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Preface

Colorectal cancer is a common source of morbidity and mortality, and as such merits study across the spectrum of its epidemiology, biology and therapy. This book aims to provide an integrated and comprehensive approach to colorectal cancer control, delivered by internationally well known and respected authors who write with clarity and authority. Rather than focus on a single topic, say genetics, we considered it important to cover all elements of disease control, given how interelated and interdisciplinary medical science has become. If we are to prevent colorectal cancer, we must first understand its aetiology and biology, describe the role of population registries in monitoring incidence and death rates to assess the impact of screening and therapy, develop molecular models of carcinogenesis which can yield novel targets for drug development.

The first half of the textbook opens with introductory chapters covering the epidemiology and basic science that underpins our understanding of carcinogenesis and the cell biology that governs the cancer phenotype. This grounding in basic and translational science provides the platform for the precision cancer medicine which we hope will come to dominate therapy over the next decade.

Our aim for the second half of the textbook was to provide a series of treatment-based chapters written by expert teams from across the planet. Each chapter takes a multi-disciplinary approach to the diagnosis and management of colorectal cancer, covering radiotherapy, medical and surgical management of specific tumour stages. We have no doubt that decision making is significantly improved when a collective view is taken and that the multidisciplinary team is the clinical engine room of colorectalcancer care.

This is a time of extraordinary innovation in oncology, with improvements seen in each of the major therapeutic areas. Drawing on the combined wisdom and experience of an extensive list of internationally renowned contributors, we believe that this updated, reformatted and revitalised book provides an essential resource for oncologists in all fields at all stages in their careers.

Acknowledgement

We would like to thank Nancy Q. Zhong for the extraordinary editorial support we have received throughout preparation of this textbook.

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Preface

Colorectal Cancer is a major problem that affects human beings wherever they may live, but it is not uniformly distributed throughout planet earth. Activities aimed at prevention, screening, surveillance and treatment of colorectal cancer also vary across the globe. The World Health Organization, via its International Agency for Research on Cancer produces Globocan that specifically tracks the varied incidence and mortality of colorectal cancer (1). The great variation in the disease burden and the diverse responses to colorectal cancer strongly suggests there are valuable lessons to be learned. This book, *Colorectal Cancer*, goes a long way towards closing the knowledge gap. *Colorectal Cancer* presents the peer reviewed work of an international cohort of preeminent authors, with a very broad view of the epidemiology, screening, surveillance, and therapeutic approaches to colorectal neoplasia.

Preventing, screening, diagnosing, treating and providing surveillance for colorectal neoplasia is best accomplished by a multidisciplinary team. Key stakeholders in the care team include primary care providers, gastroenterologists, hepatologisits, colorectal surgeons, hepatobiliary surgeons, general surgeons, radiation oncologists, medical oncologists, diagnostic radiologists, interventional radiologists, pathologists, medical geneticists, nurses, dieticians, and mental health professionals. While most patients gain the expertise of these disciplines via consultation, an interchange of ideas often occurs at multidisciplinary conferences or at formal multidisciplinary tumor boards. *Colorectal Cancer* assembles the expert input from these many disciplines, and presents a panoramic view of the clinical scenarios encountered in helping patients navigate a difficult journey.

Colorectal Cancer is a valuable tool for those seeking an overview of the field, or, more likely, focusing on a specific intervention. I plan to use this distinctive resource in targeted clinical scenarios, as a discussion springboard with colleagues in another discipline, as an aid to preparing lectures, and as a source of current carefully chosen and reviewed references.

Acknowledgement

I would like to personally thank and commend Nancy Q. Zhong, Minhua Zheng, MD, and David J. Kerr, CBE, MD, DSc, FRCP, FRCGP for their exceptional work on this important project.

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Preface

Colorectal cancer, one of the most commonly diagnosed malignancies worldwide, demonstrates substantial variation of incidence and mortality in regions around the globe and in populations of different genetic and socioeconomic characteristics. Colorectal cancer is a disease of substantial economic impact that is amenable to screening strategies and systematic efforts at prevention. Thus, the topic of colorectal cancer is particularly well suited to international efforts for scientific collaboration and knowledge sharing.

This volume represents a collection of articles that have appeared in scientific journals distributed by AME Publishing Company based in China, curated by Minhua Zheng, MD (Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China) and David J. Kerr, MD (University of Oxford, Oxford UK). The authors of each of these contributions bring considerable expertise and represent many of the most prestigious international universities and medical centers.

The first section, "*Epidemiology and Screening for Colorectal Cancer*" reviews controversies in current practices as well as newer diagnostic modalities that may improve early detection. The next section, "*Pathogenesis and Molecular Biology of Colorectal Cancer*" collects a series of articles addressing advances in colorectal cancer genetic and the emerging field of precision medicine and predictive biomarkers.

The largest sections of this volume address the initial treatment of colorectal cancer and the treatment of recurrent and metastatic disease. A wide range of technical advances are covered, including transanal endoscopic microsurgery and other minimally invasive options, as well as the roles of neoadjuvant protocols, immunotherapy, and intraoperative radiation therapy. As we enter the era of targeted therapy for colorectal cancer, overall improvements in cancer therapeutics have significantly changed the outcomes and outlook for patients with colorectal cancer. There are greater numbers of survivors living with or without disease recurrence. The final section "*Nursing and Psychological Problems of Colorectal Cancer*" covers some aspects of the challenges of survivorship.

While not a traditional textbook *per* se, this single volume effectively captures the full range of current expert thinking on the diagnosis and treatment of colorectal cancer. It will be of great interest and value to practicing physicians and surgeons, to cancer researchers, to students and trainees, and to an increasingly sophisticated lay audience.

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Epidemiology and burden of colorectal cancer in Asia-Pacific region: what shall we do now?

Mohamad Amin Pourhoseingholi

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Abstract: The burden of colorectal cancer (CRC) is in a rising trend in Asia-Pacific. There is little health authority support for CRC screening and very low public awareness of this cancer in Asian countries. The surveillance system in countries with high burden needed to provide facilities for CRC screening and public awareness education program shall be considered in national and international planes to increases the self-participation of people. Financial limitation and lack of authorities are still the main obstacles in the way of CRC screening in most Asian countries with low income status.

Keywords: Colorectal cancer (CRC); burden; Asia-Pacific; screening

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Introduction

Cancer is an increasingly problem in Asian countries, as similar as western countries because of ageing populations and changes in lifestyle. In this continent which covers approximately 60% of the world's current human population, only relatively high-income countries including Japan (1), Republic of Korea (2) and Singapore (3), led to the development and implementation of national cancer control plan.

There is an increasing burden of digestive cancer in the world and Asia-pacific region is not an exception. The list of top five most common cancers in Asian countries includes gastric cancer, liver cancer and colorectal cancer (CRC). CRC with high incidence and mortality in Western populations has been extensively studied in these countries. The comparatively low rate is observed in Asian, African, and South America countries; however the past decades have seen a rapid increase of incidence, in Asia-Pacific populations (4). This changing is attributed to environmental factors such as aging and the adoption of the Western lifestyle (5).

In this paper, the epidemiology of CRC and the status of screening strategy for Asia-Pacific region are briefly discussed.

Epidemiology of CRC in Asia-Pacific

CRC is now the third most common malignant disease in both men and women in Asia (5). In the Asia-Pacific region, the incidence varies between regions, with high incidence in Australia, and Eastern Asia, and low incidence in south-central Asia. Data from the Cancer Base of the International Agency for Research on Cancer (IARC) showed that the incidence of CRC in many Asian countries is similar to that in the western ones (6).

GLOBOCAN estimation project for 2012 indicated that, the age-specific rates (ASR) incidence for Asia was 13.7 and ASR mortality was 7.2 per 100,000. Although the incidence and mortality rate of this cancer are still higher in Western, the ratio of mortality/incidence for Asian regions are higher, which means that the poor survival (*Figure 1*) (7).

CRC is now the third most common cancer in both sexes in Asia (5) and Eastern Asian countries including China, Japan, South Korea and Singapore showed a two- to four-fold increase in incidence (5). Its incidence is higher among the Chinese (8) and this cancer is one of the three cancers with most rapidly increasing incidence in China, between 1991 and 2005 (9). In Japan and Taiwan an increasing in incidence has also been reported (10,11). In Middle East, the incidence of CRC is increased in Iran, Saudi Arabia and

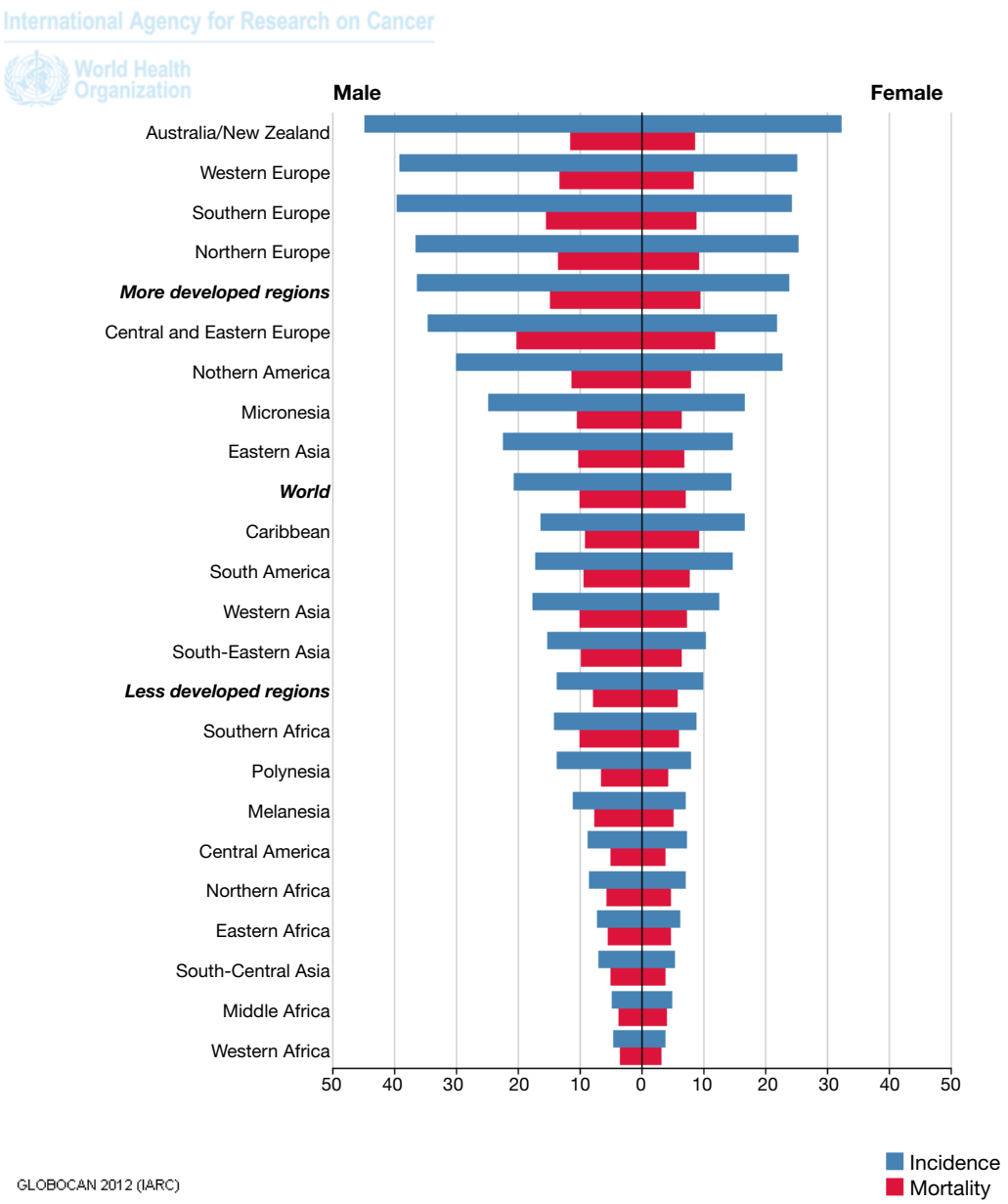

Figure 1 The mortality and incidence of colorectal cancer in the world, according to GLOBOCAN estimation project, 2012.

Jordan in recent years (12-15).

The mortality of CRC has been increasing in the last decade in Asian countries, except in Japan and Singapore (5). However, Singapore, Taiwan, and Japan have higher mortality rates for cancer of the colorectal, than the other Asian countries. Studies showed that, the CRC mortality in Hong Kong of China, Japan, South Korea, and Singapore has started to decrease, and the decrease occurred first in the younger age groups (16).

Data from the national mortality routine reporting system in China, indicated that, mortality from CRC has increased through recent decades (17). National death Statistic of Iran reported a slight increasing trend for CRC mortality, and this mortality was higher for older age and male (18,19).

Ethnicity has an important etiological role in CRC in Asia. In Singapore, where different ethnic groups livening in the same environment, the incidence of CRC is lower among the Indian and Malay populations compared to

Chinese (8,20) and Chinese people who live in Malaysia, have a significantly higher incidence of CRC than others (20).

Asia-Pacific Cohort Studies Collaboration (involving over half a million subjects from 33 cohort studies in the region of Asia-Pacific) indicated that, smoking, body mass index and lack of physical activity increased risk of CRC in Asia-Pacific region (21).

The incidence, anatomical distribution and mortality of CRC among Asian populations are not different compared with Western countries. There is a trend of proximal migration of colonic polyps and flat or depressed lesions are not uncommon (5). Other risk factors for CRC include family history and metabolic syndrome. First-degree relatives of patients with CRC have a 2-fold increased risk of CRC (22).

Screening

Although, data are lacking in countries such as India and Indonesia, the findings indicate a rapid increasing of CRC burden in Asian countries and there is a need to setup prevention program for this populated region of the world. CRC is an ideal disease for screening. But due to a lack of optimal screening strategy and public acceptance, the universal screening program has not been implemented in most countries. The facilities to access the CRC screening are an important key to reduce the burden of CRC. There are three frequently used screening modalities, namely fecal occult blood tests (FOBT), flexible sigmoidoscopy (FS) and total colonoscopy. Among these three, FOBT is the only method shown in large randomized studies to decrease mortality, using biennial guaiac-based FOBT (23).

A study on cost-effectiveness of FOBT, FS and colonoscopy in Asian countries indicated that FOBT is cost-effective compared to FS or colonoscopy in average-risk individuals aged from 50 to 80 years (24).

The Japan Public Health Center-based Prospective Study group in a cohort study showed a risk reduction in advanced CRC by almost 60% and in mortality by 30% (25). The studies which used screening colonoscopy in Asia showed that the risk of advanced neoplasm tripled after the age of 50 and most guidelines recommend screening to be started at the age of 50 years old (26).

In Asia a minority of population at risk, undergoes screening because of perceived health, access and psychological barriers (27). A survey showed that, men above 50 years of age were particularly unaware of the symptoms of CRC and the benefits of screening (5). A study on 10,078 Chinese revealed that the proportions of perceptual barriers of CRC screening were high among these participants including; financial difficulty, limited service accessibility, screening induced bodily discomfort, etc. (28) and another Chinese study indicated that the uptake of CRC tests was low in the average-risk population (29).

A study in Malaysia showed that the majority of the participants had no knowledge of digital rectal examination, colonoscopy, barium enema and fecal occult blood screening for CRC (30). In Middle East, there is no report for national CRC screening; however, Iranian study suggests it at least for the relatives of CRC patients (31).

The actual uptake and implementation of screening remain low in many Asian countries due to limited resources. National healthcare systems and health insurance are not available to majority of people (5). In most Asian countries, National health-care systems and health insurance cover only a minority of people. So, access to healthcare facilities is limited in many areas and communities of low socioeconomic status (5). Besides, there is little health authority support for CRC screening and very low public awareness of this emerging epidemic in Asia (32). Recommendation for screening by a doctor increases the participation of screening (26). Also study of Asia-Pacific Working Group in CRC revealed that physician recommendation and knowledge of screening tests were significant predictors of CRC test uptake (4). A successful screening program for CRC shall include lack of patient awareness, attitudes and acceptance, physicians' knowledge, attitudes and recommendations (33). Resource-stratified guidelines from the Asian Oncology Summit 2013 recommend that, people at increased risk of CRC (such as those with personal history or family history of CRC or adenoma) can be screened by colonoscopy. Also genetic test (dependent on the resource available) should be offered to detect increased susceptibility to CRC (34). Besides, the mechanisms involved in CRC initiation and development should be noticed to understanding the burden and prevention strategies for this malignancy. Recent data demonstrated that several genetic and epigenetic changes are important in determining patient prognosis and survival and some of these mechanisms are related to patients' response to drugs, such as aspirin, which could be used for both prevention and treatment in specific settings (35).

Conclusions

The burden of CRC is still high in Asia-Pacific region and

prevention would be one of the best methods to control the disease. It is estimated that over the next two decades the number of CRC cases will increase from 1.2 to 2.2 million worldwide, most of the increase (62%) will be in the developing countries (36) which is the results of westernized life style.

Surveillance system in countries with high burden needed to provide facilities for CRC screening (at least for population at high risks). A problem is that in countries with low facilities, there is also low coverage of cancer registry and the statistics are incomplete or underestimated. Furthermore it is necessary to establish national registry system for countries with low income or help them to estimate truly the burden and epidemiology of CRC, before adjusting any screening plan. CRC screening could be individualized based on genetic or environmental risk factors (for example, in family members of patients, or in those with environmental risk factors) but first we need reliable sufficient data from those populations with different ethnicities and lifestyles (37,38).

Also public awareness education program shall be considered in national and international plane to increases the self-participation of people. The experiences from countries with good CRC prevention system (e.g., Japan, Korea) will be particularly informative to other Asian countries; however financial limitation and lack of authorities are still the main obstacles in the way of CRC screening in most Asian countries with low income status.

