, randomized controlled trial. Plast Reconstr Surg 2012;130:57S-66S.
51. Kim JY, Davila AA, Persing S, et al. A meta-analysis of human acellular dermis and submuscular tissue expander breast reconstruction. Plast Reconstr Surg 2012;129:28-41.
52. Brooke S, Mesa J, Uluer M, et al. Complications in tissue expander breast reconstruction: a comparison of AlloDerm, DermaMatrix, and FlexHD acellular inferior pole dermal slings. Ann Plast Surg 2012;69:347-9.
53. Disa JJ, McCarthy CM, Mehrara BJ, et al. Immediate latissimus dorsi/prosthetic breast reconstruction following salvage mastectomy after failed lumpectomy/irradiation. Plast Reconstr Surg 2008;121:159e-64e.
54. Chang DW, Barnea Y, Robb GL. Effects of an autologous flap combined with an implant for breast reconstruction: an evaluation of 1000 consecutive reconstructions of previously irradiated breasts. Plast Reconstr Surg 2008;122:356-62.
55. Levine SM, Patel N, Disa JJ. Outcomes of delayed abdominal-based autologous reconstruction versus latissimus dorsi flap plus implant reconstruction in previously irradiated patients. Ann Plast Surg 2012;69:380-2.
56. Pinsolle V, Grinfeder C, Mathoulin-Pelissier S, et al. Complications analysis of 266 immediate breast reconstructions. J Plast Reconstr Aesthet Surg 2006;59:1017-24.
57. Serra-Renom JM, Munoz-Olmo JL, Serra-Mestre JM. Fat grafting in postmastectomy breast reconstruction with expanders and prostheses in patients who have received radiotherapy: formation of new subcutaneous tissue. Plast Reconstr Surg 2010;125:12-8.
58. Ribuffo D, Atzeni M, Guerra M, et al. Treatment of irradiated expanders: protective lipofilling allows immediate prosthetic breast reconstruction in the setting of postoperative radiotherapy. Aesthetic Plast Surg 2013;37:1146-52.
59. Spear SL, Wilson HB, Lockwood MD. Fat injection to correct contour deformities in the reconstructed breast.

Plast Reconstr Surg 2005;116:1300-5.
60. Gutowski KA. Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. Plast Reconstr Surg 2009;124:272-80.
61. Gfrerer L, Mattos D, Mastroianni M, et al. Assessment of patient factors, surgeons, and surgeon teams in immediate implant-based breast reconstruction outcomes. Plast Reconstr Surg 2015;135:245e-52e.
62. Seth AK, Hirsch EM, Kim JY, et al. Two surgeons, one patient: the impact of surgeon-surgeon familiarity on patient outcomes following mastectomy with immediate reconstruction. Breast 2013;22:914-8.
63. Spear SL, Ducic I, Low M, et al. The effect of radiation on pedicled TRAM flap breast reconstruction: outcomes and implications. Plast Reconstr Surg 2005;115:84-95.
64. Carlson GW, Page AL, Peters K, et al. Effects of radiation therapy on pedicled transverse rectus abdominis myocutaneous flap breast reconstruction. Ann Plast Surg 2008;60:568-72.
65. Berry T, Brooks S, Sydow N, et al. Complication rates of radiation on tissue expander and autologous tissue breast reconstruction. Ann Surg Oncol 2010;17 Suppl 3:202-10.
66. Garvey PB, Clemens MW, Hoy AE, et al. Muscle-sparing TRAM flap does not protect breast reconstruction from postmastectomy radiation damage compared with the DIEP flap. Plast Reconstr Surg 2014;133:223-33.
67. Kronowitz SJ, Robb GL. Radiation therapy and breast reconstruction: a critical review of the literature. Plast Reconstr Surg 2009;124:395-408.
68. Motwani SB, Strom EA, Schechter NR, et al. The impact of immediate breast reconstruction on the technical delivery of postmastectomy radiotherapy. Int J Radiat Oncol Biol Phys 2006;66:76-82.
69. Schaverien MV, Munnoch DA. Effect of neoadjuvant chemotherapy on outcomes of immediate free autologous breast reconstruction. Eur J Surg Oncol 2013;39:430-6.
70. Deutsch MF, Smith M, Wang B, et al. Immediate breast reconstruction with the TRAM flap after neoadjuvant therapy. Ann Plast Surg 1999;42:240-4.
71. Chang DW, Reece GP, Wang B, et al. Effect of smoking on complications in patients undergoing free TRAM flap breast reconstruction. Plast Reconstr Surg 2000;105:2374-80.
72. Kroll SS. Necrosis of abdominoplasty and other secondary flaps after TRAM flap breast reconstruction. Plast Reconstr Surg 1994;94:637-43.
73. Spear SL, Ducic I, Cuoco F, et al. Effect of obesity on flap and donor-site complications in pedicled TRAM flap breast reconstruction. Plast Reconstr Surg 2007;119:788-95.
74. Fischer JP, Nelson JA, Sieber B, et al. Free tissue transfer in the obese patient: an outcome and cost analysis in 1258 consecutive abdominally based reconstructions. Plast Reconstr Surg 2013;131:681e-92e.
75. Garvey PB, Villa MT, Rozanski AT, et al. The advantages of free abdominal-based flaps over implants for breast reconstruction in obese patients. Plast Reconstr Surg 2012;130:991-1000.
76. Garvey PB, Buchel EW, Pockaj BA, et al. Outcomes after autologous breast reconstruction in elderly patients. Plastic Surgery 2004. Philadelphia, PA, USA, 2004.
77. Masoomi H, Clark EG, Paydar KZ, et al. Predictive risk factors of free flap thrombosis in breast reconstruction surgery. Microsurgery 2014;34:589-94.
78. Mlodinow AS, Ver Halen JP, Lim S, et al. Predictors of readmission after breast reconstruction: a multi-institutional analysis of 5012 patients. Ann Plast Surg 2013;71:335-41.
79. Janis JE, Harrison B. Wound healing: part I. Basic science. Plast Reconstr Surg 2014;133:199e-207e.
80. Gherardini G, Thomas R, Basoccu G, et al. Immediate breast reconstruction with the transverse rectus abdominis musculocutaneous flap after skin-sparing mastectomy. Int Surg 2001;86:246-51.
81. Liang TJ, Wang BW, Liu SI, et al. Recurrence after skin-sparing mastectomy and immediate transverse rectus abdominis musculocutaneous flap reconstruction for invasive breast cancer. World J Surg Oncol 2013;11:194.
82. Gerber B, Krause A, Reimer T, et al. Skin-sparing mastectomy with conservation of the nipple-areola complex and autologous reconstruction is an oncologically safe procedure. Ann Surg 2003;238:120-7.
83. Munhoz AM, Montag E, Filassi JR, et al. Immediate nipple-areola-sparing mastectomy reconstruction: An update on oncological and reconstruction techniques. World J Clin Oncol 2014;5:478-94.
84. Dao TN, Verheyden CN. TRAM flaps: a reconstructive option after bilateral nipple-sparing total mastectomy. Plast Reconstr Surg 2005;116:986-92.
85. Losken A, Carlson GW, Jones GE, et al. Importance of right subcostal incisions in patients undergoing TRAM flap breast reconstruction. Ann Plast Surg 2002;49:115-9.
86. Kulkarni AR, Sears ED, Atisha DM, et al. Use of autologous and microsurgical breast reconstruction by U.S. plastic surgeons. Plast Reconstr Surg 2013;132:534-41.
87. Gurunluoglu R, Gurunluoglu A, Williams SA, et al.

Current trends in breast reconstruction: survey of American Society of Plastic Surgeons 2010. Ann Plast Surg 2013;70:103-10.
88. Gart MS, Smetona JT, Hanwright PJ, et al. Autologous options for postmastectomy breast reconstruction: a comparison of outcomes based on the American College of Surgeons National Surgical Quality Improvement Program. J Am Coll Surg 2013;216:229-38.
89. Andrades P, Fix RJ, Danilla S, et al. Ischemic complications in pedicle, free, and muscle sparing transverse rectus abdominis myocutaneous flaps for breast reconstruction. Ann Plast Surg 2008;60:562-7.
90. Garvey PB, Buchel EW, Pockaj BA, et al. DIEP and pedicled TRAM flaps: a comparison of outcomes. Plast Reconstr Surg 2006;117:1711-9; discussion 1720-1.
91. Moran SL, Serletti JM. Outcome comparison between free and pedicled TRAM flap breast reconstruction in the obese patient. Plast Reconstr Surg 2001;108:1954-60; discussion 1961-2.
92. Kanchwala SK, Bucky LP. Optimizing pedicled transverse rectus abdominis muscle flap breast reconstruction. Cancer J 2008;14:236-40.
93. Blondeel N, Vanderstraeten GG, Monstrey SJ, et al. The donor site morbidity of free DIEP flaps and free TRAM flaps for breast reconstruction. Br J Plast Surg 1997;50:322-30.
94. Chen CM, Halvorson EG, Disa JJ, et al. Immediate postoperative complications in DIEP versus free/muscle-sparing TRAM flaps. Plast Reconstr Surg 2007;120:1477-82.
95. Man LX, Selber JC, Serletti JM. Abdominal wall following free TRAM or DIEP flap reconstruction: a meta-analysis and critical review. Plast Reconstr Surg 2009;124:752-64.
96. Atisha D, Alderman AK. A systematic review of abdominal wall function following abdominal flaps for postmastectomy breast reconstruction. Ann Plast Surg 2009;63:222-30.
97. Wu LC, Bajaj A, Chang DW, et al. Comparison of donor-site morbidity of SIEA, DIEP, and muscle-sparing TRAM flaps for breast reconstruction. Plast Reconstr Surg 2008;122:702-9.
98. Chevray PM. Breast reconstruction with superficial inferior epigastric artery flaps: a prospective comparison with TRAM and DIEP flaps. Plast Reconstr Surg 2004;114:1077-83; discussion 1084-5.
99. Khoo A, Kroll SS, Reece GP, et al. A comparison of resource costs of immediate and delayed breast reconstruction. Plast Reconstr Surg 1998;101:964-8; discussion 969-70.
100. Neyt MJ, Blondeel PN, Morrison CM, et al. Comparing the cost of delayed and immediate autologous breast reconstruction in Belgium. Br J Plast Surg 2005;58:493-7.
101. Chevray PM. Timing of breast reconstruction: immediate versus delayed. Cancer J 2008;14:223-9.
102. DeBono R, Thompson A, Stevenson JH. Immediate versus delayed free TRAM breast reconstruction: an analysis of perioperative factors and complications. Br J Plast Surg 2002;55:111-6.
103. Albornoz CR, Cordeiro PG, Farias-Eisner G, et al. Diminishing relative contraindications for immediate breast reconstruction. Plast Reconstr Surg 2014;134:363e-9e.
104. Kronowitz SJ, Hunt KK, Kuerer HM, et al. Delayed-immediate breast reconstruction. Plast Reconstr Surg 2004;113:1617-28.
105. Patel KM, Albino F, Fan KL, et al. Microvascular autologous breast reconstruction in the context of radiation therapy: comparing two reconstructive algorithms. Plast Reconstr Surg 2013;132:251-7.
106. Albino FP, Patel KM, Smith JR, et al. Delayed versus Delayed-Immediate Autologous Breast Reconstruction: A Blinded Evaluation of Aesthetic Outcomes. Arch Plast Surg 2014;41:264-70.
107. Weichman KE, Broer PN, Tanna N, et al. The role of autologous fat grafting in secondary microsurgical breast reconstruction. Ann Plast Surg 2013;71:24-30.
108. Albornoz CR, Cordeiro PG, Hishon L, et al. A nationwide analysis of the relationship between hospital volume and outcome for autologous breast reconstruction. Plast Reconstr Surg 2013;132:192e-200e.

Cite this article as: Voineskos SH, Frank SG, Cordeiro PG. Breast reconstruction following conservative mastectomies: predictors of complications and outcomes. Gland Surg 2015;4(6):484-496. doi: 10.3978/j.issn.2227-684X.2015.04.13

Surgical management of breast cancer in China: the Fudan University Shanghai Cancer Center experience

Xi-Yu Liu*, Zong-Chao Gou*, Zhi-Gang Cao, Yi-Zhou Jiang, Zhi-Ming Shao

Department of Breast Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, China

Contributions: (I) Conception and design: All authors; (II) Administrative support: All authors; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work.

Correspondence to: Zhi-Ming Shao. Fudan University Shanghai Cancer Center, Shanghai 200032, China. Email: zhimingshao@yahoo.com.

Abstract: Breast cancer is the most frequently diagnosed cancer in Chinese women, and early-stage patients are significantly increasing. Surgery is the main treatment for early-stage breast cancer with the "minimally invasive procedures" concept. The modality of breast cancer surgery has changed greatly in recent years in China, especially in our cancer center, Fudan University Shanghai Cancer Center (FUSCC). Firstly, pre-surgery biopsy is a routine procedure, which is mainly assisted by imaging instruments. Secondly, the number of breast-conserving surgery (BCS) and simple mastectomy procedures with or without sentinel lymph node biopsy (SM ± SLNB) is increasing gradually; radical mastectomy (RM) and modified radical mastectomy (MRM) are decreasing annually. Thirdly, SLNB has become a routine procedure in our center; it is safe and effective for replacing axillary lymph node dissection (ALND). Finally, reconstruction surgery has progressively advanced, although the operation cases are limited. This review article looks back at the development of breast cancer surgery, highlights the hallmarks of surgical management in our center as well as in China and discusses the future necessary efforts to improve the outcome and life quality for Chinese patients.

Keywords: Breast cancer in China; Fudan University Cancer Center; surgical management

Submitted May 24, 2017. Accepted for publication Jun 26, 2017.
doi: 10.21037/tcr.2017.06.35
View this article at: http://dx.doi.org/10.21037/tcr.2017.06.35

Introduction

Breast cancer is the most frequently diagnosed malignant tumor in Chinese women (15% of all cancer), and it ranks as the sixth leading cause of cancer-related death (1). Chinese breast cancer has had a significant upward trend in age-standardized incidence rates, especially early-stage (stage 0–II) cancer (1-3). Currently, surgery still dominates the treatment for early-stage breast cancer, although adjuvant therapy has rapidly developed (4).

A transition from "acceptable maximum treatment" to "minimally invasive procedures" in the concept of surgery has been continuing since Halsted described radical mastectomy (RM) in 1894 (5). On one hand, mastectomy has been replaced by breast conservative surgery (BCS). A series of large clinical trials and meta-analyses has demonstrated that the survival of patients who underwent mastectomy did not have an advantage, and these patients instead endured more harm than those who underwent BCS followed by radiation (6-9). The breast cancer incidence of Chinese women has undergone rapid growth in recent decades as has surgical management (2,10). However, the BCS rate in China is still lower than in developed countries, and modified radical mastectomy (MRM) is still the primary surgery (11,12). On the other hand, another minimally invasive model, sentinel lymph node biopsy (SLNB), which is recommended by American Society of Clinical Oncology (ASCO) and National Comprehensive Cancer Network (NCCN) guidelines, offers better life quality for patients whose lymph nodes are negative and shows similar efficacy to axillary lymph node dissection (ALND) (4,13-15). Recent

clinical trials, such as IBCSG 23-01 and Z0011, suggest that micro-metastasis or a limited number of positive sentinel lymph nodes (SLNs) should be considered to avoid ALND (16,17). This topic is still in discussion in China because of the limited pathological diagnosis methods. In addition, reconstruction surgery has undergone great advances in China, but the proportion of reconstruction surgery in total breast cancer surgery is still far behind international progress in this field.

Chinese oncologists have made substantial efforts to maintain a balance between patient safety, minimally invasive procedures and cost-benefit considerations, although there are some obstacles of accessibility to optimal treatment in China, including the developing socioeconomic status, low rate of early detection and lack of insurance coverage for many new medications. On the other hand, many breast cancer patients are diagnosed at a relatively late stage, making their chance of less-extensive surgery low. As we conduct the highest number of breast cancer surgeries in Shanghai and the 5-year survival rate has reached 93% for operable patients, we will introduce the surgical management mode of breast cancer in our cancer center, Fudan University Shanghai Cancer Center (FUSCC), and share our experiences of clinical exploration during the last decade in this review.

Pre-surgery treatment: cross-link of imaging and surgery

Imaging techniques have been widely used in the screening and diagnosis of breast cancer. In this section, we will introduce our routine work in pre-surgery diagnosis. In screening, we utilize mammography, ultrasonography and clinical examination in combination. Core-needle biopsy (CNB) is used in the preoperative diagnosis panel. Magnetic resonance (MR) guided biopsy is also discussed in our exploration.

Combined methods of screening are employed for screening in Chinese women. Early detection and diagnosis can reduce the breast cancer mortality, help maintain the shape of the breast and improve patient quality of life. In America, both the American Cancer Society (ACS) and U.S. Preventive Services Task Force (USPSTF) recommend screening for breast cancer from 40 years of age, while for women between 40 and 49 years of age, ACS advocates for annual screening testing (qualified recommendation) and USPSTF for biennial testing (C recommendation) (18,19). The benefits of mammography are not clear for females under 50 years of age. The resolution of mammography is not satisfactory for dense breasts, and ultrasonography can detect 27% or more malignant lesions within dense breasts as a supplementary tool (20). In China, the gland tissue of breast cancer patients tends to be denser, which is partially due to the ethnic characteristics of Asian females. Additionally, there is a higher proportion of young patients, whose breasts are prone to be denser, in China. Despite an increasing shift to older age, the mean age at the diagnosis of breast cancer in China is 45–55 years, which is much younger than in developed countries (21). To test the combined method in the Chinese community, FUSCC, along with community hospitals, practiced screening in the Qibao community, Shanghai. In Qibao, we screened a total of 13,183 females and diagnosed 33 cases of breast cancer. Up to 33.3% of those diagnosed with breast cancer underwent BCS, and 27.3% avoided undergoing ALND. Meanwhile, of the total patients at FUSCC in 2009, the BCS and axillary conserving rates were 14% and 8%, respectively. Based on the population-based study, the Chinese breast cancer guidelines [China Anti-Cancer Association guidelines for breast cancer diagnosis and treatment (CACA guidelines for short)] recommend annual or biennial mammography for all females over 40 years old, and ultrasonography is suggested for females with dense breasts (22). However, further research is needed to ascertain the benefits of the screening methods. In daily practice, the combined methods facilitate the early detection of breast cancer and improve the rate of less invasive surgery.

In the diagnosis procedure, CNB features accurate diagnosis, minimally invasive biopsy and high effectiveness, reducing the waiting time during surgery for pathology reports. In FUSCC, all biopsies were performed by open surgery before 2000. The rate of CNB in all biopsies was only 5% in 2002 and increased to 30–50% after 2010 (*Figure 1*) (*Figure 1* based on data from FUSCC). Compared to open biopsy, CNB has the same accuracy and a lower coincidence rate (2–10% *vs.* <1%) (23).There are other less invasive methods in the diagnosis, such as fine-needle biopsy (FNB) and the Mammotome system. FNB shows relatively high undertriage and the possibility of false positive rates. The Mammotome system has a high accuracy rate, but it is limited due to relatively high cost and less available equipment. In all, CNB is recommended in pre-surgery diagnosis nationwide because of its accuracy and availability.

In addition to the widely used imaging methods mentioned above, we have explored MR guided biopsy in

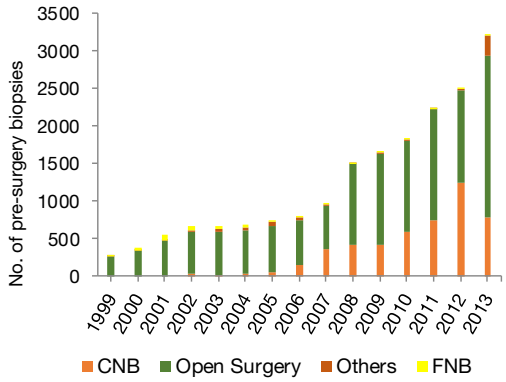


Figure 1 Pre-surgery biopsy mode in 1999–2013 in FUSCC. CNB, core needle biopsy; FNB, fine needle biopsy; FUSCC, Fudan University Shanghai Cancer Center. Figure based on data from FUSCC.

clinical practice. Imaging guided breast biopsy is divided into three categories: X-ray guided, ultrasound guided and MR guided biopsy. Ultrasound guided biopsy is usually the first choice because of its easy accessibility, and this technique is suggested for the underdeveloped area in China. X-ray guided biopsy has high diagnostic value along with low invasive lesions, and it is limited by the high equipment requirements. As a supplement to the above two methods, magnetic resonance imaging (MRI) is advantageous for its high sensitivity in detecting breast cancer (24), while its cost effectiveness requires further consideration. From 2011 to 2012, we performed MR guided biopsy in 38 cases and successfully observed five cases of invasive ductal carcinoma (IDC) and five of ductal carcinoma *in situ* (DCIS). This method can address many sub-clinical cases as well as improve the efficiency and targeting. MRI guided location evaluation and biopsy are suitable for "concealed" lesions that are unclear in mammography or ultrasonography but clear with MRI. Although MR guided biopsy has many advantages, difficulties remain, such as the disappearance of augmented loci during locating and strict requirements for the location system.

In all, the development of imaging allows for substantial work to be completed before surgery in non-invasive or minimally invasive procedures. These combined methods in screening make earlier diagnosis possible, and allow for less invasive surgery and better life quality. Additionally, CNB facilitates accurate diagnosis before surgery, with small lesions and a relatively low cost.

Surgical mode: breast conserving surgery and simple mastectomy (SM) are gradually increasing

The surgical modalities for breast cancer have undergone successive changes and revolutions with the development of adjuvant therapy and cancer biology. In this part, we will introduce the developing trend and current situation of our surgical mode, especially in terms of the increase in BCS and SM.

Since Halsted established RM in 1894, the surgery area has been undergoing a period of expansion and shrinkage. While Margottini and Urban favored removing the internal lymph node for extensive radical mastectomy (ERM), Patey and Auchincloss attempted MRM based on new anatomic knowledge about lymph vessels. Gradually, MRM began to dominate in the subsequent decades (5). In the 1970s, BCS was introduced into the breast cancer surgical field by Veronesi and Atkin, and it was successively supported by a series of prospective clinical trials and retrospective meta-analyses (6-9,24). It has been accepted by surgeons that BCS followed by radiation offers a somewhat similar survival benefit as mastectomy. Since then, BSC gradually advanced and became the first surgical choice for early breast cancer patients. For example, up to 60–70% of early-stage patients undergo BCS and 36% undergo mastectomy in the USA (11). Encouraged by BCS success, the concept of the surgical treatment mode evolved from the "acceptable maximum treatment" to "minimally invasive procedures."

In China, the "minimally effective treatment" concept has been widely accepted and respected by doctors. BCS has been used since the mid-1990s, but its rate of use has been much slower in China than in developed countries (2). The specific and detailed operation guidelines of BCS were written in the CACA guidelines since the first edition. It contains detailed necessary requirements, indications, relative contraindications and absolute contraindications for BCS. After continuous revision, an expertise group from the CACA panel encouraged all early breast cancer patients who are willing to undergo a breast-conserving procedure, without contraindications, to choose BCS. The BCS rate has been increasing in recent decades, especially in 3-A-grade hospitals (10–30%) in China (12). However, limited by the relatively low diagnostic rate of early-stage breast cancer, shortage of radiotherapy equipment and conservative concepts among patients and doctors, the total BCS rate in China is approximately 10%, while mastectomy has a rate of 89% (25). Even in large modern cities, such as

Table 1 Baseline characteristic of 18,502 operated patients from January 1999 to December 2013 in FUSCC

Characteristics	Outcomes (n=18,502)
Age, mean (IQR) (years)	50.0 (44.0–59.0)
Follow up time, median (IQR)	34.1 (15.7–59.8)
Histology types (n=18,377) (%)	
IDC	13,522 (74.7)
DCIS	2,099 (11.4)
Other	2,756 (15.0)
pT (n=16,612) (%)	
Tis	2,099 (12.6)
T1	7,573 (45.6)
T2	6,475 (39.0)
T3–4	465 (2.8)
pN (n=18,502) (%)	
N0	11,721 (63.3)
N1	3,875 (20.9)
N2	1,630 (8.8)
N3	1,276 (6.9)
pTNM stage (n=16,784) (%)	
Stage 0–I	7,166 (42.7)
Stage II	6,713 (40.0)
Stage III	2,905 (17.3)
Tumor grade (n=12,731) (%)	
I	373 (2.9)
II	8,271 (65.0)
III	4,087 (32.1)
ER status (n=17,582) (%)	
Positive	12,418 (70.6)
Negative	5,164 (29.4)
PR status (n=17,573) (%)	
Positive	11,499 (65.4)
Negative	6,074 (34.6)
HER2 status (n=15,433) (%)	
Positive	3,677 (23.8)
Negative	11,756 (76.2)

Data from FUSCC were previously published in Medicine (Baltimore) 2016;95:e4201. DCIS, ductal carcinoma in situ; ER, estrogen receptor; FUSCC, Fudan University Shanghai Cancer Center; HER2, human epidermal growth factor receptor; IDC, invasive ductal carcinoma; IQR, interquartile range; pN, pathological node stage; PR, progesterone receptor; pT, pathological tumor stage; pTNM, pathological stage.

Beijing and Shanghai, the BCS rate varies at approximately 20% (12,25).

As one of the leading cancer centers in Shanghai, the surgery mode of FUSCC represents a relatively high level in China. In our breast cancer center, the patients are fighting cancer along with a whole multidisciplinary team (MDT), including surgeons, physicians, radiologists, radiotherapy doctors and pathologists. Equipped with standard diagnostic and radiotherapy equipment as well as thorough communications with doctors, increasing numbers of eligible patients are diagnosed earlier and are willing to undergo BCS as their first choice of treatment. Here we will retrospectively summarize the surgical trend in our center from 1999 to 2013. *Table 1* summarizes the baseline characteristics of 18,502 patients who underwent breast cancer surgery from January 1999 to December 2013 in the FUSCC [*Table 1* and *Figure 2* based on data from *Medicine* (10)]. As the table shows, the median age of patients at the time of surgery was 50 years [interquartile range (IQR): 44.0–59.0], among which early breast cancer patients (stage 0–II) accounted for 82.7%. As shown in *Figure 2A*, the operation pattern in our FUSCC has continuously altered over the past 15 years [1999–2013]. In detail, MRM experienced an ascending trend before 2005 and gradually replaced ERM. Although it ranks the first among all prior types of surgeries, MRM use has been descending since 2005. In 2013, the MRM rate was lower than 50%. It should be noted that SM ± SLNB increased from 0.3% to 31.9%; meanwhile, BCS increased from 7.6% to 19.1% from 1999 to 2009. In recent years, the BCS rate remained approximately 18%, which was partially because MRI helps discover multi-focal or multi-center foci. Subgroup analysis revealed that patients under 35 years of age comprised the highest percentage group treated with BCS (29.9%, P<0.001) because young age was not a contraindication for BCS in the CACA guidelines (*Figure 2B*). Additionally, in pTNM 0–I stage patients, BCS accounted for 20.5% of cases (*Figure 2C*). The age and stage were two significant factors that influenced the surgery pattern in FUSCC. In summary, BCS and SM ± SLNB are gradually increasing, while RM and MRM are decreasing annually. The escalation of BCS and SM± SLNB represents advancement of the "minimally invasive" surgical concept in FUSCC. There are two main reasons that may explain this advancement. The first is the increasing proportion of early-stage breast cancer, which increases BCS eligible cases. The second is the promotion of SLNB in our center, which promotes SM ± SLNB surgery.

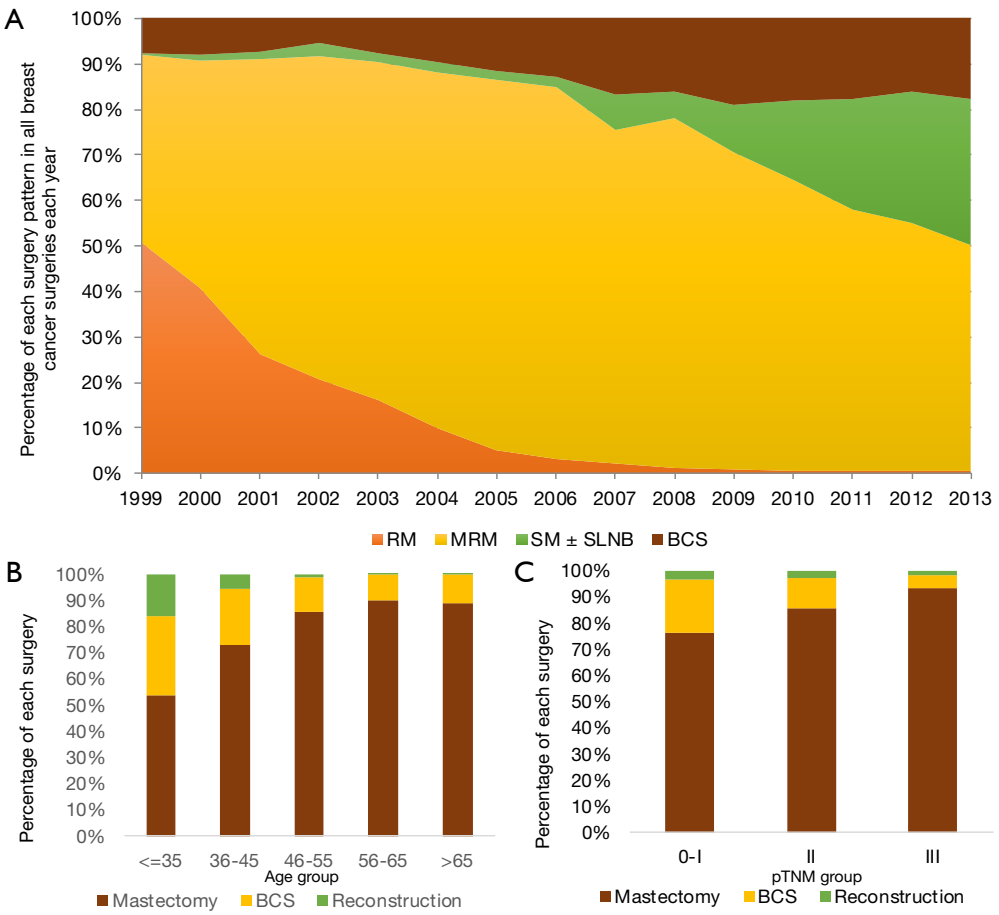


Figure 2 Surgical patterns of breast cancer in FUSCC from 1999 to 2013. (A) Surgical trend in 1999–2013 in FUSCC; (B) choice of surgical modality for different ages; (C) choice of surgical modality for different pTNM groups, 0–I stands for stage 0–I. BCS, breast conserving surgery; FUSCC, Fudan University Shanghai Cancer Center; MRM, modified radical mastectomy; pTNM, pathological stage; RM, radical mastectomy; SLNB, sentinel lymph node biopsy; SM, simple mastectomy. Figure based on data from FUSCC was previously published in Medicine (Baltimore) 2016;95:e4201.

Based on the "minimally invasive procedures" concept, doctors have been focusing on key factors that influence the BCS for decades. There are two hot topics that we will discuss below. First is the negative margin topic. For surgeons, the negative margin is always the first aim. Discussions about the standard of the negative margin are frequently the topic of international conferences. In 2014, the American Society for Radiation Oncology (ASTRO) and Society of Surgical Oncology (SSO) (ASTRO/SSO) Consensus as well as 2015 St. Gallen guidelines issued criteria on the negative margin, which was set to "no ink on tumor or DCIS" (26,27). In China, there are two leading methods for pathological evaluations of BCS margins: radial sections perpendicular to the margin or shave sections of the margin. Regardless the chosen method, the 2015 edition of CACA guidelines suggest that pathologists color each surgical margin and define "no ink on tumor" as a "negative margin" as well. For those hospitals without standard pathologic equipment, "cavity shaving" is suggested as a supplementary method. It is supported by one blockbuster study from 2015, which was on cavity shaving margins (CSM). It demonstrated that CSM could decrease the positive margin rate from 34% to 19% and the second surgery rate from 21% to 10% (28). In FUSCC, we usually follow these criteria on margins, which can increase the BCS rate and decrease the second surgery rate.

Second concern is the approach for discovering local recurrence as soon as possible after BCS. To the best of

our knowledge, true local recurrence usually occurs within 3–5 years, while the second primary tumor in the same breast usually grows after 10–15 years. Long-term follow-up studies showed that the local recurrence rate after BCS followed by radiotherapy varies between 3% to 22% (29). In FUSCC, the local recurrence rate of BCS followed by radiation was approximately 3% (30). We performed a retrospective analysis at our center in which recurrence free survival (RFS) and local recurrence free survival (LRFS) are two important endpoints. Until 2013, the 5-year RFS rate of BCS patients was 93.2% and 5-year LRFS rate was 96.5%, while for mastectomy, the rates were 87.6% and 96.0%, respectively, and they were probably influenced by stage (10). Multivariate analysis demonstrated that the lymph node status is a significant factor influencing the LRFS, especially in young patients (<50 years). The immunohistochemistry (IHC) subtypes is another factor. We found estrogen receptor positive (ER+) is a favorable characteristic for BCS patients. Additionally, when analyzing the annual recurrence pattern of mastectomy or lumpectomy, we observed a double-peak time distribution of the recurrence risk for mastectomy (a major peak at 2 years and moderate peak at 5 years), while there was only one peak at 5 years for BCS, which was confirmed by literature review (30). This finding suggests that the follow-up duration and schedule should be individually designed for BCS patients, which is also the trend for BCS management in China.

In summary, our experience, studies and guidelines facilitate our daily operation, promoting the implementation of BCS and SM ± SLNB. Investigators in FUSCC are making efforts to establish a recurrence prediction model for BCS patients, such as nomogram, to predict the recurrence possibility according to demographic and pathological characteristics. Additionally, our current goals are to customize tailored follow-up strategies, standardize salvage surgery for local recurrence patients and improve the BCS rate for patients after neoadjuvant chemotherapy (NAC).

SLNB: routinely conducted to save axilla

SLNB is another standard of care for clinical lymph node negative (cN0) patients, which involves interpreting the minimally invasive mode of local breast cancer treatment. Before 1993, ALND was the main operation for axillary staging until Krag *et al.* reported on SLNB in breast cancer treatment (31,32). The landmark Milan clinical trial, published in the NEJM in 2003, revealed that SLNB could accurately predict the axillary status, which laid the foundation for SLNB (13). Many prospective and retrospective analyses on SLNB supported that SLNB has similar efficacy to axillary dissection while offering a better quality of life for SLN negative patients (15,33). SLNB has been recommended in the ASCO guidelines and NCCN guidelines for 10 years, saving 60–75% patients from ALND and its associated side effects (14). In the 2015 edition, the CACA guidelines first suggested that SLNB should be performed as a routine procedure at the beginning of surgery as long as the hospitals have the relevant necessary equipment and techniques offered by MDT groups. Additionally, a qualified SLNB surgeon must achieve a greater than 90% success rate and less than 10% false negative rate in his or her personal SLNB experience. In China, combined methylene blue dye and radionuclide imaging are recommended, and a single marker could be used in well-practiced hospitals. Fluorescent dye has not yet been suggested as a routine method.

The CACA guidelines encourage surgeons to perform SLNB as a routine procedure. The actual percentage of patients in China who undergo ALND is up to 80%, including 60% with negative ALNs. The influences on the implementation of SLNB in China will be discussed below. First is the safety concern of the doctors. Hospitals in China with less advanced equipment are more willing to perform ALND for safety in the context of their limited techniques and cooperation with MDT team. Evaluation methods of SLN in China include touch imprint cytology (TIC) and intraoperative frozen section. In FUSCC, we use TIC as our routine method. The total accuracy rate of TIC in our center is 93.2%. In FUSCC experience, the SLNB rate is more than 50% among breast cancer surgeries, which is partially thanks to the MDT cooperation in our center. *Figure 3A* summarizes the SLNB trend of 4,992 SLNB cases in our center from 2005 to 2013 (*Figure 3* based on data from FUSCC). The SLNB rate has a two-stage pattern; before 2008, SLNB remained stable at approximately 12%, and after 2008, SLNB rapidly increased up to 51.9% in 2013. This phenomenon could be explained by the reasons given by our MDT team and our prospective trials. Three hundred patients in our center with T1–2N0 tumors were randomly divided into the SLNB and ALND groups. The 5-year RFS results showed no significant difference between these two groups irrespective of subgrouping by pT1 and pT2. *Figure 3B* shows that the SLN number is grouped into three levels: 1–2, 3–4 and ≥4 nodes. Each level maintains a high percentage: 3–4 SLNs is most frequent

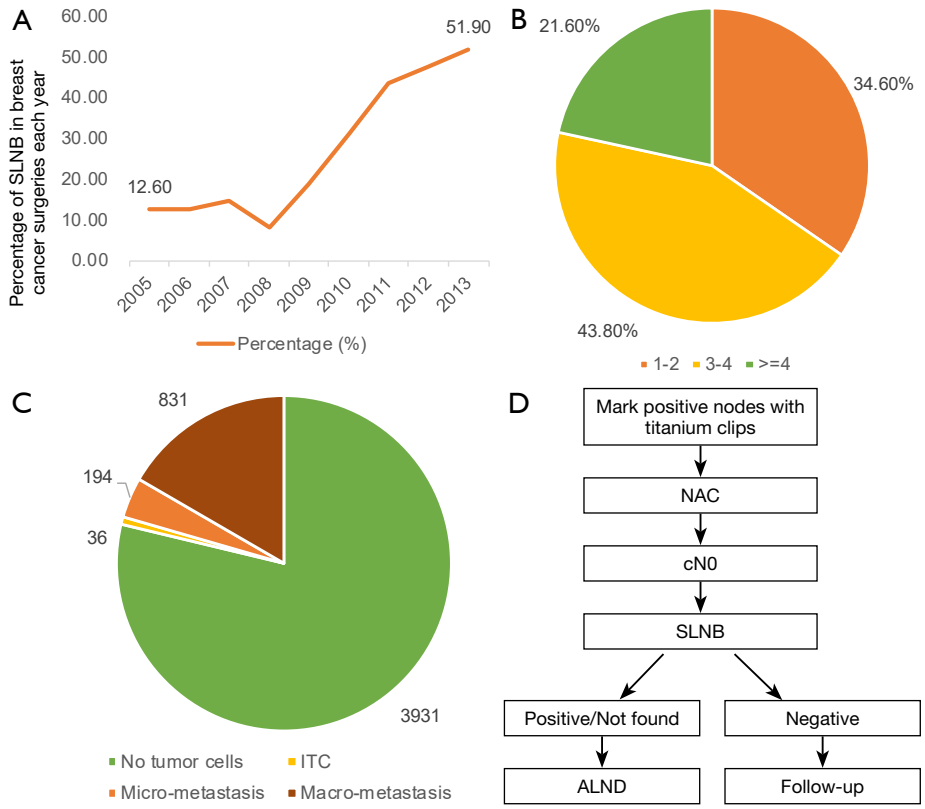


Figure 3 SLNB management in FUSCC from 2005 to 2013. (A) Trend of 4,992 SLNB cases in FUSCC in 2005–2013; (B) lymph node number from SLNB in FUSCC; (C) lymph node status from SLNB in FUSCC; The value represents case numbers; (D) flow chart of SLNB in NAC of FUSCC. ALND, axillary lymph node dissection; FUSCC, Fudan University Shanghai Cancer Center; NAC, neoadjuvant chemotherapy; SLNB, sentinel lymph node biopsy. Figure based on data from FUSCC.

one (43.8%) and ≥4 SLNs is the least frequent (21.6%). *Figure 3C* shows the final pathological diagnosis of SLNs. Up to 78.7% of reports lack a tumor in the lymph node, while the metastasis rate of SLN is 22.3% [16.6% for macro-metastasis, 3.9% for micro-metastasis and 0.7% for isolated tumor cells (ITC)]. In summary, MDT team work and relevant prospective studies help the implementation of SLNB in FUSCC. The second element that influences the widespread use of SLNB in China is salvage treatment for SLN positive patients. This topic remains under exploration and discussion. The IBCSG 23-01 trial divided SLN micro-metastasis patients into the ALND and follow-up groups (4,16). The 5-year follow-up results revealed that the local recurrence rate and overall survival had no difference between the two groups, while the ALND group had a higher chance of side effects, such as upper limb edema or movement disorder, suggesting that patients with micro-metastasis in the SLN should be relieved from ALND. Soon afterwards, the Z0011 and AMAROS (17,34) clinical trials explored the chance of exemption from ALND for SLN macro-meta stasis patients. The Z0011 trial showed no difference in recurrence events and the overall survival for patients with 1–2 SLN macro-metastasis who underwent BCS plus radiotherapy, while the AMAROS trial revealed that patients with a single SLN macro-metastasis could avoid ALND. Based on these major trials, the 2015 St. Gallen consensus supported promotion of the Z0011 experience in clinical practice. In China, the 2015 CACA guidelines showed that most CACA expertise supported the IBCSG 23-01 conclusion that micro-metastasis SLN patients with BCS followed by radiation may be spared from ALND, while ALND is still the standard of care for macro-metastasis. In FUSCC, we will consider not performing ALND for patients that undergo BCS followed by radiation to treat T1 stage and ER positive tumors. According to our summary, the ALND rate after SLNB in total SLNB

cases is 26.8%, including 93.6% in macro-metastasis cases, 64.4% for micro-metastasis ones and 25% for ITC ones respectively. The survival analysis of the ALND and no-ALND groups showed that irrespective of the type of positive SLN, the 5-year-RFS is similar, with no significant difference, which encourages further use of SLNB in positive SLN cases. The third concern is the use of SLNB for NAC patients. Published studies showed that 30–40% of patients with positive lymph node became negative after NAC (35). Our data showed that ER-poor/HER-2 positive patients treated with trastuzumab achieved the highest negative conversion rate (79.6%) of axillary lymph nodes after NAC, suggesting that ER-poor and HER2-positive status may be a potential subtype of breast cancer that does not require ALND after NAC (36). The key question is how to accurately and safely evaluate these down-staged axillary lymph nodes. The Z1071 clinical trial demonstrated that the false negative rate (FNR) of SLNB on cN1 patients after NAC was up to 12.6%, while clipping positive nodes before NAC and removing more than 2 SLNs could diminish the FNR to 6.8% (37). However, the clipping technique is still debated in China because only several cancer centers perform SLNB after NAC. The 2015 CACA guidelines do not yet propose the routine use of SLNB for cN0 patients after NAC. In FUSCC, we designed our protocol to include SLNB after NAC. As shown in *Figure 3D*, adding titanium clips on positive lymph nodes with the help of ultrasound is the first step before NAC. After NAC, SLNB will be performed on cN0 patients. For those positive (or not found) results, ALND will be performed, while negative patients only require follow-up (*Figure 3D*). Whether this method works well requires long-term evaluation.

In summary, SLNB has been widely promoted in China, and it has been safely and effectively performed in our cancer center. It is critical to choose appropriate patients for SLNB to avoid unnecessary ALND and its side effects as well as to generate new and accurate SLNB methods for NAC patients. We hope for more long-term, prospective clinical trials to guide our procedures in clinical practice.

Reconstruction surgery: latissimus dorsi myocutaneous flap ± implantation is the most common surgery

Reconstruction surgery is not a surgery to treat physiological disease; instead, it is a salvage surgery to address the psychological trauma and aesthetic defects. The first case of prosthesis implantation was reported in 1971. After 1970s, doctors started to combine local flaps and implantations together to improve the success rate. Since the 1980s, the emergence of an expander decreased surgery on the healthy breast. After 40 years of development, autologous tissue reconstruction has become the first choice for patients, including the transverse rectus abdominis musculocutaneous (TRAM) flap, free TRAM flap, latissimus dorsi myocutaneous flap (LDMF) and deep inferior epigastric artery perforator (DIEP) flap. Reconstruction surgeries could be divided into immediate breast reconstruction and delayed reconstruction according to the reconstruction time or be divided into autologous tissue reconstruction, implantation reconstruction and combined reconstruction according to the materials used for reshaping. In China, these reconstruction methods are performed, but the total rate of reconstruction surgery is only 4.5%, while it is up to 25.6% in developed countries. An investigation among 32 institutions from CACA showed that the main limitations for popularization among Chinese doctors include technical barriers to reconstruction, lack of team cooperation, a long period for training a qualified micro-surgeon and worries about local treatment safety (38). On the other hand, over-exaggerated fear of breast cancer from patients and poor economic foundation are the two main reasons patients do not choose reconstruction surgery. With the economic development in China, increasing numbers of patients are becoming concerned about their aesthetic needs. The 2015 CACA guideline introduced substantial information about reconstruction surgery to help promote it in China. *Figure 4A* displays the development curve of reconstruction surgery in our center from 2001 to 2013. The reconstruction cases increased with time, adding up to 573 immediate breast reconstruction cases. The percentage of reconstruction in total breast cancer surgery remained stable at approximately 4%. *Figure 4B* shows the composition of each reconstruction method. Autologous tissue reconstruction is the first choice (61.4%), while simple implantation is the last choice (9.6%), which is probably due to the expensive cost and complicated schedules. More specifically, LDMF with or without implantation is still the most common surgery at our center. Simple expansion/implantation surgery has rapidly increased in recent years. Pedicled-TRAM has been replaced by free-TRAM as the major abdominal flap reconstruction approach (*Figure 4C*) [*Figure 4* based on data from *Medicine* (10)]. A retrospective study at our center analyzed 118 cases of reconstruction surgeries from 2006-2013 in FUSCC using

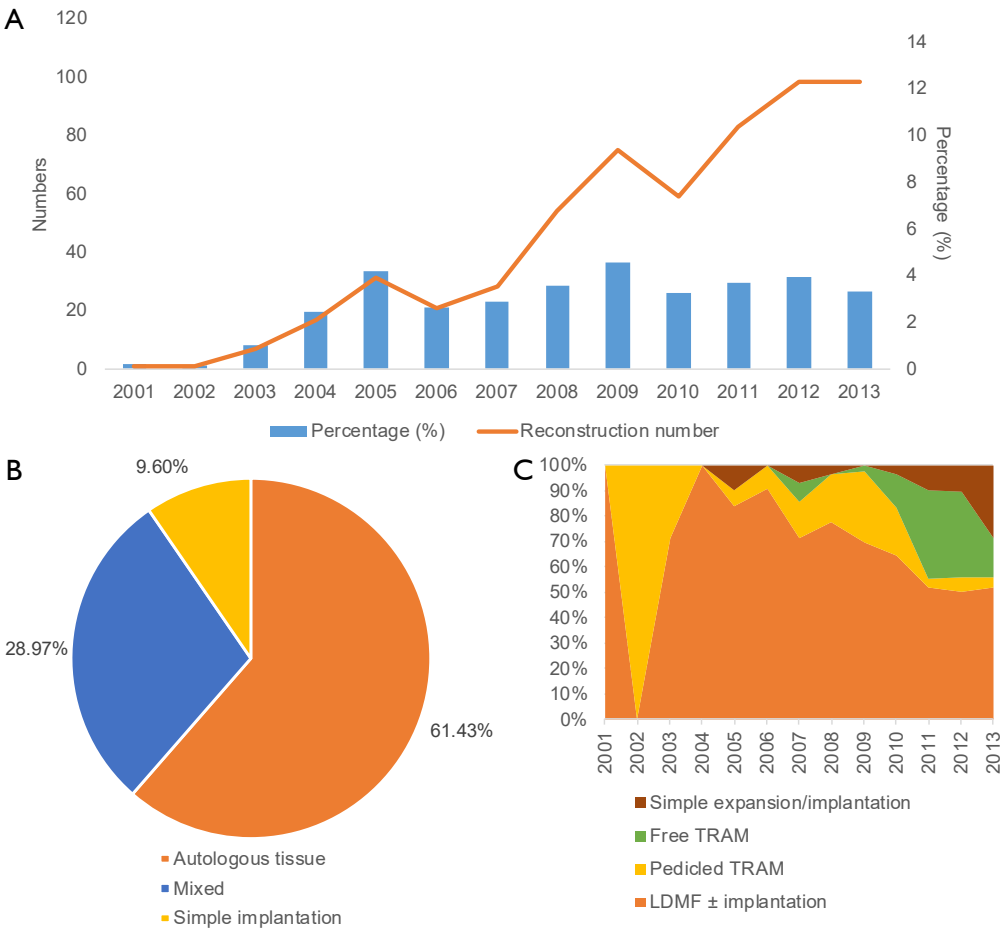


Figure 4 Reconstruction surgery management in FUSCC from 2001 to 2013. (A) Trend of reconstruction surgery in FUSCC in 2001–2013, percentage (%) represents the percentage of reconstruction surgery in total breast cancer surgery each year. (B) Proportion of each reconstruction method in FUSCC; (C) trends of each reconstruction method from 2001–2013 in FUSCC. DIEP, deep inferior epigastric artery perforator; FUSCC, Fudan University Shanghai Cancer Center; LDMF, latissimus dorsi myocutaneous flap; and TRAM, transverse rectus abdominis musculocutaneous. Figure based on data from FUSCC was previously published in Medicine (Baltimore) 2016;95:e4201.

f-TRAM techniques (39). The average surgical time is 7.72 h, and average hospitalization time after surgery is 10.73 days. In detail, the internal thoracic vessels are the first choice (72.0%). With respect to complications, only 3 cases experienced total flap necrosis. Survival analysis shows that the 5-year RFS for mastectomy is 88.3%, while the 5-year RFS of the reconstruction group is 92.3%, which is significantly higher than the mastectomy group and similar to the BCS group (92.3%). In summary, reconstruction surgery in our center is progressing at a steady pace and starting to lead on average in China, although it is far behind other International breast centers. We are making every effort in this area to make reconstruction a more viable option for more patients.

Conclusions

In conclusion, FUSCC achieves standard, distinctive surgical management experience based on guidelines, a series of studies and the context in China, which could be summarized in four parts. First, pre-surgery diagnosis in our center involves CNB guided by imaging to increase accuracy. Second, BCS and SM ± SLNB are increasing with time, which are characterized by individualized monitoring and evaluation strategies. Third, SLNB has been conducted as part of routine surgery. We developed our own protocol for SLNB after NAC with the aim of exploring how to better treat positive SLNs and down-staged SLNs after NAC. Finally, reconstruction surgery in our center is

steadily progressing. Autologous tissue reconstruction, especially LDMF ± implantation, is the major approach, while implantation has remarkably increased. Our management experience is in line with international standards and considers patient survival and quality of life.

Acknowledgements

Funding: This work was supported by grants from the Research Project of Fudan University Shanghai Cancer Center YJ201401 (YZ Jiang); Training Plan of Excellent Talents in Fudan University Shanghai Cancer Center YJYQ201602 (YZ Jiang); National Natural Science Foundation of China 81572583 (ZM Shao), 81502278 (YZ Jiang), and 81372848 (ZM Shao); Research Fund for the Doctoral Program of Higher Education of China 20130071110057 (ZM Shao); Municipal Project for Developing Emerging and Frontier Technology in Shanghai Hospitals SHDC12010116 (ZM Shao); Cooperation Project of Conquering Major Diseases in Shanghai Municipality Health System 2013ZYJB0302 (ZM Shao); Innovation Team of Ministry of Education IRT1223 (ZM Shao); and Shanghai Key Laboratory of Breast Cancer 12DZ2260100 (ZM Shao).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32.
2. Yu KD, Di GH, Wu J, et al. Development and trends of surgical modalities for breast cancer in China: a review of 16-year data. Ann Surg Oncol 2007;14:2502-9.
3. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471-4.
4. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Breast Cancer Version 2.2016. Port Washington, PA: National Comprehensive Cancer Network; 2016.
5. Plesca M, Bordea C, El Houcheimi B, et al. Evolution of radical mastectomy for breast cancer. J Med Life 2016;9:183-6.
6. Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med 2002;347:1227-32.
7. Early Breast Cancer Trialists' Collaborative G. Effects of radiotherapy and surgery in early breast cancer. An overview of the randomized trials. N Engl J Med 1995;333:1444-55.
8. Fisher B, Anderson S. Conservative surgery for the management of invasive and noninvasive carcinoma of the breast: NSABP trials. National Surgical Adjuvant Breast and Bowel Project. World J Surg 1994;18:63-9.
9. van Maaren MC, de Munck L, de Bock GH, et al. 10 year survival after breast-conserving surgery plus radiotherapy compared with mastectomy in early breast cancer in the Netherlands: a population-based study. Lancet Oncol 2016;17:1158-70.
10. Huang NS, Liu MY, Chen JJ, et al. Surgical management of breast cancer in China: A 15-year single-center retrospective study of 18,502 patients. Medicine (Baltimore) 2016;95:e4201.
11. McGuire KP, Santillan AA, Kaur P, et al. Are mastectomies on the rise? A 13-year trend analysis of the selection of mastectomy versus breast conservation therapy in 5865 patients. Ann Surg Oncol 2009;16:2682-90.
12. Zhang B, Song Q, Zhang B, et al. A 10-year (1999 ~ 2008) retrospective multi-center study of breast cancer surgical management in various geographic areas of China. Breast 2013;22:676-81.
13. Veronesi U, Paganelli G, Viale G, et al. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. N Engl J Med 2003;349:546-53.
14. Lyman GH, Giuliano AE, Somerfield MR, et al. American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol 2005;23:7703-20.
15. Mansel RE, Fallowfield L, Kissin M, et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. J Natl Cancer Inst 2006;98:599-609.
16. Galimberti V, Cole BF, Zurrida S, et al. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23-01): a phase 3 randomised controlled trial. Lancet Oncol 2013;14:297-305.
17. Jagsi R, Chadha M, Moni J, et al. Radiation field design in the ACOSOG Z0011 (Alliance) Trial. J Clin Oncol 2014;32:3600-6.
18. Oeffinger KC, Fontham ET, Etzioni R, et al. Breast

Cancer Screening for Women at Average Risk 2015 Guideline Update From the American Cancer Society. JAMA 2015;314:1599-614.
19. Siu AL; U.S. Preventive Services Task Force. Screening for Breast Cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med 2016;164:279-96.
20. Okello J, Kisembo H, Bugeza S, et al. Breast cancer detection using sonography in women with mammographically dense breasts. BMC Med Imaging 2014;14:41.
21. Fan L, Strasser-Weippl K, Li JJ, et al. Breast cancer in China. Lancet Oncol 2014;15:e279-89.
22. Committee of Chinese Breast Cancer Society. China Anti-Cancer Association guidelines for breast cancer diagnosis and treatment (2015 version). China Oncology 2015;25:692-754.
23. Bruening W, Fontanarosa J, Tipton K, et al. Systematic review: comparative effectiveness of core-needle and open surgical biopsy to diagnose breast lesions. Ann Intern Med 2010;152:238-46.
24. Newman LA, Kuerer HM. Advances in breast conservation therapy. J Clin Oncol 2005;23:1685-97.
25. Zhang BL, Sivasubramaniam PG, Zhang Q, et al. Trends in Radical Surgical Treatment Methods for Breast Malignancies in China: A Multicenter 10-Year Retrospective Study. Oncologist 2015;20:1036-43.
26. Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies-improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 2015;26:1533-46.
27. Moran MS, Schnitt SJ, Giuliano AE, et al. Society of Surgical Oncology-American Society for Radiation Oncology Consensus Guideline on Margins for Breast-Conserving Surgery With Whole-Breast Irradiation in Stages I and II Invasive Breast Cancer. Ann Surg Oncol 2014;21:704-16.
28. Chagpar AB, Killelea BK, Tsangaris TN, et al. A Randomized, Controlled Trial of Cavity Shave Margins in Breast Cancer. N Engl J Med 2015;373:503-10.
29. Huston TL, Simmons RM. Locally recurrent breast cancer after conservation therapy. Am J Surg 2005;189:229-35.
30. Yu KD, Li S, Shao ZM. Different Annual Recurrence Pattern Between Lumpectomy and Mastectomy: Implication for Breast Cancer Surveillance After Breast-Conserving Surgery. Oncologist 2011;16:1101-10.
31. Krag DN, Weaver DL, Alex JC, et al. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. Surg Oncol 1993;2:335-9; discussion 40.
32. Hsueh EC, Turner RR, Glass EC, et al. Sentinel node biopsy in breast cancer. J Am Coll Surg 1999;189:207-13.
33. Krag DN, Anderson SJ, Julian TB, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol 2010;11:927-33.
34. Donker M, van Tienhoven G, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. Lancet Oncol 2014;15:1303-10.
35. Hennessy BT, Hortobagyi GN, Rouzier R, et al. Outcome after pathologic complete eradication of cytologically proven breast cancer axillary node metastases following primary chemotherapy. J Clin Oncol 2005;23:9304-11.
36. Li JW, Mo M, Yu KD, et al. ER-poor and HER2-positive: a potential subtype of breast cancer to avoid axillary dissection in node positive patients after neoadjuvant chemo-trastuzumab therapy. PLoS One 2014;9:e114646.
37. Boughey JC, Ballman KV, Le-Petross HT, et al. Identification and Resection of Clipped Node Decreases the False-negative Rate of Sentinel Lymph Node Surgery in Patients Presenting With Node-positive Breast Cancer (T0-T4, N1-N2) Who Receive Neoadjuvant Chemotherapy: Results From ACOSOG Z1071 (Alliance). Ann Surg 2016;263:802-7.
38. Chen Y, Chen J, Chen J, et al. Current trends of breast reconstruction after mastectomy for breast cancer patients in China: a survey report. Zhonghua Zhong Liu Za Zhi 2014;36:851-7.
39. Ying C, Jia-Ying C, lin L, et al. Single-center report of 118 cases of free abdominal flaps for breast reconstruction. China Oncology 2013;8:576-83.

Cite this article as: Liu XY, Gou ZC, Cao ZG, Jiang YZ, Shao ZM. Surgical management of breast cancer in China: the Fudan University Shanghai Cancer Center experience. Transl Cancer Res 2017;6(3):588-598. doi: 10.21037/tcr.2017.06.35

Paradigm of polyendocrine therapy in endocrine responsive breast cancer: the role of fulvestrant

Valerie Nelson[1], Alfred Rademaker[2], Virginia Kaklamani[1]

[1]Department of Medicine, Division of Hematology/Oncology, Feinberg School of Medicine of Northwestern University and the Robert H Lurie Comprehensive Cancer Center, 676 N St Clair St, Suite 850, Chicago, IL 606011, USA; [2]Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, 680 N Lakeshore Drive, suite 1200, Chicago, IL 60611, USA

Correspondence to: Virginia Kaklamani. Department of Medicine, Division of Hematology/Oncology, Feinberg School of Medicine of Northwestern University and the Robert H Lurie Comprehensive Cancer Center, 676 N St Clair St, Suite 850, Chicago, IL 606011, USA. Email: v-kaklamani@northwestern.edu.

Submitted Aug 10, 2012. Accepted for publication Sep 13, 2012.
doi: 10.3978/j.issn.2304-3865.2012.09.04
View this article at: http://www.thecco.net/article/view/1111/1928

Endocrine therapy is the cornerstone of any treatment plan for endocrine-responsive breast cancer in both the adjuvant and metastatic settings (1). In the metastatic setting in post-menopausal patients aromatase inhibitors (AIs; anastrozole, exemestane, letrozole) are standard therapies, shown to demonstrate improved progression-free survival (PFS) and a favorable adverse effect (AE) profile compared to other endocrine agents such as tamoxifen (2,3). Tamoxifen, which is a selective estrogen receptor (ER) modulator (SERM), continues to have a large role in the management of endocrine receptor positive breast cancer in both the adjuvant and metastatic setting, in both pre- and post-menopausal women (4,5). The AIs reduce circulating estrogen levels in postmenopausal women by inhibiting peripheral conversion of androgens to estradiol. Fulvestrant (Faslodex, AstraZeneca) is an analogue of estradiol that binds the ER in such a way that disrupts the ER leading to increased receptor degradation and half-life (6,7), resulting in apoptosis and reduced proliferation of affected cells (8).

The sequencing and combination of endocrine therapy is an evolving area. For example, the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial showed that the combination of anastrozole with tamoxifen was not superior to single agent tamoxifen in the adjuvant setting (9). The Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) trial similarly did not show superiority of the combination compared with tamoxifen alone in decreasing the proliferation marker Ki-67 (10). Much of this data is from the pre-fulvestrant era and the sequencing and combination of endocrine therapy with this agent is not yet clear. Initial studies with fulvestrant 250 mg IM per month (the initial FDA approved dose) indicated equivalence, but not superiority, compared to tamoxifen (11), anastrozole (12), and exemestane (13). At a higher dose (500 mg every two weeks for one month followed by 500 mg monthly injections), fulvestrant was found to be at least equivalent to anastrozole alone in clinical benefit rate and overall response rate with improved time to failure by 13 months in the FIRST trial (14,15). Thus in September 2010, fulvestrant was approved at the higher dose level.

There is mixed pre-clinical rationale for the combination of fulvestrant with other anti-estrogen agents. As discussed by Weinberg *et al.* (16), multiple strategies to overcome hormonal resistance have had pre-clinical success including the combination of hormonal and growth factor blockade and dual hormonal blockade. A preclinical study in the transplanted human ER positive breast cancer cell line MCF-7, showed greater efficacy of fulvestrant compared with tamoxifen after estrogen withdrawal (17). In this model, tumor cells are injected into mice in an estrogenic environment created with an estrogen releasing subcutaneous pellet. Upon tumor formation, the estrogen pellet is removed. In this estrogen deprivation setting fulvestrant has been shown to have potent anti-tumor effects. However, if mice continue to receive estrogen, fulvestrant does not have significant anti-tumor activity (18). Later studies in the MCF-7 Ca cell model (cells that were genetically modified to express high levels of aromatase) showed equivalence of combinations of fulvestrant with anastrozole and fulvestrant with tamoxifen compared to the use of either agent alone (19). But similar studies

with various doses of fulvestrant in combination with AI showed superiority of the combination (20). For example, Macedo *et al*. (21) studied the combination of fulvestrant and anastrozole in the xenograft mouse model and showed decreased rate of tumor growth compared to either agent alone as well as down-regulation of signaling proteins such as insulin-like growth factor type I receptor beta, mammalian target of rapamycin (mTOR) and estrogen receptor alpha in tumors exposed to both agents, hinting at a mechanism for efficacy of therapy. In summary, while the preclinical picture is mixed, there is rationale for combining an anti-estrogen with an anti-estrogen receptor drug.

Given these encouraging pre-clinical results, two randomized trials were initiated to evaluate the efficacy of combined AI and fulvestrant therapy in post-menopausal breast cancer patients. The Fulvestrant and Anastrozole Combination Therapy (FACT) trial was a Phase III, open-label, prospective randomized controlled trial that evaluated a loading-dose (LD) schedule of fulvestrant 250 mg together with anastrozole versus anastrozole alone in 514 predominantly European post-menopausal women with receptor-positive breast cancer treated at first relapse (22). FACT was a negative study with no difference in primary or secondary endpoints of time to progression (TTP, defined as time from randomization to progression or death due to any cause) or overall survival (OS) between the groups.

In the face of this negative trial, the publication of the SWOG S0226 trial results in the New England Journal of Medicine, showing an improvement not just in PFS (defined as time from randomization to progression or death due to any cause) but also OS with the combination of fulvestrant and anastrozole is intriguing (23). This Phase III trial randomized 694 predominantly American postmenopausal women with previously untreated metastatic breast cancer to either fulvestrant with anastrozole or anastrozole alone with cross over to fulvestrant alone strongly encouraged at time of progression for the anastrozole alone group. The primary endpoint was PFS which was superior in the combination arm at a median of 15.0 months [95% confidence interval (CI), 13.2 to 18.4 months] in the combination group and 13.5 months (95% CI, 12.1 to 15.1 months) in the anastrozole alone group (P=0.007). OS also favored the combination arm with median of 47.7 months (95% CI, 43.4 to 55.7 months) in the combination group compared to 41.3 months (95% CI, 37.2-45.0 months) with anastrozole alone (P=0.049). The toxicity profile of the two groups was similar.

One strength of this study is the encouragement of cross-over to fulvestrant for the anastrozole only group, which occurred in 41% of the patients in the anastrozole-only group. The overall survival benefit persisted for upfront combination therapy even in those who crossed over at progression. However this must be interpreted with caution as the cross over patients received low dose fulvestrant (without the 500 mg loading dose).

Fulvestrant dosing is an issue in both FACT and SWOG S0226. Both trials use the dosing scheme of 500 mg LD on Day 1, followed by 250 mg on days 15, 29, then monthly. However, since the design and initiation of these trials, fulvestrant has been approved for use with a higher dosing scheme, specifically 500 mg on Days 0, 14, 28 and then monthly (as opposed to 250 mg monthly), based on the results of the CONFIRM trial that showed a median 1.0 month improvement in PFS with the higher dose (24).

So how should we interpret these disparate results from similarly designed trials? First of all, it is important to note that while the sample size of the two studies are different with 514 patients in the FACT trial and 694 patients in SWOG S0226, the studies were powered differently with FACT at 80% power and SWOG S0226 at 90% power resulting in similar effect size. Thus the difference in sample size is not a key feature that can explain the disparate results. However, there were significant differences both in the patient population and the duration of follow-up between these trials that likely drove the differing results.

As outlined in *Tables 1*, *2*, while the patients in both trials were similar in age and disease extent there were significant differences in the proportion of patients who were endocrine naïve. Roughly 30% of women in the FACT trial had not received prior endocrine therapy versus nearly 60% in SWOG S0226. The larger proportion of endocrine naïve patients is likely the main driver behind the positive results in the SWOG trial. Indeed, unplanned subgroup analysis of the SWOG study showed the PFS benefit may have been restricted to this large endocrine naïve group. Also notable is the increased proportion of patients who received prior chemotherapy in the FACT trial compared to SWOG S0226. Additionally, SWOG S0226 mandated that patients completed neoadjuvant or adjuvant chemotherapy more than 12 months before enrollment while FACT allowed patients who had recently progressed on chemotherapy with no "wash-out" period required. In general, then, the SWOG S0226 patient population had received less treatment - either endocrine or chemotherapy - and had a longer chemotherapy treatment free interval than the patients in FACT.

Table 1 Patient characteristics, FACT trial versus SWOG S0226 trial; References, Bergh *et al.* (22), Mehta *et al.* (23)

	FACT		SWOG 0226	
	Anastrozole alone	Anastrozole and fulvestrant	Anastrozole alone	Anastrozole and fulvestrant
Number of patients	256	258	345	349
Age (years)	63	65	65	65
Metastatic disease (%)	94.5	95.0	100	100
Disease site (%)				
Bone only	27.7	24.4	22.0	21.5
Visceral	48.4	51.9	48.4	51.9
Prior hormonal therapy (%)	65.6	69.8	40.3	40.4
Prior chemotherapy (%)	49.6	41.9	29.9	37.0

Table 2 Results, FACT trial versus SWOG S0226 trial; References, Bergh *et al.* (22), Mehta *et al.* (23)

	FACT months (95% CI)		P value	SWOG 0226 months (95% CI)		P value
	Anastrozole alone	Anastrozole and fulvestrant		Anastrozole alone	Anastrozole and fulvestrant	
TTP	10.2	10.8 (0.81 to 1.20)	0.91	---	---	---
PFS	---	---	---	13.5 (12.1 to 13.1)	15.0 (13.2 to 18.2)	0.007
OS	38.2	37.8	1.00	41.3 (37.2 to 45.0)	47.7 (43.4 to 55.7)	0.049

CI = confidence interval; TTP = time to progression; PFS = progression free survival; OS = overall survival

Another major difference between these trials is the follow-up time. Median follow up in FACT was only 8.9 months in comparison to 35 months in SWOG S0226. This becomes increasingly relevant as improvements in both PFS and OS in the SWOG trial became more pronounced over time. As reported in the New England Journal of Medicine (23), the Kaplan-Meier curves for PFS did not separate until 12 months. Similarly, the magnitude of difference in overall survival increased over time. At one year, the rate of OS was 89% with anastrozole alone and 91% with the combination. By year three, OS was 57% in the anastrozole alone group versus 62% in the combination group. Indeed, if follow-up on SWOG S0226 was only 8.9 months, it too may have been a negative study. Would longer follow-up on the FACT trial show a positive result?

The study population in SWOG S0226 was unique, including 39% of patients who had metastatic disease at presentation; in general population studies show that less than 10% (25) of patients present with metastatic disease. Perhaps there is a role for combination endocrine therapy is in this small population of previously un-treated patients with metastatic disease, however we do not have enough data to call this standard of care at this time.

What can, or should, be done to clarify the activity of combination fulvestrant and anastrozole? One possibility, particularly intriguing in light of the suggestion that combination therapy may work best in endocrine-therapy naïve patients, is investigation in the neoadjuvant setting. One small pilot study of 121 postmenopausal patients with ER-positive disease did just that, testing the combination of anastrozole plus a single 500 mg fulvestrant injection versus each agent alone. No additional Ki-67 downregulation was noted with the combination over either agent alone (26). However, in general, endocrine neoadjuvant studies have failed to produce meaningful results in part the optimal endpoint has not yet been identified.

On the other end of the disease spectrum, the South Korean SoFEA trial, reported first results at the European Breast Cancer Conference earlier this year. SoFEA is a phase III, partially blinded, randomized trial in which women with locally advanced or metastatic disease were allocated to fulvestrant 250 mg (with 500 mg LD) plus anastrozole versus fulvestrant plus placebo versus exemestane 15 mg daily after progressing on a non-steroidal aromatase inhibitor. PFS was 4.4 months (95% CI, 3.4 to 5.4 months) for the combination of fulvestrant and anastrozole, 4.8 months (95% CI, 3.6 to 5.5 months) for anastrozole alone and 3.4 months (95% CI, 3.0-4.6 months)

for exemestane alone; all differences were non-significant (27). Given the importance of longer interval follow-up as evidenced by the SWOG trial, we eagerly await more mature data from the SoFEA experience.

In conclusion, cautious optimism regarding the combination regimen of fulvestrant with anastrozole is reasonable in light of the mixed results of FACT, SWOG S0226 and the recently reported SoFEA trial. The 6.4 month improvement in OS seen in the SWOG trial is not to be taken lightly. Nor should the negative results of the FACT trial be discounted. For the time being, further study is warranted with particular attention to sub-populations that may derive the most benefit from the combination therapy, such as endocrine naïve patients with metastatic disease, admittedly a small population, or patients receiving their initial therapy for early stage breast cancer. The robust results of SWOG S0226 certainly raise hope that such a population exists.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Mauri D, Pavlidis N, Polyzos NP, et al. Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. J Natl Cancer Inst 2006;98:1285-91.
2. Nabholtz JM, Buzdar A, Pollak M, et al. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. J Clin Oncol 2000;18:3758-67.
3. Mouridsen H, Gershanovich M, Sun Y, et al. Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. J Clin Oncol 2003;21:2101-9.
4. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011;22:1736-47.
5. Beslija S, Bonneterre J, Burstein HJ, et al. Third consensus on medical treatment of metastatic breast cancer. Ann Oncol 2009;20:1771-85.
6. Wakeling AE. Similarities and distinctions in the mode of action of different classes of antioestrogens. Endocr Relat Cancer 2000;7:17-28.
7. Wakeling AE, Dukes M, Bowler J. A potent specific pure antiestrogen with clinical potential. Cancer Res 1991;51:3867-73.
8. Bundred NJ, Anderson E, Nicholson RI, et al. Fulvestrant, an estrogen receptor downregulator, reduces cell turnover index more effectively than tamoxifen. Anticancer Res 2002;22:2317-9.
9. Baum M, Budzar AU, Cuzick J, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. Lancet 2002;359:2131-9.
10. Dowsett M, Ebbs SR, Dixon JM, et al. Biomarker changes during neoadjuvant anastrozole, tamoxifen, or the combination: influence of hormonal status and HER-2 in breast cancer--a study from the IMPACT trialists. J Clin Oncol 2005;23:2477-92.
11. Howell A, Robertson JF, Abram P, et al. Comparison of fulvestrant versus tamoxifen for the treatment of advanced breast cancer in postmenopausal women previously untreated with endocrine therapy: a multinational, double-blind, randomized trial. J Clin Oncol 2004;22:1605-13.
12. Osborne CK, Pippen J, Jones SE, et al. Double-blind, randomized trial comparing the efficacy and tolerability of fulvestrant versus anastrozole in postmenopausal women with advanced breast cancer progressing on prior endocrine therapy: results of a North American trial. J Clin Oncol 2002;20:3386-95.
13. Chia S, Gradishar W, Mauriac L, et al. Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT. J Clin Oncol 2008;26:1664-70.
14. Robertson JF, Llombart-Cussac A, Rolski J, et al. Activity of fulvestrant 500 mg versus anastrozole 1 mg as first-line treatment for advanced breast cancer: results from the FIRST study. J Clin Oncol 2009;27:4530-5.
15. Robertson JFR, Lindemann JPO, Llombart-Cussac A, et al. A Comparison of Fulvestrant 500 mg with Anastrozole

as First-Line Treatment for Advanced Breast Cancer: Follow-Up Analysis from the 'FIRST' Study. Proc SABCS 2010;70:Abstract nr S1-3.
16. Weinberg OK, Marquez-Garban DC, Pietras RJ. New approaches to reverse resistance to hormonal therapy in human breast cancer. Drug Resist Updat 2005;8:219-33.
17. Osborne CK, Coronado-Heinsohn EB, Hilsenbeck SG, et al. Comparison of the effects of a pure steroidal antiestrogen with those of tamoxifen in a model of human breast cancer. J Natl Cancer Inst 1995;87:746-50.
18. Massarweh S, Osborne K, Heidi W, et al. Estrogen deprivation is crucial for the antitumor effect of fulvestrant and adding the HER inhibitor gefitinib delays acquired resistance in a xenograft model of ER-positive breast cancer. Breast Cancer Res Treat 2007;106: abstr 2090.
19. Lu Q, Liu Y, Long BJ, et al. The effect of combining aromatase inhibitors with antiestrogens on tumor growth in a nude mouse model for breast cancer. Breast Cancer Res Treat 1999;57:183-92.
20. Jelovac D, Macedo L, Goloubeva OG, et al. Additive antitumor effect of aromatase inhibitor letrozole and antiestrogen fulvestrant in a postmenopausal breast cancer model. Cancer Res 2005;65:5439-44.
21. Macedo LF, Sabnis GJ, Goloubeva OG, et al. Combination of anastrozole with fulvestrant in the intratumoral aromatase xenograft model. Cancer Res 2008;68:3516-22.
22. Bergh J, Jönsson PE, Lidbrink EK, et al. FACT: an open-label randomized phase III study of fulvestrant and anastrozole in combination compared with anastrozole alone as first-line therapy for patients with receptor-positive postmenopausal breast cancer. J Clin Oncol 2012;30:1919-25.
23. Mehta RS, Barlow WE, Albain KS, et al. Combination anastrozole and fulvestrant in metastatic breast cancer. N Engl J Med 2012;367:435-44.
24. Di Leo A, Jerusalem G, Petruzelka L, et al. Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. J Clin Oncol 2010;28:4594-600.
25. American Cancer Society. Breast Cancer Facts & Figures 2011-2012. Atlanta: American Cancer Society, Inc.2011.
26. Robertson J, Dixon J, Sibbering D, et al. Tumor biomarker changes following pre-surgical treatment with 500 mg fulvestrant plus anastrozole versus 500 mg fulvestrant and 1 mg anastozole alone. Cancer Res 2009;69: abstr 24.
27. Johnston S, KL, Ellis P, et al. Fulvestrant Alone or with Concomitant Anastrozole Vs Exemestane Following Progression on Non-Steroidal Aromatase Inhibitor- First Results of the SeFEa Trial. Eur J Cancer. Presented at 8th European Breast Cancer Conference, Vienna, Austria 2012;48: Page ii, S2).

Cite this article as: Nelson V, Rademaker A, Kaklamani V. Paradigm of polyendocrine therapy in endocrine responsive breast cancer: the role of fulvestrant. Chin Clin Oncol 2013;2(1):10. doi: 10.3978/j.issn.2304-3865.2012.09.04

Bisphosphonates in the adjuvant treatment of young women with breast cancer: the estrogen rich is a poor candidate!

Hamdy A. Azim, Nermine S. Kamal, Rafaat A. Malak

The Department of Clinical Oncology, Cairo University, Giza, Egypt

Correspondence to: Hamdy A. Azim. The Department of Clinical Oncology, Cairo University, Giza, Egypt. Email: azimonc@cairocure.com.

Abstract: During the last 2 decades the role of bisphosphonates (BPs) to reduce skeletal-related events from bone metastases in breast cancer has been well defined. Several preclinical studies have strongly suggested that BPs may also provide an anti-cancer effect in early breast cancer. Indeed, the use of adjuvant BPs represents a unique approach that attempts at eradicating occult tumor micro-metastases residing in the bone marrow via targeting the bone microenvironment to render it less favorable for cancer cell growth. Although, this concept has been tested clinically for more than 15 years, no final consensus has been reached as for the routine use of BPs in the adjuvant phase of breast cancer, owing to conflicting results of randomized studies. Nevertheless, accumulating evidence from recent trials has indicated a therapeutic benefit of adjuvant BPs—particularly zoledronic acid—in women with established menopause, with no or perhaps detrimental effects in premenopausal women. Indeed, this hypothesis has opened a new chapter on the role of estrogen-poor microenvironment as a potential pre-requisite for the anti-tumor effects of BPs in the adjuvant phase of breast cancer. In this review, we will emphasize the biological rational of using BPs to target bone microenvironment in patients with early breast cancer and we will explore mechanistic differences; related to bisphosphonates effects in premenopausal versus postmenopausal women and how the endocrine environment would influence the anticancer potential of these compounds.