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Footnote

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Improved colorectal cancer screening: a new option and opportunity

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Abstract: An important new test for colorectal cancer screening was evaluated by Imperiale *et al*. and reported in the April 4, 2014 *New England Journal of Medicine* entitled "Multitarget stool DNA testing for colorectal-cancer screening". This editorial notes the favorable trend in the reduction of colorectal cancer incidence and mortality, and explores the significant issue of suboptimal patient uptake of existing colorectal cancer screening examinations. The findings of the multitarget stool DNA test study are summarized, put into perspective, and the potential interest in this examination is considered. By expanding colorectal cancer screening uptake, the multitarget stool DNA test may further reduce the burden of colorectal cancer.

Keywords: Colon cancer; rectal cancer; colorectal cancer; colorectal neoplasia; cancer screening; cancer screening; stool DNA; multi-target stool DNA test; cancer testing; colonoscopy; fecal immunochemical test (FIT); stool occult blood; KRAS; NDRG4; BMP3; colon polyp; rectal polyp; colon adenoma; sessile serrated adenoma

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The distinctly encouraging journey toward prevention and early detection of colorectal neoplasia took another major step forward with the publication by Imperiale and colleagues of Multitarget Stool DNA Testing for Colorectal-Cancer Screening (1). The good news is that substantial progress is being made in the multi-faceted struggle with colorectal cancer. The annual update of data from the American Cancer Society published in January 2014 indicates that the incidence of colorectal cancer has been declining steadily between 2006 and 2010 by about 3.3% for men and 3.0% for women (2). Similarly, colorectal cancer mortality rates have decreased by 2.5% and 3.9%, respectively, over the same time period, and are down by 46% from their maximum (2). Long term reduction in incidence is thought to be due to reduction of risk factors and introduction of screening programs. The precipitous decline in incidence from 2008-2010, 4% per year, is thought to be due to the utilization of colonoscopy that has the ability to remove precancerous polyps (2).

Worldwide, at least 25 countries have implemented programs to screen for colorectal cancer (3). Most of these extensively use stool testing for occult blood or fecal immunochemical testing, but the United States, Germany, and Poland place a major emphasis on structural screening examinations of the colon (3,4). Several organizations in the United States publish colorectal cancer screening guidelines that are supported by virtually all healthcare insurance programs. In general, the guidelines suggest beginning of screening for average risk individuals at age 50, and include the options of colonoscopy every 10 years, flexible sigmoidoscopy every 5 years, or fecal immunochemical test (FIT) every year (5,6).

A significant problem with the current US screening recommendations is that the uptake by the population offered them is suboptimal. Quite simply, many patients who should be screened for colorectal cancer do not participate in screening programs. In the United States, the Center for Disease Control (CDC) conducts a regular national telephone survey of a representative sample of the population known as the Behavioral Risk Factor Surveillance System (BRFSS), and posts robust information about the health of the US population on its website (7). The latest results [2012] show that nationally, of those surveyed over age 50, 66.8% report having ever undergone a flexible sigmoidoscopy or colonoscopy. The greatest uptake was in Massachusetts at 76.7% and the lowest in

Alaska at 60.6%. Comparison to reports of mammogram uptake in women age 50 and above within the last two years over the very same period may shed light on an achievable public health opportunity. The Behavioral Risk Factor Surveillance System reports that nationwide, in women 50 and above, 77% have undergone mammograms in the last 2 years. The greatest uptake of breast cancer screening was in Massachusetts at 87.1%, and the lowest was in Wyoming at 64.5% (8). Although the CDC BRFSS data examines different diseases with different health optimization behaviors, an opportunity for increased colorectal screening examination uptake may exist if factors surrounding this screening, including characteristics of the examinations themselves, were enhanced.

Similar issues in colorectal neoplasia screening test uptake have been shown in other populations. When a cohort of 53,309 asymptomatic individuals aged 50-69 in Spain were offered colonoscopy or biennial FIT by a pre invitation letter, invitation letter, and two follow up letters, only 24.6% opted for colonoscopy while 34.3% selected the FIT screening program, (P<0.001) (9). Although cultural and social factors make comparisons of health optimization behaviors among different populations across the globe difficult, opportunities for improvement in colorectal cancer screening uptake may exist. The importance of the screening uptake issue is highlighted by The United States National Colorectal Cancer Roundtable. This organization, consisting of The CDC, American Cancer Society and other like-minded groups, is sponsoring a major initiative to get colorectal cancer screening rates up to 80% by 2018 (10).

When the population uptake gap of structural colorectal screening studies and the suboptimal performance characteristics of existing stool based screening strategies are considered, significant interest in development of an accurate noninvasive colorectal screening test emerged. Imperiale and colleagues used a novel multitarget stool DNA test and compared this to a commercial fecal immunochemical test (1). The new test quantitates mutant KRAS, aberrantly methylated BMP3 and NDRG4 promoter regions, controls for human DNA with beta-actin, also includes a built in immunochemical assay for human hemoglobin, and utilizes a logistic regression algorithm to provide a result. The authors studied a cohort of 9,989 asymptomatic average risk participants at 90 sites (private practice and academic) across North America having a screening colonoscopy. Of the cohort, 65 subjects (0.7%) were found to have colorectal cancer, and 757 (7.6%) had advanced lesions (adenomas or sessile serrated polyps >1 cm) on colonoscopy. The key finding was that the sensitivity of detecting colorectal cancer was 92.3% with the multitarget stool DNA testing and only 73.8% with FIT (P=0.002). Notable findings included the sensitivity of detecting advanced precancerous lesions at 42.4% with DNA testing and just 23.8% with FIT (P<0.001). The rate of detection polyps with high grade dysplasia was 69.2% with DNA testing and only 46.2% with FIT (P=0.004). The detection rate of sessile serrated polyps measuring 1cm or more was 42.4% for the DNA testing versus just 5.1% for the FIT (P<0.001). FIT had a higher specificity rate and had less subject samples rejected for technical reasons. The specificity with DNA testing and FIT were 86.6% and 94.9% (P<0.001), respectively, when subjects had no advanced or negative findings on colonoscopy, and 89.8% and 96.4% (P<0.001), among those with negative results on colonoscopy. The authors conclude that the multitarget stool DNA test detected significantly more cancers than FIT but had more false positive results.

It is clear that the multitarget stool DNA test significantly outperforms FIT on all the sensitivity based metrics evaluated: colorectal cancer detection, detection of advanced precancerous lesions, detection of polyps with high grade dysplasia, and detection of sessile serrated adenomas. As a cautionary note, the multitarget DNA stool test had lower specificity than the FIT test. The specificity of the multi-target stool DNA test correlated inversely with age. Potential reasons for declining specificity with age include lesions not detected by the index colonoscopy procedure or age related change in DNA methylation (11). Technical analytic problems resulting in subject exclusion were encountered more frequently in the DNA group than in the FIT group, both from insufficient material for analysis (213 *vs.* 34, respectively) and logistic issues with specimen shipping.

A large unanswered question is how the multitarget stool DNA test will be used in clinical practice. As the many currently unknown factors become clarified, the clinical role will be defined. On March 27, 2014 the Molecular and Clinical Genetics Panel of the Medical Devices Advisory Panel to the US Food and Drug Administration (FDA) unanimously recommended (10-0) the test for approval (12). It is quite likely the FDA will ultimately approve a more sensitive noninvasive way to screen for colorectal neoplasia than is currently available. Unknown is what the manufacturer will charge for the test in each nation that it is offered. Also unknown is what comprehensive analytic modeling studies of projected use-alone, coupled with other tests, performed at varying intervals, including sensitivity analyses of charges for each test-might show.

Guideline promulgating groups have yet to make a clinical recommendation for use of the new test, an important point as many published clinical guidelines ultimately become health insurance payment policy. In spite of the large amount of uncertainty that exists now, it seems quite likely that many patients who currently will not accept a structural screening test of the colorectum may want this exam. Patients who are at above average procedural risk for a structural exam of the colorectum are also likely to be keenly interested in this exam. Furthermore, patients looking for the most sensitive way to screen their colorectum with a nonstructural exam are likely to be asking about this test. Even without the eventual modeling studies and forthcoming guidelines, the most important stakeholder in the colorectal cancer screening decision matrix is the patient, and the current suboptimal screening uptake suggests that an improved examination option may be welcomed.

Since the initial experience in 1969, and the reports by Wolf and Shinya of successful colonoscopic polypectomy in 1973, it has been widely recognized that colorectal cancer may be prevented by removing premalignant polyps (13,14). Until better dietary advice, more research supported physical activity regimens, and effective chemoprevention strategies emerge, the main way colorectal cancer will be prevented is by colonoscopic polypectomy. Although several colorectal lesion detection strategies exist, patient adoption has been suboptimal. By development of a more accurate examination that may enable additional patients to be willing to undergo colorectal cancer screening, the multitarget stool DNA test described by Imperiale is an important step in the journey toward reduction of the burden of colorectal neoplasia. Technological refinements and advancements in colorectal cancer screening will undoubtedly continue beyond this particular significant contribution (15). Once available, this new test offers the opportunity to expand colorectal cancer screening uptake and further reduce the burden of colorectal cancer.

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Footnote

Conflicts of Interest: Dr. Heigh participated in the Multitarget Stool DNA Testing for Colorectal-Cancer Screening study at Mayo Clinic Arizona as the site Principal Investigator. Mayo Clinic has licensed technology to Exact Sciences related to stool DNA testing.

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Colorectal cancer screening: are stool and blood based tests good enough?

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Abstract: Colorectal cancer (CRC) is the third commonest cancer worldwide. As many CRC patients were identified at advanced stages, screening asymptomatic individuals has substantial clinical benefit. Most CRC arises through recognizable early stage. With the improved understanding of the biology of CRC and precancerous lesion, testing molecular aberrations in stool and blood promises novel screening approaches that are noninvasive, sensitive, and more affordable compared with traditional structural examinations.

Keywords: Colorectal cancer; screening; biomarkers; stool

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Colorectal cancer

Epidemiology

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females. Over 1.2 million new cancer cases and 600,000 deaths were estimated to have occurred in 2008 (1). The lifetime risk of CRC is approximately 6%. Risk factors for CRC include family history, male gender, smoking, alcohol consumption, physical inactivity, obesity, and red and processed meat consumption. The risk of CRC increases with age, particularly after 50. Death rates of CRC have been decreasing in several Western countries largely because of improved treatment, increased awareness and early detection (2-4). However, both the incidence and death rates of CRC are increasing in Asia because of the lack of guideline for screening and public awareness (5).

Around 15% of CRCs are inherited. The most common forms are familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). HNPCC arises because of mutations in mismatch-repair genes, including MLH1, MSH2, MSH3, MSH6, PMS1 and PMS2 (6), leading to DNA instability, such as in the length of microsatellite sequences, and results in microsatellite instability (MSI) (7). HNPCC is characterized by the early onset of colorectal tumors, particularly in proximal colon.

Around 85% of CRCs are sporadic. Based on pathological data, most sporadic CRCs are developed from adenomas (8-10). Adenomas are masses that protrude into the gut lumen, which can either be pedunculated or sessile. Adenomas can be flat or even depressed. The epithelium of adenomas can form glands (tubular adenomas), finger-like structures (villous adenomas), or a combination of both (tubulovillous adenoma). Adenomas that are larger than 1 cm, or those with severe dysplasia or a villous architecture are referred to as advanced adenomas. The development of CRC from adenoma is estimated to require 5 to 10 years, as referred to the adenoma-carcinoma sequence.

Screening

Patients with early stage CRC or precancerous lesion are mostly asymptomatic. By the time patients present with symptoms such as anemia, abdominal pain, weight loss, change in bowel habit, and rectal bleeding, the disease is likely to have reached an advanced stage. The survival from CRC is closely related to the stage of cancer when diagnosed, with late CRC having the worst outcome (11). Since most CRC develops from precancerous lesions, screening has substantial clinical benefits to patients.

Based on the guidelines from the United States, there are several options for CRC screening (12-14). Flexible sigmoidoscopy and colonoscopy are more invasive but offer the opportunity for removal of detected lesions. Stool based test represents a noninvasive approach; the most widely used is fecal occult blood test (FOBT) that tests the presence of blood in stool. With the progress in the understanding of the biology of CRC, tests based on detecting molecular abnormalities in stool offer new strategies for screening.

Using a flexible fibre-optic instrument inserted through the anus, colonoscopy allows direct visual examination of the entire colorectum, and is regarded as the gold standard for detecting colorectal lesions. It allows the option of removal and treatment of screen-detected lesions. However, colonoscopy imposes a risk of bowel perforation and bleeding, and a very low mortality risk of 1-3 death per 10,000 (14). Many patients find the procedure and the bowel preparation unpleasant. Due to its invasive nature, the cost of equipment and the demand for skilled operators, colonoscopy is not widely used as a first-line screening tool.

Stool based and blood based tests are the mainstream platforms for noninvasive CRC test. Compared to colonoscopy, both means are less sensitive and do not offer the option of immediate removal and treatment of the lesion. However, with the increased understanding in CRC biology, improved methods in stabilizing and purifying biomolecules from biological samples, these tests provide an excellent platform for testing various molecular abnormalities for CRC screening.

Stool based tests

Neoplastic features of intestinal lumen can be consistently detected in stool. Theoretically, stool based tests enable screening of the entire length of the colorectum, require no bowel preparation, and the specimens are easily transportable, which means that these tests can be obtained without the need to visit their doctors. These properties are likely to increase patient acceptability.

Fecal occult blood

The most widely used stool based test is the fecal occult blood test (FOBT). It detects blood in the stool that has leaked from disrupted vessels on the tumor or adenoma surface. FOBT has a low sensitivity as not all colorectal adenomas and tumors bleed, and those that do bleed do so intermittently (15). There is evidence that large adenomas and tumors bleed more frequently than smaller lesions (16). Asymptomatic tumors, which are the intended target of screening, also bleed less than symptomatic tumors (17). The classical FOBT involves a guaiac test for the peroxidase-like activity of heme in haemoglobin. Since heme is present in red meat, and peroxidase activity is present in fresh fruits and vegetables, false positive rate is high using this test. A diet or medication restriction is needed to optimize test performance. Sensitivity of FOBTs is typically around 50% for CRC and lower than 20% for adenomas. Despite its low sensitivity, FOBT is the only form of noninvasive test with proven efficacy in reducing CRC mortality. In three randomized controlled trials from the United States (18,19), Denmark (20,21), and the United Kingdom (22) using FOBT with annual or biennial testing has demonstrated a moderate (15-33%) reduction in CRC mortality after 10-14 years of follow-up.

A more advanced version of FOBT is the fecal immunochemical tests (FITs). FITs use antibodies specific to human hemoglobin or other blood components independent of peroxidase activity. They could be more specific in detecting blood of human origin and can eliminate the need of diet and medication restriction. Furthermore, FITs enable automated analysis for reading the test results, removing human error associated with interpretation. FITs have demonstrated a higher sensitivity towards CRC compared to guaiac based tests but its sensitivity remains low for precancerous lesions (23). In a study consisting of more than 20,000 subjects, FIT showed a sensitivity of 27% for advanced neoplasms and 66% for invasive cancer (24).

Stool DNA

Molecular alterations found in tumors can be detected in the stool because colonocytes exfoliate consistently into the lumen. The stool DNA test represents the most established noninvasive test for CRC. Various DNA mutation and methylation have been reported to be useful in discriminating CRC patients from healthy individuals. A study in an average-risk population showed that the individual marker of *APC*, *TP53*, *KRAS*, MSI and DNA integrity has a sensitivity ranging from 3.2% to 25.8% for the detection of CRC; a combined panel of these DNA markers has a sensitivity and specificity of 52% and 94%, respectively, for the detection of CRC (15). Technology used to detect DNA mutation continues to improve and the DNA panels continue to refine. Pilot studies have demonstrated the use of more sensitive approaches in testing stool based DNA mutation, such as BEAMing (which derives its name from its principal components: beads, emulsion, amplification, and magnetics) (25) and digital melt curve (26).

Better stool based DNA recovery was achieved by using EDTA-containing buffer to stabilize the stool sample (27). The addition of vimentin into the marker panel had also greatly improved the panel's performance (28). A new generation of stool DNA panel was described recently (29). It combined 4 methylation markers (*BMP3*, *NDRG4*, *vimentin*, and *TFPI2*), 7 reference mutations in *KRAS*, β-*actin* and a hemoglobin assay, achieved a sensitivity of 85% for CRC, and 54% for adenoma ≥1 cm. Each component marker typically yielded an area under the curve (AUC) value ranging from 0.61 to 0.75 towards CRC. This version of DNA test is currently seeking approval from the U.S. Food and Drug Administration.

Stool messenger RNA and protein

Stool based messenger RNA (mRNA) is another frequently exploited analyte. Several reports have shown that detecting stool based mRNA such as cyclin (30), cyclo-oxygenase 2 (COX-2) (31-34), or matrix metalloproteinase 7 (MMP-7) was able to discriminate CRC patients from healthy individuals. Notably, COX-2 mRNA was reported to be able to detect 26 out of 29 CRC cases (90% sensitivity) with 100% specificity in a Japanese study (32). Although some mRNA markers could achieve high sensitivities, the lack of stability of mRNA in stool samples has limited its application. In addition, neoplasm-derived proteins such as minichromosome maintenance proteins (35), carcinoembryonic antigen (32,36), M2 pyruvate kinase (37) and secreted clusterin isoform (38) in stool samples were also reported to be able to discriminate CRC patients from controls. Among them, stool carcinoembryonic antigen showed a sensitivity of 86% and a specificity of 93% for CRC (36). Compared with the stool DNA test, testing for RNA or protein in stool is less established. Validations in larger numbers of patients, including patients with adenomas, are warranted.

Stool microRNA

microRNA (miRNA) is a relatively new class of biomolecules being exploited as disease markers. They are 18- to 25-nucleotide non-coding RNA molecules that regulate the gene translation (39). Binding of a miRNA-loaded RNA induced silencing complex (RISC) to a complementary sequence will lead to either translational repression or decay of the targeted mRNA (40). Through this, miRNAs regulate a variety of cellular processes including apoptosis (41-43), differentiation (44) and cell proliferation (45). Altered miRNA expression profiles were found in most tumor types including CRC (46-49).

In colorectal tumors, miRNA expression profile tends to show a typical signature aberration (50). Since in 2009, several pilot studies based on small cohorts have reported the feasibility of using stool based miRNAs as biomarkers for CRC screening (51,52). In a cohort of 197 CRC patients and 119 healthy controls, Koga *et al.* investigated the sensitivities of stool based miR-17-92 cluster members, miR-21 and miR-135 in discriminating CRC patients from healthy individual (53). They reported a combined sensitivity of 74% and a specificity of 79% towards CRC; however, sensitivity towards adenoma was not investigated in this study. Wu *et al.* demonstrated stool miRNAs were relatively stable in stool and the detection by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was highly reproducible (54). Notably, miR-92a showed a sensitivity of 72% for CRC and 56% for polyps (including hyperplastic polyps and adenomas), with a specificity of 73%. The level of stool miR-92a dropped significantly after the removal of tumor or advanced adenoma. miR-92a also had a higher sensitivity towards advanced adenoma than minor polyps, and a high sensitivity in detecting distal CRC than proximal CRC.

Blood based tests

For the markers released by the tumor to be detected in blood, the mechanism of vascular invasion is required. In precancerous lesions of which vascular invasion has not yet been involved, it is expected that the amount of blood entering bloodstream is negligible. But as the staging of the cancer advances, the amount of marker detected in blood will increase as the degree of vascular invasion progresses. Compared to stool based test, blood test could be less sensitive in detecting early stage lesions but easier to implement and comply with.

Blood protein

Carcinoembryonic antigen (CEA) is a glycoprotein involved in the process of cell adhesion. It was first described as a specific CRC marker in 1969 (55). Kuusela *et al.* demonstrated its value as a diagnostic marker, in a cohort of 111 CRC patients, serum CEA showed a sensitivity of 69% and specificity of 70%. In the same cohort, cancer antigen 19-9 (CA 19-9), a cancer marker more commonly used to detect pancreatic cancer, showed a sensitivity of 36% and a

specificity of 97% for CRC (56). Until now, serum CEA level is still frequently used as a marker to monitor recurrence after surgery, but rarely as a marker in predicting the disease. Colon cancer-specific antigen (CCSA)-3 and CCSA-4 are nuclear matrix proteins. They were found to detect all 28 CRC patients (sensitivity =100%) in a study, with test specificities of 96% for CCSA-3 and 98% for CCSA-4 (57). Galectin-3 is a beta-galactoside binding protein relevant to tumor progression and metastasis. Bresalier *et al.* showed serum Galectin-3 level was able to discriminate patients with CRC from those with other colorectal diseases (hyperplastic polyps, adenomas, and inflammatory bowel disease). However, no sensitivity or specificity of Galectin-3 was reported in this study (58).

Blood messenger RNA and microRNA

Few studies had exploited blood based mRNA as CRC biomarkers. Identified by oligonucleotide microarray analysis on colorectal tissues, KIAA1199 was described as a CRC biomarker, however its function remains not clearly understood (59). Serum KIAA1199 mRNA level demonstrated a sensitivity of 74% for CRC and adenoma, and a specificity of 66%, based on a cohort of 20 CRC, 20 adenoma and 20 normal subjects. More studies had focused on plasma miRNAs, largely because they remained very stable in plasma and could be robustly quantified (60,61). Plasma based miRNA was first demonstrated to be useful as CRC biomarkers by Ng *et al.* (62). They reported plasma miR-92a, a candidate identified by miRNA array profiling, had a sensitivity of 89% and a specificity of 70% in discriminating CRC from control subjects. Notably, plasma miR-92a level dropped significantly upon the removal of tumor, showing the marker was likely to be derived from the colorectal lesions. Since then, more miRNA candidates were reported, including miR-29a (63), miR-221 (64), miR-21 (65), U2 small nuclear RNA (RNU2-1) (66), miR-601 and miR-760 (67). Among them, RNU2-1, a marker for both CRC and pancreatic ductal adenocarcinoma (PDAC), was found to have a sensitivity of 97.7% in detecting CRC and/or PDAC, at a specificity of 90.6%. But this has not yet been tested in another independent study.

Blood DNA

Because of the established mutation and methylation characterized in adenoma-carcinoma sequence, plasma DNA has been more robustly evaluated than other plasma based markers. Diehl *et al.* showed that mutant APC fragment has a 100% sensitivity in detecting Dukes D stage patients (n=6) and a sensitivity of 63% in detecting Dukes A and B stage (n=16). The test remained poor in detecting advanced adenoma (68). Hypermethylated Septin-9 is the most studied plasma DNA marker. Multiple studies had reported its sensitivity towards CRC, ranging from 52% to 73% at specificities ranging from 84% to 91%, while sensitivity towards advanced adenoma was less than 20% (69-72). Currently, Septin-9 test is the only commercially available plasma DNA test intended for CRC detection.

Blood fatty acid

Gastrointestinal tract acid-446 (GTA-446) is a long-chain polyunsaturated fatty acid. Its serum level can be detected by mass spectrometry. Serum GTA-446 level was found to be reduced in CRC patients. Ritchie *et al.* showed that among 4923 subjects who had undergone colonoscopy, 84 out of the 98 CRC cases were detected to have a low serum GTA-446 level (as defined by the lowest tenth percentile), with a test specificity of 90% (73). The reduction of serum GTA-446 level was proposed to represent a compromised ability to protect against abnormal cell growth and chronic inflammation.

Stool test *vs.* blood test

Tumor markers enter the stool and blood stream through different mechanisms. Theoretically, exfoliation of colonocytes into the lumen occurs earlier than vascular invasion. Stool based test should be more effective in detecting precancerous lesions. Ahlquist *et al.* compared two commercially available tests: the stool DNA panel test (Exact Sciences Corporation, Madison, Wisconsin) and plasma Septin-9 test (ARUP Laboratories, Salt Lake City, Utah) in the same cohort of CRC and adenoma samples (n=42) but using separate sets of normal controls (stool, n=46; plasma, n=49). They found that the stool test had a higher sensitivity in detecting CRC (87% *vs.* 60%) and large adenomas (82% *vs.* 14%) compared to the plasma Septin-9 test. The specificity for the stool test and plasma test was 93% and 73% respectively. Based on this study, the stool DNA panel test is more effective in detecting early stage lesion that the plasma Septin-9 test.

Conclusions

Colonoscopy remains to be the gold standard in detecting CRC. Stool and blood based tests could serve as first line screening tests for the screening of asymptomatic

individuals, in which only those tested positive will proceed to perform colonoscopy. Among the reported studies, many stool or blood markers had demonstrated very high sensitivity and specificity. And new biomarkers will also continue to emerge as we improve our understanding of CRC biology. However, it is always more important to validate the markers in multi-centered studies with large cohorts of samples. With vigorous testing and validation, it is foreseeable in the near future that highly sensitive noninvasive test could be achieved through combining markers of different classes of molecule (e.g., DNA, RNA, protein) sourced from different biological samples (stool, blood). Population-based CRC screening will become more common and effectively conducted.

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Footnote

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Is the ability of stool DNA test enough for practical use in colorectal cancer screening?

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Deaths from colorectal cancer (CRC) can be reduced considerably by implementing an adequate screening. Moreover, CRC screening has another merit that can detect CRC in an early stage, and can also detect precancerous lesions, resulting in the decrease in the medical cost involved in the treatment of CRC. Hence, CRC screening has been prevalent in many countries (1), and in particular, the outstanding reduction in the death from CRC in the United States is considered largely attributable to the increase in the rate of CRC screening.

As one of the screening methods of CRC, the majority of countries adopted a fecal occult blood test, which has been recently called as a fecal immunochemical test (FIT), because hemoglobin concentrations in stools are measured with an immunochemical method using an antibody specific to human hemoglobin. FITs have advantages of more sensitive and specific nature to human hemoglobin, no diet restriction requirement, and quantitative measurement with an automated analyzer. FITs, therefore, have recently replaced the formerly used guaiac-based test. Both of the two major guidelines in the United States also recommended the stool-based test as one of the CRC screening methods. The U.S. Preventive Task Force (USPTF) guideline recommended FIT, sigmoidoscopy, and colonoscopy, while the guideline of the American Gastroenterological Association suggested a stool DNA test in place of a FIT (2,3). The main reason of the avoidance of recommendation of stool DNA testing by USPTF is the lack of sufficient evidence as to the benefit and cost-effectiveness of the method. In fact, previous reports did not show that the results of a stool DNA test were always superior to those of a FIT (4,5).

The report recently published in the *New England Journal of Medicine* indicated the results of the newly developed stool DNA test used for a prospective cohort consisted of subjects at average risk of CRC (6), in comparison to the results of a FIT. The stool DNA test used in the report was comprised of one genetic marker (*K-ras* mutation), two methylation markers, and an immunochemical assay for human hemoglobin. The results indicated that the stool DNA test was superior in sensitivities for CRC (more than 90%) and advanced neoplasia (more than 40%) to a FIT. Although the results are outstanding and may reveal the new era of the CRC screening, meticulous reading of the paper revealed not only anticipation but also several problems in the methodology.

Undoubtedly, there are advantages in the DNA test based on the results of the paper. First, the DNA test is approximately 20 points more sensitive to significant colorectal neoplasia (CRC and advanced neoplasia) than a FIT. Higher sensitivity reduces false negative cases. The false negative results could easily lead the subjects to fatal status. Hence, achievement of the high sensitivity to colorectal neoplasia by the noninvasive method (without the burden of endoscopy or radiation exposure) using stools is of great value.

Second, the high sensitivities for neoplasia in the proximal colon and sessile serrated polyps are worthy to note. According to the previous reports (7,8), fecal tests including the guaiac-based tests and FITs were less sensitive to neoplasia in the proximal colon than that in the distal colon and the rectum maybe because hemoglobin in stools was diluted and/or degenerated. In this context, the new stool DNA test probably covers those lesions by detecting DNA mutation or methylation. Moreover, the high sensitivity to sessile serrated polyps deserves special

mention. Sessile serrated polyps, which are recently regarded as a precursor lesion of CRC with microsatellite instability, are usually flat-shaped, normally-colored (i.e., less likely to bleed), and located at the proximal colon. Sessile serrated polyps are usually highly methylated tumors and the DNA test probably indentified those lesions by the detection of methylation. Thus, casting spotlight to the outcasts by the current screening methodology is an excellent outcome of the article. It should have been more interesting if which genetic or epigenetic markers had contributed to the detection of such lesions had been shown in the article.

Despite such admirable outcomes, the paper harbors substantial problems. First of all, lower specificities to CRC and advanced neoplaisa should be the focus of criticism. Lower specificity indicates the increase in false positive cases. Increase in the false positive cases would also increase the number of subjects who have to undergo close examinations including colonoscopy. The raised number of the close examinations would enlarge burdens of physicians who would perform colonoscopy. In addition, the increase in close examinations would inevitably increase the medical cost. More importantly, increase in the false positive cases would make subjects become unmotivated to undergo screening tests, and reduce adherence to CRC screening, resulting in the increase in the deaths from CRC.

The lower specificity appears to be caused by the substantial problem of the DNA test, because the test is comprised of the addition of *K-ras* mutation and methylation assays to the hemoglobin immunoassay. As the authors indicated, the isolated performance of the hemoglobin immunoassay component of the multitarget DNA test was similar to that of the FIT. Therefore, both the increase in sensitivity and the decrease in specificity of the test are considered to be mainly attributable to the addition of the *K-ras* test and methylation panel. Moreover, because mutation analysis is not likely to produce false positive cases, large portion of the false positive cases would have been responsible to methylation analysis. Hence, the number and location of methylation detection sites may have room for reconsideration. Meanwhile, the lower specificity of methylation analysis may reflect methylation status of normal mucosa, because normal mucosa of patients who would develop neoplastic lesions is considerably methylated before neoplasia development (9). Therefore, it appears to be interesting to verify whether the false positive patients on the methylation panel of the test would develop colorectal neoplasia in the future.

The next drawback of this study is that the criterion of the test positivity was defined by the unique algorithm in an arbitrary manner. The black box of the arbitrarily defined algorithm must be validated by using different subject cohorts. The quality of the DNA test and the validity of the algorithm must be ascertained by future studies.

The final shortcoming of the DNA test is the high rate of invalid preparations of the material: more than 5%. Due to recent progress in the skill of colonoscopy, the intubation rate of colonoscope into the cecum has become more than 95%, maybe nearly 100%. In this sense, the current method of the collection of DNA from stools may not be sufficient for the DNA testing and the technical innovation in this field is largely anticipated.

The paper was written in complying with the sponsor, the manufacturer of the DNA test. Maybe due to the intension of the sponsor, the paper largely emphasized sensitivity of screening modalities rather than specificity in the discussion. However, what is really needed in screening tests is not sensitivity alone. Specificity and cost-effectiveness are also important factors for practical use of screening. For further verification of the DNA testing for practical use, meticulous cost-effective analysis with disclosure of the cost of the DNA test should be performed. Different from the past, recent progress of the simulation models has enabled the precise evaluation of validity and cost-effectiveness of a certain screening method (10). In the field of CRC, the personal and social costs and burdens involved in close examinations including colonoscopy and treatments including the long-term administration of expensive chemotherapeutic agents are extremely high. Hence, such simulation studies are eagerly required. The ability of other screening modalities including colonoscopy and computed tomographic colonography has also been improved. The DNA test has to compete with those modalities in practical usefulness including validity, safety, cost-effectiveness and accessibility.

The sensitivity of more than 90% achieved by the DNA test appears to be as high as it gets. No more improvement of sensitivity could be achieved without lowering specificity. The putative contributors to the increase in the screening rate of CRC in the United States are improvement of the skill of colonoscopy and political success to motivate subjects to undergo screening. Further increase in the sensitivity of one screening modality would not be of great importance in the anti-CRC strategy in the future. In this sense, it is doubtful that the development of any stool DNA test could practically surpass the screening method of the

combination of annual two-day FITs with colonoscopy of several years interval.

In conclusion, the stool DNA test reported recently has promise as one of the candidates of screening modality of CRC in the future. In particular, higher sensitivity for neoplasia in the proximal colon and sessile serrated polyps is a great advantage. However, the lower specificity and lower successful rate of the preparation of stool DNA that would result in insufficient cost-effectiveness are great obstacles for practical application. For practical use, further improvement of the methodology of the collection of DNA and meticulous analysis of cost-effectiveness in comparison to currently available screening modalities using simulation models are required.

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Footnote

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

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Leptin as a risk factor for the development of colorectal cancer

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Abstract: Obesity has been associated with many types of cancer, including colorectal cancer (CRC). The mechanisms by which obesity increases the risk for development of CRC can be explained by factors such as hyperinsulinemia, increases in proinflammatory cytokines, estrogens and insulin-like growth factors (IGFs), and alterations in adipokines levels. Leptin is the most abundant adipokine, and has also been associated with an increased risk for development of CRC, as demonstrated by innumerous *in vitro* and *in vivo* experiments, and by large epidemiological studies. By binding to its receptor, leptin activates several intracellular cascades, stimulating cell growth and proliferation, inhibiting apoptosis, and promoting angioneogenesis. Leptin has also proinflammatory effects that promote a low-grade inflammatory state and contribute to the development of CRC. A better understanding of the associations between leptin and CRC might lead to the development of novel approaches for the diagnosis, risk stratification, and treatment of CRC.

Keywords: Cancer; colon; colorectal; leptin; obesity

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Introduction

Obesity, defined by a body mass index (BMI) ≥30 kg/m^2, is a growing epidemic now affecting developing countries, as well as developed nations. The World Health Organization (WHO) reports that globally, an estimated 10% of all men and 14% of all women are obese. Indeed, the WHO estimates that half a billion people over the age of twenty worldwide are obese (1). Obesity is a major cause of global mortality and morbidity: it is well established that people who are obese are at an increased risk for developing comorbidities such as cardiovascular diseases, type 2 diabetes mellitus, end-stage renal disease, respiratory complications, depression and arthritis (2). An increasing number of studies are now demonstrating a strong association between obesity and cancer incidence (3-5). Indeed, obesity is also reported to increase cancer-associated mortality (6). Obesity has well-documented associations with many cancers such as renal and endometrial cancer (7), and those associations also extend to colon cancer. Apart from a sharing a number of common non-modifiable risk factors, obesity and colon cancer are inextricably linked with not only nutrition and metabolism, but also with a variety of hormones associated with excess fat.

Over the past decade, the adipose tissue has gained importance as not only a tissue for energy storage, but also as an endocrine organ (8). Several hormones and cytokines called adipokines are synthesized and released by the adipose tissue. Leptin is the most abundant adipokine, with key roles in controlling hunger and satiety thus regulating food intake, energy balance and body weight (9). Leptin also plays important roles in lipid and glucose metabolism, the gonadal, adrenal, somatotropic and thyroid axes, sympathetic tone, biomarkers of cardiovascular disease, immunity, and brain structure and function (10).