Keywords: Adjuvant; bisphosphonates (BPs); anti-tumor activity; premeopausal; breast cancer

Submitted May 31, 2013. Accepted for publication Jun 03, 2013.

doi: 10.3978/j.issn.2072-1439.2013.06.04

View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.06.04

Introduction

Unlike other tissues, bone is mainly composed of hard-mineralized tissue; hence it is more resistant to invasion and destruction by cancer cells compared to other metastatic sites (1). Osteoclasts have been described as the most efficient cells to induce bone resorption (1,2). Therefore, and in order to grow in bone matrix, the cancer cells must recruit and activate osteoclasts to destroy the bone matrix which is the main cellular mechanism for cancer induced bone destruction (2-4). This would provide the space in which cancer cells can grow and allow them to induce further molecular interactions with the different cytokines released during bone resorption, thus creating a microenvironment that is conducive for tumor invasion "soil and seed hypothesis" (3-6). The details of cross talks between breast cancer cells and bone microenvironment is shown in *Figure 1*. Identifying osteoclasts as the main cellular component in the development and progression of bone metastasis, has promoted the use of bisphosphonates (BPs), which are potent inhibitors of osteoclastic bone resorption, in the treatment of almost all types of bone metastases (7,8). In clinical practice, four BPs (clodronate, pamidronate, ibandronate, and zoledronic acid) have been widely used to treat breast cancer patients with bone metastases. In placebo controlled studies, these agents could significantly decrease skeletal-related events (SREs) associated with bone metastases in the treated patients, with zoledronic acid (ZA) clearly producing the greatest benefit in these patients (41% reduction in SRE versus placebo and 20% versus pamidronate) (9,10). BPs localize predominantly to skeletal areas of high bone turnover including osteolytic bone metastases. The two negatively charged phosphonate

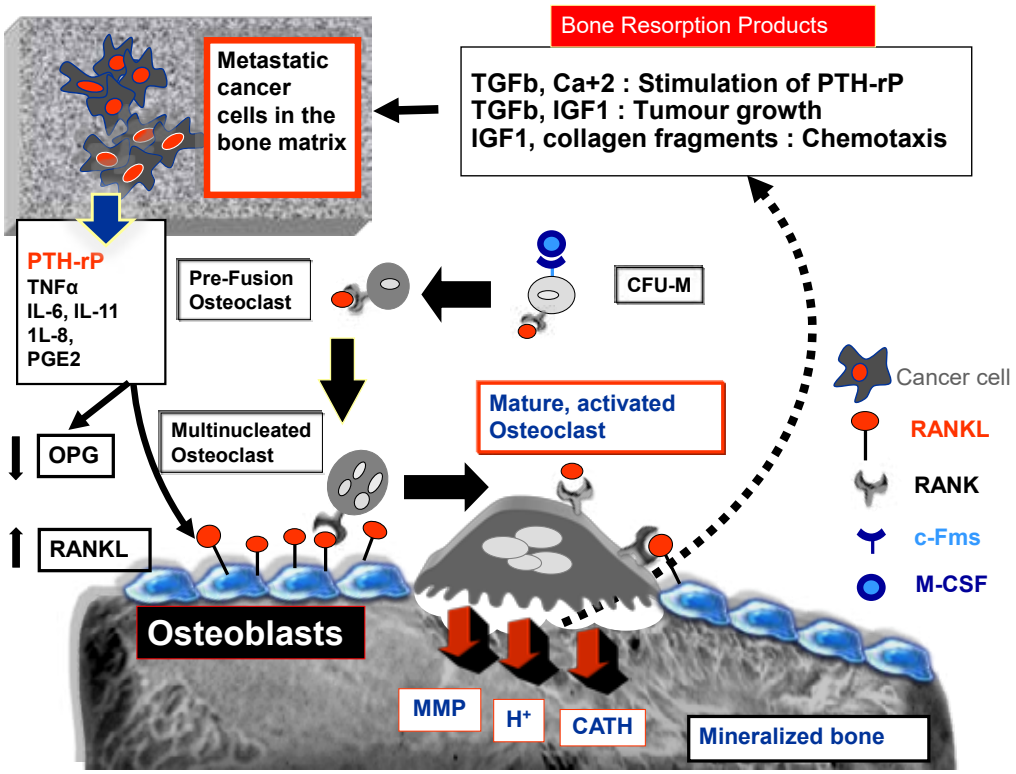


Figure 1 Molecular basis of bone metastasis in breast cancer: tumor cell-osteoclast cross talks comments. Diagrammatic illustration of Osteoclasts (OC) activation and its interplay with breast cancer cells and bone microenvironment. OC precursors differentiate from the population of monocytes/macrophages (CFU-M), by virtue of their expression of the receptor RANK. When RANKL (expressed by osteoblasts, and stromal cells) binds to this receptor in the presence of M-CSF, which in turn binds to its receptor, c-Fms, OC precursors differentiate and fuse together to form mature, multinucleated bone-resorbing OCs. Activated osteoclasts will then attach to the bone surface and via a proton pump mechanism it secrets hydrogen ions that dissolve bone minerals thus releasing calcium ions into the extracellular space. Osteoclasts also secret proteolytic enzymes like matrix metalloproteinases, collagenases, cathepsins and cysteine proteinases to induce collagen degradation and digestion of the organic matrix. Large amount of TGF-b and IGF II and other cytokines are stored within the mineralized bone matrix, and will be released during the process of OC bone resorption. When breast cancer cells colonize within the bone matrix, they start to secrete PTHrP and other osteolytic cytokines, which stimulate osteoblast production of RANKL while OPG levels are reduced, leading to enhanced osteoclastogenesis and increased bone resorption. Consequently the local milleau will be enriched by growth factors and other products of osteolysis (extracellular Ca++ and collagen fragments) which will induce: 1-stimulation of PTH-rP secretion (via TGF, Ca+ 2), 2-stimulation of tumor growth (via TGF, IGF1) and 3-chemotaxis of circulating tumor cells to arrest in bone matrix (via IGF1, collagen fragments). This evokes further PTH-rP release with worsening osteolysis, in addition to supporting the growth of breast cancer cells within the bone matrix. This reciprocal feedback between tumor cells and the bone microenvironment has been referred to as the "vicious cycle" of bone destruction. PTH-rP, parathyroid hormone related protein; TGF , transforming growth factor beta; Ca, calcium; IGF1, insulin growth factor 1; OPG, osteoprotegerin; RANKL, RANK ligand; c-Fms, colony-stimulating factor receptor 1.

groups give these compounds the ability to bind with a very high affinity to calcium ions within the hydroxyapatite crystals in mineralized bones (11,12) where they are concentrated for a very long half life that may exceed one year (as in case of ZA) (13). BPs are subsequently released from the bone mineral during bone resorption, to be internalized by the activated osteoclasts (11,12). In general all BPs inhibit osteoclast formation and migration, and promote osteoclast apoptosis. BPs also increase production of osteoprogerin (OPG) by osteoblasts (14). OPG is a secreted soluble receptor, that functions as a decoy receptor for RANKL, which is a pivotal molecule

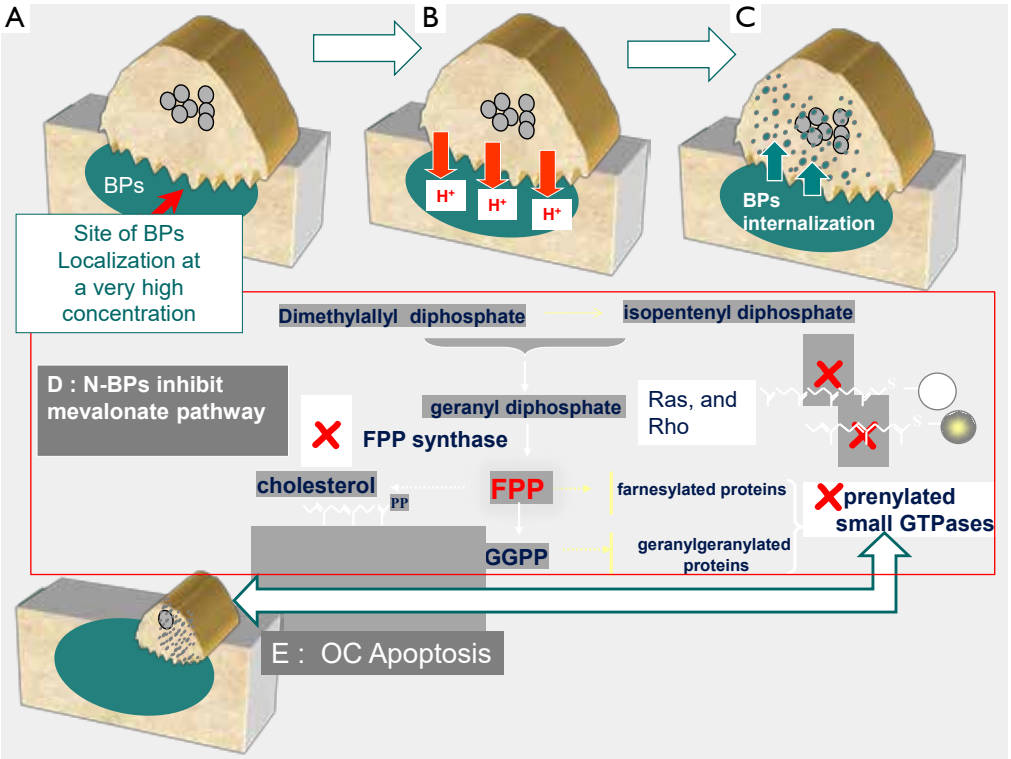


Figure 2 Nitrogen-containing bisphosphonates anti-osteoclastic and anti-tumor molecular mechanism of action. A. BPs localize with a very high affinity to skeletal areas of high bone turnover including osteolytic bone metastases where they are concentrated underneath the activated osteoclasts; B. BPs are subsequently released from the bone mineral during bone resorption; C. BPs are then internalized by the activated osteoclasts; D. Within the osteoclasts (and also breast cancer cells) the N-BPs inhibit the activity of farnesyl diphosphonate (FPP) synthase, a key enzyme in the mevalonate pathway. FPP is necessary for prenylation of small guanine triphosphatases (GTPases)--such as Ras, and Rho, which are involved in intracellular signaling; E. Inhibition of the mevalonate pathway will ultimately cause osteoclasts to undergo apoptosis. Experimental studies have shown that inhibition of this pathway by BPs, will also results in inhibition of malignant cell growth and survival in cell culture and animal models.

for osteoclastic activation. Hence, OPG is considered as a natural inhibitor of osteoclastogenesis, that induces suppression of physiological and pathological bone resorption (5,6). Of note, BPs are cleared rapidly from the blood stream via their avid binding to mineralized bone and by renal filtration of unbound drug (15). As these agents do not readily cross the plasma membrane, the intracellular concentration of BPs in most tissues is very low.

Anti-cancer effects of BPs in breast cancer

Extensive *in vitro* and animal data suggests that BPs may act as antitumor agents and can reduce skeletal tumor burden (15,16). However, and in view of their high affinity for bone mineral and very low concentration in other tissues, the evidence for their *in vivo* antitumor activity outside the bone is less convincing (17-19). BPs exert direct antitumor effects via inhibition of tumor cell adhesion, invasion, and proliferation, in addition to induction of tumor cell apoptosis (15,16). A major molecular target inhibited by nitrogen containing BP (N-BP) like ZA, pamidronate, and ibandronate is farnesyl pyrophosphate synthase (FPPS), a key enzyme in the mevalonate pathway (20,21). This is an important metabolic pathway required for producing steroids, maintaining cell-membrane integrity, regulating cellular metabolism and is also crucial for the prenylation of regulatory proteins involved in many intracellular signaling pathways that control cell proliferation. Inhibition of the mevalonate pathway will ultimately cause osteoclasts to undergo apoptosis (20,21) (*Figure 2*). The mevalonate

pathway is also an important part of the metabolic and proliferative processes in cancer cells. Compared to other BPs, ZA has been shown to be the most potent inhibitor of FPPS activity in cancer cells, which correlates with its highest anti-osteoclastic activity *in vitro* and *in vivo* . The N-BPs may also act indirectly on tumor cells through anti-angiogenic (23) and immuno-modulatory mechanisms (24-26). The later is especially attributed to their ability to accumulate in macrophages and monocytes which share the same ontogeny with osteoclasts (24). Therapeutic doses of ZA has been shown to modulate monocyte, macrophage and dendritic cell function and improve the T-cell anti-cancer properties (16,22,25,27).

Although the exact mechanism(s) responsible for the observed anti-tumor effects of BPs remains unclear, recent data from animal studies strongly suggested that the main *in vivo* effect of clinically relevant doses of BPs on breast cancer cells, is mediated via inhibition of osteoclast-mediated bone resorption rather than a direct cytotoxic effect (28). This supports the argument that tumor growth can be effectively inhibited in the clinic by targeting the bone microenvironment and not necessarily via a direct cytotoxic effect against the primary tumor.

Bone microenvironment as a rational target to prevent breast cancer relapse

It has been known for a while that dormant tumor cells (DTCs) in the bone marrow (BM), can provide a major source of late relapse in patients with early breast cancer (EBC). A significant correlation between DTC in bone marrow or circulating tumor cells in the blood stream and poor prognosis has been demonstrated in several studies (29). Indeed, the BM microenvironment can provide an ideal sanctuary site for these cancer cells to evade systemic anticancer therapy (30). Two distinct protective interactions within the bone marrow have been described as an endosteal niche and a vascular niche (31). The endosteal niche allows DTC to interact with osteoblasts, which are critical mediators of stem cell dormancy and survival. The vascular niche facilitates DTC to interact with hematopoietic stem cells. Meads *et al.* has shown that the hematopoietic stem cell can induce environmentally mediated drug resistance (EM-DR), which protects the tumor cells from the cytotoxic effects of chemotherapy as well as the physiologic mediators of cell death (32). Although, the specific signals responsible for reactivation of DTC are still unclear (33,34), yet it has been postulated that DTC in the BM can be activated by osteoclast-mediated release of bone derived growth factors (34), to form metastases at other osseous and non osseous sites, while serving also as a source of local recurrences ('tumor self-seeding' phenomenon) (35).

In several phase II clinical studies, including women with high risk, early-stage breast cancer, both ZA and ibandronate, in combination with standard adjuvant therapy, could effectively reduce DTC number and persistence in bone marrow compared with standard therapy alone (36-39). Although, the prognostic impact of such reduction of DTC has never been addressed in these studies, yet this should definitely bring enthusiasm to incorporate BPs into the adjuvant treatment regimens in EBC, in an attempt to interfere with the unique support that bone micro environment provides to cancer cell survival. Altering the BM microenvironment by adjuvant BPs therapy would--at least theoretically--render it less conducive to cancer cell survival, and therefore may provide a unique mechanism to prevent cancer recurrence in EBC (16,28,34).

The emergence of estrogen poor microenvironment as a pre-requisite to obtain a therapeutic benefit from adjuvant BPs

It is widely accepted that estrogens play a critical role in the maintenance of bone homeostasis and that the osteoclastic activation, in response to estrogen depletion is the main cellular basis of bone resorption in postmenopausal women (40,41). Importantly, it has been hypothesized that increased bone resorption would create a bone microenvironment that might serve as a homing site for DTCs, that would be subsequently associated with increase rate of relapse (42,43). Recently, this notion was indirectly supported in the clinic by some speculations from the MA27 study which was designed to compare anastrazole versus exemestane in post menopausal women with EBC. The study has reported no difference between the 2 aromatase inhibitors in terms of DFS (44). However, in a subsequent exploratory analysis, the authors have shown that patients who had osteoporosis (self reporting) and who received no therapy for their osteoporosis had the highest rate of relapse, compared to those who never had osteoporosis or those who received osteoporosis therapy (45). This strongly supports the hypothesis that an impaired bone micro-environment induced by post menopausal estrogen depletion and aromatase inhibitors (AIs) treatment would provide a fertile "soil" for DTC, and that osteoporosis (as

a surrogate marker of estrogen depletion) would negatively affect the treatment outcomes in EBC patients, which can be significantly reversed by anti-bone resorption therapy.

More recently, and in a very good animal model, that mimics the clinical situation in EBC, the group of Sheffield University has unequivocally shown that ZA could prevent breast cancer relapse only in estrogen poor microenvironment (i.e., in the ovariectomized mice), with no benefit at all in non ovariectomized mice (46). This study presented the first direct clue for a differential anti-tumor effect of ZA in the pre versus post-menopausal settings, which directly proves that the anti-cancer effect of adjuvant BPs will be exclusively seen in the post-menopausal setting, and that ZA (and probably other BPs) would mainly act by inhibiting an ovarian suppression-mediated proliferation of tumor cells resident in the BM. Therefore, estrogen poor microenvironment, with its accelerated bone resorption sequences seems to be a prerequisite to obtain a therapeutic anti-tumor benefit from adjuvant BPs (16,46,47).

Interpretation of adjuvant BPs clinical trials in early breast cancer

The first generation of clinical studies testing the anti-tumor role of BPs in early breast cancer evaluated oral clodronate in 3 randomized trials. The long term follow-up data have shown conflicting outcome, with 2 studies (48,49) demonstrating a significant benefit at some follow up periods, while in the 3rd trial the ten-year DFS was significantly lower in the clodronate group compared to the control arm (45% *vs.* 58%, P=0.01, respectively) (50). A meta-analysis of the three trials has shown that clodronate did not provide any significant benefit in bone metastasis-free survival, or DFS (51). Therefore, no real take home message could have been concluded from these trials.

Later on, the ABCSG-12 and the ZO-FAST trials, have strongly concluded for a therapeutic benefit of adjuvant ZA in women with poor estrogen microenvironment at the time of their breast cancer treatment. The ABCSG12 study (52), included 1,803 premenopausal women with stage I/II breast cancer, who were randomized to receive 3 years of ZA versus observation; added to endocrine therapy (luteinizing hormone-releasing hormone agonist to suppress the ovarian function and anastrozole or tamoxifen). The study demonstrated a 36% reduction in the relative risk of disease progression among those patients taking ZA. Importantly, and unlike the earlier clodronate studies, the therapeutic gain obtained by ZA was maintained at 84 months median follow-up, with a significant benefit in DFS (HR=0.72; *P*=0.014) and OS (HR=0.63; *P*= 0.049) (53). The ZO-FAST trial included 1,065 Stage I-IIIa, ER positive postmenopausal patients who were treated with letrozole and were randomized to either immediate or delayed ZA (54). Delayed ZA therapy was administered in case of non-traumatic fracture or crossing a bone loss threshold. At 5 years follow up, a DFS benefit (which was a secondary endpoint) of immediate ZA treatment has been reported (HR=0.66; log-rank P value=0.0375) with a trend for an OS gain (HR=0.69; P value=0.196). Of notice, the patients in the above 2 trials were treated with endocrine therapies known to induce a profound estrogen poor environment and significant bone loss. The patients in the 2 trials have received a small dose of ZA (once/6 months), that was good enough to prevent bone loss in the treated patients (which was a secondary end point for the ABCSG-12 trial and a primary end points for the ZO-FAST trial).

Unfortunately, the 2 studies cannot really answer the question related to the benefit of adjuvant BPs in other adjuvant settings (i.e., in women with estrogen rich microenvironment or in women with ER negative EBC). However, the exclusive benefit of adjuvant ZA in women with estrogen poor environment was subsequently concluded from the Azure trial, which was a randomized phase III study addressing the role of adjuvant ZA (5 years of ZA in a gradual tapering fashion) in chemotherapy treated stage II/III breast cancer. Of notice the Azure study failed to show that adding ZA to chemotherapy improves disease-free survival in the overall patient population (which was its primary endpoint). However, in a pre-specified subgroup analysis, the postmenopausal patients (5 years or more) had an significant DFS benefit with the addition of ZA (Adjusted HR=0.75; 95% CI: 0.59-0.96; P=0.02) (55). The restricted benefit of BPs adjuvant treatment in postmenopausal women was further suggested by 2 subsequent phase III studies: NSABP B-34 (3,323 patients randomized to receive oral clodronate 1,600 mg or placebo daily for 3 years and GAIN trial [3,023 randomized to receive oral ibandronate (50 mg daily for 2 years) or observation] (56,57). In line with AZURE trial, these 2 studies failed to show improvement in DFS, which was their primary end point. Still, again prespecified subgroup analysis suggested that BPs might perform better in patients who are ≥50 years (in NSABP B-34) and ≥60 years (in GAIN), or in other wards those who would have achieved complete ovarian suppression at the time of BPs treatment.

Bisphosphonates in the adjuvant treatment of young breast cancer patients: is it ready for a prime time?

With the exception of the ABCSG 12 and the ZOFAST [and its sister trials Z-FAST and E-ZO-FAST (58,59)], the majority of clinical trials addressing the anti-cancer role of adjuvant BPs in EBC, were designed on "the one size fits all" approach (*Table 1*) as they included a very heterogeneous patient population in terms of the disease phenotypes, menstrual status, and type of the standard adjuvant treatment given to their patients, which in our opinion was a major reason for their hard to interpret results. Furthermore, the 3 largest studies, AZURE, B34 and GAIN, had used different types of BPs for a variable treatment period (ranging from 2 to 5 years) and adopted different definitions of menopause. This would certainly pose many difficulties towards their combined analysis. Nevertheless, a meta-analysis of these 3 trials together with other 3 trials that specifically evaluated the effects of adjuvant BPs on DFS according to menopausal was recently presented (60). The authors reported no beneficial effect in the entire population of EBC treated by BPs compared to the control arm, with a significant DFS benefit in the subgroup of women with established menopause [HR=0.81 (0.69-0.95)]. However, an alarming conclusion was made in this meta-analysis, which suggested an apparent harm of adjuvant BPs in pre- and perimenopausal women. Importantly, this observation has been previously highlighted by AZURE study in which there was a significant detrimental effect of ZA on the rate of non-skeletal metastases in premenopausal women, that was independent of the ER status of the tumor [HR=1.32 (95% CI: 1.09-1.59)], and that was never discussed by the authors (55). Interestingly an older Finnish trial had also made a similar conclusion, when clodronate was given in the adjuvant setting, where the frequency of non-skeletal recurrences was significantly higher in the clodronate group versus the control group especially in ER negative patients (DFS at 10 years were 25% *vs.* 58%, P=0.004, respectively). Importantly, in this particular study, the only subgroup where no adverse effect of clodronate was seen, were postmenopausal ER positive patients (50). Of interest, some preclinical studies have also indicated that adjuvant BPs may enhance the development of non-skeletal metastases, if given without a concomitant anticancer drugs (like the situation in the long term BPs treatment in ER negative breast cancer) (19). This particular observation was strongly emphasized as a worrying issue when BPs are to used in the prevention setting (4,50). Till further evidence emerges, this potentially detrimental effect of adjuvant BPs in premenopausal and/or ER negative EBC could be considered due to chance. Still we wish to raise some critical questions in this context: what could be putatively tumor promoting when a high dose of ZA (as adopted in the AZURE) is given in the adjuvant phase of BC in premenopausal women? Is it the estrogen rich microenvironment or is it the ER negative phenotype or both? In fact, there is a lot of potential speculations to explain the lack of response to ZA in estrogen rich microenvironment (61). Of notice estrogen and BPs may interact at the level of BM cancer cell dormancy. The estrogen-rich bone microenvironment appears to better support the survival and expansion of DTC in the endosteal niche. This observation is supported by the findings that estrogen increases the number and activity of endosteal osteoblasts, which are critical mediators of stem cell dormancy and survival (30,62). This may imply that the ability of BPs to decrease DTC is offset by the high level of oestrogen in premenopausal women.

Finally, we believe that the altered immune profile in response to ZA that may explain a preferential benefit of this drug in relation to the disease phenotype. As mentioned earlier, standard doses of ZA have been consistently reported to induce selective stimulation of γδ T-cells which exert a beneficial anti-tumor function *in vivo* (16,22,25,26). Clinically, γδ T-cell expansion and activation has been confirmed in cancer patients after ZA administration . Recently, Benzaid *et al.* (27) showed that only the ER positive, HER2 negative breast cancer cell lines are sensitive to the immune-mediated attack by γδ T-cells. This may suggest that ER positive phenotypes are more likely to have a therapeutic benefit from adjuvant ZA. It may be assumed that premenopausal women have more ER negative disease (data not shown by the AZURE authors), which is less sensitive to γδ T-cell-mediated cytotoxicity.

Another immunologically significant molecule affected by ZA is OPG, which as mentioned earlier is a potent inhibitor of bone resorption. The ability of OPG to inhibit osteolysis suggests that OPG can have an inhibitory effect on cancer-induced bone disease and metastasis (5,6). Both ZA (in a dose dependant fashion) (14) and estrogen have been reported to increase the serum level of OPG (63-65), which is one of the suggested mechanisms for their anti-resorptive function. Interestingly, OPG may promote tumor cell survival though its ability to enhance angiogenesis and to inhibit TRAIL induced apoptosis (66-68). TRAIL

Table 1 The major trials testing the anti-tumor effects of bisphosphonates in women with EBC remarks.

TRIAL Type of BP Duration of BP	No. of patients	Age	Post menopausal	ER/PR positive	Chemo therapy	Hormone treatment	HR (DFS) Median FU in mo	P value	HR (OS)	P value
AZURE ZA (high dose) ×5 years	3,360	NA	45%	78%	95.5%	NA, mostly Tam	adj 0.98 At 60 mo	0.79	0.85	0.07
ABCSG-12 ZA (/6 mo) ×3 years	1,803	45	Induced by LHRH agonist goserelin	≥90%	5%	LHRH +TAM or Ana	0.72 At 84 mo	0.01	0.61	0.03
NSABP 34 Clodronate ×3 years	3,323	50	NA	7%	NA	Tam	0.91 At 90 mo	0.27	0.84	0.10
GAIN Ibandronate 50 mg/po ×2 years	3,023	49.5	51.6%	77%	100%	Mostly Tam	0.94 At 36 mo	0.59	1.04	0.8
ZOFAST ZA (/6 mo) ×5 years	1,065	57	100% 83% Established	100%	54%	Letrozole	0.66 At 60 mo	0.037	0.69	0.196
ZFAST* ZA (/6 mo) ×5 years	602	60	100%	100%	47%	Letrozole	NA At 61 mo	0.628	NA	
EZOFAST** ZA (/6 mo) ×5 years	527	58	100%	100%	52%	Letrozole	NA At 12 mo		NA	
Powles 2006 Clodronate ×2 years	1,069	52.8	50%	ER+: 45% PR+: 22%	60%	TAM	Bone metastasis HR 0.69	0.043	0.768	0.048
Diel 2008*** Clodronate ×2 years	290	NA	62%	73%	43%	TAM Goserelin 9%	NA		NA	
Saarto 2004 Clodronate ×3 years	282	52	50%	61% (ER) 55% (PR)	54%	TAM 63% Toremifen 37%	RR 1.52 At 120 mo	0.02	RR 0.33	0.12

*ZFAST, At month 61 DFS events were almost similar in the 2 groups [percentage (95% CI): upfront, 9.8 (6.0-10.3); delayed, 10.5 (6.6-14.4); P=0.6283]. Disease recurrence alone occurred in slightly more delayed group patients compared with the upfront group [16 patients (5.3%) vs. 21 patients (7.0%)]. **EZOFAST, At 12 months, 7 patients (2.8%) in the immediate ZOL group and 5 patients (1.9%) in the delayed ZOL group experienced distant recurrent disease. ***Diel's Study, significant OS improvement in the clodronate group at a median follow-up of 103±12 months, 79.6% in the clodronate group versus 39.3% in the control group group (P=0.04). The Significant reductions in the incidence of bony and visceral metastases and improvement in of DFS at 36- and 55-month follow-up periods were no longer seen with clodronate at a median follow-up period of 103 months.

(TNF-related apoptosis-inducing ligand) is an important molecule mediating major antitumor effects of the immune system (66). Importantly, in several cancer types, elevated levels of serum OPG were significantly associated with poor prognosis (69,70). Of note, it has been shown that OPG preferentially protects ER negative breast cancer cell lines from TRAIL-induced apoptosis *in vitro* (71). Taken together, we speculate that the premenopausal population like those treated in the AZURE trial, could have been exposed to a higher concentration of OPG in skeletal and none skeletal sites, secondary to their elevated estrogen levels and the high dose of ZA. This relative increase of OPG may shift the fine balance involved between the beneficial effects of OPG in skeletal sites, and potentially detrimental effects of inhibiting TRAIL-mediated tumor cell apoptosis and stimulation of angiogenesis (68). Actually, and in line with our assumption, premenopausal women in the AZURE did not have any detrimental effect of ZA on skeletal relapse rate. On the contrary, there was a non significant reduction of skeletal relapse in ZA treated patients compared to the control group [HR=0.86 (95% CI: 0.63-1.16)]. This would again argue for a preferential role of the immune system when a patient is exposed to high dose of ZA during the adjuvant setting: a beneficial effect in ER positive phenotype (more sensitive to γδ T cells cytotoxicity) and a potentially detrimental effect in ER negative phenotype (more protected by OPG induced Trail inhibition). Since ZA dose is critical in regulating OPG, then the positive results observed in the ABCSG-12 and ZOFAST may be also explained by the low level of OPG related to the 6 monthly ZA treatment, being given in an estrogen poor microenvironment, in a pure ER positive population which was not the case in the AZURE.

In conclusion, a number of clinical trials and animal studies have strongly suggested that the benefits of adjuvant bone targeted treatments on risks of recurrence or death in EBC are restricted to women with established menopause (72). We strongly believe that this statement is clinically and biologically correct. However, while we are focusing on 'the estrogen poor soil', as a prerequisite for a preferential benefit of adjuvant BPs, the properties of 'the seed' may be also valuable or even crucial in this context, where the ER positive and not the ER negative breast cancer phenotype may be expected to derive the maximum benefit of these agents. To this end we would certainly recommend the use of low dose of ZA (at 4 mg/6 months) in all ER positive premenopausal women whose treatment regimens includes LHRH agonist, or those who develop complete ovarian suppression following adjuvant chemotherapy. At this dose level of ZA, the associated bone loss will be effectively prevented in the treated patients, which will be the ideal approach to maintain their bone health. Furthermore ZA at this dose can effectively interrupt the cross talk between DTC and the estrogen poor bone microenvironment, a step that has been reported to potentially improve DFS in EBC. Importantly, the ABCSG-12 which is the only study that included a pure premenopausal population (median age 45 years) has recently reported in a preplanned subgroup analyses based on age (≤40 years or >40 years), that ZA significantly improved DFS by 34% in women over 40 years of age (n=1,390; HR=0.66; P=0.013), while it did not improve the DFS in women who were 40 years of age or younger (n=413) (53). The authors have attributed this to the assumption that women over 40 years of age may achieve more complete ovarian suppression. While this statement is certainly valid for women treated by adjuvant chemotherapy, it cannot be applied to the population included in the ABCSG 12 (less than 10% received chemotherapy only during the neoadjuvant phase). Furthermore, the results in women ≤40 years of age were concluded from a total of 77 DFS events at 84 months, which looks as insufficient evidence to preclude ZA benefit in these women. As the anti-tumor effects of adjuvant BPs might be exclusively observed in patients with estrogen depletion and accelerated bone loss, or in other words in those patients with a susceptible soil, then we confidently assume that it is the menopausal status rather than age that will determine the benefit of adjuvant BPs in young women. Taken together, the biological concept that one size does not fit all, seems to be very true when it comes to the role of BPs in premenopausal women with EBC.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Roodman GD. Cell biology of the osteoclast. Exp Hematol 1999;27:1229-41.

2. Guise TA, Mundy GR. Cancer and bone. Endocr Rev 1998;19:18-54.
3. Guise TA, Mohammad KS, Clines G, et al. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clin Cancer Res 2006;12:6213s-6s.
4. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2002;2:584-93.
5. Dougall WC. RANKL signaling in bone physiology and cancer. Curr Opin Support Palliat Care 2007;1:317-22.
6. Azim HA, Kamal NS, Azim HA Jr. Bone metastasis in breast cancer: the story of RANK-ligand. J Egypt Natl Canc Inst 2012;24:107-14.
7. Aapro M, Abrahamsson PA, Body JJ, et al. Guidance on the use of bisphosphonates in solid tumours: recommendations of an international expert panel. Ann Oncol 2008;19:420-32.
8. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat Rev 2001;27:165-76.
9. Pavlakis N, Schmidt R, Stockler M. Bisphosphonates for breast cancer. Cochrane Database Syst Rev 2005:CD003474.
10. Rosen LS, Gordon DH, Dugan W Jr, et al. Zoledronic acid is superior to pamidronate for the treatment of bone metastases in breast carcinoma patients with at least one osteolytic lesion. Cancer 2004;100:36-43.
11. Fleisch H. Development of bisphosphonates. Breast Cancer Res 2002;4:30-4.
12. Rogers MJ, Gordon S, Benford HL, et al. Cellular and molecular mechanisms of action of bisphosphonates. Cancer 2000;88:2961-78.
13. Brown JE, Ellis SP, Lester JE, et al. Prolonged efficacy of a single dose of the bisphosphonate zoledronic acid. Clin Cancer Res 2007;13:5406-10.
14. Viereck V, Emons G, Lauck V, et al. Bisphosphonates pamidronate and zoledronic acid stimulate osteoprotegerin production by primary human osteoblasts. Biochem Biophys Res Commun 2002;291:680-6.
15. Daubiné F, Le Gall C, Gasser J, et al. Antitumor effects of clinical dosing regimens of bisphosphonates in experimental breast cancer bone metastasis. J Natl Cancer Inst 2007;99:322-30.
16. Clezardin P. Potential anticancer properties of bisphosphonates: insights from preclinical studies. Anticancer Agents Med Chem 2012;12:102-13.
17. Sasaki A, Boyce BF, Story B, et al. Bisphosphonate risedronate reduces metastatic human breast cancer burden in bone in nude mice. Cancer Res 1995;55:3551-7.
18. Hiraga T, Williams PJ, Mundy GR, et al. The bisphosphonate ibandronate promotes apoptosis in MDA-MB-231 human breast cancer cells in bone metastases. Cancer Res 2001;61:4418-24.
19. Michigami T, Hiraga T, Williams PJ, et al. The effect of the bisphosphonate ibandronate on breast cancer metastasis to visceral organs. Breast Cancer Res Treat 2002;75:249-58.
20. Benford HL, Frith JC, Auriola S, et al. Farnesol and geranylgeraniol prevent activation of caspases by aminobisphosphonates: biochemical evidence for two distinct pharmacological classes of bisphosphonate drugs. Mol Pharmacol 1999;56:131-40.
21. Luckman SP, Hughes DE, Coxon FP, et al. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. J Bone Miner Res 1998;13:581-9.
22. Winter MC, Holen I, Coleman RE. Exploring the anti-tumour activity of bisphosphonates in early breast cancer. Cancer Treat Rev 2008;34:453-75.
23. Stresing V, Fournier PG, Bellahcène A, et al. Nitrogen-containing bisphosphonates can inhibit angiogenesis in vivo without the involvement of farnesyl pyrophosphate synthase. Bone 2011;48:259-66.
24. Rogers TL, Holen I. Tumour macrophages as potential targets of bisphosphonates. J Transl Med 2011;9:177.
25. Nussbaumer O, Gruenbacher G, Gander H, et al. DC-like cell-dependent activation of human natural killer cells by the bisphosphonate zoledronic acid is regulated by γδ T lymphocytes. Blood 2011;118:2743-51.
26. Dieli F, Gebbia N, Poccia F, et al. Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. Blood 2003;102:2310-1.
27. Benzaïd I, Mönkkönen H, Stresing V, et al. High phosphoantigen levels in bisphosphonate-treated human breast tumors promote Vgamma9Vdelta2 T-cell chemotaxis and cytotoxicity in vivo. Cancer Res 2011;71:4562-72.
28. Fournier PG, Stresing V, Ebetino FH, et al. How do bisphosphonates inhibit bone metastasis in vivo? Neoplasia 2010;12:571-8.
29. Braun S, Vogl FD, Naume B, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 2005;353:793-802.
30. Li L, Neaves WB. Normal stem cells and cancer stem cells: the niche matters. Cancer Res 2006;66:4553-7.
31. Shiozawa Y, Havens AM, Pienta KJ, et al. The bone

marrow niche: habitat to hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites. Leukemia 2008;22:941-50.
32. Meads MB, Hazlehurst LA, Dalton WS. The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. Clin Cancer Res 2008;14:2519-26.
33. Aguirre-Ghiso JA. The problem of cancer dormancy: understanding the basic mechanisms and identifying therapeutic opportunities. Cell Cycle 2006;5:1740-3.
34. Gnant M, Hadji P. Prevention of bone metastases and management of bone health in early breast cancer. Breast Cancer Res 2010;12:216.
35. Kim MY, Oskarsson T, Acharyya S, et al. Tumor self-seeding by circulating cancer cells. Cell 2009;139:1315-26.
36. Aft R, Naughton M, Trinkaus K, Effect of zoledronic acid on disseminated tumour cells in women with locally advanced breast cancer: an open label, randomised, phase 2 trial. Lancet Oncol 2010;11:421-8.
37. Rack B, Jückstock J, Genss EM, et al. Effect of zoledronate on persisting isolated tumour cells in patients with early breast cancer. Anticancer Res 2010;30:1807-13.
38. Solomayer EF, Gebauer G, Hirnle P, et al. Influence of zoledronic acid on disseminated tumor cells in primary breast cancer patients. Ann Oncol 2012;23:2271-7.
39. Hoffmann O, Aktas B, Goldnau C, et al. Effect of ibandronate on disseminated tumor cells in the bone marrow of patients with primary breast cancer: a pilot study. Anticancer Res 2011;31:3623-8.
40. Clarke BL, Khosla S. Physiology of bone loss. Radiol Clin North Am 2010;48:483-95.
41. Azim H, Azim HA Jr. Targeting RANKL in breast cancer: bone metastasis and beyond. Expert Rev Anticancer Ther 2013;13:195-201.
42. Orr W, Varani J, Gondex MK, et al. Chemotactic responses of tumor cells to products of resorbing bone. Science 1979;203:176-9.
43. Lipton A, Chapman JA, Demers L, et al. Elevated bone turnover predicts for bone metastasis in postmenopausal breast cancer: results of NCIC CTG MA.14. J Clin Oncol 2011;29:3605-10.
44. Goss PE, Ingle JN, Pritchard KI, et al. Exemestane versus anastrozole in postmenopausal women with early breast cancer: NCIC CTG MA.27--a randomized controlled phase III trial. J Clin Oncol 2013;31:1398-404.
45. Shepherd LE, Chapman JA, Ali SM, et al. Effect of osteoporosis in postmenopausal breast cancer patients randomized to adjuvant exemestane or anastrozole: NCIC CTG MA.27. J Clin Oncol 2012;30: abstr 501.
46. Holen I, Wang N, Reeves KJ, et al. Zoledronic acid specifically inhibits development of bone metastases in the post-menopausal setting-evidence from an in vivo breast cancer model. Cancer Research 2012; 72:PD07-08.
47. Winter MC, Coleman RE. Bisphosphonates in the adjuvant treatment of breast cancer. Clin Oncol (R Coll Radiol) 2013;25:135-45.
48. Powles T, Paterson A, McCloskey E, et al. Reduction in bone relapse and improved survival with oral clodronate for adjuvant treatment of operable breast cancer [ISRCTN83688026]. Breast Cancer Res 2006;8:R13.
49. Diel IJ, Jaschke A, Solomayer EF, et al. Adjuvant oral clodronate improves the overall survival of primary breast cancer patients with micrometastases to the bone marrow: a long-term follow-up. Ann Oncol 2008;19:2007-11.
50. Saarto T, Vehmanen L, Virkkunen P, et al. Ten-year follow-up of a randomized controlled trial of adjuvant clodronate treatment in node-positive breast cancer patients. Acta Oncol 2004;43:650-6.
51. Ha TC, Li H. Meta-analysis of clodronate and breast cancer survival. Br J Cancer 2007;96:1796-801.
52. Gnant M, Mlineritsch B, Schippinger W, et al. Endocrine therapy plus zoledronic acid in premenopausal breast cancer. N Engl J Med 2009;360:679-91.
53. Gnant M, Mlineritsch B, Luschin-Ebengreuth G, et al. Long-Term Follow-Up in ABCSG-12: Significantly Improved Overall Survival with Adjuvant Zoledronic Acid in Premenopausal Patients with Endocrine-Receptor-Positive Early Breast Cancer. Cancer Res 2011;71:S1-2.
54. de Boer R, Bundred N, Eidtmann H. Long-Term Survival Outcomes among Postmenopausal Women with Hormone Receptor-Positive Early Breast Cancer Receiving Adjuvant Letrozole and Zoledronic Acid: 5-Year Follow-Up of ZO-FAST. Cancer Res 2012;71:S1-3.
55. Coleman RE, Marshall H, Cameron D, et al. Breast-cancer adjuvant therapy with zoledronic acid. N Engl J Med 2011;365:1396-405.
56. Paterson AH, Anderson SJ, Lembersky BC, et al. Oral clodronate for adjuvant treatment of operable breast cancer (National Surgical Adjuvant Breast and Bowel Project protocol B-34): a multicentre, placebo-controlled, randomised trial. Lancet Oncol 2012;13:734-42.
57. Mobus V, Diel IJ, Harbeck H. GAIN Study: A Phase III Trial To Compare ETC vs. EC-TX and Ibandronate vs. Observation in Patients with Node-Positive Primary Breast Cancer-1st Interim Efficacy Analysis. Cancer Res 2012;71:S2-4.
58. Brufsky AM, Harker WG, Beck JT, et al. Final 5-year

results of Z-FAST trial: adjuvant zoledronic acid maintains bone mass in postmenopausal breast cancer patients receiving letrozole. Cancer 2012;118:1192-201.
59. Llombart A, Frassoldati A, Paija O, et al. Immediate Administration of Zoledronic Acid Reduces Aromatase Inhibitor-Associated Bone Loss in Postmenopausal Women With Early Breast Cancer: 12-month analysis of the E-ZO-FAST trial. Clin Breast Cancer 2012;12:40-8.
60. Vidal L, Ben-Aharon I, Rizel S, et al. Bisphosphonates in the adjuvant setting of breast cancer therapy: Effect on survival--A systematic review and meta-analysis. J Clin Oncol 2012;30: abstr 548.
61. Steinman RA, Brufsky AM, Oesterreich S. Zoledronic acid effectiveness against breast cancer metastases-a role for estrogen in the microenvironment? Breast Cancer Res 2012;14:213.
62. Liu CC, Howard GA. Bone-cell changes in estrogen-induced bone-mass increase in mice: dissociation of osteoclasts from bone surfaces. Anat Rec 1991;229:240-50.
63. Martini G, Gennari L, Merlotti D, et al. Serum OPG and RANKL levels before and after intravenous bisphosphonate treatment in Paget's disease of bone. Bone 2007;40:457-63.
64. Perifanis V, Vyzantiadis T, Tziomalos K, et al. Effect of zoledronic acid on markers of bone turnover and mineral density in osteoporotic patients with beta-thalassaemia. Ann Hematol 2007;86:23-30.
65. Hofbauer LC, Schoppet M, Schüller P, et al. Effects of oral contraceptives on circulating osteoprotegerin and soluble RANK ligand serum levels in healthy young women. Clin Endocrinol (Oxf) 2004;60:214-9.
66. 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.
67. Neville-Webbe HL, Cross NA, Eaton CL, et al. Osteoprotegerin (OPG) produced by bone marrow stromal cells protects breast cancer cells from TRAIL-induced apoptosis. Breast Cancer Res Treat 2004;86:269-79.
68. Reid P, Holen I. Pathophysiological roles of osteoprotegerin (OPG). Eur J Cell Biol 2009;88:1-17.
69. Eaton CL, Wells JM, Holen I, et al. Serum osteoprotegerin (OPG) levels are associated with disease progression and response to androgen ablation in patients with prostate cancer. Prostate 2004;59:304-10.
70. Jung K, Lein M, Ringsdorf M, et al. Diagnostic and prognostic validity of serum bone turnover markers in metastatic renal cell carcinoma. J Urol 2006;176:1326-31.
71. MacFarlane M, Merrison W, Dinsdale D, et al. Active caspases and cleaved cytokeratins are sequestered into cytoplasmic inclusions in TRAIL-induced apoptosis. J Cell Biol 2000;148:1239-54.
72. Winter MC, Coleman RE. Bisphosphonates in the adjuvant treatment of breast cancer. Clin Oncol (R Coll Radiol) 2013;25:135-45.

Cite this article as: Azim HA, Kamal NS, Malak RA. Bisphosphonates in the adjuvant treatment of young women with breast cancer: the estrogen rich is a poor candidate! J Thorac Dis 2013;5(S1):S27-S35. doi: 10.3978/j.issn.2072-1439.2013.06.04

Hormonal therapies in young breast cancer patients: when, what and for how long?

Alexandre Christinat, Simona Di Lascio, Olivia Pagani

Institute of Oncology of Southern Switzerland (IOSI) and Breast Unit of Southern Switzerland (CSSI), Bellinzona, Switzerland
Correspondence to: Olivia Pagani. Ospedale San Giovanni, 6500 Bellinzona, Switzerland. Email: olivia.pagani@ibcsg.org.

Abstract: Breast cancer in young women (<40 years) is a rare and complex clinical and psychosocial condition, which deserves multidisciplinary and personalized approaches. In young women with hormone-receptor positive disease, 5 years of adjuvant tamoxifen, with or without ovarian suppression/ablation, is considered the standard endocrine therapy. The definitive role of adjuvant aromatase inhibitors has still to be elucidated: the upcoming results of the Tamoxifen and EXemestane Trial (TEXT) and Suppression of Ovarian Function Trial (SOFT) trials will help understanding if we can widen our current endocrine therapeutic options. The optimal duration of adjuvant endocrine therapy in young women also remains an unresolved issue. The recently reported results of the ATLAS and aTToM trials represent the first evidence of a beneficial effect of extended endocrine therapy in premenopausal women and provide an important opportunity in high-risk young patients. In the metastatic setting, endocrine therapy should be the preferred choice for endocrine responsive disease, unless there is evidence of endocrine resistance or need for rapid disease and/or symptom control. Tamoxifen in combination with ovarian suppression/ablation remains the 1st-line endocrine therapy of choice. Aromatase inhibitors in combination with ovarian suppression/ablation can be considered after progression on tamoxifen and ovarian suppression/ablation. Fulvestrant has not yet been studied in pre-menopausal women. Specific age-related treatment side effects (i.e., menopausal symptoms, change in body image and weight gain, cognitive function impairment, fertility damage/preservation, long-term organ dysfunction, sexuality) and the social impact of diagnosis and treatment (i.e., job discrimination, family management) should be carefully addressed when planning long-lasting endocrine therapies in young women with hormone-receptor positive early and advanced breast cancer.

Keywords: Early breast cancer; young women; premenopausal; advanced breast cancer; endocrine therapy

Submitted May 02, 2013. Accepted for publication, May 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.05.25
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.05.25

Introduction

Breast cancer (BC) in women <40 years is a rare condition (1) despite an increased incidence in premenopausal women has been recently reported in several countries. In the United States, 5.5% of BCs occur in women <40 years (2). While some preliminary data suggested a higher prevalence of triple negative and Human Epidermal Growth Factor Receptor 2 (HER-2)-positive disease and found an age-related expression of key BC-associated genes, when correcting for subtype and other significant clinico-pathologic features [estrogen receptor (ER) status and histologic grade] no gene differences were retained between age-defined groups (≤45 and ≥65 years) (3).

The choice of systemic treatment for invasive BC (both early and advanced disease) should not be age-specific but driven by the biological characteristics of the tumor (including hormone receptor status, HER-2 amplification, proliferation and grade), the tumor stage and patient's comorbidities (4). Premenopausal women with invasive ER-positive (ER+) BC should be considered for adjuvant endocrine therapy (ET) regardless of age, lymph-node status or chemotherapy administration (5-7).

Several open questions of ET in premenopausal women

with ER + BC will be addressed:

(I) Is there a role for neoadjuvant endocrine therapy?
(II) Is there an optimal adjuvant endocrine therapy?
(III) Is there an optimal duration of endocrine therapy?
(IV) What's the role of ovarian function suppression/ablation?
(V) What's the role of chemotherapy-induced-amenorrhea?
(VI) What's the role of aromatase inhibitors?
(VII) What's the impact of side effects?
(VIII) What's the role of endocrine therapy in young women with metastatic breast cancer?

Five years of adjuvant tamoxifen, with or without a luteinizing hormone-releasing hormone (LH-RH) agonist, is considered standard ET for premenopausal women (5,7,8). Based on the efficacy shown in postmenopausal women (9), adjuvant aromatase inhibitors (AIs) in combination with ovarian function suppression (OFS) have been investigated in premenopausal patients with early BC. The Austrian Breast and Colorectal Cancer Study Group Trial 12 (ABCSG-12) has shown that 3-year adjuvant therapy with anastrozole plus goserelin provides a comparable disease-free survival (DFS) to that associated with tamoxifen plus goserelin (10). The upcoming results of the Suppression of Ovarian Function Trial (SOFT) (11) and Tamoxifen and EXemestane Trial (TEXT) (12) trials will provide additional evidence on the role of adjuvant AIs, if any, in premenopausal BC patients.

The optimal duration of adjuvant ET in young women remains uncertain. Recent data from the ATLAS and aTTom studies suggest that continuing tamoxifen to 10 years rather than stopping at 5 years gives a further reduction in recurrence and mortality, particularly after year 10 (13,14). Neo-adjuvant ET in premenopuasal patients has never been adequately studied.

As recommended for early BC, also in the metastatic setting age alone should not be a reason to prescribe more aggressive therapy: ET is the preferred option for ER+ disease, unless there is evidence of endocrine resistance or need for rapid disease and/or symptom control (15,16).

In young patients with ER+ metastatic breast cancer, tamoxifen in combination with OFS or ovarian ablation (OA) is the 1st-line ET of choice (17). AIs together with OFS/OA can be considered after progression on tamoxifen and OFS/OA (18,19). Fulvestrant has not yet been studied in pre-menopausal women (20,21).

In endocrine-responsive metastatic BC, most studies addressing the combination of ET and chemotherapy showed an increased overall response rate (ORR) or an increased time to progression (TTP) but no improvement in overall survival (OS) (22).

Neoadjuvant endocrine therapy

Neoadjuvant ET should not be proposed to young women outside clinical trials (4). Preliminary data suggested that neoadjuvant ET can be effective in premenopausal women (23). The use of letrozole and a LH-RH agonist as primary therapy was investigated in 32 premenopausal women with ER+ BC. The ORR was 50% (95% CI, 32-68%) and no patient progressed during treatment. Response was significantly associated with younger age ($P<0.05$) and a longer duration of treatment ($P<0.05$) (24). In the STAGE study, in 204 patients treated with 24 months' neoadjuvant therapy with goserelin plus anastrozole or tamoxifen, the combination with anastrozole achieved a significantly better ORR than goserelin plus tamoxifen (70.4% *vs.* 50.5%; 95% CI, 6.5-33.3; $P=0.004$) (25). The ORR achieved by the anastrozole group compares favorably to the ORR achieved with chemotherapy in luminal B patients (26) but a definitive randomized trial is warranted.

Tamoxifen

Tamoxifen, a selective ER modulator (SERM), is a prodrug metabolized by CYP3A4 and CYP2D6 into two active hydroxylated metabolites, 4-hydroxytamoxifen and 4-OH-N-des-methyltamoxifen (endoxifen). Endoxifen affinity for ER is about 100 times greater than tamoxifen. The effects on BC cells are produced by inhibition of both translocation and nuclear binding of the ER (27).

The benefits of adjuvant tamoxifen have been repeatedly demonstrated by the meta-analyses of the Early Breast Cancer Trialists Collaborative Group (EBCTCG). The latest overviews showed a substantial benefit both in premenopausal and postmenopausal women with ER+ BC regardless of age or the use of chemotherapy (28-30). In the 2011 overview, with a median follow-up of 13 years (30), 5 years of tamoxifen compared to no ET was associated with a reduction in BC recurrence by 39% [relative risk (RR) for recurrence 0.61, 95% CI, 0.57-0.65]. This translated into a 13% absolute reduction in the risk of recurrence at 15 years (33% versus 46%). The impact on disease recurrence was mainly seen in the first 5 years whereas the mortality reduction was significant throughout the first 15 years. A 9% absolute reduction in BC-related death was

observed at 15 years (24% versus 33%), and the risk of BC mortality was reduced by 30% (RR for death 0.70, 95% CI, 0.64-0.75). No effect of tamoxifen was reported for ER-negative disease. The magnitude of benefit was greater for women with node-positive disease and risk reductions were similar for younger as compared to older women. Several cooperative groups also reported similar benefits of adjuvant ET in very young (<35 years) women as compared to older premenopausal women because of the lower rate of permanent amenorrhea following adjuvant chemotherapy in this population (31-34).

Overall, approximately 20% of ER+ BCs are progesterone receptor (PgR)-negative: these tumors are known to have a worse prognosis than the PgR-positive counterparts (35) but the proportional benefit with tamoxifen is the same as for PgR-positive cancers (30).

HER-2 overexpression is also associated with an adverse prognosis (36). Data on HER-2 influence on adjuvant ET in younger women are limited, but in the presence of oophorectomy, the impact of adjuvant tamoxifen on outcome is comparable in patients with HER2-positive and HER2-negative tumors (37).

An association between CYP2D6 genotype and tamoxifen metabolism influencing anti-tumour activity was investigated in >20 published studies with highly inconsistent results (38). At present, CYP2D6 pharmacogenetic driven treatment decisions cannot be recommended outside clinical studies.

Is there an optimal duration of endocrine therapy?

The duration of ET has not been adequately studied in young women and is still a matter of debate. The recently published ATLAS trial included 15.244 pre- and postmenopausal women (13). Six-thousand-eight-hundred-forty-six women with ER+ disease who received tamoxifen for 5 years were randomized to continue for another 5 years (continuers group) or to stop (control group). With a median follow-up of 7.6 years, continuing tamoxifen reduced the risk for BC recurrence, compared to a 5-year treatment course (18% versus 21%, RR 0.84, 95% CI, 0.76-0.94). A persistent and more significant effect was found after year 10 (RR 0.90, 95% CI, 0.79-1.02 during years 5-9 and 0.70, 95% CI, 0.62-0.90 during subsequent years). The effect was independent of age (10% of patients were premenopausal at study entry) and nodal status (41% of patients were node-positive at diagnosis). A significant reduction in BC mortality (331 *vs.* 397 deaths; P=0.01), and overall mortality (639 *vs.* 722 deaths; P=0.01) were reported. There was a 29% reduction in the risk of BC mortality after year 10 (RR 0.71, 95% CI, 0.58-0.88). Longer therapy was associated with an increased risk for pulmonary embolism (RR 1.87, 95% CI, 1.13-3.07, P=0.01), and endometrial cancer (RR 1.74, 95% CI, 1.30-2.34, P=0.0002) with lower risk in premenopausal women, but no increase in the incidence of stroke (RR 1.06, 95% CI, 0.83-1.36), and a decrease in the incidence of ischemic heart disease (RR 0.76, 95% CI, 0.60-0.95, P=0.02).

Smaller trials in the past didn't suggest any benefit to extend tamoxifen treatment (39,40) but these negative results could have simply been due to the play of chance because of the small numbers of patients recruited.

The UK adjuvant aTTom trial randomly allocated 7,000 women, most with unknown ER status, to continue tamoxifen to 10 years or stop at 5 years: the recently reported findings confirm the ATLAS reduction in recurrence and death from breast cancer (14).

Extending tamoxifen therapy beyond 5 years should be therefore considered in premenopausal women at high risk for late relapse (i.e., pathologically involved nodes, bigger tumors size, higher tumor grade) taking into account quality-of-life issues. In the ATLAS trial 84% of women allocated to continue were still on tamoxifen 2 years after entry (i.e., at year 7 after diagnosis). On the other hand, definite long-term side effects of tamoxifen do exist, which require longer follow-up and meta-analyses of all relevant trials for balanced risk/benefit evaluation.

The NCIC-CTG MA.17/BIG 1-97 trial reported a significant advantage to extended adjuvant ET with 5 years of letrozole in postmenopausal women with ER+ tumors, who had received 5 years of tamoxifen (41). A further analysis reported that premenopausal women at initial BC diagnosis, who became definitively postmenopausal at the time of randomization after 5 years of adjuvant tamoxifen, derived significantly more benefit in terms of DFS, from the extended therapy [hazard ratio (HR) =0.25, 95% CI, 0.12-0.51] than women who were postmenopausal at initial diagnosis, independent of nodal status (42).

Ovarian function suppression/ablation

OFS/OA can be achieved by surgery, radiation, chemotherapy, or LH-RH agonists. If ovarian targeted therapy is given, there is no available evidence favoring a specific form of ovarian function manipulation.

The EBCTG overview demonstrated that OA (by

surgery or irradiation) or OFS with a LH-RH agonist significantly reduce the risk of recurrence and BC mortality in women <50 years with ER+ or ER-unknown early BC (29). However, the effects appear smaller in women who also received chemotherapy, probably because chemotherapy-induced amenorrhea (CIA) attenuated any additional effect of OFS/OA. In addition, the analysis may slightly underestimate the effects of ovarian treatment since 26% of women had ER-unknown disease, a proportion of whom had reasonably ER- disease.

The impact of adding OFS to adjuvant chemotherapy was studied in the ECOG-led Intergroup 0101 trial, where in premenopausal women with ER+ node-positive early BC the addition of both tamoxifen and goserelin improved DFS as compared to chemotherapy alone but no significant effect on DFS was shown with the addition of goserelin alone. A trend to DFS benefit from addition of goserelin to chemotherapy was demonstrated in an unplanned retrospective analysis of women <40 years (43).

Likewise, the International Breast Cancer Study Group (IBCSG) trial VIII randomized premenopausal women with node-negative ER+ early BC to either adjuvant CMF, goserelin for 2 years, or CMF followed by goserelin for 18 months. The addition of goserelin resulted in a small improvement in 5-year DFS that did not reach statistical significance (HR 0.80; 95% CI, 0.57-1.11). In an unplanned analysis according to age the subgroup of women <40 years derived a significant benefit (HR 0.34; 95% CI, 0.14-0.87) (44).

A subsequent larger EBCTG meta-analysis looked only at trials with known ER status and LH-RH agonists as method of OFS (45). OFS proved to be beneficial whether used alone (recurrence risk reduction of 28%, P=0.08), in addition to tamoxifen or chemotherapy (recurrence risk reduction of 13%, P=0.02), and as an alternative to chemotherapy. The effects of LH-RH agonists were greater in women <40 years in whom chemotherapy is less likely to induce permanent amenorrhea. However, there were few trials testing the addition of LH-RH agonists to tamoxifen (with or without chemotherapy) and no trials had compared a LH-RH agonist against chemotherapy with tamoxifen in both arms. Additionally, modern standard chemotherapies are generally less associated with premature menopause than those included in the overview and the question of whether adding a LH-RH agonist is only useful when amenorrhea is not achieved with chemotherapy is still unanswered.

Optimal duration of LH-RH agonists is also unknown, although most studies have utilized 2-3 years of LH-RH agonists with 5 years of tamoxifen.

In patients who do embark on OFS using LH-RH agonists, OFS is not always successfully achieved: cessation of menses alone is insufficient to confirm suppression, estradiol assays are often not standardized and problematic in terms of accuracy and interpretation in presence of very low levels of estradiol (46). Overall, the data available show biochemical suppression for the majority of patients but samples sizes are small and there is no data on the long-term maintenance of estradiol suppression. A pooled analysis of 193 premenopausal women with advanced BC treated with goserelin from 29 European trials showed incomplete menstrual suppression in 5% of patients (47). Anastrozole in combination with goserelin was not able to steadily suppress estradiol serum levels to the postmenopausal range in one third of 32 premenopausal patients with metastatic BC. Estradiol levels were not available in the remaining patients beyond 6 months to assure long-term suppression (18). Despite the reported limitations, estradiol levels should be checked on a regular basis (at least every 6 months), always in the same laboratory and preferably in a central reference laboratory.

When pharmacological suppression is chosen, monthly injection is the recommended way of administration, as tested in nearly all of the available trials.

The reversible OFS with LH-RH agonists can be particularly attractive to younger women, especially to those who did not complete childbearing before diagnosis, but the long-term risk of recurrence in ER+ BC should be taken into account when planning adjuvant ET in young patients (48).

Although the standard of care for ER+ premenopausal BC remains tamoxifen alone for 5 years, prospective trials to address the added role (if any) of OFS compared with tamoxifen alone have been undertaken (49). The SOFT trial will assess the role of OFS/OA in combination with the AI exemestane, compared with either OFS plus tamoxifen or tamoxifen alone. Three-thousand-sixty-six women were randomized into this study, which completed accrual in January 2011 (11). The TEXT trial assesses a LH-RH agonist with the addition of either tamoxifen or exemestane for 5 years (chemotherapy is optional): accrual of 2,672 women was completed in March 2011 (12). In the initial 890 patients entered in the TEXT trial, chemotherapy was chosen for 64% of patients, lymph node status being the predominant determinant of chemotherapy use (88% of node-positive versus 46% of node-negative) (50). The results of both these IBCSG-led trials, awaited in the course of 2014, will help the selection of the optimal ET for premenopausal women with ER+ early BC.

Table 1 Risk of chemotherapy induced amenorrhea.

Regimen	Age	Degree of risk
AC ×4 cycles - docetaxel ×4 cycles	40-49	35%
	31-39	12%
	<31	6%
AC, EC	>40	30-70%
	30-39	<20%
CMF, CEF or CAF ×6 cycles	>40	>80%
	30-39	30-70%
	<30	<20%
FEC ×6 cycles	>40	73%
	<40	38%
Methotrexate + fluorouracil		very low
Monoclonal antibodies		little evidence
Taxanes		little evidence

AC, doxorubicin, cyclophosphamide; CAF, cyclophosphamide, doxorubicin, fluorouracil; CEF, cyclophosphamide, epirubicin, fluorouracil; CMF, cyclophosphamide, Methotrexate, fluorouracil; EC, epirubicin, cyclophosphamide.

The role of surgical OA has been reconsidered with the advent of BRCA1/2 mutation testing in very young women or in patients belonging to hereditary BC families. Prophylactic oophorectomy is known to significantly reduce the risk of developing both breast and ovarian cancer in mutation carriers (51,52), who are often identified at the time of BC diagnosis. Prophylactic oophorectomy may therefore be considered when discussing adjuvant ET in this subgroup of young women.

The role of chemotherapy-induced-amenorrhea

In addition to direct cytotoxicity, adjuvant chemotherapy has an indirect endocrine effect in ER+ BC through the induction of OFS. Amenorrhea, even if transient (53), has been associated with improved treatment outcome in several trials (54).

In the IBCSG trial 13-93 of adjuvant chemotherapy ± tamoxifen in premenopausal node–positive women, patients with ER+ disease who experienced CIA had a significantly improved outcome (HR for amenorrhea *vs.* no amenorrhea =0.61; 95% CI, 0.44 to 0.86; P=0.004), whether or not they received tamoxifen (34).

In the NSABP trial B-30 in node-positive patients treated with both adjuvant anthracycline- and taxane-containing regimens, premenopausal women with ER+ tumors who had amenorrhea for ≥6 months after completion of chemotherapy had a significantly better survival (HR for death 0.52, P=0.002) and lower disease recurrence and second malignant incidence (HR 0.51, P<0.001) than those with no amenorrhea. By contrast, women with ER- tumors had a similar outcome regardless of whether they had or not amenorrhea (55,56).

The risk of CIA depends on the given regimen, total dose, dose-intensity, treatment duration, patient's age, and patient's ovarian reserve at the time of treatment initiation (54,57,58). The greatest risk is in women >40 years treated with alkylating agents (i.e., cyclophosphamide) but patient age also predicts amenorrhea in women treated with anthracycline-containing regimens (*Table 1*). The individual impact of taxanes on permanent amenorrhea is difficult to evaluate since they are usually administered sequentially or concurrently with anthracyclines and cyclophosphamide.

In the NSABP B-30 trial, four cycles of AT (doxorubicin plus docetaxel), a regimen not containing cyclophosphamide, resulted in the lowest rate of amenorrhea (59).

The limited evidence available on the addition of trastuzumab to anthracyclines and/or taxanes shows no apparent increase in the rate of permanent amenorrhea.

Aromatase inhibitors

AIs are contraindicated in premenopausal patients because the suppression of peripheral aromatase results in reduced

feedback to the hypothalamus and consequent ovarian stimulation (60). As a consequence, AIs must be used with great caution also in premenopausal women who have had CIA (61), because they can be associated with return of ovarian function and pregnancy, even in the absence of menses (62).

The ABCSG-12 trial randomized premenopausal women with ER+ early BC to receive 3 years of OFS with goserelin combined with either tamoxifen or anastrozole. Eligible patients had favorable prognosis (75% had T1, G1-2 tumors, only 30% had node-positive disease) and none received adjuvant chemotherapy (5% did receive neoadjuvant chemotherapy). At a median follow-up of 62 months, >2 years after treatment completion, there was no difference in DFS between patients on tamoxifen versus anastrozole (HR 1.08, 95% CI, 0.81-1.44; P=0.591), but OS was worse with anastrozole than with tamoxifen (HR 1.75, 95% CI, 1.08-2.83; P=0.02) (10). This latter observation could be partially related to the fact that, after disease recurrence, women receiving tamoxifen were more likely to be switched to an AI than those in the anastrozole group (61% versus 41%, respectively) who were switched to second-line, non-aromatase inhibitors, ET. In addition, overweight patients (BMI ≥25 kg/m^2) treated with anastrozole had a nearly 50% increase in the risk of disease recurrence (HR 1.49; 95% CI, 0.93-2.38; P=0.08) and a three-fold increase in the risk of death (HR 3.03; 95% CI, 1.35-6.82; P=0.004) compared with patients treated with tamoxifen (63). Anastrozole efficacy might in fact be affected by an increased total-body aromatization in the fat tissue, in which precursors are metabolized to estrogens by the enzyme aromatase and subsequent incomplete suppression of estrogen production in peripheral body fat. Of note, >90% of patients remained disease-free, suggesting that combined adjuvant ET without chemotherapy, in appropriately selected premenopausal women with endocrine-responsive tumors, can be effective. A word of caution should be raised in very young women, as only 18% of women were ≤40 years of age when randomized. The upcoming results of the SOFT and TEXT trials will provide further insight into this clinically important question.

Side effects of endocrine therapy

Side effects of tamoxifen and OFS/OA mimic menopausal symptoms, including hot flashes, sweats, weight gain and sexual dysfunction, which may negatively impact quality of life. In addition, tamoxifen has both estrogen agonist and antagonist properties with different side effect profile depending on the target organ. Hot flashes are the most common side effect of tamoxifen, reported in up to 80% of women (64). Non-hormonal and non-pharmacological therapies such as phytotherapy or acupuncture can be effective to reduce the intensity of symptoms as well as low-dose antidepressants, pregabalin and gabapentin (65-67).

The estrogen-like effect of tamoxifen on the uterus may induce endometrial hyperplasia and endometrial tumors (68,69). In the most recent EBCTCG overview, 5 years of tamoxifen were associated with a low overall incidence of uterine cancer (3.8% percent versus 1.1% in the control group) only in women aged 55 to 69 years, with no impact on mortality from uterine cancer (30). To date, no evidence-based recommendations for routine screening (i.e., transvaginal ultrasound or endometrial biopsy) in women assuming tamoxifen were published. However, abnormal bleeding should be promptly investigated and expert opinion recommendations suggest annual gynecologic examinations (7).

As previously mentioned, in the 2011 EBCTCG meta-analysis women who received tamoxifen had an increased thrombo-embolic risk (30). Similar findings were reported in BC prevention trials (70,71). Tamoxifen is therefore contraindicated in women with prior history of deep-vein thrombosis and pulmonary embolism.

Patients should be informed of the possibility of getting pregnant while on tamoxifen, despite developing amenorrhea: the relatively high frequency of severe congenital abnormalities mandates a reliable non-hormonal contraception (72).

Tamoxifen and LH-RH agonists produce hypoestrogenism with associated hyperandrogenism, which could lead to specific side effects like hair loss (73,74).

On the other hand, tamoxifen may also increase plasma estradiol concentrations by interfering with the normal negative pituitary feedback mechanisms: the resulting FSH rise drives ovarian steroidogenesis and increased incidence of ovarian cysts (75). Endogenous sex hormone levels were not correlated with outcome in a high-risk postmenopausal population prevention trial (76) but further studies may be required to explore the potential impact on the breast of increased serum estrogen levels in premenopausal BC patients treated with tamoxifen.

While in postmenopausal women tamoxifen has a well-established agonistic estrogenic effect in bone, there is some evidence that tamoxifen may decrease bone mineral

density (BMD) in premenopausal women, although the exact mechanism remains unclear. In the ZIPP (Zoladex in Premenopausal Patients) trial comparing different adjuvant ETs in early BC, a significant decline in BMD was seen after 2 years of treatment in patients receiving tamoxifen alone (77). In a Finnish survey in 111 premenopausal women with early BC treated with adjuvant chemotherapy, tamoxifen was associated with bone loss in patients who continued to menstruate after adjuvant chemotherapy whereas prevented bone loss in women who developed CIA (78). BMD has therefore to be regularly checked in premenopausal women receiving ET for BC. Bisphosphonates, although not yet approved for this indication, can prevent cancer therapy-induced bone loss and improve BMD in premenopausal women receiving therapy for BC (79-81) and should be promptly introduced at first signs of significant bone loss. Regular exercise has also a positive impact on bone mineralization and stimulation of osteogenesis (82).

Tamoxifen may adversely affect cognition (83), although few specific investigations on this side effect have been conducted and none in young women. In the ZIPP trial (6 cycles of CMF ± 2 years of goserelin, goserelin plus tamoxifen, or tamoxifen), no effect of treatment on the patients' self-evaluation of memory and concentration was shown (84). Cognitive function is being prospectively investigated in patients participating in the SOFT trial.

Treatment with LH-RH agonists is associated with menopausal side effects such as hot flushes and vaginal dryness (85) and with more severe sexual dysfunction than tamoxifen alone (86). In 293 patients enrolled in the ZIPP trial differential side effects of ET were evident only in patients who did not receive chemotherapy. Goserelin resulted in similar symptoms as CMF, whereas the side effects of tamoxifen alone were milder with the exception of vaginal discharge. Vaginal dryness from goserelin was mitigated by the addition of tamoxifen. After cessation of ET, side effects decreased in patients who had not received CMF, whereas patients treated with CMF reported ongoing problems at the 3-year follow-up (84).

Fertility issues, feasibility and safety of pregnancy should be addressed in every young patient with BC. Reproductive issues are of great importance to young women, in particular for those who did not complete their families before BC diagnosis (87). The risk of ovarian failure is associated with the chemotherapeutic agents used and patient age. In women <35 years, adjuvant chemotherapy is less likely to induce permanent amenorrhea (88,89). Fertility preservation has to be discussed early after diagnosis and patients should ideally be referred to a fertility specialist before starting therapy (88). Pregnancy following BC does not seem to negatively influence DFS or OS in ER+ premenopausal patients (90,91). The impact of a temporary interruption of adjuvant ET to allow pregnancy will be addressed in a phase II trial within the Breast International Group (BIG) and North American Breast Cancer Group (NABCG) collaboration.

Side effects of AIs when combined with LH-RH agonists are consistent with the known safety profiles for each of the agents administered (10,19).

Weight gain is often reported by women treated with tamoxifen and OFS. Randomized trials have not reported an excess in weight gain in patients treated with tamoxifen as compared to those who received placebo (92,93). In prevention trials, weight gain did not differ between anastrozole, tamoxifen and placebo (94,95).

Weight gain ≥10% after BC diagnosis was associated with a non-significant increased risk of death (HR, 1.15; 95% CI, 0.98-1.35) but not of BC-specific mortality (HR, 1.03; 95% CI, 0.84-1.26) in 12,915 patients with BC (diagnosed between 1990 and 2006) from 4 population-based prospective cohort studies examining the role of physical activity, BMI, dietary factors and interventions and quality of life in BC prognosis (96).

Overall, a woman must experience substantial weight gain before an increased risk of death is observed but normal weight women at BC diagnosis are at the highest risk of experiencing the negative effects of weight gain on overall mortality outcomes (HR, 1.24; 95% CI, 0.98-1.56). Several mechanisms have been postulated through which weight gain may influence survival, including enhanced conversion in fat tissue of androgens to estrogens (97). As a consequence, prevention of weight gain appears to be a sound public health goal for BC survivors (98).

Younger age was found in several observational studies to be a factor associated with lower rates of treatment compliance (99). In a cohort of 288 French women diagnosed with BC <40 years, 29.7% (95% CI, 24.1-36.4%) had discontinued tamoxifen after 2 years; after 3 years the proportion increased to 39.5% (95% CI, 32.9-47.0%) (100).

Endocrine therapy in metastatic breast cancer

In premenopausal women with ER+ metastatic BC, a variety of ET have proven to be effective (SERMs, OFS/OA ± tamoxifen or AIs, progestational agents (megestrol

acetate) (101). A meta-analysis comparing LH-RH agonist ± tamoxifen showed that the outcomes were significantly improved in patients who received the combination (17). On the basis of these results, a LH-RH agonist plus tamoxifen is currently recommended as the standard therapy in advanced BC.

Based on the available evidence in postmenopausal women with recurrent BC (15), the role of AIs has also been studied in premenopausal women. In a prospective, single-arm, multicenter phase II trial, 32 premenopausal patients were treated with goserelin and anastrozole achieving a clinic benefit rate of 71.9%, similar to that observed with AIs in postmenopausal women (18). Other small Phase II studies confirm the efficacy of AIs as 1st- and 2nd-line treatment in combination with a LH-RH agonist (24,102-104). In a phase II parallel group study, at median follow-up of 27.4 months, there was no statistical difference in the median TTP between premenopausal patients receiving letrozole plus goserelin and postmenopausal patients treated with letrozole alone [9.5 months (95% CI, 6.4 to 12.1 months) *vs.* 8.9 months (95% CI, 6.4 to 13.3 months)] (19).

The combination of LH-RH agonists and AIs can therefore be considered in premenopausal women with ER+ metastatic BC after progression on tamoxifen plus OFS but the exact role of this therapeutic option requires further investigation in randomized trials.

Considering the documented efficacy of fulvestrant in postmenopausal patients (105), some studies have been conducted in premenopausal patients as well (20,21). Bartsch *et al.* demonstrated a clinical benefit rate of 58% with fulvestrant plus goserelin in 26 patients pretreated with tamoxifen and aromatase inhibitors in combination with goserelin: median TTP was 6 months (95% CI, 2.4-9.6 months) and OS 32 months (95% CI, 14.28-49.72 months), respectively (106).

In endocrine-responsive metastatic BC, most studies addressing the combination of ET and chemotherapy showed an increased ORR or an increased TTP but no improvement in OS with no age-related differences (22). Trials examining concurrent versus sequential treatment with ET and chemotherapy need therefore to be conducted (107).

No specific endocrine resistance mechanisms have been identified in premenopausal patients and all the compounds developed to revert endocrine resistance have been tested only in postmenopausal patients so far.

Conclusions

Current available therapies in ER+ early and advanced BC in young women either modulate estrogen receptor activity (tamoxifen) or decrease estrogen production (OFS/OA). In early BC, several questions still need to be answered: (I) the optimal duration of pharmacological OFS; (II) the value of sequential chemotherapy and OFS, particularly in those women who do not develop CIA; (III) the utility of combined ETs (e.g., OFS + tamoxifen or AIs) and (IV) the optimal ET and treatment duration in high-risk ER+ patients

In advanced disease, ET has a major role in the long-term control of indolent disease and most of the time is associated with an acceptable toxicity profile.

Better tools to manage early menopause signs/symptoms (e.g., BMD, cognitive problems, fertility impairment and sexual disturbances) and careful monitoring of late toxicities (e.g., second cancers) need to be routinely implemented and specifically investigated.

Individualized, multidisciplinary approaches are needed to best address the complex physical and psycho-social scenario of BC at young age in order to maximize BC cure while minimizing the impact of diagnosis and treatment in women with demanding social and family commitments.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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. Samphao S, Wheeler AJ, Rafferty E, et al. Diagnosis of breast cancer in women age 40 and younger: delays in diagnosis result from underuse of genetic testing and breast imaging. Am J Surg 2009;198:538-43.
3. Anders CK, Fan C, Parker JS, et al. Breast carcinomas arising at a young age: unique biology or a surrogate for aggressive intrinsic subtypes? J Clin Oncol 2011;29:e18-20.
4. Cardoso F, Loibl S, Pagani O, et al. The European Society of Breast Cancer Specialists recommendations for the management of young women with breast cancer. Eur J Cancer 2012;48:3355-77.
5. Aebi S, Davidson T, Gruber G, et al. Primary breast

cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2011;22 Suppl 6:vi12-24.
6. Henderson IC, Berry DA, Demetri GD, et al. Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 2003;21:976-83.
7. NCCN. Breast Cancer. Version 2.2013. In National Comprehensive Cancer Network Guidelines, Edition 2013.
8. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011;22:1736-47.
9. Dowsett M, Cuzick J, Ingle J, et al. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. J Clin Oncol 2010;28:509-18.
10. Gnant M, Mlineritsch B, Stoeger H, et al. Adjuvant endocrine therapy plus zoledronic acid in premenopausal women with early-stage breast cancer: 62-month follow-up from the ABCSG-12 randomised trial. Lancet Oncol 2011;12:631-41.
11. Suppression of Ovarian Function Plus Either Tamoxifen or Exemestane Compared With Tamoxifen Alone in Treating Premenopausal Women With Hormone-Responsive Breast Cancer (SOFT). In ClinicalTrials.gov Identifier: NCT00066690.
12. Triptorelin With Either Exemestane or Tamoxifen in Treating Premenopausal Women With Hormone-Responsive Breast Cancer (TEXT). In ClinicalTrials.gov Identifier: NCT00066703.
13. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 2013;381:805-16.
14 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(suppl): abstr 5.
15. Cardoso F, Costa A, Norton L, et al. 1st International consensus guidelines for advanced breast cancer (ABC 1). Breast 2012;21:242-52.
16. Cardoso F, Fallowfield L, Costa A, et al. Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2011;22 Suppl 6:vi25-30.
17. Michaud LB, Jones KL, Buzdar AU. Combination endocrine therapy in the management of breast cancer. Oncologist 2001;6:538-46.
18. Carlson RW, Theriault R, Schurman CM, et al. Phase II trial of anastrozole plus goserelin in the treatment of hormone receptor-positive, metastatic carcinoma of the breast in premenopausal women. J Clin Oncol 2010;28:3917-21.
19. Park IH, Ro J, Lee KS, et al. Phase II parallel group study showing comparable efficacy between premenopausal metastatic breast cancer patients treated with letrozole plus goserelin and postmenopausal patients treated with letrozole alone as first-line hormone therapy. J Clin Oncol 2010;28:2705-11.
20. Robertson JF, Semiglazov V, Nemsadze G, et al. Effects of fulvestrant 250mg in premenopausal women with oestrogen receptor-positive primary breast cancer. Eur J Cancer 2007;43:64-70.
21. Young OE, Renshaw L, Macaskill EJ, et al. Effects of fulvestrant 750mg in premenopausal women with oestrogen-receptor-positive primary breast cancer. Eur J Cancer 2008;44:391-9.
22. Pritchard KI. Combining endocrine agents with chemotherapy: which patients and what sequence? Cancer 2008;112:718-22.
23. Gazet JC, Ford HT, Gray R, et al. Estrogen-receptor-directed neoadjuvant therapy for breast cancer: results of a randomised trial using formestane and methotrexate, mitozantrone and mitomycin C (MMM) chemotherapy. Ann Oncol 2001;12:685-91.
24. Torrisi R, Bagnardi V, Pruneri G, et al. Antitumour and biological effects of letrozole and GnRH analogue as primary therapy in premenopausal women with ER and PgR positive locally advanced operable breast cancer. Br J Cancer 2007;97:802-8.
25. Masuda N, Sagara Y, Kinoshita T, et al. Neoadjuvant anastrozole versus tamoxifen in patients receiving goserelin for premenopausal breast cancer (STAGE): a double-blind, randomised phase 3 trial. Lancet Oncol 2012;13:345-52.
26. Alba E, Calvo L, Albanell J, et al. Chemotherapy (CT) and hormonotherapy (HT) as neoadjuvant treatment in luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. Ann Oncol 2012;23:3069-74.
27. Osborne CK. Steroid hormone receptors in breast cancer management. Breast Cancer Res Treat 1998;51:227-38.
28. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists'

Collaborative Group. Lancet 1998;351:1451-67.
29. 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.
30. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011;378:771-84.
31. Aebi S, Gelber S, Castiglione-Gertsch M, et al. Is chemotherapy alone adequate for young women with oestrogen-receptor-positive breast cancer? Lancet 2000;355:1869-74.
32. Colleoni M, Rotmensz N, Peruzzotti G, et al. Role of endocrine responsiveness and adjuvant therapy in very young women (below 35 years) with operable breast cancer and node negative disease. Ann Oncol 2006;17:1497-503.
33. Goldhirsch A, Gelber RD, Yothers G, et al. Adjuvant therapy for very young women with breast cancer: need for tailored treatments. J Natl Cancer Inst Monogr 2001;(30):44-51.
34. International Breast Cancer Study Group, Colleoni M, Gelber S, et al. Tamoxifen after adjuvant chemotherapy for premenopausal women with lymph node-positive breast cancer: International Breast Cancer Study Group Trial 13-93. J Clin Oncol 2006;24:1332-41.
35. Arpino G, Weiss H, Lee AV, et al. Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. J Natl Cancer Inst 2005;97:1254-61.
36. Burstein HJ. The distinctive nature of HER2-positive breast cancers. N Engl J Med 2005;353:1652-4.
37. Love RR, Duc NB, Havighurst TC, et al. Her-2/neu overexpression and response to oophorectomy plus tamoxifen adjuvant therapy in estrogen receptor-positive premenopausal women with operable breast cancer. J Clin Oncol 2003;21:453-7.
38. Fleeman N, Martin Saborido C, Payne K, et al. The clinical effectiveness and cost-effectiveness of genotyping for CYP2D6 for the management of women with breast cancer treated with tamoxifen: a systematic review. Health Technol Assess 2011;15:1-102.
39. Fisher B, Dignam J, Bryant J, et al. Five versus more than five years of tamoxifen for lymph node-negative breast cancer: updated findings from the National Surgical Adjuvant Breast and Bowel Project B-14 randomized trial. J Natl Cancer Inst 2001;93:684-90.
40. Stewart HJ, Forrest AP, Everington D, et al. Randomised comparison of 5 years of adjuvant tamoxifen with continuous therapy for operable breast cancer. The Scottish Cancer Trials Breast Group. Br J Cancer 1996;74:297-9.
41. Jin H, Tu D, Zhao N, et al. Longer-term outcomes of letrozole versus placebo after 5 years of tamoxifen in the NCIC CTG MA.17 trial: analyses adjusting for treatment crossover. J Clin Oncol 2012;30:718-21.
42. Higgins MJ, Liedke PE, Goss PE. Extended adjuvant endocrine therapy in hormone dependent breast cancer: the paradigm of the NCIC-CTG MA.17/BIG 1-97 trial. Crit Rev Oncol Hematol 2013;86:23-32.
43. Davidson NE, O'Neill AM, Vukov AM, et al. Chemoendocrine therapy for premenopausal women with axillary lymph node-positive, steroid hormone receptor-positive breast cancer: results from INT 0101 (E5188). J Clin Oncol 2005;23:5973-82.
44. International Breast Cancer Study Group (IBCSG), Castiglione-Gertsch M, O'Neill A, et al. Adjuvant chemotherapy followed by goserelin versus either modality alone for premenopausal lymph node-negative breast cancer: a randomized trial. J Natl Cancer Inst 2003;95:1833-46.
45. LHRH-agonists in Early Breast Cancer Overview group, Cuzick J, Ambroisine L, et al. Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials. Lancet 2007;369:1711-23.
46. Dowsett M, Folkerd E. Deficits in plasma oestradiol measurement in studies and management of breast cancer. Breast Cancer Res 2005;7:1-4.
47. Blamey RW, Jonat W, Kaufmann M, et al. Goserelin depot in the treatment of premenopausal advanced breast cancer. Eur J Cancer 1992;28A:810-4.
48. Jatoi I, Anderson WF, Jeong JH, et al. Breast cancer adjuvant therapy: time to consider its time-dependent effects. J Clin Oncol 2011;29:2301-4.
49. Griggs JJ, Somerfield MR, Anderson H, et al. American Society of Clinical Oncology endorsement of the cancer care Ontario practice guideline on adjuvant ovarian ablation in the treatment of premenopausal women with early-stage invasive breast cancer. J Clin Oncol 2011;29:3939-42.
50. Regan MM, Pagani O, Walley B, et al. Premenopausal endocrine-responsive early breast cancer: who receives

chemotherapy? Ann Oncol 2008;19:1231-41.
51. Eisen A, Lubinski J, Klijn J, et al. Breast cancer risk following bilateral oophorectomy in BRCA1 and BRCA2 mutation carriers: an international case-control study. J Clin Oncol 2005;23:7491-6.
52. Kauff ND, Domchek SM, Friebel TM, et al. Risk-reducing salpingo-oophorectomy for the prevention of BRCA1- and BRCA2-associated breast and gynecologic cancer: a multicenter, prospective study. J Clin Oncol 2008;26:1331-7.
53. Pagani O, O'Neill A, Castiglione M, et al. Prognostic impact of amenorrhoea after adjuvant chemotherapy in premenopausal breast cancer patients with axillary node involvement: results of the International Breast Cancer Study Group (IBCSG) Trial VI. Eur J Cancer 1998;34:632-40.
54. Walshe JM, Denduluri N, Swain SM. Amenorrhea in premenopausal women after adjuvant chemotherapy for breast cancer. J Clin Oncol 2006;24:5769-79.
55. Swain SM, Jeong JH, Geyer CE Jr, et al. Longer therapy, iatrogenic amenorrhea, and survival in early breast cancer. N Engl J Med 2010;362:2053-65.
56. Swain SM, Jeong JH, Wolmark N. Amenorrhea from breast cancer therapy--not a matter of dose. N Engl J Med 2010;363:2268-70.
57. Meirow D, Biederman H, Anderson RA, et al. Toxicity of chemotherapy and radiation on female reproduction. Clin Obstet Gynecol 2010;53:727-39.
58. Torino F, Barnabei A, De Vecchis L, et al. Recognizing menopause in women with amenorrhea induced by cytotoxic chemotherapy for endocrine-responsive early breast cancer. Endocr Relat Cancer 2012;19:R21-33.
59. Ganz PA, Land SR, Geyer CE Jr, et al. Menstrual history and quality-of-life outcomes in women with node-positive breast cancer treated with adjuvant therapy on the NSABP B-30 trial. J Clin Oncol 2011;29:1110-6.
60. Dowsett M, Folkerd E, Doody D, et al. The biology of steroid hormones and endocrine treatment of breast cancer. Breast 2005;14:452-7.
61. Ortmann O, Pagani O, Jones A, et al. Which factors should be taken into account in perimenopausal women with early breast cancer who may become eligible for an aromatase inhibitor? Recommendations of an expert panel. Cancer Treat Rev 2011;37:97-104.
62. Smith IE, Dowsett M, Yap YS, et al. Adjuvant aromatase inhibitors for early breast cancer after chemotherapy-induced amenorrhoea: caution and suggested guidelines. J Clin Oncol 2006;24:2444-7.
63. Pfeiler G, Königsberg R, Fesl C, et al. Impact of body mass index on the efficacy of endocrine therapy in premenopausal patients with breast cancer: an analysis of the prospective ABCSG-12 trial. J Clin Oncol 2011;29:2653-9.
64. Day R, National Surgical Adjuvant Breast and Bowel Projet P-1 study (NSABP-1). Quality of life and tamoxifen in a breast cancer prevention trial: a summary of findings from the NSABP P-1 study. National Surgical Adjuvant Breast and Bowel Project. Ann N Y Acad Sci 2001;949:143-50.
65. Loprinzi CL, Qin R, Balcueva EP, et al. Phase III, randomized, double-blind, placebo-controlled evaluation of pregabalin for alleviating hot flashes, N07C1. J Clin Oncol 2010;28:641-7.
66. Bordeleau L, Pritchard KI, Loprinzi CL, et al. Multicenter, randomized, cross-over clinical trial of venlafaxine versus gabapentin for the management of hot flashes in breast cancer survivors. J Clin Oncol 2010;28:5147-52.
67. Loprinzi CL, Sloan J, Stearns V, et al. Newer antidepressants and gabapentin for hot flashes: an individual patient pooled analysis. J Clin Oncol 2009;27:2831-7.
68. Fisher B, Costantino JP, Redmond CK, et al. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst 1994;86:527-37.
69. Braithwaite RS, Chlebowski RT, Lau J, et al. Meta-analysis of vascular and neoplastic events associated with tamoxifen. J Gen Intern Med 2003;18:937-47.
70. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90:1371-88.
71. Cuzick J, Forbes J, Edwards R, et al. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. Lancet 2002;360:817-24.
72. Braems G, Denys H, De Wever O, et al. Use of tamoxifen before and during pregnancy. Oncologist 2011;16:1547-51.
73. Ayoub JP, Valero V, Hortobagyi GN. Tamoxifen-induced female androgenetic alopecia in a patient with breast cancer. Ann Intern Med 1997;126:745-6.
74. Puglisi F, Aprile G, Sobrero A. Tamoxifen-induced total alopecia. Ann Intern Med 2001;134:1154-5.
75. Metindir J, Aslan S, Bilir G. Ovarian cyst formation in patients using tamoxifen for breast cancer. Jpn J Clin Oncol 2005;35:607-11.
76. Beattie MS, Costantino JP, Cummings SR, et al.