Leptin has been implicated in the pathogenesis of several types of obesity-related cancers, including colon cancer (11). This effect can be explained by leptin's effect on the regulation of specific intracellular pathways that control cell growth, differentiation, apoptosis and angiogenesis, involved in the pathogenesis of cancer (12). Furthermore, leptin is a crucial inflammatory mediator, owing to its homology with well-characterized cytokines and its ability to stimulate the secretion of other inflammatory factors (13), which also are contributors to colon cancer

Table 1 Summarized findings from Bardou *et al*. 2013 (23) of five meta-analyses reporting increased risk of colon cancer in obese males and females

Study	RR (95% CI) for CRC in obese individuals	
	Men	Women
Guh *et al*. (24)	1.95 (1.59-2.39)	1.66 (1.52-1.81)
Harriss *et al*. (25)	1.24 (1.2-1.28)	1.09 (1.04-1.14)
Dai *et al*. (26)	1.71 (1.33-2.19)	1.10 (0.92-1.32)
Moghaddam *et al*. (27)	1.46 (1.36-1.56)	1.15 (1.06-1.24)
Larsson *et al*. (28)	1.30 (1.25-1.35)	1.12 (1.07-1.18)

pathogenesis (12). It is therefore hypothesized that leptin, through its action on the regulation of body weight, specific intracellular pathways, and inflammation, influences the pathogenesis and progression of colon cancer. Whilst maintaining a focus on recent publications, this review will examine the links between obesity and colon cancer, and between leptin and colon cancer, focusing on the molecular mechanisms by which leptin is thought to contribute to cancer etiology. It is hoped that better understanding of the functions of leptin and its involvement in colon cancer pathogenesis will help to unravel novel biomarkers to improve current screening programs, and new potential therapeutic drug targets to prevent or treat the condition.

Obesity-related colon cancer

Colon or colorectal cancer (CRC) is an obesity-related cancer that affects more than 1 million people worldwide. It is associated with a mortality rate of 33% in developed countries, and a 5-year survival rate of less than 60% in most European countries (14). Colon cancer is associated with many risk factors including increasing age, male sex, genetic predisposition, previous colonic polyps or previous incidence of CRC, diabetes mellitus, and inflammatory bowel disease; and with environmental risk factors such as sedentary behavior, consumption of processed red meats, inadequate intake of fiber, tobacco smoking, and heavy alcohol consumption (15-17).

It is widely accepted that obesity supports many adverse hormonal, metabolic and immunological alterations in the body. These alterations, in turn, result in an increased risk for the development and progression of colon cancer. Several studies demonstrate an association between obesity and colon cancer (18-22). In a recent report synthesizing a number of meta-analysis examining obesity and colon cancer risk, Bardou *et al*. reported that all studies found that obesity was associated with an increased risk of colon cancer in both males and females. The results of the study are summarized in *Table 1* (23). These data comprehensively suggest an association between obesity and colon cancer risk.

The molecular mechanisms by which obesity influences colon cancer development are not completely understood, though several promising streams of investigation are emerging. Obesity is often associated with increased expression of the enzyme fatty acid synthase (FASN), a key regulator of lipogenesis (29) that is upregulated in CRC. In a cohort study comprising 647 CRC patients, the overexpression of FASN in those with a BMI >27 kg/m^2 was associated with a poorer outcome (30). Therefore, it has been suggested that FASN plays a role in the pathogenesis of CRC, by maintaining membrane integrity in the endoplasmic reticulum of tumor cells (31).

Obesity is associated with increases in serum concentrations of insulin-like growth factor-1 (IGF-1) (32), which mediates the effects of growth hormone and is a potent inhibitor of apoptosis. In this way, studies have shown that IGF-1 can support tumor cell growth and metastasis, and the prevention of apoptosis (33). It has been shown that IGF-1 levels, as well as its bioavailability (regulated by its binding proteins), are directly associated with CRC risk, by disrupting growth factor regulation and leading to uncontrolled cell proliferation (34). Also, polymorphisms in the IGF-1 gene can regulate the risk for CRC development: in a case-control study of Singaporean-Chinese individuals (as a measure into the effects of the "Western lifestyle"), Wong and colleagues examined polymorphisms in the IGF-1 gene promoter region that affect its viability (35). Of the 298 cases of CRC, a single nucleotide polymorphism in the IGF-1 promoter region "IGF1-2995 C/A" was associated with a 40% decrease in colon cancer risk (35). This suggests that regulation of IGF-1 may be an important mechanism by which colon cancer development is restricted in some cases. Interestingly, the decrease in risk was accentuated in those patients who were physically active. Indeed, in mice on a calorie-restricted diet, decreased systemic IGF-1 resulted in an improved outcome, attributed to the regulation of nuclear factor-κβ (NFκβ) and modulation of inflammatory genes (36).

Estrogen levels are often increased in obese postmenopausal women (37), and can also play a role in the pathogenesis of obesity-related colon cancer, depending on the estrogen receptor (ER) that is predominant in the

Table 2 Obesity-related factors influencing colon cancer development and progression

Study	Model	Obesity-related biological element	Effect on colon cancer development
Ogino *et al*. (30)	Human CRC patients	FASN	Supports membrane biosynthesis of tumors
Wong *et al*. (35)	*In vitro* human tissue	IGF-1	Inhibits apoptosis
Flores *et al*. (40)	Murine diet induced obesity	TNF-alpha	Promotes inflammatory milieu; Impairs insulin signaling
EPIC studies (44-46)	Human CRC patient blood samples	HDL, apoA, HbA1c, IGFs, CRP, TNF-α, IL-6, adipokines	Statistically associated with colon cancer. Supports metabolic and cellular dysregulation and chronic inflammation stressing colonic cells predisposes them to carcinogenesis

tissue. In normal colon cells, ER-β is the receptor that is most predominantly expressed, and its activation is protective against colon cancer through the induction of apoptosis. However, in malignant colonic cells, ER-α is the most abundant receptor, and its activation by estrogen promotes cell growth (38). Therefore, increased estrogen in obesity may have protective effect via ER-β activation, whilst activation of ER-α in the later stage of colon cancer may promote cancer development.

An important part of the obesity-associated milieu is the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α), which is overexpressed in obese human and animal adipose tissues (39). In murine models of diet-induced obesity and genetic obesity, Flores and colleagues found that obesity-related colonic inflammation, witnessed through TNF-α overexpression, increased expression and activity of c-jun N terminal kinase (JNK) and inhibitor of nuclear factor κβ kinase (IKK) pathways (40). Stimulation of these pathways resulted in impairment of insulin signaling, and TNF-α neutralization reversed obesity-induced tumor growth. This finding is in accordance with previous epidemiological studies which have demonstrated that hyperinsulinemia and insulin resistance are a potentially crucial mechanism by which obesity increases the risk of colon cancer development, through the activation of the PI3K/Akt pathway (41,42).

The European Prospective Investigation into Cancer and Nutrition (EPIC) yielded many associations between obesity and increased risk of colon cancer development. Obesity is linked to a high-fat diet and alterations in the circulating lipid profile, with decreases in concentrations of high-density lipoproteins (HDL) and increases of low-density lipoproteins (LDL) and triglycerides. As elevated levels of HDL-cholesterol have been associated with a reduced risk of colon cancer, its decrease could potentially predispose to the development of CRC (43).

Obesity is also associated with increased blood glucose and glycated hemoglobin (HbA1c), which is a marker of circulating glucose concentrations. EPIC investigators reported a statistically significant association between high HbA1c and increased colon cancer risk. This suggests that alterations in glucose/insulin homeostasis, most likely due to hyperinsulinemia and insulin resistance, may be an important risk factor for the development of obesity-related cancer (44). The EPIC and other studies also found many associations between other inflammatory factors and cytokines such as IL-6 and IL-17, adipokines (including leptin), IGFs and increased risk of colon cancer (45,46). Moreover, in obesity, circulating levels of the adipokine adiponectin are often decreased, which has also been associated with an increased risk for colon cancer by activating the PI3K/Akt pathway (47). *Table 2* summarizes studies associating obesity-related alterations and colon cancer.

Molecular biology of leptin

Leptin is a 16 kDa protein synthesized mainly by the adipose tissue. It is encoded by the ob gene, and shares structural homology with the cytokines IL-6 (an important inflammatory mediator), IL-11, IL-12 and IL-2, indicating its inflammatory roles (13). Leptin regulates feeding behavior by binding to its receptor (Ob-R), expressed in many areas within the central nervous system, mainly in the acuate nucleus of the hypothalamus. Ob-R is a tyrosine kinase-associated receptor that signals through JAK and STAT, and is expressed as at least four different isoforms in humans: Ob-Ra, Ob-Rb, and Ob-Rc (membrane-anchored), and Ob-Re (soluble) (9). In the hypothalamus, the activation

of Ob-Rb stimulates the expression of the anorexigenic neurotransmitters pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART). Also, leptin inhibits the orexigenic neurons that express neuropeptide Y (NPY) and Agouti-related peptide (AgRP). Therefore, through its actions on anorexigenic and orexigenic neurons, leptin stimulates satiety and inhibits hunger.

Peripherally, Ob-R and its isoforms are widely expressed in most tissues that have been tested, including the colon (48). Through its central and peripheral actions, leptin is thought to have proinflammatory activities, evidenced by its ability to increase production of TNF and IL-6 in monocytes and to stimulate the production of various CC-motif chemokines (49). The proinflammatory state that is seen in obese individuals can be, at least in part, explained by the high levels of leptin that are seen in those individuals, who do not benefit from the anorexigenic effects of leptin due to central leptin resistance (50). Besides regulating energy balance and having proinflammatory effects, leptin also regulates endocrine systems such as the thyrotropic, gonadotropic and corticotropic axes, and affects glucose homeostasis, hematopoiesis, angiogenesis, osteogenesis, and wound healing (10).

Inflammatory factors increasing leptin

Since there appears to be a connection between leptin and CRC, it is relevant to summarize the factors that contribute to hyperleptinemia. Circulating leptin levels correlate well with body fat, and high levels of circulating leptin is one of the consequences of being obese. Therefore, it is crucial to consider the possible role of leptin in comorbidities related to obesity. As obesity is characterized as a chronic low-inflammatory grade disorder (51,52), contributing factors maintaining and/or enhancing obesity-related inflammation including elevation of circulating leptin levels should be considered. Indeed, it is well known that inflammatory challenges increase leptin synthesis and release (53,54), and that chronic inflammatory conditions promote cancer development (55,56).

High levels of circulating leptin could be deleterious, as leptin has proinflammatory actions. Leptin and its long-isoform functional receptor (Ob-Rb) share tridimensional and sequence homologies respectively, with cytokines of the IL-6 family and gp130, the signal transducing component of the IL-6-type receptor (57,58). Presumably, high circulating leptin levels found in obese individuals could contribute to the low-grade inflammation that characterizes obesity. In fact, circulating leptin levels display a circadian rhythm in parallel to that of NO_3/NO_2 [measured as an index nitric oxide (NO) synthesis, a powerful oxidant agent] (59). In previous *in vitro* and *in vivo* studies, we showed that leptin increased not only NO_3/NO_2, but also TNF-α, a prototypical proinflammatory cytokine (59). In clinical studies, others have shown that circulating leptin also correlates with proinflammatory factors such as IL-6, a cytokine that has been largely correlated with metabolic syndrome (60). Thus, leptin could play a crucial role bridging the gaps among obesity, inflammation and presumably cancer.

Other two contributing factors to the low-grade inflammatory state occurring during obesity that might also increase leptin synthesis and release are (I) high-calorie intake-induced macrophage infiltration in adipose tissue; and (II) increased intestinal permeability (61). Excessive calorie overload causes hypertrophic adipocytes to release monocyte chemoattractant protein (MCP)-1, which in turn favors increased macrophages infiltration into the adipose tissue (62). Subsequently, infiltrating macrophages increase the synthesis and release of several proinflammatory factors such as TNF-α, IL-1β and IL-6 (63,64), all of which are known to increase leptin synthesis and release.

Gut health plays a key role as a barrier to prevent translocation of intestinal bacteria and bacterial lipopolysaccharide (LPS) into the blood stream. Emerging evidence suggests that obesity causes increased gut permeability, which contributes to the low-grade chronic inflammatory state observed during obesity (65,66). The key role that gut microbiota plays in obesity has been recently shown in preclinical studies (61,67,68). In mice studies, it was shown that high-fat diet increased circulating endotoxin and proinflammatory factors (61). These changes appeared to be due, at least in part, by changing gut microbiota composition (increasing Firmicutes to Bacteroidetes ratio), which favored endotoxin translocation into the bloodstream (61). As leptin synthesis and release can be increased by LPS, this mechanism might account for the increased hyperleptinemia that occurs during obesity.

Other studies carried out in mice mimicking Roux-en Y gastric bypass (RYGB), currently the most effective treatment for obesity, also strengthened the concept that gut microbiota can contribute to the obese/lean phenotype (68). In the latter study, the authors provided support to a new emerging concept: conserved post-operative changes in gut microbiota played a key role to reduced weight and adiposity after RYGB surgery (68).

In summary, leptin shares tridimensional similarities

with the cytokine family, it can increase proinflammatory factors, and it might be regulated by gut microbiota and macrophage infiltration in adipose tissue. As a relationship between hyperleptinemia and CRC appears to be evident, inflammatory factors that chronically increase leptin have to be considered for overcoming the deleterious consequeces of hyperleptinemia.

Associations between leptin and colon cancer

Serum leptin levels are markedly increased in obese individuals, where obesity is an important risk factor for colon cancer development. Since the demonstration of leptin's effect as a growth factor for colonic epithelial cancer (48), several studies have hypothesized an association between increased leptin levels and colon cancer risk. The leptin receptor is found in colonic epithelium, which has functional importance in regulating cell processes (48). Dysregulation of these processes, as a result of the obesity-related hyperleptinemia, can lead to neoplasia.

In vitro colon cancer cell line studies have shown that stimulation of these cells by leptin leads to tyrosine phosphorylation of Ob-R, activating major signal transduction pathway elements including p42/44 mitogen-activated protein kinase, JNK, mitogen-activated protein kinase, Src/phosphoinositide, 3-kinase/protein kinase B and extracellular-signal-regulated kinase (69,70). Leptin stimulation has also been reported to inhibit apoptosis of human CRC cells via several mechanisms involving extracellular-signal-regulated kinase, p38 mitogen-activated protein kinase activation and nuclear translocation of NF-κB (71).

Some rodent studies have contradicted findings from *in vitro* studies: whilst leptin stimulation *in vitro* led to increases in signal transduction, in nude mice leptin stimulation failed to promote the growth of cancer xenografts (72). Furthermore, in Apc/Min+ mice (a murine model of colon cancer), leptin stimulation failed to induce tumor growth (72). Interestingly, in the absence of leptin, ob/ob mice showed increased sensitivity to colon cancer carcinogens. In contrast to these negative findings, a high-fat diet promoted colonic epithelium proliferation in mice, which would imply that obesity-associated hyperleptinemia is associated with colon cancer (73). However, carcinogen-induced tumor growth in leptin-deficient mice was slower than in leptin-resistant *db/db* mice, suggesting the mechanism of carcinogenesis is more complex than simple rises in circulating leptin concentrations. Indeed, Endo and colleagues observed that Ob-R is overexpressed in colonic tumors, and that leptin is linked to the activation of Wingless-related integration site (Wnt) signaling (an important paracrine signaling mechanism) (73). This observation, together with differences in tumor growth seen between substrate and receptor deficient models, reveals a potential molecular mechanism for colon cancer development.

On an inflammatory level, leptin induces the secretion of the inflammatory cytokines IL-6, IL-1β and CXCL1 in humans, which have all been implicated in colon carcinogenesis (74). In particular, the secretion of CXCL1 supports *in vitro* studies, which reveal that leptin promotes vascular endothelial growth factor (VEGF) activity by epithelial cells, and thus provides a mechanism for tumor-associated angiogenesis, promoting tumor survival and proliferation (70). Indeed, by stimulating angiogenesis, leptin facilitates tumor growth and invasion of adjacent organs (75). Furthermore, Ob-R overexpression suggests that tumors may be sensitive/responsive to leptin, thus providing further means for cancer survival and growth, described below in *Figure 1*.

On the other hand, hypoxia (a common finding in malignant tissues), has also been shown to induce cancer epithelial cells to produce endothelial growth factor (EGF), which regulates the levels of leptin and VEGF (70). The effect of hypoxia on leptin levels can also be indirectly mediated by hypoxia-inducible factor 1-alpha (HIF-1α), which binds to target genes that contain a specific hypoxia-responsive element (HRE) (76). The leptin gene contains eight HRE regions, and thus it is likely to be regulated by hypoxia through HIF-1α. Koda and colleagues explored this relationship in a cohort of CRC patients, and found a significant positive correlation between leptin levels and the amount of HIF-1α ($r=0.243$, $P=0.005$), and between Ob-R and HIF-1α ($r=0.325$, $P<0.001$). As expected, leptin and Ob-R also shared a positive correlation ($r=0.426$, $P<0.001$) (75). These results further support a role for hypoxia in neoplasia, and demonstrate that leptin has a role in cancer progression through an auto-/paracrine mechanism. The co-expression of leptin with Ob-R suggests that local activity of the leptin/Ob-R axis is responsible for colon cancer cell responses to an hypoxic environment.