Endogenous sex hormones, breast cancer risk, and tamoxifen response: an ancillary study in the NSABP Breast Cancer Prevention Trial (P-1). J Natl Cancer Inst 2006;98:110-5.
77. Sverrisdóttir A, Fornander T, Jacobsson H, et al. Bone mineral density among premenopausal women with early breast cancer in a randomized trial of adjuvant endocrine therapy. J Clin Oncol 2004;22:3694-9.
78. Vehmanen L, Elomaa I, Blomqvist C, et al. Tamoxifen treatment after adjuvant chemotherapy has opposite effects on bone mineral density in premenopausal patients depending on menstrual status. J Clin Oncol 2006;24:675-80.
79. Aft R. Protection of bone in premenopausal women with breast cancer: focus on zoledronic acid. Int J Womens Health 2012;4:569-76.
80. Body JJ. Prevention and treatment of side-effects of systemic treatment: bone loss. Ann Oncol 2010;21 Suppl 7:vii180-5.
81. Hadji P, Gnant M, Aapro M, et al. Dosing of zoledronic acid throughout the treatment continuum in breast cancer. Crit Rev Oncol Hematol 2011;79:175-88.
82. Hojan K, Milecki P, Moli ska-Glura M, et al. Effect of physical activity on bone strength and body composition in breast cancer premenopausal women during endocrine therapy. Eur J Phys Rehabil Med 2013;49:331-9.
83. Paganini-Hill A, Clark LJ. Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen. Breast Cancer Res Treat 2000;64:165-76.
84. Nystedt M, Berglund G, Bolund C, et al. Side effects of adjuvant endocrine treatment in premenopausal breast cancer patients: a prospective randomized study. J Clin Oncol 2003;21:1836-44.
85. Colleoni M, Giobbie-Hurder A. Benefits and adverse effects of endocrine therapy. Ann Oncol 2010;21 Suppl 7:vii107-11.
86. Berglund G, Nystedt M, Bolund C, et al. Effect of endocrine treatment on sexuality in premenopausal breast cancer patients: a prospective randomized study. J Clin Oncol 2001;19:2788-96.
87. Ruddy KJ, Gelber S, Ginsburg ES, et al. Menopausal symptoms and fertility concerns in premenopausal breast cancer survivors: a comparison to age- and gravidity-matched controls. Menopause 2011;18:105-8.
88. Christinat A, Pagani O. Fertility after breast cancer. Maturitas 2012;73:191-6.
89. Davis AL, Klitus M, Mintzer DM. Chemotherapy-induced amenorrhea from adjuvant breast cancer treatment: the effect of the addition of taxanes. Clin Breast Cancer 2005;6:421-4.
90. Azim HA Jr, Kroman N, Paesmans M, et al. Prognostic impact of pregnancy after breast cancer according to estrogen receptor status: a multicenter retrospective study. J Clin Oncol 2013;31:73-9.
91. Pagani O, Azim H Jr. Pregnancy after Breast Cancer: Myths and Facts. Breast Care (Basel) 2012;7:210-4.
92. Cuzick J, Sestak I, Baum M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 2010;11:1135-41.
93. Kumar NB, Allen K, Cantor A, et al. Weight gain associated with adjuvant tamoxifen therapy in stage I and II breast cancer: fact or artifact? Breast Cancer Res Treat 1997;44:135-43.
94. Sestak I, Harvie M, Howell A, et al. Weight change associated with anastrozole and tamoxifen treatment in postmenopausal women with or at high risk of developing breast cancer. Breast Cancer Res Treat 2012;134:727-34.
95. Cuzick J, Forbes JF, Sestak I, et al. Long-term results of tamoxifen prophylaxis for breast cancer--96-month follow-up of the randomized IBIS-I trial. J Natl Cancer Inst 2007;99:272-82.
96. Caan BJ, Kwan ML, Shu XO, et al. Weight change and survival after breast cancer in the after breast cancer pooling project. Cancer Epidemiol Biomarkers Prev 2012;21:1260-71.
97. Chlebowski RT, Aiello E, McTiernan A. Weight loss in breast cancer patient management. J Clin Oncol 2002;20:1128-43.
98. Pierce JP, Stefanick ML, Flatt SW, et al. Greater survival after breast cancer in physically active women with high vegetable-fruit intake regardless of obesity. J Clin Oncol 2007;25:2345-51.
99. Hershman DL, Kushi LH, Shao T, et al. Early discontinuation and nonadherence to adjuvant hormonal therapy in a cohort of 8,769 early-stage breast cancer patients. J Clin Oncol 2010;28:4120-8.
100. Huiart L, Bouhnik AD, Rey D, et al. Early discontinuation of tamoxifen intake in younger women with breast cancer: is it time to rethink the way it is prescribed? Eur J Cancer 2012;48:1939-46.
101. Carlson RW, Anderson BO, Burstein HJ, et al. Invasive breast cancer. J Natl Compr Canc Netw 2007;5:246-312.
102. Cheung KL Winterbottom L, Owers R. Goserelin plus anastrozole as first-line endocrine threrapy for premenopausal women with oestrogen receptor (ER) positive advanced breast cancer (ABC). J Clin Oncol

2005;23:abstr 731.
103. Forward DP, Cheung KL, Jackson L, et al. Clinical and endocrine data for goserelin plus anastrozole as second-line endocrine therapy for premenopausal advanced breast cancer. Br J Cancer 2004;90:590-4.
104. Nishimura R, Anan K, Yamamoto Y, et al. Efficacy of goserelin plus anastrozole in premenopausal women with advanced or recurrent breast cancer refractory to an LH-RH analogue with tamoxifen: results of the JMTO BC08-01 phase II trial. Oncol Rep 2013;29:1707-13.
105. Croxtall JD, McKeage K. Fulvestrant: a review of its use in the management of hormone receptor-positive metastatic breast cancer in postmenopausal women. Drugs 2011;71:363-80.
106. Bartsch R, Bago-Horvath Z, Berghoff A, et al. Ovarian function suppression and fulvestrant as endocrine therapy in premenopausal women with metastatic breast cancer. Eur J Cancer 2012;48:1932-8.
107. Cardoso F, Bedard PL, Winer EP, et al. International guidelines for management of metastatic breast cancer: combination vs sequential single-agent chemotherapy. J Natl Cancer Inst 2009;101:1174-81.

Cite this article as: Christinat A, Di Lascio S, Pagani O. Hormonal therapies in young breast cancer patients: when, what and for how long? J Thorac Dis 2013;5(S1):S36-S46. doi: 10.3978/j.issn.2072-1439.2013.05.25

A balancing act for breast cancer? Everolimus for hormone receptor positive patients

Stephanie B Wheeler[1,2,3], Katherine Reeder-Hayes[2,3,4], Anne-Marie Meyer[2,3]

[1]University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Health Policy and Management, USA; [2]University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, USA; [3]University of North Carolina at Chapel Hill, Cecil G Sheps Center for Health Services Research, USA; [4]University of North Carolina at Chapel Hill, School of Medicine, Department of Hematology and Oncology, USA
Correspondence to: Stephanie B Wheeler, PhD, MPH. 1103C McGavran-Greenberg, CB# 7411, 135 Dauer Drive, Chapel Hill, NC 27599-7411, USA. Email: stephanie_wheeler@unc.edu.

Submitted Mar 02, 2012. Accepted for publication Mar 21, 2012.
doi: 10.3978/j.issn.2218-676X.2012.03.02
View this article at: http://tcr.thepbpc.org/article/view/414/827

Recent results from a Phase III randomized trial comparing everolimus [a mammalian target of rapamycin (mTOR) inhibitor] plus exemestane versus placebo plus exemestane provide encouraging evidence of a new option for treatment of advanced, hormone receptor positive (HR+) breast cancer (1). Oral endocrine therapy (ET) is the first line therapeutic strategy of choice for most women with metastatic HR+, Her2 negative breast cancer (2). ET options include aromatase inhibitors (AI) such as exemestane, letrozole, and anastrozole, and selective estrogen receptor modifiers such as tamoxifen and fulvestrant. However, some tumors do not respond to ET, and many others become refractory to ET over time. Activation of the mTOR pathway has been associated with ET resistance in preclinical studies, leading investigators to explore mTOR inhibition as a treatment strategy in endocrine-refractory HR+ breast cancer (3-7). In this quickly changing therapeutic environment, understanding the comparative effectiveness of single and combination targeted therapies for breast cancer is essential (8).

The BOLERO-2 study, a multisite, international trial conducted by Baselga *et al.*, recruited breast cancer patients who were postmenopausal, with advanced, estrogen receptor-positive (ER+) and HER2-negative disease that was refractory to endocrine therapy (1). ET refractory disease was defined as recurrence during adjuvant therapy or within 12 months of completion of adjuvant therapy or progression during or within 1 month of treatment for advanced disease with a non-steroidal aromatase inhibitor. Early progression-free survival data strongly suggest an advantage for combination therapy-6.9 months for everolimus/exemestane compared to 2.8 months for placebo/exemestane, producing a hazard ratio for progression or death of 0.43 (95% Confidence Interval, 0.35-0.54; P<0.001) (1). This finding is remarkable given the limited therapeutic options available to women with advanced, HR+ disease whose tumors have become refractory to ET. However, substantial side effects and differential discontinuation of therapy were observed in the everolimus/exemestane arm, leading to some concerns about safety and tolerability of this combined therapeutic regimen. The authors' findings are consistent with other trials combining everolimus with ET for breast cancer, and preclinical data suggesting that everolimus and letrozole work synergistically to inhibit angiogenesis and tumor cell growth, while minimizing the potential for ET non-response (3,4,6,9). Because BOLERO-2 data are not yet fully mature, it remains to be seen whether combination therapy improves overall survival.

Patients in the BOLERO-2 trial were quite sick, admittedly by design; at recruitment, 56% had visceral disease, and over half had been previously exposed to at least three lines of therapy (1). Serious adverse events were recorded in 23% of patients in the everolimus/exemestane arm (11% attributed to therapy), compared to 12% in the exemestane/placebo arm (of which 1% were attributed to therapy), leading to higher discontinuation rates in the everolimus/exemestane arm. Disproportionately high rates of stomatitis, anemia, dyspnea, hyperglycemia, fatigue, and pneumonitis were observed in the everolimus/

exemestane arm, consistent with other studies (6). Unfortunately, the authors did not report statistical tests for significant differences in adverse events between the study arms, but they did acknowledge that adverse events were substantially higher in the combination therapy arm and that the majority of patients who discontinued everolimus did so because of lack of tolerability. Perhaps more concerning, seven deaths in the everolimus/exemestane arm were directly attributable to study-related adverse events and included deaths from sepsis, tumor hemorrhage, cerebrovascular incident, renal failure, suicide, and pneumonia. The authors concluded that "careful monitoring of patients and increase physician awareness of the safety profile of everolimus are warranted" (1). But the question remains: Given the tolerability concerns, does everolimus in combination with exemestane offer greater benefit (or reduced harms) compared to other available targeted therapies for ET refractory patients, such as traditional cytotoxic chemotherapy?

To answer these questions, a decision analytic model may be useful. In a decision model, by explicitly taking account of the comparative risk of harms and the corresponding utility of those harms (whether they result in morbidity or mortality), researchers can quantify and compare potential harms against potential benefits. Moreover, costs can be built into the equation - not just costs of everolimus (which are substantial) and AIs or other anti-estrogen therapies, but also costs of managing adverse events, costs of subsequent hospitalizations and emergency department visits, and costs of follow-up care. In light of the recent controversy surrounding novel therapies such as bevacizumab which may confer modest progression free survival benefits in combination with standard breast cancer therapies at significantly increased cost, such analyses are likely to become increasingly relevant in clinical practice. Although the BOLERO-2 trial suggests that progression-free survival may be improved with the addition of everolimus to exemestane *in a clinical trial setting*, it is well recognized that clinical trial populations are different from real-world populations in that clinical trial populations tend to be younger, healthier, wealthier, more educated and more health literate. In the real-world setting, patients may prefer to forego the significant risk of serious side effects in order to maintain quality of life, particularly when combination therapy may gain them a few additional months of less than ideal health (in the event that an adverse event occurs, which is likely in approximately 1 in 4 patients) but ultimately, is unlikely to save their lives. The early BOLERO-2 data indicate no difference in "time to deterioration of quality of life" as measured by European Organization for Research and Treatment of Cancer quality of life questionnaires (1). The authors are to be commended for including quality of life measures in their assessment of outcomes. However, providing access to the absolute quality of life data and collecting additional validated quality of life measures would make a more convincing case that there is no decrement in quality of life associated with the combination of everolimus/exemestane. Given the high incidence of adverse events in the combined therapy arm, it is possible that with longer follow-up quality of life between the two arms would diverge. Tolerability and quality of life associated with new cancer therapeutic regimens are essential components of informed decision making around cancer treatment. Survival benefits are only part of the complex equation that patients and physicians must implicitly consider in making decisions about cancer treatment. Building and parameterizing a comprehensive decision analytic model based on the BOLERO-2 trials results and other data will help patients and their physicians better understand the balance among all potential costs, harms, and benefits associated with this exciting and innovative mTOR therapy.

In addition to using decision models to understand the multiple dimensions of clinical and financial harms and benefits of everolimus, it will be important to better understand predictors of ET resistance in general and potential population heterogeneity in mTOR inhibitor effectiveness. In the adjuvant setting, evidence has indicated that as many as 50% of women who initiate ET discontinue the regimen prematurely (before 5 years) or do not take therapy as clinically prescribed, the reasons for which are unclear (10-14). Non-adherence, in theory, may diminish the active properties of AIs and anti-estrogen therapies. The extent of ET non-adherence in the metastatic setting and the contribution of non-adherence to real-world effectiveness of ET remain unexplored at this point. We also do not yet know whether and how population heterogeneity modulates the effectiveness of mTOR inhibitors. Clearly this complicated pathway has become increasingly identified as a significant player in oncogenic processes (5,7,15). However, there are no biomarkers clinically available to predict which patients will respond to mTOR inhibitors (7). At the same time, there appears to be some evidence that upstream mutations may decrease the effectiveness of mTOR

inhibitors (16,17). Given the potential therapeutic value of mTOR inhibitors but also non-trivial side-effects, it would be of great importance to identify biomarkers associated with heterogeneity in treatment response.

In conclusion, the BOLERO-2 preliminary data provide an exemplary picture of translational research at its best. In a very short period of time, everolimus as an active agent for the treatment of HR+ breast cancer has been catapulted from in vitro studies to human trials. The BOLERO-2 study also represents an exciting example of the potential benefit of combination therapy targeted to overcome specific resistance pathways in HR+ breast cancer. However, given the proliferation of therapies demonstrating progression free survival benefits in this disease setting, and the implausibility of testing each new therapeutic combination versus all its clinically reasonable comparators in randomized controlled trials, we must continue to develop not only novel therapies, but correspondingly more sophisticated methods for evaluating real world effectiveness and weighing risks and benefits for individual patients.

Acknowledgments

SBW has been supported in part by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health and an Agency for Healthcare Research and Quality Mentored Clinical Scientists Comparative Effectiveness Development Award, Grant No. 1-K-12 HS019468-01 (Weinberger). KRH has been supported in part by an Agency for Healthcare Research and Quality National Research Service Award T-32 Post-doctoral Traineeship at the Cecil G Sheps Center for Health Services Research, Grant No. 5-&-32 HS000032-20 (Carey). SBW and AM have been supported in part by the University Cancer Research Fund. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, AHRQ, or the University Cancer Research Fund.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 2012;366:520-9.
2. Wilcken N, Hornbuckle J, Ghersi D. Chemotherapy alone versus endocrine therapy alone for metastatic breast cancer. Cochrane Database Syst Rev 2003;2:CD002747.
3. Baselga J, Semiglazov V, van Dam P, et al. Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer. J Clin Oncol 2009;27:2630-7.
4. Awada A, Cardoso F, Fontaine C, et al. The oral mTOR inhibitor RAD001 (everolimus) in combination with letrozole in patients with advanced breast cancer: results of a phase I study with pharmacokinetics. Eur J Cancer 2008;44:84-91.
5. Efeyan A, Sabatini DM. mTOR and cancer: many loops in one pathway. Curr Opin Cell Biol 2010;22:169-76.
6. Lane HA, Lebwohl D. Future directions in the treatment of hormone-sensitive advanced breast cancer: the RAD001 (Everolimus)-letrozole clinical program. Semin Oncol 2006;33:S18-25.
7. Zaytseva YY, Valentino JD, Gulhati P, et al. mTOR inhibitors in cancer therapy. Cancer Lett 2012;319:1-7.
8. Perez EA, Spano JP. Current and emerging targeted therapies for metastatic breast cancer. Cancer 2012;118:3014-25.
9. Treeck O, Wackwitz B, Haus U, et al. Effects of a combined treatment with mTOR inhibitor RAD001 and tamoxifen in vitro on growth and apoptosis of human cancer cells. Gynecol Oncol 2006;102:292-9.
10. Partridge AH, Wang PS, Winer EP, et al. Nonadherence to adjuvant tamoxifen therapy in women with primary breast cancer. J Clin Oncol 2003;21:602-6.
11. McCowan C, Shearer J, Donnan PT, et al. Cohort study examining tamoxifen adherence and its relationship to mortality in women with breast cancer. Br J Cancer 2008;99:1763-8.
12. Kimmick G, Anderson R, Camacho F, et al. Adjuvant hormonal therapy use among insured, low-income women with breast cancer. J Clin Oncol 2009;27:3445-51.
13. Hershman DL, Kushi LH, Shao T, et al. Early discontinuation and nonadherence to adjuvant hormonal therapy in a cohort of 8,769 early-stage breast cancer patients. J Clin Oncol 2010;28:4120-8.
14. Owusu C, Buist DS, Field TS, et al. Predictors of tamoxifen discontinuation among older women with estrogen receptor-positive breast cancer. J Clin Oncol

2008;26:549-55.
15. Bhaskar PT, Hay N. The two TORCs and Akt. Dev Cell 2007;12:487-502.
16. Vermaat JS, Nijman IJ, Koudijs MJ, et al. Primary colorectal cancers and their subsequent hepatic metastases are genetically different: implications for selection of patients for targeted treatment. Clin Cancer Res 2012;18:688-99.
17. Wang LE, Ma H, Hale KS, et al. Roles of genetic variants in the PI3K and RAS/RAF pathways in susceptibility to endometrial cancer and clinical outcomes. J Cancer Res Clin Oncol 2012;138:377-85.

Cite this article as: Wheeler SB, Reeder-Hayes K, Meyer AM. A balancing act for breast cancer? Everolimus for hormone receptor positive patients. Transl Cancer Res 2012;1(2):109-112. doi: 10.3978/j.issn.2218-676X.2012.03.02

Treatment of breast cancer in young women: do we need more aggressive therapies?

Giuseppe Cancello, Emilia Montagna

Division of Medical Senology, European Institute of Oncology, Milan, Italy

Correspondence to: Giuseppe Cancello, MD, PhD. Division of Medical Senology, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy. Email: giuseppe.cancello@ieo.it.

Abstract: Breast cancer diagnosed in young patients has been reported to have a more aggressive biologic behaviour and to be associated with a more unfavorable prognosis compared with the disease in older patients. However controversies exist regarding the optimal treatment and if more aggressive therapies are really crucial in this population. Very young women with this disease are faced with personal, family, professional, and quality-of-life issues that further complicate the phase of treatment decision-making. Moreover it's mandatory in young patients to consider the impact of acute but also late toxicities in relation to long life-expectancy, too. Dose-dense and high-dose chemotherapy are two examples of more aggressive therapies that failed to show a clear beneficial in a feasible way compared to standard regimens also in young patients. The benefit evidenced in patients with ER-positive disease raises the hypothesis that efficacy of dose-intensive chemotherapy might simply be related to its endocrine effects. The study of the biology and of the oncogenic pathways should be a research priority so to aid management of young patients with breast cancer, and more important, to better tailor treatments that could be offered to young women or, simply to use better the modalities available today. For the time being, young age alone should not be a reason to prescribe more aggressive therapies and there are no evidence to recommend a specific chemotherapy regimen for young women.

Keywords: Young; breast cancer; chemotherapy; treatment; biology

Submitted April 30, 2013. Accepted for publication Jun 05, 2013.
doi: 10.3978/j.issn.2072-1439.2013.06.10
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.06.10

Introduction

Breast cancer in young patients is an important topic for manifold reasons. First of all, the prevalence of breast cancer in pre-menopausal women has been steadily increasing in several countries over the last years (1,2). Moreover, the management of breast cancer in young patients (<35 or <40 years) solicits an integrated approach taking into account relevant issues such as fertility preservation and pregnancy, apart from a long-life expectancy.

Overall, young patients have been reported to be associated with an increased risk of recurrence and death, as well as with unfavorable clinical and biological characteristics when compared to older patients (3-7).

Although it's clear that breast cancer in young women presents more frequently an aggressive phenotype with a consequently adverse outcome, controversies exist regarding the optimal treatment in this population and if more aggressive therapies are really crucial.

Furthermore very young women with this disease are faced with personal, family, professional, and quality-of-life issues that further complicate the phase of treatment decision-making.

Focus on adjuvant chemotherapy of breast cancer in young women

Age is not clearly associated to a specific response to chemotherapy. There are in fact some controversial data about the potential role of age as predictive factor.

The meta-analysis performed by Early Breast Cancer Trialists' Collaborative Group showed that polychemotherapy in women less than 50 years was associated with a recurrence rate of 41% compared to 53% of control group with a 15-year gain of 12%, while the 15-year gain was 4% for women aged more than 50 years. The effect of chemotherapy on recurrence rate and mortality was independent of age. The data have been subdivided into 10-year bands of age at entry; the mean annual reduction of risk of relapse attributable to chemotherapy (mainly CMF and anthracyclines) was 40% in patients less than 40, 36% in patients 40-49 and 23% in patients 50-59 (8).

In women younger than 50 years and oestrogen receptor positive (ER+) tumors, adjuvant polychemotherapy is associated with an annual reduction in mortality of 31% [standard error (SE) =0.10]. In this subgroup of patients, tamoxifen is also very effective with an annual reduction in mortality ranging from 39% (SE=0.12) in women younger than 40 years to 24% in women aged 40-49 years (8).

However, when ER status is taken into account, age disappears as an independent prognostic factor for the benefit of chemotherapy with all ER-negative patients benefiting from chemotherapy at the same extent (9).

Data with more recent regimens including taxanes are much more controversial, with some studies suggesting a higher and others a lower benefit in younger women. Obviously, these data have to be carefully interpreted, the effects observed being in part related to the degree of amenorrhea induced by the diverse regimens (10).

The most recent meta-analysis of EBCTCG compared different polychemotherapy regimens, including also the taxanes. In all meta-analyses involving taxane-based or anthracycline-based regimens, proportional risk reductions were little affected by age. Hence, largely independent of age (up to at least 70 years) or the tumour characteristics currently available to us for the patients selected to be in these trials, some taxane-plus-anthracycline-based or higher-cumulative-dosage anthracycline-based regimens (not requiring stem cells) reduced breast cancer mortality by, on average, about one-third (11).

Chemotherapy in ER-positive breast cancer in young women: endocrine effect of chemotherapy

Available adjuvant treatments for premenopausal endocrine-responsive breast cancer patients include chemotherapy and/or tamoxifen and luteinizing hormone-releasing hormone (LH-RH) agonists.

Chemotherapy exerts some of its effect via an endocrine mechanism in premenopausal women with ER-positive tumors (12).

It is important to focus on endocrine effects (suppression of endocrine ovarian function) of chemotherapy in premenopausal women. The endocrine effects of chemotherapy vary with age. Goodwin *et al.* examined factors predicting onset of menopause in a cohort of premenopausal women with newly diagnosed breast cancer receiving either adjuvant CMF, cyclophosphamide, epirubicin, and fluorouracil (CEF), tamoxifen, or no treatment. They demonstrated that two factors, age and use of systemic chemotherapy, are important predictors of menopause onset in premenopausal women with newly diagnosed breast cancer, with the risk that began to increase at age 35 (13).

Moreover it is known that the incidence of amenorrhea is proportional to the duration of chemotherapy (14).

Data in the literature support a role for ovarian function suppression in the adjuvant program of pre-menopausal patients.

Between 1978 and 1993 the International Breast Cancer Study Group (IBCSG) treated 3,700 premenopausal and perimenopausal patients with various timing and duration of adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF with or without low-dose prednisone and oophorectomy) in Trials I, II, V and VI. 314 of these women were less than 35 years old at randomisation. In these trials patients were not routinely offered hormonal therapy following chemotherapy. Trial I investigated the addition of low-dose prednisone to a cyclophosphamide-methotrexate-fluorouracil (CMF) combination in patients with one to three positive axillary nodes. In trial II, patients with four or more positive axillary nodes were randomised to 1 year of CMF and low-dose prednisone (CMFP) or to a surgical oophorectomy and CMFP. In Trial V and VI patients received only chemotherapy but no any kind of hormonal therapy. The failure to achieve chemotherapy-induced amenorrhea was associated with an increased risk of relapse among pre-menopausal patients with ER-positive tumors [hazard ratios (HR), 1.67; 95% confidence interval (CI), 1.19-2.34; P=0.003] in this retrospective analysis of IBCSG Trials I, II, V and VI. Moreover in these trials, younger patients with ER-positive tumors had a significantly worse prognosis than did younger patients with ER-negative tumors (10-year DFS was 25% for ER-positive tumors versus 47% for ER-negative tumors; P=0.014). In contrast, among older patients, the prognosis was similar

for patients with ER-positive tumors compared to patients with ER-negative tumors (10-year DFS was 45% versus 46%; P=0.27). The interaction between age and ER status on outcome was statistically significant (P=0.002) (15).

A retrospective cohort study of a National Cancer Institute of Canada Clinical Trials Group indicated that the achievement of amenorrhea at 12 months was significantly associated with relapse-free survival and overall survival (16).

Finally, the results of IBCSG trail 13-93 showed that premenopausal patients with ER-positive tumors who achieved chemotherapy-induced amenorrhea had a significantly improved outcome (HR for amenorrhea v no amenorrhea =0.61; 95% CI, 0.44 to 0.86; P=0.004), whether or not they received tamoxifen (17).

A pooled analysis of patients 40 years old or younger enrolled in different EORTC trials demonstrated that hormone receptor-positive patients experienced no survival advantage of prolonged adjuvant CMF chemotherapy compared with hormone receptor-negative patients. However, in patients who did not receive adjuvant chemotherapy, hormone receptor-positive status was associated with improved survival rates compared with hormone receptor-negative status. In overall multivariate analyses, both ER-positive status and PgR-positive status remained independent prognostic factors of OS. Young patients with hormone receptor-positive tumors benefit less from adjuvant systemic chemotherapy than patients with hormone receptor-negative tumors. These results confirm that chemotherapy alone cannot be considered optimal adjuvant systemic treatment in breast cancer patients 40 years old or younger with hormone receptor-positive tumors (18).

These analyses of treatment outcome leads to the hypothesis that the endocrine effects of chemotherapy alone were insufficient for patients in the younger age group with endocrine-responsive tumors, for whom suppression of estradiol production might be essential.

However, recent epidemiological data seem to show and confirm the recent attitude to use chemotherapy but also better/optimal endocrine treatment (i.e., LHRH-analogue plus Tamoxifen) for young patients with endocrine-responsive disease (19).

A recent SEER population-base study in fact showed that HR for mortality in women 40-50 with ER positive BC but also in women <40 with ER positive had improvements over time.

In ER negative patients, the degree of improvements over time was less than that seen in ER positive women. Authors conclude that therefore, mortality improvements in young women with ER positive BC may be attributed to treatment advances with endocrine agents (19).

However the question of whether additional benefit can be obtained from ovarian suppression in premenopausal patients receiving tamoxifen is now being directly addressed by the global Suppression of Ovarian Function Trial (SOFT) coordinated by the IBCSG on behalf of the Breast International Group and the North American Breast Cancer Intergroup. SOFT compares tamoxifen alone versus ovarian function suppression plus tamoxifen versus ovarian function suppression plus exemestane for patients with steroid hormone receptor-positive tumors who remain premenopausal after adjuvant chemotherapy or for whom tamoxifen alone is considered reasonable treatment option.

Chemotherapy in ER-negative breast cancer in young women

Regardless of the age of premenopausal patients with ER-negative tumors, adjuvant chemotherapy appears to be a very important component of a successful treatment regimen.

Evaluation of data from NSABP, IBCSG and SWOG trails, showed that the difference in outcome with respect to age group (young versus old patients) is much smaller for patients with ER-negative tumors compared to patients with ER-positive tumors. No difference was found about relative risk of relapse comparing patients less than 35 years old with those 35 years of age and older with ER-negative disease who received adjuvant chemotherapy. Therefore the beneficial effects of chemotherapy might be similar for younger and older premenopausal women for the ER-negative cohort (20).

In the NSABP Trial B-13, specifically designed for ER-negative disease, the effect of chemotherapy compared with no adjuvant treatment in women less than 50 years old is overwhelming, corresponding to a 38% reduction in the risk of relapse. In this setting of ER-negative disease, the magnitude of the estimated effect of chemotherapy is the same for younger as for older patients although, because of the smaller sample size, the result for the younger group is statistically uncertain (21).

Colleoni *et al.* evaluated biological features, treatment recommendations and prognosis for 841 premenopausal patients with pT1-3, pN0 and M0, operated at European Institute of Oncology, Milan, Italy from 1997 to 2001. Treatment modalities were well balanced between the

young and older patients in the subgroup of endocrine unresponsive disease; in this subgroup, a statistically significant difference in DFS but not OS was observed for very young patients (below 35 years) versus older patients in univariate analysis (HR=3.26, 95% CI, 1.14 to 9.33, P<0.0196 for DFS; HR=2.12, 95% CI, 0.60 to 7.51; P=0.24 for OS). The association disappeared in multivariate analysis (17).

Recent data of GeparTrio neoadjuvant study suggest that young age is constantly associated with greater benefit from preoperative anthracycline-taxane-based chemotherapy. In this trial about 17.4% of the patients were below the age of 40 years, pCR was significantly higher in patients under the age of 40 years compared to those 40 years or older. The highest pCR rate could be detected for those under 40 years with an ER/PgR negative (P=0.001) tumor. When a tumor was triple negative pCR rates were as high as 57% in the <40 years population compared to 34% in the patients ≥40 years (P<0.0001). In the triple negative setting, age was the only independent predictive factor for chemotherapy response in this setting (22).

Another neoadjuvant trial evaluated cisplatin in twenty-eight patients with triple-negative breast cancer to identify specific biomarkers predictors of response.

The study showed a strong association between younger age and good response (P=0.001 based on quartiles of age, according to Miller-Payne score; significant even after Bonferroni adjustment for multiple comparisons; when the two BRCA1 mutations carriers were excluded, P=0.001).

However age was not significantly associated with pCR (P=0.13) or clinical response (P=0.46) (23).

A more aggressive therapy: two examples

Dose-dense chemotherapy

Two systematic reviews and meta-analyses of the existing data from randomized controlled trials regarding the efficacy and toxicity of the dose-dense adjuvant chemotherapy were published.

The first meta-analysis showed that patients who received dose-dense chemotherapy had better overall survival [HR of death =0.84, 95% CI =0.72 to 0.98, P=0.03] and better disease-free survival (HR of recurrence or death =0.83, 95% CI, 0.73 to 0.94, P=0.005) than those on the conventional schedule; no benefit was observed in patients with hormone receptor-positive tumors (24).

The second meta-analysis demonstrated that dose-dense therapy can improve DFS (3,356 patients; HR=0.83; 95% CI, 0.73e0.95; P=0.005), independent of hormone receptor expression status; there was no OS benefit with dose-dense therapy (25).

However both meta-analyses didn't perform efficacy analyses according to the age of patients.

In the study of Venturini *et al.* 1,214 patients with early-stage breast cancer were randomly assigned to receive six cycles of FEC 14 (administered every 14 days) or of FEC 21 (administered every 21 days). At a median follow-up of 10.4 years, no statistically significant difference in the hazard of death [hazard ratio (HR) =0.87, 95% CI, 0.67 to 1.13] or recurrence (HR=0.88, 95% CI, 0.71 to 1.08) was found between FEC 14 and FEC 21 groups after adjustment by multivariable analysis. Although the study was underpowered for subset analysis, authors observed a suggestion of higher efficacy associated with the FEC 14 regimen than with the FEC 21 regimen among patients younger than 50 years; these patients had a statistically significant 34% reduced risk of recurrence (HR=0.66, 95% CI, 0.46 to 0.94) and a non-statistically significant 27% reduced risk of death (HR=0.73, 95% CI, 0.46 to 1.16). This greater efficacy seems not to be mediated by a greater activity of FEC 14 in suppressing ovarian function, because the rate of chemotherapy-induced amenorrhea was virtually identical in the two arms (26).

The INT 9742 trial randomized about 2,000 patients to receive sequential or concurrent chemotherapy with anthracyclines and taxanes every two or three weeks. Dose-dense treatment improved the primary end point, DFS [risk ratio (RR) =0.74; P=0.010], and OS (RR=0.69; P=0.013). Multivariate analysis didn't show a different risk of death among pre-menopausal and post-menopausal patients (27).

Finally, no studies specifically evaluated or analyzed the impact of the dose-dense chemotherapy in very young patients (below 35 or 40 years), apart from a controversial exploratory data about premenopausal status; therefore, even if the dose-dense regimens are apparently feasible about acute and late toxicities, they cannot be considered a standard approach in very young patients with early breast cancer.

Dose-intensive /high-dose chemotherapy

In 1995, the International Breast Cancer Study Group (IBCSG) initiated a clinical trial (Trial 15-95) to examine the role of dose-intensive epirubicin and cyclophosphamide (DI-EC) versus conventional adjuvant chemotherapy for

patients with high-risk early breast cancer. After prolonged follow-up, DI-EC significantly improved DFS, but the effect was observed only in patients with ER-positive disease, leading to the hypothesis that efficacy of DI-EC may relate to its endocrine effects.

A STEPP analysis, conducted in order to ascertain the magnitude of the effect of DI-EC in patients with ER-positive tumors according to age, showed a visual trend suggesting a larger effect for DI-EC in younger patients, therefore supporting a possible correlation between the achievement of ovarian function suppression and efficacy of DI-EC., even if the interaction of age and treatment was not statistically significant (P=0.54) (28).

Other studies exploring the activity of high-dose chemotherapy described a more pronounced effect of high dose chemotherapy in younger patients.

A trend towards an advantage for younger women (age <35 years) and women with four to nine involved axillary lymph nodes was also shown in the Italian study of the Michelangelo Group, in spite of a lack of an overall benefit after median 5 years of follow-up (29).

In general, adjuvant high-dose chemotherapy (HDC) with autologous hematopoietic stem-cell transplantation (AHST) for high-risk primary breast cancer has not been shown to prolong survival. Moreover individual trials have had limited power to show overall benefit or benefits within subsets.

However some retrospective subgroup analyses showed benefit from high-dose chemotherapy independent of hormone receptor status and age. Effects were more pronounced in young patients, but no data are available on the effect according to age, amenorrhea in endocrine-responsive disease, and in those with hormone receptor-negative disease (29,30).

A recent meta-analysis of individual patient data from 15 randomized adjuvant breast cancer trials including 6,210 patients showed that after a median follow-up of 6 years high-dose chemotherapy prolong relapse-free survival [hazard ratio (HR), 0.87; 95% CI, 0.81 to 0.93; P=0.001] but not overall survival (OS; HR, 0.94; 95% CI, 0.87 to 1.02; P=0.13). Younger patients had a significantly better RFS on HDC than did older patients. However for overall survival, no covariates had statistically significant interactions with treatment effect, and no subsets evinced a significant effect of high-dose chemotherapy (31).

The sum of these results limit the use of high-dose chemotherapy in breast cancer.

The advantage in some subsets of patients was restricted to some retrospective analyses with low study power.

In clinical decision making, any benefit in recurrence or survival must be weighted against the greater toxicities of HDC.

Individual studies have reported that the quality of life among patients receiving HDC is lower during treatment than that among the patients receiving control (32).

These findings appear more relevant in young patients in whom we have to consider the impact of acute but also late toxicities in relation to long life-expectancy, too. Reliable evidence of benefit is required to justify the burden and expense of dose-intensive therapy and the results in patients with ER-positive disease raise the hypothesis that efficacy of DI-EC may relate to its endocrine effects. There are surely less costly ways of offering endocrine therapy to very young patients with endocrine-responsive breast cancer.

Biology of breast cancer in young women

Recent studies have examined the distribution of breast cancer immunohistochemical and molecular subtypes and gene expression signatures to evaluate if breast cancer in young women is enriched with aggressive subtypes and also to question whether breast cancer diagnosed at a young age has a unique biology. The findings of these field researches could be relevant to discriminate prognostic subgroups in young patients, but also to understand if young age alone can be an indicator for adjuvant chemotherapy.

Breast cancer is a heterogeneous disease and gene expression studies have identified molecularly distinct subtypes with prognostic implications across multiple treatment settings. The immunohistochemical evaluation of ER, progesterone receptor (PgR), Ki-67 and HER2 may be considered a surrogate means for identifying the molecular subtypes of breast cancer.

Cancello *et al.* investigated the prognosis of very young patients (below 35 years) compared to older premenopausal patients (aged 35-50) using an immunohistochemical classification. The analysis was based on data from 2,970 patients, of whom 315 were aged less than 35 years. According to the immunohistochemical classification, in the group of patients aged <35 years, there were less tumors identified as Luminal A (9.2% versus 21.2%) and more Triple Negative tumors (16.2% versus 7.5%; P<0.0001) than in older patients, apart from a higher prevalence of high grade tumors and a higher percentage of tumors with peri-vascular invasion.

More importantly, in the same study, patients <35 years

of age presented a significantly increased risk of recurrence and death [hazards ratio HR =1.65, 95% CI 1.30-2.10 and HR=1.78, 95% CI, 1.12-2.85, respectively] when compared with older patients with similar characteristics of disease. Very young patients with tumors classified as Luminal B, HER2 and Triple Negative were at increased risk of poorer DFS (HR=1.62, 95% CI, 1.21-2.18; HR=2.37, 95% CI, 1.12-5.02 and HR=2.04, 95% CI, 1.11-3.72, respectively), while in the Luminal B and Triple Negative subtypes, patients <35 years had a twofold higher risk of death compared with older patients (*Figure 1*). In this series very young patients with triple negative and HER2-subtype breast cancer received the same percentage of chemotherapy compared with older patients, while patients aged less than 35 years with Luminal B tumors receive more chemotherapy and more LHRH-analogue + tamoxifen combination therapy than older patients with the same subtype disease (33).

In 2008 a large-scale genomic analysis was published. In this study two age-specific cohorts (young: ≤45 years, n=200; older: ≥65 years, n=211) were compared by prognosis, clinicopathologic variables, mRNA expression values, single gene analysis, and gene set enrichment analysis (GSEA). Tumors arising in young women had significantly lower ER mRNA (P≤0.0001), ER (P=0.02), and progesterone receptor (PR) expression (P<0.0001), but higher HER-2 (P<0.0001) and epidermal growth factor receptor (EGFR) expression (P<0.0001). Exploratory analysis (GSEA) revealed 367 biologically relevant gene sets significantly distinguishing breast tumors arising in young women. Combining clinicopathologic and genomic variables tumors arising in young women demonstrated that younger age and lower ER and higher EGFR mRNA expression were significant predictors of inferior DFS (34).

However after some years same authors chose to reanalyze their previous data set to evaluate the relationship between age and breast cancer subtype, and to account for potential confounding variables not previously included. First of all, they found that there was a significant association between subtype and age (P=3.8e-06). Specifically, a higher proportion of younger women were diagnosed with basal-like [odds ratio (OR), 12.27; 95% CI, 3.96 to 45.0] and HER2-enriched (OR, 4.63; 95% CI, 1.50 to 16.48) breast tumors. More interesting, the correction for the significant clinicopathologic features (grade, subtype, sample source) with the adjusted model yielded zero gene differences (q<0.05) between breast tumors of previously defined age groups in two different data sets (35).

More recently, a comprehensive analysis was conducted to clarify the relevance of several published prognostic gene signatures in young women (≤40) and to determine whether young age is truly associated with unique disease biology.

In about 2,901 patients, authors observed a significantly higher risk of relapse in patients of 40 years or less than in older age groups (P<0.0001).

More interestingly, authors identified a total of 41 genes and 13 gene sets as potential candidate age-related genes and pathways aberrations reported in previous literature data. Within a cohort of untreated patients the expression of 16 genes and gene sets were found to be significantly age dependent after adjustment. In the cohort of treated patients authors found that 12 out of the 16 were still significantly associated with age after adjustment. The common themes associated with young age were enrichment of biological processes related to immature mammary cell populations (RANKL, c-kit, BRCA1-mutated phenotype, mammary stem cells, and luminal progenitors cells), and growth factor signaling [mitogen—activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)-related]. There was also downregulation of apoptosis-related genes (36).

The few studies published on intrinsic biology of breast cancer in young women showed as only conclusive result that breast cancer in young women present more frequently an aggressive phenotype.

The analysis of immuno-defined subtypes seems to show that the behaviour of a specific subtype in a young patient is intrinsically more aggressive than in an older patient (33).

However the gene-signature analyses showed contradictory results, on the one hand showing that age alone does not appear to provide an additional layer of biologic complexity above that of breast cancer subtype and grade, on the other hand suggesting that breast cancer arising at a young age is biologically distinct beyond subtype distribution and is enriched with unique molecular processes (35,36).

Final considerations and conclusions

The available published data don't suggest a specific medical treatment approach for the very young patients with breast cancer.

The indications for and the choice of type of adjuvant systemic treatment for invasive breast cancer should be driven, as in other age categories, by the biological characteristics of the tumours, as the immunohistochemical-defined subtypes, the tumour stage and patient's

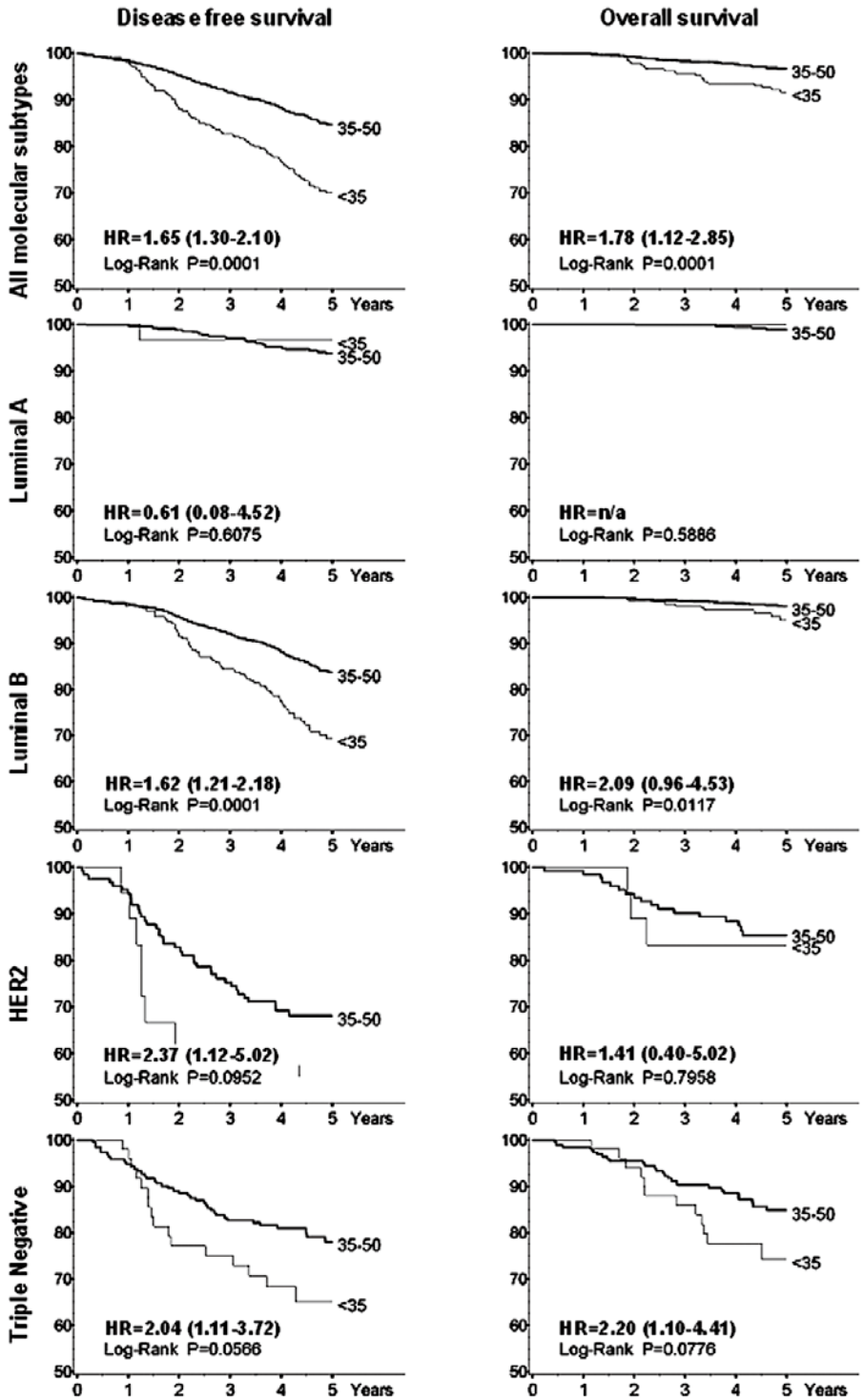


Figure 1 Disease free survival and overall survival in breast cancer patients according to age at diagnosis and IHC classification. With permission from (34). HR and 95% CI obtained from multivariable Cox proportional hazards regression model adjusted for hormonal receptor status, proliferative index (ki-67), peritumoral vascular invasion, tumor size, nodal status and Her2Neu overexpression, chemiotherapy (none/CMF/Anthracycline containing therapy, other regimen) and hormonotherapy (none, LHRH or Tamoxifen alone, LHRH + Tamoxifen, other regimen). N/a, not available.

comorbidities and preferences. Furthermore the type of systemic treatment of early breast cancer is independent of BRCA or any other constitutional genetic status.

Therefore, for the time being, young age alone should not be a reason to prescribe more aggressive therapies and there are no evidence to recommend a specific chemotherapy regimen for young women.

The association with a more aggressive biology should be better understand so to aid management of young patients with breast cancer, and more important, to tailor treatment investigations so to clarify if we need new modalities of treatment or, simply we have to use better the modalities available today.

Additionally, a better understanding of the oncogenic signaling pathways of breast cancer arising in young women so to elucidate if breast cancer in youth is a unique biologic entity could enable us to better tailor treatments that could be offered to young women.

Prospective data from the randomized trials probably will help to re-assess the prognosis and benefit of chemotherapy according to age and tumour biology in the modern era.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Bouchardy C, Fioretta G, Verkooijen HM, et al. Recent increase of breast cancer incidence among women under the age of forty. Br J Cancer 2007;96:1743-6.
2. Brinton LA, Sherman ME, Carreon JD, et al. Recent trends in breast cancer among younger women in the United States. J Natl Cancer Inst 2008;100:1643-8.
3. Walker RA, Lees E, Webb MB, et al. Breast carcinomas occurring in young women (< 35 years) are different. Br J Cancer 1996;74:1796-800.
4. Chung M, Chang HR, Bland KI, et al. Younger women with breast carcinoma have a poorer prognosis than older women. Cancer 1996;77:97-103.
5. Winchester DP, Osteen RT, Menck HR. The National Cancer Data Base report on breast carcinoma characteristics and outcome in relation to age. Cancer 1996;78:1838-43.
6. Albain KS, Allred DC, Clark GM. Breast cancer outcome and predictors of outcome: are there age differentials? J Natl Cancer Inst Monogr 1994;(16):35-42.
7. Colleoni M, Rotmensz N, Robertson C, et al. Very young women (<35 years) with operable breast cancer: features of disease at presentation. Ann Oncol 2002;13:273-9.
8. 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.
9. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Clarke M, Coates AS, et al. Adjuvant chemotherapy in oestrogen-receptor-poor breast cancer: patient-level meta-analysis of randomised trials. Lancet 2008;371:29-40.
10. Swain SM, Jeong JH, Geyer CE Jr, et al. Longer therapy, iatrogenic amenorrhea, and survival in early breast cancer. N Engl J Med 2010;362:2053-65.
11. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Peto R, Davies C, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet 2012;379:432-44.
12. Pagani O, O'Neill A, Castiglione M, et al. Prognostic impact of amenorrhoea after adjuvant chemotherapy in premenopausal breast cancer patients with axillary node involvement: results of the International Breast Cancer Study Group (IBCSG) Trial VI. Eur J Cancer 1998;34:632-40.
13. Goodwin PJ, Ennis M, Pritchard KI, et al. Risk of menopause during the first year after breast cancer diagnosis. J Clin Oncol 1999;17:2365-70.
14. Goldhirsch A, Gelber RD, Castiglione M. The magnitude of endocrine effects of adjuvant chemotherapy for premenopausal breast cancer patients. The International Breast Cancer Study Group. Ann Oncol 1990;1:183-8.
15. Aebi S, Gelber S, Castiglione-Gertsch M, et al. Is chemotherapy alone adequate for young women with oestrogen-receptor-positive breast cancer? Lancet 2000;355:1869-74.
16. Parulekar WR, Day AG, Ottaway JA, et al. Incidence and prognostic impact of amenorrhea during adjuvant therapy in high-risk premenopausal breast cancer: analysis of a National Cancer Institute of Canada Clinical Trials Group Study--NCIC CTG MA.5. J Clin Oncol 2005;23:6002-8.

17. International Breast Cancer Study Group, Colleoni M, Gelber S, et al. Tamoxifen after adjuvant chemotherapy for premenopausal women with lymph node-positive breast cancer: International Breast Cancer Study Group Trial 13-93. J Clin Oncol 2006;24:1332-41.
18. van der Hage JA, Mieog JS, van de Vijver MJ, et al. Efficacy of adjuvant chemotherapy according to hormone receptor status in young patients with breast cancer: a pooled analysis. Breast Cancer Res 2007;9:R70.
19. Ademuyiwa FO, Groman A, Hong CC, et al. Time-trends in survival in young women with breast cancer in a SEER population-based study. Breast Cancer Res Treat 2013;138:241-8.
20. Goldhirsch A, Gelber RD, Yothers G, et al. Adjuvant therapy for very young women with breast cancer: need for tailored treatments. J Natl Cancer Inst Monogr 2001;(30):44-51.
21. Fisher B, Redmond C, Wickerham DL, et al. Systemic therapy in patients with node-negative breast cancer. A commentary based on two National Surgical Adjuvant Breast and Bowel Project (NSABP) clinical trials. Ann Intern Med 1989;111:703-12.
22. Huober J, von Minckwitz G, Denkert C, et al. Effect of neoadjuvant anthracycline-taxane-based chemotherapy in different biological breast cancer phenotypes: overall results from the GeparTrio study. Breast Cancer Res Treat 2010;124:133-40.
23. Silver DP, Richardson AL, Eklund AC, et al. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. J Clin Oncol 2010;28:1145-53.
24. Bonilla L, Ben-Aharon I, Vidal L, et al. Dose-dense chemotherapy in nonmetastatic breast cancer: a systematic review and meta-analysis of randomized controlled trials. J Natl Cancer Inst 2010;102:1845-54.
25. Lemos Duarte I, da Silveira Nogueira Lima JP, Passos Lima CS, et al. Dose-dense chemotherapy versus conventional chemotherapy for early breast cancer: a systematic review with meta-analysis. Breast 2012;21:343-9.
26. Venturini M, Del Mastro L, Aitini E, et al. Dose-dense adjuvant chemotherapy in early breast cancer patients: results from a randomized trial. J Natl Cancer Inst 2005;97:1724-33.
27. Citron ML, Berry DA, Cirrincione C, et al. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/ Cancer and Leukemia Group B Trial 9741. J Clin Oncol 2003;21:1431-9.
28. Colleoni M, Sun Z, Martinelli G, et al. The effect of endocrine responsiveness on high-risk breast cancer treated with dose-intensive chemotherapy: results of International Breast Cancer Study Group Trial 15-95 after prolonged follow-up. Ann Oncol 2009;20:1344-51.
29. Gianni A, Bonadonna G. Five-year results of the randomized clinical trial comparing standard versus high-dose myeloablative chemotherapy in the adjuvant treatment of breast cancer with >3 positive nodes. Proc Am Soc Clin Oncol 2001;20:abstr 80.
30. Rodenhuis S, Bontenbal M, van Hoesel QG, et al. Efficacy of high-dose alkylating chemotherapy in HER2/neu-negative breast cancer. Ann Oncol 2006;17:588-96.
31. Berry DA, Ueno NT, Johnson MM, et al. High-dose chemotherapy with autologous hematopoietic stem-cell transplantation in metastatic breast cancer: overview of six randomized trials. J Clin Oncol 2011;29:3224-31.
32. Farquhar CM, Marjoribanks J, Lethaby A, et al. High dose chemotherapy for poor prognosis breast cancer: systematic review and meta-analysis. Cancer Treat Rev 2007;33:325-37.
33. Cancello G, Maisonneuve P, Rotmensz N, et al. Prognosis and adjuvant treatment effects in selected breast cancer subtypes of very young women (<35 years) with operable breast cancer. Ann Oncol 2010;21:1974-81.
34. Anders CK, Hsu DS, Broadwater G, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. J Clin Oncol 2008;26:3324-30.
35. Anders CK, Fan C, Parker JS, et al. Breast carcinomas arising at a young age: unique biology or a surrogate for aggressive intrinsic subtypes? J Clin Oncol 2011;29:e18-20.
36. Azim HA Jr, Michiels S, Bedard PL, et al. Elucidating prognosis and biology of breast cancer arising in young women using gene expression profiling. Clin Cancer Res 2012;18:1341-51.

Cite this article as: Cancello G, Montagna E. Treatment of breast cancer in young women: do we need more aggressive therapies? J Thorac Dis 2013;5(S1):S47-S54. doi: 10.3978/j.issn.2072-1439.2013.06.10

Neoadjuvant bevacizumab and chemotherapy in breast cancer

Suparna B. Wedam, Sherry X. Yang

Medical Oncology Branch, Center for Cancer Research; National Clinical Target Validation Laboratory, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Correspondence to: Dr. Sherry X. Yang. National Clinical Target Validation Laboratory, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD 20892, USA. Tel: 301-496-6711. Email: Sherry.Yang@nih.gov.

Submitted Mar 03, 2012. Accepted for publication Mar 23, 2012.
doi: 10.3978/j.issn.2218-676X.2012.03.04
View this article at: http://tcr.thepbpc.org/article/view/375/724

Bevacizumab (Avastin, Roche-Genentech) is a humanized monoclonal antibody targeting all isoforms of the vascular endothelial growth factor A (VEGF-A), an important regulator of angiogenesis. It stimulates endothelial cell proliferation/migration as well as induces vascular leakage or vasodilatation, which are essential in a variety of physiological and pathological conditions (1). It has been found that VEGF-A expression is upregulated in various human tumors. Over the past decade, bevacizumab has been incorporated into chemotherapy for the treatment of cancer patients with advanced diseases including colorectal cancer, non-squamous non-small cell lung cancer, renal cell carcinoma and glioblastoma.

Recently, two randomized phase III clinical trials evaluated neoadjuvant bevacizumab plus chemotherapy for early and locally advanced HER2-negative breast cancer - one is the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-40 trial and the other is the GeparQuinto (GBG44) trial (2,3). The primary endpoint of both studies was to compare pathological complete response (pCR) rate, a surrogate endpoint for neoadjuvant therapy efficacy, of neoadjuvant chemotherapy with or without bevacizumab in patients with HER2-negative primary breast cancer. Patients were eligible if they had a tumor stage T1c to T3, nodal stage N0 to N2a and no distant metastases specified in the NSABP B-40 trial or the criteria as NSABP B-40 plus stage 4a to 4d and nodal stage N3 in the GeparQuinto trial. The addition of bevacizumab to chemotherapy significantly and moderately increases pCRrate (34.5% *vs*. 28.2%, P=0.02) in the breast by the NSABP B-40, and in the breast and nodes (18.4% *vs*. 14.9%, P=0.04) by the GeparQuinto. Additionally, pCR rate was not significantly increased by adding capecitabine or gemcitabine to docetaxel compared to docetaxel monotherapy in NSABP B40 study. Both study results were published in the Journal of *New England Journal of Medicine* in the January 26, 2012 issue amid the controversy or debate over bevacizumab in the treatment of HER2-negative locally recurrent or metastatic breast cancer.

In November 2011, based on the lack of survival benefit or improvement on the quality of life, and small improvement in progression-free survival (less than three months) by adding bevacizumab to capecitabine or a taxane/an anthracycline from the two double-blind randomized phase III studies (AVADO and RIBBON-1) in HER2-negative metastatic breast cancer (4,5), the Food and Drug Administration (FDA) revoked the approval of bevacizumab in combination with paclitaxel as first-line treatment for metastatic breast cancer in the United States (http://1.usa.gov/v3KYnY). The FDA initially granted an accelerated approval for bevacizumab in metastatic breast cancer in February 2008 based on the Eastern Cooperative Oncology Group (ECOG) 2100 trial results, in which the addition of bevacizumab to weekly paclitaxel chemotherapy significantly improved progression-free survival (median, 11.8 *vs*. 5.9 months) and nearly doubled objective response rate (6). However, it is yet to be confirmed whether the choice of chemotherapy agents or regimens for use in combination with bevacizumab could be more or less effective or has an impact on the magnitude of improvement of progression-free survival.

In both neoadjuvant trials, bevacizumab plus chemotherapy versus chemotherapy alone increased grade 3/4 adverse events that have been associated with bevacizumab such as hypertension, proteinuria, headache, and/or left ventricular dysfunction or aggregating the toxicities of chemotherapy agents, for example, neutropenia, mucositis, hand-foot syndrome or infection similar largely

to the reports from previous bevacizumab trials (4-6). It was the concerns on its safety profile that has partially contributed to the decision of withdrawal of the drug for metastatic breast cancer (http://1.usa.gov/v3KYnY). Interestingly, the surgical complications of only 2% and 14.7% in NSABP B-40 and GeparQinto, respectively, were much lower than what would have predicted. In our own study using neoadjuvant bevacizumab in 21 patients with inflammatory and locally advanced breast cancer, we experienced a 24% complication rate in all patients and 39% complication rate in patients that underwent surgery (7). This is similar to a 43% surgical complication rate reported by Golshan *et al.* in another small neoadjuvant trial administering cisplatin in combination with bevacizumab to 51 patients (8). The type of events that were considered "complications" was not elaborated in detail in the two Phase III studies. Nonetheless, this is a toxicity that will need to be followed as the use of bevacizumab increases in the neoadjuvant setting.

In the subset analyses, the divergent occurs on the subgroups of patients who benefited from the combination treatment. The addition of bevacizumab significantly increases pCR rate in patients with hormone receptor-positive tumors by NSABP B-40 whereas in those with the triple-negative subset by GeparQuinto (2,3). The discrepancy itself may suggest that hormone receptors might not be the critical factors for bevacizumab efficacy or benefit in HER2-negative population in the neoadjuvant setting. We anticipate that both trials will provide opportunities to delineate the molecular markers, which have emerged from single arm bevacizumab chemotherapy clinical trials that are associated with overall survival (9), using the tissues collected before the initiation of treatment. In addition, the biopsies or specimens collected on therapy or after surgery following neoadjuvant treatment could facilitate the identification of the biomarkers of resistance. It would be the identification of subset patients who maximally benefit from the administration of bevacizumab that holds the key for successful use of this drug. At this point, the data for overall survival is premature and it is matter of time before we know whether pCR will translate into an increase in overall survival.

Cite this article as: Wedam SB, Yang SX. Neoadjuvant bevacizumab and chemotherapyin breast cancer. Transl Cancer Res 2012;1(1):57-58. doi: 10.3978/j.issn.2218-676X.2012.03.04

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997;18:4-25.
2. Bear HD, Tang G, Rastogi P, et al. Bevacizumab added to neoadjuvant chemotherapy for breast cancer. N Engl J Med 2012;366:310-20.
3. von Minckwitz G, Eidtmann H, Rezai M, et al. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. N Engl J Med 2012;366:299-309.
4. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. J Clin Oncol 2010;28:3239-47.
5. Robert NJ, Diéras V, Glaspy J, et al. RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. J Clin Oncol 2011;29:1252-60.
6. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357:2666-76.
7. Wedam SB, Low JA, Yang SX, et al. Antiangiogenic and antitumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. J Clin Oncol 2006;24:769-77.
8. Golshan M, Garber JE, Gelman R, et al. Does neoadjuvant bevacizumab increase surgical complications in breast surgery? Ann Surg Oncol 2011;18:733-7.
9. Yang SX, Steinberg SM, Nguyen D, et al. p53, HER2 and tumor cell apoptosis correlate with clinical outcome after neoadjuvant bevacizumab plus chemotherapy in breast cancer. Int J Oncol 2011;38:1445-52.

Impact of preoperative magnetic resonance imaging in breast cancer patients candidates for an intraoperative partial breast irradiation

Agnès Tallet[1], Sandrine Rua[2], Aurélie Jalaguier[3], Jean-Marie Boher[4], Mathieu Minsat[1], Monique Cohen[2], Gilles Houvenaeghel[2], Eric Lambaudie[2], Elisabeth Chereau[2], Camille Jauffret[2], Max Buttarelli[2], Martine Poncet[3], Emmanuelle Charafe-Jauffret[5], Michel Resbeut[1]

[1]Department of Radiation Oncology, [2]Department of Surgical Oncology, [3]Breast imaging Unit, [4]Department of Biostatistics, [5]Department of Biopathology, Institut Paoli Calmettes, 232 BVD Ste Marguerite, 13273 Marseille cedex 09, France

Correspondence to: Agnès Tallet, M.D. Radiation Oncology Department, 232 Boulevard de Sainte Marguerite, 13009 Marseille, France. Email: talleta@ipc.unicancer.fr.

Objective: Partial breast irradiation (PBI) could be a reasonable option in patients with early breast cancer (BC) provided that an adequate patient selection, based on robustly established criteria is performed. A preoperative magnetic resonance imaging (MRI) in patient selection for PBI is not consensual. The aim of this retrospective study was to assess the impact of preoperative MRI on patient eligibility for PBI.

Methods: Since March 2012, patients with early BC, meeting the Inca's criteria for PBI were offered the possibility of shortened treatment through intra-operative radiation therapy, either in a prospective trial or off protocol. Eligibility criteria based on physical examination, mammography and ultrasound, and a pathological exam of biopsy, were as follows: menopaused woman 55 years or older with a T1, N0, hormonal-receptor-positive and HER2-negative, invasive, non-lobular epithelioma, without extensive intraductal component (defined as more than 25% of ductal component on biopsy), non-fast-growing tumor, without lymphovascular invasion (LVI), without criteria for adjuvant chemotherapy. A contrast-enhanced MRI was not routinely performed, but at the discretion of the physician as was the rule in TARGIT-A trial. We assessed the rate of additional cancer revealed by the preoperative MRI, remote in the same breast not detected by mammography and/or ultrasound.

Results: Between March 2012 and February 2014, 179 early BC patients meeting the required criteria were planned for an intraoperative radiotherapy (IORT)-PBI. Seventy nine percent of them (141/179) underwent a breast MRI as part of preoperative assessment. ACR3-ACR4 abnormalities not detected by mammograms or ultrasound were found in 44 patients (31%), which prompted a focused mammary ultra-sound, and a biopsy was realized in 29/141 patients (21%). A second breast carcinoma was found in 10 patients (7% of patients with a preoperative MRI, 4 ipsilateral lesions, 5 contralateral lesions, and one both ipsi- and contralateral lesion, precluding IORT-PBI in 5/141 patients (4%).

Conclusions: The use of preoperative MRI in patient staging leads to diagnosis of an ipsilateral second BC in 4% of cases, which appears substantial in a highly selected population. We therefore support the routine use of this exam for the staging of patient candidate for a PBI.

Keywords: Breast cancer (BC); breast cancer recurrence; intraoperative radiotherapy (IORT); magnetic resonance imaging (MRI); partial breast irradiation (PBI)

Submitted Feb 01, 2015. Accepted for publication Apr 10, 2015.
doi: 10.3978/j.issn.2218-676X.2015.04.04
View this article at: http://dx.doi.org/10.3978/j.issn.2218-676X.2015.04.04

Introduction

Breast conserving surgery (BCS) followed by whole breast irradiation (WBI) is recognized as the standard of care for early breast cancer (BC). Based upon local failure patterns, partial breast irradiation (PBI) (targeting the tumor bed) has been considered for early BC patients, in order to shorten the treatment time, reduce the radio-induced toxicity, therefore improving quality of life. However, treatment's de-escalation remains to be proven equivalent in terms of local control to the gold standard WBI, and PBI is currently still debated due to lack of results of randomized trials with sufficient follow-up. Nonetheless, PBI is increasingly used due to its convenience for patients and its advantages related to radiation facilities access. If this shortened treatment would become standard, only highly selected patients would benefit from this kind of treatment. Currently, many questions regarding appropriate patient selection criteria still exist, despite the fact that both the American and European societies of Radiology and Oncology (ASTRO, GEC-ESTRO) provided recommendations for patients treated off clinical trials, based on tumor- and patient characteristics, recommendations that slightly differ between each society (1,2). These patient selection criteria are likely the cornerstone of PBI's success. The challenge is to avoid this "lightest" treatment for patients with a high ipsilateral recurrence risk. Magnetic resonance imaging (MRI) is a highly sensitive exam in detecting clinically occult BC, not highly specific, and has never been proven improve local recurrence rates (LRR) when used as part of preoperative staging in patients treated with BCS followed by WBI (3,4). On the contrary, the routine use of breast MRI for preoperative staging is not recommended since it may modify the surgery type thus increasing the mastectomy rate (3). In patients planned for a PBI, conditions are different because an occult cluster, remote in the ipsilateral breast will be ignored by the partial breast treatment and may result in an increase of LRR. That is why the value of breast MRI for preoperative staging in this setting deserves assessment. ASTRO Task Force does not support the routine use of preoperative breast MRI in patient selection for PBI. Neither in the TARGIT-A trial, nor in the ELIOT trial, preoperative breast MRI was required, and was just performed at the discretion of the physician. Intraoperative radiotherapy (IORT) using low-energy X-rays of 50 kV is one of the methods of PBI used in France, currently under investigation in clinical trials and used sometimes off protocol. Patient selection for PBI varies according to the clinical trial, but is usually based on recommendations close to those provided by both ASTRO and GEC-ESTRO. This retrospective study was conducted in order to assess the impact of preoperative MRI on patient eligibility for PBI, and to determine the rate of unsuspected ipsilateral second cancer on mammography, in a highly selected population. It was also to assess the number of additional exams prompted by MRI abnormalities and MRI's relevance, in order to avoid useless exams for those patients at supposed very low risk of second ipsilateral cancer.

Methods

Patients

From March 2012 through February 2014, BC patients meeting the Inca's criteria for PBI were offered the possibility of a shortened treatment through IORT, either in a prospective trial or off protocol (all patients gave written or oral consent, registered in the medical chart). Data were monitored prospectively. The eligibility criteria, based on physical examination, mammography and ultrasound, and a biopsy pathological exam, were as follows: menopaused woman, 55 years or older with T1, N0, hormonal-receptor-positive and HER2-negative, invasive non-lobular epithelioma, without extensive intraductal component (defined as more than 25% of ductal component on biopsy), without fast-growing tumor, without lymphovascular invasion (LVI), without criteria for adjuvant chemotherapy. Preoperative assessment included a tumor biopsy for pathological assessment that had to provide as many information as possible for selecting patients meeting the required criteria.

Breast MRI

Breast MRI was performed on a 1.5-T GE MR scanner HDxT (General Electric, Milwaukee, WI) using a dedicated phased array bilateral breast coil. Patients were imaged in the prone position. First, morphologic sequences were acquired using an axial echo gradient 3D T1-weighted sequence (TR/TE: 8/4; flip angle: 15°; no gap; field of view: 35 cm; matrix: 512/320; number of excitations: 0.7; scanning time: 90 sec) and a 2D T2-weighted sequence (TR/TE: 3220/85; flip angle: 90°; no gap; field of view: 35 cm; matrix: 352/352; number of excitations: 2; scanning time: 4 min 40 sec). Then, an axial 3D dynamic contrast-enhanced T1-weighted fat-saturated gradient-echo sequences was acquired (TR/TE: 5.4/2.6; flip angle: 15°; slice thickness: 2 mm; no gap; field of view: 35 cm; matrix: 416/416; number of excitations:

0.7; scanning time: 7 min 25 sec). These sequences were acquired before and six times after bolus injection of gadolinium chelate (Dotarem, Guerbet, France) (0.1 mmol·kg^{-1} body weight) given via a power injector (Mallinckrodt, St Louis, MO, USA). All of the MR images were reviewed on the ADW console (General Electric). A board certified radiologist with 10 years of experience in breast MRI, reviewed all the breast MRI. The radiologist first interpreted the MR images and looked for the presence or absence of additional lesions. An additional lesion was defined as a lesion separate from the index tumor undetected by conventional methods (mammography and ultrasonography) but detected by sequentially performed contrast-enhanced breast MRI. If additional lesions were detected on MRI, the reader classified the lesions based on the second edition of BI-RADS MR lexicon and assigned a BI-RADS category.

Treatments

IORT was performed for all patients in a single surgical procedure. The sentinel node biopsy as well as instantaneous pathological analysis were performed before IORT which was maintained if the results were negative. Surgical excision was performed from the skin up to the pectoral fascia in every case. Margin status was assessed intraoperatively through a fast frozen section analysis to ensure clear margins before the IORT procedure; if this margin was positive or close, a re-resection was performed before IORT. The skin was spared from radiation dose by properly applied water-coated gauze and was maintained at more than 10 mm from the applicator surface. A radiation shield material was applied on the pectoral muscle to avoid a high single radiation dose in the ribs.

Data collection and procedures

For this retrospective analysis, we reviewed all MRI reports from patients scheduled for PBI (having had a preoperative MRI), and searched for abnormalities not detected by mammograms or ultrasound. We recorded the location of the occult foci (same quadrant or remote in the breast), their distance relative to the index lesion and the results of pathologic evaluation when subsequent biopsy was performed. The supplemental foci classified as ACR 3-4, prompted a second-look focused ultrasound which, either has invalidated a supplemental lesion, or confirmed a suspicious lesion which therefore has led to a pathologic assessment through a micro-biopsy. The supplemental foci, classified ACR5, were systematically submitted to biopsy. Multifocal disease was defined as one (or more) additional lesion, biopsy-proven, in the same quadrant, whereas multicentric disease was defined as one (or more) additional lesion, biopsy-proven, at more than 4 cm from the index lesion or in another quadrant. Mammographic breast density was described for all patients using the BIRADS lexicon of the American College of Radiology. The breast density was divided in four categories: a breast entirely fatty, was defined as a "density 1"; Breast with scattered fibroglandular densities (approximately 25-50% glandular): "density 2"; Breast with scattered fibroglandular densities (approximately 51-75% glandular): "density 3" and finally, an extremely dense breast tissue more than 75% glandular was defined as a "density 4".

Statistical analysis

Patient's treatments and tumors' characteristics were summarized using means, standard deviations, medians and ranges for quantitative variables and counts and percentages for categorical variables. The incidence of additional ipsilateral BC in the present study population was defined as the primary evaluation criterion. The proportion of patients presenting an ipsilateral additional BC was estimated with 95% confidence intervals using an exact binomial method (5). Statistical analysis was performed using SAS v9.3 sofware (SAS institute Inc. Cary, NC, USA). This retrospective study was approved by our institutional review board.

Results

Between March 2012 and February 2014, 179 early BC patients meeting the required criteria were planned for PBI. Seventy nine percent of them (141/179) underwent an MRI as part of preoperative staging, and constituted the study population. Thirty eight patients did not performed a pre-operative MRI, mainly due to surgeon's preference (27/38 patients) or due to MRI contraindication (5 patients), or patient refusal (4 patients) or other cause (2 patients).

Patient characteristics

Clinical and pathological characteristics of the 141 patients who underwent a preoperative MRI are detailed in *Table 1*. Median age was 67 years (range, 55-90 years). Three fourth of patients were classified as T0N0 and median pathologic

Table 1 Patient characteristics

Characteristics	Clinical	Pathological
Age (median, range)	67 [55-90]	
Tumor size	T	pT
0	104 (72%)	0 (0)
1a	0 (0)	16 (11%)
1b	12 (9%)	40 (29%)
1c	27 (19%)	69 (49%)
2	0 (0)	16 (11%)
Median pT		12 mm
Grade		Post-op
1		63 (45%)
2		65 (46%)
3		13 (9%)
LVI		Pos-top
Yes		23 (16%)
no		118 84%)
pN		
0		107 (76%)
0 (i+)		4 (3%)
1mi		8 (5%)
1		18 (13%)
2		4 (3%)

LVI, lymphovascular invasion.

Table 2 New abnormalities detected by MRI, ignored by conventional exam

Side	ACR3-ACR4 abnormalities' rate: 31% (44/141 pts)			
	N (%)	Biopsy (%)	Second cancer (%)	IORT cancelled (%)
Ipsilateral	33 [23]	21 [15]	5 [4]	5 [4]
ACR3	21	12	2	
ACR4	12	8	3	
Contralateral	19 [13]	15 [11]	6 [4]	1
ACR3	10	8	1	
ACR4	8	6	4	
ACR5	1	1	1	

MRI, magnetic resonance imaging; IORT, intraoperative radiotherapy.

size was 12 mm. The tumor grade was correctly assessed on biopsy. LVI was missed by biopsy in 16% of patients and lymph-node involvement has also been ignored by clinical exam and imaging in 16% of patients.

Breast MRI new abnormalities and second cancer rate

The preoperative breast MRI has identified ACR3-4 new abnormalities in 31% of patients (44/141), either in ipsilateral or contralateral breast or both (*Table 2*). Overall, subsequently to focused ultrasound, a biopsy has been required for 29/141 patients (21%). Suspicious ipsilateral lesions were found in 33/141 patients (23%), distributed as shown in *Table 2*, and were invalidated by focused ultrasound in 12 patients; thus the ipsilateral MRI abnormalities have prompted a biopsy for 21/141 patients (15%). A second ipsilateral cancer was confirmed in one fourth of biopsied patients, and led to the IORT-PBI cancellation in 4% of patients having had a preoperative MRI. Suspicious contralateral lesions were found in 19/141 patients (13%). Focused ultrasound have invalidated four of them, having required a biopsy for 15 patients (11%; some of them have already been accounted due to bilateral additional abnormalities). A contra-lateral BC was found in 6/141 patients (4.3%; 95% CI: 1.5-9.0), and IORT-PBI was cancelled only for the patient presenting both an ipsilateral and contralateral second cancer. In other words, a contralateral synchronous BC has not precluded the planned IORT treatment, which has sometimes been performed for the two sides.

Additional ipsilateral cancer location

The distance between the external edges of the two ipsilateral lesions was found to be 45 to 90 mm (mean 50 mm), with two bifocal lesions, in the same quadrant, becoming bi-centric due to the distance of more than 40 mm between the two lesions, two bi-centric lesions (in two different quadrants) and one multicentric lesion. Breast density for these patients was two in most of cases.