Leptin affects many cellular signal transduction pathways, and can act as an important gene expression regulator. Nowakowska-Zajdel performed a microarray analysis using samples obtained from 11 CRC patients, targeted at analyzing genes that encode proteins that are affected by leptin. The genes *AKT1*, *STAT3* and *MCL1* were upregulated at the early stage of disease, and the gene

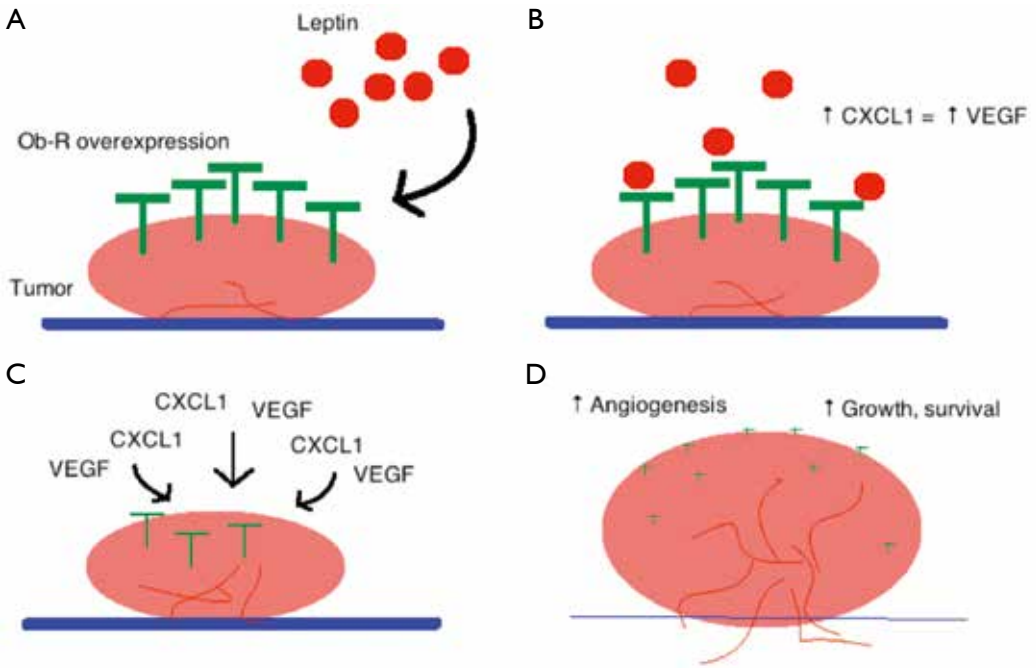

Figure 1 Leptin promotes tumor survival by upregulating the expression of CXCL1 and VEGF, which promote angiogenesis and tumor growth in the neoplasm. (A) Colon tumor cells overexpress Ob-R and are responsive to leptin; (B) Activation of Ob-R by leptin leads, directly and indirectly, to CXCL1 and VEGF expression; (C) CXCL1 and VEGF act on the neoplasm; (D) Tumor grows and increases blood vessel invasion. Adapted from (7,74).

STAT5B was silenced. Furthermore, the genes *VEGFC* and *CCND1* were overexpressed and the *VEGFA* gene was silenced (77). Differences in the gene expression profile between early and late stage cancers suggest that leptin plays a role in the dynamic and changing system of the neoplasm, and that leptin may, at least in part, be responsible for tumor progression by means of transcription activation and repression or silencing. Of clinical value, the genes revealed to be overexpressed at an early stage of the disease may have the potential to be used as part of a colon cancer screening program, in an effort to identify patients for treatment at an earlier stage, thereby improving the prognosis.

Similar to Nowakowska-Zajdel's study, in a mouse model of colon cancer, leptin was found to upregulate the proinflammatory cytokine gene profile (74). Real-time polymerase chain reaction (RT-PCR) assays were performed on colonic tissue harvested from wild-type and leptin-deficient mice. Compared to basal gene expression, a few genes were differentially expressed. Following leptin administration, several more genes encoding products affected by leptin were significantly upregulated, summarized in *Table 3*.

Additionally, cytokines, including those previously mentioned to promote colonic neoplasia, were significantly altered. Leptin administration altered the proinflammatory cytokine profile more substantially in ob/ob mice than in wild-type mice (summarized in *Table 4*) (74). These findings fit with the growing hypothesis that leptin is a major immune regulator, and substantiates the notion of adipose tissue as an immunoendocrine organ. In the presence of increase leptin sensitivity (i.e., in the leptin-deficient mouse), leptin's effects on the upregulation of proinflammatory markers are enhanced.

The same study has shown that IL-6 and CXCL1 were rapidly upregulated 1-hr after leptin administration, and returned to near basal levels after a further three hours (74). This time-dependent response indicates that these genes may be involved in an early response. Time-dependent changes, though over a longer time period, were also seen by Nowakowska-Zajdel and colleagues (44). Interestingly, the authors have not observed the localization of leptin-regulated proinflammatory cytokines with macrophage markers (F4/80 and CD11c) (74). This suggests that leptin stimulation may directly or indirectly result in an

Table 3 Differences in gene expression following leptin administration in wild-type and leptin-deficient mice

Comparison ob/ob *vs*. wild-type	Gene	Fold difference change in expression normalized to GAPDH	P value
Basal	IGFBP3	0.67	0.089
	IGF2	0.66	0.009
1-hr post leptin administration	IGFBP3	0.57	0.024
	ObR	0.67	0.043
	ObR-b	0.56	0.062
	AKT2	0.84	0.049
	MUC2	1.83	0.029

Adapted from (74).

Table 4 Differences in cytokine gene expression following leptin administration in wild-type and leptin-deficient mice

Cytokine gene	Fold difference change in expression 1-hr post leptin administration, normalized to GAPDH	
	Wild-type	ob/ob
IL-6	8.23 (<0.0001)	14.77 (<0.0001)
IL-1β	2.35 (0.049)	ns
CXCL1	3.8 (0.024)	6.14 (0.003)
INSR	1.21 (0.009)	ns
ICAM	ns	1.7 (0.013)

Adapted from (74). ns, not significant.

upregulation of IL-6, IL-1β and CXCL1 in cells already resident within colonic tissue, possibly independent of other inflammatory mechanisms. Furthermore, Padidar and colleagues showed visible changes in response to leptin in cells embedded in the epithelium, lamina propria and muscularis layers of the colon (74). As previously mentioned, CXCL1 is an important angiogenic factor and, together with VEGF (a powerful pro-angiogenic growth factor), this could be a potential mechanism by which Ob-R–expressing tumors support their growth and survival, when stimulated by leptin. Overall, this study provides *in vivo* evidence of the direct effect of leptin on colon cancer pathogenesis.

Human epidemiological studies have further demonstrated the association between hyperleptinemia and colon cancer. Epidemiological studies carried out in two different cohorts, one from Norway (78) and another from Sweden (79), have shown increased risk of colon cancer in individuals with high levels of leptin. In a case-control study of more than 100 volunteers, Hillenbrand and colleagues examined the adipokine profile of CRC patients, morbidly obese (MO) patients and healthy blood donor (BD) participants. As expected, CRC and MO patients had a systemic increase in inflammatory mediators, in line with the theory that inflammation contributes to obesity and colon cancer (80). However, there were significant differences between CRC and MO adipokine profiles. Median leptin concentrations were lower in CRC patients as compared with MO. In contrast to the leptin findings, adiponectin, another adipose tissue-derived cytokine, was increased in CRC patients as compared with MO. Furthermore, there was no difference in adiponectin levels between CRC and BD individuals. These differences were sex-dependent, where females tended to have higher levels of both leptin and adiponectin compared to males in all three groups of volunteers. Overall, this study suggested that CRC and MO individuals have similar cytokine profiles, but with discrepancies in the concentration of leptin, suggesting that leptin does contribute to CRC risk, independent of obesity. However, this study failed to take into account the possible role for soluble leptin receptor (Ob-Re) as a potential mechanism of circulating leptin sequestration thus reducing its bioavailability (80).

The role of Ob-Re on colon cancer was addressed by Aleksandrova and colleagues (46). In a large prospective study of approximately 520,000 participants, leptin was negatively correlated with Ob-Re. Furthermore, leptin was not significantly associated with an increased risk of CRC, but Ob-Re was strongly inversely associated with CRC, meaning that CRC is associated with a low circulating concentration of Ob-Re (which lead to higher bioavailable leptin levels) (46). Indeed, higher levels of Ob-Re were found to be associated with an advanced stage of tumor development (81). These studies do, however, report cancer site specific differences in adipokine concentrations. Hillenbrand and colleagues report higher levels of leptin in

Table 5 Effects of leptin in the development and progression of colon cancer in cell, animal and humans studies

Study	Model	Major effects on colon cancer development
Cascio *et al*. (70), Aparicio *et al*. (72)	*In vitro* colon cancer cell lines	Leptin stimulated tyrosine phosphorylation of Ob-R and activated major elements in signal transduction pathways • p42/44 mitogen-activated protein kinase • c-Jun N-terminal kinase • mitogen-activated protein kinase • Src/phosphoinositide • 3-kinase/protein kinase B • extracellular-signal-regulated kinase Leptin regulates VEGF signaling
Endo *et al*. (73)	Mice on high-fat diet	Colonic epithelium proliferation; Leptin linked to Wnt signaling; Leptin upregulated Ob-R and supported pro-angiogenic factor secretion
Koda *et al*. (75)	Human colon cancer tissue samples	HIF-1a correlated with leptin and Ob-R levels, supported hypoxia-related changes in tumors
Nowakowska-Zajdel *et al*. (77)	*In vitro* human cancer cells	Upregulated leptin-associated pathways: • AKT1 • STAT3 • MCL1 • VEGFC • CCND1 Downregulated: • STATB • VEGFA
Padidar *et al*. (74)	*In vitro* human cancer cells	Leptin stimulation promoted the upregulation of genes • IGFBP3 • ObR • ObRb • AKT2 • MUC2 • IL6 • CXCL1 • ICAM1
Hillenbrand *et al*. (80)	Human colon cancer patient blood samples	Increased leptin in CRC patients compared with morbidly obese and healthy blood donors
Aleksandrova *et al*. (46)	Human colon cancer patient blood samples	No difference in leptin levels in CRC compared with controls. Increased soluble Ob-R in CRC patients

patients with colonic cancer as compared with rectal cancer in both males and females, and Aleksandrova and colleagues report an increased risk of colonic cancer at the highest quintile of leptin concentration compared to no significant increases in risk in developing rectal cancer (46,80). It is important to note that the study by Alesandrova and colleagues was a multi-center trial which included over half a million participants from nine countries, and thus greater emphasis should be placed on their conclusions. Taken together, these studies indicate a role for the leptin/Ob-Re axis in colon cancer development, and highlight the need to establish the action of leptin in colon cancer pathogenesis. Furthermore, these studies demonstrate the need to clarify the role of Ob-Re in either stimulating leptin signaling or sequestering leptin and reducing its bioavailability. Finally, it would be of clinical value to determine the reasons for gender-related differences in leptin levels and colon cancer risk. The effects of leptin in the development and progression of colon cancer in cell, animal and humans studies are summarized in *Table 5*.

Conclusions and future directions

Obesity is a risk factor for several cancer types, including colon cancer. This can be explained by several changes in hormonal and cytokine profiles that stimulate cell growth, inhibit apoptosis, and promote angioneogenesis. Leptin is increased in obesity, and has been shown to play an important role in the pathophysiology of obesity-related colon cancer by affecting cell growth, apoptosis and angioneogenesis. Several human and animal trials have explored the possible association between leptin and colon cancer, though the exact mechanisms remain unclear. Some human studies have yielded contradictory findings in terms of a clear association between the adipokine and increased CRC risk (46,80), and it is possible that the links between obesity, inflammation and colon cancer extend beyond the traditional adipokines leptin and adiponectin. The adipose tissue is emerging as a major endocrine organ and with more research focusing into the immunoendocrine nature of that tissue, many novel adipokines have been discovered. These adipokines, for example visfatin, omentin-1 and vaspin have now also been associated with CRC in an obesity-independent manner (82).

Like any cancer, CRC has a multifactorial etiology, and several factors affecting cancer development and progression need to be taken into account. As colon cancer develops and progresses over several decades, lifestyle interventions can be an important adjunct to medical therapies to effectively treat and suppress cancer development and metastasis. Better understanding of the mechanisms by which leptin is associated with CRC can potentially lead to the development of novel approaches for the diagnosis, risk stratification, and treatment of colon cancer.

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Footnote

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

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Risk of colorectal cancer after detection and removal of adenomas at colonoscopy

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In the United States, a large proportion of endoscopists are conducting surveillance examinations after polypectomy along the American Gastroenterological Association guidelines (1).

In the guideline, patients can be stratified more definitely at their baseline colonoscopy into those at lower risk or increased risk for a subsequent advanced neoplasia. People at increased risk have either 3 or more adenomas, or advanced adenomas which is an adenoma with high-grade dysplasia, or with villous features, or an adenoma 1 cm or larger in size. It is recommended that they have a 3-year follow-up colonoscopy. People at lower risk who have 1 or 2 small (<1 cm) tubular adenomas with no high-grade dysplasia can have a follow-up evaluation in 5-10 years. People with hyperplastic polyps only should have a 10-year follow-up evaluation, as for average-risk people. After this guideline published, several studies have examined the risk of advanced colorectal neoplasia in patients with previously endoscopically resected colorectal adenomas to quantify their risk of developing a subsequent advanced adenoma or cancer. A pooled analysis of eight prospective studies (with a total of 9,167 subjects) estimated that the risk of advanced colorectal neoplasia was 12 percent during a median follow-up of four years; 58 patients (0.6 percent) developed invasive cancer (2). The strongest risk factors were advanced neoplasia in the initial polypectomy, older age, and the number and size of prior adenomas.

However, in most of studies, evidence for surveillance intervals continues to be based primarily on adenoma recurrence rather than on Colorectal Cancer (CRC) incidence. In this study, authors aimed to assess risk of CRC rather than adenoma recurrence. They showed that patients with a history of detection and removal of at least one adenoma had a strongly and significantly reduced risk of CRC up to 5 years after colonoscopy compared with people who had never undergone large-bowel endoscopy. They concluded that extension of surveillance intervals to 5 years should be considered, even after detection and removal of high-risk polyps, whereas it is the common understanding that a surveillance interval of 3 years is needed after detection and removal of high-risk adenomas, which is mainly based on studies that focused on risk of advanced adenomas following colonoscopic polypectomy (3-7).

This study was conducted retrospectively. However, the authors sought to raise evidence level. This is multi-center study with 22 hospitals, and they could recruit 6,422 persons, and this is five fold samples of their previous report (8). Personal interviews were conducted by trained interviewers who visited the patients during hospitalization or, if they had already left the hospital, at their homes. The standardized interviews lasted for about 1 hour. Furthermore, they sought to validate the obtained information by medical records from the participants' physicians.

There is evidence of substantial overuse of surveillance colonoscopies, especially after detection and removal of low-risk adenomas (9-12). So, it is important to evolve the adequate time interval to surveillance colonoscopy after adenoma removal.

Recently, several new risk factors have suggested in many studies. An increased body mass index (BMI) is associated with an increased risk of colorectal adenomas (13). COX-2 agents demonstrated significant reductions in advanced and metachronous adenomas (14-16). Aspirin also reduces the

incidence of metachronous adenomas and probably cancer (17). Ursodeoxycholic acid reduces the risk of adenomas with high-grade dysplasia (18).

Further and even larger studies are needed to more precisely define surveillance intervals with enhanced risk stratification.

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Footnote

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

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Acromegaly and colorectal cancer

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Abstract: Several studies have suggested increased risk of colon cancer and polyps. Prospective studies using colonoscopy showed a three times higher prevalence of intestinal polyps and up to four times increased presence of colorectal cancer (CRC) in acromegaly than in normal controls, independently of sex, age, duration of disease and clinical status of the patients. Guidelines recommend early colonoscopic screening starting at the time of diagnosis. Interval colonic surveillance depends on the findings from the baseline colonoscopy and on insulin-like growth factor 1 (IGF-1) levels. The mechanisms involved in cancer initiation and progression in acromegalic patients remain unclear. Several hypotheses have been investigated and they may be related to sustained increase of growth hormone (GH) and IGF-1 levels, metabolic disorders, and genetic factors.

Keywords: Acromegaly; growth hormone (GH); insulin-like growth factor 1 (IGF-1); colorectal cancer (CRC); colonoscopy; screening

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Introduction

Acromegalic patients are exposed to chronic growth hormone (GH) hypersecretion mostly associated to pituitary adenomas (1,2). The disease has a subclinical course, and the delay on diagnosis is associated with high morbidity and with premature mortality related to increased cardiovascular risk, sleep apnea, metabolic comorbidities, and cancer (3-5).

Overall and cancer mortality in acromegaly have been shown to correlate with the degree of GH control. Several studies have suggested increased risk of colon cancer and polyps in acromegalic patients (6-9). Prospective studies using colonoscopy showed a three times higher prevalence of intestinal polyps and up to four times increased presence of colorectal cancer (CRC) in acromegaly than in normal controls, independently of sex, age, duration of disease and clinical status of the patients (8). Data from registry-based cohorts in Europe showed increased risks for digestive system cancers [standardized incidence ratio (SIR) =2.1, 95% CI, 1.6-2.7), notably of the small intestine (SIR =6.0, 95% CI, 1.2-17.4), colon (SIR =2.6, 95% CI, 1.6-3.8), and rectum (SIR =2.5, 95% CI, 1.3-4.2) (10).

CRC is one of the most prevalent malignancy worldwide (11) and among patients with acromegaly (7-10), in whom it implies a mortality rate higher than that expected for the general population (6). The main objective of the current article is to review the most relevant aspects concerning prevalence, pathogenesis and screening of CRC in acromegalic patients.

Pathogenesis of colorectal cancer (CRC) in acromegaly

The mechanisms involved in cancer initiation in acromegalic patients remain unclear. Several hypothesis have been investigated and they may be related to sustained increase of GH and insulin-like growth factor 1 (IGF-1) levels, metabolic disorders, and genetic factors (7,8,12) (*Figure 1*).

GH-IGF-1 axis

Overall and cancer mortality in acromegaly have been shown to correlate with the degree of GH control (2,6,7).