Discussion

BCS followed by WBI is recognized as the standard of care for early BC. WBI consists of 50 Gy/2 Gy per fraction/25 fractions/5 weeks in the whole breast followed by a 10-16 Gy boost to the tumor bed. This regimen has been proven being able to allow a 5- and 10-year LRR of 4% and 8% respectively for unselected patients more than 50 years old (6). In selected patients, >50 years old with favorable tumor characteristics, lower LRR could be expected. Any change in radiotherapy schedule must guarantee the same LRR without increasing toxicity. The rationale for PBI is based upon the fact that although more than half of

specimens of mastectomies undertaken for small BC harbor occult cancer foci remote from the index lesion (7,8), at least three fourth of LR are true recurrences (occurring in the initial tumor bed) (9). This fact has been observed in patients treated with BCS followed by WBI, as well as in patients treated with BCS alone (without WBI) (9). Nonetheless, BC recurrence outside the index quadrant appears to be significantly lowered by adjuvant WBI, from 1.5-3.5% to 0.5-1% (9-11). In other words, the role of WBI is to reduce the recurrence risk in the tumor bed, as well as remote in the breast. It could be argue that the risk of recurrence outside of the initial tumor bed is so low that slightly increasing this risk is acceptable when, on the other side 3 to 5 weeks of radiation treatment is avoided. But this 1.5% to 3.5% recurrence risk, remote from the index lesion, observed when adjuvant WBI is omitted, is not so far from the 4% of occult second cancer in the ipsilateral breast revealed by breast MRI, and can therefore be avoided by a preoperative MRI. And it has also been suggested that patients are willing to accept treatments inconvenience for an expected 1% benefit on recurrence risk (12). Noteworthy, in this situation, it's not treatment convenience which is discussed but only an additional exam to lower the recurrence risk. The use of preoperative MRI has been accused to unnecessarily increase the mastectomy rate. This is actually true only when a histologic confirmation is not done. The TARGIT-A, a prospective randomized trial, have reported the non-inferiority of intraoperative PBI compared to WBI in terms of LR (5-y LR: 2.1% in the IORT arm *vs*. 1.1% in the WBI arm; P=0.31, for the subgroup of 2298 patients treated with IORT concurrently with lumpectomy or lumpectomy followed by WBI), with a median follow-up of 2 years and 5 months for the whole cohort (13). Patients included in this trial had to be ≥45 years old, T2-3 unifocal invasive ductal BC (lobular carcinoma was not permitted), suitable for breast-conserving surgery, N0-1 nodal status. Preoperative breast MRI was not mandatory. This non-inferiority study was powered to detect a 2.5% absolute difference in local recurrence between the two arms. The authors concluded that IORT concurrent with lumpectomy within a risk-adapted approach should be considered as an option for eligible patients with BC carefully selected as per the TARGIT-A trial protocol, as an alternative to postoperative WBI. It seems that the recurrence rate remote from the index lesion is until then the same in the two arms, with a relatively short follow-up, not long enough to definitely conclude. The results of the ELIOT trial have recently been reported (14). This randomized controlled trial has included 1,305 patients randomly assigned to IORT or WBI. Patient selection was less stringent than in TARGIT-A trial, (more advanced disease). The authors have detailed the sites of recurrence and reported an increased ipsilateral recurrence rate both in the tumor bed and remote from the index lesion. Patients receiving WBI did not experienced any recurrence outside the tumor bed at 5 years, whereas patients in the IORT group had a 1.9% recurrence rate remote from the index lesion (P=0.0001). Several studies have examined the potential of preoperative breast MRI to improve patient's selection for PBI (15-20). The rate of ipsilateral additional BC was reported ranging from 2.8% to 10%, depending of the robustness of patient selection and correlated with known prognostic factors for local recurrence such as tumor size, age less than 50 years, LVI and HER2-positive tumors (19). Our data show that in highly selected patients considered as candidates for IORT, 4% presented with a bifocal tumor, which could result in a 4% recurrence rate in the follow-up (excluding true LR), higher than that expected. These results compare favorably with those from other series having tested the role of preoperative MRI in patient selection for PBI. MRI seems therefore an interesting exam in selecting candidates for PBI and should be performed systematically despite its high level of false positive, in order to not sentencing PBI for the wrong reasons. This practice has been approved by the EUSOMA working group with a high level (B) of recommendation (21). The limitations of this study include its retrospective nature, the small study population and small number of events precluding an analysis of clinical or pathological factors correlated with the presence of additional cancer. We would be for example interested in defining or exclusion of the risk depending on the breast density. Finally, the real significance of these occult additional lesions is not known and it is not clear if these lesions could be indolent lesions. That is why comparison of patients treated with PBI with or without the routine use of preoperative MRI would answer this question.

Conclusions

The use of preoperative MRI in patient staging leads to diagnosis of an ipsilateral second BC in 4% of cases, which appears substantial in a highly selected population. We therefore support the routine use of this exam for the staging of patient candidate for a PBI.

Acknowledgements

We thank all the authors for their contribution in the recruitment, treatment, follow-up of the patients and for having participated in the elaboration and writing of this manuscript.

Authors' contributions: AT and MR collected the data and wrote the article; SR collected the data, participated to the writing of the article; AJ and MP collected the data and participated to the writing of the article; JMB participated to the writing of the article and proceeded to the statistical analysis; MM participated to the writing of the discussion; MC, GH, EL, RC, CJ, MB have recruited patients for PBI and revised the manuscript thus improving the discussion; ECJ have analyzed the surgical specimens and revised the article.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Smith BD, Arthur DW, Buchholz TA, et al. Accelerated partial breast irradiation consensus statement from the American Society for Radiation Oncology (ASTRO). Int J Radiat Oncol Biol Phys 2009;74:987-1001.
2. Polgár C, Van Limbergen E, Pötter R, et al. Patient selection for accelerated partial-breast irradiation (APBI) after breast-conserving surgery: recommendations of the Groupe Européen de Curiethérapie-European Society for Therapeutic Radiology and Oncology (GEC-ESTRO) breast cancer working group based on clinical evidence (2009). Radiother Oncol 2010;94:264-73.
3. Houssami N, Hayes DF. Review of preoperative magnetic resonance imaging (MRI) in breast cancer: should MRI be performed on all women with newly diagnosed, early stage breast cancer? CA Cancer J Clin 2009;59:290-302.
4. Houssami N, Turner R, Macaskill P, et al. An individual person data meta-analysis of preoperative magnetic resonance imaging and breast cancer recurrence. J Clin Oncol 2014;32:392-401.
5. Feller W. An introduction to probability theory and its applications Vol1, 3rd edition. New York: Wiley, 1968.
6. Bartelink H, Horiot JC, Poortmans PM, et al. Impact of a higher radiation dose on local control and survival in breast-conserving therapy of early breast cancer: 10-year results of the randomized boost versus no boost EORTC 22881-10882 trial. J Clin Oncol 2007;25:3259-65.
7. Holland R, Veling SH, Mravunac M, et al. Histologic multifocality of Tis, T1-2 breast carcinomas. Implications for clinical trials of breast-conserving surgery. Cancer 1985;56:979-90.
8. Vaidya JS, Vyas JJ, Chinoy RF, et al. Multicentricity of breast cancer: whole-organ analysis and clinical implications. Br J Cancer 1996;74:820-4.
9. Veronesi U, Marubini E, Mariani L, et al. Radiotherapy after breast-conserving surgery in small breast carcinoma: long-term results of a randomized trial. Ann Oncol 2001;12:997-1003.
10. Clark RM, McCulloch PB, Levine MN, et al. Randomized clinical trial to assess the effectiveness of breast irradiation following lumpectomy and axillary dissection for node-negative breast cancer. J Natl Cancer Inst 1992;84:683-9.
11. Liljegren G, Holmberg L, Adami HO, et al. Sector resection with or without postoperative radiotherapy for stage I breast cancer: five-year results of a randomized trial. Uppsala-Orebro Breast Cancer Study Group. J Natl Cancer Inst 1994;86:717-22.
12. Ravdin PM, Siminoff IA, Harvey JA. Survey of breast cancer patients concerning their knowledge and expectations of adjuvant therapy. J Clin Oncol 1998;16:515-21.
13. Vaidya JS, Wenz F, Bulsara M, et al. Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: 5-year results for local control and overall survival from the TARGIT-A randomised trial. Lancet 2014;383:603-13.
14. Veronesi U, Orecchia R, Maisonneuve P, et al. Intraoperative radiotherapy versus external radiotherapy for early breast cancer (ELIOT): a randomised controlled equivalence trial. Lancet Oncol 2013;14:1269-77.
15. Al-Hallaq HA, Mell LK, Bradley JA, et al. Magnetic resonance imaging identifies multifocal and multicentric disease in breast cancer patients who are eligible for partial breast irradiation. Cancer 2008;113:2408-14.
16. Godinez J, Gombos EC, Chikarmane SA, et al. Breast MRI in the evaluation of eligibility for accelerated partial breast irradiation. AJR Am J Roentgenol 2008;191:272-7.
17. Tendulkar RD, Chellman-Jeffers M, Rybicki LA, et al. Preoperative breast magnetic resonance imaging in early breast cancer: implications for partial breast irradiation. Cancer 2009;115:1621-30.
18. Horst KC, Fero KE, Ikeda DM, et al. Defining an optimal role for breast magnetic resonance imaging when evaluating patients otherwise eligible for accelerated partial

breast irradiation. Radiother Oncol 2013;108:220-5.
19. Dorn PL, Al-Hallaq HA, Haq F, et al. A prospective study of the utility of magnetic resonance imaging in determining candidacy for partial breast irradiation. Int J Radiat Oncol Biol Phys 2013;85:615-22.
20. Kühr M, Wolfgarten M, Stölzle M, et al. Potential impact of preoperative magnetic resonance imaging of the breast on patient selection for accelerated partial breast irradiation. Int J Radiat Oncol Biol Phys 2011;81:e541-6.
21. Sardanelli F, Boetes C, Borisch B, et al. Magnetic resonance imaging of the breast: recommendations from the EUSOMA working group. Eur J Cancer 2010;46:1296-316.

Cite this article as: Tallet A, Rua S, Jalaguier A, Boher JM, Minsat M, Cohen M, Houvenaeghel G, Lambaudie E, Chereau E, Jauffret C, Buttarelli M, Poncet M, Charafe-Jauffret E, Resbeut M. Impact of preoperative magnetic resonance imaging in breast cancer patients candidates for an intraoperative partial breast irradiation. Transl Cancer Res 2015;4(2):148-154. doi: 10.3978/j.issn.2218-676X.2015.04.04

First year experience with IORT for breast cancer at the Geneva University Hospitals

Vincent Vinh-Hung[1,2], Virginie Nepote[1], Alexandre Rozenholc[1], Hansjoerg Vees[1], Sindy Monnier[1], Monica Castiglione-Gertsch[1], Odile Fargier-Bochaton[1], Youri Popowski[1], Michel Rouzaud[1], Patrick Petignat[1], Philippe Nouet[1], Angele Dubouloz[1], Raymond Miralbell[1]

[1]Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland; [2]Centre Hospitalier Universitaire de Martinique, Fort-de-France, Martinique, France
Correspondence to: Vincent Vinh-Hung. Hôpital Clarac, Bd Pasteur, 97200 Fort-de-France, Martinique, France. Email: anhxang@gmail.com.

Purpose: The Breast Centre at the Geneva University Hospitals implemented guidelines for breast cancer intraoperative radiotherapy (IORT) in 2012. The present study evaluates early breast and skin toxicities observed during the first year of the implementation.

Material and methods: From February 2012 to January 2013, 52 women received IORT for primary breast cancer treated with conservative surgery. IORT was delivered with the Intrabeam® system. The prescribed dose was 20 Gy at the applicator's surface. No further radiation was to be given according to the following criteria: age ≥50 years old, histopathology of invasive ductal, mucinous, tubular, medullar or colloid carcinoma, unifocal tumor, absence of lymphovascular invasion (LVI), absence of extensive in situ component, tumor size ≤30 mm, pathological nodal status pN0 by sentinel node biopsy or pN1mi by axillary dissection, and clear resection margins ≥2 mm. If the criteria were not met, additional whole breast radiotherapy (WBRT) to an equivalent dose of 50 Gy in 2-Gy fractions was to be given post-operatively. Toxicity grades were based on LENT-SOMA scoring tables.

Results: IORT was given as exclusive radiation therapy in 34 (65%) patients, whereas 18 (35%) patients received an additional hypofractionated WBRT of 40-47.25 Gy in 15-21 fractions, one of whom further received after IORT-WBRT an additional electron boost of 13.5 Gy to the tumor bed in 6 fractions. At 1-month post-IORT, no heart toxicity was recorded. Six (11.5%) patients presented grade 2 lung symptoms. Breast/skin most frequent toxicities were seroma, grade 3 in 13 of 52 (25%) patients. One patient had a wound dehiscence requiring suture, and one patient had hematoma after immediate bilateral breast reconstruction with implants. Regarding the 18 patients who received additional WBRT, subsequent reevaluation after the WBRT found no heart toxicity, grade 1 lung symptoms in 1 (5%) patient, and grade 3 breast and skin toxicity in 2 (11%) patients (1 persistent seroma and 1 skin dryness). The breast and skin toxicity observed in patients after additional WBRT was not significantly increased as compared with the toxicity earlier observed after IORT, P=0.631.

Conclusions: Early evaluation of IORT found mild to moderate breast and skin toxicity. Toxicity was not significantly increased in patients receiving additional WBRT.

Keywords: Breast; lumpectomy; intraoperative; radiotherapy; observational study

Submitted Dec 23, 2013. Accepted for publication Jan 20, 2014.
doi: 10.3978/j.issn.2218-676X.2014.01.07
View this article at: http://www.thetcr.org/article/view/2095/2760

Introduction

With 5,250 new diagnoses each year, and correspondingly an age standardized rate (ASR) European standard of 111.3 per 100,000 women, breast cancer incidence in Switzerland ranks 15th in Europe (1,2). Within the country, regional disparities have been observed regarding diagnosis and management of the disease (3). In the canton of Geneva, high breast cancer incidence (ASR 128.5, surpassed only

by the canton of Vaud's ASR of 129.8), high proportion of tumors with favorable characteristics, and commensurately low mortality have been ascribed to running programs of mammography screening (3,4). A survey of 1,404 women with operable invasive breast cancer diagnosed in the canton in 2000-2005 found that the majority presented with early stage disease, 50% stage I, 40% stage II (5). Breast conserving surgery was the preponderant surgical procedure. Most women received post-operative radiotherapy. The Geneva University Hospitals (HUG)'s Breast Centre is the public breast cancer unit where two third of these cantonal cases were managed. Radiotherapy has been routinely delivered using fractionation schedules considered safe (6,7), at the cost of extending treatment time over seven weeks. Since many cases in our practice presented with early stage disease, we considered the possibility of reducing the radiation treatment burden by using hypofractionation and partial breast irradiation. The publication of two large series of intra-operative radiotherapy (IORT), by Vaidya *et al*. (8) and by Veronesi *et al*. (9), provided good evidence to support the use of IORT. We argued in our national medical journal that it was reasonable to propose IORT to patients with low risk of recurrence (10). IORT was later implemented in our hospital in 2012. The purpose of the present study is to evaluate the characteristics of patients who received IORT and to evaluate early toxicity.

Methods

We retrospectively reviewed the medical records of all women who underwent IORT, from the beginning of its availability at the HUG in February 2012, until January 2013.

Selection of patients

Prior to any therapy, all patients with a newly diagnosed breast cancer referred to the HUG were discussed at a multidisciplinary meeting organized weekly ("concertation d'oncologie sénologie préthérapeutique", COSP) (5). IORT was proposed to patients after consensus on the *a priori* eligibility of the patient for breast conserving surgery with IORT, either as exclusive radiation treatment, or as a boost. The HUG eligibility criteria for IORT as exclusive radiation treatment were adapted from the 2009's recommendations of the Groupe Européen de Curiethérapie-European Society for Therapeutic Radiology and Oncology (GEC-ESTRO) regarding accelerated partial breast irradiation (11): age ≥50 years old, histopathology of invasive ductal, mucinous, tubular, medullar or colloid carcinoma, unifocal-unicentric tumor, absence of LVI, absence of extensive *in situ* component, tumor size ≤30 mm, pathological nodal status pN0 by sentinel node biopsy or pN1mi by axillary dissection, and clear resection margins ≥2 mm. If the criteria were not met, additional whole breast radiotherapy (WBRT) was to be given post-operatively. Patients were excluded from IORT in case of invasive lobular carcinoma (ILC), ductal carcinoma in situ (DCIS), extensive intraductal component (EIC), LVI, or neoadjuvant chemotherapy.

Surgical procedure

IORT was scheduled with the surgery only when the patient provided written informed consent. The patient was admitted to the gynecological surgery ward on the day prior to surgery. Breast harpoon localization by mammography or by ultrasound was done for non-palpable lesions. Lymphoscintigraphy through peri-areolar injection with SPECT/CT was done for the mapping of sentinel nodes. On the day of surgery, the surgical procedure under general anesthesia began with sentinel nodes biopsy. Thereafter, excision of the breast tumor was done, typically through a separate incision except for tumors located in or near the axillary tail of the breast. The resected tissue was inspected by palpation and by radiography. Additional resection of breast tissue was done if it was considered that the tumor or the harpoon was close to a margin. Frozen section pathological examination was done for sentinel nodes, but not for resection margins.

IORT procedure

IORT was done immediately after completion of the tumor excision using the Intrabeam system (Carl Zeiss Surgical GmbH, Oberkochen, Germany) with a spherical applicator. The size of the applicator was chosen according to the size of the resection cavity. The breast tissue surrounding the resection cavity was mobilized in order to appose the tissue on the applicator and was fastened with a purse-string stitch. Skin distance was controlled visually. A moistened gauze was inserted between the skin and the applicator if the applicator's distance to the hypodermis was estimated to be less than 5 mm. Shielding was not used. A dose of 20 Gy at the surface of the applicator was prescribed. Radiation delivery and anesthesia were monitored outside

of the operating room. The applicator was removed after the radiation delivery. The surgery proceeded with an axillary dissection in case of pathological involvement of sentinel nodes. Intravenous antibiotic perfusion was given perioperatively. The IORT procedure, from tumor excision till removal of the applicator, lasted on average one hour. The radiation oncologist jointly participated with the surgeon during the application, and supervised the radiation delivery by a dosimetrist.

External radiotherapy

Definitive pathological results were discussed at a separate multidisciplinary meeting organized weekly for post-surgery cases ("colloque d'oncogynécologie"). For IORT patients, IORT was validated as the sole radiation treatment if the post-operative pathological examination confirmed the eligibility criteria. Otherwise, external WBRT was recommended, with or without regional lymph node irradiation according to pathological lymph node status. External beam radiotherapy was scheduled four and six weeks after surgery-IORT if no adjuvant chemotherapy were given, or four weeks after the last cycle of adjuvant chemotherapy if it was given. External radiotherapy was delivered to the breast at a prescribed dose considered equivalent to 46-50 Gy in 2 Gy fractions through tangential beams. Patients were treated prone if treatment planning showed improved lung sparing with comparable breast coverage (12). In case of supine treatment, right side breasts were treated in free breathing, whereas left side breasts were treated in deep inspiration breath hold under videoscopic control (13). Field-in-field compensation was used as needed to ensure that the 107% isodose volume did not exceed 2 mL (14,15). Radiation was delivered to the ipsilateral axillary supra-clavicular areas if >3 or >20% axillary lymph nodes were involved. Radiation was not delivered to the internal mammary chain.

Data analyses

Patients' initial data were retrieved from a core database that maintained the list of patients for whom the multidisciplinary COSP proposed IORT. The medical records were abstracted for demographic, clinical, pathological, and treatment characteristics. Toxicity was retrospectively scored from the records at two time points: for all patients, at the first follow-up consultation after surgery-IORT which was nominally scheduled at four weeks post-surgery-IORT, and, for patients who received external beam radiotherapy, at the first follow-up after the end of the radiotherapy which was nominally scheduled at six weeks post-radiotherapy. Toxicity scoring used the Subjective, Objective, Management and Analytic/Late Effects Normal Tissues (SOMA/LENT) system for breast, skin, lung and heart, but without the functional examinations (16-18). The scores were crosschecked with the physicians who examined the patients at different time points. For the purpose of reporting, we graded toxicity as the maximum score observed in any item. We also combined the breast and skin scores retaining only the highest recorded score. Descriptive presentation of the data used cross-tabulations. Significance testing of contingency tables used the Chi-square test. Comparison of means used the Student *t*-test.

Results

IORT was proposed to 60 patients but was delivered only in 52 cases. The IORT was not done in 8 patients, for preoperative reasons in 3 patients, and was cancelled at the time of operation in the other 5 patients. Preoperatively, 1 patient did not wish to receive any additional information other than the date of her surgery, 1 patient elected to have surgery in another hospital, and 1 patient participated in a preoperative FDG PET/CT trial, the examination found a multifocal tumor. At the time of operation, 1 patient had tumor close to skin, the overlying skin had been resected, the remaining skin was overstretched by approximation of tissues; 1 patient had tumor adherent to pectoralis muscle, there was no specification that the distance of the tumor from the skin and from the muscle should countermand the IORT, but it was considered in this patient that the flat surface at the bottom of the resection cavity would not receive adequate irradiation; 3 patients had extended lumpectomy cavities that did not allow appropriate apposition of breast tissue to the applicators. For the patients receiving IORT, the applicators' sizes were 2.5 (7.7%), 3 (23%), 3.5 (48%), 4 (9.6%), 4.5 (7.7%), and 5 cm (4%).

As could be expected from the selection procedure, the 52 women receiving IORT presented a good concordance between pathological characteristics and eligibility for exclusive IORT (*Table 1*): 88% were older than 50, 94% were invasive ductal carcinoma or other non-lobular types, 90% had resection margins of 2 mm or more, 96% had unifocal breast tumor. One patient had positive resection margin, re-operation found no residual disease, she was considered as fulfilling the margin criteria. Thirty-four

Table 1 Patients' characteristics

Characteristic	All [N=52 (col%)]	IORTXCL full eligibility (N=27)	IORTXCL incomplete criteria (N=7)	IORT + WBRT (N=18)
Age				
>50	46 (88.5)	27	7	12
≤50	6 (11.5)	0	0	6
Histopathology				
Invasive ductal/ other	49 (94.2)	27	6	16
Invasive lobular	3 (5.8)	0	1	2
DCIS extensive				
Absent	44 (84.6)	27	4	13
Present	8 (15.4)	0	3	5
Margin				
≥2 mm	47 (90.4)	27	4	16
<2 mm	5 (9.6)	0	3	2
Multifocal				
No	50 (96.2)	27	7	16
Yes	2 (3.8)	0	0	2
Lymphovascular invasion				
Absent	42 (80.8)	27	5	10
Present	10 (19.2)	0	2	8
Tumor size				
T1b	12 (23.1)	8	1	3
T1c	32 (61.5)	19	5	8
T2	8 (15.4)	0	1	7
pN				
N0	37 (71.2)	21	3	13
N1 (1-3 positive nodes)	4 (7.7)	1	0	3
N2 (4-9 positive nodes)	1 (1.9)	0	0	1
Nx (no biopsy)	10 (19.2)	5	4	1
Tumor grade				
G1	29 (55.8)	17	5	7
G2	18 (34.6)	8	2	8
G3	5 (9.6)	2	0	3
Hormone receptors				
ER–/PR–	1 (1.9)	1	0	0
ER+/PR–	6 (11.5)	4	1	1
ER+/PR+	45 (86.5)	22	6	17
ki-67				
<14%	36 (69.2)	18	6	12
>20%	4 (7.7)	2	0	2
14-20%	12 (23.1)	7	1	4
HER2				
Negative	48 (92.3)	24	7	17
Positive	4 (7.7)	3	0	1
Chemo/hormone therapy				
No/No	4 (7.7)	2	1	1
No/Yes	42 (80.8)	23	6	13
Yes/No	1 (1.9)	1	0	0
Yes/Yes	5 (9.6)	1	0	4

IORTXCL, exclusive intraoperative radiotherapy (IORT); WBRT, whole breast radiotherapy; DCIS, ductal carcinoma in situ.

(65%) patients had no additional radiotherapy after IORT, of whom 27 fulfilled all eligibility criteria, and 7 did not. Eighteen (35%) received additional WBRT, of whom 1 fulfilled all eligibility criteria for exclusive IORT, and 1 received an additional boost to tumor bed.

Delivery of WBRT was significantly related to the number of unmet criteria. The proportion of patients receiving WBRT was 3.6%, 58.3%, 80%, and 100% with 0, 1, 2, and 3 unmet criteria, respectively, P<0.0001 (*Table 2*). There was no case of nodal irradiation. The one patient with four involved axillary nodes had a low lymph node ratio of 16% (4 positive out of 25 examined lymph nodes). WBRT setup was prone in 6 patients, supine free breathing in 9 patients, and supine deep inspiration breath hold in 3 patients. Doses delivered were 15×2.67 Gy (1 patient), 16×2.5 Gy (3 patients), 16×2.66 Gy (6 patients), 20×2.2 Gy (1 patient), 20×2.25 Gy (4 patients), and 21×2.25 Gy (3 patients, 1 with boost 6×2.25 Gy).

Regarding the seven patients who did not receive WBRT although they did not met full requirement for exclusive IORT, the mean age was 74 years, as compared with mean age of 68 years in the exclusive IORT group with fulfilled criteria, and mean age of 59 years in the IORT with WBRT group, P=0.006. The unmet criteria among these seven

Table 2 Treatment delivered according to number of unmet IORT criteria

Number of unmet criteria	IORTXCL N=34 (%)	IORT + WBRT N=18 (%)
0	27 (96.4)	1 (3.6)
1	5 (41.7)	8 (58.3)
2	2 (20.0)	7 (80.0)
3	0 (0.0)	2 (100.0)

IORTXCL, exclusive intraoperative radiotherapy (IORT); WBRT, whole breast radiotherapy.

Table 3 Breast and skin toxicity

Breast/skin toxicity grading	Post IORT N=52 (%)	Post IORT+WBRT N=18 (%)
Grade 0	8 (15.4)	0 (0.0)
Grade 1	23 (44.2)	11 (61.1)
Grade 2	6 (11.5)	5 (27.8)
Grade 3	13 (25.0)	2 (11.1)
Grade 4	2 (3.8)	0 (0.0)

IORT, intraoperative radiotherapy; WBRT, whole breast radiotherapy.

Table 4 Breast and skin toxicity in 18 patients after IORT and after WBRT

Post IORT (N=18) Grade	Post IORT + WBRT (N=18) Grade	Change	N (%)
0	1	1	3 (16.7)
1	1	0	7 (38.9)
1	2	1	3 (16.7)
2	3	1	1 (5.6)
3	1	–2	1 (5.6)
3	2	–1	1 (5.6)
3	3	0	1 (5.6)
4	2	–2	1 (5.6)

IORT, intraoperative radiotherapy; WBRT, whole breast radiotherapy.

patients were: extensive DCIS in 3, resection margin <2 mm in 2, presence of LVI in 1, and combined unmet criteria of ILC combined with resection margin <2 mm in 1 patient. The latter patient was 92 years old. None had multifocal disease, the largest tumor size was 2.4 cm, and axillary lymph node exploration was omitted in 4 of the 7 patients.

Early toxicities were evaluated in all patients at a median of 27 days (range, 13-70 days) after IORT. There were no heart-related complications. Lung symptoms of cough, dyspnea and chest discomfort scored as Grade 2 were noted in 6 of 52 (11.5%) patients. The symptoms were mild and abated in the following weeks, chest X-rays or CT were not performed. The most frequent breast/skin toxicities were seroma, scored as grade 3 in 13 of 52 (25%) patients (*Table 3*). Two cases were scored as grade 4: one patient presented wound dehiscence requiring suture; the other patient had immediate bilateral breast augmentation with implants following her tumor resection and IORT, she presented with bilateral hematoma requiring re-operation.

Early post-WBRT evaluation was done at a median of 40 days (range, 19-81 days) after completion of WBRT. There were no heart complication, and only 1 patient presented with mild symptoms of cough and dyspnea. Breast/skin evaluation recorded 2 patients as presenting Grade 3 toxicity, one for persistence of seroma, the other for intense skin dryness. We compared how the grades changed in these 18 patients relatively to their earlier post-IORT evaluation (*Table 4*). The elapsed time was median 90 days (range, 41-244 days) between the IORT and WBRT evaluations, respectively 86 days among 14 patients without chemotherapy, and 228 days among 4 patients receiving chemotherapy. Grade post-WBRT was increased in 7 patients, comparable in 8 patients, and decreased in 3 patients. Matched pairs analysis showed no significant relationship between the grades at the two time points, P=0.631.

Prior to submission of the present report, we updated the verification of our patients' files. As of December 20, 2013, the median follow-up was 370 days (min-max range 27-637 days, interquartile range 227-490 days), there were no recurrences, no grade 4 toxicities.

Discussion

Earlier on like several others we gathered large evidence showing a survival advantage with radiotherapy in breast cancer (19-21). We noted that the proportional reduction of mortality would yield a quite small absolute survival benefit in the case of small node-negative tumors (20). We argued then for partial breast irradiation. The simple rationale is that in low risk tumors, reducing radiotherapy would reduce toxicity. Tumor recurrence would also increase but

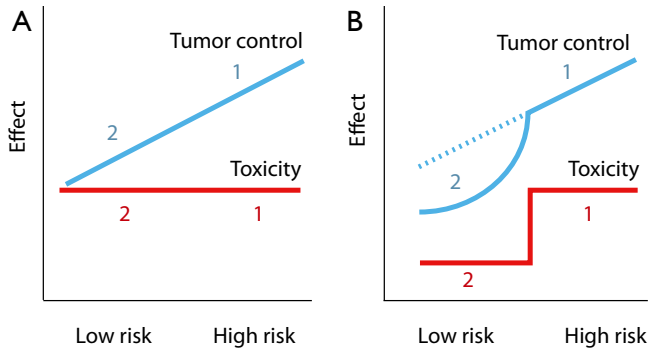


Figure 1 Graphical display of the putative effects of switching from whole breast radiotherapy (WBRT) to intraoperative radiotherapy (IORT). (A) Radiotherapy is known to have a proportional effect on tumor control. As risk decreases, the effect on tumor control decreases: blue line, from 1 for high risk tumors, to 2 for low risk tumors. But the toxicity on normal tissues is unchanged: red line, same toxicity from 1 to 2. The absolute overall gain is the difference between the blue and the red line. The gain decreases with low risk tumors; (B) Switching from WBRT for high risk tumors to IORT for low risk tumors decreases toxicity: red line, toxicity decreases from level 1 to level 2. High risk tumors receive the same WBRT, the effect on tumor control is the same as in (A): blue line, part 1. Low risk tumors receive IORT which incurs a loss of tumor control, the effect on tumor control is less than in (A): blue curve, part 2. However, the absolute overall gain represented by the difference between the blue curve and the red line does not decrease. A potential caveat is for intermediate risk tumors.

moderately, the net effect would be a survival gain (*Figure 1*). The 2010's publications of the TARGIT-A trial (8) and the Milan's experience (9) gave confidence to proceed toward implementing IORT in our hospital. The recent update of the TARGIT-A trial demonstrates that overall survival is maintained, and even tend to improve, despite a small increase in breast recurrences (22). A very similar finding was also reported in the ELIOT trial (23).

We opted for a soft X-ray based system on consideration that our selection of patient would be low risk disease that would not require highly penetrating radiation. Indeed our patients' characteristics presented good prognostic tumor profiles matching well epidemiological surveys of the canton. The number of patients receiving IORT in the present report appears however much lower than what might be expected.

The different WBRT hypofractionation schedules that we used reflect uncertainties during our learning curve. Over the last five years, our department has progressively phased out WBRT of 50 Gy in 25 fractions. Older patients received the Whelan schedule of 42.5 Gy in 16 fractions (24,25) but given four times a week, whereas younger patients received a moderately hypofractionated schedule of 47.25 Gy in 21 fractions given four times a week (6). With IORT delivering 20 Gy in a single fraction, there were concerns that adding WBRT according to these schedules would considerably increase the risk of toxicity. Consequently the prescribed dose was reduced, either by substracting 1 fraction or by reducing the dose per fraction. We took into account that the UK START trial B gave 1 fraction less than the Whelan schedule (26), which suggested a margin for dose reduction of up to 6.25%. Dose reduction was applied to 5 of the first 5 patients then to 3 of the next 6 patients. Thereafter as no unexpected acute toxicities were observed, we applied our usual WBRT schedules except for 1 patient out of 7 who received 40 Gy in 15 fractions.

The IORT dose of 20 Gy in a single shot followed by fractionated WBRT of 50 Gy in 2 Gy fraction-equivalent deserves a particular comment. A single dose of 20 Gy has been considered equivalent to 1.5-2.5 times the same total dose of fractionated external beam radiotherapy (27). That is, a patient given 20 Gy IORT followed by 50 Gy fractionated WBRT would supposedly have received the equivalent of 80-100 Gy, far in excess of the conventional 50 Gy WBRT +16 Gy boost. However, such equivalence approximation does not take into consideration that, unlike intraoperative electron, brachytherapy or external beam radiotherapy which are prescribed on a volume, firstly the Intrabeam dose is prescribed at the surface of the applicator, secondly the dose decreases monotonously with the tissue distance from the applicator, and consequently the radiobiological modelling differs (28). Assuming an applicator size of 4 cm, assuming that the distance from the applicator's surface where it matters most is 1.0 to 1.5 cm (28), not taking into account the applicator's handle, the corresponding volume of breast tissue encompassed by the irradiation is 80 to 146 mL, the estimated mean dose to the breast tissue around the applicator is 10.4 Gy (volume within 1 cm) to 7.8 Gy (volume within 1.5 cm). These doses represent 52% to 39% of the nominal value of 20 Gy. Regarding the ipsilateral breast as an organ at risk, Aziz *et al.* have shown in an anthropormophic phantom dosimetric study that 20 Gy at the surface of a 4 cm applicator delivered to the breast a mean dose of 2.2 Gy (29). By contrast, conventional fractionated boost doses of

16 Gy to tumor bed have been shown to deliver a mean dose of 16 Gy to an average planning target volume (PTV) of 101 +/– 47 mL, and a mean dose to the ipsilateral breast of 7.8-10.5 Gy (30). Accordingly the dose delivered to the breast is reduced four- to five-fold with IORT as compared with conventional boosts. We did not make a direct clinical comparison with conventional external beam radiotherapy. But historically in a group of 50 consecutive patients treated with moderately hypofractionated external beam radiotherapy that we evaluated four years ago, acute G1, G2 and G3 skin toxicity occurred in the boost area of 26%, 60% and 14% patients, respectively (6). That is to say, in line with the dosimetric studies, external beam radiotherapy was associated with slightly more acute toxicity than IORT + WBRT.

The present study has several limitations: small number of patients, retrospective, potential recollection bias, very short follow-up, no patient's self-assessment. There were no functional lung or heart explorations, neither cosmesis nor quality of life evaluation, there was no formal comparison group. Lack of functional lung-heart explorations might have missed subclinical toxicities (31). Quality of life evaluation would have been valuable to confirm other authors who found less pain, breast and arm symptoms in IORT alone patients as compared with external beam radiotherapy (32). Nevertheless despite the limitations of our study, we believe that sharing one's experience can be useful, to identify issues and to formulate hypotheses for further researches. One possible issue might be the role of medical imaging. Similarly to Tuschy *et al.* (33), IORT had to be cancelled in several cases. The happenstance of a patient who underwent a PET/CT raises the question of whether or not it can have a role in the selection of patients. Likewise, we could reflect on the utility of breast MRI prior to IORT (34).

Arguably our use of SOMA/LENT for grading of early toxicities can be considered not optimal. The SOMA/LENT is intended for evaluation of late toxicities. However we plan to evaluate our patients in a few years. We felt using the same scoring system throughout in order to compare the toxicity grades over time would facilitate that follow-up.

Although the current follow-up is short, we found that Intrabeam IORT is a safe technique that did not prevent further radiotherapy. Our experience is in line with other authors who have reported low rates of late toxicities with longer follow-up when using IORT as boost (35,36). Compared to the TARGIT-A trial in which the rate of additional WBRT as per treatment was 15.2% (22), our 35% rate of WBRT was considerably larger. This might be related to different selection criteria. We noted that age and the number of unmet criteria were significant factors in the delivery of additional WBRT. The importance of age as a potential issue will have to be debated in the selection of patients (37). Other issues are the role of hormone receptors, which we did not take into consideration in the current guidelines, and the role of LVI. We considered LVI as an exclusion criterion for IORT. However, LVI or other high risk prognostic factors could in fact be major indication for IORT in order to deliver radiation at the time of surgery. This could be a challenging hypothesis that might be tested in future researches.

Conclusions

In our early experience, we found that Intrabeam IORT was a safe procedure. Toxicity of IORT was moderate. It was not significantly increased in patients receiving WBRT. The technique deserves to be made more readily available to our patients.

Acknowledgements

IORT as a reality at the HUG owes to late Georges Vlastos, founder and head of the onco-senology unit.

Heartfelt thanks to Olena Gorobets of the Cité Hospitalière de Mangot-Vulcin for her precious editorial assistance, to Maryam Ackermann-Zare of the HUG for her great help with the follow-up of patients, and to Edite Richard for her careful maintenance of the patients database.

Footnote

Virginie Nepote was partly funded by private funds for IORT raised by Georges Vlastos and Vincent Vinh-Hung and managed by the HUG. The authors have no conflicts of interest to declare.

References

1. Bouchardy C, Lutz JM, Kühni C. Cancer in Switzerland: situation and development from 1983 to 2007. Neuchatel: FSO, 2011.
2. 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.
3. Ess S, Savidan A, Frick H, et al. Geographic variation in breast cancer care in Switzerland. Cancer Epidemiol 2010;34:116-21.
4. NICER. National statistics on cancer incidence. Interactive statistics map. Zurich, Switzerland: National Institute for Cancer Epidemiology and Registration, 2013.
5. Taban F, Rapiti E, Fioretta G, et al. Breast cancer management and outcome according to surgeon's affiliation: a population-based comparison adjusted for patient's selection bias. Ann Oncol 2013;24:116-25.
6. Vock J, Peguret N, Balmer-Majno S, et al. 254 Four times weekly adjuvant breast radiotherapy with a moderately intensified boost to the tumour bed - feasibility and acute toxicity. EJC Suppl 2010;8:132-3.
7. Van de Steene J, Soete G, Storme G. Adjuvant radiotherapy for breast cancer significantly improves overall survival: the missing link. Radiother Oncol 2000;55:263-72.
8. Vaidya JS, Joseph DJ, Tobias JS, et al. Targeted intraoperative radiotherapy versus whole breast radiotherapy for breast cancer (TARGIT-A trial): an international, prospective, randomised, non-inferiority phase 3 trial. Lancet 2010;376:91-102.
9. Veronesi U, Orecchia R, Luini A, et al. Intraoperative radiotherapy during breast conserving surgery: a study on 1,822 cases treated with electrons. Breast Cancer Res Treat 2010;124:141-51.
10. Vlastos G, Monnier S, Vinh-Hung V. Innovations in locoregional treatments of breast cancer. Rev Med Suisse 2010;6:2016, 2018-23.
11. Polgár C, Van Limbergen E, Pötter R, et al. Patient selection for accelerated partial-breast irradiation (APBI) after breast-conserving surgery: recommendations of the Groupe Européen de Curiethérapie-European Society for Therapeutic Radiology and Oncology (GEC-ESTRO) breast cancer working group based on clinical evidence (2009). Radiother Oncol 2010;94:264-73.
12. Fargier O, Grozema F, Laouiti M, et al. DVH comparison of whole breast radiotherapy (WBRT) in prone and supine position. Abs OC-0349. Radiother Oncol 2013;106:S136-7.
13. Vinh-Hung V, Grozema F, Lee YE, et al. Single Institution Review of Setup Errors with Deep-Inspiration Breath Hold (DIBH) for Left Sided Breast RT. Abstract 41. SASRO, Geneva, Mar 31 - Apr 2, 2011.
14. Pignol JP, Olivotto I, Rakovitch E, et al. A multicenter randomized trial of breast intensity-modulated radiation therapy to reduce acute radiation dermatitis. J Clin Oncol 2008;26:2085-92.
15. Barnett GC, Wilkinson J, Moody AM, et al. A randomised controlled trial of forward-planned radiotherapy (IMRT) for early breast cancer: baseline characteristics and dosimetry results. Radiother Oncol 2009;92:34-41.
16. LENT SOMA tables. Radiother Oncol 1995;35:17-60.
17. Rubin P, Constine LS 3rd, Fajardo LF, et al. EORTC Late Effects Working Group. Overview of late effects normal tissues (LENT) scoring system. Radiother Oncol 1995;35:9-10.
18. Pavy JJ, Denekamp J, Letschert J, et al. EORTC Late Effects Working Group. Late effects toxicity scoring: the SOMA scale. Radiother Oncol 1995;35:11-5.
19. Whelan TJ, Julian J, Wright J, et al. Does locoregional radiation therapy improve survival in breast cancer? A meta-analysis. J Clin Oncol 2000;18:1220-9.
20. Vinh-Hung V, Verschraegen C. Breast-conserving surgery with or without radiotherapy: pooled-analysis for risks of ipsilateral breast tumor recurrence and mortality. J Natl Cancer Inst 2004;96:115-21.
21. Clarke M, Collins R, Darby S, et al. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;366:2087-106.
22. Vaidya JS, Wenz F, Bulsara M, et al. Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: 5-year results for local control and overall survival from the TARGIT-A randomised trial. Lancet 2014;383:603-13.
23. Veronesi U, Orecchia R, Maisonneuve P, et al. Intraoperative radiotherapy versus external radiotherapy for early breast cancer (ELIOT): a randomised controlled equivalence trial. Lancet Oncol 2013;14:1269-77.
24. Whelan T, MacKenzie R, Julian J, et al. Randomized trial of breast irradiation schedules after lumpectomy for women with lymph node-negative breast cancer. J Natl Cancer Inst 2002;94:1143-50.
25. Whelan TJ, Pignol JP, Levine MN, et al. Long-term results of hypofractionated radiation therapy for breast cancer. N Engl J Med 2010;362:513-20.
26. START Trialists' Group, Bentzen SM, Agrawal RK, et al. The UK Standardisation of Breast Radiotherapy (START) Trial B of radiotherapy hypofractionation for treatment of early breast cancer: a randomised trial. Lancet 2008;371:1098-107.
27. Calvo FA, Meirino RM, Orecchia R. Intraoperative

radiation therapy first part: rationale and techniques. Crit Rev Oncol Hematol 2006;59:106-15.
28. Herskind C, Griebel J, Kraus-Tiefenbacher U, et al. Sphere of equivalence--a novel target volume concept for intraoperative radiotherapy using low-energy X rays. Int J Radiat Oncol Biol Phys 2008;72:1575-81.
29. Aziz MH, Schneider F, Clausen S, et al. Can the risk of secondary cancer induction after breast conserving therapy be reduced using intraoperative radiotherapy (IORT) with low-energy x-rays? Radiat Oncol 2011;6:174.
30. Toscas JI, Linero D, Rubio I, et al. Boosting the tumor bed from deep-seated tumors in early-stage breast cancer: a planning study between electron, photon, and proton beams. Radiother Oncol 2010;96:192-8.
31. Verbanck S, Hanon S, Schuermans D, et al. Small airways function in breast cancer patients before and after radiotherapy. Breast Cancer Res Treat 2012;135:857-65.
32. Welzel G, Boch A, Sperk E, et al. Radiation-related quality of life parameters after targeted intraoperative radiotherapy versus whole breast radiotherapy in patients with breast cancer: results from the randomized phase III trial TARGIT-A. Radiat Oncol 2013;8:9.
33. Tuschy B, Berlit S, Nasterlack C, et al. Intraoperative radiotherapy of early breast cancer using low-kilovoltage x-rays-reasons for omission of planned intraoperative irradiation. Breast J 2013;19:325-8.
34. Horst KC, Fero KE, Ikeda DM, et al. Defining an optimal role for breast magnetic resonance imaging when evaluating patients otherwise eligible for accelerated partial breast irradiation. Radiother Oncol 2013;108:220-5.
35. Wenz F, Welzel G, Blank E, et al. Intraoperative radiotherapy as a boost during breast-conserving surgery using low-kilovoltage X-rays: the first 5 years of experience with a novel approach. Int J Radiat Oncol Biol Phys 2010;77:1309-14.
36. Sperk E, Welzel G, Keller A, et al. Late radiation toxicity after intraoperative radiotherapy (IORT) for breast cancer: results from the randomized phase III trial TARGIT A. Breast Cancer Res Treat 2012;135:253-60.
37. Azria D, Lemanski C. Intraoperative radiotherapy for breast cancer. Lancet 2014;383:578-81.

Cite this article as: Vinh-Hung V, Nepote V, Rozenholc A, Vees H, Monnier S, Castiglione-Gertsch M, Fargier-Bochaton O, Popowski Y, Rouzaud M, Petignat P, Nouet P, Dubouloz A, Miralbell R. First year experience with IORT for breast cancer at the Geneva University Hospitals. Transl Cancer Res 2014;3(1):65-73. doi: 10.3978/j.issn.2218-676X.2014.01.07

Radiotherapy

CyberKnife stereotactic body radiotherapy and CyberKnife accelerated partial breast irradiation for the treatment of early breast cancer

Sandra S. Vermeulen[1], Jonathan A. Haas[2]

[1]Radiation Oncology, Executive Director - Swedish Radiosurgery Center, Swedish Medical Center, Seattle Washington, USA; [2]Radiation Oncologist, Chief of Radiation Oncology, Winthrop-University Hospital, Mineola, New York, USA

Correspondence to: Sandra Vermeulen, MD. Executive Director, Swedish Radiosurgery Center, 550 17th Ave, A-10 Seattle, WA 98122, USA. Email: Sandra.Vermeulen@swedish.org.

Background: Stereotactic body radiotherapy (SBRT) and accelerated partial breast irradiation (APBI) delivered using the CyberKnife radiosurgery system allows coverage of the lumpectomy cavity comparable to brachytherapy without being invasive. Here we review our combined experience treating 46 stage I post-lumpectomy patients with this approach.

Methods: Twenty-one patients at the Swedish Medical Center in Seattle were treated with total doses ranging from 25-36 Gy delivered in 5 to 10 equal fractions. Twenty-six patients at Winthrop University Hospital were treated with 30 Gy in 5 equal fractions. Margin and isodose schemes differed between sites, but were chosen to assure lumpectomy cavity coverage, including a margin to account for potential microscopic disease and a small margin to account for residual uncertainty, and low doses to organs at risk. Patient setup methods varied between sites but were devised to assure reproducibility and optimal beam delivery angles. Radiation was delivered while tracking and correcting for respiratory motion with the Synchrony respiratory motion management system.

Results: Mean follow-up was 31 months (range, 6-57 months) at Swedish and median follow-up was 22 months (range, 7-39 months) at Winthrop. Local control was obtained and continues in all patients. One patient reported minor pain at the lumpectomy site 10 months post-treatment, a second had palpable, non-painful firmness at the lumpectomy site, and a single patient showed Grade 1 dry skin desquamation. No serious toxicity has been observed. The cosmesis was good-excellent in all 46 patients using the Harvard cosmesis scale.

Conclusions: CyberKnife SBRT/APBI appears safe with low toxicity and excellent short-term local control. Centers interested in CyberKnife SBRT/APBI for their patients should consider treating on protocol in Investigational Review Board-approved studies, or at least according to the American Society for Radiation Oncology (ASTRO) eligibility guidelines for women with early-stage breast cancer.

Keywords: Accelerated partial breast irradiation (APBI); breast conserving therapy (BCT); cosmesis; CyberKnife; quality of life (QOL); stereotactic body radiotherapy (SBRT); whole breast radiation therapy (WBRT)

Submitted Mar 18, 2014. Accepted for publication Jul 08, 2014.
doi: 10.3978/j.issn.2218-676X.2014.07.06
View this article at: http://dx.doi.org/10.3978/j.issn.2218-676X.2014.07.06

Introduction

Phase I and II studies and some preliminary Phase III studies have challenged the standard of care [fully fractionated post-lumpectomy whole breast radiation therapy (WBRT)] for patients with early-stage breast cancer by delivering radiation to a restricted breast volume in fewer (i.e., 10 *vs.* 25) high-dose fractions, a technique known as accelerated partial breast irradiation (APBI) (1-3). Unlike WBRT, APBI limits the radiation to the region around the tumor bed in the hopes of reducing toxicity while maintaining equivalent cancer control rates. A more extreme form of APBI, stereotactic body radiotherapy (SBRT), aims to complete treatment in as few as five sessions. Here we describe, in a single report, our independent experiences using the CyberKnife System (Accuray Incorporated, Sunnyvale, CA, USA) for the delivery of APBI and SBRT to patients undergoing breast-conserving therapy.

The pathological argument for APBI

Poorer resolution mammography, non-universal pathologic margin standards, elementary radiation equipment and a naive bias toward a belief that cancer spreads broadly through the breast understandably resulted in post-lumpectomy WBRT becoming the early standard of care in breast conservation therapy (4). However, published data documents that 90% of breast cancer recurrences in women with early stage disease (stage 0-III) treated with lumpectomy with clear 2 mm or greater margins occur within 10 mm of the resection cavity (5-9). Others have shown 65-100% of breast cancer recurrences after conservative surgery and WBRT are in the same quadrant as the initial tumor and have the same histology as the primary tumor (10-12). Even without adjuvant radiotherapy, recurrence is located within the region of the tumor bed in the vast majority of cases (4,13-15). Because whole breast irradiation is not without side effects (16), radiation oncologists now question if it is necessary to treat the entire breast following a lumpectomy in all cases. Since side effects are related to fraction size and volume of normal tissue irradiated, reducing the volume is postulated to lower the risks. Also, by reducing the volume of normal tissue included within the radiation treatment field, the dose per fraction can be higher and overall treatment times reduced. Indeed, current APBI is commonly delivered in 5-10 fractions over 1-2 weeks.

APBI techniques

Interstitial multi-catheter brachytherapy

The oldest APBI technique, with the most published experience, is interstitial multi-catheter brachytherapy. Excellent control rates and acceptable toxicities are well documented with multi-catheter brachytherapy (3,8). Unfortunately, the procedure is invasive, carries the risk of infection and, similar to other multi-catheter brachytherapy techniques, is complex to perform. MammoSite (Proxima Therapeutics, Inc., Alpharetta, GA, USA) brachytherapy is a more user-friendly technique in which a single balloon is placed in the lumpectomy cavity. Although many have described the procedure as more comfortable for the patient compared to the multi-catheter approach, the balloon may not fit an irregularly shaped cavity or cannot be used if its placement is too close to the skin or chest wall. In addition, the catheter entry point is a source for infection requiring prophylactic antibiotics. On the other hand, a report from the American Society of Breast Surgeons MammoSite Breast Brachytherapy Registry Trial reports a 91% good-to-excellent cosmetic result at a mean follow-up of 54 months in the treatment of 1,449 women with early breast cancer (17).

Intra-operative radiotherapy (IORT)

IORT is an elegant and efficient treatment approach to APBI, delivered at the time of the lumpectomy. The main criticism of this technique is that the final pathologic review of the specimen occurs a day or more after the treatment has been delivered prohibiting the re-excision in patients with a positive surgical margin. Nevertheless, IORT has been delivered to more than 5,000 patients in the TARGIT-A trial and in the Eliot Trial. Veronesi *et al.* (18) reported the outcomes of 1,822 patients who underwent breast conservation surgery and IORT. At 36 months mean follow-up, the local recurrence was 2.3%, local liponecrosis toxicity 4.2% and fibrosis 1.8%.

External beam techniques

Three-dimensional conformal radiotherapy (3D-CRT) and intensity-modulated radiotherapy (IMRT) have gained popularity for early breast cancer patients seeking APBI. Both techniques are available at most radiation facilities and, unlike the brachytherapy modalities, are non-invasive. The disadvantage, however, is that the delivery of the beam is not as accurate. To compensate for the set-up uncertainty

Table 1 Patient and tumor characteristics for all patients

	Swedish	Winthrop
Mean age, years [range]	58 [46-82]	68 [48-85]
Tumor type	DCIS: 8 patients; IDC: 13 patients	DCIS: 13; IDC: 13
Tumor TMN stage	Tis: 8 patients; T1a: 1 patients; T1b: 5 patients; T1c: 7 patients	Tis: 13; T1a: 4; T1b: 5; T1c: 4
Mean tumor diameter (range), cm	DCIS: 1.6 (0.8-2.2); IDC: 1.2 (0.8-1.8)	DCIS: 1 (0.1-1.8); IDC: 0.975 (0.2-2.0)
Side	Right: 10; left: 11	Right: 18; left: 8
Quadrant	UOQ: 4; C: 8; LIQ: 2; UIQ: 5; LOQ: 2	UOQ: 8; C: 8; LIQ:2; UIQ: 2; LOQ: 6
Nodal stage	8 DCIS NX; 13 IDC N0	13 DCIS NX; 13 IDC N0
ER positive	8 DCIS; 13 IDC	4 DCIS; 13 IDC

DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; UOQ, upper outer quadrant; C, central quadrant; LIQ, lower inner quadrant; LOQ, lower outer quadrant; NX, node(s) not sampled; N0, node(s) sampled were negative; ER, estrogen receptor.

and respiratory motion during treatment, a larger margin to cover the 10-mm minimum risk area surrounding the cavity is required. Unfortunately this margin can result in greater coverage of normal structures such as the lung, chest wall and skin, and the heart particularly for left-sided lesions. Indeed, recent publications have shown greater toxicities with unacceptable cosmesis in women who elected a 3D-CRT or IMRT, APBI approach (19,20). In 5-year follow-up from a single-institution trial, Liss *et al*. reported a long-term rate of fair-to-poor cosmesis of 26.7% (21).

Disease control for APBI is promising. A recent study reported 5-year follow-up of patients stratified by risk according to the criteria established by the National Surgical Adjuvant Breast And Bowel Project (NSABP) B39/ Radiation Therapy Oncology Group (RTOG) 0413 trial (in which women with early breast cancer are randomized to WBRT *vs.* APBI). In this study patients were treated with either MammoSite or multi-catheter HDR brachytherapy. No significant differences in tumor control rate (97.8% *vs.* 93.6%) or overall survival (92.1% *vs.* 89.5%) between low and high risk groups were obtained (22).

Stereotactic body radiotherapy and APBI

SBRT brings together the potential benefits of breast brachytherapy APBI with the non-invasiveness of external beam radiation therapy. SBRT delivers a highly conformal dose that mimics the dosimetry of a breast brachytherapy implant. The CyberKnife is a frameless robotic stereotactic radiosurgery system which provides image-guidance for continuous tracking of respiratory target motion and automatic correction of beam aim in real-time as the patient breathes. This results in dose placement accuracy to within about a millimeter for moving targets (23), which allows uncertainty margins to be very narrow, thus making it easier to keep doses to organs at risk low. In a treatment planning study researchers at the University of Texas Southwestern Medical compared CyberKnife SBRT, APBI and 3D-CRT treatment plans. They noted that the SBRT, APBI treatment plans achieved highly conformal target coverage and reduced the dose to nearby organs at risk relative to 3D-CRT plans (24). At Fox Chase Cancer Institute, a similar treatment planning comparison concluded that the CyberKnife's more conformal dose could result in reduced toxicity by a reduction in dose to surrounding breast tissue (25) and patient movement including respiration (26).

CyberKnife APBI/SBRT: treatment methods

Twenty-one patients at Swedish Medical Center (Swedish) and 26 at Winthrop University Hospital (Winthrop) were treated. Two Swedish patients were treated in a 5-fraction regimen, but due to insurance limitations most patients were treated using a 10-fraction APBI protocol. Winthrop patients were treated with 5-fraction SBRT as part of an IRB-approved protocol. Patient selection criteria closely followed the American Society for Radiation Oncology (ASTRO) consensus statement for "suitable" or "cautionary" candidates (27). Women older than 45 years of age with Tis, T0, T1, T2 non-lobular carcinomas less than 3 cm, with negative margins (>2 mm) and lymph nodes, were eligible (*Table 1*). APBI was initiated within 9 weeks of the patient's last breast cancer surgery.

Fiducial implantation

At Swedish 4-5 gold fiducials were placed in the walls of the cavity at the time of the lumpectomy to allow CyberKnife tracking of respiratory motion. For 25 Winthrop patients fiducial markers were placed by the treating radiation oncologist under image guidance on a CT simulator with coordinate placement determined by the physics/dosimetry staff for optimal location. One patient had fiducial markers placed by the surgeon.

Treatment planning, immobilization

At Swedish non-contrast computed tomographic (CT) scans (1.0-mm slice thickness) were acquired with the patient wearing a support bra and placed in an alpha cradle with arms at her side supported below the chest. The CT images started at the mandible and extended several centimeters below the inframammary fold. Non-contrast magnetic resonance images (MRI) were fused to CT when the lumpectomy cavity was ill-defined on CT due to the adjacent breast tissue density or artifact scatter from the fiducials. The lumpectomy cavity was best delineated on the T2 axial or STIR MRI images. The fiducials were seen on the 2dT2 (STAR) sequence and used to verify the correctness of the fusion with the CT. At Winthrop similar practices were followed except patients were immobilized either using a thermoplastic cast across the chest with a hole removed around the areola to facilitate repositioning, or in an alpha cradle with the breast in its natural position. At Winthrop treatment planning was based on CT imaging only.

Treatment volumes, dose and fractionation

The clinical target volume (CTV) was defined as the lumpectomy cavity plus 15 mm. The planning target volume (PTV) was defined as the CTV plus a 2-mm margin while ensuring a 5-mm sparing distance from the skin and chest wall. Also, a field within a field was created to force the dose maximum into the lumpectomy cavity. The 2-mm CTV margin was added to accommodate for the possible tracking error of the fiducials. No additional volumes were considered necessary to account for variability in day-to-day set-up or patient mobility.

At Swedish, the first two patients were treated with an SBRT regimen of 5 fractions of 5 Gy each. Difficulty securing insurance for SBRT forced adoption of a 10-fraction APBI approach. Patients initially received 34 Gy in 10 fractions delivered to the PTV, prescribed to the 65-75% isodose. After 12 patients were treated without toxicity, the peripheral dose was increased to 36 Gy in 10 fractions. One patient's overall treatment time was decreased to 6 fractions because of co-morbidities and a difficult commute to the center. The dose at the cavity wall was 38.5 Gy or greater. Treatment was typically performed twice daily, although when scheduling conflicts arose we extended the treatment time but ensured its completion within 2 weeks. At Winthrop all patients were treated under an SBRT protocol delivering 30 Gy in 5 equal, 6-Gy fractions to a median prescription isodose of 71%. These isodoses were chosen to allow for a more rapid fall-off of dose beyond the target volume, thus more closely emulating HDR brachytherapy treatment. Treatment times averaged 46 min, ranging from about 36 to 55 min.

The dose constraints at both sites were based upon the NSABP/RTOG protocol (*Table 2*). For very medial inner quadrant or lower inner quadrant lesions, acceptance of a higher dose point, not volume, was allowed for the contralateral breast, the heart and lung. The volumes allowable for these structures were well below the acceptable limits by one third to one half. As an example, the largest contralateral breast point in our series was 8 Gy. The volume of the breast that received 0.5 Gy, however, was only 1.5%.

In addition to examination of dosimetry, acute and late toxicity, and disease control, cosmesis was judged using the Harvard cosmesis scale at multiple time points post-treatment. An excellent outcome was defined as "*minimal or no difference*" in appearance and good cosmesis was defined as "*a slight difference*". Fair or poor cosmesis defined as "*obvious differences...involving a quarter or less of the breast*" or "*as marked change involving more than a quarter of the breast tissue*".

Results

Swedish

The mean PTV for the whole group was 114 cm^3 (range, 39-241 cm^3) and mean percent isodose prescription line was 70% (range, 65-76%). The mean percent of the whole breast reference volume receiving 100% and 50% of the dose (V100 and V50) was 12% (range, 7-17%) and 26% (range, 16-39%), respectively. Treatment plans generally met dose constraints, although in a few cases upper ranges exceeded some constraints [see *Table 2*; for a fuller account of APBI dosimetry see (28)]. Dosimetry for the patients at Winthrop (not shown) did not differ substantially from that

Table 2 Dose limitations for normal tissue based on the NSABP/RTOG protocol and for patients treated at Swedish cancer institute with CyberKnife APBI to a dose of 34-36 Gy delivered in 10 fractions (n=16)

NSABP/RTOG structure	Constraint (3D-CRT)	CyberKnife treatment (mean, range)
Ipsilateral breast	V34 <35%; V17 <60%	Volume: 12%, 7-17%; volume: 26%, 16-39%
Contralateral breast	Dmax <1 Gy	Max dose: 1 Gy, 0.04-8 Gy
Ipsilateral lung	V10 <15%	Volume: 3%, 0-12%
Contralateral lung	V1.7 <15%	Volume: 4%, 0-19%
Heart (RT breast)	V1.7 <5%	Volume: 5%, 0-19%
Heart (LT breast)	V1.7 <40%	Volume: 10%, 0-54%
Thyroid	Dmax <1 Gy	Max dose: <1 Gy, 0-0.6 Gy
Skin	Dmax <49.3 Gy	Max dose: 37 Gy, 27-44 Gy
Chest wall	Dmax <40.8 Gy	Max dose: 35 Gy, 29-41 Gy

APBI, accelerated partial breast irradiation; NSABP, National Surgical Adjuvant Breast And Bowel Project; RTOG, Radiation Therapy Oncology Group; 3D-CRT, three-dimensional conformal radiotherapy.

depicted in *Table 2*. The beam number mean was 151 (range, 95-250). Two patients not counted among the 21 treated were simulated but not treated. One had an enlarging seroma that twice altered the positions of the fiducials from the planning CT. The second patient had poor breast integrity which also resulted in changes in fiducial position. Both patients were sent for whole breast irradiation.

At a mean follow-up of 31 months (range, 6-57 months), no breast cancer recurrence has been identified. Acutely, minimal erythema involving a small portion of the breast was reported by two patients and minimal fatigue was observed by half of the patients treated. No treatment was given for these acute toxicities which subsided by 2 and 3 weeks respectively. One patient had minor pain at the lumpectomy site at 10 months since treatment. One patient has palpable non-painful firmness at the lumpectomy site but the shape of the breast was excellent and skin fibrosis minimal. The size, shape and texture of a patient's treated breast was compared to the breast's original appearance after surgery and from pictures taken at the time of simulation. Cosmetic outcome were excellent or good in all 21 patients treated.

Winthrop

The mean PTV for the whole group was 113 cm^3 (range, 25-274 cm^3). The mean percent of the of the whole breast reference volume receiving 100% and 50% of the dose (V100 and V50) was 14% and 29%, respectively. The median number of beams was 122 (range, 89-187).

With a median follow-up of 21 months (range, 7-39 months) all 26 patients (100%) remain locally controlled with no evidence of disease following treatment. Acutely, RTOG Grade 1 dry skin desquamation occurred in 1 of 25 patients. The cosmesis was good-excellent in all 25 patients using the Harvard cosmesis scale. *Figures 1-3* show examples of maintained breast cosmesis.

Discussion

Based on these preliminary results we are optimistic that with stereotactic tracking ability and a low prescription isodose, issues involving patient motion, set-up reproducibility and toxicity are of less concern with CyberKnife APBI than for patients receiving 3D-CRT. Indeed, the PTV is similar to that seen in patients treated with multi-catheter or balloon catheter brachytherapy. The mean ipsilateral breast volumes receiving 100% and 50% of the prescribed dose were less than half that allowable in the NSABP/RTOG study. Without any observable acute side effects and excellent/good cosmetic outcomes, and the fact that normal tissue constraints are easily met, we conclude that the CyberKnife provides a suitable non-invasive approach for delivering APBI for women with early breast cancer.

Disadvantages of this approach include the need for fiducial-based tracking. The cooperation of lumpectomy surgeons or straightforward fiducial implantation procedures can lessen the difficulty this poses for physicians and patients. The fiducials array must also stray minimally from their positions during planning CT scanning to allow accurate tracking in all six dimensions, which

Figure 1 Excellent breast cosmesis at 12 months post-SBRT. SBRT, stereotactic body radiotherapy.

Figure 2 Excellent breast cosmesis at 13 months post-SBRT. SBRT, stereotactic body radiotherapy.

Figure 3 Very good breast cosmesis at 7 months post-SBRT. Note this woman's pacemaker in her upper chest, which did not have to be relocated during CyberKnife SBRT. SBRT, stereotactic body radiotherapy.

puts a premium on effective implantation and patient setup procedures. It also requires that changes in breast morphology during treatment be minimal, which can usually be achieved given the short treatment times, but note again the unusual circumstances with the patient from Swedish. In addition, treatment session times are considerably longer than those required for conventionally fractionated WBRT. This is usually not a difficult tradeoff for patients, however, as 5-10 sessions are generally much more convenient than 25. Although at Swedish we were compelled to use a 10-fraction APBI approach, we believe that 5-fraction SBRT with the CyberKnife is feasible and is likely to be a highly convenient, effective adjuvant to lumpectomy with low toxicity and very good to excellent cosmetic results. Still, long-term follow-up from well-controlled prospective studies is required to make strong claims about the value of the approach. In addition, as is clear from this report, sites evaluating APBI/SBRT with the CyberKnife are developing different treatment planning methods, doses and fractionation, and workflows; some attention to optimizing practices would be necessary to develop multi-institutional trials.

Conclusions

CyberKnife SBRT/APBI is currently under investigation at many centers for the treatment of early breast cancer. SBRT/APBI offers patients radiation treatment in a much shorter time than WBRT and without the invasiveness of a brachytherapy implant. In-breast tumor recurrence is the primary endpoint of SBRT/APBI studies. Quality of life (QOL) endpoints are also measured and include cosmesis, fatigue, breast-related symptoms and perceived convenience of care. Continued follow-up is needed to confirm that SBRT/APBI goals measured in these ways are met. As a result, all centers considering CyberKnife SBRT/APBI for their patients are encouraged to submit to national or Investigational Review Board-approved studies. Off-study patients should be treated according to the ASTRO eligibility guidelines published in 2009 for women considering ABPI for early breast cancer.

Acknowledgements

The authors thank David Schaal, PhD, of Accuray Incorporated, for helpful comments and Astrid Sanchez for accurate and timely data management on the manuscript.

Footnote

Dr. Vermeulen has no conflicts of interest; Dr. Haas has received speaker's honoraria from Accuray Incorporated.

References

1. Formenti SC, Truong MT, Goldberg JD, et al. Prone accelerated partial breast irradiation after breast-conserving surgery: Preliminary clinical results and dose–volume hist analysis ogram. Int J Radiat Oncol Biol Phys 2004;60:493-504.
2. Keisch M, Vicini F, Kuske RR, et al. Initial clinical experience with the MammoSite breast brachytherapy applicator in women with early-stage breast cancer treated with breast-conserving therapy. Int J Radiat Oncol Biol Phys 2003;55:289-93.
3. Vicini FA, Baglan KL, Kestin LL, et al. Accelerated treatment of breast cancer. J Clin Oncol 2001;19:1993-2001.
4. Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. N Engl J Med 2002;347:1233-41.
5. Clark RM, McCulloch PB, Levine MN, et al. Randomized clinical trial to assess the effectiveness of breast irradiation following lumpectomy and axillary dissection for node-negative breast cancer. J Natl Cancer Inst 1992;84:683-9.
6. Holland R, Connolly JL, Gelman R, et al. The presence of an extensive intraductal component following a limited excision correlates with prominent residual disease in the remainder of the breast. J Clin Oncol 1990;8:113-8.
7. Rosen PP, Fracchia AA, Urban JA, et al. "Residual" mammary carcinoma following simulated partial mastectomy. Cancer 1975;35:739-47.
8. Vicini FA, Kestin L, Chen P, et al. Limited-field radiation therapy in the management of early-stage breast cancer. J Natl Cancer Inst 2003;95:1205-10.
9. Vicini FA, Kestin LL, Goldstein NS. Defining the clinical target volume for patients with early-stage breast cancer treated with lumpectomy and accelerated partial breast irradiation: a pathologic analysis. Int J Radiat Oncol Biol Phys 2004;60:722-30.
10. Fisher ER, Sass R, Fisher B, et al. Pathologic findings from the National Surgical Adjuvant Breast Project (protocol 6). II. Relation of local breast recurrence to multicentricity. Cancer 1986;57:1717-24.
11. Fowble B, Solin LJ, Schultz DJ, et al. Breast recurrence following conservative surgery and radiation: patterns of failure, prognosis, and pathologic findings from mastectomy specimens with implications for treatment. Int J Radiat Oncol Biol Phys 1990;19:833-42.
12. Liljegren G, Holmberg L, Adami HO, et al. Sector resection with or without postoperative radiotherapy for stage I breast cancer: five-year results of a randomized trial. Uppsala-Orebro Breast Cancer Study Group. J Natl Cancer Inst 1994;86:717-22.
13. Fisher B, Bryant J, Dignam JJ, et al. Tamoxifen, radiation therapy, or both for prevention of ipsilateral breast tumor recurrence after lumpectomy in women with invasive breast cancers of one centimeter or less. J Clin Oncol 2002;20:4141-9.
14. Hughes KS, Schnaper LA, Berry D, et al. Lumpectomy plus Tamoxifen with or without Irradiation in Women 70 Years of Age or Older with Early Breast Cancer. N Engl J Med 2004;351:971-977
15. Veronesi U, Luini A, Del Vecchio M, et al. Radiotherapy after breast-preserving surgery in women with localized cancer of the breast. N Engl J Med 1993;328:1587-91。
16. Effects of radiotherapy and surgery in early breast cancer. An overview of the randomized trials. Early Breast Cancer Trialists' Collaborative Group. N Engl J Med 1995;333:1444-55.
17. Vicini F, Beitsch P, Quiet C, et al. Five-year analysis of treatment efficacy and cosmesis by the American Society of Breast Surgeons MammoSite Breast Brachytherapy Registry Trial in patients treated with accelerated partial breast irradiation. Int J Radiat Oncol Biol Phys 2011;79:808-17.
18. Veronesi U, Orecchia R, Luini A, et al. Intraoperative radiotherapy during breast conserving surgery: a study on 1,822 cases treated with electrons. Breast Cancer Res Treat 2010;124:141-51.
19. Jagsi R, Ben-David MA, Moran JM, et al. Unacceptable cosmesis in a protocol investigating intensity-modulated radiotherapy with active breathing control for accelerated partial-breast irradiation. Int J Radiat Oncol Biol Phys 2010;76:71-8.
20. Hepel JT, Tokita M, MacAusland SG, et al. Toxicity of three-dimensional conformal radiotherapy for accelerated partial breast irradiation. Int J Radiat Oncol Biol Phys 2009;75:1290-6.
21. Liss AL, Ben-David MA, Jagsi R, et al. Decline of cosmetic outcomes following accelerated partial breast irradiation using intensity modulated radiation therapy: results of a single-institution prospective clinical trial. Int J Radiat Oncol Biol Phys 2014;89:96-102.

22. Patel RR, Christensen ME, Hodge CW, et al. Clinical outcome analysis in "high-risk" versus "low-risk" patients eligible for national surgical adjuvant breast and bowel B-39/radiation therapy oncology group 0413 trial: five-year results. Int J Radiat Oncol Biol Phys 2008;70:970-3.
23. Hoogeman M, Prévost JB, Nuyttens J, et al. Clinical accuracy of the respiratory tumor tracking system of the cyberknife: assessment by analysis of log files. Int J Radiat Oncol Biol Phys 2009;74:297-303.
24. Heinzerling JH, Ding C, Ramirez E, et al. Comparative cose-volume analysis for Cyberknife and 3D conformal partial breast irradiation treatment of early stage breast cancer. Int J Radiat Oncol Biol Phys 2010;78:S825-6.
25. Fan J, Hayes S, Freedman G, et al. Planning the breast boost: Dosimetric comparison of Cyberknife, photo mini tangents, IMRT and electron techniques. Int J Radiat Oncol Biol Phys 2010;78:S788-S789.
26. Kilby W, Dooley JR, Kuduvalli G, et al. The CyberKnife Robotic Radiosurgery System in 2010. Technol Cancer Res Treat 2010;9:433-52.
27. Smith BD, Arthur DW, Buchholz TA, et al. Accelerated partial breast irradiation consensus statement from the American Society for Radiation Oncology (ASTRO). Int J Radiat Oncol Biol Phys 2009;74:987-1001.
28. Vermeulen S, Cotrutz C, Morris A, et al. Accelerated Partial Breast Irradiation: Using the CyberKnife as the Radiation Delivery Platform in the Treatment of Early Breast Cancer. Front Oncol 2011;1:43.

Cite this article as: Vermeulen SS, Haas JA. CyberKnife stereotactic body radiotherapy and CyberKnife accelerated partial breast irradiation for the treatment of early breast cancer. Transl Cancer Res 2014;3(4):295-302. doi: 10.3978/j.issn.2218-676X.2014.07.06

Brest experience in intraoperative radiotherapy for breast cancer

Petra Miglierini[1], Stéphane Key[1], Pierre-François Dupré[2], Emmanuelle Le Fur[1], Omar Miranda[1], Anne-Sophie Lucia[1], Sarah Quillevere[1], Olivier Pradier[1]

[1]Department of Radiotherapy, [2]Department of Gynecology and Obstetrics, Regional University Hospital Morvan, 29 200 Brest, France

Correspondence to: Petra Miglierini, MD. Department of Radiotherapy, Institute of Oncology and Haematology, Regional University Hospital Morvan, 2 Avenue Foch, 29 200 Brest, France. Email: petra.miglierini@chu-brest.fr.

Background: Targeted intraoperative radiotherapy (TARGIT) of an early breast cancer is a novel and promising treatment approach. For carefully selected patients, it permits to omit external beam breast radiotherapy (EBRT) and thus reduce the treatment duration. Moreover it offers an excellent precision without risk of tumour site miss and normal tissue sparing. Various techniques of intraoperative radiotherapy (IORT) are available. The first technique being tested in randomised trial was the TARGIT using a device developed by clinical academics in collaboration with the industry, Intrabeam® system that consists of a small low-energy X-ray generator. Strict criteria for TARGIT eligibility need to be respected, i.e., age ≥45 years and invasive ductal carcinoma that is unifocal on conventional imaging ≤3.5 cm, without gross lymph node involvement.

Methods: Our inclusion criteria were significantly stricter than those of the TARGIT-A trial and we only included patients ≥55 years with unifocal ductal invasive carcinoma of grade 1 or 2, tumour size ≤2 cm (based upon clinical and ultrasound evaluation), significant expression of hormone receptors (≥10%), no ErbB-2 expression. Intrabeam® system has been established within Regional University Hospital in Brest on April 2011. Between Mai 2011 and September 2013, 74 female patients were scheduled for TARGIT of an early stage breast cancer. Patients submitted a breast-conserving surgery (BCS) and sentinel lymph node (SLN) search. In case of respect of inclusion criteria patients benefited of TARGIT using a spherical applicator of a diameter depending upon the tumour and breast size. A total dose of 20 Gy was prescribed to the surface of the applicator and delivered into the surgical cavity. If any unfavorable histological modification appeared in the final pathological examination, a further EBRT needed to be done with a dose of 46-50 Gy. Patients were evaluated clinically 3-4 weeks post-operatively and possible side effects were documented.

Results: Sixty five patients received TARGIT. For 66% of them, the TARGIT was the only radiation treatment. For 33% also a complementary EBRT was required and thus the TARGIT has replaced the boost, only. Among the first side effects observed induration of surgical bed, radiation dermatitis, seroma, and delayed healing were the most frequent ones that have appeared in 20%, 14%, 12.5%, and 12.5% of patients, respectively.

Conclusions: Intraoperative irradiation during BCS is a feasible and promising alternative to conventional external fractionated radiotherapy. Strict eligibility criteria need to be taken into account before TARGIT is proposed to the patients. At present, only women with an early-stage breast cancer with low risk of recurrence can be candidates for this treatment modality.

Keywords: Breast cancer; intraoperative radiotherapy (IORT); tolerance; side effects

Submitted Dec 20, 2013. Accepted for publication Mar 04, 2014.
doi: 10.3978/j.issn.2218-676X.2014.03.01
View this article at: http://www.thetcr.org/article/view/2368/2963

Introduction

Breast cancer is a leading cancer site and the first cause of death from cancer in women in the whole Europe. It represented the third most frequent cause of death from cancer in general population in 2012 (1). Appropriate treatment approaches are required according to every disease stage. Regardless of particular cases, main breast cancer management consists of breast-conserving surgery (BCS), adjuvant chemotherapy if indicated, and external beam radiotherapy (EBRT) of whole ipsilateral breast. These might be further associated with hormonal therapies depending upon tumour pathological characteristics.

Since almost 90% of local recurrences appear within the tumour bed, its complementary irradiation, i.e., boost, aims to reduce the risk of this relapse and became an inevitable part of the breast irradiation protocol in invasive breast carcinomas. This can be delivered simultaneously together with the whole breast EBRT or consecutively in 5 to 8 fractions of 2 Gy by reduced fields using photons or electrons.

Recently, numerous clinical research teams dealt with a possibility of a complementary boost delivery into the tumour bed during BCS in order to shorten the duration of subsequent radiotherapy. Another advantage of an targeted intraoperative radiotherapy (TARGIT) is to avoid a "geographic miss" of tumour site during later EBRT (2). For the purpose of TARGIT different techniques are available up today, e.g., linear accelerators, brachytherapy or mobile devices generating low-energy X-rays such as Intrabeam® system from Carl Zeiss Surgical company. This miniature X-ray source with 50 kV maximum has already been tested on large series of patients with very promising results in terms of recurrence rates, acute or late side effects, cosmetic outcomes, or quality of life (3-9). The Intrabeam® device permits acceleration of electrons that are forwarded towards a gold target and induce a formation of radiation field with an isotropic dose distribution. Thus, low-energy X-rays are generated and spread homogenously upon the whole surface of a spherical applicator which is particularly adapted for mammary gland treatment. Because of its steep dose fall-off it requires relatively modest precautions of radiation protection.

As it was demonstrated by Vaidya *et al*. (6), in some very carefully selected patients with early-stage breast cancer a restriction of radiation therapy exclusively to the tumour bed during surgery, i.e., TARGIT alone, should be considered as an alternative to EBRT delivered over several weeks.

An Intrabeam® system has been established at our Regional University Hospital in Brest on April 2011. At present, the irradiation using this device is performed in two main indications, breast cancer and vertebra bone metastasis. For the latter, TARGIT is followed by a kyphoplasty which is done at the same operation time. A trained staff consisting of three radiation oncologists, three surgeons (gynecologists), and three physicists performs breast intraoperative radiotherapy (IORT) treatments once or twice a week since May 2011.

In this paper, we describe our experience with this low-energy X-ray generator device in patients with early-stage breast cancer.

Methods

Between May 2011 and September 2013, 74 female patients were scheduled for breast TARGIT using a mobile device Intrabeam® (Carl Zeiss Surgical, Oberkochen, Germany) according to strict inclusion criteria. BCS as well as TARGIT were performed in a dedicated operation room with leaded walls although these are not essential. For this study in our center, we included only female menopausal patients 55-year old and more. Prior to BCS, tumour histological characteristics were evaluated on a microbiopsy piece. Our inclusion criteria were significantly stricter than those of the TARGIT-A trial and we only included patients with the following tumour characteristics: unifocal ductal invasive carcinoma of grade 1 or 2, tumour size ≤20 mm (based upon clinical or ultrasound evaluation), significant expression of hormone receptors (≥10%), no ErbB-2 expression. Sentinel lymph node (SLN) was inevitably explored during every BCS and we performed TARGIT IORT only if this was negative although this is not in the TARGIT-A trial protocol. The TARGIT exclusion criteria in this study in out center were the following: ductal invasive carcinoma with diffuse microcalcifications, multifocal or bilateral carcinoma, lobular invasive carcinoma, lymph node involvement, personal history of malignant disease and life expectancy <10 years, previous thoracic irradiation (m. Hodgkin), homolateral breast cancer or BRCA mutation.

Intraoperatively and prior to TARGIT, frozen section analysis of tumour piece was performed at the Department of Pathological Anatomy of the Regional University Hospital Brest for each patient and permitted to confirm the retention of TARGIT indication or not. Depending on initial tumour and breast size, the choice of spherical

applicator diameter was done by the surgeon. This could vary from 2 to 5 cm. A total dose of 20 Gy was prescribed to the surface of the applicator. Although this is no longer considered necessary, in our center, prior to positioning of the applicator itself, a small tungsten impregnated plaque is placed at the bottom of the surgical cavity in order to ensure a radiation protection of the ribs, lung, and heart.

Once the final pathological examination of tumour and SLN was achieved, the decision of further additional external breast irradiation or not was discussed at a regular multidisciplinary meeting. If the TARGIT inclusion criteria were still respected, no complementary EBRT was performed and TARGIT was considered as an exclusive breast treatment. On contrary, if at least one of the above mentioned criteria was not met, or intraductal component was >25%, or there was a lymphovascular invasion (LVI), or positive surgical margins, a whole breast EBRT was done at a dose of 46 to 50 in 2 Gy per fraction. Under these conditions, the TARGIT was considered as a boost only.

Demographic and histological parameters of eligible patients were reviewed retrospectively and collected within an Excel™ system (Microsoft Corporation, Redmond, Seattle, USA). The first clinical post-operative evaluation was done 3-4 weeks after the BCS. Cosmetic outcomes and potential post-treatment toxicities were evaluated without grading at this time. No statistical analysis was performed.

Results

Seventy four patients with an early-stage breast cancer were initially eligible for TARGIT. Mean age of these patients was 68.1 years (56-86 years). Mean chest diameter of all patients was 98 cm (85-119 cm) and mean body mass index value was of 25.7 (18.7-35).

Since one of the advantages of TARGIT is to spare time consuming and several weeks lasting classic EBRT of breast cancer we have evaluated the social situation of our patients as well. Eighteen percent of patients were still actively working at the moment of cancer diagnosis and treatment, 82% were already retired. Mean distance between patient's home and Regional University Hospital in Brest was 35 km (0-135 km).

Concerning tumour characteristics, 59% of them were located in a left breast and 41% in a right one. Regarding the tumour size, no clinically palpable tumour (T0), tumour inferior to 2 cm (T1) or tumour of 2-5 cm (T2) was found in 53%, 43%, and 4% of patients, respectively. No patient presented a clinically palpable lymph node at the moment of diagnosis. Histological grade was of 1 for 70% of tumours and of 2 for the rest 30%.

As for treatment procedure, mean time between the moment of diagnosis and treatment itself was 6 weeks and 3 days. The BCS lasted in average 2.5 hours while the mean duration of intraoperative irradiation was 28 minutes. In majority of cases (92% of patients) spherical applicators of 3-4 cm were used.

Finally, 65 out of 74 eligible patients could benefit of TARGIT. Nine patients did not receive the intraoperative treatment because of the following reasons: positive surgical margins despite the second reexcision, histological doubt in SLN, no SLN found, positive SLN, tumour not found in the piece, bifocal tumour, and one case of technical impossibility to recover the applicator by the mammary gland.

According to the final histological examination of tumour piece, in 66% of cases TARGIT was considered as an exclusive radiation treatment and in 33% of it needed an addition whole breast EBRT and, thus it was considered as a boost only. Twenty three percent of patients required EBRT because of unfavorable final histological profile, e.g., positive lymph nodes, higher histological grade, ErbB-2 overexpression. For 10% of these patients, the involved margins required a reexcision and subsequent EBRT. In the TARGIT-A trial, apart from positive margins, the other factors on their own, would not necessarily prompt the addition of EBRT.

Negative SLN was achieved in 77% of cases whereas it was positive in the remaining 23%. Micrometastases were found in 10.2%, macrometastases in another 10.2%, and 2.6% were of unknown status.

Presence of side effects was evaluated during the first post-operative medical visit 3-4 weeks after the BCS and TARGIT. Induration of the surgical bed, being the most frequent side effect, was present in 13 patients (20%). This was followed by radiation dermatitis in 9 patients (14%). In eight patients the wound was slightly inflamed and sensible even 4 weeks after the surgery. This phenomenon was assigned in our conditions as a delayed wound healing although a true dehiscence could be observed only in one patient. The approximate duration of the healing process in these 8 patients was 1.5-2 months.

Seroma within the surgical bed was seen in eight patients, from whom two presented simultaneously a delayed wound healing. Three patients suffered from a surgical site hematoma and one patient experienced an infection of the surgical bed.

Discussion

A decline in breast cancer mortality was reported in recent years as a consequence of the combined effects of earlier cancer detection and a range of improvements in its treatment (1). Post-operative radiotherapy of mammary gland is a recommended and inevitable approach in treatment of invasive and intraductal breast cancers. Because invasive carcinomas have a particular tendency to induce a recurrence at the initial tumour site, an additional boost targeting tumour bed is required. At present, this one can be delivered in several different radiation forms using either photons or electrons. The use of a mobile low-energy X-ray generator Intrabeam® presents a novel and promising technique. Its major advantage is the possibility of direct irradiation of tumour bed without a risk of target miss as it can happen during EBRT boost. Furthermore, intraoperative irradiation permits immediate treatment of surgical bed avoiding a delay between surgery and EBRT. In selected patients with favorable early stage breast cancer, the studies demonstrated non-inferiority of TARGIT to the conventional EBRT with respect to the local control, safety and cosmetic outcomes (6,10).

Early and late side effects of IORT using Intrabeam® system have already been evaluated by several research teams (3,5,11). Sperk *et al.* (5) observed that concerning late radiation toxicities in patients treated with IORT exclusively compared to external breast radiotherapy there were no significant differences in terms of fibrosis, breast edema, ulceration, hyperpigmantation, lymphedema or pain incidence. As for early complications, Tuschy *et al.* (3) noticed particularly the appearance of surgical bed induration, seroma, erythema of grade I and II, and mastitis in 24%, 17.3%, 13%, and 3.4% respectively. These results seem comparable to early side effects observed among the group of patients treated at our institution.

Regarding the local control rates, the first results were already published. Vaidya *et al.* (12) observed in their TARGIT-A trial that the 5-year risk of local recurrence in conserved breast was 3.3% for IORT versus 1.3% for EBRT. These outcomes may appear statistically significant but the P value of 0.04 was above the pre-defined P value of 0.01; also they were simultaneously acceptable in terms of the threshold of the pre-defined non-inferiority margin. The authors recommend the use of TARGIT during the initial lumpectomy rather than as a delayed procedure by reopening the wound. When used in this manner, the recurrence rates were 2.2% versus 1.2%, and the difference was not statistically significant. In addition, while breast cancer mortality was similar, non-breast cancer mortality was significantly reduced with TARGIT.

The recently published ELIOT study carried by Veronesi *et al.* (13) showed that the 5-year event rate for ipsilateral breast tumour recurrence (IBTR) was 4.4% for IORT with electrons and 0.4% for a whole-breast irradiation. Although the rate for IBTR in the IORT group was within the prespecified equivalence margins, the rate was significantly greater than with the EBRT. No difference in terms of the overall survival was found.

In our study, the late radiation complications incidence and local control rates are yet difficult to define because of a relatively short clinical experience so far.

The IORT during BCS is a promising alternative to the conventional external whole-breast irradiation in a carefully selected group of patients with early breast cancer.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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. Herskind C, Steil V, Kraus-Tiefenbacher U, et al. Radiobiological aspects of intraoperative radiotherapy (IORT) with isotropic low-energy X rays for early-stage breast cancer. Radiat Res 2005;163:208-15.
3. Tuschy B, Berlit S, Romero S, et al. Clinical aspects of intraoperative radiotherapy in early breast cancer: short-term complications after IORT in women treated with low energy x-rays. Radiat Oncol 2013;8:95.
4. Tuschy B, Berlit S, Romero S, et al. Influence of age on short-term complications after intraoperative radiotherapy in women after breast-conserving surgery. Anticancer Res 2013;33:3995-9.
5. Sperk E, Welzel G, Keller A, et al. Late radiation toxicity after intraoperative radiotherapy (IORT) for breast cancer: results from the randomized phase III trial TARGIT A.

Breast Cancer Res Treat 2012;135:253-60.
6. Vaidya JS, Joseph DJ, Tobias JS, et al. Targeted intraoperative radiotherapy versus whole breast radiotherapy for breast cancer (TARGIT-A trial): an international, prospective, randomised, non-inferiority phase 3 trial. Lancet 2010;376:91-102.
7. Vaidya JS, Baum M, Tobias JS, et al. The novel technique of delivering targeted intraoperative radiotherapy (Targit) for early breast cancer. Eur J Surg Oncol 2002;28:447-54.
8. Welzel G, Boch A, Sperk E, et al. Radiation-related quality of life parameters after targeted intraoperative radiotherapy versus whole breast radiotherapy in patients with breast cancer: results from the randomized phase III trial TARGIT-A. Radiat Oncol 2013;8:9.
9. Kraus-Tiefenbacher U, Scheda A, Steil V, et al. Intraoperative radiotherapy (IORT) for breast cancer using the Intrabeam system. Tumori 2005;91:339-45.
10. Ruano-Ravina A, Cantero-Muñoz P, Eraso Urién A. Efficacy and safety of intraoperative radiotherapy in breast cancer: a systematic review. Cancer Lett 2011;313:15-25.
11. Kraus-Tiefenbacher U, Bauer L, Scheda A, et al. Long-term toxicity of an intraoperative radiotherapy boost using low energy X-rays during breast-conserving surgery. Int J Radiat Oncol Biol Phys 2006;66:377-81.
12. Vaidya JS, Wenz F, Bulsara M, et al. Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: 5-year results for local control and overall survival from the TARGIT-A randomised trial. Lancet 2014;383:603-13.
13. Veronesi U, Orecchia R, Maisonneuve P, et al. Intraoperative radiotherapy versus external radiotherapy for early breast cancer (ELIOT): a randomised controlled equivalence trial. Lancet Oncol 2013;14:1269-77.

Cite this article as: Miglierini P, Key S, Dupré PF, Le Fur E, Miranda O, Lucia AS, Quillevere S, Pradier O. Brest experience in intraoperative radiotherapy for breast cancer. Transl Cancer Res 2014;3(2):175-179. doi: 10.3978/j.issn.2218-676X.2014.03.01

Intraoperative radiotherapy with electrons (ELIOT) for early breast cancer: the European Institute of Oncology experience

Roberto Orecchia[1,2], Maria Cristina Leonardi[1], Patrick Maisonneuve[3], Anna Morra[1], Roberta Lazzari[1], Federica Cattani[1], Veronica Dell'Acqua[1], Nicole Rotmensz[3], Giuseppe Viale[2,4], Alberto Luini[5], Paolo Veronesi[2,5], Viviana Galimberti[5], Stefano Zurrida[2,5], Oreste Gentilini[5], Mattia Intra[5], Umberto Veronesi[6]

[1]Division of Radiation Oncology, European Institute of Oncology, Milan, Italy; [2]University of Milan, Italy; [3]Division of Epidemiology and Biostatistics, [4]Division of Pathology, [5]Division of Breast Surgery, [6]Scientific Directorate, European Institute of Oncology, Milan, Italy

Correspondence to: Roberto Orecchia. Division of Radiotherapy-Advanced Radiation Centre, European Institute of Oncology, Via G. Ripamonti 435, 20141 Milan, Italy. Email: roberto.orecchia@ieo.it.

Abstract: Partial breast irradiation (PBI) as an alternative to whole breast irradiation (WBI) is an attractive approach tested in several phase II and a few phase III studies, using different modalities of irradiation. Intraoperative radiotherapy (RT) with electrons allows the reduction of a whole course of WBI to a single session during surgery. The European Institute of Oncology (Milan, Italy) conducted a series of investigations aimed proving the safety and the effectiveness of intraoperative RT as a full dose PBI. The single dose of 21 Gy was calculated to be theoretically equivalent to a full course of conventional WBI. This ultimate form of hypofractionation carried some concern regarding the long-term impact on breast parenchyma. Phase I and II studies and a number of off- protocol patients have shown feasibility and good short-term results in both disease control and cosmesis. The results of the ELIOT randomized phase III trial comparing intraoperative RT to conventional WBI are discussed in the context of a worldwide scenario including other four randomized studies and one metanalysis, so far. From the analysis of the available data, the adequate patient selection emerges as mandatory to make intraoperative RT and other PBI modalities a reasonable alternative to conventional WBI. Further investigations are required to fully understand the weight of clinical and histologic variables classically associated with increased risk of local recurrence. In addition, the role of molecular classification in the pattern of recurrence after PBI will be more and more predominant.

Keywords: Partial breast irradiation (PBI); intraoperative electrons; breast cancer (BC)

Submitted Dec 23, 2013. Accepted for publication Feb 17, 2014.
doi: 10.3978/j.issn.2218-676X.2014.02.04
View this article at: http://www.thetcr.org/article/view/2094/2759

Partial breast radiation therapy (PBI) as a component of breast conservation therapy is an emerging paradigm in the treatment of women with early breast cancer (BC) (1). Over the past two decades there has been a radical change in BC radiation. In the nineties almost all the women received whole breast irradiation (WBI), which was almost always performed with conventional fractionation. Nowadays, a number of radiation oncologists are delivering alternate breast radiotherapy (RT) strategies from WBI with hypofractionated schemes to PBI. In a survey (2) where physicians were asked how often they used the breast irradiation regimens, Balloon-PBI was the second most common irradiation technique after conventionally fractionated WBI, although this technique is not currently supported by clinical phase III trials. PBI is an attractive treatment approach that offers patients shortened overall treatment times and a potential decrease in the radiation dose delivered to non-target portions of the breast and adjacent tissues. Over the past decade, PBI has spread quickly, showing a 10-fold increase between 2002 and 2007 (3) and thousands of women are being currently treated with different modalities in clinic practice. At the 13th St Gallen expert consensus meeting, the majority of the Panel recognized the safety and efficacy of some forms of PBI in

selected patients, although the issue about the definition of a suitable group still exists (4). The NSABP B-39/RTOG 0413 trial, closed in April 2013, enrolled a large number of patients with estrogen negative status, 1 to 3 positive axillary lymph nodes and younger than 50 years old, which might clarify the appropriateness of PBI in this setting (5).

The rationale for PBI is based on the observation that the majority of local recurrences (LR) were close to the region of the primary tumor (6). Therefore, limiting the radiation target volume to the originally involved portion of the breast would achieve local control equivalent to WBI in selected cases. The ideal patients are the ones with low risk of harboring distant tumor cells or with distant tumor cells which remain dormant, because of the intrinsic indolent nature and/or the effect of systemic therapies. The previous studies on PBI failed to achieve acceptable local control because of the poor patient selection, the inadequate target definition and dose prescription. Over time, the eligibility criteria and the radiation technique have been refined, achieving annual LR rate lower than 1%, with best figures about 0.5% (7). So far, five phase III randomized trials and one metanalysis evaluating PBI have been published. In the first two randomized studies, the Christie Hospital (8) and the Yorkshire Breast Cancer Group trials (9), PBI showed poor local control, because both of them were inadequate to modern standard. Conversely, the Hungarian trial based its success on strict selection criteria, including only low-risk BC patients. At ten years follow-up no difference was found regarding LR and any survival endpoint (10). In the more recent Targit-A trial, where more than 80% of patients fell into the ASTRO suitable group, the preliminary results, after two years and a half, showed similar LR rate among PBI and WBI patients. After five years, LR in the intraoperative arm was greater than that in WBI arm (3.3% *vs.* 1.3%, P=0.042) (11). In the ELIOT trial (12), both true LR and new ipsilateral BC were significantly more common in the intraoperative RT arm than in WBI arm, while no differences in any survival endpoint was noticed. The correlation between PBI and an increased risk for both local and regional recurrence, without any impact on survival, was also outlined by the metanalysis (13). Different modalities of PBI have been used, each of them with their own advantages and drawbacks. Intraoperative RT with electrons with one single fraction of 21 Gy has the advantage of one short procedure that includes both surgery and RT at the same time. Extending the operation by few minutes (the whole procedure, in fact, from preparing the tumor bed to delivering the prescribed dose, takes not more than 15 minutes), avoids long treatment course and solves the practical question of travelling back and forth from the RT centre, which in some countries or circumstances might be an obstacle. In addition, intraoperative RT with electrons allows a great decrease in the radiation dose delivered to non-target tissues, since skin is moved away from the radiation field and ribs, lungs and heart are properly shielded. Furthermore, the intraoperative modality allows a precise delineation of the tumour bed, which is identified under visual control, avoiding any geographic miss. The development of this technique was made possible by the availability of new mobile linear accelerators, which are able to enter the operating theatre to administer the treatment. The Milan experience started in 1999 at the European Institute of Oncology (14).

After short phase I and II studies, a single dose of 21 Gy was selected. The technical details have been previously described (15).

The dose of 21 Gy, prescribed at the 90% isodose in a single fraction, was delivered immediately after the tumor removal, through a round Perspex applicator tube. The diameter of the collimator was chosen according to the site and the size of the tumor. The energy of the electron beams was selected according to the measured thickness of the reconstructed gland. To protect the underlying critical structures (ribs, lung, heart), an aluminum and lead disc was placed between the mammary gland and the superficial fascia of the major pectoral muscle.

From a radiobiological point of view, the treatment of 21 Gy in a single fraction was supposed to be equivalent to the conventional treatment of 60 Gy in 30 fractions, by using the linear quadratic equation. Assuming that the alpha-beta ratio of breast tumor cells and early side effects is equal to 10, giving a single-dose treatment of 21 Gy should result in the same local control and acute toxicity as conventionally fractionated doses of 65 Gy. Conversely, assuming that the alpha-beta ratio of breast tumor cells is equal to 4, 21 Gy in a single dose should be equivalent to 131 Gy in 2 Gy fractions. However, more severe side effects (such as fibrosis) in late responding tissues (which have alpha-beta ratios of 3 or lower) might be expected from the single-fraction treatment, since biologically equivalent dose higher than 168 Gy is achieved (16). Although the LQ-model seems not to fit well in a high dose per fraction region, at present, it remains the most reliable reference model (17). From a clinical point of view, IEO Phase I and II studies (14) have shown feasibility and good short-term results in both disease control and cosmesis. Out of

101 patients who took part in the dose escalation study, 16 patients (16%) developed breast fibrosis that was mild in 15 and severe in one, while two patients reported mild pain on the tumor bed, with a mean follow-up of 42 months. Patients who did not enter the phase III ELIOT trial, although being treated according to the same schedule of 21 Gy, were analyzed apart in a report (18) with a median follow-up of 36.1 months. Among them, 34 (1.9%) reported breast fibrosis, which was severe in two cases, and 14 (0.8%) experienced moderate skin retraction. The ELIOT phase III study, comparing the intraoperative PBI with conventional WBI, started in November 2000 and the accrual continued till December 2007 (12). At that time, the eligibility criteria considered as adequate for selecting patients for intraoperative treatment were based on simply clinical and tumor features: small tumors, up to 2.5 cm , clinically negative axillary nodes and age over 48. This age cut-off was set to include only women in peri- or postmenopausal status, for whom the risk of LR throughout the breast is considered lower than in young patients. A total of 1,305 BC patients were randomized before surgery in the study (654 in the conventional WBI arm and 651 in the intraoperative RT arm). Due to ineligibility after surgery or protocol violation, 119 patients were excluded and a total of 1,186 patients were available for analysis (601 in the conventional WBI arm and 585 in the intraoperative RT arm). The primary endpoint was the incidence of in-breast reappearances, including true local relapse (defined as any recurrence near the site of the primary tumor) and ipsilateral BC. The study was designed as an equivalent trial. The equivalence was based on the expected 5-year rate local relapses in the conventional arm of about 3% and in the intraoperative RT arm of no more than 7.5%.

Among the ELIOT phase III patients, acute side effects were limited with a statistically significant difference in favor of the intraoperative RT arm (P=0.0002), except for a higher incidence of fat necrosis. In particular, fewer skin side effects were observed in the intraoperative RT arm, compared to WBI arm, because of the skin sparing. No differences between the two arms were observed for mammary fibrosis, mammary retraction, pain or burning.

Based on these data, the expected toxicity seems not to be confirmed by clinical observations. However, as late morbidity can increase over time (19), the final assessment should be made after follow-up period longer than five years.

Regarding local control, among off-protocol patients at 36 months (18), a LR rate of 3.6% was observed, of which more than 60% were true recurrences, whereas the remaining was considered second ipsilateral cancers, occurring outside the index quadrant. This group of patients, excluded from the ELIOT trial because they did not fully satisfied the strict eligibility criteria, was at higher risk of failure compared to in protocol patients. In fact, the number of patients aged 50 or under, with tumor size larger than 2 cm, more than three positive lymph nodes, grade 3 and high Ki-67 was greater than in ELIOT trial patients. With an annual rate of in-breast reappearances of 1.21%, the cumulative incidence would achieve 6.05% at five years. Most of the factors deemed prognostic for LR are well-known. In univariate analysis, the risk of LR increased with the increase of tumour size, number of positive lymph nodes and proliferative index (Ki-67). In addition, the presence of LVI and HER2 over expression, the absence of ER/PR receptor status, and the young age confirmed to be risk factors. In multivariate analysis, age <50 and tumour size >2 cm remained independent predictors of local relapse.

Combined with increasing evidence that WBI improves long-term overall survival, BC experts have been striving to identify the proper eligibility criteria to safely select patients for PBI. Several consensus statements from different breast experts panels have been published. The most expansive recommendations were released in 2009 by the American Society for Radiation Oncology (ASTRO) (20), and in 2010 by the European Society for Radiation Oncology (GEC-ESTRO) (21). These recommendations outlined three patient groups, based on clinical and pathologic risk factors. Whether these guidelines optimally define the risk categories remain in question. Numerous studies have failed to find a correlation between risk stratification and rates of LR. A pooled analysis including more than two thousand patients, showed a similar 5-year rate of local, regional and distant failure between PBI and WBI patients categorized according the ASTRO groupings (22).

We are aware that these guidelines for PBI cannot be fully applied to intraoperative RT, since they are based mainly on histopathologic features, which are not entirely available at the time of delivering intraoperative irradiation. This is without doubt one of the greatest issues connected with intraoperative techniques, because the definitive pathologic report can show histologic or biomolecular features for which WBI would be the best choice. TARGIT-A trial included the possibility to complete the treatment by adding WBI, in case of critical pathological findings. However, some efforts to improve the pre-irradiation pathologic tumour evaluation can be made. Being able to rely on a good quality standard of

preoperative and intraoperative pathologic assessment, many of the tumour features requested by ASTRO and GEC-ESTRO recommendations might be satisfied. In fact, true-cut or core biopsy specimens and intraoperative frozen sections can show the type of histology, grading, hormonal receptor status, margin resection involvement and sentinel lymph node status. We applied the ASTRO and GEC-ESTRO recommendations for the use of PBI to off-protocol patients treated with intraoperative electrons, to evaluate the ability to predict clinical outcome (23,24).

ASTRO groupings observed stricter criteria compared to ESTRO and this difference affected the correct identification of the risk categorizes. The "suitable" or "good" candidates showed a very low rate of in-breast recurrences, which was 1.5% and 1.7% according to ASTRO and ESTRO, respectively. While both the consensus guidelines successfully pinpointed this subgroup of patients with low-risk of LR, there was no agreement in the identification of the higher risk subgroups. ASTRO, due to strict selection criteria, kept on detecting differences between the intermediate and high risk groups, (4.4% and 8.8%, respectively), while ESTRO, with looser selection criteria, failed to notice any differences between the groups (7.4% and 7.8%, respectively). In the ASTRO and ESTRO favourable groups, patients reported a low risk of LR both near and distant from the original tumor site. Conversely, in the more unfavorable groups, patients developed high LR rate both in the index quadrant and in the remaining breast. This finding may be the expression of a form of radioresistance and the presence of a great amount of distant tumor cells associated with more aggressive tumors.

In the ELIOT phase III trial (12), the majority of patients shared the same tumor features as the suitable ASTRO group. The two arms were perfectly balanced at baseline, except for a higher frequency of G1 tumors in the intraoperative RT arm. After median follow-up of 5.8 years for all patients, 35 in-breast reappearances, with a 5-year LR rate of 4.4%, were observed in the intraoperative RT arm compared to four cases, with a 5-year LR rate of 0.4%, in the conventional WBI arm (P=0.0001). Breaking down the in-breast reappearance incidence according to the site of recurrence, an excess of "true local relapses" was found in the intraoperative RT arm (21 cases, 2.5%) compared to the conventional WBI arm (4 cases, 0.4%) (P=0.0003). The occurrence of a new tumor in the ipsilateral breast, at a distance from the index quadrant, was observed only in the intraoperative RT arm, with 14 events (1.9%, P=0.0001). This finding supports the effect of WBI on preventing LR, already highlighted by some randomized studies (25). Therefore, in the intraoperative RT arm an excess of recurrences in the ipsilateral breast was detected, both in the index quadrant and in the other quadrants of the same breast compared to the conventional WBI arm. Interestingly, in the Hungarian study, the relapse rate in the arm with PBI was 5.5% at five years, which was similar to that recorded in the intraoperative RT arm of ELIOT study (26). In the latter one, the observed LR rate was within the prespecified equivalence margin of 7.5%, but it was significantly greater compared to that observed in the conventional WBI arm. Because of this great difference between the two arms, ELIOT phase III trial failed to demonstrate the equivalence.

An important point to emphasize is that, in spite of the increased LR incidence in the intraoperative RT arm, the 5-year overall survival was similar in the two arms (96.8% in the intraoperative RT arm and 96.9% in the WBI arm), with an equal number of distant metastases and deaths after a median follow-up of 5.7 years.

The analysis aimed at identifying characteristics associated with the rate of local relapse was restricted to patients treated with intraoperative RT, since the low number of recurrences in WBI arm prevented any further investigation. In multivariate analysis (12), tumor size greater than 2 cm (HR 2.24), ≥4 positive lymph nodes (HR 2.61), high grade tumor (HR 2.18) and triple negative subtype (HR 2.40) presented a significantly increased risk of in- breast reappearances. Patients receiving intraoperative RT with at least one of these high-risk factors had a significant increase in the 5-year LR risk, from 1.5% to 11.3%. Several studies have investigated the association of the molecular subtypes with rates of local recurrence, but the impact is still unclear. Some studies have shown that the basal or triple-negative and HER2+ subtypes are associated with an increased risk of LR (27). Among the ELIOT trial patients, molecular subtypes remain independent predictors of local relapse. In fact, compared to Luminal A patients, the other subtypes showed a significant increase in local recurrence rate.

A stratification of LR according to site of in-breast failure was carried out among the ELIOT out-trial patients (18). Patients in the Luminal A category had a very low risk of both true local relapse and new ipsilateral BC, luminal B and triple negative subtypes had higher incidence of LR in both the index quadrant and in the remaining breast, while for HER2+ patients the true recurrences were prevalent.

When we applied the ASTRO guidelines to patients

enrolled in the ELIOT phase III trial, the suitable patients according to ASTRO treated with intraoperative electrons presented a local relapse rate as low as those treated with WBI, whereas the cautionary and the unsuitable groups showed better local control when treated with WBI. It means that aggressive tumors have a larger amount of distant microscopic disease, which might be controlled by extended radiation fields. Since 2011, the NCCN guidelines (28) recognized the use PBI for the ASTRO suitable group. The results from the ELIOT phase III trial strengthen the indication of the use of PBI for this subgroup of patients. It should be pointed out that patients belonging to the ASTRO "cautionary" or "unsuitable" category are not necessarily at higher risk of LR, but should be encouraged to take part in specifically addressed clinical trials (29). However, for the time being, the safe applicability of intraoperative breast irradiation should be limited to patients classified "suitable "according to ASTRO, as emerged by the results of ELIOT phase III.

Acknowledgements

We thank the Italian Association for Cancer Research, the Jacqueline Seroussi Memorial Foundation for Cancer Research, the Umberto Veronesi Foundation, the American Italian Cancer Foundation, the Lombardy Region for their contribution and support to the ELIOT study.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Offersen BV, Overgaard M, Kroman N, et al. Accelerated partial breast irradiation as part of breast conserving therapy of early breast carcinoma: a systematic review. Radiother Oncol 2009;90:1-13.
2. Hoopes DJ, Kaziska D, Chapin P, et al. Patient preferences and physician practice patterns regarding breast radiotherapy. Int J Radiat Oncol Biol Phys 2012;82:674-81.
3. Husain ZA, Mahmood U, Hanlon A, et al. Accelerated partial breast irradiation via brachytherapy: a patterns-of-care analysis with ASTRO consensus statement groupings. Brachytherapy 2011;10:479-85.
4. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013;24:2206-23.
5. Radiation Therapy Oncology Group 0413/National Surgical Adjuvant Breast and Bowel Project B-39. Available online: http://www.rtog.org/clinicaltrials/protocoltable.aspx, accessed December 12, 2013.
6. Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med 2002;347:1227-32.
7. Polgár C, Van Limbergen E, Pötter R, et al. Patient selection for accelerated partial-breast irradiation (APBI) after breast-conserving surgery: recommendations of the Groupe Européen de Curiethérapie-European Society for Therapeutic Radiology and Oncology (GEC-ESTRO) breast cancer working group based on clinical evidence (2009). Radiother Oncol 2010;94:264-73.
8. Ribeiro GG, Magee B, Swindell R, et al. The Christie Hospital breast conservation trial: an update at 8 years from inception. Clin Oncol (R Coll Radiol) 1993;5:278-83.
9. Dodwell DJ, Dyker K, Brown J, et al. A randomised study of whole-breast vs tumour-bed irradiation after local excision and axillary dissection for early breast cancer. Clin Oncol (R Coll Radiol) 2005;17:618-22.
10. Polgár C, Fodor J, Major T, et al. Breast-conserving therapy with partial or whole breast irradiation: ten-year results of the Budapest randomized trial. Radiother Oncol 2013;108:197-202.
11. Vaidya JS, Wenz F, Bulsara M, et al. Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: 5-year results for local control and overall survival from the TARGIT-A randomised trial. Lancet 2014;383:603-13.
12. Veronesi U, Orecchia R, Maisonneuve P, et al. Intraoperative radiotherapy versus external radiotherapy for early breast cancer (ELIOT): a randomised controlled equivalence trial. Lancet Oncol 2013;14:1269-77.
13. Valachis A, Mauri D, Polyzos NP, et al. Partial breast irradiation or whole breast radiotherapy for early breast cancer: a meta-analysis of randomized controlled trials. Breast J 2010;16:245-51.
14. Luini A, Orecchia R, Gatti G, et al. The pilot trial on intraoperative radiotherapy with electrons (ELIOT): update on the results. Breast Cancer Res Treat 2005;93:55-9.
15. Veronesi U, Gatti G, Luini A, et al. Intraoperative

radiation therapy for breast cancer: technical notes. Breast J 2003;9:106-12.
16. Rosenstein BS, Lymberis SC, Formenti SC. Biologic comparison of partial breast irradiation protocols. Int J Radiat Oncol Biol Phys 2004;60:1393-404.
17. Kirkpatrick JP, Meyer JJ, Marks LB. The linear-quadratic model is inappropriate to model high dose per fraction effects in radiosurgery. Semin Radiat Oncol 2008;18:240-3.
18. Veronesi U, Orecchia R, Luini A, et al. Intraoperative radiotherapy during breast conserving surgery: a study on 1,822 cases treated with electrons. Breast Cancer Res Treat 2010;124:141-51.
19. Gorodetsky R, Lotan C, Piggot K, et al. Late effects of dose fractionation on the mechanical properties of breast skin following post-lumpectomy radiotherapy. Int J Radiat Oncol Biol Phys 1999;45:893-900.
20. Smith BD, Arthur DW, Buchholz TA, et al. Accelerated partial breast irradiation consensus statement from the American Society for Radiation Oncology (ASTRO). Int J Radiat Oncol Biol Phys 2009;74:987-1001.
21. Polgár C, Van Limbergen E, Pötter R, et al. Patient selection for accelerated partial-breast irradiation (APBI) after breast-conserving surgery: recommendations of the Groupe Européen de Curiethérapie-European Society for Therapeutic Radiology and Oncology (GEC-ESTRO) breast cancer working group based on clinical evidence (2009). Radiother Oncol 2010;94:264-73.
22. Wilkinson JB, Beitsch PD, Shah C, et al. Evaluation of current consensus statement recommendations for accelerated partial breast irradiation: a pooled analysis of William Beaumont Hospital and American Society of Breast Surgeon MammoSite Registry Trial Data. Int J Radiat Oncol Biol Phys 2013;85:1179-85.
23. Leonardi MC, Maisonneuve P, Mastropasqua MG, et al. How do the ASTRO consensus statement guidelines for the application of accelerated partial breast irradiation fit intraoperative radiotherapy? A retrospective analysis of patients treated at the European Institute of Oncology. Int J Radiat Oncol Biol Phys 2012;83:806-13.
24. Leonardi MC, Maisonneuve P, Mastropasqua MG, et al. Accelerated partial breast irradiation with intraoperative electrons: using GEC-ESTRO recommendations as guidance for patient selection. Radiother Oncol 2013;106:21-7.
25. Gujral DM, Sumo G, Owen JR, et al. Ipsilateral breast tumor relapse: local recurrence versus new primary tumor and the effect of whole-breast radiotherapy on the rate of new primaries. Int J Radiat Oncol Biol Phys 2011;79:19-25.
26. Polgár C, Fodor J, Major T, et al. Breast-conserving treatment with partial or whole breast irradiation for low-risk invasive breast carcinoma--5-year results of a randomized trial. Int J Radiat Oncol Biol Phys 2007;69:694-702.
27. Nguyen PL, Taghian AG, Katz MS, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and HER-2 is associated with local and distant recurrence after breast-conserving therapy. J Clin Oncol 2008;26:2373-8.
28. NCCN Clinical Practice Guidelines in Oncology for Breast cancer. Available online: ww.nccn.org/professionals/physician_gls/f_guidelines.asp
29. Vicini F, Arthur D, Wazer D, et al. Limitations of the American Society of Therapeutic Radiology and Oncology Consensus Panel guidelines on the use of accelerated partial breast irradiation. Int J Radiat Oncol Biol Phys 2011;79:977-84.

Cite this article as: Orecchia R, Leonardi MC, Maisonneuve P, Morra A, Lazzari R, Cattani F, Dell'Acqua V, Rotmensz N, Viale G, Luini A, Veronesi P, Galimberti V, Zurrida S, Gentilini O, Intra M, Veronesi U. Intraoperative radiotherapy with electrons (ELIOT) for early breast cancer: the European Institute of Oncology experience. Transl Cancer Res 2014;3(1):59-64. doi: 10.3978/j.issn.2218-676X.2014.02.04

Comparison of the proliferative and clonogenic growth capacity of wound fluid from breast cancer patients treated with and without intraoperative radiotherapy

Marlon R. Veldwijk[1], Christian Neumaier[1], Axel Gerhardt[2], Frank A. Giordano[1], Marc Sütterlin[2], Carsten Herskind[1*], Frederik Wenz[1*]

[1]Department of Radiation Oncology, [2]Department of Gynecology and Obstetrics, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, D-68135 Mannheim, Germany

*These authors shared senior authorship to this work.

Correspondence to: Marlon R. Veldwijk, Ph.D. Department of Radiation Oncology, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, Mannheim D-68135, Germany. Email: Marlon.Veldwijk@medma.uni-heidelberg.de.

Abstract: The physiological wound healing process after breast-conserving surgery is believed to contribute to a microenvironment promoting motility and invasive activity of residual malignant cells. In intraoperative radiotherapy (IORT), a high single dose of radiation is applied to the tumor bed directly after surgical removal of the tumor and this has been reported to abrogate the stimulatory effects of wound fluid (WF). In the present study, we tested whether IORT alters the influence of WF on the proliferative and clonogenic growth of human MCF7 breast cancer cells. Breast cancer patients were recruited from our IORT studies. WF from 12 patients who underwent IORT, and 18 control patients without IORT, were collected for 24 h. Proliferation was tested in a short-term (MTT) assay and the colony formation assay. A non-significant trend for reduced proliferation was seen in the MTT assay when WF from IORT-treated patients was added at 1% (P=0.07) but not at 3% (P=0.16). No significant effect of IORT-treated WF on the clonogenic growth capacity of MCF7 cells (P=0.79) was found. Our short-term proliferation results with the ER/PgR$^+$-Her2/neu$^-$ cell line MCF7 complement previously published data with ER/PgR$^-$-Her2/neu$^-$ and ER/PgR$^-$-Her2/neu$^+$ breast cancer cell lines showing no significant effect of IORT on short-term proliferation. In addition, for the first time, a lack of an effect of IORT on the clonogenic growth capacity of WF was shown. It should be noted that the present results do not exclude potential other effects of IORT on the cytokine composition and functional activity of WF.

Keywords: Intraoperative radiotherapy (IORT); surgery; wound fluid (WF); cell proliferation; clonogenic cell assay

Submitted Jan 23, 2015. Accepted for publication Mar 31, 2015.
doi: 10.3978/j.issn.2218-676X.2015.04.01
View this article at: http://dx.doi.org/10.3978/j.issn.2218-676X.2015.04.01

Introduction

The ability of the cellular microenvironment to influence cell behavior has been known for quite some time. In malignancies, the microenvironment was shown to regulate tumor cell fate even suggesting that disruption of its homeostasis may drive tumor progression (1). Induction of the wound healing response after surgery with the ensuing microenvironment reorganization and tissue reconstruction may, therefore, potentially influence recurrence (2). After damage, wound healing, including inflammation, tissue repair, and remodeling, is essential to ensure host integrity in multicellular eukaryotic organisms (3). As previously shown in experimental systems (4,5), it is to be expected that growth factors secreted during wound healing can also affect growth of malignant and non-malignant cells clinically. It was previously observed that wound fluid (drained after surgery; WF) collected from breast cancer patients can indeed stimulate proliferation of breast cancer cells (6).

In intraoperative radiotherapy (IORT), a high single dose of radiation is applied to the tumor bed directly after surgical removal of the tumor, in contrast to conventional external-beam radiotherapy (EBRT) which is applied after wound healing is completed. In a previous study, WF obtained from patients treated with IORT within the TARGIT-A trial (7) was reported to produce a reduction in WF-stimulated proliferation and invasion of breast cancer cell lines *in vitro* compared to WF from non-IORT patients (8,9). However, no significant effect of IORT on the proliferative capacity of WF was observed in a short-term proliferation assay (2-D) using ER/PgR$^-$-Her2/neu$^-$ and ER/PgR$^-$-Her2/neu$^+$ breast cancer cell lines, Furthermore, although significant effects of IORT were found in invasion (3-D Matrigel) and migration assays, clonogenic proliferation was not tested.

Therefore, the purpose of the present study was to validate the effect of IORT on WF-stimulated short-term proliferation in an ER/PgR$^+$-Her2/neu$^-$ human breast cancer cell line (MCF7), and for the first time test the effect on clonogenic, long-term proliferation.

Methods

Cell culture

The human breast carcinoma cell line MCF7 (ER/PgR$^+$-Her2/neu$^-$; American Type Culture Collection, LGC Standards GmbH, Wesel, Germany) was propagated in DMEM supplemented with 10% fetal bovine serum (FBS; all from Biochrom AG, Berlin, Germany). Three days before each experiment, cells were cultured in 3% FBS-containing medium. Cells were kept at 37 ℃ in a humidified incubator with 95% air/5% CO_2.

Collection and preparation of WF

Thirty patients with low-risk breast cancer were treated with breast-conserving surgery, of which 12 received IORT with a single dose of 20 Gy prescribed to the applicator surface (10-12). After surgery, WF was drained from the wound for 24 h. Thereafter, WF samples were collected, centrifuged at 800 ×g for 5 min and the supernatant was filtered through 40 µm filters (BD Falcon, Heidelberg, Germany). After a second centrifugation step (3,500 ×g for 5 min), the supernatant was subsequently filtered through 5, 0.8 and 0.22 µm filters and aliquots stored at –80 ℃. These steps ensured sufficient removal of cells and debris from the WF that would otherwise interfere with cell growth. The study was approved by the Medical Ethics Commission II of the Medical Faculty of Mannheim, Heidelberg University and was conducted according to Declaration of Helsinki principles. Of note, although all patients received a perioperative antibiotic treatment, this was prolonged (3 days) for patients receiving IORT *vs*. a single application for non-IORT-treated patients.

Proliferation assay

MCF7 cells were seeded in 96 well plates (8 wells per group, 5×10^3 cells per well in 50 µL serum-free DMEM medium). Samples were supplemented with 50 µL DMEM medium containing 3% FBS and 1% or 3% WF. After 48 h, 20 µL MTT [5 mg/mL, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and incubated for 3 h at 37 ℃. Viable cells reduce the yellow MTT to a non-hydrosoluble purple formazan. Thereafter, 100 µL of 10% SDS (Sodium dodecyl sulfate)/10 mM HCl in PBS was added to each well and plates incubated o/n at 37 ℃ to allow dissolving of the formazan. The next day, the absorbance at 590 nm (reference 690 nm) was quantified using a spectrophotometer (Tecan Infinite M200). Results are shown as percentage of controls.

Colony formation assay

MCF7 cells were seeded at 150 cells/T25 culture flask (triplicates) in 4 mL DMEM medium supplemented with 3% FBS and 3% WF and incubated for 2 weeks in a humidified incubator with 95% air/5% CO_2 at 37 ℃. Thereafter, cells were fixed with methanol/acetic acid and stained with crystal violet as described previously (13). Colonies (≥50 cells) were scored and the plating efficiency determined: plating efficiency = number of colonies obtained/number of cells seeded.

Statistics

Replicates were performed at least in triplicate and data are presented as mean ± standard error, unless otherwise noted. Wilcoxon/Kruskal-Wallis (non-parametric) tests and linear regression were performed with JMP11 statistical software (SAS Institute GmbH, Böblingen, Germany). Graphs were plotted using SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany).

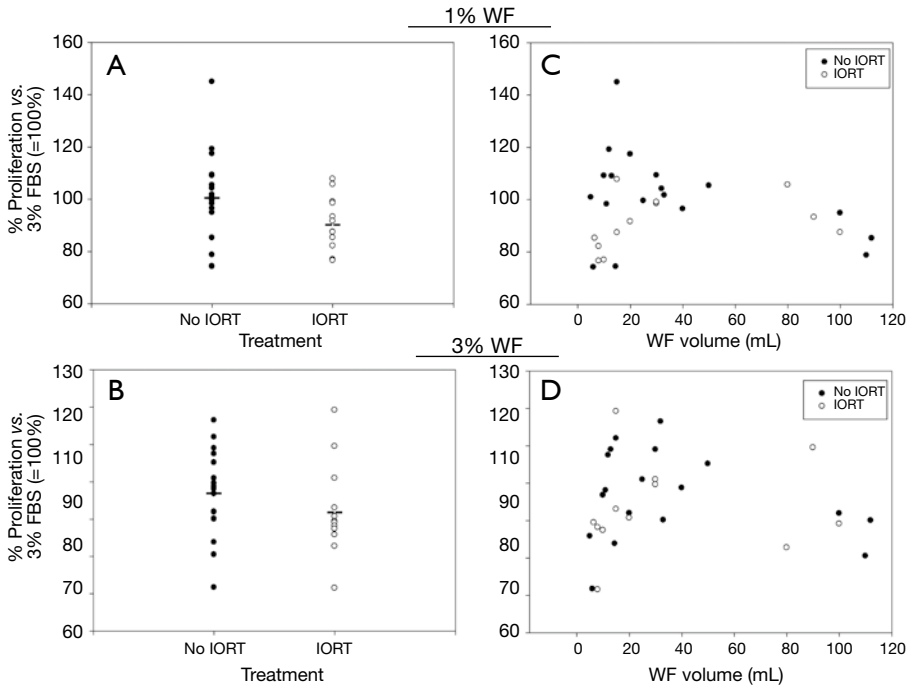


Figure 1 Proliferation of MCF7 cells (MTT assay) after 48h incubation with WF from breast cancer patients treated with or without IORT. Cells were either treated with 1% (A,C) or 3% WF (B,D). All samples were treated with 3% FBS which was also used as control (3% FBS=100%). Whereas for 1% WF a strong (A. P=0.07) and for 3% WF a weak trend (B. P=0.16) for an inhibitory effect of IORT on the proliferative capacity of WF could be observed, for neither group this was significant. In addition, it was tested whether there was a correlation between the WF volume and the effect of WF on the proliferation (C,D). Here no significant correlation between the proliferation rates and the WF volume was observed (C. 1% WF: $R^2_{No\ IORT}$=0.138, R^2_{IORT}=0.119; D. 3% WF: $R^2_{No\ IORT}$=0.045, R^2_{IORT}=0.015). Please note the scaling of the graphs. $n_{No\ IORT}$=18, n_{IORT}=12. IORT, intraoperative radiotherapy; WF, wound fluid; FBS, fetal bovine serum.

Results

Effects of IORT on the short-term proliferative capacity of WF

Pilot experiments showed that MCF7 cells did not proliferate when supplemented with pure WF and that 3% FBS was required to warrant proper proliferation (data not shown). To test potential concentration effects, experiments were performed with either 1% or 3% WF added to medium containing 3% FBS and normalized to the proliferation rates of cells receiving only 3% FBS. For 1% WF a trend for a modest inhibiting effect of IORT on the proliferative capacity of WF was observed (P=0.07; no IORT: 101.3%±4.0% *vs.* IORT: 91.0%±3.0%; *Figure 1A*) and the difference was also not significant using 3% WF (P=0.16; no IORT: 97.4%±2.8% *vs.* IORT: 92.0%±3.6%; *Figure 1B*). As large variations in the volumes of the WF occurred, a potential correlation between the volume and the effect on proliferation of MCF7 cells was tested. Here, no correlation could be detected, irrespective of using 1% ($R^2_{No\ IORT}$=0.138, R^2_{IORT}=0.119; *Figure 1C*) or 3 % WF ($R^2_{No\ IORT}$=0.045, R^2_{IORT}=0.015; *Figure 1D*).

Effects of IORT on the clonogenic growth capacity of WF

As repopulation of residual tumor cells to form recurrences depends on the capacity of cells to reproduce themselves, the effect of IORT on WF-stimulated clonogenic growth was tested in the colony formation assay. 3% WF from patients receiving IORT had no significant effect on the plating efficiency after 14 days incubation compared to that of WF from patients not receiving IORT (P=0.79; no IORT: 29.0%±6.1% *vs.* IORT: 32.0%±8.9%; *Figure 2A*). Also, no significant correlation between the volume of the WF and the plating efficiency was observed ($R^2_{No\ IORT}$=0.04, R^2_{IORT}=0.05; *Figure 2B*).

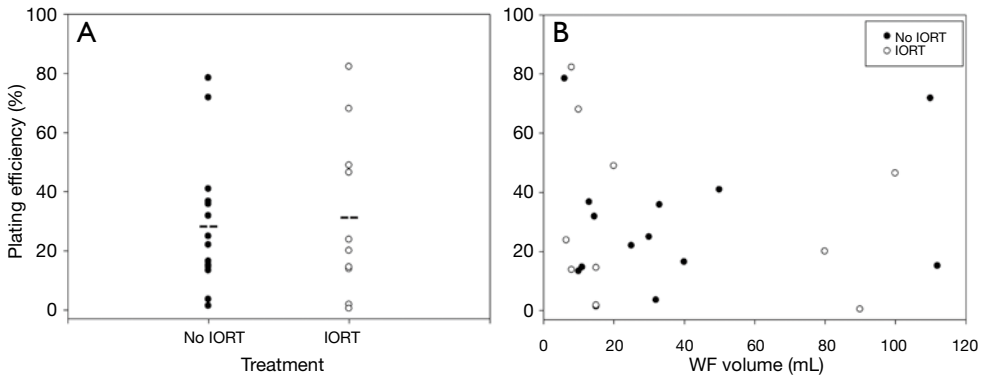


Figure 2 (A) Clonogenic proliferation of MCF7 cells after 14 day incubation during colony formation with 3% WF from breast cancer patients treated with or without IORT. (B) All cells were treated with 3% WF and 3% FBS. No correlation between the WF volume and the effect of WF on the plating efficiency was observed. For none of the groups, a significant difference between the effect of IORT or lack of IORT on clonogenic growth (A. P=0.79) or a correlation between the clonogenic growth and the WF volume was observed (B. $R^2_{No\ IORT}$=0.04, R^2_{IORT}=0.05). $n_{No\ IORT}$=14, n_{IORT}=10. IORT, intraoperative radiotherapy; FBS, fetal bovine serum; WF, wound fluid.

Discussion

In this work the effect of IORT on the proliferation and clonogenic growth capacity of WF obtained from breast cancer patients treated with or without IORT was investigated. Using 1% WF, a non-significant trend for an inhibiting effect of the IORT on the proliferative capacity was observed (*Figure 1A*) but not for 3% WF (P=0.16; *Figure 1B*). Similarly, no significant effect of the IORT on the clonogenic growth capacity of WF was observed either (*Figure 2A*). Our results from the short-term proliferation assay (MTT) with ER/PgR$^+$ and Her2/neu$^-$ MCF7 cells complement previous data by Belletti *et al.* (8) on MDA-MB-231 (ER/PgR$^-$-Her2/neu$^-$), MDA-MB-453 and SKBR-3 (both ER/PgR$^-$-Her2/neu$^+$) and are broadly in line with the absence of a significant effect of IORT on WF-stimulated proliferation in 2-D cultures in these cell lines. Although stimulation of proliferation by WF was found to be higher in Her2/neu positive than in negative cell lines (6), IORT did not seem to have a significant effect on the proliferative capacity of WF irrespective of the estrogen, progesterone, and Her2 receptor status of the breast cancer cell line. However, it should be noted that in the present study MCF7 cells did not proliferate when supplemented with pure WF but required addition of 3% FBS in both assays. This is consistent with recent evidence from head and neck tumor cell lines that the stimulatory effect of WF on proliferation may be cell-line specific (14).

As WF volumes varied greatly, we speculated that this may potentially modulate the effects of the WF on the tested proliferation and clonogenic growth of MCF7 cells. The rationale behind this was that increased/decreased content of diluting liquid (blood/ lymph) will affect the concentration of growth modulating molecules in the WF. No correlation was detected between the WF volume and proliferation of MCF7 cells in either assay (*Figure 1C,D* and *Figure 2B*), thereby arguing against this hypothesis.

A limitation of the present study may be that WF did not stimulate proliferation of MCF7 cells as found previously for other breast cancer cell lines (6,8). However, the previous studies used WF in serum-free medium with peripheral blood serum as controls whereas MCF7 cells required 3% FBS for short-term and clonogenic proliferation. Furthermore, a significant reduction of WF-stimulated proliferation by IORT was not observed in short-term 2-D cultures of MDA-MB-231 (slight decrease; P=0.11), MDA-MB-453 (slight increase; P=0.2), or SKBR-3 (slight increase; P=0.1) in the study by Belletti *et al.* (8). Notably, they did not show data for MCF7 in 2-D culture but the non-significant decrease (P=0.07-0.16) observed for MCF7 in the present study together with the previous findings supports the conclusion that WF from IORT patients does not impair proliferation in 2-D culture. This was corroborated by the absence of an effect of IORT on WF-stimulated entry into the S-phase of the cell cycle (8).

It should be noted that the present results do not rule out that there may be differences in the cellular microenvironment and the cytokine composition of WF after IORT compared to no IORT. Thus, Belletti *et al.* (8)

showed that IORT reduced WF-stimulated migration (chemotaxis assay) and invasion (Matrigel Transwell assay). This was associated with changes in the cytokine profile of WF by intraoperative tumor-bed irradiation as performed according to the TARGIT protocol (8). In addition, the size of MCF7 colonies grown in a Matrigel 3-D matrix was reduced. However, since 2-D area rather than cell numbers per colony or yield of colonies per cell seeded was measured, proliferation may have been confounded by invasion and migration in this assay.

In summary, the present study did not support an effect of WF from IORT patients on clonogenic or short-term proliferation of MCF7 breast cancer cells.

Acknowledgements

We would like to thank the staff of the Department of Radiation Oncology and the Department of Gynecology and Obstetrics (UMM, Mannheim) for their support. The original study on IORT was funded by the German Federal Ministry of Education and Research (BMBF; grant FKZ01ZP0508).

Footnote

Conflicts of Interest: Radiobiological research in our department is supported by Carl Zeiss Surgery, Oberkochen, Germany. FG, MS and FW receive speaker's fees and honoraria by Carl Zeiss Meditec AG. For all other authors, there are no conflicts of interest.

References

1. Cox TR, Erler JT. Molecular pathways: connecting fibrosis and solid tumor metastasis. Clin Cancer Res 2014;20:3637-43.
2. Baum M, Demicheli R, Hrushesky W, et al. Does surgery unfavourably perturb the "natural history" of early breast cancer by accelerating the appearance of distant metastases? Eur J Cancer 2005;41:508-15.
3. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999;341:738-46.
4. Demicheli R, Valagussa P, Bonadonna G. Does surgery modify growth kinetics of breast cancer micrometastases? Br J Cancer 2001;85:490-2.
5. Tsuchiya Y, Sawada S, Yoshioka I, et al. Increased surgical stress promotes tumor metastasis.Surgery 2003;133:547-55.
6. Tagliabue E, Agresti R, Carcangiu ML, et al. Role of HER2 in wound-induced breast carcinoma proliferation. Lancet 2003;362:527-33.
7. Vaidya JS, Wenz F, Bulsara M, et al. Risk-adapted targeted intraoperative radiotherapy versus whole-breast radiotherapy for breast cancer: 5-year results for local control and overall survival from the TARGIT-A randomised trial. Lancet 2014;383:603-13.
8. Belletti B, Vaidya JS, D'Andrea S, et al. Targeted intraoperative radiotherapy impairs the stimulation of breast cancer cell proliferation and invasion caused by surgical wounding. Clin Cancer Res 2008;14:1325-32.
9. Herskind C, Wenz F. Radiobiological aspects of intraoperative tumour-bed irradiation with low-energy X-rays (LEX-IORT). Transl Cancer Res 2014;3:3-17.
10. Vaidya JS, Joseph DJ, Tobias JS, et al. Targeted intraoperative radiotherapy versus whole breast radiotherapy for breast cancer (TARGIT-A trial): an international, prospective, randomised, non-inferiority phase 3 trial. Lancet 2010;376:91-102.
11. Sperk E, Welzel G, Keller A, et al. Late radiation toxicity after intraoperative radiotherapy (IORT) for breast cancer: results from the randomized phase III trial TARGIT A. Breast Cancer Res Treat 2012;135:253-60.
12. Neumaier C, Elena S, Grit W, et al. TARGIT-E(lderly)--prospective phase II study of intraoperative radiotherapy (IORT) in elderly patients with small breast cancer. BMC Cancer 2012;12:171.
13. Liu Q, Schneider F, Ma L, et al. Relative Biologic Effectiveness (RBE) of 50 kV X-rays measured in a phantom for intraoperative tumor-bed irradiation. Int J Radiat Oncol Biol Phys 2013;85:1127-33.
14. Ekblad L, Lindgren G, Persson E, et al. Cell-line-specific stimulation of tumor cell aggressiveness by wound healing factors - a central role for STAT3. BMC Cancer 2013;13:33.

Cite this article as: Veldwijk MR, Neumaier C, Gerhardt A, Giordano FA, Sütterlin M, Herskind C, Wenz F. Comparison of the proliferative and clonogenic growth capacity of wound fluid from breast cancer patients treated with and without intraoperative radiotherapy. Transl Cancer Res 2015;4(2):173-177. doi: 10.3978/j.issn.2218-676X.2015.04.01

The use of postoperative radiation after nipple sparing mastectomy

Roberto Orecchia[1,2]

[1]University of Milan, Milan, Italy; [2]European Institute of Oncology, Milan, Italy

Correspondence to: Roberto Orecchia. European Institute of Oncology, Scientific Directorate, Via Ripamonti 435, 20141 Milan, Italy. Email: roberto.orecchia@ieo.it.

Abstract: Nipple-sparing mastectomy (NSM) is a surgical procedure designed to reduce the disabling psychological effects of radical mastectomy. The preservation of the nipple-areola complex (NAC) produces a better result of the breast reconstruction, but some concerns exist of increasing the risk for local recurrence (LR). To reduce this risk a restricted inclusion criteria is suggested. In case of patients with high or intermediate risk the use of radiation therapy (RT) is also recommended, but a current standard for radiation after NSM is not available. In the literature, with few exceptions, there are few reports detailing indication and technique of radiation after NSM. There is a general consensus that post-mastectomy radiation therapy (PMRT) should be considered for patients with four or more positive axillary lymph nodes, primary tumour size 5 cm or more, T4 disease for skin involvement and positive margin. Almost all of these patients are candidate to receive external beam radiotherapy to the chest wall and to the supraclavicular/axillary region, less to the internal mammary chain. PMRT could be omitted in elderly patients with poor clinical conditions or co-morbidities that substantially reduce the life expectancy. Because the indications of NSM have been progressively extended also to larger or multi-centric tumours, this procedure has been criticised because of the increased risk of recurrence behind the areola due to the remaining glandular tissue, especially the terminal ducts, kept to preserve its blood supply, and especially in case of more advanced tumours. To reduce this concern, adjuvant RT after NSM should be administered in high risk patients who meet the criteria for current recommendations, but in other cases, such as in patients with intermediate risk or lower stage, the indication should be discussed on individual.

Keywords: Nipple-sparing mastectomy (NSM); breast cancer; post-mastectomy radiation therapy (PMRT)

Submitted Sep 16, 2015. Accepted for publication Oct 20, 2015.
doi: 10.3978/j.issn.2227-684X.2015.11.01
View this article at: http://dx.doi.org/10.3978/j.issn.2227-684X.2015.11.01

Introduction

Post-mastectomy radiation therapy (PMRT) has become increasingly common for women with locally advanced breast cancer. Irradiation is typically delivered to the chest wall and regional lymphatics. Current recommendations include high risk patients with a tumour size of 5 cm or larger (the cancer can be one lump, or a series, or even microscopic disease that together reach this size) and/or with at least four positive nodes in the axilla (1). Also patients with positive margin of resection (R1/R2, without possibility of achieving clear margins) or with skin involvement are suggested to receive postoperative irradiation.

Conservative surgery has also become more frequent, in order to reduce the psychological drawbacks of the mastectomy in breast cancer treatment. Conservative surgery is now well accepted not only for small tumours but also for larger tumours when oncoplastic surgery can be applied to reshape the breast despite the large defect. However, to date 25% or more of the women with breast cancer are still candidate to mastectomy because of the large size of the tumour (or the small size of the breast), or the multi-centricity of the cancer, or the local recurrence after a previous conservative treatment. To reduce the

detrimental psychological effect of mastectomy, the skin-sparing mastectomy have been introduced and demonstrated to be effective and safe (2). In order to further improve the aesthetics and psychological results, the additional preservation of the nipple-areola complex (NAC) has been also described, introducing the concept of nipple-sparing mastectomy (NSM) (3). Because the indications of NSM have been progressively extended to larger or multi-centric tumours (4), this procedure has been criticised because of the increased risk of recurrence behind the areola due to the remaining glandular tissue, especially the terminal ducts, kept to preserve its blood supply, especially in case of more advanced tumours (5). To reduce this concern adjuvant radiation therapy (RT) after NSM should be administered in high risk patients who meet the criteria for current recommendations, but in other cases, such as in patients with intermediate risk or lower stage, there is no general consensus regarding indications and its role is still unclear (6).

The aim of this paper is to review the use of postoperative irradiation after NSM and try to focus on the still open questions in this setting.

Indications for adjuvant radiation therapy (RT) after mastectomy

PMRT has been known to substantially reduce the risk of loco-regional failure (LRF), and but also to increase disease specific survival and overall survival, particularly in patients with positive lymph nodes and adequate axillary surgery and even when systemic therapy is given (7). The absolute benefit gained from PMRT is believed greatest for those at high risk of LRF. There is a general consensus that PMRT should be considered when risk of LRF is greater than 20%, such as for patients with four or more positive axillary lymph nodes, primary tumour size 5 cm or more, T4 disease for skin involvement and positive margin (1). Almost all of these patients are candidate to receive external beam radiotherapy to the chest wall and to the supraclavicular/axillary region, less to the internal mammary chain. PMRT could be omitted in elderly patients with poor clinical conditions or co-morbidities that substantially reduce the life expentancy. These indications should be applied independently from the type of mastectomy, and also in presence of reconstruction.

Other factors may contribute to increase the risk of LRF, and particularly when more than one are present (8). These include young age, less than 40 years, premenopausal status, histological grade 3 tumours, invasive lobular cancer, presence of lympho-vascular invasion, less than six nodes removed at axillary dissection, positive lymph node ratio >20%, and significant nodal extracapsular invasion. Waiting for a definitive assessment of the impact of different molecular subtypes on LRF, currently still unclear, PMRT should be also considered in patients with earlier stages, but with two or more risk factors. Considerations of adverse histo-pathological factors have been included in the recent recommendations from the 2015 Saint Gallen Breast Cancer Conference (9).

It remains unclear whether patients with one to three axillary nodes positive benefit significantly from PMRT. A subset analysis of the Danish 82 b and c studies including only those patients with eight or more axillary nodes removed reported a significant and equal reductions in LRF and overall survival at 15 years with PMRT in both the one to three and greater than four involved node groups (10). Also in the Early Breast Cancer Trialists' Collaborative Group (EDCTCG) analysis on a subgroup of more than 1,300 patients with one to three positive nodes included in the randomized trials conducted between 1964 and 1986, a decrease in LRF and breast cancer mortality was found (7). Based on these data, patients with positive axillary nodes, irrespective of the number of involved lymph nodes, considered are mandatory to be treated with PMRT in some guidelines (11).

In spite of these recommendations, the role of PMRT in this setting of patients remains controversial in practice, especially in the current era of more effective systemic therapies. A very recent study showed that the effectiveness of PMRT in terms of survival for breast cancer patients even in intermediate risk category (pT1-2 and one to three tumour positive lymph nodes) is not for all patients, but depends on the combination between the number of positive lymph nodes and the tumour size (12). Using data from the National Cancer Database (NCDB) and the Surveillance, Epidemiology, and End Results (SEER) program between 1998 and 2008, this study respectively identified 93,793 and 36,299 women in this stages who underwent mastectomy. The association of PMRT use with overall and cause-specific survival was examined using multivariable Cox models in subgroups defined by tumour stage. In the NCDB cohort, PMRT was associated with a 14% relative risk reduction in all-cause mortality among the patients with two positive lymph nodes and tumours 2-5 cm in size or three positive nodes [hazard ratio (HR), 0.86; 95% confidence interval (CI), 0.81-0.91; P<0.0001], but PMRT had no beneficial effect for the patients with one

positive node or two positive nodes and tumours 2 cm in size or smaller. Analysis of the SEER cohort confirmed this heterogeneous effect, showing PMRT to be associated with a 14% relative risk reduction in breast cancer cause-specific mortality among the patients with two positive nodes and tumours 2-5 cm in size or three positive nodes (HR, 0.86; 95% CI, 0.77-0.96; P=0.007) but not in the other subgroup. To try to resolve the question of patients with one to three positive axillary nodes a phase III randomised control trial—the Selective Use of Post-operative Radiotherapy after Mastectomy (SUPREMO) trial—is currently being conducted in Europe.

Also the role of irradiation of the internal mammary nodal region is controversial. Clinical evidences of benefit have been shown in the recent results from the European Organisation for Research and Treatment of Cancer (EORTC) 22922 and the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) MA20 randomised trials. In the EORTC trial women who had a medially or centrally located primary tumour, irrespective of axillary involvement, or node-positive axilla and receiving internal mammary irradiation improved their disease-free survival (+3%; P=0.02) and reduced breast-cancer mortality (–1.9%; P=0.02), with a marginal effect on overall survival at 10-year (P=0.06) (13). In the MA-20 study, among women with node-positive or high-risk node-negative breast cancer, the addition of regional nodal irradiation to whole breast irradiation increased the rate of disease-free survival (+5%; P=0.01), again not improving overall survival rate (14). The report of these trials can be translated in a larger number of patients who traditionally have not received regional RT (high risk LN– and 1-3 LN+) in the next future will be receiving regional RT (including internal mammary chain).

Specific risk factors for local recurrence (LR) in NSM and role of radiation

Today, no prospective and comparative studies between NSM and other types of mastectomy have been published, comparing not only the cosmetic outcome with other reconstructive techniques, but also and more important, the safety and the risk of LR, especially in case of the use of NSM in locally advanced stages. The validity of a comparison of results between various hystorical series of patients who underwent NSM is questionable because of the differences in terms of selection criteria (invasive or *in-situ* disease), surgical technique (one-stage surgery or delayed), and use or not of adjuvant RT.

Also the use of radiation in the setting of NSM has been sparsely reported, and often without providing details with respect to indication and technique. The presence of neoplastic cells in the retro areolar area is correlated to the distance between the tumour and the areola, therefore the conservation of the areola could be proposed only for peripheral tumours, but the margins of the tumour are sometimes difficult to evaluate preoperatively. Intraoperative frozen section examination of retro areolar tissue is considered as an important step to determine the eligibility of NSM procedure. In a series of patients treated at the European Institute of Oncology in Milan 88 cases with false-negative frozen section and ten with close margins were reported (15). Despite the frozen-section negativity, the definitive histology of retro areolar tissue revealed the presence of atypia in 11 NAC (11.2%), LCIS in 20 (20.4%), invasive carcinoma in 19 (19.4%), ductal carcinoma in situ (DCIS) in 38 (38.8%), and close margins in 10 (10.2%). The median follow-up was 64 (range, 18-113) months, and median age was 44 (range, 29-64) years. The 5-year cumulative incidence of LR and NAC recurrence was 11.2% (10/98 patients) and 2.4% (2/98 patients), respectively. The two cases of NAC recurrence consisted of Paget's disease. Analyzing the definitive results of retro areolar tissue, the 5-year cumulative incidence of LR was 42.9% (n=4) for atypia, 8.7% (n=3) for DCIS, 10% (n=2) for LCIS, 10% (n=1) for close margins, and 0% for invasive carcinoma. Intraoperative irradiation was given on the NAC in 93 cases (94.9%). Such results could be an argument in favor of a good efficacy of radiotherapy in this group of patients, at higher risk for LR, especially in case of invasive cancer where none of 19 cases with positive margins manifested a LR.

As mentioned before, the most extensive experience on the use of radiation after NSM has been conducted in Milan, where more than 2,000 patients have been treated with intraoperative irradiation. The technique has been extensively used in breast conserving surgery (16,17). The procedure starts immediately after the subcutaneous mastectomy and before the reconstruction. An electron beam is used intra operatively with an energy level appropriately chosen, more frequently 6 MeV. A total dose of 16 Gy (prescribed at the point of maximum dose) is delivered in the region of the NAC. The biologic equivalent of a single intraoperative dose is felt to be 1.5-2.5 higher than the dose delivered with conventional fractionated external irradiation and a single dose of 16 Gy corresponds to a fractionated dose of about 45 Gy for

early-responding tissue (tumour cells) and of 70-80 Gy for late-responding tissues (vessels, fat, nerves). Two shielding aluminium and lead disks are placed between the NAC and the pectoralis muscle to minimize the irradiation of the thoracic wall. The chest wall protection is guaranteed both by the absorption properties of the lead and aluminium and their thickness. The sterile collimator of the accelerator is placed in the correct position in contact with the NAC in order to guarantee the coverage of the entire target volume and simultaneously to avoid any surgical wound contamination. The area that is to be irradiated ("clinical target volume") includes the remaining glandular tissue behind the NAC and corresponds to the NAC diameter and its periphery. The placement of a layer of gauze over the areola with a hole in the middle corresponding to the nipple is also recommended, because the thickness of the gauze further improves the homogenous distribution of the dose to the nipple and to the areola. The breast reconstruction is performed immediately after the NAC irradiation using either a prosthesis or a flap.

The results of combining NSM with intraoperative radiotherapy were reported in one thousand and one patients, treated from March 2002 to November 2007 for invasive carcinoma in 82% of the cases and *in situ* carcinoma in 18% (18). The median follow-up time was 20 (range, 1-69) months. The total NAC necrosis was observed in 35 cases (3.5%) and partially in 55 (5.5%). In 50 patients (5%) it was removed. The median rate of the patients for global cosmetic result on a scale ranging from 0 (worst) to 10 (excellent) was 8. Only 15% of the patients reported a partial sensitivity of the NAC. Of the 14 (1.4%) LR, ten occurred close to the tumour site, all far from the NAC corresponding to the field of radiation. No recurrences were observed in the NAC. A comparison was also performed between the 800 patients who received intraoperative irradiation and the 201 who underwent delayed one-shot radiotherapy, with the same dose by electrons, on the days following the operation, and no significant outcome difference was observed.

Using this large series of patients, the same group also identified some risk factors of recurrences in the breast and the nipple areola complex (19). The more significant risk factors of LR in the breast for the patients with invasive cancer were high grade, overexpression/amplification of HER2/neu and molecular subtype luminal B. In patients with intraepithelial neoplasia the risk factors of LR in the breast and in the NAC were age (<45 years), absence of estrogen receptors, high grade, HER2/neu overexpression and high Ki-67.

In addition to the contribution of Milan experience, there are other few reports useful to define the role of RT after NSM. Currently there is only a series showing a difference in decreasing the risk of recurrence that highlights the role of RT (20). In these patients who received subcutaneous mastectomy with or without adjuvant RT, the LR rate at a median follow-up of 13 years was 8.5% and 28.4%, respectively. This percentage of LR without RT is much higher than expected, but the selection criteria included tumours larger than 3 cm, and lymph-node metastases were found in 40.3% of patients, making these patients high risk for LR.

Also complications arising as a result of NSM treatment have been studied. In a very recent report outcomes of NSM plus immediate reconstruction from 2007 to 2013 have been evaluated (21). There were 982 NSM: 816 had no radiation, 69 had prior radiation, and 97 had PMRT. Compared to breasts with no RT, both prior RT and PMRT increased overall complications (10.2% *vs.* 21.7% and 17.5%, P=0.003, P=0.03, respectively) and nipple loss (0.9% *vs.* 4.3% and 4.1%, P=0.04, P=0.02, respectively), while PMRT increased rate of reconstruction failure (2.2% *vs.* 8.2%, P=0.003). On multivariate regression analysis, prior RT [odds ratio (OR), 2.53, P=0.006], PMRT (OR, 2.29, P=0.015), age >55 years (OR, 2.03, P=0.04), breast volume ≥800 cm^3 (OR, 1.96, P=0.04), smoking (OR, 2.62, P=0.001), and periareolar incision (OR, 1.74, P=0.03) were independent risk factors for complications requiring surgical revision. In irradiated breasts, complication rates were 13.4% without further risk factors and 17.5%, 50%, and 66.7% when 1, 2, and ≥3 additional independent risk factors were present, respectively (P<0.001). The conclusion was that although complication rates were higher in irradiated breasts, reconstruction failure and nipple/areola necrosis was infrequent and radiation RT should not be a contraindication to NSM.

Finally, the current use of radiation after NSM has been investigated in a recent report (22). Female patients who underwent NSM or non-NSM for breast cancer from 2006 to 2010 were isolated from the SEER database. A total of 112,817 patients were included: 470 (0.4%) underwent NSM and 112,347 (99.6%) underwent non-NSM. NSM patients with 0 nodes/size ≤2 cm, 0 nodes/size 2-5 cm, and unexamined axilla/size ≤2 cm had higher odds of radiation when compared with size- and node-matched mastectomy patients. Multivariate logistic regression showed that NSM patients had higher odds of radiation (OR, 2.01, P<0.001)

Table 1 Studies reported the use of radiation therapy (RT) in NSM

Author, year	Stages & RT	LLR recurrence (%)	NAC recurrence (%)	NAC necrosis (%)
Gerber *et al*., 2003	0-IIB, RT 27%	NA	11.7	NA
Benediktsson *et al*., 2008	0-III, RT 21.8%	8.5 with RT	NA	NA
Petit *et al*., 2009	DCIS, T1-T4, N0-N1 80% IORT, 20% RT delayed	1.4	0	9.0
Sakamoto *et al*., 2010	0-IIIA, 15% N+, RT 30%	0	0	10
Harness *et al*., 2011	0-IV, RT 27.5% (locoregional)	0	0	5
Moyer *et al*., 2012	0-III, RT 11.5% (close margin)	NA	NA	37.5 (partial)
Shi *et al*., 2012	I-III, RT optional	5.7	2.8	5.7
Warren Peled *et al*., 2012	0-IV, RT 26.7%	2.4	1.5	1.5
Rulli *et al*., 2013	DCIS, T1-T2, 0-3N+, RT 16.6%	3.3	3.3	0
Burdge *et al*., 2013	IIB-III, RT 100% >5 cm/N+	10.3	0	NA
Fortunato *et al*., 2013	DCIS, I-III, RT 19%	0.8	0	4.3
Sbitany *et al*., 2014	I-III, RT 19% (before or after)	NA	NA	4
Reish *et al*., 2015	I-III, RT 13%	NA	NA	10

NSM, nipple-sparing mastectomy; RT, radiation therapy; LLR, loco regional rate of recurrence; NAC, nipple-areola complex; DCIS, ductal carcinoma in situ; IORT, intra operative radiation therapy.

than mastectomy patients. Radiation was given to 18% of NSM patients who did not meet NCCN guidelines according to size or lymph node involvement, compared with 6% of mastectomy patients. This may reflect a concern with leaving ductal tissue in the NAC.

In *Table 1* a series of clinical studies reporting the use of RT after NSM is listed. In the great majority of these studies the details on indication and technique of the delivery of radiation is very limited. All these studies are retrospective.

Conclusions

Recommendations for radiation delivery in breast cancer patients after mastectomy suggest that radiation is generally indicated in high-risk patients, such as those with tumour size >5 cm, positive lymph nodes in the axilla, or positive tumour margins. The definitive results of recent trials on regional irradiation can enlarge these indications to patients with intermediate risk who traditionally have not received regional radiation (high risk LN– and 1-3 LN+). The NSM is a new approach of the well known subcutaneous mastectomy which spares a small amount of glandular tissue behind the areola to protect its blood supply. Some concerns exist about the safety of these procedures, especially in case of more advanced breast tumours. The postoperative radiotherapy could complete the cancer treatment by reducing the risk of LR beneath the areola, but the use of radiation in NSM patients has been variable in the reported literature. There is a clear need for large cooperative perspective studies in this setting.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Breast Cancer, Version 2015. Available online: http://www.nccn.org
2. Lanitis S, Tekkis PP, Sgourakis G, et al. Comparison of skin-sparing mastectomy versus non-skin-sparing mastectomy for breast cancer: a meta-analysis of observational studies. Ann Surg 2010;251:632-9.
3. Gerber B, Krause A, Reimer T, et al. Skin-sparing mastectomy with conservation of the nipple-areola

complex and autologous reconstruction is an oncologically safe procedure. Ann Surg 2003;238:120-7.
4. Coopey SB, Tang R, Lei L, et al. Increasing eligibility for nipple-sparing mastectomy. Ann Surg Oncol 2013;20:3218-22.
5. Petit JY, Veronesi U, Lohsiriwat V, et al. Nipple-sparing mastectomy--is it worth the risk? Nat Rev Clin Oncol 2011;8:742-7.
6. Janssen S, Holz-Sapra E, Rades D, et al. Nipple-sparing mastectomy in breast cancer patients: The role of adjuvant radiotherapy (Review). Oncol Lett 2015;9:2435-2441.
7. EBCTCG (Early Breast Cancer Trialists' Collaborative Group), McGale P, Taylor C, et al. Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: meta-analysis of individual patient data for 8135 women in 22 randomised trials. Lancet 2014;383:2127-35.
8. Rowell NP. Radiotherapy to the chest wall following mastectomy for node-negative breast cancer: a systematic review. Radiother Oncol 2009;91:23-32.
9. Coates AS, Winer EP, Goldhirsch A, et al. -Tailoring therapies-improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 2015;26:1533-46.
10. Overgaard M, Nielsen HM, Overgaard J. Is the benefit of postmastectomy irradiation limited to patients with four or more positive nodes, as recommended in international consensus reports? A subgroup analysis of the DBCG 82 b&c randomized trials. Radiother Oncol 2007;82:247-53.
11. Wenz F, Sperk E, Budach W, et al. DEGRO practical guidelines for radiotherapy of breast cancer IV: radiotherapy following mastectomy for invasive breast cancer. Strahlenther Onkol 2014;190:705-14.
12. Huo D, Hou N, Jaskowiak N, et al. Use of Postmastectomy Radiotherapy and Survival Rates for Breast Cancer Patients with T1-T2 and One to Three Positive Lymph Nodes. Ann Surg Oncol 2015;22:4295-304.
13. Poortmans PM, Collette S, Kirkove C, et al. Internal Mammary and Medial Supraclavicular Irradiation in Breast Cancer. N Engl J Med 2015;373:317-27.
14. Whelan TJ, Olivotto IA, Parulekar WR, et al. Regional Nodal Irradiation in Early-Stage Breast Cancer. N Engl J Med 2015;373:307-16.
15. Kneubil MC, Lohsiriwat V, Curigliano G, et al. Risk of locoregional recurrence in patients with false-negative frozen section or close margins of retroareolar specimen in nipple-sparing mastectomy. Ann Surg Oncol 2012;19:4117-23.
16. Leonardi MC, Maisonneuve P, Mastropasqua MG, et al. Accelerated partial breast irradiation with intraoperative electrons: using GEC-ESTRO recommendations as guidance for patient selection. Radiother Oncol 2013;106:21-7.
17. Veronesi U, Orecchia R, Maisonneuve P, et al. Intraoperative radiotherapy versus external radiotherapy for early breast cancer (ELIOT): a randomised controlled equivalence trial. Lancet Oncol 2013;14:1269-77.
18. Petit JY, Veronesi U, Orecchia R, et al. Nipple sparing mastectomy with nipple areola intraoperative radiotherapy: one thousand and one cases of a five years experience at the European institute of oncology of Milan (EIO). Breast Cancer Res Treat 2009;117:333-8.
19. Petit JY, Veronesi U, Orecchia R, et al. Risk factors associated with recurrence after nipple-sparing mastectomy for invasive and intraepithelial neoplasia. Ann Oncol 2012;23:2053-8.
20. Benediktsson KP, Perbeck L. Survival in breast cancer after nipple-sparing subcutaneous mastectomy and immediate reconstruction with implants: a prospective trial with 13 years median follow-up in 216 patients. Eur J Surg Oncol 2008;34:143-8.
21. Tang R, Coopey SB, Colwell AS, et al. Nipple-Sparing Mastectomy in Irradiated Breasts: Selecting Patients to Minimize Complications. Ann Surg Oncol 2015;22:3331-7.
22. Agarwal S, Agarwal J. Radiation delivery in patients undergoing therapeutic nipple-sparing mastectomy. Ann Surg Oncol 2015;22:46-51.

Cite this article as: Orecchia R. The use of postoperative radiation after nipple sparing mastectomy. Gland Surg 2016;5(1):63-68. doi: 10.3978/j.issn.2227-684X.2015.11.01

Optimal management of sentinel lymph node positive biopsy patients in early breast cancer

Geraldine M. Jacobson[1], Jessica F. Partin[2], Mohamad A. Salkeni[3]

[1]Departments of Radiation Oncology, [2]Departments of Surgery, [3]Departments of Internal Medicine, West Virginia University, Robert C. Byrd Health Sciences Center, Morgantown, West Virginia 26506-9000, USA

Correspondence to: Geraldine M. Jacobson, MD, MBA, MPH. Department of Radiation Oncology, West Virginia University, P.O. Box 9234, Health Sciences Center, Morgantown, West Virginia 26506–9234, USA. Email: gmjacobson@hsc.wvu.edu.

Submitted Jan 29, 2015. Accepted for publication Feb 05, 2015.
doi: 10.3978/j.issn.2305-5839.2015.02.33
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.02.33

What is the optimal management of a positive sentinel lymph node (SLN) in patients with early stage breast cancer? Prior to the widespread adoption of SLN biopsy, axillary lymph node dissection (ALND) was considered to have both therapeutic and prognostic benefit. Multiple studies have shown the accuracy and predictive value of the SLN procedure (1) and randomized trials confirmed that patients with negative SLN could forgo ALND (2,3).

For patients with a positive SLN, a completion ALND was considered beneficial for optimizing regional control and for potentially improving survival. Yet some retrospective studies showed low axillary recurrence in women with positive SLN who did not have an ALND (4).

In the past decade several randomized studies have addressed whether ALND is indicated following a positive SLN biopsy in patients with early breast cancer and clinically negative lymph nodes (LNs). Other therapeutic modalities, including systemic therapy and radiation, may contribute to regional control. In addition, clinical and biologic markers are widely used as prognostic indicators.

EORTC 10981-2203, the AMAROS trial, was initiated in 2001 to assess whether patients with a positive SLN could be treated with radiotherapy instead of ALND, with comparable medical benefit and fewer side effects (5). Eligible patients with invasive breast cancers measuring ≤3 cm and clinically negative LNs were randomized to ALND or axillary radiation following a positive SLN biopsy. Local treatment included mastectomy or breast conservation surgery. ALND included anatomical levels I and II to include at least 10 LNs. Axillary radiotherapy included all three axillary levels and the medial supraclavicular fossa. The prescribed dose was 50 Gy in 25 fractions of 2 Gy per fraction. Additional metastatic LNs were found in 33% of patients undergoing ALND.

With a median follow up of 6.1 years, there was no statistically significant difference in axillary recurrence, disease free survival (DFS) or overall survival between the two groups. Five-year axillary recurrence was 0.43% after ALND *vs.* 1.19% after axillary radiotherapy. Lymphedema was significantly greater in the ALND group. The AMAROS trial showed that ALND and axillary radiotherapy provided comparable axillary control in the study population with significantly less morbidity in the radiotherapy group.

The AMAROS trial was the first study to prospectively compare axillary radiation therapy (RT) against ALND in early stage breast cancer patients with positive SLN. Its value lies in demonstrating low axillary recurrence following radiation, alongside with decreased morbidity compared to ALND. The results of this trial should be viewed in the context of historic data on risks of clinical axillary recurrence, other recent trials addressing positive SLND, and contemporary breast cancer management.

In the NSABP B-04 (6) trial about 40% of patients with clinically negative nodes treated by radical mastectomy were found to have positive LNs. Patients treated with total mastectomy (no ALND) without axillary radiation were followed. Only about half of these women developed a clinically positive axillary node as a first event. The data from NSABP B-04 suggests that leaving positive nodes unresected did not significantly increase the rate of distant recurrence or breast cancer specific mortality. At 25 years of follow up there was no survival advantage from RT after total mastectomy in women with clinically negative nodes.

A French trial, initiated before the introduction of SLN

biopsy, randomized patients with breast cancer <3 cm and clinically negative LNs to ALND or axillary radiotherapy (7). 21% of the ALND patients had positive LNs. At 15 years follow up there was no difference in long-term survival between the two groups. There was a small difference in axillary LN recurrence 1% in the ALND group *vs.* 3% in the RT group.

The American College of Surgeons Oncology Group Z0011 study (8) was a prospective trial, which evaluated survival of patients with clinically negative LNs randomized to an ALND *vs.* no further treatment after a positive SLN biopsy. Patients were treated with breast conserving surgery, lumpectomy followed by radiation. All patients received opposed tangential field whole breast radiation; third field radiation to the regional nodes was not permitted. Of patients undergoing an ALND, 27.3% had an additional positive lymph node. At 6.3 years follow up there was no difference in local or regional recurrence between the two groups. The use of SLND alone did not result in inferior survival.