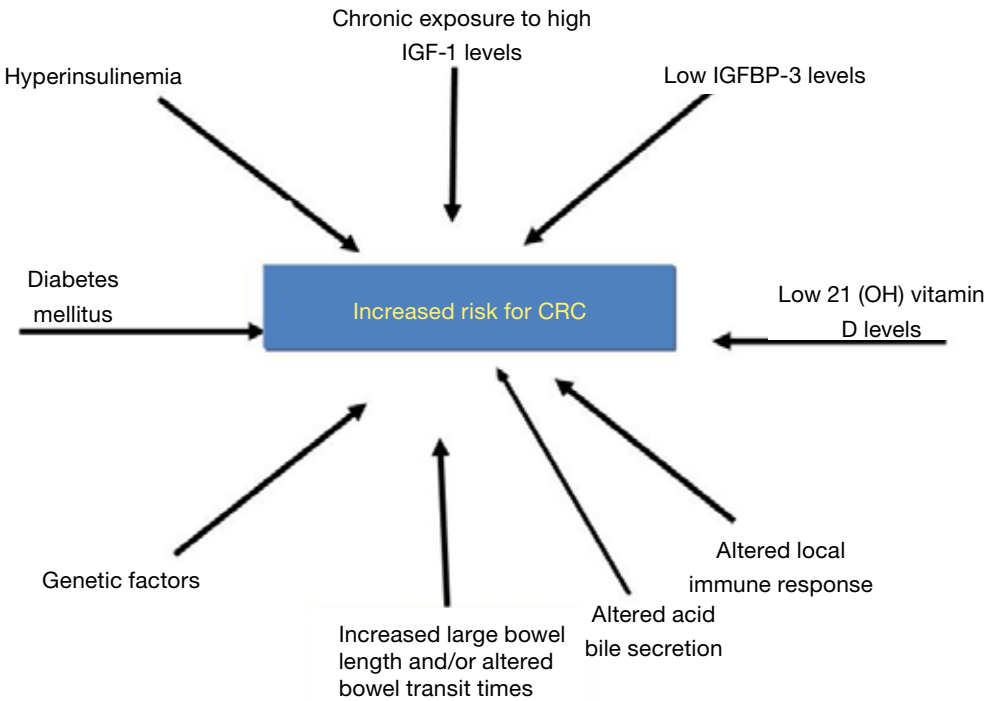

Figure 1 Potential factors involved in the pathogenesis of colorectal carcinoma (CRC) in acromegaly. Chronic exposure to high IGF-1 levels seems to be the most important.

Some authors have described increased risk for benign and malignant tumors in digestive tract in acromegalics, and they found that the odds ratio for the presence of hyperplastic polyps was 8.3, for adenomas 4.2 and for colon carcinomas 9.8, showing an association with higher serum GH levels (12).

A recent study performed in a cohort of Japanese acromegalic patients has shown that increased mean area under the curve (AUC) for GH was associated with an increased risk for colon adenocarcinomas (13).

An attractive explanation for the increased risk of CRC in acromegaly has been the link to IGF-1. Plasma GH triggers the production of IGF-1 from the liver, which in turn stimulates the growth of organs and tissues, through its known mitogenic and antiapoptotic properties. Any imbalance in the tight control between epithelial cell turn-over and cell death could result in epithelial hyperproliferation, promoting the formation of hyperplastic polyps and colorectal adenomas (14). IGF-1 receptors, as well as IGF-1 mRNA, have been identified in human CRC cell lines (15).

IGF-1 can stimulate growth of CRC cells *in vitro*, whereas the blockade of its effect by the alpha IR3, a neutralizing monoclonal antibody against the human IGF-1 receptor, inhibits cell growth in the same model (15,16). Cats *et al.* (17) reported that patients with acromegaly had an increased proliferation index of colonic epithelium proportional to their circulating IGF-1 levels. A recent study found that elevated levels of serum IGF-1 are associated with increased proliferation in the superficial crypt cells and stronger immunostaining to Ki67 in colonic epithelial cells (18). These results suggest that colonic neoplasia in acromegaly would result from increased proliferation rather than deceased apoptosis (18).

The role of chronic exposure to elevated IGF-1 levels and cancer development involves several hypotheses. Cohen *et al.* (19) discussed three important mechanisms. First, an effect of IGF-1 causing symptomatic benign tissue hyperplasia may result in an ascertainment bias leading to an initiation of procedures resulting in the diagnosis of asymptomatic cancers. Second, elevated serum IGF-1 in cancer patients may originate within the tumor (as suggested by some animal studies). Thirdly, serum IGF-1 may actually be a surrogate marker of tissue IGF-1 levels or of nutritional factors, which are not under GH control and may be involved in cancer initiation (19).

Studies on the IGF-1 signal transduction pathways have suggested that the IGF-1 receptor and the activation of tyrosine kinase may be a potential substrate for steroid receptor coactivator (SRC) oncogenes and may be associated to the mechanisms of dedifferentiation (15,18,19).

IGF binding protein-3 (IGFBP-3) regulates the bioavailability of IGF-1 an IGF-2 and has both anti-proliferative and pro-apoptotic properties (7,20). Elevated plasma IGFBP-3 has therefore been associated with reduced risk of CRC. By contrast, excess GH causes an elevated IGF-I to IGFBP-3 ratio, which is expected to increase cancer risk (15,20). Increased circulating levels of IGF-2 and IGFBP-2 are also believed to play a role in the pathogenesis of colonic neoplasms in acromegaly (15,16).

Metabolic disorders

Factors such as hyperinsulinaemia, diabetes mellitus, altered acid bile secretion, altered local immune response, increased large bowel length and/or altered bowel transit times could also contribute to an adenoma occurrence/recurrence in patients with acromegaly (7,15).

In vivo experimental studies (21,22) demonstrated growth-promoting effects of exogenous insulin, dietary-induced hyperinsulinemia, and hypertriglyceridemia on colon cancer and aberrant crypt foci, a putative precursor of colon cancer. Moreover, insulin has been shown to increase the growth of colon epithelial and carcinoma cells *in vitro* (23).

It has also been suggested that insulin may promote colorectal carcinogenesis directly by activating its own receptor, the receptors for IGF-1, or hybrid insulin/IGF-1 receptors (24), all of which are expressed by colorectal epithelial and carcinoma cells (25). In addition, chronic hyperinsulinemia may indirectly promote colorectal carcinogenesis by inducing pathophysiologic changes in concentrations of circulating IGF-1 and IGF binding proteins (IGFBPs) (20,26).

Recent prospective observational studies (27,28) have shown that colorectal adenomas and cancer are positively, albeit moderately, associated with type 2 diabetes. Accordingly, there have been some reports that hyperglycemia is associated with an increased risk of CRC (29,30). These results have led to the suggestion that hyperinsulinemia might underlie the link between type 2 diabetes and CRC (7,15). Indeed, both cross-sectional and prospective population studies have found that CRC is more common in people with hyperinsulinemia and its metabolic correlates, including type 2 diabetes and hypertriglyceridemia (27,28). The study by Colao *et al*. (31) suggested that increase in fasting insulin levels is associated with an 8.6- to 14.8-fold increased risk of presenting with colonic adenomas in acromegaly. Diabetes or impaired glucose tolerance was also a risk factor for the development of colonic lesions (31). However, in another Italian study, fasting insulin, 25(OH)-D3, folate, and homocysteine levels did not differ in acromegaly patients with or without colonic adenomas (32).

Epidemiological studies have revealed that low serum 25(OH) D levels, i.e., vitamin D deficiency/insufficiency, are associated with higher incidence in colon cancer, which is associated with poor prognosis (33,34). The protective role of vitamin D3 against cancer has been attributed to its influence of on cell proliferation, differentiation, apoptosis, DNA repair mechanisms, inflammation and immune function (33,34). However, clinical studies so far have not demonstrated any effects of vitamin D supplementation on cancer incidence or prognosis (11,33).

Genetic factors

The investigation of cancer-related proteins may identify protein biomarkers or therapeutic targets. A recent study described the proteogenomic characterization of human colon and rectal cancers, and highlighted potential candidates at chromosome 20, including HNF4A (hepatocyte nuclear factor 4), TOMM34 (translocase of outer mitochondrial membrane 34) and SRC proto-oncogene (35).

Other authors have suggested the association of CRC and chromosomal instability and using SNP microarrays (36).

In acromegalic patients, few studies have been performed to associate polymorphisms or gene mutations and colorectal tumors (CRT). A recent study evaluated the polymorphism of C677T in methylenetetrahydrofolate reductase (MTHFR) gene, which is a well-documented risk factor for CRT in the general population. It was found that patients with TT genotype showed a 2.4 higher odd ration for CRT (95 % CI, 0.484-11.891; P = NS) than C-allele carriers among patients with low plasma folate levels (37).

The association of the Ser326Cys polymorphism in the 8-oxoguanine glycosylase (OGG1) gene with a colon carcinoma and diabetes mellitus has been examined and results suggest that the Cys allele may influence the colon polyp risk in acromegalic patients (38).

Germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene, known to be a tumor

Table 1 Potential genetic disorders involved in the development of colorectal cancer and polyps in acromegaly

Phenotype	Genetic disorder	Gene	Odds ratio	Reference
Colon cancer	C677T polymorphism	Methylenetetrahydrofolate reductase (MTHFR)	2.4	Torre *et al*. (37)
Polyps	Ser326Cys polymorphism	8-oxoguanine glycosylase 1 (OGG1)	2.1	Zengi *et al*. (38)
Colon cancer	Missense R16H (47G > A)	Aryl hydrocarbon receptor interacting protein (AIP)	Not available	Georgitsi *et al*. (40)

suppressor gene, are related to pituitary adenoma predisposition, and may be involved in the pathogenesis of prolactin (PRL) or GH over secreting pituitary adenomas (39). However, somatic AIP mutations are not common in colorectal, breast, and prostate cancers (40). Indeed, among the 52 CRCs samples initially screened, a heterozygous missense change, R16H (47G > A) in exon 1 was detected in two samples (40).

Potential genetic disorders involved in the occurrence of CRC and polyps in patients with acromegaly are summarized in *Table 1*.

Epidemiologic findings

Acromegalic patients may be at an increased risk for malignancies in several systems including the thyroid, digestive tract, brain, kidney, breast and prostate (8,26-30). CRC incidence (2,6-10,41-44) and mortality rates (6,7,43) have been reported to be higher in acromegalics than expected. However, reported relative risks of CRC vary significantly depending on the study population and the study design. Moreover, the reported higher indices of colorectal neoplasia in acromegalics have not been a universal finding (45-47).

Several studies have demonstrated that in acromegalic patients there is a considerable incidence of colonic neoplasms, included the CRC (48-56). Among the first studies published on these topic, two may be highlighted. The first one found an incidence of colonic neoplasms of 41%, among which 29% of adenomatous polyps and 12% of CRC (48). Ituarte *et al*. (49) demonstrated that in a total of 33 acromegalics, 12 were submitted to a colonoscopy. Among the colonoscopy findings, three patients presented adenomatous polyps and three had colon cancer (49).

These alarming results raised various posterior studies about the incidence of the colonic neoplasia in acromegaly (6,13,31,37,47,50-56). These findings are summarized in *Table 2*. Overall, the prevalence of CRC ranged from 1.07% to 20%. In the series by Renehan *et al*. (47), of the 115 patients with complete examinations, adenocarcinomas were discovered in 3 (2.6%), and at least 1 adenoma was found in 11, giving an overall prevalence of neoplasia of 12% (14 of 115). Prevalence rates for age bands 30-40, 40-49, 50-59, 60-69, and 70+ yr were 0%, 8%, 12%, 20%, and 21%, respectively. Compared with the two control models, the prevalence of occult CRC was not significantly increased (acromegalics *vs*. models 1 and 2, 2.6% *vs*. 2.3% and 0.9%), nor was there an increase in the prevalence of adenomas in any age band. Pathological characteristics showed some differences, in that adenomas in acromegalics tended to be right sided (68% *vs*. 57% and 56%), larger (for ≥10 mm, 27% *vs*. 13% and 9%), and of advanced histology (for tubulovillous, 27% *vs*. 4% and 22%) (47).

A retrospective chart analysis was performed on 140 patients with active acromegaly who had attended a single Japanese institute and confirmed that patients with acromegaly have an increased risk of colon cancer and polyps (9). Indeed, colon cancer was found in 10 patients, thyroid cancer in 5, breast cancer in 4 and gastric cancer in 2. When compared with the local population, the SIRs for thyroid cancer in patients with acromegaly were 61.74 [95% confidence interval (CI), 0.51-114.63] for females and 272.4 (95% CI, 29.12-876.71) for males. The SIRs for colon cancer in the acromegalic patients were 17.4 (95% CI, 4.74-44.55) for females and 19.0 (95% CI, 5.18-48.64) for male patients in comparison with the local population. Of the benign tumors, multinodular goiter and colonic, gastric and gallbladder polyps were observed in 57% (47/83), 40% (35/87), 23% (10/43), and 14% (11/77) of the patients, respectively (9).

In the meta-analysis done by Rokkas *et al*. (57), data from 701 patients with acromegaly and 1,573 controls were gathered. The pooled results of this study clearly showed that acromegalic patients are at a significantly increased risk of developing colorectal adenomatous and hyperplastic polyps as well as CRC compared with controls. They also highlighted the true increased prevalence of colon cancer compared to adenoma in acromegaly (57). These findings

Table 2 Epidemiological findings of colon cancer in acromegaly

Authors (Ref) [year]	Country	Number of patients	Median age	% male in the study	% male with cancer	Control group	Adenomatous polyps prevalence (%)	Cancer prevalence (%)
Klein *et al*. (48) [1982]	USA	17	49	–	–	No	29.0	12.0
Ituarte *et al*. (49) [1984]	USA	12	56	–	–	No	15.0	20.0
Brunner *et al*. (50) [1990]	USA	29	–	–	–	Yes	14.0	6.9
Jenkins *et al*. (51) [1997]	United Kingdom	155	63	–	–	No	26.0	6.5
Orme *et al*. (6) [1998]	United Kingdom	1,362	–	–	–	No	–	1.3
Renehan *et al*. (47) [2000]	United Kingdom	115	54.8	56.6	33.3	Yes	12.2	2.6
Mestron *et al*. (53) [2004]	Spain	1,219	45	39.2	–	Yes	9.5	1.2
Terzolo *et al*. (52) [2005]	Italy	235	48.9	49.1	46.1	Yes	23.0	4.3
Bogazzi *et al*. (56) [2006]	Italy	82	56	41.4	–	Yes	32.9	3.7
Colao *et al*. (31) [2007]	Italy	210	44	47.1	83.3	No	20.0	2.8
Kurimoto *et al*. (9) [2008]	Japan	87	–	–	50.0	No	40.0	11.5
Baldys-Waligórksha *et al*. (54) [2010]	Poland	101	51.8	30.0	–	No	13.0	2.0
Vallette *et al*. (55) [2013]	Canada	649	45	50.7	–	No	–	1.1
Torre *et al*. (37) [2014]	Italy	51	50	34.8	100.0	No	44.0	2.6
Yamamoto *et al*. (13) [2014]	Japan	57	50. 3	42.1	–	Yes	31.6	5.3

might support the hypothesis of an increased risk of malignant transformation in acromegaly.

Analysis of prospective colonoscopic screening studies involving almost 700 subjects with acromegaly has shown a 2.4-fold increased risk of colonic adenomas and a 7.4-fold greater risk of cancer with an overall prevalence of CRC of 3.7 % (58,59).

Among consecutive 57 acromegalic patients who had undergone full-length colonoscopy at the time of diagnosis, 22 (38.6%), 18 (31.6%) and 3 (5.3%) patients were diagnosed with hyperplastic polyps, adenomas, and adenocarcinomas, respectively and the prevalence was significantly higher than in a historical control group, Chinese patients with irritable bowel syndrome (the odds ratio was 4.0, 8.7, and 17.5, respectively) (13). The prevalence of adenocarcinomas was also significantly higher in these patients than in the general Japanese population (odds ratio 14.5). Patients with acromegaly who had colorectal neoplasms had longer disease duration than those without colorectal neoplasms (13).

In the study by Wassenaar *et al*. (60), colonic diverticula were present in 37% of patients, dolichocolon in 34%, and adenomatous polyps in 34%, which was increased compared with controls (odds ratio 3.6, 95% CI, 1.4-5.7; 12.4, 95% CI, 6.8-18.0; 4.1, 95% CI, 1.9-6.4, respectively).

By contrast, two studies have failed to demonstrate an increased prevalence of neoplasia in acromegaly (45,47). In both studies patients were predominantly younger and colonoscopy was incomplete with caecal intubation rate of 70% (45,47).

Acrochordons (skin tags) are markers for the colonic lesions and have been found in most patients harboring these lesions (2,8).

Colorectal cancer (CRC) screening in acromegaly

Why to screen?

CRC is the third most frequent cancer in men, after lung and prostate cancer, and is the second most frequent cancer in women after breast cancer (11,61). It is also the third cause of death in men and women separately, and

is the second most frequent cause of death by cancer if both genders are considered together (61,62). In 2014, an estimated 71,830 men and 65,000 women will be diagnosed with CRC and 26,270 men and 24,040 women will die of the disease in USA (62). CRC accounts for approximately 10% of deaths by cancer (11).

Several studies have shown an increased prevalence of pre-cancerous and cancerous colonic lesions in patients with acromegaly compared to the general population (irrespective of diet, age of onset, disease duration and ethnicity, increased propensity for malignant transformation, and worse case prognosis of CRC) (7,15,41-44,48-53).

In non-acromegalic individuals, the majority of colon cancers develop as a result of multi-step malignant transformation of benign adenomatous colonic polyps, which takes approximately 10-15 years (15). The onset of GH hypersecretion is difficult to ascertain, but usually precedes the diagnosis of acromegaly by at least 7 to 10 years (1,2,7). Therefore, one may speculated that if acromegaly is associated with increased incidence of colon polyps, there is ample time for premalignant lesions to transform into a cancer (15).

A large retrospective cohort study (n=1,362) has shown that overall cancer mortality was not increased but patients with acromegaly through concurrent colon cancer had nearly a 2.5-fold higher colon cancer specific mortality rate compared to the general population [standardized mortality rate (SMR) =2.47] (6).