IBCSG-2301, a multi-center, randomized phase 3 study of ALND *vs.* no ALND in patients with sentinel-node micro-metastases (<2 mm) concluded that axillary dissection could be avoided in patients with early breast cancer and limited sentinel LN involvement (9).

The AMAROS results suggest that radiation provides equivalent outcomes to SLN positive patients as ALND with less morbidity. The ACOG Z011 study demonstrates comparable outcomes with standard whole breast radiation, without the addition of regional node irradiation. Discussants of the ACOG Z011 trial suggest that standard opposed tangential fields irradiate the SLND site, much of the level I axilla and a portion of the level II axilla. The reference cited, published in 2001 (10) is based on two dimensional imaging and planning, with clips used as surrogates for inclusion of axillary contents. A more recent review of axillary lymph node coverage in standard tangential fields based on CT-based 3D planning shows that only about 55% of level I-II axillary LNs are covered by 95% of the prescribed dose (11). Despite the lack of complete axillary coverage by the radiation field, the axillary recurrence rate in the Z011 trial was extremely low, less than 2%. The additional therapeutic benefit of treating the entire axillary lymph node volumes as described in the AMAROS study is likely to be minimal. The larger field would increase the volume of normal tissue irradiated and potentially the morbidity and cost of treatment.

Progress in locoregional therapy for early stage breast cancer has resulted in decreasing the morbidity of breast cancer treatment. The NSABP B-04 trial with 25-year follow-up data demonstrated equivalent overall survival between radical mastectomy, mastectomy with radiation, and mastectomy alone in clinically node-negative women (6). Subsequently, the SLN biopsy procedure has been established as the staging procedure of choice for women with early-stage, node-negative breast cancer, allowing for accurate staging of the axilla while decreasing the rates of lymphedema, arm dysfunction, and pain. There has been an effort to identity node-positive women in whom the morbidity of a completion axillary dissection may be avoided with acceptably low risk of axillary recurrence. The SLN biopsy is falsely negative in about 5% of node-positive patients (12), but this does not appear to have a corresponding axillary recurrence rate. Despite presumably untreated disease in the axilla, the axillary recurrence rate following a negative SLNB is far lower than expected based on the FNR and suggests that such disease is less likely to produce clinical disease.

Improved mortality from breast cancer can be attributed not only to increased screening but also improvements in therapy. Women in the NSABP B-04 trial did not receive systemic therapy, while in more recent trials with shorter follow-up, the majority of women [95-97% in the IBCSG 23-01 trial (9) and 96-97% in Z0011 (8)] received at least some form of adjuvant systemic therapy. The use of genomic profiling has greatly advanced direction of systemic therapy with better tailored therapy, and some women within the traditionally considered "low-risk" group (node-negative women with tumors that are hormone receptor-positive and HER2-neu-negative) have been identified as having tumors with higher risk for distant recurrence and appropriately offered systemic therapy. One such assay, the Oncotype Dx assay, has demonstrated an association between higher score and locoregional recurrence risk (13). In addition, neoadjuvant systemic therapy can effectively treat axillary nodal disease in up to 30-40% of patients (14). While the ACOSOG Z1071 trial showed an inferior false negative rate of 12.6% for SLNB following neoadjuvant chemotherapy, multiple studies nevertheless highlight the ability of systemic therapy to eradicate some disease in the axilla and may allow for alternate therapy to the axilla (15).

Recently, more women are avoiding a completion axillary dissection in the event of a positive sentinel node biopsy. This is both patient- and surgeon-driven and therefore represents selection bias; however, these studies demonstrate low risks of axillary recurrence following sentinel node

biopsy only. Nomogram tools have been developed to assist surgeons and patients in selection of patients for completion axillary dissection, and these rely on predictors such as tumor size, tumor grade, estrogen receptor status, and lymphovascular invasion; using such nomograms has been shown to decrease the rate of completion axillary dissection in a subset of women with more favorable tumor factors with only a marginally higher rate of axillary recurrence (2% *vs.* 0.4% at 23-30 months) (16). The AMAROS trial did not evaluate hormonal status or LVI status, but the low level of axillary recurrence suggests that radiation represents an acceptable means of disease control in the axilla regardless of tumor type. While the ACOSOG Z0011 trial included only women undergoing breast conservation with adjuvant RT, both the IBCSG 23-01 and the AMAROS trial allowed patients to undergo either breast conservation or mastectomy (9% in the IBCSG trial and 17-18% in the AMAROS trial) for their local treatment. Interestingly, 19% of patients undergoing breast conservation in the IBCSG trial received intraoperative RT only, thereby missing the previously offered suggestion of axillary treatment with standard tangential fields. This may also represent the efficacy of systemic treatment in eradication of axillary disease.

While there is little, if any, controversy to the prognostic value of axillary LNs, not everyone is in agreement to the therapeutic benefit of axillary nodal dissection. The impact of axillary nodal dissection on survival is not well established. Most of the data showing improved survival are derived from either retrospective studies, or from studies that justifiably allowed adjuvant chemotherapy for patients post-dissection if they were found to have positive nodes. Adjuvant chemotherapy is expected to positively impact survival, which can lead to a biased improved survival in patients undergoing ALND compared to those who did not (7,17,18). On the other hand, the NSABP B04 has demonstrated no improvement in survival with removal of occult axillary metastases (8). In addition, a meta-analysis of three large trials comparing axillary dissection *vs.* no dissection, found no improvement in overall survival, axillary recurrence or ipsilateral breast recurrence in axillary dissection groups (19).

The National Comprehensive Cancer Network (NCCN) has taken an early step toward reducing the number of axillary dissections for clinically negative axilla (version 3.2014). Patients with clinically negative axilla, who underwent lumpectomy and received no neoadjuvant chemotherapy, and were found to have less than three positive sentinel nodes and T1 or T2 tumor, have the option of forgoing completion axillary dissection, given that they will be proceeding with adjuvant radiotherapy.

In order to address this debate, a prospective and well-powered trial that places the benefits and adverse events on two arms of a scale is needed. Management of axillary nodes has been evolving in a logical, albeit slow, pattern; that is, towards minimizing long term complications, without compromising outcome.

In this regard, the AMAROS trial represents a landmark article that may potentially impact standard of care practices. Needless to say that longer follow up is needed for more robust conclusions.

Nevertheless, there still remain several inevitable questions that need to be addressed. First, can we forgo ASLN procedure in patients who have clinically node negative axilla? What about the infrequent patient that is found to have three or more positive sentinel nodes? And finally, can neoadjuvant systemic therapy eliminate the need for axillary dissection for clinically node positive patients who has good response. This is what the ongoing NSABP-B51/RTOG-1304 is designed to address (20).

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. van der Ploeg IM, Nieweg OE, van Rijk MC, et al. Axillary recurrence after a tumour-negative sentinel node biopsy in breast cancer patients: A systematic review and meta-analysis of the literature. Eur J Surg Oncol 2008;34:1277-84.
2. Veronesi U, Paganelli G, Viale G, et al. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. N Engl J Med 2003;349:546-53.
3. Mansel RE, Fallowfield L, Kissin M, et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. J Natl Cancer Inst 2006;98:599-609.
4. Fant JS, Grant MD, Knox SM, et al. Preliminary outcome analysis in patients with breast cancer and a positive sentinel lymph node who declined axillary dissection. Ann

Surg Oncol 2003;10:126-30.
5. Donker M, van Tienhoven G, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. Lancet Oncol 2014;15:1303-10.
6. Fisher B, Jeong JH, Anderson S, et al. Twenty-five-year follow-up of a randomized trial comparing radical mastectomy, total mastectomy, and total mastectomy followed by irradiation. N Engl J Med 2002;347:567-75.
7. Louis-Sylvestre C, Clough K, Asselain B, et al. Axillary treatment in conservative management of operable breast cancer: dissection or radiotherapy? Results of a randomized study with 15 years of follow-up. J Clin Oncol 2004;22:97-101.
8. Giuliano AE, McCall L, Beitsch P, et al. Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: the American College of Surgeons Oncology Group Z0011 randomized trial. Ann Surg 2010;252:426-32;discussion 432-3.
9. Galimberti V, Cole BF, Zurrida S, et al. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23-01): a phase 3 randomised controlled trial. Lancet Oncol 2013;14:297-305.
10. Schlembach PJ, Buchholz TA, Ross MI, et al. Relationship of sentinel and axillary level I-II lymph nodes to tangential fields used in breast irradiation. Int J Radiat Oncol Biol Phys 2001;51:671-8.
11. Reed DR, Lindsley SK, Mann GN, et al. Axillary lymph node dose with tangential breast irradiation. Int J Radiat Oncol Biol Phys 2005;61:358-64.
12. Kim T, Giuliano AE, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in early-stage breast carcinoma: a metaanalysis. Cancer 2006;106:4-16.
13. Mamounas EP, Tang G, Fisher B, et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. J Clin Oncol 2010;28:1677-83.
14. Mamounas EP. Timing of determining axillary lymph node status when neoadjuvant chemotherapy is used. Curr Oncol Rep 2014;16:364.
15. Boughey JC, Suman VJ, Mittendorf EA, et al. Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. JAMA 2013;310:1455-61.
16. Park J, Fey JV, Naik AM, et al. A declining rate of completion axillary dissection in sentinel lymph node-positive breast cancer patients is associated with the use of a multivariate nomogram. Ann Surg 2007;245:462-8.
17. Truong PT, Bernstein V, Wai E, et al. Age-related variations in the use of axillary dissection: a survival analysis of 8038 women with T1-ST2 breast cancer. Int J Radiat Oncol Biol Phys 2002;54:794-803.
18. Joslyn SA, Konety BR. Effect of axillary lymphadenectomy on breast carcinoma survival. Breast Cancer Res Treat 2005;91:11-8.
19. Sanghani M, Balk EM, Cady B. Impact of axillary lymph node dissection on breast cancer outcome in clinically node negative patients: a systematic review and meta-analysis. Cancer 2009;115:1613-20.
20. National Institutes of Health. Standard or Comprehensive Radiation Therapy in Treating Patients With Early-Stage Breast Cancer Previously Treated With Chemotherapy and Surgery. Available online: https://clinicaltrials.gov/ct2/show/NCT01872975

Cite this article as: Jacobson GM, Partin JF, Salkeni MA. Optimal management of sentinel lymph node positive biopsy patients in early breast cancer. Ann Transl Med 2015;3(7):87. doi: 10.3978/j.issn.2305-5839.2015.02.33

Should trastuzumab be administered concomitantly with anthracycline in women with early, HER2-positive breast cancer?

Filippo Montemurro

Unit of Investigative Clinical Oncology, Division of Medical Oncology, Fondazione del Piemonte per l'Oncologia, Candiolo Cancer Center (IRCCs), Candiolo 10060, Italy

Correspondence to: Filippo Montemurro, MD. Unit of Investigative Clinical Oncology, Division of Medical Oncology, Fondazione del Piemonte per l'Oncologia, Candiolo Cancer Center (IRCCs), Strada Provinciale 142, Km 3.95, Candiolo 10060, Italy. Email: filippo.montemurro@ircc.it.

Abstract: Clinical targeting of the human epidermal growth factor receptor 2 (HER2) has dramatically improved the outlook of a subset of about 15% of breast cancers carrying HER2 gene amplification and/or HER2 protein overexpression. Since the initial experiences with the anti-HER2 monoclonal antibody trastuzumab, it was clear that combination with conventional chemotherapy was required to exploit the full potential of HER2-targeting. However, prohibitive rates of cardiac toxicity were observed when trastuzumab was given concurrently with anthracyclines, which are compounds that have played a pivotal role in the treatment of breast cancer for decades. While most of the anti-HER2 programs have been designed as to avoid concomitance with anthracyclines, high rates of pathological complete remission (pCR) were obtained in carefully selected patients with operable breasts cancer receiving concomitant trastuzumab and anthracycline in the preoperative setting. A recently published randomized study compared directly the current standard of sequential anthracycline followed by concomitant taxane and trastuzumab with a reversed sequence of taxanes followed by anthracyclines with trastuzumab administered concurrently with the whole program as neoadjuvant treatment for patients with early, operable breast cancer. The practical question asked by this study was whether a potential increase in the efficacy of trastuzumab based regimens could be worth the risk of giving it in concomitance with anthracyclines. This editorial will review the background of this study and discuss the impact of its results on the current clinical practice and on future research in the field.

Keywords: Breast neoplasms; human epidermal growth factor receptor 2 (HER2); trastuzumab; pertuzumab; lapatinib; chemotherapy; anthracycline; metastatic; adjuvant; neo-adjuvant

Submitted Jun 01, 2014. Accepted for publication Jun 03, 2014.
doi: 10.3978/j.issn.2218-676X.2014.06.09
View this article at: http://dx.doi.org/10.3978/j.issn.2218-676X.2014.06.09

The monoclonal antibody trastuzumab, the first anti-human epidermal growth factor receptor 2 (HER2) therapy introduced in the clinic, has represented a major step forward in the treatment of breast cancer (1). *HER2* gene amplification, which almost invariably results in overexpression of its product, a transmembrane tyrosine-kinase receptor, is found in about 15% of breast cancers. This abnormality drives an aggressive clinical phenotype characterized, in the absence of specific targeting, by increased risk of relapse after surgery of localized disease, tendency to spread to distant organs with frequent visceral and central nervous system involvement, resistance to endocrine manipulation and short survival times in patients with metastatic disease (2). HER2-targeting with trastuzumab has resulted in a dramatic improvement in the life expectancy of women with metastatic disease and, with its introduction in adjuvant programs for operable disease, in a significant increase in cure rate. While newer anti HER2-agents are improving the clinical outlook of HER2-positive breast cancer patients beyond what was once hardly conceivable, much of the critical information on how to integrate anti HER2-therapy in the management

of these patient comes from the early experiences with trastuzumab. As single agent, trastuzumab showed modest activity in women with HER2-positive metastatic breast cancer (1). Response rates ranging from 15% to 35% were observed according to the load of previous treatments for metastatic disease. However, as preclinical studies suggested, and for reasons that are still not completely understood, the full potential of HER2-targeting with this monoclonal antibody could be exploited by combining it with conventional chemotherapy (3). This rationale was explored in a pivotal randomized trial that led to the approval of the monoclonal antibody trastuzumab (4). In this study, women with HER2 positive breast cancer were randomized to chemotherapy with or without concomitant trastuzumab as first line therapy for metastatic disease. The chemotherapy schema was different according to whether patients had received anthracyclines in the adjuvant setting. In case of no prior exposure, the chemotherapy schema consisted of AC (doxorubicin 60 mg/m^2 or epi-doxorubicin 75 mg/m^2 and cyclophosphamide 600 mg/m^2 every 3 weeks for six or more cycles). Those who had received an anthracycline in the adjuvant setting received paclitaxel at the dose of 175 mg/m^2 every 3 weeks for 6 or more cycles. This study provided evidence that trastuzumab could improve response rate, progression-free survival (PFS) and overall survival (OS), compared with chemotherapy alone. However, and unexpectedly, trastuzumab resulted associated with significant cardiac dysfunction, with a 27% incidence of left ventricular ejection fraction (LVEF) depression, including a 16% incidence of heart failure (New York Heart Association Class III and IV) in the anthracycline-containing arm. Notably, the corresponding figures in the AC alone arm were 8% and 3%, respectively. Although significantly less frequently, cardiac toxicity was observed also in the paclitaxel plus trastuzumab arm (overall 13%, NYHA class III and IV 2%). These findings prompted a systematic retrospective analysis of all the trastuzumab trials conducted at the time and research to elucidate the role of HER2 in cardiac function (5). Trastuzumab was confirmed to induce LVEF depression. However, differently from anthracycline-induced cardiomyopathy which is characterized by irreversible myofibrillar damage, trastuzumab effect was mostly reversible upon withdrawal of this antibody (5). Molecular biology studies revealed that HER2 is involved in embryonic cardiac development (6). Furthermore, the epidermal growth factor receptor (EGFR) family, which includes HER2, is involved in repairing the oxidative damage related to anthracycline exposure (7). Therefore, pharmacological inhibition of the physiological function of HER2 in the heart could account for the toxicity observed when anthracycline and trastuzumab were administered together (7). The lessons from these initial experiences have been carried forward over the years up to present time, having had a profound influence on the design of any anti HER2 therapeutic strategy. Screening for pre-existing cardiac conditions that could predispose patients to cardiac toxicity, avoidance of concomitance with anthracyclines, regular cardiac monitoring and proactive cardiac pharmacologic intervention to support LVEF are considered both in clinical trial design and in the current clinical practice, regardless of the anti HER2-compound used (8). Tackling trastuzumab-related cardiac toxicity was a relevant issue when trastuzumab was studied in the adjuvant setting, where anthracycline are an important component of the chemotherapy regimens. In fact, a meta-analysis of studies comparing anthracycline-based *vs.* cyclophosphamide, methotrexate, 5-fluorouracil (CMF)-like adjuvant treatments found that the major efficacy of the former regimens was restricted to women with HER2-positive breast cancer (9). Beyond strict cardiac inclusion criteria, trialists dealing with trastuzumab-based experimental arms used different approaches (8): administering trastuzumab after anthracyclines and concomitantly with taxanes, developing anthracycline-free adjuvant chemotherapy regimens, where trastuzumab could be administered concomitantly with the complete chemotherapy program, or administering trastuzumab after the completion of chemotherapy, regardless of the regimen used. Because partial or no overlap between trastuzumab and chemotherapy could be seen as a potential limitation to the full exploitation of trastuzumab-based therapy, a number of authors tried to evaluate the feasibility of anthracyclines, either conventional or liposomal, administered concomitantly with trastuzumab in the metastatic setting (10). While these experiences could not convincingly demonstrate the safety and convenience of these regimens, the results of a small study conducted ad M.D. Anderson Cancer Center caused quite a stir in the field (11). Women with HER2-positive operable breast cancer were randomized to neoadjuvant chemotherapy consisting of four cycles of paclitaxel (225 mg/m^2 as a 24-hour continuous infusion every 3 weeks) followed by four cycles of FEC_{75} (5-fluorouracil 500 mg/m^2, epi-doxorubicin 75 mg/m^2, and cyclophosphamide 500 mg/m^2 every 3 weeks for four cycles) with or without concomitant trastuzumab (24 weeks of treatment). The study was prematurely

Table 1 Selected neoadjuvant trials with anti HER2-therapy

Author	Study arms	N	pCR[1] rate (%)	LVEF drop >10% points to below an LVEF of 50%	CHF (%)
Buzdar (11)	T→FEC	19	25.0	26.00[2]	0
	TH→FECH	23	66.7	30.00[2]	0
Gianni (12)	AT→T→CMF	118	19.0	17.00[2]	0
	ATH→TH→CMF	117	38.0	27.00[2]	2.0
Untch (13)	ECH→DH	308	30.2	0.40	2.6
	ECL→DL	307	44.6	1.40	0.3
Guarneri (14)	TH→FECH	36	25.0	3.00	0
	TL→FECL	36	26.3	0	0
	THL→FECHL	46	46.7	0	0
Buzdar (15)	FEC-TH	138	56.5[3]	7.90[4]	0
	TH-FECH	142	54.2[3]	10.60[4]	0
Schneeweiss (16)	FECHP→DHP	72	50.7	5.60	0
	FEC→DHP	75	45.3	5.30	2.7
	DCHP	76	51.9	3.90	0
Ismael (17)	DH→FECH	263	34.2	2.12	0
	DscH→FECscH	260	39.2	2.40	0.75
Baselga (18)	TH	149	27.6	0.60	0
	TL	154	20.0	0.60	0
	THL	152	46.8	0.60	0
Gianni (19)	DH	107	21.5	0.90	
	DHP	107	39.3	2.80	0
	HP	107	11.2	0.90	1.0
	DP	96	17.7	1.00	0

[1], no residual disease in the breast and the axilla; [2], overall incidence of LVEF drop of >10 percentage points; [3], pCR defined as no residual invasive disease in the breast; [4], as reported by the cardiac review panel; [5], one case of New York Heart Association grade II CHF. pCR, pathological complete remission; LVEF, left ventricular ejection fraction; CHF, congestive heart failure; T, paclitaxel; FEC, 5-fluorouracil, epirubicin and cyclophosphamide; H, trastuzumab; A, doxorubicin; CMF, cyclophosphamide, methotrexate and 5-fluorouracil; L, lapatinib; D, docetaxel, P, pertuzumab; scH, subcutaneous trastuzumab.

closed after the accrual of just 42 patients because of an unprecedented rate of pCR in the "all concomitant" trastuzumab and chemotherapy arm of 66.7%, compared with 25% in the chemotherapy alone arm (*Table 1*). Subsequent follow-up of patients treated with that regimen showed a high rate of freedom from disease progression (20). Most importantly, authors did not find a strong signal towards cardiac toxicity. After the MD. Anderson study other groups studied concomitant regimens confirming high rates of pCR and an acceptable profile of cardiac toxicity (*Table 1*) (12-14,16,17). Furthermore, a small, but provocative study from Finland used an "all concomitant" adjuvant regimen of taxanes or vinorelbine followed by FEC and trastuzumab given for 9 weeks only (21). Hazard ratios for event-free survival (EFS) and OS were in the same range of those reported in the registration trials, where trastuzumab was not combined with anthracycline and given in general for one year. Also in this case, no signal for increased cardiac toxicity was observed in the trastuzumab arm of the study.

These results suggested revisiting the concept of full concomitance between trastuzumab and anthracycline-based therapy, as an approach to optimize the efficacy of trastuzumab in the setting of early breast cancer. One important consideration regarding the cardiac concerns associated with trastuzumab treatment is that patients in

small trials of neoadjuvant chemotherapy may represent a selected population that only partially overlaps with the "real world" patients. Population based studies reveal that, because of frequent cardiac conditions which, by themselves, do not represent a contraindication to treatment, the incidence of cardiac toxicity despite all the precautions is higher than that reported in clinical trials (22). For all these reasons, whether increasing the potential efficacy of trastuzumab based regimens is worth the risk of giving it in concomitance with anthracyclines has remained an open issue until recently. A randomized study from the Institution that first showed the potentiality of the "all concomitant" approach has provided a convincing response (15). The Z1041 trial randomized a total of 282 women with early, HER2-positive breast cancer to either a sequential arm of four cycles of FEC_{75} followed by weekly paclitaxel (80 mg/m^2/week for 12 weeks) plus 12 weekly administrations of trastuzumab (4 mg/kg loading dose, followed by weekly doses of 2 mg/kg) or to a concomitant arm of weekly paclitaxel (same schedule as above) followed by four cycles of FEC_{75} with weekly trastuzumab started with paclitaxel and administered for a total of 24 weeks. Upon completion of treatment patients were schedule to undergo surgery and, then, advised to continue trastuzumab for up to one year. The primary study end-point was pCR in the breast and the study was powered on the hypothesis that the concomitant schedule could increase the pCR rate by 20% or more, from an expected 25% in the sequential arm. Patients were meticulously selected on the basis of strict cardiac criteria, including no history of myocardial infarction, congestive heart failure (CHF), cardiomyopathy, or cardiac disease requiring drug treatment; severe conduction abnormality, valvular disease, cardiomegaly, ventricular hypertrophy on electrocardiography, or poorly controlled hypertension. The striking finding of this trial was that doubling the duration of trastuzumab and giving it in full concomitance with a taxane and anthracycline-based chemotherapy yielded a high pCR that was similar to that achieved by patients in the more conventional sequential arm, were trastuzumab duration was just a half (12 weeks) (*Table 1*). In fact, pCR rate in the latter arm was largely higher than expected. The study also confirmed the cardiac feasibility of trastuzumab with anthracyclines, with caveats to consider regarding the selection of patients (see above). The results of the Z1041 trial mirror those of the previously published TRYPHAENA trial (*Table 1*) (17), where HER2-inhibition consisted of trastuzumab and the other anti HER2 monoclonal antibody pertuzumab and confirm that whether anti HER2 therapy should be administered in full concomitance with a sequence of anthracycline and taxanes is no longer an issue. On a more general level, they also suggest that further manipulation of the classical ingredients of the HER2-therapy recipe (anthracycline, taxanes, concomitance and duration) will hardly result in an increase in pCR, and possibly, in cure rate. In fact, two studies using single agent chemotherapy and double HER2 targeting with either the tyrosine-kinase inhibitor lapatinib or pertuzumab showed impressive rates of pCR obtained after just 16 weeks of treatment (*Table 1*). In those two studies, an anthracycline-based regimen was administered after surgery, before resuming HER2-targeting. A relevant scientific issue would be assessing the role of further anthracycline therapy in those patients achieving pCR in the neoadjuvant setting. In fact, not all patients who achieve a pCR show long-term EFS, but for a proportion of them anthracycline and their fearsome general and cardiac toxicity could be possibly avoided. On the other hand, as a slightly better DFS and OS favouring AC followed by docetaxel plus trastuzumab over the anthracycline-free docetaxel, carboplatin and trastuzumab was observed in the Breast Cancer International Research Group (BCIRG) 006 trial (23). This suggests the existence of a subset of HER2 positive tumors that may be better cured with anthracycline-containing regimens combined with HER2 targeting.

For now, clinicians managing operable HER2-breast cancer patients at risk of relapse are reinforced in their prescribing patterns by the results of the Z1041 study. However, the real challenge, especially in the era of multiple HER2-targeting strategies, is to tailor treatment intensity to clinical and biological features of the tumor. There is increasing recognition that breast cancer in general, and HER2 positive breast cancer in particular can be further grouped on the basis of their molecular heterogeneity (24). Deciphering this heterogeneity has several obvious implications in the process of optimizing the toxicity/benefit and cost/effectiveness ratios of treatment for HER2-positive breast cancer. In this respect, considering that adjuvant anti HER2 therapy is increasingly offered to patients with small, node-negative, HER2 positive tumors, depotentiation of the chemotherapy component of programs should became a major focus for research. Once again, however, translational research is called into cause in the eternal quest for biomarkers that could help stratify patients according to the likelihood to derive benefit from a specific treatment approach. Unfortunately, the jury is still out for the most promising candidate biomarker of potential

sensitivity to anthracycline, the topoisomerase 2 gene status or protein expression (25). Yet, further research in this direction should be pursued to define those patients for whom anthracycline could be safely omitted and those, conversely, for whom these drugs still represent a life-saving option.

Acknowledgements

None.

Footnote

Conflicts of Interest: I am member of the speaker's bureau for Hoffmann La Roche S.p.A, and consultant and member of the speaker's bureau for GlaxoSmithKline S.p.A. The author declares no conflict of interest.

References

1. Montemurro F, Valabrega G, Aglietta M. Trastuzumab-based combination therapy for breast cancer. Expert Opin Pharmacother 2004;5:81-96.
2. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177-82.
3. Pegram M, Hsu S, Lewis G, et al. Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene 1999;18:2241-51.
4. 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.
5. Telli ML, Hunt SA, Carlson RW, et al. Trastuzumab-related cardiotoxicity: calling into question the concept of reversibility. J Clin Oncol 2007;25:3525-33.
6. Lee KF, Simon H, Chen H, et al. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature 1995;378:394-8.
7. Crone SA, Zhao YY, Fan L, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. Nat Med 2002;8:459-65.
8. Rossi V, Redana S, Milani A, et al. Trastuzumab in the adjuvant setting: a practical review. Therapy 2011;8:161-77.
9. Gennari A, Sormani MP, Pronzato P, et al. HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: a pooled analysis of randomized trials. J Natl Cancer Inst 2008;100:14-20.
10. Rayson D, Richel D, Chia S, et al. Anthracycline-trastuzumab regimens for HER2/neu-overexpressing breast cancer: current experience and future strategies. Ann Oncol 2008;19:1530-9.
11. Buzdar AU, Ibrahim NK, Francis D, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 2005;23:3676-85.
12. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 2010;375:377-84.
13. Untch M, Loibl S, Bischoff J, et al. Lapatinib versus trastuzumab in combination with neoadjuvant anthracycline-taxane-based chemotherapy (GeparQuinto, GBG 44): a randomised phase 3 trial. Lancet Oncol 2012;13:135-44.
14. Guarneri V, Frassoldati A, Bottini A, et al. Preoperative chemotherapy plus trastuzumab, lapatinib, or both in human epidermal growth factor receptor 2-positive operable breast cancer: results of the randomized phase II CHER-LOB study. J Clin Oncol 2012;30:1989-95.
15. Buzdar AU, Suman VJ, Meric-Bernstam F, et al. Fluorouracil, epirubicin, and cyclophosphamide (FEC-75) followed by paclitaxel plus trastuzumab versus paclitaxel plus trastuzumab followed by FEC-75 plus trastuzumab as neoadjuvant treatment for patients with HER2-positive breast cancer (Z1041): a randomised, controlled, phase 3 trial. Lancet Oncol 2013;14:1317-25.
16. Schneeweiss A, Chia S, Hickish T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). Ann Oncol 2013;24:2278-84.
17. Ismael G, Hegg R, Muehlbauer S, et al. Subcutaneous versus intravenous administration of (neo)adjuvant trastuzumab in patients with HER2-positive, clinical stage I-III breast cancer (HannaH study): a phase 3, open-label, multicentre, randomised trial. Lancet Oncol 2012;13:869-78.
18. Baselga J, Bradbury I, Eidtmann H, et al. Lapatinib

with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 2012;379:633-40.
19. Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25-32.
20. Buzdar AU, Valero V, Ibrahim NK, et al. Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. Clin Cancer Res 2007;13:228-33.
21. Joensuu H, Bono P, Kataja V, et al. Fluorouracil, epirubicin, and cyclophosphamide with either docetaxel or vinorelbine, with or without trastuzumab, as adjuvant treatments of breast cancer: final results of the FinHer Trial. J Clin Oncol 2009;27:5685-92.
22. Bowles EJ, Wellman R, Feigelson HS, et al. Risk of heart failure in breast cancer patients after anthracycline and trastuzumab treatment: a retrospective cohort study. J Natl Cancer Inst 2012;104:1293-305.
23. Slamon D, Eiermann W, Robert N, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011;365:1273-83.
24. Montemurro F, Di Cosimo S, Arpino G. Human epidermal growth factor receptor 2 (HER2)-positive and hormone receptor-positive breast cancer: new insights into molecular interactions and clinical implications. Ann Oncol 2013;24:2715-24.
25. Press MF, Sauter G, Buyse M, et al. Alteration of topoisomerase II-alpha gene in human breast cancer: association with responsiveness to anthracycline-based chemotherapy. J Clin Oncol 2011;29:859-67.

Cite this article as: Montemurro F. Should trastuzumab be administered concomitantly with anthracycline in women with early, HER2-positive breast cancer? Transl Cancer Res 2014;3(6):541-546. doi: 10.3978/j.issn.2218-676X.2014.06.09

Pertuzumab in metastatic breast cancer: unanswered questions

Ana Elisa Lohmann[1], Kamal S. Saini[2], Otto Metzger-Filho[3]

[1]Breast Fellow, Department of Medical Oncology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; [2]Scientific Co-Director, Breast International Group, Jules Bordet Institute, Brussels, Belgium; [3]Research Fellow, Breast Oncology Center, Dana-Farber Cancer Institute, Boston, USA
Correspondence to: Ana Elisa Lohmann, MD. BC Cancer Agency, 600 W, 10th Avenue, Vancouver, Canada, V5Z4E6.
Email: ana.lohmann@bccancer.bc.ca.

Submitted Mar 29, 2012. Accepted for publication Apr 25, 2012.
doi: 10.3978/j.issn.2218-676X.2012.04.03
View this article at: http://tcr.thepbpc.org/article/view/380/743

Recently, Baselga and colleagues published in *New England Journal of Medicine* the analyses of the Clinical Evaluation of Pertuzumab and Trastuzumab Study (CLEOPATRA) (1). CLEOPATRA is a phase III trial in 808 patients with human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer receiving first line therapy with docetaxel and trastuzumab with placebo or pertuzumab until disease progression or unacceptable toxicity.

After 19.3 months of median follow-up (during which 165 events occurred), the interim results showed that the addition of pertuzumab improved progression-free-survival (PFS) by 6 months in comparison with placebo arm (18.5 versus 12.4 months; hazard ratio [HR] for progression 0.62; 95% CI 0.51 to 0.75; P<0.001). Among patients with measurable disease, overall response rate was significantly higher with pertuzumab (80.2% versus 69.3%, 95% CI 4.2 to17.5; P=0.001) with manageable toxicity. Even though the improvement in overall survival (OS) was not statistically significant, there was a trend toward prolonged survival with pertuzumab (HR=0.64, 95% CI 0.47 to 0.88; P=0.005; but it did not meet the O'Brien-Fleming stopping boundary of the Lan-DeMets alpha spending function) (1).

The addition of pertuzumab was associated with increased incidence of diarrhea, rash, mucosal inflammation, febrile neutropenia and dry skin. Interestingly, febrile neutropenia was more common in patients from Asia (26% in the pertuzumab group and 12% in the control group) than from other regions (approximately 10% in both groups). On the other hand, the toxicity observed more often in the control arm was edema and constipation. The incidence of cardiac toxicities was comparable between treatment arms. Finally, there was no difference in treatment related death (1).

In the last decade, many new drugs have demonstrated activity in metastatic breast cancer, with the anti-HER2 therapies seemingly having the greatest impact on survival (2,3). Approximately 20-25% of early-stage breast cancers over-express HER2 and are associated with poor outcome (4). This subtype of breast cancer is currently treated with a combination of chemotherapy and HER2-targeted agents, and a significant increase in OS has been noted since the introduction of these targeted agents. Trastuzumab, a monoclonal antibody directed against domain IV of the HER2 receptor, and lapatinib, a small molecule tyrosine kinase inhibitor binding both HER1 and HER2, are currently available for clinical use in combination with chemotherapy or hormotherapy in metastatic breast cancer (3,5,6).

Primary and secondary resistance to anti-HER2 therapies is frequently encountered in the metastatic setting, underscoring the need to identify new targeted treatments for advanced disease. Many mechanisms of resistance have been proposed, including HER2 crosstalk with other HER members or insulin-like growth factor-1 receptor, and increased phosphatidylinositol 3-kinase (PI3K)/Akt pathway activation due to PTEN deficiency or *PIK3CA* activating mutations (7).

Several new therapeutic agents are currently in development, including pertuzumab, a human monoclonal antibody that binds to domain II of the HER2 receptor and inhibits the ligand-dependent dimerization and signaling of HER2 (8). Given the fact that pertuzumab and trastuzumab bind different epitopes on the HER2 receptor, the two antibodies are thought to be complementary in action. A phase II trial in which the combination of trastuzumab and pertuzumab was given (without chemotherapy) to patients who had progressed on trastuzumab, showed an objective response rate of 24% and acceptable toxicity (9). In the neoadjuvant

NeoSphere study, the addition of pertuzumab to docetaxel and trastuzumab significantly increased pathological complete response rate compared to trastuzumab plus docetaxel alone (45.8% versus 29.0% respectively, P=0.0141) (10).

BIG 4-11 (APHINITY) is an ongoing large, randomized phase III, double-blind, placebo-controlled study comparing the efficacy and safety of chemotherapy plus trastuzumab and placebo with that of chemotherapy plus trastuzumab and pertuzumab as adjuvant therapy in patients with operable, HER2-positive, primary breast cancer (11). It is expected to enroll 3806 patients from about 44 countries worldwide.

While pertuzumab has shown impressive activity and acceptable toxicity in studies conducted so far, it is crucial to consider some additional points. Firstly, in the interim analysis of the CLEOPATRA trial, although there was a trend toward improvement in OS, the actual OS did not reach statistical significance. The final OS analysis is eagerly awaited, and could be available in 2013. Secondly, the economic impact on the healthcare system of administering two targeted therapies concomitantly should be carefully evaluated. Thirdly, results of other new anti-HER2 agents, such as trastuzumab-DM1 (antibody-drug conjugate), neratinib, and afatinib (both HER2 tyrosine kinase inhibitors) etc are expected in the near future, and it will be a challenge to know how best to optimally use the many available anti-HER2 therapeutic options (12-14). Fourthly, long-term safety needs to be robustly established. Finally, it is extremely important to identify new biomarkers in order to prospectively select those patients that are expected to derive benefit from targeted agents.

The development of many new anti-HER2 molecules in the last two decades have lead to a paradigm shift in the treatment of this subgroup of patients and has vastly improved their clinical outcome. Pertuzumab may turn out to be another effective option for these patients. However the molecular biology, drug resistance, cost effectiveness, and drug side effects are not yet fully understood. HER2-positive breast cancer has been intensively studied in the recent but it continues to be an intriguing subtype.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Baselga J, Cortés J, Kim SB, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012;366:109-19.
2. Perez EA, Romond EH, Suman VJ, et al. Four-Year Follow-Up of Trastuzumab Plus Adjuvant Chemotherapy for Operable Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer: Joint Analysis of Data From NCCTG N9831 and NSABP B-31. J Clin Oncol 2011;29:3366-73.
3. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006;355:2733-43.
4. Pegram MD, Pauletti G, Slamon DJ. HER-2/neu as a predictive marker of response to breast cancer therapy. Breast Cancer Res Treat 1998;52:65-77.
5. Johnston S, Pippen J, Pivot X, et al. Lapatinib Combined With Letrozole Versus Letrozole and Placebo As First-Line Therapy for Postmenopausal Hormone Receptor–Positive Metastatic Breast Cancer. J Clin Oncol 2009;27:5538-46.
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. Pohlmann PR, Mayer IA, Mernaugh R. Resistance to Trastuzumab in Breast Cancer. Clin Cancer Res 2009;15:7479-91.
8. Saini KS, Azim HA Jr, Metzger-Filho O, et al. Beyond trastuzumab: new treatment options for HER2-positive breast cancer. Breast 2011;20:S20-S27.
9. Baselga J, Gelmon KA, Verma S, et al. Phase II Trial of Pertuzumab and Trastuzumab in Patients With Human Epidermal Growth Factor Receptor 2–Positive Metastatic Breast Cancer That Progressed During Prior Trastuzumab Therapy. J Clin Oncol 2010;28:1138-44.
10. Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25-32.
11. A Study of Pertuzumab in Addition to Chemotherapy and Herceptin (Trastuzumab) as Adjuvant Therapy in Patients With HER2-Positive Primary Breast Cancer. Available

online:http://clinicaltrials.gov/ct2/show/NCT01358877
12. Burris HA, Rugo HS, Vukelja SJ, et al. Phase II Study of the Antibody Drug Conjugate Trastuzumab-DM1 for the Treatment of Human Epidermal Growth Factor Receptor 2 (HER2) –Positive Breast Cancer After Prior HER2-Directed Therapy. J Clin Oncol 2011;29:398-405.
13. Burstein HJ, Sun Y, Dirix LY, et al. Neratinib, an Irreversible ErbB Receptor Tyrosine Kinase Inhibitor, in Patients With Advanced ErbB2-Positive Breast Cancer. J Clin Oncol 2010;28:1301-7.
14. Lin NU, Winer EP, Wheatley D, et al. A phase II study of afatinib (BIBW 2992), an irreversible ErbB family blocker, in patients with HER2-positive metastatic breast cancer progressing after trastuzumab. Breast Cancer Res Treat 2012. [Epub ahead of print].

Cite this article as: Lohmann AE, Saini KS, Metzger-Filho O. Pertuzumab in metastatic breast cancer: unanswered questions. Transl Cancer Res 2012;1(2):122-124. doi: 10.3978/j.issn.2218-676X.2012.04.03

Pertuzumab: a step forward in treating HER2-positive breast cancer

Dimitrios Mavroudis

Department of Medical Oncology, University Hospital of Heraklion, University of Crete, Greece

Correspondence to: Dimitrios Mavroudis, MD. Department of Medical Oncology, University Hospital of Heraklion, University of Crete, Greece. Email: mavrudis@med.uoc.gr.

Submitted Mar 04, 2012. Accepted for publication Mar 27, 2012.
doi: 10.3978/j.issn.2218-676X.2012.03.05
View this article at: http://tcr.thepbpc.org/article/view/377/739

HER2-positive breast cancer represents an aggressive subtype of the disease occurring in approximately 20% of patients. While trastuzumab has revolutionized the treatment of HER2-positive breast cancer, a proportion of patients have *de novo* trastuzumab-resistant disease and even those who initially respond will eventually develop trastuzumab-resistance. The recent publication in the *New England Journal of Medicine* of the promising results of the CLEOPATRA trial for the combination of pertuzumab plus trastuzumab plus docetaxel in HER2-positive metastatic breast cancer (MBC) represented a significant advancement in the treatment of this illness (1).

Pertuzumab (formerly called Omnitarg or 2C4) is a humanized monoclonal antibody that targets a different extracellular domain of the HER2 receptor than does trastuzumab and inhibits heterodimerization of HER2 with HER1 and especially with HER3 which is a more critical partner for HER2 pathway activation (2). In a seminal publication it was shown that the combination of trastuzumab and pertuzumab induced increased apoptosis in the HER2-overexpressing BT474 breast cancer cell line via reduced levels of total and phosphorylated HER-2 protein and blocked receptor signalling through Akt (3). Moreover, in a mouse HER2-positive xenograft model, the combination was synergistic with anti-estrogen therapy and gefitinib, an EGFR tyrosine kinase inhibitor, in delaying tumor progression (4). Based on these results the combination of pertuzumab and trastuzumab was initially tested in a phase II study in women with HER2-positive MBC that had progressed during prior trastuzumab therapy; an encouraging objective response rate of 24% and a clinical benefit rate of 50% were observed thus verifying that the combination of the two monoclonal antibodies was active in trastuzumab-resistant disease (5). This effect was primarily due to the synergistic action of both antibodies since pertuzumab alone had minimal activity in this setting (6).

The therapeutic value of pertuzumab plus trastuzumab in combination with docetaxel chemotherapy was evaluated in a randomized, double-blind, placebo-controlled phase III study and compared with the "standard" regimen of docetaxel plus trastuzumab as first-line treatment in patients with HER2-positive MBC (1). The study was powered to detect a 33% improvement in independently-assessed median progression-free survival (PFS) in the pertuzumab group as the primary endpoint. Four hundred patients were randomized on each arm according to geographic region and prior therapy in the adjuvant or neoadjuvant setting. Surprisingly, only half of patients had prior exposure to chemotherapy and 10% had received trastuzumab as adjuvant or neoadjuvant treatment. At least six cycles of docetaxel were administered while the monoclonals were continued until disease progression. After a median follow up period of 19 months in both groups, there was a 6.1 month improvement in independently-assessed PFS in the pertuzumab group with 38% reduction in the odds of disease progression or death ($P<0.001$). This effect was observed across all predefined subgroups including the small subgroup of patients who had previously received trastuzumab with chemotherapy as adjuvant or neoadjuvant treatment. In the interim analysis of overall survival there was a strong trend toward a survival benefit as well. The objective response rate, which was

a secondary endpoint, was also 10% higher for the pertuzumab group. All these improvements of the efficacy endpoints came at a low price of increased toxicity. Although several side effects such as rash, mucositis, diarrhea, febrile neutropenia were more common, only the latter two of grade 3 or above were increased with pertuzumab. Additional cardiac toxicity was not observed with pertuzumab despite a very close monitoring.

To put the results of this trial into perspective we should consider that response to trastuzumab-based therapy of HER2-positive breast cancer is indeed variable and may depend on the high or low levels of HER2 homodimers (7). For those patients with HER2 pathway activation primarily due to ligand binding, the formation of HER2-HER3 heterodimers is critical (2) and can be effectively inhibited by the co-administration of pertuzumab (1). This has now been proven in MBC and also validated in the neoadjuvant setting with the recently published NeoSphere trial where the co-administration of pertuzumab plus trastuzumab with docetaxel chemotherapy achieved a significantly improved pathologic complete response rate compared to either monoclonal alone (8). Taken together, it appears that the more comprehensive blockade of HER2 with the two antibodies has the potential to improve survival of HER2-positive breast cancer and represents once more a paradigm shift in the treatment of this disease.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

Cite this article as: Mavroudis D. Pertuzumab: a step forward in treating HER2-positive breast cancer. Transl Cancer Res 2012;1(2):117-118. doi: 10.3978/j.issn.2218-676X.2012.03.05

References

1. Baselga J, Cortés J, Kim SB, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012;366:109-19.
2. Lee-Hoeflich ST, Crocker L, Yao E, et al. A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. Cancer Res 2008;68:5878-87.
3. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. Cancer Res 2004;64:2343-6.
4. Arpino G, Gutierrez C, Weiss H, et al. Treatment of human epidermal growth factor receptor 2-overexpressing breast cancer xenografts with multiagent HER-targeted therapy. J Natl Cancer Inst 2007;99:694-705.
5. Baselga J, Gelmon KA, Verma S, et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. J Clin Oncol 2010;28:1138-44.
6. Cortés J, Fumoleau P, Bianchi GV, et al. Pertuzumab Monotherapy After Trastuzumab-Based Treatment and Subsequent Reintroduction of Trastuzumab: Activity and Tolerability in Patients With Advanced Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer. J Clin Oncol 2012;30:1594-600.
7. Ghosh R, Narasanna A, Wang SE, et al. Trastuzumab has preferential activity against breast cancers driven by HER2 homodimers. Cancer Res 2011;71:1871-82.
8. Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25-32.

Bevacizumab: Where do we go from here in breast cancer?

Faina Nakhlis, Mehra Golshan

Department of Surgery Brigham and Women's/Faulkner Hospital and Dana Farber Cancer Institute; Harvard Medical School: Boston, MA, USA
Correspondence to: Mehra Golshan, MD, FACS. Department of Surgery; Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA. Tel: 6176322174; Fax: 6175827740. Email: mgolshan@partners.org.

Submitted Mar 08, 2012. Accepted for publication Mar 15, 2012.
doi: 10.3978/j.issn.2218-676X.2012.03.01
View this article at: http://tcr.thepbpc.org/article/view/374/723

Bevacizumab is a monoclonal antibody against circulating vascular endothelial growth factor A, which received accelerated FDA approval in 2008 for first line treatment of HER2-negative metastatic breast cancer. The initial approval was based on studies suggesting a progression-free survival and not overall survival. This observation served as the basis for looking into the use of bevacizumab as an adjunct to neoadjuvant chemotherapy in the treatment of HER2-negative breast cancer.

In the January 2012 issue of the NEJM Bear *et al*. reported the results of the NSABP B-40 trial in which 1,206 patients with clinical T1c-T3, N0-N2, M0 HER2-negative tumors were randomized to receive one of three neoadjuvant chemotherapy regimens (regimen 1: docetaxel, 100 mg/m^2 on day 1; regimen 2: docetaxel, 75 mg/m^2 on day one plus capecitabine, 825 mg/m^2 on days 1 and 14; regimen 3: docetaxel, 75 mg/m^2 on day one plus gemcitabine, 1,000 mg/m^2 on days 1 and 8; all given for four cycles, followed by 4 cycles of doxorubicin-cyclophosphamide) (1). Within each treatment arm patients were further randomized to receive or not to receive bevacizumab, 15 mg/m^2, for the first 6 cycles of chemotherapy. The primary end-point was pathologic complete response in the breast; the secondary end-points were clinical complete response after completion of docetaxel portion of chemotherapy and at the completion of the entire neoadjuvant therapy regimen, pathologic complete response in the breast and the lymph nodes and incidence of the New York Heart Association (NYHA) class III or IV congestive heart failure and of other cardiac events.

The study demonstrated that the addition of neither capecitabine nor gemcitabine contributed to an improvement of the pathologic complete response rate (29.7% and 31.8%, respectively, *vs*. 32.7%; P=0.69). However, the addition of bevacizumab significantly increased the rate of pathologic complete response in the breast alone (28.2% *vs*. 34.5%, P=0.02). Although statistically significant this benefit was modest and not seen when the breast and axilla response were combined. This came at the cost of increased toxic side effects. In the same issue a companion article by von Minckwitz et al. reporting for the GeparQuinto trial showed benefit in the hormone-receptor negative population as opposed to Bear *et al*., reporting its greatest benefit in the hormone receptor positive cancers (2). The role of bevacizumab in the treatment of breast cancer remains to be fully elucidated. There may be a subset of patients who would benefit from bevacizumab in the metastatic, neoadjuvant or adjuvant setting. Unfortunately current data do not give guidance to the group that may benefit the most.

The results from the GeparQuinto study were initially presented at the 33rd San Antonio Breast Cancer Symposium in December of 2010 and at the Annual Meeting of the American Society of Clinical Oncology (ASCO) in June 2011, while the NSABP B-40 trial results were presented I at the June 2011 ASCO meeting as well. These studies were subsequently published in January of 2012 in the NEJM, following a crucial recommendation issued by the Food and Drug Administration (FDA) on November 18th 2011 to revoke the agency's previous accelerated approval of bevacizumab for the treatment of breast cancer. The basis for this decision stems from the toxicity data on the drug in the setting of metastatic breast cancer, which demonstrate that its risks, some of which are potentially life-threatening, are outweighed the drug's limited beneficial impact on disease-free and overall

survival. While the results of the two studies suggest that bevacizumab may have a role in the treatment of HER2-negative breast cancer in the neoadjuvant setting, these data are unlikely to find clinical application in the US at this time due to the afore-mentioned decision by the FDA, but it remains to be seen with maturation of data from these trials and others that are nearing completion will result in any appeals to the FDA to reverse its decision to revoke bevacizumab's approval.

Acknowledgements

None.

Cite this article as: Nakhlis F, Golshan M. Bevacizumab: Where do we go from here in breast cancer? Transl Cancer Res 2012;1(1):55-56. doi: 10.3978/j.issn.2218-676X.2012.03.01

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Bear HD, Tang G, Rastogi P, et al. Bevacizumab added to neoadjuvant chemotherapy for breast cancer. N Engl J Med 2012;366:310-20.
2. von Minckwitz G, Eidtmann H, Rezai M, et al. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. N Engl J Med 2012;366:299-309.

Lapatinib as a therapeutic option in brain metastases from HER2+ breast cancer

Maurie Markman

Cancer Treatment Centers of America, Philadelphia, PA, USA

Correspondence to: Maurie Markman, M.D. Eastern Regional Medical Center, 1331 East Wyoming Avenue Philadelphia, PA 19124, USA. Email: maurie.markman@ctca-hope.com.

Submitted Dec 23, 2012. Accepted for publication Jan 04, 2013.
doi: 10.3978/j.issn.2224-5820.2013.01.01
View this article at: http://www.amepc.org/apm/article/view/1428/2049

The treatment of brain metastases is one of the most challenging management issues in solid tumor oncology. In addition to the well-recognized problem with the adequacy of drug delivery to the central nervous system, even moderate increases in tumor size in this closed space can result in devastating symptoms and substantially shorten survival.

The major treatment modality for brain metastasis from solid tumors has been external beam radiation, although in some circumstances (e.g., single metastatic lesion in a patient with a good performance status) surgical resection is a reasonable option or even the favored strategy. Unfortunately, while radiation is frequently quite effective in providing short-term palliation of distressing symptoms, the modality can also result in neuro-cognitive dysfunction. In addition, the benefits from central nervous system radiation are generally only modest in duration with most patients progressing after a fairly limited period of stabilization of the disease process.

The development of brain metastases in patients with advanced breast cancer is a relatively common event. In particular, as many as one-half of women with HER2 positive metastatic disease will be found to have developed brain metastases during the course of their illness.

It is uncertain whether this high substantial risk of metastatic disease in the brain is due to the relative lack of activity of the available anti-neoplastic agents against breast cancer within the brain (including cytotoxic chemotherapy and trastuzumab), the relatively poor penetration of such agents into brain tissue, or may actually be somewhat of an artifact of the longer survival being experienced by patients due to the increasing effectiveness of therapy in non-brain sites. As a result, patients with HER2 positive metastatic breast cancer will have a greater opportunity to develop both symptomatic and asymptomatic brain metastases.

Limited prior experience had revealed modest activity for lapatinib again metastatic breast cancer within the brain in a setting where radiation had been previously delivered to this site. These data led to the initiation of a prospective phase 2 trial examining the combination of lapatinib with capecitabine in patients with HER2 positive breast cancer metastatic to the brain where brain radiation had not been previously delivered (1).

Patients received lapatinib (1,250 mg daily) and capecitabine (2,000 mg/m^2 day 1-14 in a 21 day cycle). An objective response was defined as at least a 50% reduction in the total volume of the central nervous system metastatic lesions. To be declared a response, steroid use was not permitted and there could be no deterioration in the neurological status. Further, the response had to last at least 4 weeks and there could be no evidence of progression outside the central nervous system.

A total of 44 patients were eligible for an evaluation of response to this treatment regimen, of which 66% (29 patients) achieved a partial response (no complete response observed). Overall, 84% of the patient population exhibited some reduction in their tumor volume within the brain compared to the baseline determination.

The median follow-up in this patient population was 21 months. The median time to disease progression for the entire population was 5.5 months, with the time to progression in the responding population (median: 6 months) being superior to the group who failed to respond (median: 2.8 months). As anticipated, the most common site of initial progression was the central nervous system (78% of

patients). The median survival for the treated population was 17 months, with 91% of patients surviving for at least 6 months. Finally, the median time to subsequent radiation was 8.3 months, with the majority of patients (82%) ultimately requiring brain radiation.

Side effects were common with this therapeutic program, particularly diarrhea and the "hand-foot syndrome". Approximately one-third of the treated population experienced at least one serious adverse event. Fortunately, there were no deaths felt to be caused by the program of lapatinib and capecitabine.

As this was a non-randomized phase 2 trial it remains uncertain if the activity observed in this trial is superior to what would have been achieved if these patients had been treated with primary whole brain radiation, perhaps with the addition of chemotherapy or lapatinib. However, it is certainly fair to label these results as being quite interesting in that more than one-half of the treated population appeared to exhibit an element of genuine clinical benefit. In addition, the data certainly support the hypothesis that the anti-cancer drug therapy achieved sufficient concentrations within the central nervous system to produce both a biologic and clinical effect.

A phase 3 trial is planned, comparing this strategy to whole brain radiation therapy, which will hopefully provide a definitive answer regarding the role of lapatinib as a primary strategy for the management of metastatic HER2 positive breast cancer within the brain.

Cite this article as: Markman M. Lapatinib as a therapeutic option in brain metastases from HER2+ breast cancer. Ann Palliat Med 2013;2(1):35-36. doi: 10.3978/j.issn.2224-5820.2013.01.01

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Bachelot T, Romieu G, Campone M, et al. Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. Lancet Oncol 2013;14:64-71.

The ALTERNATE trial: assessing a biomarker driven strategy for the treatment of post-menopausal women with ER+/Her2– invasive breast cancer

Vera Jean Suman[1], Matthew J. Ellis[2], Cynthia X. Ma[3]

[1]Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA; [2]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX, USA; [3]Division of Oncology, Siteman Cancer Center, Washington University School of Medicine, St Louis, MO, USA
Correspondence to: Vera Jean Suman, PhD. Professor of Biostatistics & Alliance Faculty Statistician, Mayo Clinic-CCS, 200 First Street SW, Rochester, MN 55905, USA. Email: suman@mayo.edu.

Abstract: The Alliance for Clinical Trials in Oncology cooperative group has designed a phase III neoadjuvant clinical trial (ALTERNATE trial) which randomizes women with cT2-4 N0-3 M0 ER+/Her2– invasive breast cancer to either anastrozole, fulvestrant or its combination to assess a biomarker-driven treatment strategy to identify women with a low risk of disease recurrence. This strategy incorporates the findings that: higher expression of the proliferation marker, Ki67, after 2 weeks of neoadjuvant endocrine therapy (ET), is associated with poor recurrence-free survival, and that patients with surgical findings of pT1/2, pN0 disease, Ki67 ≤2.7% and ER Allred score of 3-8 after neoadjuvant ET have extremely low recurrence rates. We present a description and rationale for the design of this trial.

Keywords: Breast cancer; aromatase inhibitors (AIs); postmenopausal

Submitted Jul 19, 2015. Accepted for publication Aug 18, 2015.
doi: 10.3978/j.issn.2304-3865.2015.09.01
View this article at: http://dx.doi.org/10.3978/j.issn.2304-3865.2015.09.01

Introduction

Estrogen contributes to the development and progression of breast cancer through the carcinogenic effects of estrogen metabolites and stimulation of the estrogen receptor (ER) signaling pathways (1). In post-menopausal women, estrogens are largely a result of the conversion of adrenal androgens by the aromatase enzyme in adipose tissues. Estrogen signaling is dependent upon the binding of estrogen to its receptors, ERα and ERβ (2,3). Approximately 70-80% of all breast tumors express ERα protein (4). Endocrine treatment strategies for postmenopausal women with ER positive (ER+) breast cancer have been developed: to bind to the ERs without activating it but preventing estrogen from binding to these receptors [selective ER modulators (SERM)], to degrade ER (fulvestrant), or to block estrogen biosynthesis by inhibiting aromatase [aromatase inhibitors (AIs)]. These strategies been demonstrated to be efficacious in neo-adjuvant, adjuvant, and metastatic disease settings for postmenopausal women with ER+ breast cancer (3,5,6); in the adjuvant setting when combined with ovarian function suppression for premenopausal women with early stage ER+ breast cancer (6-8) and in the prevention setting for postmenopausal women at high risk of developing breast cancer (6,9,10). However, there is variability in both tumor response and treatment tolerability. There is an unmet clinical need to identify patients with endocrine sensitive disease for whom adjuvant chemotherapy could be avoided and to investigate the underlying drivers of endocrine resistant tumors to inform the development of targeted agents to prevent disease recurrence.

Neoadjuvant endocrine therapy (ET) in postmenopausal women with locally advanced ER+ breast cancer

Neoadjuvant ET in postmenopausal women with locally advanced ER+ breast cancer may result in a reduction

in tumor size thereby either improving the chances of breast conserving surgery or rendering an inoperable tumor operable. Moreover, neoadjuvant ET provides the opportunity for interrogation of pre- and post-treatment tumor specimens to assess tumor responsiveness to ET in the early disease setting.

Two randomized neoadjuvant clinical trials (IMPACT and PROACT) enrolled postmenopausal women with newly diagnosed ER+ invasive breast cancer whose extent of disease was considered to require a mastectomy or to be inoperable to assess whether: (I) the clinical response rate; or (II) the rate of conversions to breast conserving surgery among women differed between types of endocrine therapies (11,12). The PROACT trial randomized patients between tamoxifen (20 mg daily) and anastrozole (1 mg daily) for 16 weeks prior to surgery and then post-surgery for a total of 5 years. Concurrent chemotherapy was allowed. The IMPACT trial randomized patients to tamoxifen (20 mg daily), anastrozole (1 mg daily) or the combination of tamoxifen and anastrozole for 12 weeks prior to surgery then post-surgery for a total of 5 years. Concurrent chemotherapy was not allowed. The findings among the patients who received only ET on the PROACT trial were similar to that of the IMPACT trial. No significant differences were seen in the clinical response rate or the percentage of women who became candidates for breast-conserving surgery between anastrozole and tamoxifen.

Suppression of the proliferation marker, Ki67 after short term exposure to NET

The design of the IMPACT trial mirrored that of the ATAC trial which randomized postmenopausal women with newly diagnosed breast cancer to 5 years of adjuvant treatment with tamoxifen (20 mg daily), anastrozole (1 mg daily) or their combination (13). One of the aims of the IMPACT study was to assess whether changes in the proliferation maker, Ki67, after 2 or 12 weeks of neoadjuvant ET would reflect differences in long term outcomes seen in the ATAC trial. The IMPACT trial found that the suppression of the proliferation marker Ki67 after 2 and 12 weeks of treatment was significantly greater with anastrozole than with tamoxifen (14). This finding mirrored that of the ATAC trial where both disease-free survival and time to recurrence were found to be significantly increased with anastrozole relative to tamoxifen. IMPACT trial also demonstrated that high Ki67 expression levels after 2 weeks of neoadjuvant ET (anastrozole, tamoxifen or their combination) was associated with poorer recurrence-free survival (15), raising the possibility that changes in tumor biomarkers after short-term exposure to ET may improve our ability to predict long term outcomes in individual patients.

Greater suppression of the proliferation marker Ki67 after 16 weeks of neoadjuvant treatment with an AI relative to a SERM was also seen in P024, a randomized neo-adjuvant randomized, double-blind clinical trial comparing letrozole (2.5 mg daily) to tamoxifen (20 mg daily) in postmenopausal women with hormone receptor positive primary invasive breast cancer who were not eligible for breast conserving surgery (16). Patients enrolled onto P024 continued to receive their assigned endocrine treatment for a total of 5 years post-surgery. Utilizing the long term outcomes of these patients, Ellis *et al.* found that pathologic tumor stage, pathologic nodal stage, surgical specimen Ki67 level, and ER Allred score were independently associated with both relapse-free survival (RFS) and breast cancer specific survival (17). The preoperative endocrine prognostic index (referred to as the PEPI score, *Table 1*) was developed based on these findings. Outcome data from the IMPACT trial was used to assess the validity of the PEPI score as a prognostic index for RFS. Patients were classified into 3 PEPI risk groups (0 *vs.* 1-3. *vs.* 4+). RFS was indeed found to differ significantly among these groups (log rank P=0.002). Moreover, in both trials, patients with a PEPI score of 0 (pT1/2, pN0, Ki67 ≤2.7%, Allred score 3-8) had an extremely low risk of relapse.

From these studies, questions arose as to whether postmenopausal women with ER positive invasive breast cancer that was either operable or potentially operable who have a PEPI score of 0 after neoadjuvant AI therapy could forgo adjuvant chemotherapy

Z1031 was a randomized neoadjuvant phase II screening trial in post-menopausal women with clinical stage II/III ER+ breast cancer designed to determine which endocrine agent (anastrozole, letrozole or exemestane) or subset of agents should be recommended for future testing against chemotherapy in the neo-adjuvant setting based on differences in clinical response rates (using WHO criteria) after 16 weeks of treatment (18). Both letrozole and anastrozole met the criteria for recommendation for further study. However, no significant differences were found among these 3 endocrine treatments in terms of Ki67 suppression after 16 weeks of treatment or PEPI-0 rate.

Table 1 Post neoadjuvant endocrine therapy residual disease factors comprising the PEPI score* for RFS and BCSS

Factor	RFS risk points	BCSS risk points
Pathologic T stage		
pT0-2	0	0
pT3/4	3	3
Pathologic N stage		
pN0	0	0
pN1-3	3	3
ER Allred score		
0-2	3	3
3-8	0	0
Ki67 level		
0-2.7%	0	0
2.8-7.3%	1	1
7.4-19.7%	1	2
19.8-53.1%	2	3
53.2% or more	3	3

*Determining PEPI score: a patient's PEPI score is determined by summing risk points corresponding to pT stage, pN stage, Ki67 and Allred score from their surgical specimen disease following neo-adjuvant endocrine therapy. For example, a patient with a pT2 pN1 tumor with Ki67 =5% and ER Allred score =5 would be assigned a PEPI score of 4 (0+3+1+0) for both RFS and BCSS. RFS, relapse-free survival; BCSS, breast cancer specific survival.

An extension of Z1031 (Z031B) examined whether postmenopausal women with clinical stage II/III ER+ breast cancer and a tumor Ki67 >10% after 4 weeks of anastrozole or letrozole treatment would benefit from switching to neoadjuvant chemotherapy (19). A second objective was to examine long-term outcomes in women with a tumor Ki67 ≤10% after 4 weeks of NET and a PEPI score =0 at the completion of 16 weeks of NET who do not receive adjuvant chemotherapy. Both the 4-week tumor biopsy specimen and surgical specimen were submitted to a central laboratory for a Ki67 determination and results were returned to the submitting sites within 14 days of submission to ensure timely decision making. Among the 245 women enrolled on Z1031B, 35 had a 4 week Ki67 >10% and switched to neoadjuvant chemotherapy. There were two (5.7%, 95% CI: 0.7-19.1%) pathologic complete responses among these 35 women. Long term outcome data are maturing. This study demonstrated the feasibility of conducting biomarker directed triage trials in the neoadjuvant setting.

The impact of neoadjuvant ET on the proliferation marker, Ki67, has also been studied in the estrogen-receptor antagonist, fulvestrant, which binds, blocks and accelerates the degradation of the ER. Trial 0057, a double-blind, randomized phase II neoadjuvant clinical trial in postmenopausal women with ER+ primary cT1-3 breast cancer examined the changes in ER and Ki67 expression pre and post 2 to 3 weeks of treatment with either the combination of fulvestrant (500 mg day 1) and anastrozole (1 mg/day days 14-21); fulvestrant with anastrozole placebo; or anastrozole with fulvestrant placebo (20). Ki67 expression was found to be significantly reduced from pre-treatment measurements for each of these treatment groups. Moreover, amount of reduction in Ki67 expression was not found to differ with respect to treatment. Also, the reduction in ER expression was significantly greater with fulvestrant alone or in combination with anastrozole than with anastrozole alone.

ALTERNATE trial design considerations

The Alliance for Clinical Trials in Oncology cooperative group designed a randomized phase III clinical trial (ALTERNATE trial) in women with cT2-4 N0-3 M0 ER+/Her2– invasive breast cancer to assess a biomarker driven treatment strategy based on Ki67 values following 4 and 12 weeks of neoadjuvant ET and the PEPI score to identify women at low risk of disease recurrence (*Figures 1*,*2*). The treatment strategies under investigation in this trial are: (I) anastrozole administered by mouth 1 mg days 1-28 for 6 28 day-cycles, surgery, and then anastrozole by mouth 1 mg daily for 4.5 years; (II) fulvestrant 500 mg administered intramuscularly days 1 and 15 of the first 28 day cycle and then day 1 of the 5 remaining 28 day neoadjuvant ET cycles; surgery; fulvestrant day 1 of first 18 months post-surgery followed by anastrozole by mouth 1 mg daily for 3 years; and (III) the combination of anastrozole and fulvestrant employing the same administrative schedule as in the single agent arms for the neoadjuvant portion; surgery and then fulvestrant day 1 of first 18 months post-surgery and anastrozole by mouth 1 mg daily for 3 years. It is recommended that women with tumor Ki67 >10% on breast biopsy after 4 weeks (mandatory) or 12 weeks (optional) of neoadjuvant ET switch to neo-adjuvant chemotherapy. Also, women having completed 6 months of neoadjuvant ET and found to have pT3/4 or pN1-3 or

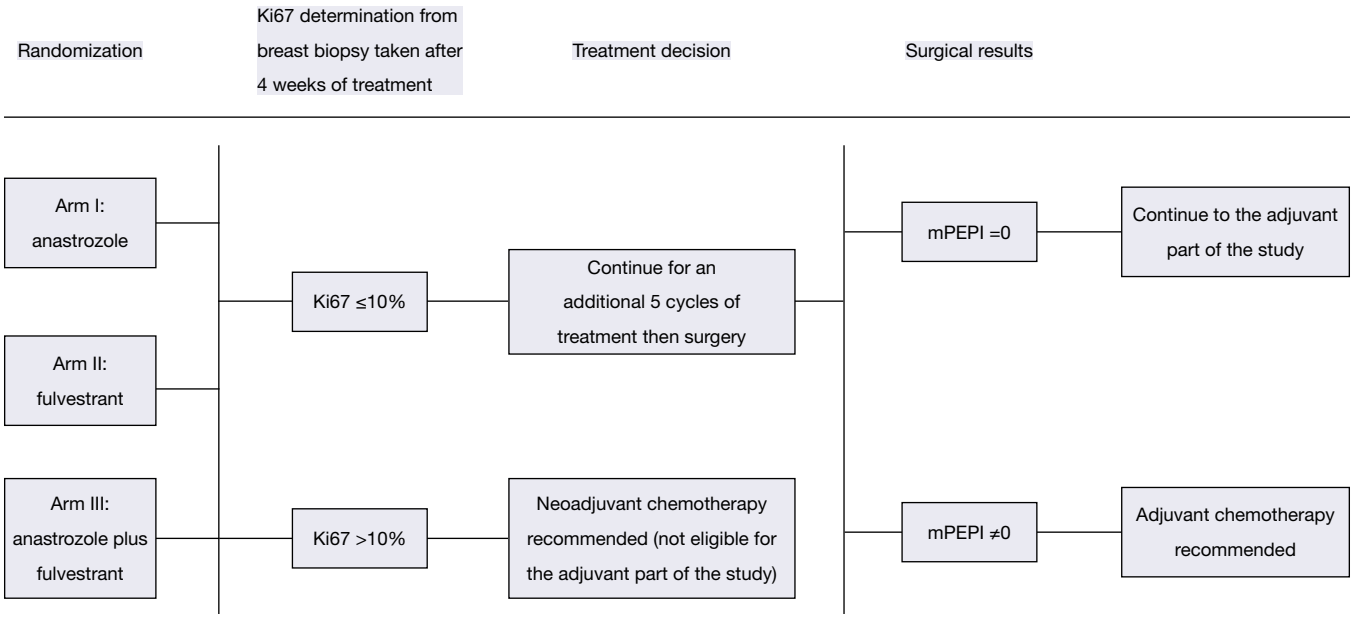


Figure 1 Schema for neoadjuvant portion of ALTERNATE trial. mPEPI, modified PEPI score.

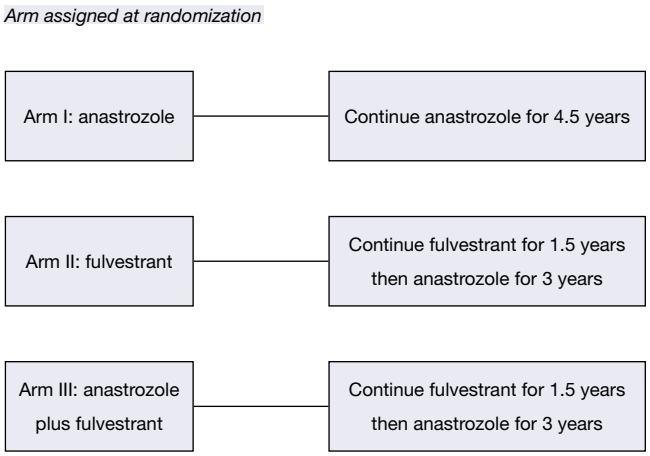


Figure 2 Schema for adjuvant portion of ALTERNATE trial.

Ki67 >2.7% residual disease at surgery are recommended to receive adjuvant chemotherapy of their physician's choosing.

Within this overarching goal, a number of questions concerning endocrine resistance in both the neoadjuvant and adjuvant settings will be addressed. Endocrine resistance in the neoadjuvant disease setting is defined as one of the following events: (I) Ki67 >10% after 4 weeks of neoadjuvant ET; (II) Ki67 >10% after 12 weeks of neoadjuvant ET; (III) radiographic confirmation of progressive disease during neoadjuvant ET; (IV) surgical findings of pT3/4 or pN1-3 or Ki67 >2.7% residual disease; or (V) discontinuation of neoadjuvant ET for any reason. The primary objective for the neoadjuvant portion of the ALTERNATE trial is to determine whether endocrine resistant rate (ERR) with fulvestrant alone or with fulvestrant plus anastrozole is less than that for anastrozole alone. Secondary aims include examining differences in surgical outcomes, clinical and radiographic response rates and safety profile between the three treatment arms. Correlative objectives include examining the degree of tumor Ki67 suppression in each treatment arm as well as evaluating tumor tissue, serum, and plasma specimens collected prior to neoadjuvant ET, after 4 weeks of neoadjuvant ET, and at surgery to gain insights into signaling pathways associated with endocrine resistance.

A comparison of the endocrine sensitivity rate (1-ERR or ESR) among the first 440 patients randomized to each treatment arm will be used to determine whether any of the fulvestrant containing arms should be closed to further enrollment. Consideration for retaining a fulvestrant containing treatment arm will be based on whether its ESR is at least 10% greater than that for anastrozole alone. The sample size for these 2 pairwise comparisons was determined assuming the ESR for the anastrozole arm would be similar to that the anastrozole arm of Z1031B,

namely, 34%. For a given fulvestrant containing arm, a one-sided alpha =0.025 chi-square test of the difference in two independent binomial proportions will have a 82% change of detecting a 10% or greater increase in ESR with this fulvestrant containing regimen, when the ESR with anastrozole alone is at most 34%. In addition, 3 interim analyses are planned to assess futility based on the conditional probability (under the alternative hypothesis) of declaring a fulvestrant containing regimen having at least a 10% higher endocrine sensitivity rate than anastrozole at the final analysis (440 pts per regimen) given the endocrine sensitivity findings to that interim analysis time point.

The adjuvant portion of the ALTERNATE trial addresses questions concerning the clinical outcomes of patients considered to be at low risk of disease recurrence after neoadjuvant ET who do not receive adjuvant chemotherapy but continue to receive ET for an additional 4.5 years. Determination of whether a patient is at low risk of disease recurrence is based on 4-week Ki67 level and a modification of the PEPI score. Since fulvestrant down-regulates ER expression and a tumor rendered ER– by fulvestrant may not reflect a poor prognosis, ER Allred score results are not be included in the modified PEPI score (mPEPI). Women are classified as being at a low risk for disease recurrence if their Ki67 ≤10% after 4 weeks of neoadjuvant ET and mPEPI =0 (that is, pT0-2/pN0/ Ki67 ≤2.7% residual disease). The primary endpoint of the adjuvant portion of this trial is recurrence-free survival (RFS) defined as the time from surgery to the first of the following disease events: invasive ipsilateral breast tumor recurrence, local/regional recurrence, distant recurrence or death due to any cause.

The enrollment period for this trial will depend upon whether none, one or both of the fulvestrant containing arms are found to have a favorable ESR relative to that of anastrozole as well as the finding from the Z1031B trial that 34% of the women receiving neoadjuvant ET had both a week 4 Ki67 ≤10% and mPEPI =0. The benchmark for considering this this biomarker driven strategy to be effective for a given treatment arm relies on the results of Southwest Oncology Group 8814 trial which reported that the 5-year disease-free survival rate with tamoxifen was 91% among women with pT1-3, N1 ER+/Her2– breast cancer and an Oncotype DX recurrence score ≤25 (21). As such, this biomarker driven strategy will be considered effective for a given treatment arm if its 5-year recurrence-free survival is not less than 90%. If only the anastrozole arm is to be assessed and 940 are women are randomized to anastrozole over a 5-year period (yielding 320 women with both a week 4 Ki67 ≤10% and mPEPI =0) and followed a minimum of 4 years after the close of enrollment, a one sample α =0.025 non-parameter Brookmeyer-Crowley type one sample test will have a 90% chance of rejecting that the 5-year RFS rate is 95% or more when the true 5-year RFS rate is at most 90% (22,23). If one or more of the fulvestrant containing arms are to be assessed as well, the enrollment period and/or the follow-up will be increased to ensure sufficient power to assess this primary endpoint. No interim analyses are planned for the adjuvant phase of this trial.

Trial status

This trial opened to enrollment on December 13, 2013, shortly after the merger of the American College of Surgeon Oncology Group (ACSOG), Cancer and Leukemia Group B (CALGB) and the North Central Cancer Treatment Group (NCCTG) into the Alliance for Clinical Trials in Oncology. A number of changes accompanied this merger which increased the need to provide participating sites additional materials to increase awareness of newly instituted systems and procedures impacting the conduct of this trial. An educational slide set was prepared to provide the rationale behind the clinical and correlative objectives. An instructional video was produced to illustrate tissue and blood sample procurement and processing procedures; discuss the biopsy/shipping kit contents and review shipping instructions. Both a Physician Fact Sheet and a Patient Brochure were developed. A monthly newsletter is sent to all participating sites describing any protocol changes, addressing frequently asked questions, and providing updated enrollment numbers. The Study Chair, Community Oncology Co-chair, statistical team, and data manager also hold a teleconference monthly with the Clinical Research Associates of participating sites to answer questions and gather information concerning issues they are encountering as they navigate the protocol procedures and the new Medidata RAVE data submission application.

The accrual rate was been climbing with 236 women have enrolled as of August 10, 2015.

Conclusions

The ALTERNATE trial provides the unique opportunity

to prospectively validate a biomarker driven strategy for the treatment of post-menopausal women with ER+/Her2– invasive breast cancer and to examine the signaling pathways that lead to endocrine resistance.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Bhatnagar AS. The discovery and mechanism of action of letrozole. Breast Cancer Res Treat 2007;105 Suppl 1:7-17.
2. Heldring N, Pike A, Andersson S, et al. Estrogen receptors: how do they signal and what are their targets. Physiol Rev 2007;87:905-31.
3. Chumsri S, Howes T, Bao T, et al. Aromatase, aromatase inhibitors, and breast cancer. J Steroid Biochem Mol Biol 2011;125:13-22.
4. Keen JC, Davidson NE. The biology of breast carcinoma. Cancer 2003;97:825-33.
5. Fabian CJ. The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. Int J Clin Pract 2007;61:2051-63.
6. Ingle JN, Dowsett M, editors. Advances in Endocrine Therapy of Breast Cancer. New York: Marcel Dekker, 2004.
7. Goss PE, Ingle JN, Alés-Martínez JE, et al. Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med 2011;364:2381-91.
8. Cuzick J, Sestak I, Forbes JF, et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomised placebo-controlled trial. Lancet 2014;383:1041-8.
9. Pagani O, Regan MM, Walley BA, et al. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. N Engl J Med 2014;371:107-18.
10. Francis PA, Regan MM, Fleming GF, et al. Adjuvant ovarian suppression in premenopausal breast cancer. N Engl J Med 2015;372:436-46.
11. Cataliotti L, Buzdar AU, Noguchi S, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. Cancer 2006;106:2095-103.
12. Smith IE, Dowsett M, Ebbs SR, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol 2005;23:5108-16.
13. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet 2005;365:60-2.
14. Dowsett M, Smith IE, Ebbs SR, et al. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. Clin Cancer Res 2005;11:951s-8s.
15. Dowsett M, Smith IE, Ebbs SR, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J Natl Cancer Inst 2007;99:167-70.
16. Ellis MJ, Ma C. Letrozole in the neoadjuvant setting: the P024 trial. Breast Cancer Res Treat 2007;105 Suppl 1:33-43.
17. Ellis MJ, Tao Y, Luo J, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. J Natl Cancer Inst 2008;100:1380-8.
18. Ellis MJ, Suman VJ, Hoog J, et al. Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype--ACOSOG Z1031. J Clin Oncol 2011;29:2342-9.
19. Ellis MJ, Suman V, McCall L, et al. Abstract PD07-01: Z1031B Neoadjuvant Aromatase Inhibitor Trial: A Phase 2 study of Triage to Chemotherapy Based on 2 to 4 week Ki67 level > 10%. Cancer Res 2012;72:PD07-01.
20. Robertson JF, Dixon JM, Sibbering DM, et al. A randomized trial to assess the biological activity of short-term (pre-surgical) fulvestrant 500 mg plus anastrozole versus fulvestrant 500 mg alone or anastrozole alone on primary breast cancer. Breast Cancer Res 2013;15:R18.
21. Albain KS, Barlow WE, Shak S, et al. Prognostic and

predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol 2010;11:55-65.

22. Brookmeyer R, Crowley JJ. A confidence interval for the median survival time. Biometrics 1982;38:29-41.
23. One Sample-Nonparametric Survival. Available online: http://www.swogstat.org/stat/public/one_nonparametric_survival.htm

Cite this article as: Suman VJ, Ellis MJ, Ma CX. The ALTERNATE trial: assessing a biomarker driven strategy for the treatment of post-menopausal women with ER+/Her2– invasive breast cancer. Chin Clin Oncol 2015;4(3):34. doi: 10.3978/j.issn.2304-3865.2015.09.01

Theranostic nanoparticles for enzyme-activatable fluorescence imaging and photodynamic/chemo dual therapy of triple-negative breast cancer

Jaehee Choi*, Hyunjin Kim*, Yongdoo Choi

Molecular Imaging & Therapy Branch, Division of Convergence Technology, National Cancer Center, Goyang, Gyeonggi-do, Korea
*These authors contributed equally to this work.
Correspondence to: Yongdoo Choi, PhD. Molecular Imaging & Therapy Branch, Division of Convergence Technology, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang, Gyeonggi-do, Korea. Email: ydchoi@ncc.re.kr.

Background: Triple-negative breast cancer (TNBC) is a highly diverse group of cancers characterized by tumors that does not express estrogen and progesterone receptors, as well as human epidermal growth factor receptor 2 (*HER2*) gene expression. TNBC is associated with poor prognosis due to high rate of recurrence and distance metastasis, lack of response to hormonal or *HER2*-targeted therapies, and partial response to chemotherapy. Hence, development of new therapeutic strategies to overcome such limitations is of great importance. Here we describe the application of photosensitizer-conjugated and camptothecin (CPT)-encapsulated hyaluronic acid (HA) nanoparticles as enzyme-activatable theranostic nanoparticles (EATNP) for near-infrared (NIR) fluorescence imaging and photodynamic/chemo dual therapy of TNBC.

Methods: For the preparation of EATNPs, chlorin e6 (Ce6), a second generation photosensitizer, was covalently conjugated to a monomethoxy poly(ethylene glycol)-grafted HA backbone. Ce6-conjugated HA (Ce6-HA) formed self-assembled nanoparticles (i.e., Ce6-HA NPs) in an aqueous solution. Subsequently, CPT, a topoisomerase 1 inhibitor with remarkable anticancer efficacy but with low water solubility, was encapsulated inside the hydrophobic core of Ce6-HA NPs thereby forming EATNPs.

Results: Fluorescence and singlet oxygen generation (SOG) of EATNPs are quenched in its native state. Treatment of EATNPs with hyaluronidase (HAdase) induces enzyme concentration-dependent activation of NIR fluorescence and SOG. Moreover, HAdase-mediated degradation of the nanoparticles also triggers the release of CPT from the EATNPs. *In vitro* confocal microscopy and cytotoxicity tests confirmed that EATNPs were efficiently introduced into MDA-MB-231 TNBC cell line, thereby inducing better cytotoxicity than that by free CPT. Additional light irradiation onto the EATNP-treated cells significantly increased therapeutic efficacy in TNBC, which indicates that EATNP plays an important role in enzyme-activated NIR fluorescence imaging and photodynamic/chemo dual therapy of TNBC.

Conclusions: We found that HAdase may switch on NIR fluorescence and SOG of EATNPs. Moreover, CTP release from the nanoparticles is triggered by the enzyme HAdase. *In vitro* cell study showed potential utility of EATNPs for fluorescence imaging and photodynamic/chemo dual therapy of TNBC.

Keywords: Chemotherapy; fluorescence imaging; photodynamic therapy (PDT); theranostic nanoparticles; triple-negative breast cancer (TNBC)

Submitted Aug 05, 2015. Accepted for publication Aug 27, 2015.
doi: 10.3978/j.issn.2223-4292.2015.08.09
View this article at: http://dx.doi.org/10.3978/j.issn.2223-4292.2015.08.09

Introduction

Triple-negative breast cancers (TNBC) are characterized by tumors that does not express estrogen and progesterone receptor, as well as human epidermal growth factor receptor 2 (*HER2*) gene expression (1,2). TNBC patients account for approximately 15% of total breast cancer patients. It is associated with poor prognosis due to high rate of recurrence and distance metastasis, lack of response to hormonal or *HER2*-targeted therapies, and partial response to chemotherapy (3). Therefore, development of new strategies to overcome therapeutic limitations of TNBC is of great importance. In the recent studies, nanomedicine-based chemo/gene dual therapy and chemo/photothermal/gene triple therapy of TNBC showed high potential as a new therapeutic option for TNBC (4,5).

Photodynamic therapy (PDT) using combinations of chemical photosensitizers, light, and molecular oxygen has emerged as an effective therapeutic option for various cancers (6,7). Photosensitizers are considered to be potential theranostic agents as they simultaneously generate fluorescence signals for imaging and singlet oxygen for therapy upon excitation by a specific wavelength of light. However, limited tumor selectivity, unfavorable pharmacokinetics, and prolonged skin photosensitivity of water-insoluble PDT agents have been the main obstacles to their clinical applications. In PDT, most of the anticancer drugs used in chemotherapy show low water solubility and severe side effects due to their poor tumor selectivity. Therefore, various drug delivery systems such as nanoparticles and polymer-drug conjugates have been tried to enhance the specificity of photosensitizers and chemotherapeutic drugs to cancer sites (8-15).

Here, we propose the application of photosensitizer-conjugated and anticancer drug-loaded polymeric nanoparticles as an enzyme-activatable theranostic nanoparticle (EATNP) for selective near-infrared (NIR) fluorescence imaging and photodynamic/chemo dual therapy of TNBC (*Figure 1*). For the preparation of EATNPs, chlorin e6 (Ce6), a second generation photosensitizer, was covalently conjugated to a monomethoxy poly(ethylene glycol)-grafted hyaluronic acid (HA) backbone. Ce6-conjugated HA (Ce6-HA) formed self-assembled nanoparticles (i.e., Ce6-HA NPs) in an aqueous solution. Subsequently, camptothecin (CPT), a topoisomerase 1 inhibitor with remarkable anticancer efficacy but with low water solubility (16,17), was encapsulated inside the hydrophobic core of Ce6-HA NPs thereby forming EATNPs. We hypothesized that the aggregated photosensitizers inside EATNPs are optically quenched and therefore its fluorescence and singlet oxygen generation (SOG) are turned off while circulating in the blood. However, the preferential accumulation of the nanoparticles in tumors via enhanced permeability and retention (EPR) effect, followed by endocytosis into cancer cells, might cause degradation of the HA backbones by intracellular hyaluronidase (HAdase), resulting in triggered release of both Ce6s and CTPs from the nanoparticles. Subsequent fluorescence emission and SOG of the released Ce6s as well as chemotherapeutic action by the released CPTs may enable not only selective fluorescence detection with high target-to-background ratio but also subsequent photodynamic/chemo dual therapy of TNBC. HA, a linear polysaccharide abundant in the extracellular matrix, is

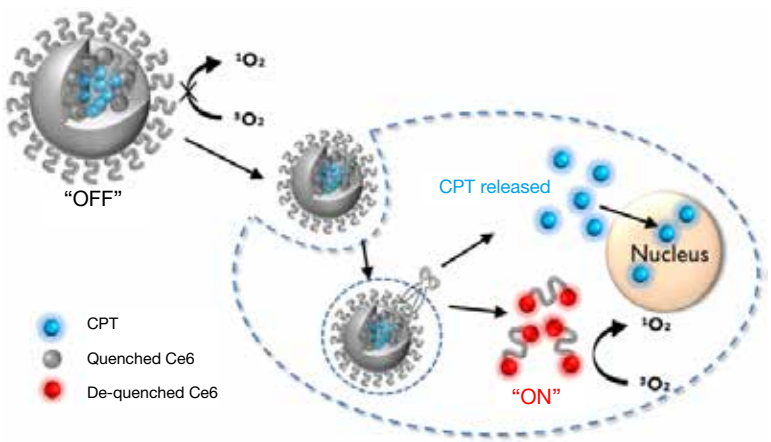


Figure 1 Schematic diagram of enzyme-activatable fluorescence imaging and photodynamic/chemo dual therapy of cancer. CPT, camptothecin; Ce6, chlorin e6.

degradable by tumor-associated enzyme HAdase. Studies have shown positive correlation between the HAdase levels and tumor progressions (18,19); especially HAdase levels of breast metastatic tumors were shown to be 4 times higher than the primary breast cancer tumors (20,21).

Materials and methods

Materials

HA (MW 6.63×10^4 Da) was purchased from Lifecore Biomedical (Chaska, MN, USA). 1-Ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC), sulfo-N-hydroxysulfosuccinimide (sulfo-NHS), adipic acid dihydrazide (ADH), (S)-(+)-CPT, and HAdase (1,228 unit/mg) were purchased from Sigma-Aldrich (MO, USA). Monomethoxy poly(ethylene golycol)-amine (mPEG-amine, MW =5,000 Da) was purchased from Sunbio (Anyang, Korea). Ce6 and dialysis membranes (MWCO: 10,000 and 50,000 Da) were purchased from Frontier Scientific (UT, USA) and Spectrum Laboratories (CA, USA), respectively. Singlet Oxygen Sensor Green (SOSG) was purchased from Invitrogen (NY, USA). Amicon ultra centrifugal filter tube was obtained from Merk Millipore Corp (Darmstadt, Germany).

The MDA-MB-231 human breast cancer cell line was obtained from the American Type Culture Collection (MD, USA). The cell line was maintained in RPMI 1640 medium (GIBCO®, ThermoFischer Scientific, NY, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution in a humidified incubator (37 ℃, 5% CO).

Synthesis of Ce6-HA

Ce6-HA was synthesized using a standard EDC/NHS chemistry (*Figure 2*). At first, amine-functionalized and mPEG-grafted hyaluronic acid (ADH-mPEG-HA) was prepared by conjugating both ADH and mPEG-amine with the carboxylic acids of HA. Briefly, 200 mg HA was dissolved in sodium phosphate buffer (pH 7.4, 20 m); EDC (240 mM, 0.5 mL) and sulfo-NHS (250 mM, 0.5 mL) were sequentially added to the HA solution and stirred for 30 min. Both ADH (27 mg) and mPEG-amine (150 mg) dissolved in sodium phosphate buffer (pH 7.4, 1 mL) and were added to the activated HA solution. The conjugation reaction was allowed to proceed overnight at room temperature. The reactant was dialyzed against deionized (DI) water for purification and then lyophilized by freeze-drying. Conjugation of ADH and mPEG in a HA polymer backbone was analyzed by ^{1}H-NMR analysis (*Figure S1*).

Next, second generation photosensitizer Ce6 was conjugated with ADH-mPEG-HA. At first, the carboxylic acid of Ce6 (34 mg, 1 mL) was activated with EDC (2 mM) and sulfo-NHS (5 mM) in DMSO. Then, ADH-mPEG-HA (122 mg) was dissolved in DMF:H_2O cosolvent (1:1 v/v, 6 mL) and mixed with the activated Ce6 solution, and then conjugation reaction was allowed to proceed overnight at room temperature. Ce6-conjugated mPEG-HA (i.e., Ce6-HA) was purified by dialysis method against phosphate buffer (pH 7.4, 10 mM) and DI water for several times, and then the final product was freeze-dried. The degree of substitution of ADA and mPEG molecules per unit (2 glucose rings) of HA was analyzed with ^{1}H-NMR. To calculate the concentration of Ce6, the absorbance of Ce6-HA (dissolved in 0.1 M NaOH/0.1% SDS) was measured at 400 nm. Ce6 has a molar extinction coefficient of 1.5×10^5 $M^{-1}cm^{-1}$ at 400 nm (22,23).

Preparation of CPT-loaded Ce6-HA nanoparticles

Ce6-HA forms self-assembled nanoparticles in aqueous solution during dialysis procedure as it consists of hydrophilic mPEG-grafted HA backbones and hydrophobic Ce6s. Therefore, enzyme-activatable theranostic nanoparticles (EATNP) were prepared by encapsulating anticancer drug CPT with self-assembled Ce6-HA nanoparticles by using the dialysis method. Ce6-HA (9 mg), dissolved in DMF:H_2O (1:1 v/v, 6 mL) cosolvent, was mixed with CPT (1 mg, 200 μL DMSO) and stirred for 1 h for complete dissolution. Then, the solution underwent dialysis against DI water to form self-assembled nanoparticles. Ce6-HA nanoparticles without CPT (i.e., Ce6-HA) were also prepared by dialysis method for comparison. Final products were freeze-dried and preserved at refrigerator for further use.

UV/Vis absorption spectra of Ce6-HA NPs and EATNPs were analyzed by UV/Vis spectrophotometry (DU730, Beckman Coulter, Brea, CA, USA). The hydrodynamic size of CPT/Ce6-HA NPs and Ce6-HA NPs were characterized using a zeta potential/particle sizer (Malvern Instrument, Malvern, UK). The CPT/Ce6-HA NPs were dispersed in phosphate buffered saline (PBS) (6.7 mM, pH 7.4, NaCl 154 mM) and free Ce6 was dissolved in 0.1 M NaOH/ 0.1% SDS solution to analyze the optical characters. Fluorescence spectra were recorded on a multifunctional microplate (Tecan, Safire 2, Switzerland) with excitation at 400 nm.

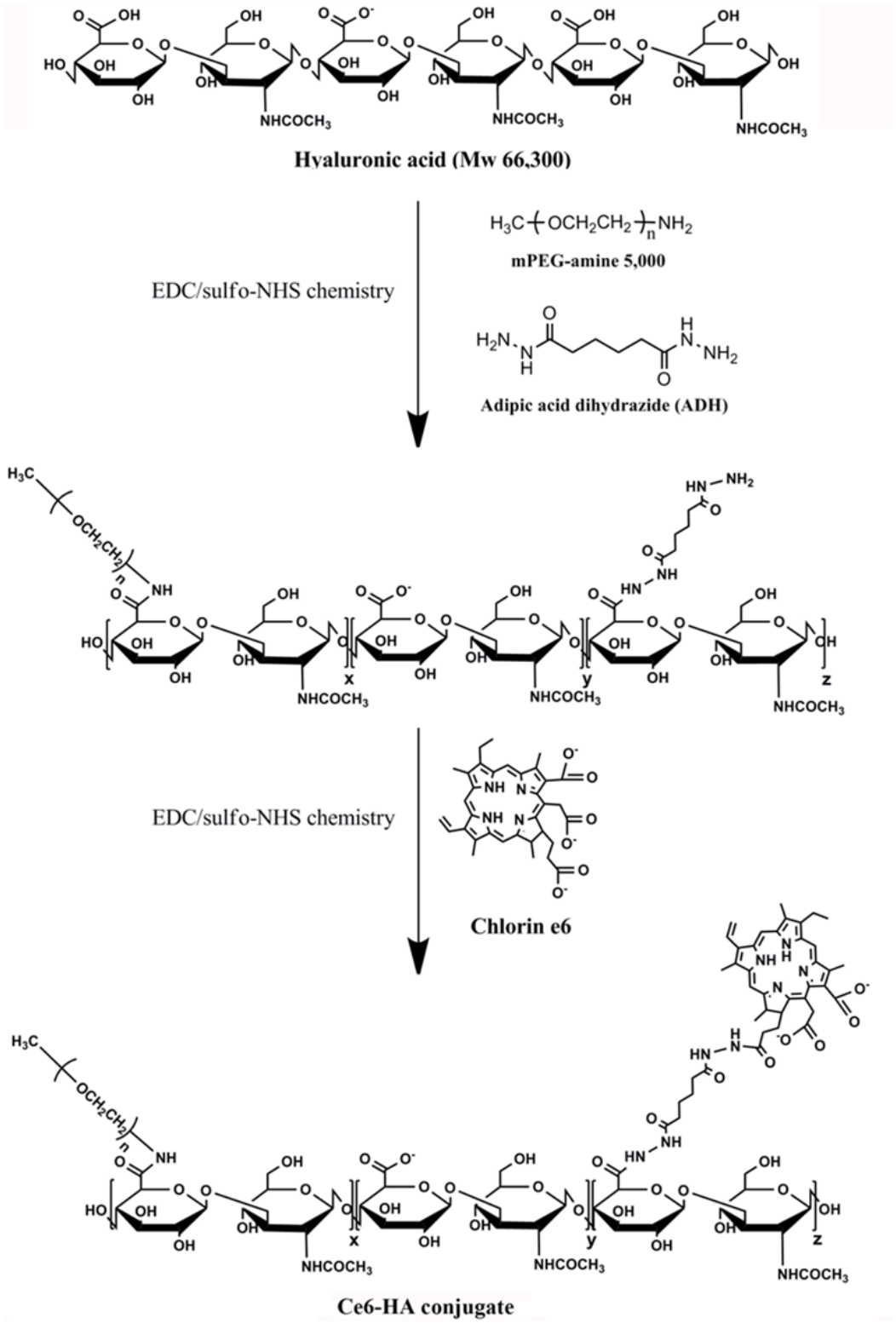


Figure 2 Synthesis of enzyme-activatable theranostic nanoparticles (EATNPs) by EDC/NHS chemistry. EDC, carbodiimide; NHS, N-hydroxysulfosuccinimide; HA, hyaluronic acid; Ce6-HA, Ce6-conjugated HA.

Analysis of fluorescence and SOG

To observe fluorescence quenching and recovery, free Ce6 was dissolved in 1% (v/v) Tween20/PBS to prevent the self-quenching effect resulting from aggregation. Then EATNPs were dissolved in PBS without Tween20. Degradation of HA backbones by HAdase released Ce6 with an increased HAdase concentration that resulted in the recovery of the fluorescence signal. After the addition of HAdase (0-1,200 unit/mL), the fluorescence response of EATNP solution (1 μM Ce6 equivalent, 100 μL) was measured at 120 min (excitation: 400 nm, emission: 430-800 nm).

To evaluate the inhibitory and recovery characteristics with respect to SOG, EATNPs were dispersed in PBS solution (saturated with oxygen gas) and then treated with various concentrations of HAdase for 2 h (n=4). Next, singlet-oxygen-detecting-reagent (SOSG) was dissolved in HAdase-treated EATNP solution. The final concentration of SOSG reagent in the test solution was maintained at 1 μM. Each solution was irradiated with a 670 nm CW laser (irradiation dose rate: 68 mW/cm^2). Relative SOG of EATNPs with and without HAdase treatment was analyzed by measuring the increase in SOSG fluorescence during 120 s light illumination with laser.

Drug release test

EATNPs dispersed in PBS solution was mixed with either acetate buffer solution (pH 4.5, 100 mM) or HAdase-contained acetate buffer solution (1,200 U/mL), and then enzyme reaction was carried out at 37 ℃ for 24 h. At each time point, the sample solutions were collected and transferred to amicon ultra centrifugal filter tube (MWCO 3k), and then centrifuged for 10 min at 14,000 ×g. After addition of Tween20 (1 v/v %), the absorbance (UV/V) of the solution was measured at 365 nm and compared with a standard curve of free CPT to calculate the amount of released CPTs.

In vitro cytotoxicity and phototoxicity test

The cells were seeded onto a 96-well plate at 1×10^4 cells/well and incubated for 24 h. Then, free CPT and EATNPs were diluted in RPMI 1640 culture medium (GIBCO®) containing 10% FBS to obtain different concentrations of 0-20 μM CPT equivalent (that was corresponding to 0-10 μM Ce6 equivalent for EATNPs). The culture medium was replaced with fresh medium containing free CPT or EATNPs, and the cells were incubated for 6 h. Thereafter the cells were washed three times and fresh cell culture medium was added. The cells in the PDT-treated group were irradiated with 670 nm CW laser (dose rate: 50 mW/cm^2, dose: 10 J/cm^2). After incubating the cells for an additional 18 h, viability of cells was analyzed using a CCK-8 solution. Absorbance was measured at 450 nm (reference =650 nm) using a microplate reader (Tecan Safire 2). Untreated control cells served as 100% viable cells and the medium served as the background. Data are expressed as the mean (SD) of four data samples.

Confocal fluorescence images

For confocal images, MDA-MB-231 cells were seeded at a density of 1×10^5 cells/well onto a LabTek II Chambered Coverglass (ThermoFischer Scientific, NY, USA) and incubated for 24 h for cell attachment. EATNPs were dispersed and diluted with RPMI 1640 medium (GIBCO®) containing 10% FBS to obtain 1 μM Ce6 equivalent. The cell culture medium was replaced with EATNPs-containing cell culture medium. After incubation for 6 h, the cells were washed three times and were transferred to a fresh culture medium. Fluorescence images of the cells (excitation: 405 nm, emission: 650 nm long-pass filter) were captured using a confocal scanning-laser microscopy (CSLM, ZEISS LSM 510 META).

Results and discussion

Ce6-HA NPs were prepared with Ce6-HA, as mentioned above. Ce6-HA NPs were round shaped as observed using scanning electron microscope (SEM) (*Figure 3*), and its hydrodynamic size and zeta potential were 170.9±34.67 nm and –27.9±5.55 mV, respectively, confirming the formation of self-assembled nanoparticles. Negative zeta potential value of the nanoparticles indicated that the hydrophilic HA backbones are localized at the outer layer of Ce6-HA NPs. Significant broadening of the Soret band region of HA-Ce6 NP UV/V is spectrum (Beckman Coulter, CA, USA) is the hallmark of Ce6 aggregation, and explains fluorescence quenching of HA-Ce6 NPs compared with the free Ce6 at the same equivalent concentration.

Next, CPT-loaded HA-Ce6 NPs (i.e., EATNPs) were prepared by encapsulating hydrophobic CPT drugs with the self-assembled Ce6-HA NPs using dialysis method. Ce6-HA (9 mg) was dissolved in dimethylformamide:H_2O cosolvent (1:1 v/v, 6 mL) and mixed with CPT (1 mg,

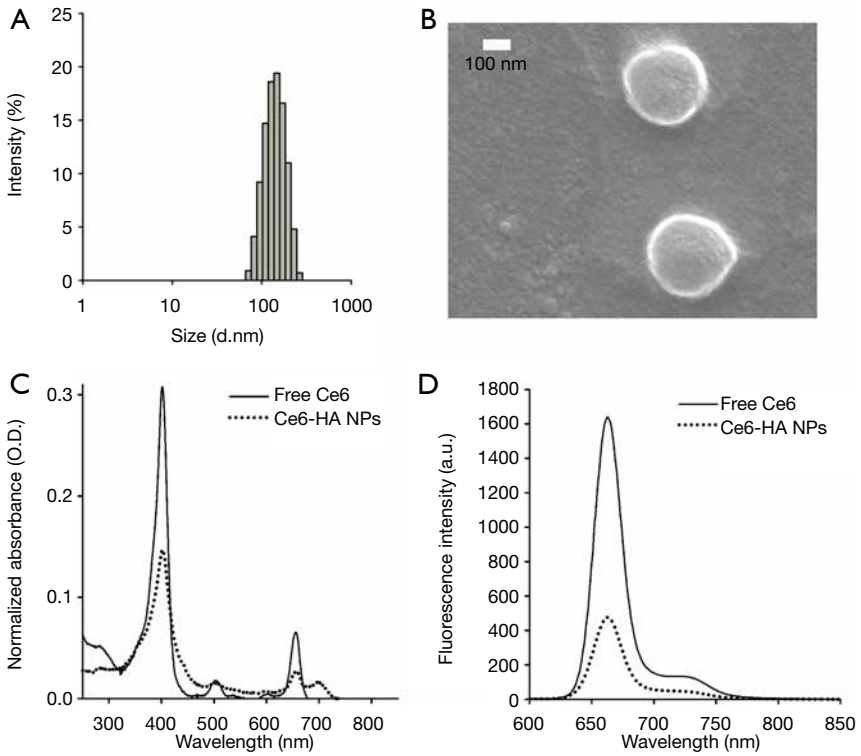


Figure 3 Characterization of Ce6-conjugated HA nanoparticles (Ce6-HA NPs). (A) Hydrodynamic size and (B) scanning electron microscopic (SEM) image of Ce6-HA NPs before encapsulation of CPT; (C) comparison of UV/Vis spectrum and (D) fluorescence intensity with free Ce6 and Ce6-HA NPs at 2 μM Ce6 equivalent. Ce6, chlorin e6.

200 μL dimethyl sulfoxide) and stirred for 1 h. Then the solution underwent dialysis against distilled water to form CPT-loaded nanoparticles. The hydrodynamic size and zeta potential of the prepared EATNPs in aqueous solution were 88.78±6.49 nm and −25.4±4.48 mV, respectively. Hydrodynamic size of EATNPs was about two times smaller than Ce6-HA NPs, while its zeta potential value was slightly higher than that of Ce6-HA NPs. Hydrophobic interactions between Ce6s and CPTs inside the nanoparticles tighten the core of the self-assembled nanoparticles, thereby reducing its hydrodynamic size (*Figure 4A*). Fluorescence of CPT as well as Ce6 was significantly quenched inside EATNPs as shown in *Figure 4B* (inset image).

As mentioned above, HAdase are overexpressed in various tumors and its levels are much higher in breast metastatic tumors than the primary breast tumors (20,21), indicating that HAdase might be used as a molecular switch for selective imaging and therapy of metastatic TNBCs. Therefore, we confirmed whether treatment of EATNPs with HAdase stimulates recovery of NIR fluorescence and SOG in addition to releasing CTP from the nanoparticles. NIR fluorescence intensity of EATNPs increased with increasing concentration of HAdase, indicating degradation of the nanoparticle by HAdase and subsequent recovery of Ce6 fluorescence, as shown in *Figure 5A,B*. The fluorescence intensity of EATNPs was three-fold higher than that of buffer-treated nanoparticles when treated with 1,200 unit/mL HAdase. Then, SOG from EAPNPs was measured in the absence and presence of HAdase, using SOSG as a singlet-oxygen-detecting reagent (*Figure 5C,D*). As in the fluorescence experiment, treatment with HAdase triggered enzyme concentration-dependent recovery of SOG. About 4.1-fold increase in SOG was obtained by treatment with 1,200 unit/mL HAdase.

We tested the effect of HAdase on CPT release from the EATNPs (*Figure 6A*). In the absence of HAdase, only 17% and 29% of CPT were released from the nanoparticles at 6 h and 24 h, respectively. In contrast, treatment with HAdase (i.e., 1,200 unit/mL) induced 51% release of CPT within 6 h, which is about three times higher than that of non-enzyme treated control. After 24 h of HAdase treatment 58% CTP was released from EATNPs. This

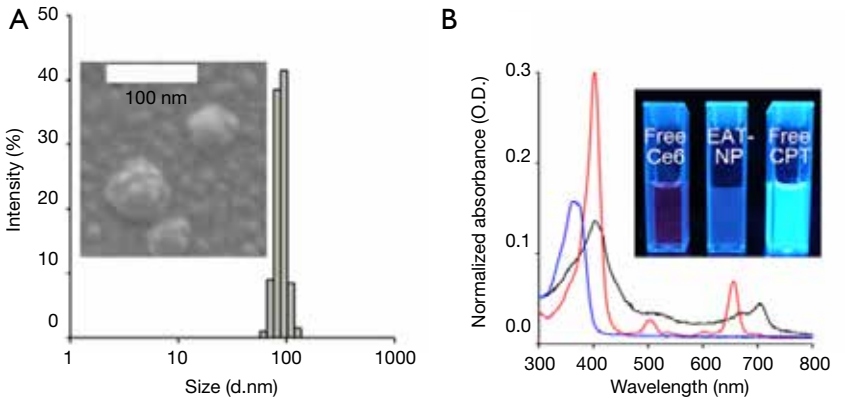


Figure 4 Characterization of EATNP. (A) Hydrodynamic size distribution and scanning electron microscopic (SEM) image of enzyme-activatable theranostic nanoparticle (EATNP); (B) comparison of UV/Vis absorption spectra of free Ce6 (red), EATNP (black), and free CPT (blue) at 2 μM Ce6 and 5 μM CPT equivalent concentrations. Inset image: Fluorescence images of free Ce6, EATNPs, and free CPT in aqueous solutions under 380 nm UV light. Ce6, chlorin e6; CPT, camptothecin.

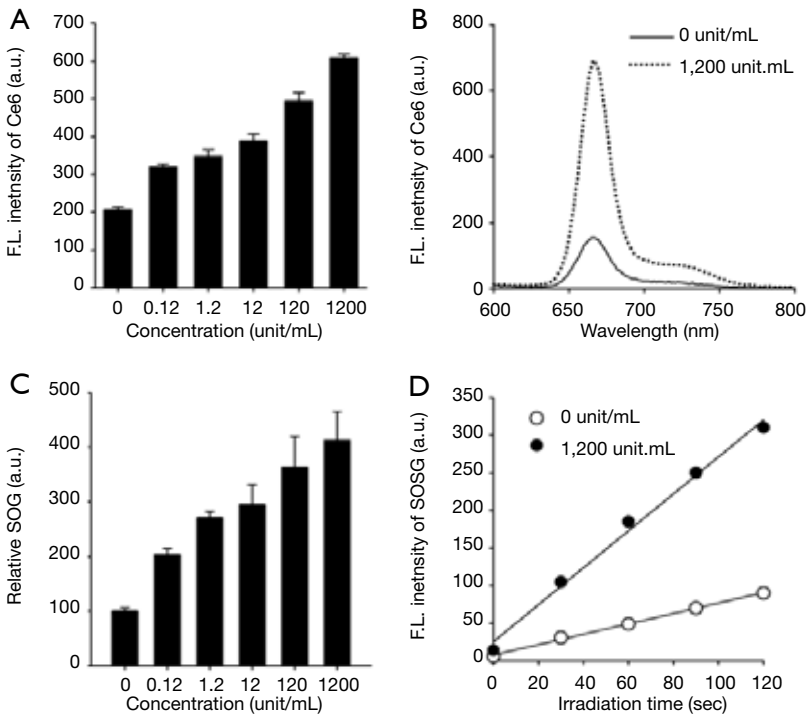


Figure 5 HAdase-mediated recovery of fluorescence and SOG of EATNP. (A) Fluorescence intensity of enzyme-activatable theranostic nanoparticles (EATNPs) (1 μM Ce6 equivalent) measured after 2 h of HAdase treatment at various concentrations; (B) fluorescence spectra of EATNPs (1 μM Ce6 equivalent) treated with buffer solution or HAdase (1,200 unit/mL) for 2 h; (C) relative SOG *vs.* HAdase concentration. EATNPs were treated with HAdase at various concentrations and then SOG of the EATNPs was measured after laser irradiation (670 nm laser); (D) time-dependent SOG of HAdase-treated EATNPs during irradiation (670 nm CW laser, 68 mW/cm², n=4). Ce6, chlorin e6; SOG, singlet oxygen generation.

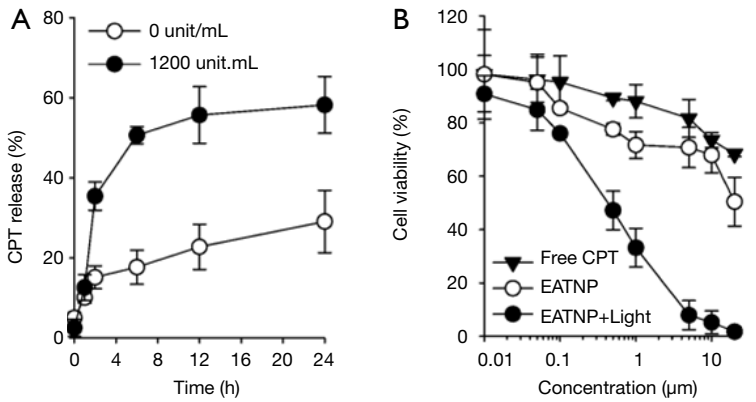


Figure 6 HAdase-activatable drug release and PDT effect. (A) HAdase-dependent release profile of CPT from enzyme-activatable theranostic nanoparticles (EATNPs) (2 μM CPT equivalent, n=4); (B) viability of MDA-MB-231 cells after treatment with free CPT and EATNP at various concentrations of CPT equivalent (n=4). CPT, camptothecin.

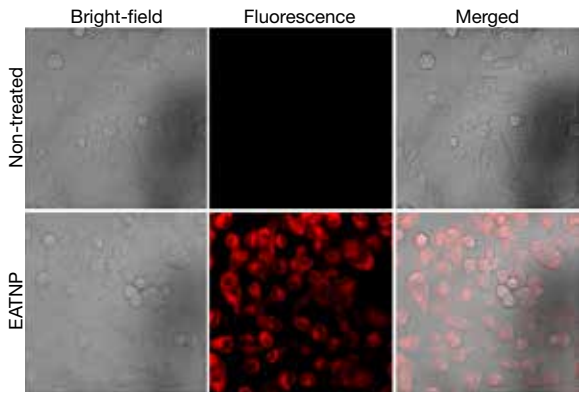


Figure 7 Confocal laser fluorescence images (Ex. 405 nm, Em. 650 nm long-pass filter) of the cells treated with cell culture media and enzyme-activatable theranostic nanoparticles (EATNP) (1 μM Ce6 equivalent) for 6 h. Red colour indicates fluorescence signals from Ce6 inside the cells. Ce6, chlorin e6.

data matched well with fluorescence recovery of HAdase-treated EATNPs indicating HAdase-mediated nanoparticle degradation and subsequent release of both Ce6s and CTPs.

We then evaluated the therapeutic efficacy of EATNPs in TNBC cell line, MDA-MB-231 cells (*Figure 6B*). The cells were treated with free CPT and EATNPs at various concentration of CPT equivalent for 6 h and washed three times with fresh cell culture media. MDA-MB-231 cells in the EATNP plus light group additionally received light irradiation with a 670 nm CW laser (dose rate: 68 mW/cm², dose: 10 J/cm^2). After incubating the cells for an additional 18 h, viability of the cells was analyzed. As a result, about 28% of the cells were dead after treating the cells with EATNPs at 1 μM CPT equivalent, while 12% of cell death was recorded with free CPT treatment at the same concentration. Upon additional light illumination for PDT, 67% of the cells were killed. Also, cell viability of the EATNP plus light group (20 μM CPT equivalent) was reduced to 2%, while cell viability of EATNP- and free CPT-treated groups were 50% and 68%, respectively.

Strong fluorescence signals of the EATNP-treated cells were observed in the images obtained using a confocal laser scanning microscope, which may indicate efficient cellular uptake of EATNPs by the cells and fluorescence recovery inside the cells (*Figure 7*).

Conclusions

In summary, we found that HAdase may switch on NIR fluorescence and SOG of EATNPs. Moreover, CTP release from the nanoparticles is triggered by the enzyme HAdase. *In vitro* cell study showed potential utility of EATNPs for fluorescence imaging and photodynamic/chemo dual therapy of TNBC.

Acknowledgements

Funding: This work was supported by the National Research Foundation of Korea (NRF) grant (NRF-2014R1A2A1A11050923) funded by the Korea government (MSIP), and by a National Cancer Center grant (1310160), Republic of Korea.

Footnote

Conflicts of Interest: The authors have no conflicts of interest

to declare.

References

1. Davis SL, Eckhardt SG, Tentler JJ, Diamond JR. Triple-negative breast cancer: bridging the gap from cancer genomics to predictive biomarkers. Ther Adv Med Oncol 2014;6:88-100.
2. Oakman C, Viale G, Di Leo A. Management of triple negative breast cancer. Breast 2010;19:312-21.
3. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. Cancer Biol Med 2015;12:106-16.
4. Deng ZJ, Morton SW, Ben-Akiva E, Dreaden EC, Shopsowitz KE, Hammond PT. Layer-by-layer nanoparticles for systemic codelivery of an anticancer drug and siRNA for potential triple-negative breast cancer treatment. ACS Nano 2013;7:9571-84.
5. Su S, Tian Y, Li Y, Ding Y, Ji T, Wu M, Wu Y, Nie G. "Triple-punch" strategy for triple negative breast cancer therapy with minimized drug dosage and improved antitumor efficacy. ACS Nano 2015;9:1367-78.
6. Celli JP, Spring BQ, Rizvi I, Evans CL, Samkoe KS, Verma S, Pogue BW, Hasan T. Imaging and photodynamic therapy: mechanisms, monitoring, and optimization. Chem Rev 2010;110:2795-838.
7. Josefsen LB, Boyle RW. Unique diagnostic and therapeutic roles of porphyrins and phthalocyanines in photodynamic therapy, imaging and theranostics. Theranostics 2012;2:916-66.
8. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm 2015;93:52-79.
9. Mohamed S, Parayath NN, Taurin S, Greish K. Polymeric nano-micelles: versatile platform for targeted delivery in cancer. Ther Deliv 2014;5:1101-21.
10. Park D, Cho Y, Goh SH, Choi Y. Hyaluronic acid-polypyrrole nanoparticles as pH-responsive theranostics. Chem Commun (Camb) 2014;50:15014-7.
11. Canal F, Sanchis J, Vicent MJ. Polymer--drug conjugates as nano-sized medicines. Curr Opin Biotechnol 2011;22:894-900.
12. Jang B, Park JY, Tung CH, Kim IH, Choi Y. Gold nanorod-photosensitizer complex for near-infrared fluorescence imaging and photodynamic/photothermal therapy in vivo. ACS Nano 2011;5:1086-94.
13. Cho Y, Kim H, Choi Y. A graphene oxide-photosensitizer complex as an enzyme-activatable theranostic agent. Chem Commun (Camb) 2013;49:1202-4.
14. Wang YG, Kim H, Mun S, Kim D, Choi Y. Indocyanine green-loaded perfluorocarbon nanoemulsions for bimodal (19)F-magnetic resonance/nearinfrared fluorescence imaging and subsequent phototherapy. Quant Imaging Med Surg 2013;3:132-40.
15. Kim J, Tung CH, Choi Y. Smart dual-functional warhead for folate receptor-specific activatable imaging and photodynamic therapy. Chem Commun (Camb) 2014;50:10600-3.
16. Liu YQ, Li WQ, Morris-Natschke SL, Qian K, Yang L, Zhu GX, Wu XB, Chen AL, Zhang SY, Nan X, Lee KH. Perspectives on biologically active camptothecin derivatives. Med Res Rev 2015;35:753-89.
17. Yokoyama M, Opanasopit P, Okano T, Kawano K, Maitani Y. Polymer design and incorporation methods for polymeric micelle carrier system containing water-insoluble anti-cancer agent camptothecin. J Drug Target 2004;12:373-84.
18. Choi KY, Saravanakumar G, Park JH, Park K. Hyaluronic acid-based nanocarriers for intracellular targeting: interfacial interactions with proteins in cancer. Colloids Surf B Biointerfaces 2012;99:82-94.
19. Beech DJ, Madan AK, Deng N. Expression of PH-20 in normal and neoplastic breast tissue. J Surg Res 2002;103:203-7.
20. Bertrand P, Girard N, Duval C, d'Anjou J, Chauzy C, Ménard JF, Delpech B. Increased hyaluronidase levels in breast tumor metastases. Int J Cancer 1997;73:327-31.
21. Udabage L, Brownlee GR, Nilsson SK, Brown TJ. The over-expression of HAS2, Hyal-2 and CD44 is implicated in the invasiveness of breast cancer. Exp Cell Res 2005;310:205-17.
22. Hamblin MR, Miller JL, Rizvi I, Ortel B, Maytin EV, Hasan T. Pegylation of a chlorin(e6) polymer conjugate increases tumor targeting of photosensitizer. Cancer Res 2001;61:7155-62.
23. Kim H, Mun s, Choi Y. Photosensitizer-conjugated polymeric nanoparticles for redox-responsive fluorescence imaging and photodynamic therapy. J Mater Chem B 2013;1:429-31.

Cite this article as: Choi J, Kim H, Choi Y. Theranostic nanoparticles for enzyme-activatable fluorescence imaging and photodynamic/chemo dual therapy of triple-negative breast cancer. Quant Imaging Med Surg 2015;5(5):656-664. doi: 10.3978/j.issn.2223-4292.2015.08.09

Fertility counseling of young breast cancer patients

Matteo Lambertini[1], Paola Anserini[2], Alessia Levaggi[3], Francesca Poggio[3], Lucia Del Mastro[3]

[1]S.C. Oncologia Medica A, IRCCS AOU San Martino-IST, Genova, Italy; [2]Centro di Fisiopatologia della Riproduzione, Unità Operativa Clinico-Ostetrico-Ginecologica, IRCCS AOU San Martino-IST, Genova, Italy; [3]S.S. Sviluppo Terapie Innovative, IRCCS AOU San Martino-IST, Genova, Italy
Correspondence to: Matteo Lambertini. IRCCS AOU San Martino-IST, S.C. Oncologia Medica A, Largo Rosanna Benzi 10, Genova 16132, Italy. Email: matteo.lambertini@hsanmartino.it.

Abstract: Approximately 6% of women with breast cancer are diagnosed before the age of 40. Young age is an independent predictor of adverse outcome and most young breast cancer patients receive systemic treatment with chemotherapy, hormonal therapy or both. The loss or impairment of fertility is a potential side effect of antineoplastic treatments. Due to the rising trend to delaying pregnancy in life, an increasing proportion of young cancer patients who are yet to have a pregnancy will face the problem of iatrogenic menopause in the future. The incidence of anticancer-treatment-related ovarian failure depends on the type of chemotherapy regimen administered, the use of tamoxifen and the age of patients. It rises with increasing age, in the range of 22-61% and 61-97% in women aged <40 years and >40 years respectively. Although there is a clear trend to increasing incidence of ovarian failure with the rise in aging, there may be a small proportion of patients who became amenorrhoeic despite the very young age, thus indicating that also individual factors still unknown may affect the probability of treatment-related ovarian failure. A prompt referral of patients to reproductive counseling and a multidisciplinary team including Oncology and Reproductive Units are essential to face the management of fertility issues in cancer patients. Fertility counseling should include a detailed description of all the available techniques to preserve fertility. The main available fertility preservation techniques, standard and experimental, for young breast cancer patients include: temporary ovarian suppression during chemotherapy with gonadotropin-releasing hormone analogues, embryo cryopreservation, cryopreservation of oocytes and cryopreservation of ovarian tissue. Research efforts are still necessary to improve the efficacy and safety of the available fertility preservation strategies as well as an efficient collaboration between oncologists and gynecologists is necessary to improve patients' access to the strategies themselves.

Keywords: Fertility preservation; counseling; breast cancer

Submitted Apr 21, 2013. Accepted for publication May 29, 2013.
doi: 10.3978/j.issn.2072-1439.2013.05.22
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.05.22

Introduction

Approximately 3% of all tumours are diagnosed in patients younger than 40 years: the most common types of cancer in young women are breast carcinoma, tumours of the thyroid, melanoma, carcinoma of the cervix and carcinoma of the colon-rectum (1). Concerning breast cancer incidence, approximately 6% of women with breast carcinoma are diagnosed before the age of 40 (1); recent data showed that the incidence of breast cancer diagnosed in young women is increasing (2).

Although the majority of anticancer treatments (surgery, radiotherapy, chemotherapy, endocrine therapy and biologic therapy) have a substantial impact on gonadal function and may lead to loss of fertility (3), therapies performed to treat thyroid cancer and melanoma, generally, do not impair gonadal function. As reported by Stensheim *et al.* in a large population based study, the pregnancy rates in survivors of malignant melanoma or thyroid cancer are similar to that of general population (4). Conversely, a lower pregnancy rate occurred in survivors of breast cancer, cervical cancer and leukemia (4). The available evidence suggests that

Table 1 Congenital abnormalities of infants born to women with history of breast cancer.

Authors	Type of study	Previous anticancer treatment	No. of pregnancies	No. of live births	No. of congenital abnormalities (%)
Azim *et al*. (22)	Retrospective study	Chemotherapy → Trastuzumab	45	33	1 (3.0%)
Sutton *et al*. (23)	Retrospective review	FAC	33	19	0 (0%)
Dalberg *et al*. (24)	Population-based cohort study	N.R.	N.R.	331	24 (7.2%)
Langagergaard *et al*. (25)	Population-based cohort study	N.R.	N.R.	216	7 (3.4%)

FAC, fluorouracil/doxorubicin/cyclophosphamide; N.R., not reported.

fertility preservation is becoming a primary issue for young cancer patients, and that infertility resulting from cancer treatment may be associated with psychosocial distress (3,5). The access to fertility counseling has a growing importance both for the improved prognosis of cancer patients and for the delaying of child-bearing that is a social problem in western nations (6). As recommended by the American Society of Clinical Oncology (ASCO), all oncologists should refer young cancer patients for fertility counseling: particularly, all patients should receive an assessment for and communication regarding risk of treatment-related infertility, and all patients at risk of infertility and interested in fertility preservation should be referred to a specialist with expertise in fertility preservations methods (3). Nevertheless, at least half of patients have no memory of a discussion about fertility at the time of their treatment disposition (7-11). The likelihood that oncologists discuss fertility preservation with newly diagnosed patients may be affected by patients' characteristics such as prognosis, sex, age, marital status, sexual orientation and finances, but few data are available on this topic (7,12). Furthermore, some studies have suggested that oncologists may not know the clinical recommendations related to this issue or that their knowledge on the subject has little update (12,13); other studies report the negative effect of the lack of ad hoc multidisciplinary team (7,14). Fortunately, in recent years there is an improved understanding of the risks of infertility and of the available strategies to reduce its incidence, and a greater dissemination of information to both medical doctors and patients leading to more informed decision making and improved quality of care (15,16). As confirmed by a recent German study, the proportion of patients who do not remember any discussion about the issues related to fertility prior to treatment is gradually decreasing over time from 67% in the period 1980-1984 to 50% in the period 2000-2004 (17).

The main purpose of the present review is to encourage a reliable fertility counseling as a key moment in the decision-making process of young patients candidates for anticancer treatments. Data about pregnancy after breast cancer, the effect of anticancer treatments on gonadal function, the key points to keep in mind to perform a correct fertility counseling, and data about the available strategies for fertility preservations in breast cancer patients, will be reviewed.

Pregnancy after breast cancer

The proportion of patients with at least one full-term pregnancy after breast cancer diagnosis reported in the literature is very low: only 3% of women younger than 45 years at diagnosis (8% if considering only women aged less than 35 years) (18-21). This result is due to several factors including the damage derived from gonadotoxic therapy and the fear related to a negative impact of pregnancy on the evolution of breast cancer. There are two main concerns for young cancer patients to experience pregnancy after cancer diagnosis and treatment: the occurrence of congenital abnormalities and the potential obstetric and birth complications due to previous cancer treatments, and the possibility that pregnancy might have negative consequences on the prognosis of the patient herself.

Regarding the first point, data from four studies are available (22-25) (*Table 1*). The reported rate of congenital abnormalities of infants born to women with history of breast cancer ranges from 0% (23) to 7.2% (24). Considering that the percentage of congenital abnormalities in general population is nearly 4%, the rate observed in women with history of breast cancer is similar to that of general population in all (22,23,25) but one (24) of available studies. In the study by Dalberg *et al.* the congenital abnormalities reported were: ten cardiac defects (including

Table 2 Spontaneous abortion rate and induced abortion rate for pregnancies after breast cancer diagnosis and treatments.

Authors	Type of study	Study population	No. of patients with pregnancy	No. of pregnancies	No. of spontaneous abortion (%)	No. of induced abortion (%)
Kroman *et al*. (26)	Retrospective study	5,725	173	211	22 (10.4%)	92 (43.6%)
Gelber *et al*. (28)	Retrospective study	N.R.	94	137	12 (8.7%)	33 (24%)
Blakely *et al*. (18)	Retrospective study	383	47	47	4 (8.5%)	10 (21.2%)
Ives *et al*. (20)	Retrospective study	2,539	123	175	15 (8.5%)	42 (24%)
Cordoba *et al*. (30)	Retrospective study	115	18	18	0	8 (44.4%)
Kranick *et al*. (32)	Retrospective cohort study	451	107	107	11 (10.2%)	39 (36.4%)
Azim *et al*. (33)	Retrospective cohort study	1,207	333	333	N.R.	135 (41.7%)*

*The number of spontaneous or induced abortion was not specified. N.R., not reported.

three children with patent ductus arteriosus and four with septal defects), three kidney/ureteragenesis defects, two undescended testes in full-term infants, two unspecified limb malformations, two ear malformations, two skin malformations, one chromosome anomaly (trisomy 21), one congenital hydrocephaly, and one orofacial cleft (24).

Regarding to obstetric and birth complications a relatively higher abortion rate (20-44%) was reported in patients with history of breast cancer as compared to the untreated population (20,26-31). Such a higher abortion rate reflects the uncertainties and fear faced not only by patients but also by their threating physicians about the safety of pregnancy after the diagnosis and treatment of breast cancer (*Table 2*). Indeed, two recent cohort studies in a large population of women previously treated for breast cancer are reassuring (24,25), although the study by Dalberg *et al.* reported a higher incidence of birth complications, such as caesarean section, preterm birth, babies with low birth weight, in women previously treated for breast cancer as compared to controls (24). Therefore, a close monitoring of pregnancy in women previously treated for cancer is recommended.

With regard to the concern about the potential negative impact of pregnancy on patients' prognosis, in the past, on the basis of purely theoretical assumptions, pregnancy after breast cancer was contraindicated. The available clinical data do not confirm such hypothesis: so far, it is well established that women who became pregnant after breast cancer do not have a worse prognosis (18-20,26,28,31-38). A meta-analysis of 14 retrospective control-matched studies that assessed the impact of pregnancies on overall survival (OS) of women with history of breast cancer, showed that women who got pregnant following breast cancer diagnosis had a 41% reduced risk of death compared to women who did not get pregnant [pooled relative risk (PRR): 0.59; confidence interval (CI): 0.50-0.70] (39). Even after correcting data for the so called "healthy mother effect", in the subgroup analysis where the outcome of women with history of breast cancer who became pregnant was compared to breast cancer patients who did not get pregnant and were known to be free of relapse, there was no significant differences in survival between groups (PRR: 0.85; 95% CI: 0.53-1.35) (39).

To better clarify the impact of pregnancy on disease-free survival (DFS) in women with history of breast cancer according to estrogen receptor status, Azim *et al.* performed a multicenter retrospective cohort study (33). Patients who became pregnant any time after breast cancer were matched to patients with breast cancer with similar estrogen receptor, nodal status, adjuvant therapy, age and year at diagnosis: the primary objective was DFS in patients with estrogen receptor-positive breast cancer. No difference in DFS was observed between pregnant and non-pregnant patients in the estrogen receptor positive group [hazard ratio (HR): 0.91; 95% CI: 0.67-1.24] or the estrogen receptor negative cohort (HR: 0.75; 95% CI: 0.51-1.08). However the pregnant group had better overall survival (HR: 0.72; 95% CI: 0.54-0.97) with no interaction according to estrogen receptor status (33). So far, the historical contraindication to pregnancy in patients with previous history of breast cancer should be considered permanently dropped out, even if it is not clear yet the ideal interval to wait between the end of anticancer treatments and the conception. There are no biological rationale or supporting evidences to define a "gold standard time" for women to become subsequently pregnant (40). However, experts recommend avoiding early pregnancy within 2 years from diagnosis in case of high risk of early relapse (41). Timing could be "personalized" taking into accounts patient age, risk of relapse, previous

treatments and need for adjuvant hormonal therapy (18,19,42). On this issue, a project carried on by the Breast International Group and North American Breast Cancer Group (BIG-NABCG) is going to start: it is a prospective study directed to young women with endocrine sensitive early breast cancer who desire to become pregnant and who are disease free after 2 years of adjuvant endocrine therapy (38). The major aims of the projects are to assess patients and offspring outcomes, focusing on pregnancy (abortion, miscarriage, ectopic stillbirth, live birth rates), birth (preterm birth, low birth weight, birth defects rates) and breast cancer outcomes (DFS, OS). The trial is divided in two phases: (I) the observational phase investigates the feasibility and impact of a temporary treatment interruption to allow conception; (II) the subsequent experimental phase will investigate the optimal duration of subsequent endocrine treatment after delivery (38).

Reassurance on the safety of pregnancy in patients who experienced breast cancer is increasing the number of couples who have access to the Centers of Reproductive Medicine because of infertility after cancer treatments. Even though assisted reproduction may be an option for those couples with other infertility factors (such as tubal factor, endometriosis, male factor, etc.) when infertility is due to reduced ovarian function because of gonadotoxic therapies, reduced success are obtained compared with non-cancer patients (43).

Effect of anticancer treatments on gonadal function

Infertility is defined as the inability to conceive after 1 year of intercourse without contraception.

Anticancer treatment may have a negative impact on gonadal function and may lead to loss of fertility and early menopause. Acute amenorrhea occurring during treatment, may be affected permanently or temporary and results from loss of the growing follicle population. The majority of patients younger than 40 years recover menses within 1 year from cessation of treatment; incidence of permanent amenorrhea after systemic treatment for breast cancer is estimated to be between 33% and 76% in women age 50 or younger (44). However since the primordial follicle pool is bound to be reduced also in women who resume menses, patients should be advised of a higher risk of infertility and premature menopause to let them make a well-timed family planning. It has been demonstrated that women who continue to menstruate after treatment with chemotherapy for breast cancer remain at an increased risk of entering menopause early and that a significative reduction of fertility potential anticipate menopause of about 5 years (45).

The effects of anticancer treatments on reproductive organs may be direct (e.g., pelvic surgery or irradiation, chemotherapy) or may derive by hormonal alteration (e.g., a cranial irradiation damaging the pituitary axis) (16). The rate of anticancer treatment-related infertility is variable and depends on several factors: class, dose, dose-intensity of the drug used, method of administration (oral versus intravenous), size and location of the radiation field, the radiation delivered dose and its fragmentation, age of the patient, disease, history of previous treatment for infertility, comorbidities (3).

Particularly, the incidence of anticancer-treatment-related ovarian failure in breast cancer patients depends mainly on the type of chemotherapy regimen administered, the use of tamoxifen and the age of patients at diagnosis. It rises with increasing age, in the range of 22-61% and 61-97% in women aged <40 years and >40 years respectively (46). Among chemotherapy agents, the greatest risk is associated with alkylating agents (particularly cyclophosphamide) (47-49); also carboplatin and cisplatin can have a negative effect. A low risk of treatment-related ovarian failure is associated with methotrexate (M) and fluorouracil (F) (3). Few data are available for newer agents such as taxanes. Fornier *et al.* reported a case series of 230 women younger than 40 years treated with the addition of taxanes to anthracycline-containing chemotherapy for breast cancer showing a similar rate of amenorrhea for this women compared to historical controls (50). However, available date about the risk of amenorrhea with taxanes are still not conclusive (51).

Focusing on clinical studies in breast cancer patients, the incidence of chemotherapy-induced amenorrhea by regimen ranged from 9% to 75% (*Table 3*) (50,52-56). Ganz and colleagues provided results of the menstrual history (MH) and quality-of-life (QoL) outcomes in breast cancer patients treated with adjuvant therapy within the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-30 trial (56). The NSABP B-30 trial was a three-arm multicenter study carried on in 5,300 women with early-stage, node-positive breast cancer: it demonstrated that adjuvant therapy with sequential doxorubicin (A) and cyclophosphamide (C) followed by docetaxel (T; AC→T), compared with four cycles of AT or TAC, improved DFS and OS (57). MH and QoL were secondary outcomes of

Table 3 Incidence of chemotherapy induced amenorrhea by regimen reported in breast cancer clinical trials.

References	Regimen	% patients developing amenorrhea
Bines *et al*. (52)	CMF ×6	20-75
Bines *et al*. (52)	AC ×4	34
Bines *et al*. (52)	MF ×6	9
Venturini *et al*. (53)	CEF ×6	50-60
Levine *et al*. (54)		
Martin *et al*. (55)	FAC ×6	51
Martin *et al*. (55)	TAC ×6	61
Fornier *et al*. (50)	AC ×4 → T ×4	15*
Ganz *et al*. (56)	AC ×4 → T ×4	70
Ganz *et al*. (56)	AT ×4	38
Ganz *et al*. (56)	TAC ×4	58

*only ≤40 years patients; amenorrhea ≥12 months. CMF, cyclophosphamide/methotrexate/fluorouracil; AC, doxorubicin/cyclophosphamide; MF, methotrexate/fluorouracil; CEF, cyclophosphamide/epirubicin/fluorouracil; FAC, fluorouracil/cyclophosphamide/doxorubicin; TAC, docetaxel/doxorubicin/cyclophosphamide; T, docetaxel; AT, doxorubicin/docetaxel.

the trial and were assessed with standardized questionnaires at baseline and at follow-up visits every 6 months (56). Pre-specified analyses evaluated rates of amenorrhea by treatment arm, the relationship between amenorrhea and QoL, and QoL by treatment arm. Prolonged amenorrhea was defines as having at least 6 months without a menstrual cycle. The rates of prolonged amenorrhea at 12 months after the start of therapy was significantly different between treatment arms: 69.8% for AC→T, 37.9% for AT, and 57.7% for TAC (P<0.001). The amenorrhea rates were higher with the addition of tamoxifen; the AT group without tamoxifen showed the lowest rate of amenorrhea, hovering around 20-30% across the 24-month period of observation. Approximately 61% of women under the age of 40 experienced at least 24 months of amenorrhea contrasting with nearly 100% among patients older than 40 years (56). This study highlighted that, among chemotherapy agents, alkylating agents are associated with a high gonadal toxicity. There are two major mechanisms associated with chemotherapy induced ovarian toxicity, the direct induction of follicle and oocyte apoptosis (58) and the vascular damage to the ovary (59). Compared to untreated women, patients receiving chemotherapy showed a significantly lower follicle counts (58). Such an effect was more pronounced in patients receiving alkylating agents than in patients who did not receive these agents (58). Moreover, chemotherapy regimens, regardless of whether they include an alkylating agent, showed to alter also ovarian stromal function: ovarian cortical pieces that were previously exposed to chemotherapy secreted significantly less estradiol compared with controls (58). Injury to blood vessels and focal damage to the ovarian cortex are considered other important mechanisms for chemotherapy induced ovarian toxicity (59). Ovarian tissue previously exposed to chemotherapy, showed to have severe narrowing and obliteration of the vascular lumen of cortical blood vessels, due to hyalinization of the vessel, intimal fibrosis and thickening of the muscular layer; furthermore, ovaries exposed to chemotherapy revealed several areas of subcapsular focal cortical fibrosis with preservation of the ovarian surface epithelium (59).

Concerning adjuvant endocrine therapy, tamoxifen alone is associated with a low risk of premature menopause, which is strictly dependent on age: over the age of 45, the risk of infertility is 10% higher than in controls (60). The administration of tamoxifen sequentially to chemotherapy causes a statistically significant increase in the risk of infertility compared to chemotherapy alone (53,61). The analogues of luteinizing hormone (LHRHa) or gonadotropin-releasing hormone (GnRHa) lead to a temporary ovarian suppression; however, the reversibility of such effect is strongly influenced by patients' age: the resumption of menstrual cycles is expected in 90% of patients under the age of 40, and in 70% of women older than 40 years (19,60).

Age, the specific chemotherapy regimen administered and marginal tamoxifen use have a very important impact on ovarian function in young cancer patients. This finding has been confirmed by Petrek and colleagues in

a prospective observational study that assessed ovarian function after breast cancer treatment in 595 premenopausal patients (62). Ovarian function was assessed using the surrogate of monthly bleeding; median follow-up was 45 months. Patients of all ages experienced disruptions in their menstrual activity: however, the majority of women aged 40 years or older had no menstrual bleeding at the end of chemotherapy and no recovery of bleeding in the follow up years compared with younger women. Patients younger than 35 years had rapid menstrual cycling recovery with the proportion with bleeding rising to approximately 85% at 6 months following the end of chemotherapy, and remaining relatively constant; the recovery was less pronounced for patients between the ages of 35 and 40. Concerning the chemotherapy regimen administered, treatment with AC alone resulted in an important decrease in the proportion of patients with periods; paclitaxel or T added to AC led to a small further decline in the number of patients with bleeding, while CMF resulted in a greater proportion of patients with monthly bleeding in the initial months but with a progressive decrease in the follow-up years. Finally, the addition of tamoxifen resulted in a decrease in the proportion of patients with monthly bleeding by 1 year following chemotherapy, but this effect became non significant by 3 years. In conclusion, significantly different proportions of women had monthly bleeding depending on their age ($P<0.001$), chemotherapy regimen ($P<0.001$), and time since chemotherapy. Using monthly bleeding as surrogate to assess ovarian function, authors showed the important impact of age, chemotherapy regimen and tamoxifen use, on gonadal function of young breast cancer patients (62).

Key issues during fertility counseling

The possible impact of anticancer treatment on fertility and menstrual function should be addressed in all breast cancer patients in reproductive age (3). The choice of the best time to discuss issues related to chemotherapy induced infertility risks is complex. In general, an early discussion facilitates the planning of a fertility preservation technique. Particularly, oocyte or embryo cryopreservation needs a couple of weeks from the beginning of a menstrual cycle to be accomplished with the consequence that therapy initiation can be delayed for more than a month. Moreover a young patient who finds out to have a cancer and at the same time is plugged into the decision of whether undergoing a fertility preservation technique, needs some time to make her decision. On the other hand, a premature referral to reproductive counseling may overestimate the need of fertility preservation strategies in the cases that will not require chemotherapy, increasing unnecessarily the psychological burden for these patients. Physician first addresses fertility issues in cancer patients must be aware of the above mentioned pitfalls related to various counseling timing (last menstrual period and expected final diagnosis).

It is responsibility of the radiologist, surgeon and, mainly, of the oncologist to make patients aware of the impact of cancer treatment on fertility and to evaluate if they wish a thorough reproductive counseling (*Figure 1*). Oncologists with enough experience and knowledge in this field, may carry on a complete reproductive counseling themselves and refer to Reproductive Units only those patients who choose to undergo cryopreservation fertility techniques. Oncologists need to have a cooperation with one or more Reproductive Units to give their patients the opportunity to undergo a well-timed and complete counseling (12,13). Therefore, a well-organized linkage between oncology and Reproductive Units is the first step to be accomplished to face the management of fertility issues in cancer patients (*Figure 1*).

According to results of a recent survey on post-treatment QoL that included 1,041 women aged 18-40 years who were counseled either by the oncology team (61%) or by fertility specialists (5%), specialized counseling about reproductive loss and pursuing fertility preservation is associated with less regret and greater QoL for survivors (63). In this study 36 (4%) patients took action to preserve fertility (63).

Fertility counseling should be patient-tailored, since both the impact of chemotherapy on reproductive potential and the success of fertility preservation techniques are strongly linked to patient's age and ovarian reserve. Ovarian reserve is a widely used term to indicate the ovary reproductive potential due to the number and the quality of its oocytes asset (64). Many factors, in addition to age, may affect ovarian reserve and consequently the expected damage induced by chemotherapy, and the success of fertility preservation techniques. Some of these factors, such as multiple ovarian surgery, heavy smoking, progressively shorter cycle duration, family history of premature menopause, are suggested by a proper clinical history collection and should be searched for (65). Ovarian reserve is assessed by hormonal assays and evaluation of antral follicular count (AFC) with transvaginal ultrasound (66). Among hormonal markers, anti-mullerian hormone (AMH) has been proven the more accurate in predicting

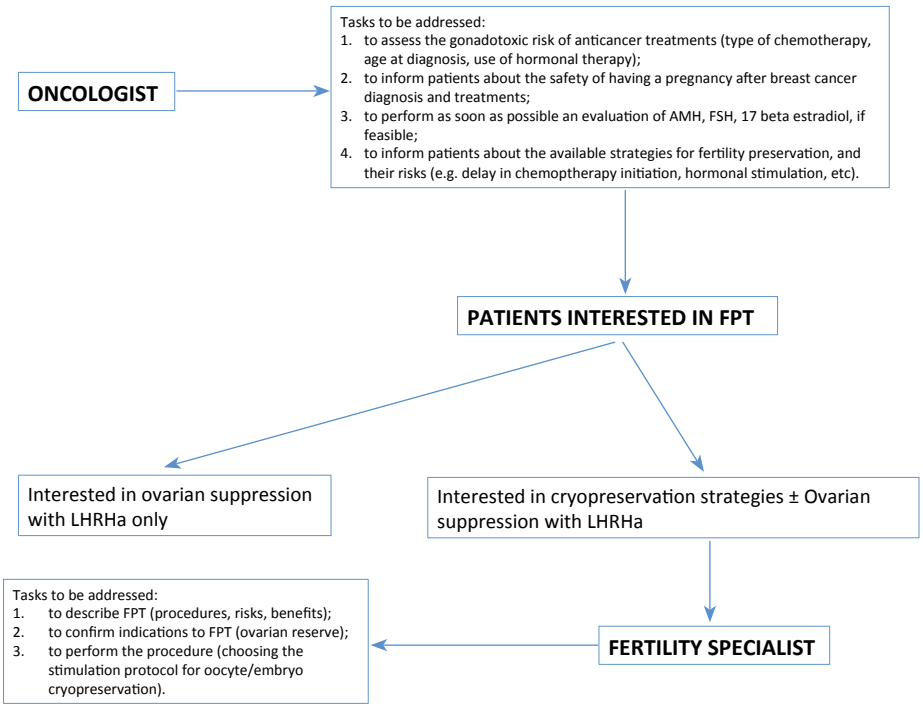


Figure 1 Fertility counseling: major steps of counseling to be accomplished by oncologists and fertility specialists. AMH, anti-mullerian hormone; FSH, follicle-stimulating hormone; FPT, fertility preservation techniques; LHRHa, luteinizing hormone-releasing hormone analogues.

ovarian response to stimulation both in IVF than in fertility preservation cycles (67,68). It is a dimeric glycoprotein produced by granulosa cells, from pre-antral and antral follicles and reflects the ovarian follicular pool. AMH concentration measurements are useful in the evaluation of chemotherapy induced ovarian damage and may become a tool for the comparison of ovarian toxicity of different chemotherapy regimens (69-72). Since AMH concentrations are stable throughout the menstrual cycle, differently from other hormonal markers such as basal follicle-stimulating hormone (FSH) and 17 beta estradiol which must be dosed early in the follicular phase (day 2-4), AMH evaluation should be done as soon as possible to make results available at the time of consultation. Patients' age and ovarian reserve markers measurement are essential to estimate expected damage of anticancer therapies on ovarian function and to decide about fertility preservation techniques (73).

Adequate efficiency of both oocyte/embryo and ovarian tissue cryopreservation can be expected in patients below 38 years of age and with an age-appropriate ovarian reserve. In patients aged between 38 and 40, fertility preservation techniques may be efficacious only in cases with a good ovarian reserve. It has been reported a low response to stimulation with letrozole and gonadotropins for oocytes recovery in breast cancer patients when the AMH level is ≤1.2 ng/mL (68).

Patients should be informed that chemotherapy to treat breast cancer implies a risk of ovarian function compromise that include acute ovarian failure, infertility and early menopause, which probably are three different signs of the same mechanism. It is essential that patients understand that their reproductive potential may be impaired also in the presence of regular menses (74).

Fertility counseling should include a detailed description of all the available techniques to preserve fertility which are appropriate for that particular patient including procedures, timing, possible complications, expected results. It is mandatory to make clear to the patient what is well-known and what is still experimental about these techniques. In some cases, more than one technique can be applied at the same patient or, when chemotherapy can be postponed, more cycles of ovarian stimulation can be performed to storage a larger number of oocytes or embryos rising the chances of future pregnancies. There

Table 4 Available strategies for fertility preservation in breast cancer patients.

Strategy	Definition	Ovarian stimulation required	Preservation of ovarian function	Limits
Oocyte cryopreservation	Harvesting and freezing of unfertilized eggs	Yes	No	❖ Requires 10-14 days of ovarian stimulation; ❖ outpatient surgical procedure; ❖ expensive procedure.
Embryo cryopreservation	Harvesting eggs, in vitro fertilization, and freezing of embryos	Yes	No	❖ Requires 10-14 days of ovarian stimulation; ❖ outpatient surgical procedure; ❖ requires partner or donor sperm; ❖ expensive procedure; ❖ ethical conflict on the fate of the embryos in case the mother dies before implantation.
Ovarian tissue cryopreservation and transplantation	Freezing of ovarian tissue and reimplantation after cancer treatment	No	Yes*	❖ Outpatient surgical procedure; ❖ expensive procedure; ❖ not suitable when risk of ovarian involvement is high; ❖ available in few centers.
Ovarian suppression with LHRHa	Use of hormonal therapies to protect ovarian tissue during chemotherapy	No	Unknown	❖ Conflicting results from phase III trials; ❖ few data on long-term outcomes in patients with endocrine-sensitive breast cancer; ❖ few data about the incidence of pregnancy after breast cancer treatment with the use of such strategy.

*no data are available about the long-term recovery of ovarian function. LHRHa, luteinizing hormone-releasing hormone analogues.

are some circumstances which may increase complications or contraindicate a technique such as thromboembolic risk, severe abdominal adhesions which must be taken into consideration during fertility counseling.

The percentage of patients who choose to undergo oocyte/embryo or ovarian tissue cryopreservation after fertility counseling reported in the literature varies from 4% to over 50% (63,75). In our experience approximately 22% of breast cancer patients accepted to undergo fertility counseling performed by the reproductive physician and 8% underwent surgical fertility preservation techniques (oocytes cryopreservation or ovarian tissue cryopreservation) (76). A better understanding of factors that influence patients' choice will help physicians to improve the quality of fertility counseling.

Strategies for fertility preservation

The choice between the available fertility preservation strategies for young women candidates for cancer treatments depends on several factors: patient's age and ovarian reserve, type of cancer treatment planned, whether she has a partner, the time available, and the possibility that cancer has metastasized to her ovaries (77).

So far, the main available fertility preservation techniques, standard and experimental, for young breast cancer patients are: temporary ovarian suppression, embryo cryopreservation, cryopreservation of oocytes and cryopreservation of ovarian tissue (*Table 4*). Among the cryopreservation techniques, to date, cryopreservation of embryos and of mature oocytes are the only strategies that have shown reliable results, while cryopreservation of ovarian tissue or cryopreservation of immature oocyte or of oocytes matured *in vitro* are still in the early experimental phase.

Ovarian suppression with LHRHa

The rationale for the use of LHRHa to reduce the gonadal toxicity of chemotherapy is the observation that cytotoxic drugs mostly affect tissues with a rapid cellular turnover; then, a state of induced gonadal inhibition during

exposure to chemotherapy may protect the ovaries (78). Because chronic administration of LHRHa decreases FSH secretion and suppresses gonadal function, it has been hypothesized that it may reduce chemotherapy toxicity on the gonads (79). Four phase III studies have recently been published in breast cancer patients candidates for chemotherapy to investigate the efficacy of such strategy to preserve ovarian function (80-83). In these studies, breast cancer patients were randomly assigned to receive adjuvant or neoadjuvant chemotherapy in combination with LHRHa or chemotherapy alone. These studies reported conflicting results. Major limits of these studies are: heterogeneous target population and differences in patients' age at treatment, chemotherapy regimens used, selection of patients, duration of follow-up, and end points utilized to assess treatment efficacy. A recent meta-analysis to evaluate the role of LHRHa in the prevention of chemotherapy-induced premature ovarian failure (POF) has been presented: a total of seven randomized clinical trials involving 745 premenopausal patients randomly assigned to receive chemotherapy or chemotherapy plus LHRHa were included in the analysis; 5 trials were carried out in breast cancer patients and two trials in lymphoma patients (84). The pooled odds ratio estimate for chemotherapy induced POF was 0.46 (95% CI: 0.3-0.72) showing an important benefit of this strategy in reducing the gonadal toxicity of cytotoxic therapy in premenopausal cancer patients (84). Recently, a meta-analysis designed to assess the efficacy of LHRHa administration to prevent chemotherapy induced ovarian toxicity specifically in premenopausal breast cancer women has been published (85). Five randomized clinical trials (total number of patients: 528) were included in the analysis: significantly fewer women treated with LHRHa during chemotherapy experienced post-treatment POF (RR: 0.40; 95% CI: 0.21-0.75). However, both treatment groups had similar rates of resumed menses (RR: 1.31; 95% CI: 0.93-1.85) and spontaneous pregnancy (RR: 0.96; 95% CI: 0.20-4.56) (85).

This strategy, in contrast to embryo and oocyte cryopreservation, can preserve the overall ovarian function and not only fertility; furthermore, this technique can be performed in combination with cryopreservation strategies, thus increasing the chance of fertility recovery after cancer treatments.

This strategy has two major limits: few data are available on the long term efficacy and safety of the technique, and there is no reimbursement of these treatment by the National Health System of most countries even if the cost of the treatment is lower (about 1,000 euros for 6 months of treatment) than the cost of cryopreservation strategies.

Embryo or oocyte cryopreservation

One of these two strategies is recommended as fertility preservation option in breast cancer patients (86). Cryopreservation of embryos has been the only established procedure for fertility preservation for many years; since January 2013, cryopreservation of oocytes is no longer considered experimental (87). Cryopreservation of oocytes can be applied also in patients without a male partner and in countries where embryo cryopreservation is prohibited. Both techniques may be offered when it is medically reasonable to delay chemotherapy by 2 to 6 weeks because they require a phase of ovarian stimulation lasting about 9-15 days which is usually started at the onset of menses (3). Moreover, since efficacy of oocyte and embryo cryopreservation depends on the number of recovered oocytes, these procedures may be proposed only to patients below the age of 38-40 years and with the possibility to recover a sufficient number of oocytes (approximately 8-15).

To overcome the need to wait the onset of menses and allow more patients the chance of embryo/oocyte cryopreservation without delaying initiation of chemotherapy, there are some attempts with the initiation of ovarian stimulation in the luteal or late follicular phases. Preliminary experiences with these "emergency protocols" showed promising results in terms of oocyte recovery (88-90).

There are still some concerns about the impact of the ovarian stimulation required for oocyte and embryo cryopreservation, on hormone responsive tumors. To reduce the potential risk of short-term exposure to high estrogen levels alternative approaches for ovarian stimulation with letrozole or tamoxifen has been developed (91-93). The largest experience with the use of cryopreservation strategies in breast cancer patients is reported by Azim and colleagues (94). Authors prospectively evaluated for fertility preservation 215 breast cancer patients before adjuvant chemotherapy: a total of 79 women underwent embryo or oocyte cryopreservation, and the remained 136 patients did not undergo any fertility-preserving procedures and served as controls (94). At a median follow up of 23.4 months after chemotherapy the HR for recurrence after *in vitro* fertilization (IVF) was 0.56 (95% CI: 0.17-1.9) and the survival of patients that underwent cryopreservation strategies was not compromised compared with controls (P=0.36). As reported in the conclusion of the paper, "further

research, including longer-term follow-up is needed to confirm these findings" (94).

There are few data on pregnancies obtained with oocyte and embryo cryopreserved in cancer patients: therefore, to estimate pregnancy rate potential of these fertility preservation techniques it is necessary to consider data derived from the age-matched infertile population (95). Moreover, during fertility counseling, clinic-specific success rate should be considered, since results varies among different laboratories.

Among emerging strategies to be considered still experimental, cryopreservation of immature oocyte or of oocytes matured *in vitro* should be mentioned. Through this techniques, oocytes' collection can be obtained without hormonal stimulation or with a short stimulation lasting 3-5 days; immature oocytes can be cryopreserved after maturation *in vitro* or cryopreserved at the immature stage and then matured *in vitro* after thaw before insemination. So far, the results obtained with these strategies are lower than those obtained with oocytes matured *in vivo* (96,97).

Ovarian tissue cryopreservation

This is a promising technique but should be considered still experimental (3). Major advantages are that it does not require neither a sperm donor nor hormonal stimulation, and that it offers the opportunity to preserve both fertility and the overall ovarian function. This strategy can be performed at any time of the menstrual cycle, thus avoiding the delay in chemotherapy initiation; however, it requires a laparoscopic surgery for the removal of fragments of ovarian cortex (98,99). This strategy can be offered to patients younger than 38 years with adequate ovarian reserve: the success rate of the technique in older women is uncertain due to the reduced number of primordial follicles at that age (100). Ovarian tissue is removed through a laparoscopic procedure requiring general anesthesia, and then frozen (3). A large biopsy is needed because many follicles are lost during freezing/thawing/transplantation procedures (101). The ovarian tissue, once the patient has completed cancer treatment, can be transplanted orthotopically to the pelvis (102-106) or heterotopically to subcutaneous areas (for example forearm, lower abdomen) (107,108). To date, more than 25 pregnancies have been reported, all of them after orthotopic grafting, either spontaneously or with assisted reproductive technique. However, it is not possible to express the success rate for autotransplantation of cryopreserved ovarian tissue, as it is not well known how many attempts have been made of reimplantation of thawed frozen ovarian tissue in women.

In a single center experience, four of the seven patients who underwent ovarian tissue transplantation, conceived with assisted reproduction techniques (57%) (109). The percentage of ovarian function recovery is high (90-100%) even if its duration is still limited (up to a few years) (110).

One important concern about the application of this technique is the potential reintroduction of cancer cells (111-113). In a recent large study aiming to assess the incidence of malignant cells in ovarian tissue before cryopreservation, 1.3% (5/391) of ovarian tissue samples were found positive for malignant cells at light microscopy evaluation (114). All positive samples belonged to patients with haematologic disease while so far, no malignant cells have been found in ovarian tissue from breast cancer patients by immunohistochemistry (115,116). However, it is essential to provide an adequate preoperative screening to rule out a possible cancer involvement of the ovary and to perform an accurate histological examination of the ovarian tissue removed before replanting it (115-119).

So far, ovarian tissue cryopreservation has to be considered still experimental and should be performed only in centers with the necessary expertise under approved clinical protocols; furthermore, particular attention should be paid to the follow-up of these patients for recurrent cancer (3).

Conclusions

Loss of reproductive potential as a consequence of anticancer treatment negatively impacts QoL in young survivors (120,121). As showed in recent studies, the potential iatrogenic loss of fertility, which also means loss of a potential child, has a profound impact on young women and in some ways may be more stressful than the cancer diagnosis itself (122,123). So far, all oncologists should refer young cancer patients for fertility counseling: receiving counseling about reproductive loss before anticancer therapies significantly improved QoL after cancer treatment for reproductive-age women (63). Particularly, all patients with newly diagnosed cancer should receive an assessment for and communication regarding risk of treatment-related infertility, and all patients interested in fertility preservation should be referred to a specialist with expertise in fertility preservations methods (3). Since the historical contraindication to pregnancy in patients with previous history of breast cancer should be considered

permanently dropped out, the same recommendation should be applied to breast cancer patients. As showed by Rippy *et al*., an active approach to counseling makes a huge psychological difference (124). Authors assessed in women under the age of 45 at the time of diagnosis of breast cancer, how many of them wanted and tried to become pregnant after breast cancer treatment, the effect of pre-treatment counseling and their prognosis. They showed a higher rate of pregnancy than expected, possibly due to newer treatments including fertility preservation and also possibly due to the active counseling program in the unit. Authors concluded that "the positive attitude of the breast team towards pregnancy may also help reduce the fear of pregnancy after breast cancer and consequently also reduce the elective abortion rate" (124).

Oncologists should feel empowered to discuss the possible fertility loss due to anticancer treatments and the available strategies to reduce such effect. Patients should have active counseling about fertility when planning treatment, and fertility preservation can then be incorporated into a treatment plan. An informed choice about whether to access any available fertility preservation strategy can only be made after a proper discussion of their risks, success rates and costs. On the other hand, being some fertility preservation strategies still experimental and difficult to access in some centers, there is an imperative for oncologists and gynecologists to conduct more research efforts in this important field (125). Major attention should be performed to obtain data on the long term follow up of breast cancer patients that underwent one or more fertility preservation strategies at the time of cancer diagnosis and treatment. More research are needed to improve the efficacy and safety of the available strategies, and an effective collaboration between oncologists and gynecologists should be implemented to improve patients access to reproductive technologies.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Surveillance, Epidemiology and End Results (SEER) Web site. Available online: http://www.seer.cancer.gov. April 2010, based on the November 2009 submission.
2. Merlo DF, Ceppi M, Filiberti R, et al. Breast cancer incidence trends in European women aged 20-39 years at diagnosis. Breast Cancer Res Treat 2012;134:363-70.
3. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006;24:2917-31.
4. Stensheim H, Cvancarova M, Møller B, et al. Pregnancy after adolescent and adult cancer: a population-based matched cohort study. Int J Cancer 2011;129:1225-36.
5. Reh AE, Lu L, Weinerman R, et al. Treatment outcomes and quality-of-life assessment in a university-based fertility preservation program: results of a registry of female cancer patients at 2 years. J Assist Reprod Genet 2011;28:635-41.
6. Johnson JA, Tough S, Society of Obstetricians and Gynaecologists of Canada. Delayed child-bearing. J Obstet Gynaecol Can 2012;34:80-93.
7. Schover LR, Brey K, Lichtin A, et al. Knowledge and experience regarding cancer, infertility, and sperm banking in younger male survivors. J Clin Oncol 2002;20:1880-9.
8. Schover LR, Rybicki LA, Martin BA, et al. Having children after cancer. A pilot survey of survivors' attitudes and experiences. Cancer 1999;86:697-709.
9. Zebrack BJ, Casillas J, Nohr L, et al. Fertility issues for young adult survivors of childhood cancer. Psychooncology 2004;13:689-99.
10. Duffy CM, Allen SM, Clark MA. Discussions regarding reproductive health for young women with breast cancer undergoing chemotherapy. J Clin Oncol 2005;23:766-73.
11. Snyder KA, Pearse W. Discussing fertility preservation options with patients with cancer. JAMA 2011;306:202-3.
12. Quinn GP, Vadaparampil ST, Gwede CK, et al. Discussion of fertility preservation with newly diagnosed patients: oncologists' views. J Cancer Surviv 2007;1:146-55.
13. Köhler TS, Kondapalli LA, Shah A, et al. Results from the survey for preservation of adolescent reproduction (SPARE) study: gender disparity in delivery of fertility preservation message to adolescents with cancer. J Assist Reprod Genet 2011;28:269-77.
14. Quinn GP, Vadaparampil ST, Lee JH, et al. Physician referral for fertility preservation in oncology patients: a national study of practice behaviors. J Clin Oncol 2009;27:5952-7.
15. Partridge AH, Ruddy KJ, Kennedy J, et al. Model program to improve care for a unique cancer population: young women with breast cancer. J Oncol Pract 2012;8:e105-10.