Data from St. Bartholomew's Hospital, in London, demonstrated that patients with an initial adenoma at the initial screening had a 4.4 and 8.8 fold increased risk of developing a new adenoma at the second and the third colonoscopy respectively, while patients with a normal initial colonoscopy and elevated IGF-1 level had 7.5-fold risk of a subsequent adenoma compared to those with a normal colonoscopy at the initial screening and inactive disease (15,58). Notably, despite a normal baseline colonoscopy 50-100% of patients went on to have adenoma detected at interval colonoscopies. Of all patients who had an adenoma at the second, third and fourth colonoscopy, 50%, 75% and 100% respectively had an adenoma at the initial colonoscopy implying that 50% and 25% of patients with new polyps at the second and third colonoscopy respectively, had a normal initial colonoscopy (15,58,59). These findings strongly support an evidence base for a regular surveillance programme in all patients with acromegaly, irrespectively of the findings from the initial colonoscopy (15).

By contrast, Bogazzi *et al.* (56) have shown that if colonic adenomas were not present initially, it was unlikely that they develop thereafter, regardless the metabolic control of acromegaly. Conversely, new lesions were frequent (and often multiple) in patients who already had colonic adenomas at baseline, particularly if acromegalic disease was poorly controlled by treatment (56).

How to screen?

Fecal occult blood testing (FOBT) is the most common mass screening test for CRC (63,64). It is a simple, cheap and safe laboratory test that relies on the assumption that asymptomatic CRC and large adenomas may bleed (63,64). False negative results may be due to incorrect storage of sample or drug assumption, whereas hemorrhoids, diet and medications are causes of false positive results (63-65).

Other screening tests, such as optical colonoscopy (OC) and computed tomography colonography (CTC) are highly accurate for examining the entire colon for adenomas and CRC (63,64). OC is widely accepted as the gold standard procedure for detection of colorectal neoplasia, and there are indirect data showing that this strategy may contribute to a 76% to 90% decrease of the incidence for CRC (15,63,64). Moreover, screening with OC in selected cohorts of subjects by detection and removal of most advanced adenomas could allow long screening intervals (15,63,64).

Colonoscopy was shown to be superior to FOBT in detecting colonic lesions at the first diagnosis of acromegaly. In the study by Bogazzi *et al.* (66) FOBT, which was positive in 16 (18.8%) out of 85 patients, identified 2 patients with colonic adenocarcinoma and 2 with adenoma; the remaining 12 patients had no detectable colonic lesions. Colonoscopy revealed colonic lesions in 29 patients: 3 (3.5%) cancers, 11 (12.9%) adenomas, and 15 (17.6%) hyperplastic polyps. The remaining 56 acromegalic patients had no detectable lesions. A patient with cancer and 9 patients with adenoma were missed if screened only by FOBT (66).

Unlike the general population, 25% of adenomas and 50% of carcinomas seem to occur in the ascending and transverse colon in patients with acromegaly, therefore a total colonoscopy is required rather than sigmoidoscopy or limited colonoscopy (15,47,57).

The major disadvantages of OC as a screening test are its complications, including bleeding and perforation, and the discomfort due to both full bowel preparation and the procedure itself (63,67,68). Moreover, there are some technical challenges for colonoscopy in acromegalic

patients. Indeed, colonic transit time in these subjects is more than twice that of normal subjects, so that standard bowel preparation is often inadequate leading to suboptimal assessment (52,69,70). Furthermore, the increased bowel length and the intestinal loop complexity seen in acromegalic patients may lead to higher levels of technical difficulties and increase the risks of complications at conventional colonoscopy (15,70,71). Finally, the estimate death rate associated with the colonoscopic procedure in acromegalic patients can be as high as 1 in 2,898 exams (1 in 10,000 for the general population) (72,73).

An alternative procedure to OC is the CTC, also named virtual colonoscopy, whose main disadvantages are the fact that it does not allow polyp resection or biopsy, and delivers a significant amount of radiation therefore unsuitable for a screening programme (15,63). However, it is a safe and very accurate procedure (74,75). A review and meta-analysis assessing the sensitivity of both CTC colonography and OC for CRC detection found that primary CT colonography may be more suitable than OC for initial investigation of suspected CRC (76). Nevertheless, according to most experts, CTC should be reserved for patients with incomplete or unfeasible colonoscopy (15,63,77).

In the study by Ramos *et al*. (74), which evaluated 21 acromegalic patients, CTC showed 88% sensitivity, 75% specificity and 81% accuracy in detection of colonic polyps. This procedure was performed without complications and a complete and safe colorectal evaluation was possible in all acromegalic patients (74). Similar results were reported by Resmini *et al*. (78).

Other new technology such as colon capsule endoscopy may aid endoscopists in the challenge of completing the evaluation of the colon in those patients with an incomplete colonoscopy (63). Finally, there have been large studies which examine the performance characteristics of the so-called non-invasive CRC screening tests such as fecal immunochemical test (FIT) and fecal DNA (11,63). The performance of these new technologies in acromegalic patients has not yet been demonstrated.

When to screen?

Repeated colonoscopic screening of patients with acromegaly has demonstrated that they are at high risk of developing a new colonic neoplasia, especially in those with an adenoma at the initial screening and/or who have uncontrolled disease with persistently abnormal GH and IGF-1 levels (15,52,58). Furthermore, acromegalic patients are at an increased risk of malignant transformation of benign adenomatous colon polyps to CRC, which then reaches a higher mortality rate compared to the general population (15,57). For all these reasons, the guidelines from different institutions and societies recommend early colonoscopic screening starting at the time of diagnosis (or at the age of 40 years considering this is the mean age at diagnosis) (15).

The most commonly referenced guidelines for colonoscopic screening and surveillance in patients with acromegaly are those published by the Acromegaly Consensus Group (ACG) in 2009 (47), a group of experts from the St. Bartholomew's Hospital (Barts) in 2010 (79), the British Society of Gastroenterology (BSG) in 2010 (80), the American Association of Clinical Endocrinologists (AACE) in 2011 (5) and the Pituitary Society in 2013 (1). According to Barts and the BSG guidelines colonoscopic surveillance should be commenced at the age of 40 (47,80). The ACG, the Pituitary Society and AACE state, however, that the baseline colonoscopy should be performed at the time of acromegaly diagnosis (1,5,47). If a patient has normal findings on initial colonoscopy and normal IGF-1 levels, all guidelines but those from ACG recommend that further colonoscopies should be performed every 10 years (every 5-10 years for ACG). However, if baseline or subsequent surveillance colonoscopy reveals the presence of an adenoma, Barts, the Pituitary Society and the AACE recommend 5-yearly surveillance, BSG recommend 3-yearly colonoscopy while the ACG guidelines recommend further colonoscopies every 3-5 years depending of the number and size of adenoma (1,5,47,79,80). Recently, these guidelines were elegantly reviewed by Lois *et al*. (15). The current recommendations for surveillance colonoscopy in acromegaly are summarized in *Table 3*.

Management and prevention

The options for the management of CRC include surgery, chemotherapy and radiotherapy, and they do not differ in patients with or without acromegaly. CRC largely can be prevented by the early detection and removal of adenomatous polyps (11). Indeed, several cohort studies demonstrate that polyps removal lowers the incidence of CRC by 76-90% (11).

As the incidence of polyps and CRC is higher in patients with active acromegaly, normalization of IGF-1 levels, regardless the kind of treatment (surgery or medical therapy), is always beneficial (7,8,51,57,58).

Table 3 Current recommendations for surveillance colonoscopy in subjects with acromegaly

	Barts guidelines, 2010 (79)	ACG guidelines, 2009 (47)	BSG guidelines, 2010 (80)	AACE guidelines, 2011 (5)	Pituitary Society guidelines, 2013 (1)
Age at initial colonoscopy	40 yrs	At the time of diagnosis	40 yrs	At the time of diagnosis	At the time of diagnosis
Normal initial colonoscopy and normal IGF-1	Every 10 yrs	Every 10 yrs	Every 5-10 yrs	Every 10 yrs	Every 10 yrs
Adenoma(s) in the initial colonoscopy and/or elevated IGF-1	Every 5 yrs	Every 3-5 yrs depending on the number and size of adenomas*	Every 3 yrs	Every 5 yrs	Every 5 yrs

*, no specific recommendations were made depending on IGF-1 levels; Barts, St. Bartholomew's Hospital; BSG, British Society of Gastroenterology; AACE, American Association of Clinical Endocrinologists; ACG, Acromegaly Consensus Group. Adapted from Lois *et al*. (15).

The role of aspirin in the prevention of the development of colonic neoplasms in acromegaly has not yet been fully evaluated. However, the results of a Cochrane review, which included three randomized control trials (RCTs), showed that aspirin (acetylsalicylic acid) significantly lowers the recurrence of adenomas after a three-year follow-up in the general population (RR =0.77; 95% CI, 0.61-0.96) (81). Moreover, the joint analysis of the British doctors aspirin trial and the UK-TIA aspirin trial indicates that taking aspirin in doses of ≥300 mg/day for at least five years is an effective primary prevention method against CRC with a 10-year latency period (82). Although the pharmacological mode of aspirin action is unclear, inhibition of COX-1 and/or COX-2 is most likely involved (11,82).

Conclusions

Patients with acromegaly are at high risk for benign and malignant colonic neoplasms (6,7,58,59). Furthermore, these patients are at an increased risk of malignant transformation of benign adenomatous colon polyps to CRC, whose mortality rate in higher than that seen in the general population (6,57). Therefore, guidelines from different institutions and societies recommend early colonoscopic screening starting at the time of diagnosis (or at the age of 40 years, considering this is the mean age at diagnosis). Interval colonic surveillance depends on the findings from the baseline colonoscopy and on IGF-1 levels. Firm evidence and outcome based confirmation of the best approach is still lacking (15).

The mechanisms involved in cancer development and progression in acromegalic patients are still unclear. Chronic exposure to elevated IGF-1 levels seems to be the most important (15,47,56,58). In addition, hyperinsulinemia, diabetes mellitus, altered acid bile secretion, altered local immune response, increased large bowel length and/or altered bowel transit times, and genetic factors could also play a role (7,15,31).

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Footnote

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Evolution of imaging in rectal cancer: multimodality imaging with MDCT, MRI, and PET

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Abstract: Magnetic resonance imaging (MRI), multidetector computed tomography (MDCT), and positron emission tomography (PET) are complementary imaging modalities in the preoperative staging of patients with rectal cancer, and each offers their own individual strengths and weaknesses. MRI is the best available radiologic modality for the local staging of rectal cancers, and can play an important role in accurately distinguishing which patients should receive preoperative chemoradiation prior to total mesorectal excision. Alternatively, both MDCT and PET are considered primary modalities when performing preoperative distant staging, but are limited in their ability to locally stage rectal malignancies. This review details the role of each of these three modalities in rectal cancer staging, and how the three imaging modalities can be used in conjunction.

Keywords: Rectal cancer; staging; magnetic resonance imaging (MRI); computed tomography (CT); positron emission tomography (PET)

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Introduction

Colorectal cancer is incredibly common, representing the 4th leading cause of cancer mortality and the 2nd most common malignancy worldwide, with nearly 1 million newly diagnosed colorectal cancers each year (1,2). Of all colorectal cancers, rectal cancer comprises over 1/3 of cases, with over 40% arising within 6 cm of the anal verge (1,3). While there is little doubt that colonoscopy and biopsy are, and will remain for the foreseeable future, the gold standard modalities for the initial diagnosis of rectal cancer, traditional radiologic imaging modalities are of vital importance with regard to the local staging of patients with a known diagnosis and the identification of distant metastatic disease (i.e., distant staging).

The importance of diagnostic imaging in accurate distant staging is beyond doubt, with multidetector computed tomography (MDCT), magnetic resonance imaging (MRI), and positron emission tomography (PET) all offering valuable means of identifying tumor spread to the liver, lungs, and distant lymph nodes; the three most common sites of distant metastatic disease (2,4). Traditionally, metastatic colorectal cancer at presentation has been treated solely with chemotherapy, although it is increasingly thought that this patient population might also benefit from local resection of their tumor, with associated increased quality of life measures and longer survival (even despite the presence of distant metastases), and in some cases, resection of metastases (particularly to the liver or lungs) may also be a feasible option. Accordingly, the identification of distant metastatic disease has a profound impact on the management algorithm employed for this group of patients, making accurate distant radiologic staging vital (4,5). However, local staging has become equally critical in patient management, particularly given the increasing incorporation of neoadjuvant chemoradiation into treatment protocols. More specifically, while the increasing adoption of total mesorectal excision (i.e., 'en-bloc' resection of the mesorectum) has significantly reduced the incidence of post-operative local recurrence within the surgical bed

(once as high as 38%), locally advanced tumors are still far more likely to recur, and these locally advanced tumors are increasingly being treated with preoperative radiation and chemotherapy prior to total mesorectal excision, requiring radiology to be accurate in determining the local extension of tumors (T-stage), the relationship of a tumor to the mesorectal fascia, and the presence of suspicious locoregional lymph nodes (N-stage) (6). This review will describe the role of the three most important radiologic modalities in the local and distant staging of rectal cancer, namely MDCT, MRI, and PET or PET-CT, all of which serve complementary roles in the initial accurate staging of patients.

Magnetic resonance imaging (MRI)

Local staging

Technique

From a technique standpoint, while the protocols utilized in rectal MRI will vary slightly from institution to institution, high resolution T2 weighted images (with a slice thickness of 3 mm) with a small field of view (FOV) focusing on the rectum are the most critical to accurate diagnosis, as they provide the best means of evaluating the rectal wall and perirectal fat (allowing optimal discrimination of T2 from T3 tumors), and should be acquired in the axial, sagittal, and coronal planes. While the radiologist may choose to primarily focus on the axial images, the coronal and sagittal images become increasingly important when confronted by an infiltrative tumor involving larger portions of the rectum, or an excessively tortuous rectum. In addition, the coronal plane tends to be the most useful for establishing the relationship of a tumor with the internal and external anal sphincters, as tumoral involvement of the sphincter complex could potentially necessitate the performance of an abdominoperineal resection with en bloc resection of the sphincter complex. While the small FOV high-resolution T2 weighted images are the most important imaging sequences, most protocols will incorporate larger FOV T2 weighted images of the pelvis and pre- and post-gadolinium 3-dimensional fast spoiled gradient echo sequence (FSPGR) images to evaluate for the presence of pelvic lymphadenopathy (outside of the mesorectum) and to identify other salient pelvic abnormalities. Moreover, while the T2 weighted images are the most important to evaluate the tumor itself and its relationship with the rectal wall and mesorectal fat, the post-gadolinium images may be helpful in some select cases. In addition, diffusion weighting imaging (DWI) has increasingly been incorporated into these protocols, and can serve as a means for accentuating the primary tumor and locoregional lymph nodes. While DWI and post-gadolinium images are not absolutely critical for evaluation of the primary tumor, most rectal cancers will enhance avidly and demonstrate restricted diffusion (3,6).

Typically, the rectum will be 'cleansed' prior to the study using a standard preparation of sodium bisphosphonate or a sodium phosphate enema, in order to avoid fecal material interfering with study interpretation. Subsequently, many practices will administer a small volume (usually 60 cc) of a rectal contrast agent, which can either be ultrasound gel (a 'positive' contrast agent that is T2 hyperintense) or a mixture of barium sulfate and ferumoxsil (a 'negative' contrast agent that is T2 hypointense). These agents can help accentuate small or polyploid tumors that might be difficult to identify without adequate rectal distension, particularly in tumors that are higher in the rectum. Some, but not all, practices utilize a bowel paralytic such as glucagon, which can reduce artifacts related to bowel motion (3,6). The utilization of an endorectal coil has increasingly decreased, particularly as positioning of the coil can be problematic in higher rectal tumors, as well as those lesions that cause significant narrowing of the rectum, and moreover, it places limits on the field of view that may hinder complete assessment of a tumor's involvement of the mesorectal fascia and slightly more distant mesorectal lymph nodes (7). While the use of an endorectal coil was originally advocated in the belief that it offered improved image quality and signal to noise ratio (SNR), there is very little evidence that the endorectal coil offers any substantial benefit over a standard phased array coil in terms of diagnostic quality.

T-stage and local tumor extension

A T1 tumor extends through the muscularis mucosa and into the submucosa, while a T2 tumor extends through the submucosa into the muscularis propria. In most cases, these two T-stages are treated equivalently, without the addition of preoperative chemotherapy or radiation, and distinguishing T1 and T2 tumors is not possible on MRI with a high degree of accuracy given that the submucosa and muscularis propria of the rectal wall cannot be consistently differentiated on MRI (8). However, T3 tumors (which extend beyond the muscularis propria) have been shown to have better outcomes (with a lesser risk of local recurrence) when treated with preoperative chemoradiation and these lesions can be distinguished from

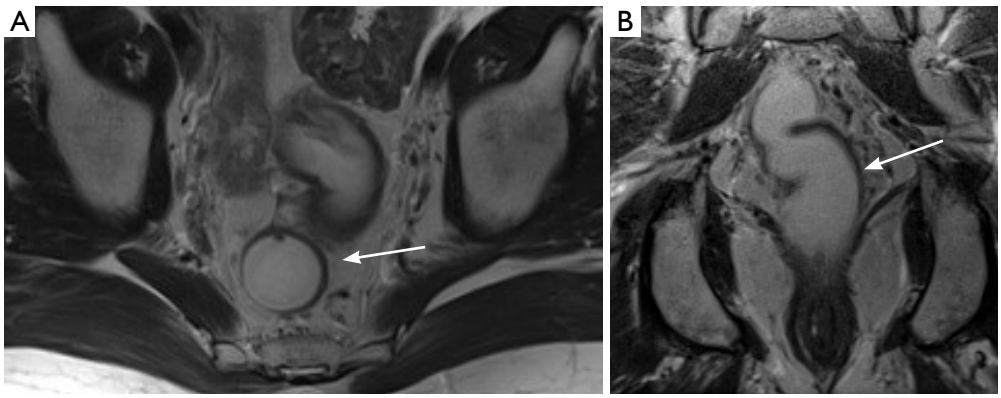

Figure 1 Normal appearance of the rectum on T2 weighted images. In both images, there is a clearly defined, T2 hypointense line (arrow) around the margins of the rectum, representing the intact muscularis propria.