16. Ruddy KJ, Partridge AH. Fertility (male and female) and menopause. J Clin Oncol 2012;30:3705-11.
17. Hohmann C, Borgmann-Staudt A, Rendtorff R, et al. Patient counselling on the risk of infertility and its impact on childhood cancer survivors: results from a national survey. J Psychosoc Oncol 2011;29:274-85.
18. Blakely LJ, Buzdar AU, Lozada JA, et al. Effects of pregnancy after treatment for breast carcinoma on survival and risk of recurrence. Cancer 2004;100:465-9.
19. Del Mastro L, Catzeddu T, Venturini M. Infertility and pregnancy after breast cancer: current knowledge and future perspectives. Cancer Treat Rev 2006;32:417-22.
20. Ives A, Saunders C, Bulsara M, et al. Pregnancy after breast cancer: population based study. BMJ 2007;334:194.
21. Mueller BA, Simon MS, Deapen D, et al. Childbearing and survival after breast carcinoma in young women. Cancer 2003;98:1131-40.
22. Azim HA Jr, Metzger-Filho O, de Azambuja E, et al. Pregnancy occurring during or following adjuvant trastuzumab in patients enrolled in the HERA trial (BIG 01-01). Breast Cancer Res Treat 2012;133:387-91.
23. Sutton R, Buzdar AU, Hortobagyi GN. Pregnancy and offspring after adjuvant chemotherapy in breast cancer patients. Cancer 1990;65:847-50.
24. Dalberg K, Eriksson J, Holmberg L. Birth outcome in women with previously treated breast cancer--a population-based cohort study from Sweden. PLoS Med 2006;3:e336.
25. Langagergaard V, Gislum M, Skriver MV, et al. Birth outcome in women with breast cancer. Br J Cancer 2006;94:142-6.
26. Kroman N, Jensen MB, Melbye M, et al. Should women be advised against pregnancy after breast-cancer treatment? Lancet 1997;350:319-22.
27. Velentgas P, Daling JR, Malone KE, et al. Pregnancy after breast carcinoma: outcomes and influence on mortality. Cancer 1999;85:2424-32.
28. Gelber S, Coates AS, Goldhirsch A, et al. Effect of pregnancy on overall survival after the diagnosis of early-stage breast cancer. J Clin Oncol 2001;19:1671-5.
29. Lawrenz B, Henes M, Neunhoeffer E, et al. Pregnancy after successful cancer treatment: what needs to be considered? Onkologie 2012;35:128-32.
30. Córdoba O, Bellet M, Vidal X, et al. Pregnancy after treatment of breast cancer in young women does not adversely affect the prognosis. Breast 2012;21:272-5.
31. von Schoultz E, Johansson H, Wilking N, et al. Influence of prior and subsequent pregnancy on breast cancer prognosis. J Clin Oncol 1995;13:430-4.
32. Kranick JA, Schaefer C, Rowell S, et al. Is pregnancy after breast cancer safe? Breast J 2010;16:404-11.
33. Azim HA Jr, Kroman N, Paesmans M, et al. Prognostic impact of pregnancy after breast cancer according to estrogen receptor status: a multicenter retrospective study. J Clin Oncol 2013;31:73-9.
34. Kroman N, Jensen MB, Wohlfahrt J, et al. Pregnancy after treatment of breast cancer--a population-based study on behalf of Danish Breast Cancer Cooperative Group. Acta Oncol 2008;47:545-9.
35. Sankila R, Heinävaara S, Hakulinen T. Survival of breast cancer patients after subsequent term pregnancy: "healthy mother effect". Am J Obstet Gynecol 1994;170:818-23.
36. Malamos NA, Stathopoulos GP, Keramopoulos A, et al. Pregnancy and offspring after the appearance of breast cancer. Oncology 1996;53:471-5.
37. Ariel IM, Kempner R. The prognosis of patients who become pregnant after mastectomy for breast cancer. Int Surg 1989;74:185-7.
38. Pagani O, Partridge A, Korde L, et al. Pregnancy after breast cancer: if you wish, ma'am. Breast Cancer Res Treat 2011;129:309-17.
39. Azim HA Jr, Santoro L, Pavlidis N, et al. Safety of pregnancy following breast cancer diagnosis: a meta-analysis of 14 studies. Eur J Cancer 2011;47:74-83.
40. Litton JK. Breast cancer and fertility. Curr Treat Options Oncol 2012;13:137-45.
41. Cardoso F, Loibl S, Pagani O, et al. The European Society of Breast Cancer Specialists recommendations for the management of young women with breast cancer. Eur J Cancer 2012;48:3355-77.
42. Royal College of obstetricians and gynaecologists. Pregnancy and breast cancer. Guideline No. 12; Jan 2004. Available online: http://www.rcog.org.uk/index.sap?PageID=529
43. Barton SE, Missmer SA, Berry KF, et al. Female cancer survivors are low responders and have reduced success compared with other patients undergoing assisted reproductive technologies. Fertil Steril 2012;97:381-6.
44. Hulvat MC, Jeruss JS. Maintaining fertility in young women with breast cancer. Curr Treat Options Oncol 2009;10:308-17.
45. Partridge A, Gelber S, Gelber RD, et al. Age of menopause among women who remain premenopausal following treatment for early breast cancer: long-term results from International Breast Cancer Study Group Trials V and VI. Eur J Cancer 2007;43:1646-53.

46. Del Mastro L, Venturini M, Sertoli MR, et al. Amenorrhea induced by adjuvant chemotherapy in early breast cancer patients: prognostic role and clinical implications. Breast Cancer Res Treat 1997;43:183-90.
47. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinol Metab Clin North Am 1998;27:927-43.
48. Fisher B, Dignam J, Mamounas EP, et al. Sequential methotrexate and fluorouracil for the treatment of node-negative breast cancer patients with estrogen receptor-negative tumors: eight-year results from National Surgical Adjuvant Breast and Bowel Project (NSABP) B-13 and first report of findings from NSABP B-19 comparing methotrexate and fluorouracil with conventional cyclophosphamide, methotrexate, and fluorouracil. J Clin Oncol 1996;14:1982-92.
49. Longhi A, Macchiagodena M, Vitali G, et al. Fertility in male patients treated with neoadjuvant chemotherapy for osteosarcoma. J Pediatr Hematol Oncol 2003;25:292-6.
50. Fornier MN, Modi S, Panageas KS, et al. Incidence of chemotherapy-induced, long-term amenorrhea in patients with breast carcinoma age 40 years and younger after adjuvant anthracycline and taxane. Cancer 2005;104:1575-9.
51. Okanami Y, Ito Y, Watanabe C, et al. Incidence of chemotherapy-induced amenorrhea in premenopausal patients with breast cancer following adjuvant anthracycline and taxane. Breast Cancer 2011;18:182-8.
52. Bines J, Oleske DM, Cobleigh MA. Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer. J Clin Oncol 1996;14:1718-29.
53. Venturini M, Del Mastro L, Aitini E, et al. Dose-dense adjuvant chemotherapy in early breast cancer patients: results from a randomized trial. J Natl Cancer Inst 2005;97:1724-33.
54. Levine MN, Bramwell VH, Pritchard KI, et al. Randomized trial of intensive cyclophosphamide, epirubicin, and fluorouracil chemotherapy compared with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer. National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 1998;16:2651-8.
55. Martin M, Pienkowski T, Mackey J, et al. Adjuvant docetaxel for node-positive breast cancer. N Engl J Med 2005;352:2302-13.
56. Ganz PA, Land SR, Geyer CE Jr, et al. Menstrual history and quality-of-life outcomes in women with node-positive breast cancer treated with adjuvant therapy on the NSABP B-30 trial. J Clin Oncol 2011;29:1110-6.
57. Swain SM, Jeong JH, Geyer CE Jr, et al. Longer therapy, iatrogenic amenorrhea, and survival in early breast cancer. N Engl J Med 2010;362:2053-65.
58. Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer 2007;110:2222-9.
59. Meirow D, Dor J, Kaufman B, et al. Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury. Hum Reprod 2007;22:1626-33.
60. Goodwin PJ, Ennis M, Pritchard KI, et al. Risk of menopause during the first year after breast cancer diagnosis. J Clin Oncol 1999;17:2365-70.
61. Lee S, Kil WJ, Chun M, et al. Chemotherapy-related amenorrhea in premenopausal women with breast cancer. Menopause 2009;16:98-103.
62. Petrek JA, Naughton MJ, Case LD, et al. Incidence, time course, and determinants of menstrual bleeding after breast cancer treatment: a prospective study. J Clin Oncol 2006;24:1045-51.
63. Letourneau JM, Ebbel EE, Katz PP, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer 2012;118:1710-7.
64. Gleicher N, Weghofer A, Barad DH. Defining ovarian reserve to better understand ovarian aging. Reprod Biol Endocrinol 2011;9:23.
65. Abusief ME, Missmer SA, Ginsburg ES, et al. Relationship between reproductive history, anthropometrics, lifestyle factors, and the likelihood of persistent chemotherapy-related amenorrhea in women with premenopausal breast cancer. Fertil Steril 2012;97:154-9.
66. Lutchman Singh K, Muttukrishna S, Stein RC, et al. Predictors of ovarian reserve in young women with breast cancer. Br J Cancer 2007;96:1808-16.
67. La Marca A, Sighinolfi G, Radi D, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010;16:113-30.
68. Lee S, Ozkavukcu S, Heytens E, et al. Anti-Mullerian hormone and antral follicle count as predictors for embryo/oocyte cryopreservation cycle outcomes in breast cancer patients stimulated with letrozole and follicle stimulating hormone. J Assist Reprod Genet 2011;28:651-6.
69. Anderson RA, Themmen AP, Al-Qahtani A, et al. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal

women with breast cancer. Hum Reprod 2006;21:2583-92.
70. Lie Fong S, Lugtenburg PJ, Schipper I, et al. Anti-müllerian hormone as a marker of ovarian function in women after chemotherapy and radiotherapy for haematological malignancies. Hum Reprod 2008;23:674-8.
71. Anderson RA, Cameron DA. Pretreatment serum anti-müllerian hormone predicts long-term ovarian function and bone mass after chemotherapy for early breast cancer. J Clin Endocrinol Metab 2011;96:1336-43.
72. Morgan S, Anderson RA, Gourley C, et al. How do chemotherapeutic agents damage the ovary? Hum Reprod Update 2012;18:525-35.
73. Rosendahl M, Andersen CY, la Cour Freiesleben N, et al. Dynamics and mechanisms of chemotherapy-induced ovarian follicular depletion in women of fertile age. Fertil Steril 2010;94:156-66.
74. Letourneau JM, Ebbel EE, Katz PP, et al. Acute ovarian failure underestimates age-specific reproductive impairment for young women undergoing chemotherapy for cancer. Cancer 2012;118:1933-9.
75. Lawrenz B, Jauckus J, Kupka MS, et al. Fertility preservation in >1,000 patients: patient's characteristics, spectrum, efficacy and risks of applied preservation techniques. Arch Gynecol Obstet 2011;283:651-6.
76. Lambertini M, Anserini P, Fontana V, et al. Prospective observational study on fertility preservation in young early breast cancer patients: the PREFER (PREgnancy and FERtility) trial. 2013 ASCO Annual Meeting (abstract: e17548).
77. Roberts JE, Oktay K. Fertility preservation: a comprehensive approach to the young woman with cancer. J Natl Cancer Inst Monogr 2005;(34):57-9.
78. Rivkees SA, Crawford JD. The relationship of gonadal activity and chemotherapy-induced gonadal damage. JAMA 1988;259:2123-5.
79. Del Mastro L, Giraudi S, Levaggi A, et al. Medical approaches to preservation of fertility in female cancer patients. Expert Opin Pharmacother 2011;12:387-96.
80. Badawy A, Elnashar A, El-Ashry M, et al. Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: prospective randomized study. Fertil Steril 2009;91:694-7.
81. Del Mastro L, Boni L, Michelotti A, et al. Effect of the gonadotropin-releasing hormone analogue triptorelin on the occurrence of chemotherapy-induced early menopause in premenopausal women with breast cancer: a randomized trial. JAMA 2011;306:269-76.
82. Gerber B, von Minckwitz G, Stehle H, et al. Effect of luteinizing hormone-releasing hormone agonist on ovarian function after modern adjuvant breast cancer chemotherapy: the GBG 37 ZORO study. J Clin Oncol 2011;29:2334-41.
83. Munster PN, Moore AP, Ismail-Khan R, et al. Randomized trial using gonadotropin-releasing hormone agonist triptorelin for the preservation of ovarian function during (neo)adjuvant chemotherapy for breast cancer. J Clin Oncol 2012;30:533-8.
84. Del Mastro L, Levaggi A, Poggio F, et al. Role of temporary ovarian suppression obtained with GNRH analogue in reducing premature ovarian failure (POF) induced by chemotherapy in premenopausal cancer patients: a meta-analysis of randomized studies. ESMO Congress 2012, Ann Oncol 2012;23:Suppl 9 (abstract: 1551PD).
85. Yang B, Shi W, Yang J, et al. Concurrent treatment with gonadotropin-releasing hormone agonists for chemotherapy-induced ovarian damage in premenopausal women with breast cancer: a meta-analysis of randomized controlled trials. Breast 2013;22:150-7.
86. ISFP Practice Committee, Kim SS, Donnez J, et al. Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet 2012;29:465-8.
87. Practice Committees of American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril 2013;99:37-43.
88. Michaan N, Ben-David G, Ben-Yosef D, et al. Ovarian stimulation and emergency in vitro fertilization for fertility preservation in cancer patients. Eur J Obstet Gynecol Reprod Biol 2010;149:175-7.
89. Sönmezer M, Türkçüo lu I, Co kun U, et al. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. Fertil Steril 2011;95:2125.e9-11.
90. Bedoschi GM, de Albuquerque FO, Ferriani RA, et al. Ovarian stimulation during the luteal phase for fertility preservation of cancer patients: case reports and review of the literature. J Assist Reprod Genet 2010;27:491-4.
91. Oktay K. Further evidence on the safety and success of ovarian stimulation with letrozole and tamoxifen in breast cancer patients undergoing in vitro fertilization to cryopreserve their embryos for fertility preservation. J Clin Oncol 2005;23:3858-9.
92. Oktay K, Buyuk E, Libertella N, et al. Fertility preservation in breast cancer patients: a prospective

controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol 2005;23:4347-53.
93. Oktay K, Buyuk E, Davis O, et al. Fertility preservation in breast cancer patients: IVF and embryo cryopreservation after ovarian stimulation with tamoxifen. Hum Reprod 2003;18:90-5.
94. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol 2008;26:2630-5.
95. Chung K, Donnez J, Ginsburg E, et al. Emergency IVF versus ovarian tissue cryopreservation: decision making in fertility preservation for female cancer patients. Fertil Steril 2013;99:1534-42.
96. Cao YX, Chian RC. Fertility preservation with immature and in vitro matured oocytes. Semin Reprod Med 2009;27:456-64.
97. Fadini R, Dal Canto MB, Mignini Renzini M, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. Reprod Biomed Online 2009;19:343-51.
98. von Wolff M, Donnez J, Hovatta O, et al. Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy--a technique in its infancy but already successful in fertility preservation. Eur J Cancer 2009;45:1547-53.
99. Donnez J, Dolmans MM. Cryopreservation and transplantation of ovarian tissue. Clin Obstet Gynecol 2010;53:787-96.
100. Oktay K. Evidence for limiting ovarian tissue harvesting for the purpose of transplantation to women younger than 40 years of age. J Clin Endocrinol Metab 2002;87:1907-8.
101. Newton H, Aubard Y, Rutherford A, et al. Low temperature storage and grafting of human ovarian tissue. Hum Reprod 1996;11:1487-91.
102. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med 2000;342:1919.
103. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364:1405-10.
104. Meirow D, Levron J, Eldar-Geva T, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005;353:318-21.
105. Radford JA, Lieberman BA, Brison DR, et al. Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet 2001;357:1172-5.
106. Tryde Schmidt KL, Yding Andersen C, Starup J, et al. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer - follicular growth, steroid production and oocyte retrieval. Reprod Biomed Online 2004;8:448-53.
107. Oktay K, Buyuk E, Rosenwaks Z, et al. A technique for transplantation of ovarian cortical strips to the forearm. Fertil Steril 2003;80:193-8.
108. Oktay K, Economos K, Kan M, et al. Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm. JAMA 2001;286:1490-3.
109. Meirow D, Raanani H, Brengauz M, et al. Results of one center indicate that transplantation of thawed ovarian tissue is effective. Repeated IVF reveals good egg quality and high pregnancy rate. Hum Reprod 2012;27:ii115-ii117.
110. Kim SS, Lee WS, Chung MK, et al. Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. Fertil Steril 2009;91:2349-54.
111. Kim SS, Radford J, Harris M, et al. Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. Hum Reprod 2001;16:2056-60.
112. Oktay K, Rodriguez-Wallberg K, Schover L. Preservation of fertility in patients with cancer. N Engl J Med 2009;360:2681; author reply 2682-3.
113. Meirow D, Hardan I, Dor J, et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum Reprod 2008;23:1007-13.
114. Dolmans MM, Jadoul P, Gilliaux S, et al. A review of 15 years of ovarian tissue bank activities. J Assist Reprod Genet 2013;30:305-14.
115. Sánchez-Serrano M, Novella-Maestre E, Roselló-Sastre E, et al. Malignant cells are not found in ovarian cortex from breast cancer patients undergoing ovarian cortex cryopreservation. Hum Reprod 2009;24:2238-43.
116. Rosendahl M, Timmermans Wielenga V, Nedergaard L, et al. Cryopreservation of ovarian tissue for fertility preservation: no evidence of malignant cell contamination in ovarian tissue from patients with breast cancer. Fertil Steril 2011;95:2158-61.
117. Azem F, Hasson J, Ben-Yosef D, et al. Histologic evaluation of fresh human ovarian tissue before cryopreservation. Int J Gynecol Pathol 2010;29:19-23.

118. Fabbri R, Venturoli S, D'Errico A, et al. Ovarian tissue banking and fertility preservation in cancer patients: histological and immunohistochemical evaluation. Gynecol Oncol 2003;89:259-66.
119. Bittinger SE, Nazaretian SP, Gook DA, et al. Detection of Hodgkin lymphoma within ovarian tissue. Fertil Steril 2011;95:803.e3-6.
120. Loprinzi CL, Wolf SL, Barton DL, et al. Symptom management in premenopausal patients with breast cancer. Lancet Oncol 2008;9:993-1001.
121. Tschudin S, Bitzer J. Psychological aspects of fertility preservation in men and women affected by cancer and other life-threatening diseases. Hum Reprod Update 2009;15:587-97.
122. Schover LR. Patient attitudes toward fertility preservation. Pediatr Blood Cancer 2009;53:281-4.
123. Partridge AH, Gelber S, Peppercorn J, et al. Web-based survey of fertility issues in young women with breast cancer. J Clin Oncol 2004;22:4174-83.
124. Rippy EE, Karat IF, Kissin MW. Pregnancy after breast cancer: the importance of active counselling and planning. Breast 2009;18:345-50.
125. Gracia CR, Jeruss JS. Lives in the balance: women with cancer and the right to fertility care. J Clin Oncol 2013;31:668-9.

Cite this article as: Lambertini M, Anserini P, Levaggi A, Poggio F, Del Mastro L. Fertility counseling of young breast cancer patients. J Thorac Dis 2013;5(S1):S68-S80. doi: 10.3978/j.issn.2072-1439.2013.05.22

Sexuality and breast cancer: prime time for young patients

Ana Catarina Pinto[1,2]

[1]Medicine Department, Institut Jules Bordet, Université libre de Bruxelles, Brussels, Belgium; [2]Br.E.A.S.T. Data Centre, Institut Jules Bordet, Brussels, Belgium

Correspondence to: Ana Catarina Pinto, MD, MSc. Boulevard de Waterloo, 121 (7th floor), 1000 Brussels, Belgium. Email: ana-catarina.pinto@bordet.be.

Abstract: Sexuality and sexual functioning is a cardinal domain of health-related quality of life in breast cancer patients, namely in the younger population. Young women below 40 years of age go through a time in their lives where sexual self-identity has recently matured, their professional obligations are demanding and they bear interpersonal and childbearing expectations, all of which can suffer a devastating turnaround with cancer diagnosis and its physical and psychological aftermath. Although these women's sexuality and directed interventions have remained largely unaddressed so far, concepts are evolving and treatment options are becoming diversified, chiefly on the field of non-hormonal pharmacological therapy of sexual dysfunction. This review will examine the definitions of female sexual dysfunction, the etiology of the disorders in young breast cancer patients, the assessment methods, the non-pharmacological and pharmacological treatment options and the challenges that lie ahead.

Keywords: Sexuality; sexual dysfunction; female; breast cancer; chemotherapy; breast surgery

Submitted Apr 23, 2013. Accepted for publication, May 19, 2013.
doi: 10.3978/j.issn.2072-1439.2013.05.23
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2013.05.23

Introduction

Sexuality is a basic and important domain of human experience (1) that can be damaged during and following cancer treatment (2). Several studies refer to sexual functioning as an important domain of health-related quality of life (HRQoL) of oncologic patients (3-6), that, nonetheless, is lacking proper undertaking by healthcare providers (4).

The risk of sexual dysfunction is even of greater importance among young cancer patients and survivors (4,7), with young breast cancer (BC) patients at particularly high risk (4,5,7). The reasons are: (I) the growing number of diagnosis of BC among premenopausal women all over the world (8), who already comprise 25% of all diagnosis of breast cancer (9); (II) a higher fragility of young women regarding their sexual self-conception and body image (versus their older counterparts); (III) the closely related comorbidities of premature menopause and infertility, caused by the disease and its treatments, that frequently develop concomitantly with sexual dysfunction; (IV) the developmental and relational period in these women's lives, where they are struggling to form stable peer and intimate partner relationships, highly engaged in studies or early professional careers, gaining/reinforcing their personal and financial independence, and having to manage and integrate all this with BC diagnosis and treatment (5,8-12).

The scope of this review will be the pathophysiology of female sexual function, emphasizing the specific risk factors for abnormal functioning in young breast cancer patients, its assessment tools, management (pharmacological and non-pharmacological treatment interventions) and global strategies for improvement of sexual health management in breast cancer oncology practice.

Sexual health and sexual dysfunction in young women with breast cancer

The sexual experience is complex, involving many internal and external factors. There are various definitions of sexuality and sexual functioning, namely the one by the World Health Organization (13), "as a state of physical, mental and social well-being in relation to sexuality… as

Table 1 Categories of female sexual dysfunction (17,18).

Diagnostic categories
Sexual desire/interest disorder
Subjective sexual arousal disorder
Combined sexual arousal disorder
Genital arousal disorder
Persistent sexual arousal disorder
Orgasmic disorder
Vaginismus
Dyspareunia

well as the possibility of having pleasurable and safe sexual experiences, free of coercion, discrimination and violence".

The most widely-know model of sexual functioning (for both sexes) was developed by Masters and Johnson in 1966 (14,15) and consists of four phases: (I) excitement phase (initial arousal) with feelings of sexual pleasure accompanied by physiologic changes (genital vasocongestion, increase in respiratory rate, heart rate and blood pressure); (II) plateau phase with maximal arousal and muscular tension; (III) orgasm, with the peak of sexual pleasure, accompanied by rhythmic contractions of the pelvic musculature and reproductive organs; (IV) resolution phase, with muscular relaxation and an overall sense of well-being. Cleary, Hegarty and McCarthy have very recently proposed a more comprehensive approach of sexual health (16), encompassing the dimensions of Sexual Self-Concept (sexual self-esteem, body image and sexual framing schema), Sexual Functioning (sexual response cycle) and Sexual Relationships (intimacy and communication).

From the conceptual standpoint, for a sexual problem to be considered a sexual dysfunction, according to the American Psychiatric Association (APA), it has to recur or persist in time and cause marked personal distress or interpersonal difficulty (14).

APA defined 5 categories: hypoactive sexual desire disorder (HSDD), female sexual arousal disorder (FSAD), female orgasmic disorder, dyspareunia and vaginismus (14). This classification was, nevertheless, based on the classical four-stage model of sexual response (15), and so it does not encompass the more subjective dimension of the female sexual experience (17). Consequently, a consensus panel organized by the International Committee of the American Foundation for Urological Disease (17,18) devised more comprehensive diagnostic criteria (*Table 1*). For diagnosis, other medical conditions or physiological drug-effects need to be ruled out (17,19) and the sexual problem needs to have a negative impact in the woman's functioning, be it psychological or interpersonal (14,17).

The prevalence of each disorder is not accurate, mostly due to the fact that some disorders have only recently been identified (17,18) and accordingly lack evidence. As the majority of the studies used the APA classification, it is advised to consider the major categories of the disorders (desire/interest, arousal, orgasm, vaginismus/dyspareunia) when referring to them.

In the general adult female population, between 9-43% report having sexual problems (6,20), being the most commonly reported low desire (39%; causing distress in 10-14%), followed by low arousal (26%; causing distress in 5%) and orgasmic difficulties (21%; causing distress in 5%) (20). The reported rates of dyspareunia and vaginismus are about 16% (21,22).

Many of the studies conducted in BC patients were small, had sample biases, were retrospective or lacked a control group, but some suggest even that nearly all women present a problem in sexual functioning after BC treatment (3,6). The prevalence of the most commonly reported sexual problems in BC patients follows the aforementioned trend in the general population (4), generally with higher percentages, especially regarding dyspareunia and vaginismus (35-38%) (4). It is important to stress that most studies do not report the magnitude of sexual impairment before the cancer diagnosis, so part of the problem could already be pre-existent and hence not be fully explained by the oncologic experience (3,6).

Impact of the oncological diagnosis and treatment in the sexual health of young women with breast cancer

Change in the hormonal milieu

Abrupt menopause caused by cancer treatment, with the clustering of vasomotor symptoms, sleep disturbances, and vaginal dryness and atrophy, is more impairing and symptomatic than when following natural menopause (9). The transient or permanent ovarian failure induced by chemotherapy, hormonal therapy or ovarian suppression, causes depletion of the circulating level of estrogens and testosterone, two steroid hormones that play an important role in sexual functioning. Lack of estradiol is associated with diminished libido and sexual responsivity,

hypoestrogenization impairs vulvovaginal vasocongestion during arousal and causes vulvovaginal atrophy and dryness which can lead to pain during intercourse (7,23,24). Studies evaluating the correlation between androgens [testosterone, androstenedione, dihydrotestosterone and dehydroepiandrosterone (DHEA)] and female sexual function have reported conflicting results (18,24,25). The fact testosterone is a precursor for estrogen formation renders difficult the distinction between the physiological effects of the two hormones (18); still, testosterone has vasodilatory effects, and may be linked to vaginal health and also an increase in libido and arousal, mainly in the postmenopausal population (26-28).

Breast cancer surgery

The association between the type of surgery, body image and sexual functioning has provided inconsistent results (3,10,29,30). One confounding factor in most studies (due to the multimodal treatment often necessary in young women with BC) might be chemotherapy, as women who received it do systematically worse in sexual functioning than women not having been submitted to it (3,9,11,25,29). The complexity also arises from the different "breast concepts" at play. Langellier and Sullivan identified four different, yet closelly related, "types of breasts" present on the speech and experience of BC patients (31): (I) the "medicalized breast"—the diseased body part, whose removal is usually accompanied by relief; (II) the "functional breast"—symbol of the nurturing quality of women, particular important in her relationship to her children; (III) the "gendered breast"—synonym of femininity, beauty and sexual attractiveness, on the personal and social spheres; (IV) the "sexualized breast"—pertaining to the tactile and visual sensations of the organ itself (30,31). It is, therefore, understandable that women can have dichotomous feelings about the breast and its changes during the cancer diagnosis and treatment. As an example, in some cultures and ethnic environments, undergoing a mastectomy and losing one breast is regarded as becoming "half a woman" (32).

Evidence shows that, overall, women with a better bodily self-image prior to the BC diagnosis cope better and have higher sexual satisfaction scores than women with a worse prior body image; the same holds true for women without and with previous mood disorders (anxiety, depression), respectively (25,29,30); also the HRQoL impact of the body alterations seems to be higher in the first year post-diagnosis, improving thereafter (30,33).

With respect to the type of surgical procedure, despite some controversial results (29,34,35), a growing body of evidence states that body image is significantly better in women who have undergone breast conserving surgery, as opposed to mastectomy, but this is the only aspect where it impacts sexuality (3,10,30). Indeed, giving the patient an active role in the choice of surgery, rather than the extent of the surgery itself, seems to be a major determinant of satisfaction with self-image; the empowerment of the woman on the preferences about her own body is far more crucial than the outcome itself (3,5,7).

In patients having undergone breast reconstruction, it is provoking to acknowledge that, although the final results are considered satisfactory by most, they report not having been properly informed about loss of nipple and breast sensation (4,36). Newer oncoplastic surgery techniques promise better overall cosmetic results (7).

There is no consensus regarding the impact on sexual functioning of the interval between surgery and the resumption of sexual intercourse (29) although some have found the longer the deferment, the higher the chance of sexual dysfunction (8).

Breast cancer systemic therapy

The alterations in sexual functioning brought about by antineoplastic drugs may be temporary or permanent and depend greatly on the drug class, total dose delivered, schedule of administration and time-length of therapy, as well as concomitant use of other antineoplastics or drugs that can modulate their action (37).

Globally, chemotherapy is a major determinant of sexual dysfunction, affecting all the phases of the sexual response cycle (30,38). This repercussion is particularly stern and catastrophic for young women, who are frequently also dealing with the grief of infertility (5,9,29,30). Cytotoxic chemotherapy, besides the chemotherapy-induced amenorrhea and ovarian failure entailing the endocrine consequences earlier described, also causes alopecia, nails changes and weight gain or loss (7,8,29,37,39), affecting women's sexual self-concept and, consequently, their sexual interactions.

Primary ovarian failure is more common with alkylating agents, antimetabolites, vinca alkaloids, combination protocols and dose-dense regimens (37,39).

Drugs that act on the immune system, such as the targeted monoclonal antibodies trastuzumab and pertuzumab, or colony-stimulating factors may cause tiredness, flu-like symptoms and bone pain, decreasing sexual desire

and altering body image (7). Other targeted agents, such tyrosine kinase inhibitors (lapatinib), mammalian target of rapamycin inhibitors (everolimus), or antiangiogenic agents (bevacizumab) have adverse effects such as fatigue, diarrhea, rash and hypertension, which, besides body-image transformation, can potentially lead to diminished interest in engaging in sexual activity and social isolation (7).

Hormonal treatments, such as antiestrogens, estrogen receptor antagonists, aromatase inhibitors (seldom used in premenopausal BC patients) and gonadotropin-releasing hormone (GnRH) agonists all have similar effects on sexual function (7,9), ultimately originating lack of genital lubrication and subsequent dyspareunia (nonetheless reversible after end of treatment), hot flashes, decreased interest in sex, weight gain and mood changes. Tamoxifen is an exception regarding vulvovaginal health, since its estrogenic effect in the vaginal epithelium may actually prevent vaginal dryness (7,9).

Comorbid conditions, concomitant medications and relationship factors

Mood disturbances, as anxiety and depression, are highly prevalent in BC patients, with depressive symptoms being particularly marked after diagnosis and during the active treatment phase (40). They have also shown to be strong correlates of sexual dysfunction, especially in the domains of low desire and anorgasmia, in several studies in both sexes and in cancer and non-cancer patients (20,25,41). In women submitted to chemotherapy as high as 30-40% can experience a severe and disabling degree of distress that can last for years following diagnosis (41). The psychotropic medications used in the treatment of these conditions have well recognized side effects on sexual function (7,41,42). This stems from their interference with neurotransmitters that act on the central modulation of sexual response: they inhibit dopamine and norepinephrine, which are involved in the arousal phase, and may also increase prolactin, eliciting gonadal suppression (18).

The most common drugs used to treat chemotherapy side effects, such as nausea and vomiting, also have anti-serotonin and anti-dopaminergic effects (there is wide pharmacological class overlap), and hence evoke similar consequences to antidepressants and anxiolytics (7,41,42). Beta-blockers, sometimes prescribed for anxiety, also have a detrimental impact on sexuality (41,43).

In partnered patients, the quality of their relationship is a critical and concordant predictor of sexual functioning (30), surpassing physical changes as a determinant for sexual health (8,30). The partner's understanding and acceptance, a strong intimate bond and good communication and affection help in the sexual renegotiation process that follows the oncologic experience (7,8,30). Young women and their partners seem to need additional focus from healthcare providers, since young husbands are less skilled to cope with illness and, not rarely, the care of young children (8).

Assessment of sexual dysfunction in young women with breast cancer

The diagnostic assessment of patients combines the identification of the diagnostic criteria, elicited by conducting a thorough medical and sexual history, with a pelvic examination (the former is mandatory for sexual pain disorders, but should be performed in all patients with sexual complaints, because it can reveal possible loco-regional etiological factors and co-morbidities).

Presently, there is no gold standard for the evaluation of sexual health problems in cancer patients (4).

The initial step is to discuss the issue. Evidence shows that oncologists and ancillary care providers often feel reluctant to raise the subject, because of inadequate training in sexual matters, personal or patient awkwardness or lack of time (4,7). On the other hand, this leaves the patient uninformed, with unmet needs, conveying the message that sexual dysfunction is meaningless, or that it is a treatment side effect to which there are no solutions and that must be endured in silence (4,7). In spite of the communication barriers and mismatch in expectations between patients and providers, some data suggest patients are quite willing to debate the subject, even though they prefer the healthcare provider to initiate the discussion (7) and sometimes bring it up more easily with their general practitioner than their oncologist (5). Including sexual health as a part of the initial routine oncological treatment plan and follow-up would obviate many of these obstacles and should be an imperative in all practices providing breast cancer treatment (4,9).

One of the most widely accepted screening sexual models that could be useful in oncology is the PLISSIT model created by Annon (7,41): Permission (to discuss the subject), Limited Information (not to overwhelm the patient), Specific Suggestions (to-the-point pragmatic information) and Intensive Therapy (in the case of expert referral needed) (7,41). Concerning patient-reported outcomes, the most widely used questionnaire for women is the Female Sexual Function Index (FSFI), a 19-item instrument covering

Table 2 Pharmacological treatments of sexual dysfunction in young BC patients.

Agent	Targeted sexual problem/dysfunction	Tested in cancer patients (Yes/No)	Level of Evidence[a]
Hormonal			
Estrogens (transdermal estradiol; vaginal estradiol tablets)	Vulvovaginal atrophy and dryness; dyspareunia	Yes[b]	1a
Testosterone (topical cream; transdermal patch)	Low sexual desire	Yes[b]	1b
Tibolone	Sexual desire and arousal	Yes[c]	2b
DHEA (intravaginal cream)	Vulvovaginal atrophy; sexual desire and arousal	Yes	Unknow
Non-hormonal			
Flibanserin	Low sexual desire and distress	No	Unknow
Phosphodiesterase type 5 (PDE-5) inhibitors (sildenafil)	Genital arousal	No	2b
Bremelanotide	Arousal	No	1b
Phentolamine	Vulvovaginal lubrication	No	Unknow
Prostaglandins	Arousal		2b
Bupropion	Reduction of sexual dysfunction; depression	No	Unknow
L-arginine	Vasomotor symptoms; sexual desire	Yes	2b

[a]Level of Evidence based on the classification of the Oxford Centre for Evidence-based Medicine—Levels of Evidence (March 2009) (http://www.cebm.net/?o=1025, last accessed on 15.04.2013); [b]Its use in BC cancer patients lacks long-term safety data and should be discussed on a case-by-case basis (46,47). Alternatives for vaginal dryness and dyspareunia include water-based lubricants and moisturisers; [c]It should not be used in BC patients (one study closed prematurely due to safety concerns).

6 domains of sexual functioning: sexual desire, sexual arousal, lubrication, orgasm, satisfaction and pain (44). The provider should devoid his speech of technical jargon and colloquialisms, avoid cultural and ethnical stereotyping and also avert being judgmental (7); this is particularly true regarding singled patients, whose sexuality is often dismissed by the provider, and that may have or be attempting sexual interactions and have questions and needs that require equal addressing (4).

Management of sexual dysfunction in young women with breast cancer

There is a dearth for evidence-based treatments for sexual dysfunction in the context of breast cancer, even more so for young patients, due to the lack of attention received by this subgroup in the majority of studies conducted thus far (4).

Setting realistic tailored treatment goals, using a multidisciplinary team approach (oncologist, nurse, psychologist, psychiatrist, sex therapist, pelvic physical therapist) and treating associated conditions that might be at the origin of the problem (for e.g., changing an antidepressant by another of a different class with a better profile regarding sexual side effects) should be principles of intervention. In partnered relationships the partners should be involved in the intervention, by some form of couple's therapy, which has been proven to be one of the most effective non-pharmacological strategies (4,45). Brief counseling or short-term sex therapy programs can yield positive results (4). For younger patients, community-based support, retreats and social programs look more appealing and suitable (7).

Pharmacological treatments are summarized in *Table 2* (4,9,18,48).

Future directions: an integrative approach for the improvement of sexual health management in breast cancer oncology practice

Whereas BC treatment does not radically differ according

to the patients' age group, ideal management of BC patients below 40 years of age demands attention to certain issues that are specific of that age group (8,49) and, thereupon, certain resources that may not be widely available in oncology clinics. As a proof, one can look at the low rates of compliance to the American Society of Clinical Oncology guidelines pertaining to the aspects of discussion of infertility and proper referral to reproductive specialists before start of therapy (50). Dedicated holistic programs such as PYNK (49), involving a multidisciplinary committee, and joining patient psychosocial support, gatherings, a sexual health and rehabilitation clinic with research and educational efforts, are in urgent need of dissemination (49). Meanwhile, oncologic clinics should pay greater attention to patients' sexual health and, in the event of not being endowed with the appropriate resources, provide external referral (4,5). Ethnic diversity with differing sexual constructs and linguistic factors also needs examination, notably in a growing multicultural society (4). Sexual functioning in the cancer continuum is a topic of increasing relevance and it claims competent addressing in the growing communities of younger patients and cancer survivors.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA 1999;281:537-44.
2. Sadovsky R, Basson R, Krychman M, et al. Cancer and sexual problems. J Sex Med 2010;7:349-73.
3. Ganz PA. Sexual functioning after breast cancer: a conceptual framework for future studies. Ann Oncol 1997;8:105-7.
4. Bober SL, Varela VS. Sexuality in adult cancer survivors: challenges and intervention. J Clin Oncol 2012;30:3712-9.
5. Kedde H, van de Wiel HB, Weijmar Schultz WC, et al. Sexual dysfunction in young women with breast cancer. Support Care Cancer 2013;21:271-80.
6. Panjari M, Bell RJ, Davis SR. Sexual function after breast cancer. J Sex Med 2011;8:294-302.
7. Krebs LU. Sexual health during cancer treatment. Adv Exp Med Biol 2012;732:61-76.
8. Cardoso F, Loibl S, Pagani O, et al. The European Society of Breast Cancer Specialists recommendations for the management of young women with breast cancer. Eur J Cancer 2012;48:3355-77.
9. Schover LR. Premature ovarian failure and its consequences: vasomotor symptoms, sexuality, and fertility. J Clin Oncol 2008;26:753-8.
10. Lam WW, Li WW, Bonanno GA, et al. Trajectories of body image and sexuality during the first year following diagnosis of breast cancer and their relationship to 6 years psychosocial outcomes. Breast Cancer Res Treat 2012;131:957-67.
11. Ganz PA, Desmond KA, Leedham B, et al. Quality of life in long-term, disease-free survivors of breast cancer: a follow-up study. J Natl Cancer Inst 2002;94:39-49.
12. Odo R, Potter C. Understanding the needs of young adult cancer survivors: a clinical perspective. Oncology (Williston Park) 2009;23:23-7, 33.
13. Organization WH. What constitutes sexual health? 2010.
14. Association AP. eds. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition - Text Revision (DSM-IV-TR). Washington, DC: American Psychiatric Association editions, 2000.
15. Masters W, Johnson V. eds. Human sexual response. Toronto, New York: Bantam Books, 1966.
16. Wylie K, Mimoun S. Sexual response models in women. Maturitas 2009;63:112-5.
17. Basson R. Women's sexual dysfunction: revised and expanded definitions. CMAJ 2005;172:1327-33.
18. Fooladi E, Davis SR. An update on the pharmacological management of female sexual dysfunction. Expert Opin Pharmacother 2012;13:2131-42.
19. Basson R, Leiblum S, Brotto L, et al. Definitions of women's sexual dysfunction reconsidered: advocating expansion and revision. J Psychosom Obstet Gynaecol 2003;24:221-9.
20. Shifren JL, Monz BU, Russo PA, et al. Sexual problems and distress in United States women: prevalence and correlates. Obstet Gynecol 2008;112:970-8.
21. Pitts MK, Ferris JA, Smith AM, et al. Prevalence and correlates of three types of pelvic pain in a nationally representative sample of Australian women. Med J Aust 2008;189:138-43.
22. Harlow BL, Stewart EG. A population-based assessment of chronic unexplained vulvar pain: have we underestimated

the prevalence of vulvodynia? J Am Med Womens Assoc 2003;58:82-8.
23. Shafer L. Sexual dysfunction. In: Carlson K, Eisenstat S. eds. Primary care of women. St. Louis, MO: Mosby, 2002:415.
24. Dennerstein L, Dudley EC, Hopper JL, et al. Sexuality, hormones and the menopausal transition. Maturitas 1997;26:83-93.
25. Speer JJ, Hillenberg B, Sugrue DP, et al. Study of sexual functioning determinants in breast cancer survivors. Breast J 2005;11:440-7.
26. Shifren JL, Braunstein GD, Simon JA, et al. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 2000;343:682-8.
27. Simon J, Braunstein G, Nachtigall L, et al. Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 2005;90:5226-33.
28. Shifren JL, Davis SR, Moreau M, et al. Testosterone patch for the treatment of hypoactive sexual desire disorder in naturally menopausal women: results from the INTIMATE NM1 Study. Menopause 2006;13:770-9.
29. Fobair P, Stewart SL, Chang S, et al. Body image and sexual problems in young women with breast cancer. Psychooncology 2006;15:579-94.
30. Gilbert E, Ussher JM, Perz J. Sexuality after breast cancer: a review. Maturitas 2010;66:397-407.
31. Langellier KM, Sullivan CF. Breast talk in breast cancer narratives. Qual Health Res 1998;8:76-94.
32. Manderson L, Stirling L. The absent breast: speaking of the mastectomised body. Feminism Psychol 2007;17:75-92.
33. Joly F, Espie M, Marty M, et al. Long-term quality of life in premenopausal women with node-negative localized breast cancer treated with or without adjuvant chemotherapy. Br J Cancer 2000;83:577-82.
34. Parker PA, Youssef A, Walker S, et al. Short-term and long-term psychosocial adjustment and quality of life in women undergoing different surgical procedures for breast cancer. Ann Surg Oncol 2007;14:3078-89.
35. Han J, Grothuesmann D, Neises M, et al. Quality of life and satisfaction after breast cancer operation. Arch Gynecol Obstet 2010;282:75-82.
36. Snell L, McCarthy C, Klassen A, et al. Clarifying the expectations of patients undergoing implant breast reconstruction: a qualitative study. Plast Reconstr Surg 2010;126:1825-30.
37. Azim HA Jr, Peccatori FA, de Azambuja E, et al. Motherhood after breast cancer: searching for la dolce vita. Expert Rev Anticancer Ther 2011;11:287-98.
38. Ochsenkühn R, Hermelink K, Clayton AH, et al. Menopausal status in breast cancer patients with past chemotherapy determines long-term hypoactive sexual desire disorder. J Sex Med 2011;8:1486-94.
39. Rodriguez-Wallberg KA, Oktay K. Options on fertility preservation in female cancer patients. Cancer Treat Rev 2012;38:354-61.
40. Pinto AC, de Azambuja E. Improving quality of life after breast cancer: dealing with symptoms. Maturitas 2011;70:343-8.
41. Dizon DS. Quality of life after breast cancer: survivorship and sexuality. Breast J 2009;15:500-4.
42. Rees PM, Fowler CJ, Maas CP. Sexual function in men and women with neurological disorders. Lancet 2007;369:512-25.
43. Addis IB, Ireland CC, Vittinghoff E, et al. Sexual activity and function in postmenopausal women with heart disease. Obstet Gynecol 2005;106:121-7.
44. Rosen R, Brown C, Heiman J, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther 2000;26:191-208.
45. Taylor S, Harley C, Ziegler L, et al. Interventions for sexual problems following treatment for breast cancer: a systematic review. Breast Cancer Res Treat 2011;130:711-24.
46. Santen RJ, Allred DC, Ardoin SP, et al. Postmenopausal hormone therapy: an Endocrine Society scientific statement. J Clin Endocrinol Metab 2010;95:s1-s66.
47. Davis SR, Moreau M, Kroll R, et al. Testosterone for low libido in postmenopausal women not taking estrogen. N Engl J Med 2008;359:2005-17.
48. Shifren JL. Sexual dysfunction in women: Management. In: Rose BD. eds. Waltham, UpToDate in Waltham, MA, 2013.
49. Ali A, Warner E. pynk: Breast Cancer Program for Young Women. Curr Oncol 2013;20:e34-9.
50. Quinn GP, Vadaparampil ST, Lee JH, et al. Physician referral for fertility preservation in oncology patients: a national study of practice behaviors. J Clin Oncol 2009;27:5952-7.

Cite this article as: Pinto AC. Sexuality and breast cancer: prime time for young patients. J Thorac Dis 2013;5(S1):S81-S86. doi: 10.3978/j.issn.2072-1439.2013.05.23

Breast cancer and sleep disturbance: more than simply a quality of life concern

Halle C.F. Moore

The Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA

Correspondence to: Halle C.F. Moore, M.D. The Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA. Email: Mooreh1@ccf.org.

Submitted Sep 18, 2012. Accepted for publication Oct 19, 2012.

doi: 10.3978/j.issn.2224-5820.2012.10.02

View this article at: http://www.amepc.org/apm/article/view/1156/1804

As most practicing oncologists are well aware, problems with sleep are frequent among women with breast cancer. Insomnia affects approximately one quarter of the general adult population with women most commonly affected (1). Sleep problems are particularly prevalent in perimenopausal and postmenopausal women (2) and sleep patterns change with normal aging (3). Other factors associated with sleep disturbance include pain, anxiety and stressful life events - all of which are likely to be present in individuals newly diagnosed with breast cancer.

In the article accompanying this editorial, Van Onselen *et al.* (4) present a prospective evaluation of sleep disturbance and daytime sleepiness over time in female patients undergoing surgery for breast cancer. The initial assessment is done prior to breast surgery with subsequent assessments performed monthly for 6 months. The authors examine self-reported changes in sleep disturbance and evaluate characteristics associated both with baseline levels of sleep disturbance and with how symptoms change over time. Not surprisingly, sleep disturbance was common at baseline in these women with recently diagnosed breast cancer. Although symptoms generally improved over time, mean measures of sleep disturbance remained at clinically significant levels throughout the study period.

Factors associated with baseline sleep disturbance included anxiety, difficulty with coping, fatigue, hot flashes and having received preoperative chemotherapy. Fatigue and anxiety also correlated with daytime sleepiness. Interestingly, other factors associated with baseline daytime sleepiness were somewhat different and included the type of surgery planned whereas difficulty coping did not appear to correlate with baseline scores of daytime sleepiness. The findings indicate that daytime sleepiness, which often correlates with fatigue, may be due to factors other than night-time sleep disturbance. It is also clear that problems with sleep and sleepiness may have a variety of contributing factors as well as different manifestations among individuals.

Due to the nature of this study, a true baseline measure of sleep quality is not available as all patients were enrolled following a recent cancer diagnosis, although many had not yet initiated cancer treatment. The presurgical assessment, however, does provide a snapshot of the high level of sleep related symptoms present in newly diagnosed breast cancer patients. The initial assessment also allows the investigators to evaluate factors associated with change over time. For instance, the presence of comorbid medical conditions, which is a well established risk factor for sleep problems (1), did not appear to influence the trajectory of sleep symptoms over time. In contrast, higher education levels did not appear to affect baseline sleep disturbance scores but did correlate with higher subsequent levels of sleep disturbance that took longer to improve.

The current study does not provide information on use of adjuvant endocrine therapy among study subjects. Aromatase inhibitors in particular have been associated with insomnia (5) and it is unclear to what extent endocrine therapy or endocrine changes among participants may also have contributed to the changes in sleep disturbance over time. Clearly women about to undergo surgery for early stage breast cancer represent a heterogeneous group both in terms of baseline demographics and treatment received.

With this study, Van Onselen *et al.* add to our understanding of the prevalence and time course of sleep problems associated with a breast cancer diagnosis and the findings highlight some of the factors that appear to contribute to sleep concerns. A better understanding of

patient sleep concerns may lead to improvements in quality of life as well as overall health. There is evidence that the relationship between sleep and fatigue, anxiety and medical comorbidity may be bidirectional, meaning that not only do these factors contribute to sleep disturbance but may be consequences of sleep disturbance as well (1). This interaction may explain the propensity for sleep problems to persist even after the initial cause is no longer a factor.

It is also likely that excessive fatigue secondary to sleep disturbance may affect a patient's tolerance of chemotherapy and that insomnia due to endocrine therapy may compromise treatment adherence. Recognition of factors that may predict for persistent sleep difficulties and early intervention to improve sleep and reduce hindrances to sleep has the potential to improve breast cancer related outcomes as well as other physical and psychological health outcomes.

Acknowledgements

None.

Cite this article as: Moore HC. Breast cancer and sleep disturbance: more than simply a quality of life concern. Ann Palliat Med 2012;1(3):211-212. doi: 10.3978/j.issn.2224-5820.2012.10.02

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Morin CM, Benca R. Chronic insomnia. Lancet 2012;379:1129-41.
2. Kravitz HM, Joffe H. Sleep during the perimenopause: A SWAN story. Obstet Gynecol Clin North Am 2011;38:567-86.
3. Collop NA, Salas RE, Delayo M, et al. Normal sleep and circadian processes. Crit Care Clin 2008;24:449-60, v.
4. Van Onselen C, Paul SM, Lee K, et al. Trajectories of Sleep Disturbance and Daytime Sleepiness in Women Before and After Surgery for Breast Cancer. J Pain Symptom Manage 2012. [Epub ahead of print].
5. van de Velde CJ, Rea D, Seynaeve C, et al. Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): a randomised phase 3 trial. Lancet 2011;377:321-31.

Brain metastases in HER2 positive breast cancer: the next hurdle

Samuel Ow[1], Soo-Chin Lee[1,2]

[1]Department of Hematology-Oncology, National University Cancer Institute, National University Health System, Singapore; [2]Cancer Science Institute, National University of Singapore, Singapore

Correspondence to: Soo-Chin Lee, M.D. Department of Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore, Level 7, Tower Block, 1E Kent Ridge Road, Singapore 119228. Email: csilsc@nus.edu.sg.

Submitted Oct 11, 2012. Accepted for publication Oct 27, 2012.
doi: 10.3978/j.issn.2224-5820.2012.10.09
View this article at: http://www.amepc.org/apm/article/view/1324/1800

Advances in our understanding of the biological subtypes of breast cancer have revolutionized its treatment landscape and prognosis. This is particularly so in the field of HER2 positive tumours, where targeted therapy with anti-HER2 agents such as trastuzumab and lapatinib, are now approved options. Since the introduction of trastuzumab, patients with HER2 positive breast cancers are experiencing longer disease- and progression-free intervals as well as better overall survival. Paralleling the problems with an ageing population, the increasing life expectancy of such patients have resulted in a new set of medical issues that oncologists and palliative physicians have to grapple with, such as brain metastases.

Trastuzumab is a humanized monoclonal antibody directed against the extracellular domain of HER2, approved by the United States Food and Drug Administration (FDA) for use in the adjuvant and palliative treatment of HER2 positive breast cancer. It is well established that despite its anti-tumour efficacy, it does not penetrate the blood-brain barrier well, with one study showing serum to cerebrospinal fluid trastuzumab level being 420:1 (1). As such, the brain becomes an important sanctuary site for breast cancer cells to seek refuge in and replicate. Retrospective studies have also shown an increase in the incidence of brain metastases in patients treated with trastuzumab (2,3). Biological factors, in addition to treatment factors, contribute to the predisposition of HER2 positive tumours to disseminate to the brain compared to other subtypes (4). In an analysis of 10 adjuvant trials examining the sites of metastases in 9524 patients with early stage breast cancers treated without anthracyclines, taxanes or trastuzumab in the pre-trastuzumab era (5), the 10-year incidence of central nervous system (CNS) relapse at any time was almost double in patients with HER2 positive disease compared to those with HER2 negative breast cancer (6.8% versus 3.5%; $P<0.01$), supporting the hypothesis that HER2 positive breast cancer is biologically inclined to develop brain metastases. Furthermore, the improved prognosis of HER2 positive breast cancer patients with trastuzumab treatment 'unmasks' brain metastases which may not have been detected had the patients succumb to the disease earlier.

Brain metastases pose a great challenge clinically due to their associated morbidity and significant impact on patients' quality of life. Interestingly, anti-HER2 agents continue to show efficacy in controlling the extra-cranial tumour burden in patients with brain metastases, which may account for the longer time from brain metastases to death observed in HER2 positive metastatic breast cancer patients treated with trastuzumab compared to those who did not receive treatment or have HER2 negative disease (6,7). However, overall survival is still compromised as half of them will eventually die from CNS disease progression (2). Current treatment options for brain metastases in breast cancer include steroids, neurosurgery, stereotactic radiosurgery and whole brain radiotherapy (WBRT), depending on the size and number of lesions (8). WBRT, probably the most commonly employed palliative treatment for brain metastases, is associated with radio-induced neurocognitive impairment that can occur early, or present late with irreversible decline (9). These potentially debilitating side effects are a constant reminder that development of alternative therapies with lower morbidity is still required.

Although trastuzumab, a monoclonal antibody, is unable to permeate the CNS, numerous studies have shown that

lapatinib, a small dual tyrosine-kinase inhibitor of HER1 and HER2, has activity against brain metastases in HER2 positive breast cancer patients. In the landmark study by Geyer *et al.* which proved the superiority of lapatinib and capecitabine over capecitabine alone in patients with advanced HER2 positive breast cancer who had progressed on trastuzumab, a smaller albeit non-significant number of patients developed brain metastases in the combination arm, providing hints that lapatinib could prevent or delay the onset of CNS involvement (10). Since then, lapatinib has been studied prospectively in phase 2 trials in HER2 positive breast cancer patients with brain metastases, as monotherapy (11,12) and in combination with capecitabine (12-14). However, most of these trials were small and involved patients who have previously received WBRT. Objective CNS responses were heterogeneous, ranging from 3% with lapatinib alone to up to 38% for combination therapy. In a small number of patients who had CNS progression on lapatinib monotherapy, 20% experienced partial CNS response when capecitabine was added, suggesting that combination therapy has a role to play even if patients had previous treatment with lapatinib (12). Subgroup analysis of two trials showed that capecitabine-naïve patients had better response than those who had prior exposure to capecitabine (14,15).

The recent online publication by Bachelot *et al.* in Lancet Oncology describes the LANDSCAPE study, a prospective single-arm phase 2, open label, multicentre study in which HER2 positive breast cancer patients with brain metastases without prior exposure to whole brain irradiation, capecitabine or lapatinib, were treated with lapatinib (1,250 mg daily) combined with capecitabine (2,000 mg/m^2 daily from day 1 to day 14) in 21-day cycles (16). The primary endpoint was the proportion of patients with an objective CNS response, which was defined as a 50% or greater volumetric reduction of CNS lesions in the absence of increased steroid use, progressive neurological symptoms and progressive extra-CNS disease. Of the 45 patients enrolled, 44 were assessable for efficacy with a median follow-up of 21.2 months (range, 2.2-27.6 months). 29 patients had an objective CNS response (65.9%, 95% CI, 50.1-79.5), all of which were partial responses.

LANDSCAPE is the first prospective study examining the combination of lapatinib and capecitabine in HER2 positive breast cancer patients with brain metastases who were WBRT-naïve. The study results are encouraging, with a high CNS response rate and fairly short median time to first documented response of 1.8 months (95% CI, 1.1-5.8 months). As expected, the regimen resulted in the additional benefit of extra-CNS disease control, with 44.1% of evaluable patients having an objective extra-CNS response. The importance of CNS control in the overall prognosis of patients with brain metastases was further substantiated in subgroup analysis showing significant improved time to progression in responders (6.0 months; 95% CI, 5.5-7.4 months) compared to non-responders (2.8 months; 95% CI, 1.4-4.2 months; P<0.0001). Median time to WBRT was a meaningful 8.3 months (95% CI, 5.4-9.1 months) in the study population whose median overall survival was reported to be 17 months (95% CI, 13.7-24.9 months). Amongst the patients who progressed on treatment, four-fifths relapsed first in the CNS alone, and almost all ultimately received WBRT as a palliative measure.

These data provide strong evidence that combination of lapatinib and capecitabine is a feasible alternative to delay whole brain radiotherapy and its associated side effects. The combination is especially relevant for patients with significant extra-cranial disease and who also require systemic therapy. Convenience of oral administration makes this an appealing option compared to WBRT which could be a logistical challenge in patients with limited mobility or poor performance status. However, there are still limitations and many questions left unanswered. The applicability of LANDSCAPE is constrained by its phase 2 design and small sample size. In addition, 43% had asymptomatic brain metastases and all had good Graded Prognostic Assessment scores. Patients with ECOG status of 2 made up less than 5% of study participants, implying that patients may have been naturally self-selected to account for the good outcome observed in the trial. This is in contrast with real life situation where patients often present with seizures and other neurological disability and may not have good performance status that would be required for trial entry.

Although the authors concluded that the regimen was tolerable, almost half the patients (49%) actually experienced grade 3 or 4 adverse events, with diarrhoea and hand-foot syndrome being most common. One-third of patients required dose reduction for lapatinib, and slightly more than half had dose reductions for capecitabine, while treatment was discontinued in 9%, suggesting that toxicities must be clinically significant in these patients. Lapatinib is also not readily available in many less developed healthcare systems, compared to facilities for palliative radiation, making these findings irrelevant in certain countries. Importantly, barring resource restriction issues, the cost of lapatinib and capecitabine for an average woman in the

United States is USD$2,919 per cycle (17), or USD$21,406 for 5.5 months, the median progression-free interval seen in LANDSCAPE, which is more than three-fold the USD$6,500 for WBRT reported in a cost-effectiveness analysis (18). However, one may argue that systemic treatment with an anti-HER2 agent such as lapatinib would still be warranted post-WBRT, thereby negating the cost difference in developed countries where both options are readily available.

In the LANDSCAPE study, 78% of the 41 patients with available data had CNS disease alone as the first site of progression, underscoring the fact that many patients may require several lines of brain metastases-specific treatments as overall survival rates improve. One pertinent question is the optimal sequence of treatment for brain metastases in HER2 positive breast cancer, i.e. lapatinib and capecitabine before WBRT, or vice versa, which needs to be addressed in a phase 3 clinical trial, now being planned by the LANDSCAPE investigators.

Brain metastases are now an important site of disease progression and a major cause that limits quality of life and survival in HER2 positive breast cancer. We are now entering an era where anti-HER2 treatment is no longer limited to trastuzumab and lapatinib. Pertuzumab, a monoclonal antibody against HER2, has recently received FDA approval (19) while T-DM1, a trastuzumab-cytotoxic conjugate, is seeking approval, for metastatic HER2 positive breast cancer (20). These large molecules, while unlikely to be active against brain metastases, are expected to further prolong survival, making treatment of brain metastases an ever more pertinent issue. Other anti-HER2 agents in active development in breast cancer include afatinib, a small molecule tyrosine kinase inhibitor that has promising activity against brain metastases (21), and may further expand the treatment options in these patients.

Once a domain which was largely excluded from major therapeutic trials, brain metastases are increasingly being acknowledged by clinicians and scientists as the next major hurdle to prolonging survival in HER2 positive breast cancer. The results from the LANDSCAPE study brings home the point that good clinical outcome is achievable in selected patients with brain metastases with systemic therapy. Beyond solely targeting the HER2 receptor, research into therapies blocking novel pathways such as bevacizumab, phosphoinositide-3-kinase inhibitors, and poly (ADP ribose) polymerase (PARP) inhibitors in brain metastases in breast cancers is on-going. It is hopeful that in the near future, oncologists will be equipped with an armamentarium of different agents which can be deployed in succession to treat patients with HER2 positive breast cancer with brain metastases. The days where local therapies such as WBRT are these patients' only option are over.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Stemmler J, Schmitt M, Willems A, et al. Brain metastases in HER2-overexpressing metastatic breast cancer: Comparative analysis of trastuzumab levels in serum and cerebrospinal fluid. J Clin Oncol 2006;24:64S.
2. Bendell JC, Domchek SM, Burstein HJ, et al. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. Cancer 2003;97:2972-7.
3. Lin NU, Bellon JR, Winer EP. CNS metastases in breast cancer. J Clin Oncol 2004;22:3608-17.
4. Lin NU, Winer EP. Brain metastases: the HER2 paradigm. Clin Cancer Res 2007;13:1648-55.
5. Pestalozzi BC, Zahrieh D, Price KN, et al. Identifying breast cancer patients at risk for Central Nervous System (CNS) metastases in trials of the International Breast Cancer Study Group (IBCSG). Ann Oncol 2006;17:935-44.
6. Dawood S, Broglio K, Esteva FJ, et al. Defining prognosis for women with breast cancer and CNS metastases by HER2 status. Ann Oncol 2008; 19:1242-8.
7. Yap YS, Cornelio GH, Devi BC, et al. Brain metastases in Asian HER2-positive breast cancer patients: anti-HER2 treatments and their impact on survival. Br J Cancer 2012;107:1075-82.
8. National Comprehensive Cancer Network (NCCN) clinical practice guidelines in oncology. Central nervous system cancers. Version I, 2012. Available online: www.nccn.org/professionals/physician_gls/pdf/cns
9. Tallet AV, Azria D, Barlesi F, et al. Neurocognitive function impairment after whole brain radiotherapy for brain metastases: actual assessment. Radiat Oncol 2012;7:77.
10. Geyer CE, Forster J, Lindquist D et al. Lapatinib plus

capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006;355:2733-43.
11. Lin NU, Carey LA, Liu MC, et al. Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol 2008;26:1993-9.
12. Lin NU, Diéras V, Paul D, et al. Multicenter phase II study of lapatinib in patients with brain metastases from HER2- positive breast cancer. Clin Cancer Res 2009;15:1452-9.
13. Boccardo F, Kaufman B, Baselga J, et al. Evaluation of lapatinib (Lap) plus capecitabine (Cap) in patients with brain metastases (BM) from HER2þ breast cancer (BC) enrolled in the Lapatinib Expanded Access Program (LEAP) and French Authorisation Temporaire d'Utilisation (ATU). J Clin Oncol 2008;26; abstr 1094.
14. Ro J, Park S, Kim S, et al. Clinical outcomes of HER2-positive metastatic breast cancer patients with brain metastasis treated with lapatinib and capecitabine: an open-label expanded access study in Korea. BMC Cancer 2012;12:322.
15. Sutherland S, Ashley S, Miles D, et al. Treatment of HER2-positive metastatic breast cancer with lapatinib and capecitabine in the lapatinib expanded access programme, including efficacy in brain metastases—the UK experience. Br J Cancer 2010;102:995-1002.
16. Bachelot T, ROmieu G, Campone M, et al. Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. Lancet Oncol 2012. pii: S1470-2045(12)70432-1.
17. Le QA, Hay JW. Cost-effectiveness analysis of lapatinib in HER2-positive advanced breast cancer. Cancer 2009;115:489-98.
18. Mehta M, Noyes W, Craig B, et al. A cost-effectiveness and cost-utility analyses of radiosurgery vs. resection for single-brain metastases. Int J Radiat Oncol Biol. Phys 1997;39:445-54.
19. United States Food and Drug Administration [Internet]. Approved Drugs [updated 2012 Nov 6; cited 2012 Dec 15]. Pertuzumab;[1 screen]. Available online: http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm307592.htm
20. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367:1783-91.
21. 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.

Cite this article as: Ow S, Lee SC. Brain metastases in HER2 positive breast cancer: the next hurdle. Ann Palliat Med 2012;1(3):198-201. doi: 10.3978/j.issn.2224-5820.2012.10.09

Brain metastases in HER2-positive breast cancer: challenges and opportunities

Davis Torrejón[1], Serena Di Cosimo[2]

[1]Department of Medical Oncology, Vall d'Hebron University Hospital, Barcelona, Spain; [2]Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Correspondence to: Davis Torrejón. Department of Medical Oncology, Vall d'Hebron University Hospital, Passeig de la Vall d'Hebron 119-129, 08035 Barcelona, Spain. Email: dytorrej@vhebron.net.

Submitted Oct 01, 2012. Accepted for publication Oct 26, 2012.
doi: 10.3978/j.issn.2224-5820.2012.10.11
View this article at: http://www.amepc.org/apm/article/view/1326/1799

The development of metastases in the central nervous system (CNS) is one of the most devastating consequences of breast cancer progression (1). Although epidemiologic studies estimate that the incidence of brain metastases (BM) in women with metastatic breast cancer (MBC) is 10-16% (2,3), reports from autopsies suggest rates of up to 30% (2,4,5).

Life expectancy for patients with breast cancer has risen thanks to advances in efficient systemic treatments, such as trastuzumab in HER2-positive patients that together with the detection of subclinical disease, has led to an increase in the incidence of BM (6), which is even greater than hormone receptor-positive tumors. In the RegistHER study, a prospective observational study of 1,012 patients with newly diagnosed HER2-positive MBC, 37.3% of patients developed BM after a median follow-up of 29 months (7). Herein lies the importance of the current interest in determining new therapeutic strategies in patients with BM phenotype HER2-positive, which not only come down to local treatments such as whole brain radiotherapy (WBRT) with its associated late toxicity (8), but which also offer optimal CNS responses.

Why is there more BM in HER2-positive tumors? Some have attributed this to an inherent biological tropism for the CNS independent of treatment and other prognostic factors (9-11). Therefore it is fundamental to identify molecular signatures predictive of organ-specific metastases. The hypothesis of an increase in BM in the post-trastuzumab era has also been proposed, since it does not cross the blood-brain barrier (BBB) similarly in many chemotherapeutic agents used in the conventional treatment of MBC.

In recent years, a limited number of newer chemotherapeutic agents have demonstrated activity in prospective studies of MBC-related BM. Limited activity with temozolomide (12-14) or cisplatin (15-17) has been demonstrated as a single agent or in combination with other chemotherapies and WBRT. Similarly, there are provocative retrospective data with capecitabine, an agent with well established efficacy in breast cancer, which has been proposed to cross the BBB via the human concentrative nucleoside transporter (hCNT) (18,19).

Nevertheless, possibilities are emerging within anti-Her2-therapies: the role of trastuzumab is being considered as a probable radiosensitizer (20,21) or the penetration of the BBB - still unconfirmed for lapatinib (22). Ongoing phase II studies with afatinib (NCT01441596; LUX-breast3), neratinib (NCT01494662) and everolimus (NCT01305941) are trying to find new paradigms in treatments for patients with HER2-positive MBC with BM.

The LANDSCAPE study (23) has emerged in this situation and was published last November in the *The Lancet Oncology*, a phase II study to determinate if patients with HER2-positive MBC associated previously untreated multiple BM who receive lapatinib plus capecitabine can avoid or delay WBRT, support a high objective CNS response (65.9%; 95% CI, 50.1-79.5%) among 44 evaluable patients, and nine patients (20%) had a volumetric reduction of at least 80%. Efficacy with the combination is similar to that with WBRT, but with the possibility of less neurological toxicity. Median time to progression was 5.5 months (95% CI, 4.3-6.0 months) and median time to WBRT of 8.3 months (95% CI, 5.4-9.1 months), which is clinically relevant for a population with short overall survival.

It is important to note that the individual contributions of lapatinib versus capecitabine versus the combination are unknown, as many of these patients had not received prior capecitabine, which appears to have independent CNS activity. Additionally, it is not a comparative study with other therapeutic regimes, such as monotherapy, other combination treatments, and WBRT. A previous study (24) comparing lapatinib plus capecitabine to lapatinib plus topotecan for patients with HER2-positive breast cancer BM progressing after trastuzumab and radiotherapy, was stopped before full enrollment, although marked CNS activity was observed with the combination lapatinib/capecitabine.

In the population studied, 50% were Hormonal Receptor negative (ER negative, PR negative). Taking into consideration the recent Sant Gallen classification, efficacy results of the combination according to hormone receptor expression should be known, since many of them could be classified in the Luminal B phenotype. Furthermore, it is important to control systemic disease. Seven patients (16%) of the study had CNS progression, of which two patients had progression outside of the CNS.

Considered inclusive by the authors, it is necessary to mention the limitations preferentially related to their extrapolation to the general population. More than 95% of all patients presented with Eastern Cooperative Oncology Group performance status of 0-2 and 43% of the patients had asymptomatic BM, which is better than would be expected in an unselected population of patients with BM, without providing quality of life information and neurocognitive functions.

From the pharmacological perspective, no measurement of the concentration of lapatinib or capecitabine in the cerebrospinal fluid has been done, so it is not possible to affirm its penetration of the BBB. Other factors should also be taken consideration such side effects: diarrhea (20%) and hand-foot syndrome (20%) grade 3-4, requiring dose reductions of lapatinib in 16 (36%) of 45 patients and dose reductions of capecitabine were necessary in 26 (58%) of 45 patients.

Considering said limitations, this study can be considered a first important advance in the search for treatment strategies for BM in BMC. The incidence of BM will probably rise within the clinical handling of HER2-positive patients. As a result, it is a necessity to continue research for new drugs focusing on obtaining CNS activity, in addition to sufficient BBB penetration.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Mayer M. A patient perspective on brain metastases in breast cancer. Clin Cancer Res 2007;13:1623-4.
2. Tsukada Y, Fouad A, Pickren JW, et al. Central nervous system metastasis from breast carcinoma. Autopsy study. Cancer 1983;52:2349-54.
3. Patanaphan V, Salazar OM, Risco R. Breast cancer: metastatic patterns and their prognosis. South Med J 1988;81:1109-12.
4. Hagemeister FB Jr, Buzdar AU, Luna MA, et al. Causes of death in breast cancer: a clinicopathologic study. Cancer 1980;46:162-7.
5. Cho SY, Choi HY. Causes of death and metastatic patterns in patients with mammary cancer. Ten-year autopsy study. Am J Clin Pathol 1980;73:232-4.
6. Bendell JC, Domchek SM, Burstein HJ, et al. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. Cancer 2003;97:2972-7.
7. Brufsky AM, Mayer M, Rugo HS, et al. Central nervous system metastases in patients with HER2-positive metastatic breast cancer: incidence, treatment, and survival in patients from registHER. Clin Cancer Res 2011;17:4834-43.
8. Chang EL, Wefel JS, Hess KR, et al. Neurocognition in patients with brain metastases treated with radiosurgery or radiosurgery plus whole-brain irradiation: a randomised controlled trial. Lancet Oncol 2009;10:1037-44.
9. Pestalozzi BC, Zahrieh D, Price KN, et al. Identifying breast cancer patients at risk for Central Nervous System (CNS) metastases in trials of the International Breast Cancer Study Group (IBCSG). Ann Oncol 2006;17:935-44.
10. Kallioniemi OP, Holli K, Visakorpi T, et al. Association of c-erbB-2 protein over-expression with high rate of cell proliferation, increased risk of visceral metastasis and poor long-term survival in breast cancer. Int J Cancer 1991;49:650-5.
11. Gabos Z, Sinha R, Hanson J, et al. Prognostic significance of human epidermal growth factor receptor positivity for the development of brain metastasis after newly diagnosed breast cancer. J Clin Oncol 2006;24:5658-63.
12. Christodoulou C, Bafaloukos D, Linardou H, et al.

Temozolomide (TMZ) combined with cisplatin (CDDP) in patients with brain metastases from solid tumors: a Hellenic Cooperative Oncology Group (HeCOG) Phase II study. J Neurooncol 2005;71:61-5.
13. Addeo R, De Rosa C, Faiola V, et al. Phase 2 trial of temozolomide using protracted low-dose and whole-brain radiotherapy for nonsmall cell lung cancer and breast cancer patients with brain metastases. Cancer 2008;113:2524-31.
14. Siena S, Crinò L, Danova M, et al. Dose-dense temozolomide regimen for the treatment of brain metastases from melanoma, breast cancer, or lung cancer not amenable to surgery or radiosurgery: a multicenter phase II study. Ann Oncol 2010;21:655-61.
15. Viñolas N, Graus F, Mellado B, et al. Phase II trial of cisplatinum and etoposide in brain metastases of solid tumors. J Neurooncol 1997;35:145-8.
16. Franciosi V, Cocconi G, Michiara M, et al. Front-line chemotherapy with cisplatin and etoposide for patients with brain metastases from breast carcinoma, nonsmall cell lung carcinoma, or malignant melanoma: a prospective study. Cancer 1999;85:1599-605.
17. Cassier PA, Ray-Coquard I, Sunyach MP, et al. A phase 2 trial of whole-brain radiotherapy combined with intravenous chemotherapy in patients with brain metastases from breast cancer. Cancer 2008;113:2532-8.
18. Ekenel M, Hormigo AM, Peak S, et al. Capecitabine therapy of central nervous system metastases from breast cancer. J Neurooncol 2007;85:223-7.
19. Chargari C, Kirova YM, Diéras V, et al. Concurrent capecitabine and whole-brain radiotherapy for treatment of brain metastases in breast cancer patients. J Neurooncol 2009;93:379-84.
20. Liang K, Lu Y, Jin W, et al. Sensitization of breast cancer cells to radiation by trastuzumab. Mol Cancer Ther 2003;2:1113-20.
21. Chargari C, Idrissi HR, Pierga JY, et al. Preliminary results of whole brain radiotherapy with concurrent trastuzumab for treatment of brain metastases in breast cancer patients. Int J Radiat Oncol Biol Phys 2011;81:631-6.
22. Polli JW, Olson KL, Chism JP, et al. An unexpected synergist role of P-glycoprotein and breast cancer resistance protein on the central nervous system penetration of the tyrosine kinase inhibitor lapatinib (N-{3-chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furyl]-4-quinazolinamine; GW572016). Drug Metab Dispos 2009;37:439-42.
23. Bachelot T, Romieu G, Campone M, et al. Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. Lancet Oncol 2013;14:64-71.
24. Lin NU, Eierman W, Greil R, et al. Randomized phase II study of lapatinib plus capecitabine or lapatinib plus topotecan for patients with HER2-positive breast cancer brain metastases. J Neurooncol 2011;105:613-20.

Cite this article as: Torrejón D, Di Cosimo S. Brain metastases in HER2-positive breast cancer: challenges and opportunities. Ann Palliat Med 2012;1(3):195-197. doi: 10.3978/j.issn.2224-5820.2012.10.11

图书在版编目（CIP）数据

乳腺癌：英文/邵志敏，[美]彼得·G.科尔代鲁（Peter G. Cordeiro），[美]查尔斯·M.鲍尔奇（Charles M. Balch）主编. —长沙：中南大学出版社，2017.9

ISBN 978-7-5487-3015-6

Ⅰ.①乳… Ⅱ.①邵… ②彼… ③查… Ⅲ.①乳腺癌—诊疗—英文 Ⅳ.①R737.9

中国版本图书馆CIP数据核字(2017)第238429号

AME 科研时间系列医学图书 1A021

乳腺癌

Breast Cancer

邵志敏，[美]彼得 G. 科尔代鲁(Peter G. Cordeiro)，
[美]查尔斯 M. 鲍尔奇(Charles M Balch) 主编

□**丛书策划** 郑 杰 汪道远 李 媚

□**整理编辑** 郑思华

□**责任编辑** 廖莉莉

□**责任校对** 石曼婷

□**责任印制** 易红卫 谢础圆

□**版式设计** 陈贝贝 林子钰

□**出版发行** 中南大学出版社

社址：长沙市麓山南路 邮编：410083

发行科电话：0731-88876770 传真：0731-88710482

□**策 划 方** AME Publishing Company 易研出版公司

地址：香港沙田石门京瑞广场一期，16 楼 C

网址：www.amegroups.com

□**印 装** 天意有福科技股份有限公司

□**开 本** 880×1230 1/16 □印张 20 □字数 856 千字 □插页

□**版 次** 2017 年 9 月第 1 版 □ 2017 年 9 月第 1 次印刷

□**书 号** ISBN 978-7-5487-3015-6

□**定 价** 685.00 元

AME Journals

AME Publishing Company

update on August 02, 2017

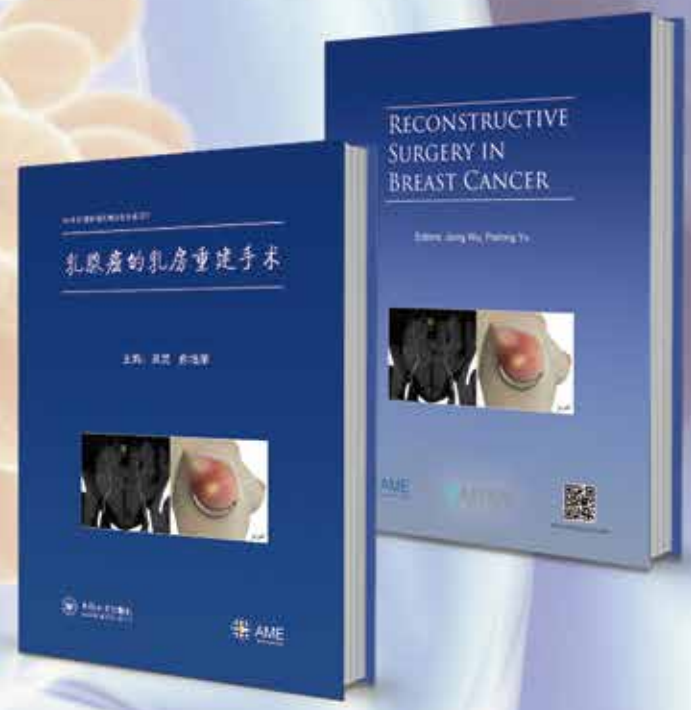
AME Books

AME Publishing Company

update on August 02, 2017

Reconstructive Surgery in Breast Cancer

Editors: Jiong Wu, Peirong Yu



中南大學出版社
www.csupress.com.cn

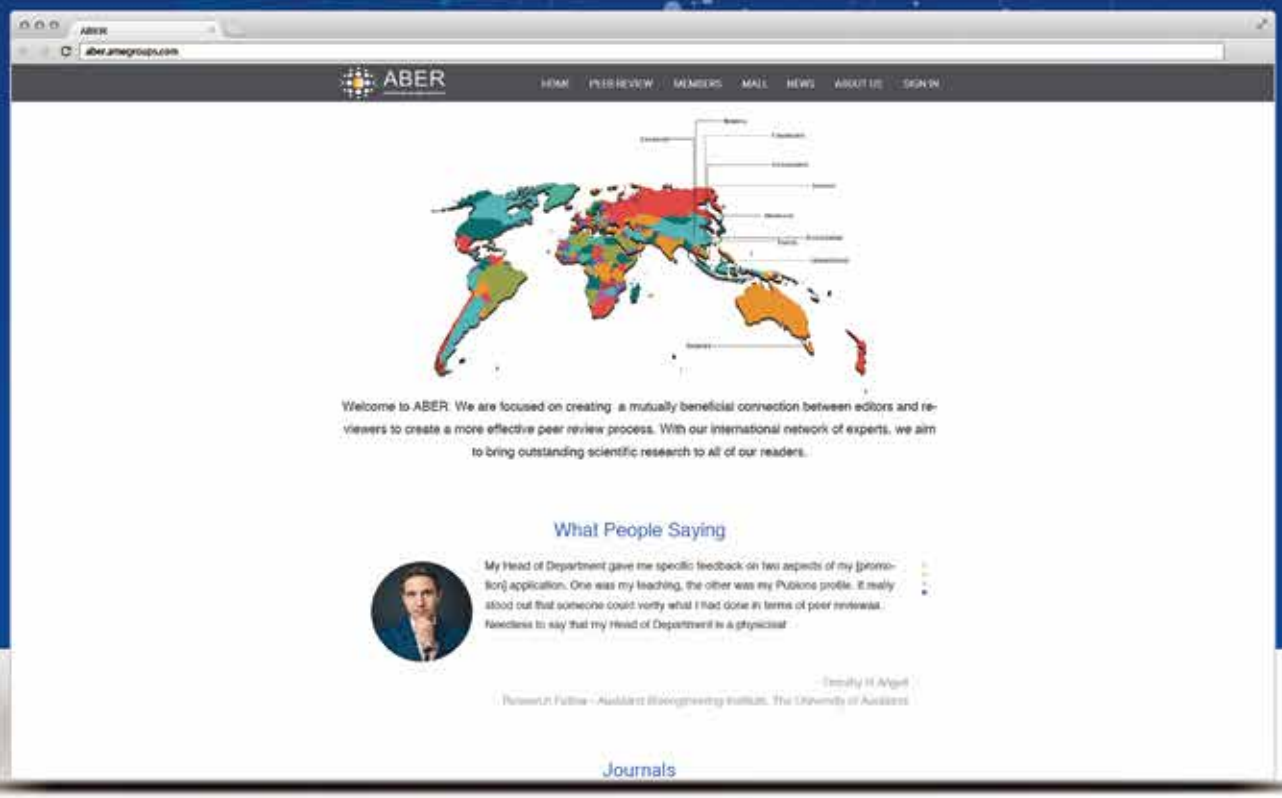
AME
Publishing Company

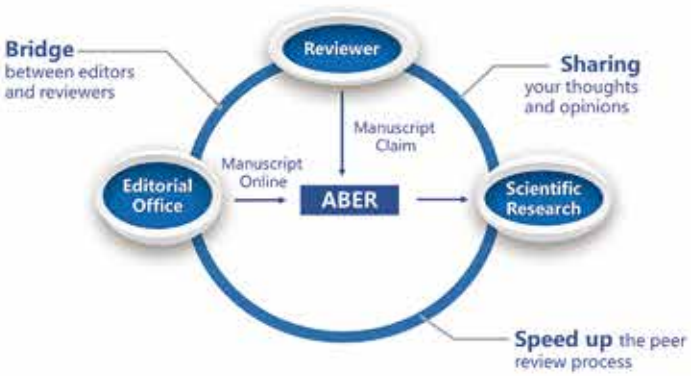
Book Introduction

amazon

ABER

AME Bond between Editor and Reviewer





Scan the QR code to learn more about ABER.

Contact us:
aber@amegroups.com


TRANSLATIONAL
CANCER RESEARCH
ISSN 2218-676X
VOL 6, NO 4
AUG 2017
2016
IMPACT FACTOR
1.167
AME
Translational Cancer Research