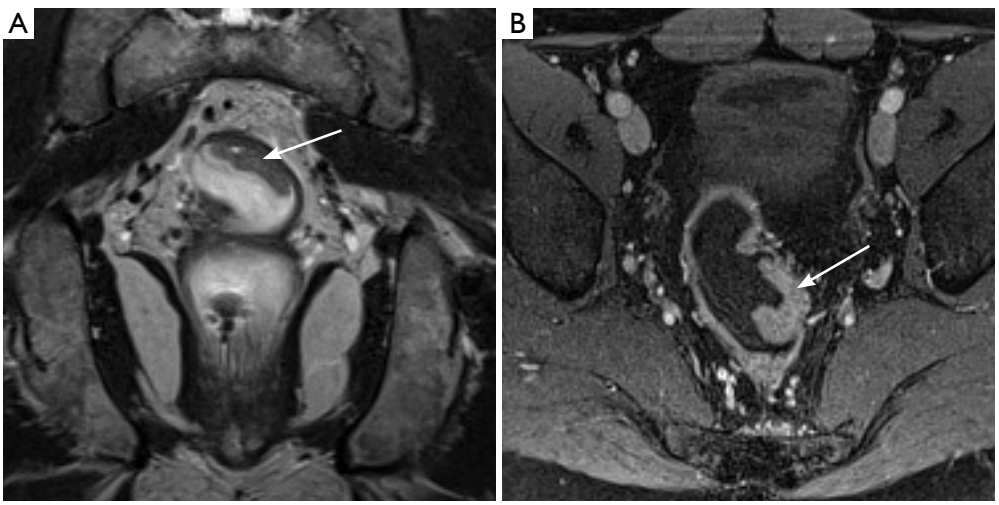

Figure 2 Example of a T2N0 rectal cancer. Coronal (A) T2 weighted image demonstrates a small polyploid mass (arrow) arising from the wall of the rectum. Importantly, the overlying hypointense line demarcating the muscularis propria remains intact, suggesting this is not a T3 lesion. Axial post-gadolinium image (B) nicely demarcates the mass (arrow), although evaluating extension through the muscularis is not possible on this sequence.

T1 and T2 tumors on MRI. T4 tumors are characterized by their spread into the visceral peritoneum, adjacent organs, or the levator musculature (3,6). On MRI, the three layers of the rectal wall are usually clearly discernible on T2-weighted images, with the mucosa and submucosa appearing relatively hyperintense, the muscularis appearing relatively hypointense in the middle of the wall, and a layer of hyperintense perirectal fat on the outside of the wall. Careful evaluation of the T2 hypointense muscularis throughout the areas abutting the rectal cancer is critical, and this thin hypointense line should be intact and clearly visible throughout the rectum for a tumor to be described as a T1 or T2 lesion. A tumor that has breached the T2 hypointense layer of the rectal wall (i.e., the muscularis is not clearly visualized adjacent to the tumor) can be considered to be at least a T3 tumor, necessitating preoperative chemoradiation (*Figures 1-4*) (3,6).

Once a tumor is characterized as either a T1/T2 or T3 lesion, the extent of involvement of the surrounding mesorectum and the adjacent pelvic structures can also have an important impact on patient prognosis. T3 tumors can be further subdivided into T3a (<5 mm extension beyond the muscularis) and T3b (>5 mm extension beyond the muscularis), and MRI has been shown to be relatively accurate in distinguishing these small differences in involvement. Such a distinction between T3a and T3b

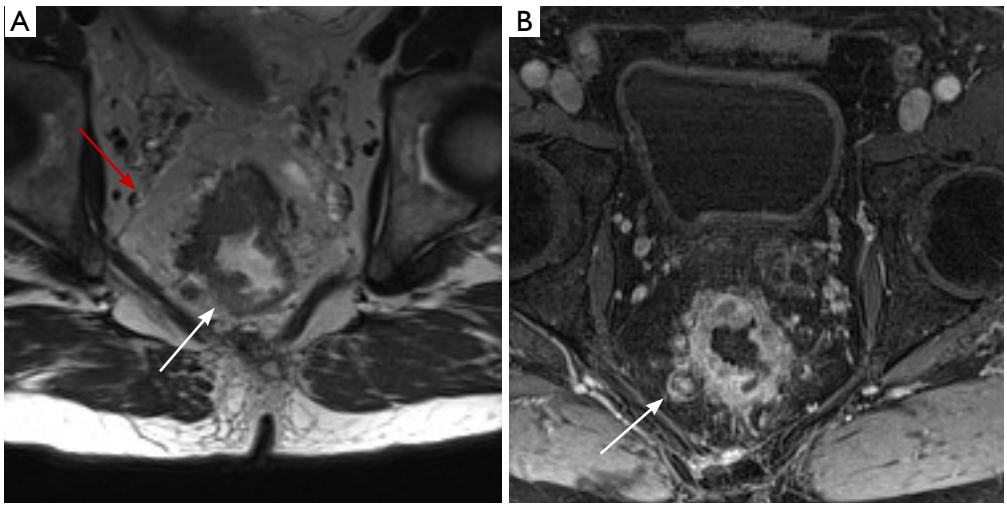

Figure 3 Axial high-resolution T2 weighted image (A) demonstrates circumferential thickening (white arrow) around the entirety of the rectum, in keeping with the patient's malignancy. In this case, the T2 hypointense muscularis is absent underlying the mass, suggesting this represents a T3 malignancy. Red arrow illustrates the intact mesorectal fascia or circumferential resection margin (CRM). Axial post-gadolinium axial image (B) demonstrates a heterogeneously enhancing malignant lymph node (arrow) in the 7 o'clock position.

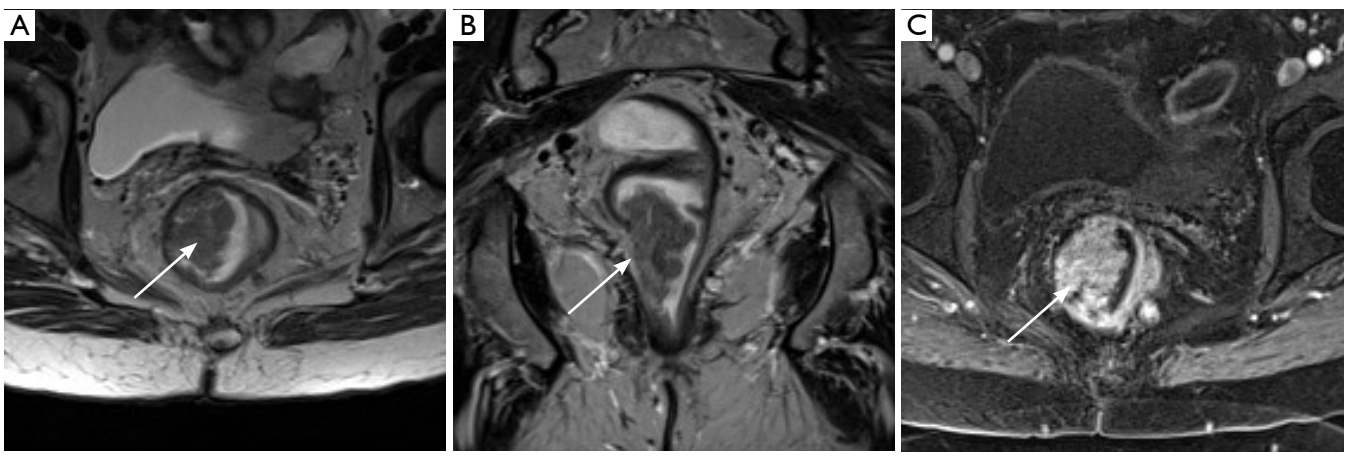

Figure 4 Axial (A) and coronal (B) T2 weighted images demonstrate a polyploid mass (arrow) arising from the right lateral aspect of the rectum, with complete loss of the underlying T2 hypointense muscularis (best visualized on the coronal image), in keeping with a T3 lesion. The mass (arrow) (C) demonstrates avid enhancement on the post-gadolinium image.

tumors may be of clinical importance, as >5 mm extension into the mesorectum has been found to be associated with a significantly lower 5-year survival rate (54% *vs.* 85%) (9). Just as important as the tumor's T-stage, however, is the proximity of the tumor to the margins of the mesorectal fascia (also described as the 'circumferential resection margin' or 'CRM'), as tumors that are 1 mm or less from the mesorectal fascia are at substantially higher risk of local recurrence (*Figure 5*) (8). A tumor's relationship to the fascia is relatively easy to perceive on MRI, but is not usually possible to delineate with endoscopic ultrasound (EUS). Finally, particularly for advanced tumors, MRI offers an accurate means of assessing involvement of adjacent pelvic organs (including the prostate, seminal vesicles, uterus, vagina, etc.), the sacrum, the anal sphincters, the pelvic sidewalls, and adjacent vasculature (*Figure 6*) (3,6).

Locoregional lymph node staging

While the superior soft tissue resolution of MRI does facilitate the identification of local lymph nodes (both in the

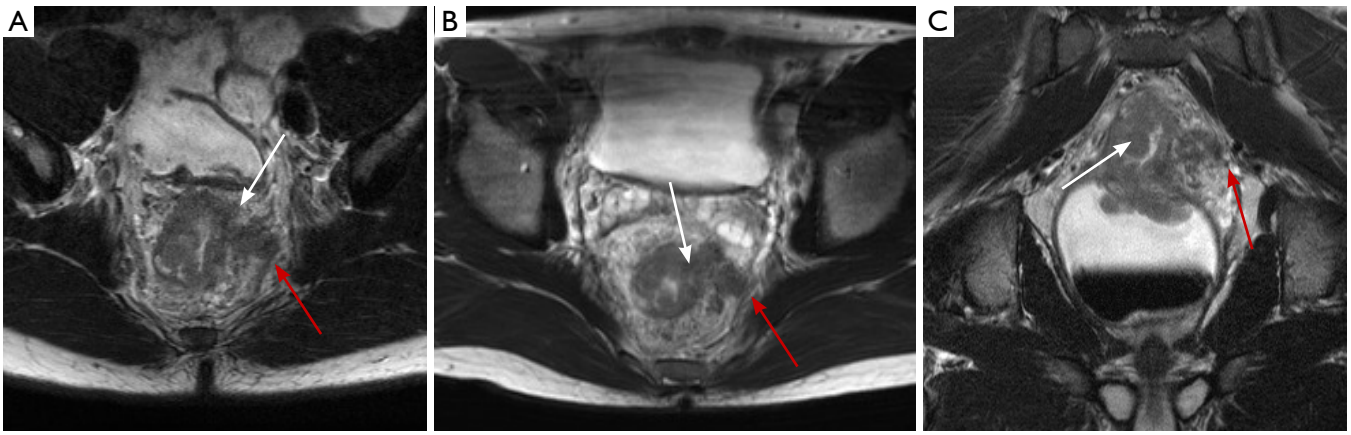

Figure 5 Axial (A,B) and coronal (C) T2 weighted images demonstrate a rectal mass (white arrows) extending through the rectal wall at the 3 o'clock position into the mesorectal fat. In this case, the mass involves the CRM at this position (red arrow).

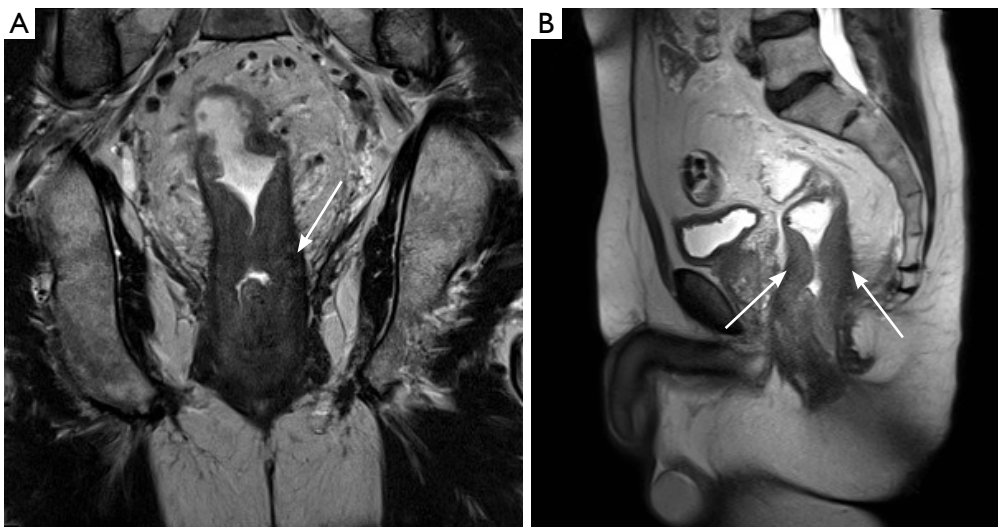

Figure 6 T4 low rectal cancer (arrows) with involvement of both the internal and external sphincters illustrated on coronal (A) and sagittal (B) T2 weighted images.

mesorectum and the pelvis), the ability to discern a benign from a malignant lymph node is still partially based upon size criteria, inherently limiting sensitivity and specificity. The most commonly used size criteria, particularly in the mesorectum, is 5 mm, which provides a sensitivity of only 68% and a specificity of only 78%, as a sizeable number of ultimately metastatic nodes at histopathology measure under 5 mm in size. Morphologic data, including irregular lymph node margins and abnormal signal or enhancement may also be useful ancillary features. The presence of suspicious nodes is important for treatment planning, as mesorectal lymph nodes (which are typically resected with the surgical excision) close to the mesorectal fascia may necessitate wider surgical margins at that site, while lymph nodes outside of the mesorectum (which are not usually resected with the surgical specimen) may necessitate wider radiation, an extended surgical resection, or even upstaging to M1 disease (lymph nodes in the external iliac chains, obdurator chains, or the retroperitoneum) (3,6).

Accuracy of MRI for local staging

There is little doubt that MRI is an accurate modality for establishing the T-stage of a tumor and delineating its relationship with the mesorectal fascia (CRM). A meta-analysis by Al-Sukhni *et al.* in 2012 (10) encompassing 21 different studies found excellent

sensitivities and specificities for establishing involvement of the CRM (up to 77% and 94% respectively), with a slightly lower performance for determining T-stage (87% and 75% respectively). The excellent performance of MRI in evaluating CRM involvement has been consistent across multiple studies in the literature, including a study by the MERCURY study group that found 92% specificity in predicting a negative surgical margin (11-13). However, as one would expect given the limitations of any anatomic imaging modality in evaluating lymph nodes, sensitivities and specificities for lymph node involvement in the study by Al-Sukhni *et al.* were only 77% and 71% respectively (10). While some had hoped that the inclusion of DWI into imaging protocols might help distinguish benign from malignant lymph nodes, this has not turned out to be the case: Metastatic lymph nodes do demonstrate lower mean ADC values, but ADC values have not proven particularly sensitive or specific for metastatic lymphadenopathy (14,15).

When compared to EUS, another modality commonly utilized for local staging, there is little doubt that EUS is superior in distinguishing T0, T1, and T2 tumors, a distinction that is not possible on MRI, and that may be clinically important in a small group of patients who might undergo local resection (T0 or T1 tumor) rather than total mesorectal excision (with a T2 tumor). In general, both modalities are probably relatively similar in their ability to distinguish T1 or T2 tumors from T3 tumors, and both modalities have similar limitations in distinguishing metastatic from benign lymph nodes in the mesorectum (although EUS can likely identify more lymph nodes than MRI given its spatial resolution). MRI can clearly better identify lymph nodes distant from the tumor (including the upper rectum), and the ability to evaluate CRM involvement is clearly an advantage of MRI (8).

Distant staging

In most cases, MDCT represents the best primary option for distant staging of rectal cancer, particularly given the propensity for tumors to metastasize to the lungs (where MRI is highly limited). Moreover, even with regards to evaluation of the liver (usually considered the greatest strength of MRI), in the vast majority of cases the routine preoperative addition of MRI to MDCT is likely to be of little benefit, as a study by Wiggans *et al.* found that the addition of MRI did not make a significant difference in patients with colorectal cancer to lesion detection, recurrence rates, or patient survival (16).

Nevertheless, the primary role of MRI in distant staging is as a trouble-shooting modality when confronted with an indeterminate lesion on MDCT, particularly in the liver. It is not at all uncommon to be confronted with a 'too-small-to characterize' lesion on MDCT measuring under 1 cm in size, which cannot be definitively characterized as either benign (i.e., cyst or hemangiomas) or malignant (i.e., metastasis) (17). Given the superior soft tissue resolution of MRI, as well as the ability to use several imaging sequences in conjunction to arrive at a diagnosis, the specificity of MRI for small liver lesions is superior to MDCT (18). In most cases, metastases will be T1 hypointense and T2 hyperintense (although lower in signal compared to cysts or hemangiomas) and will demonstrate peripheral enhancement. Moreover, the increasing utilization of diffusion weighted images in liver protocols offers another means of both identifying lesions which might not be conspicuous on either CT or standard MRI pulse sequences, as well as the risk stratification of liver lesions (as liver metastases will tend to have lower ADC values) (19,20).

Multidetector computed tomography (MDCT)

Technique

In cases with a known primary rectal malignancy, most institutions employ a single-phase technique, with the acquisition of venous phase images at roughly 60-70 s after the rapid injection of intravenous contrast (3-5 cc/s). In some instances, when seeking to better define subtle abnormal enhancement or delineate a subtle bowel lesion, the incorporation of arterial phase images may have some value in certain select cases (typically at 25-30 s after the injection of IV contrast). Particularly in those cases when the primary tumor has not yet been resected, and there is the intention to evaluate local tumor extension and mesorectal lymphadenopathy, neutral contrast agents (such as VoLumen) are utilized to distend the bowel without creating unnecessary streak or beam-hardening artifacts. Accordingly, positive oral contrast is usually avoided in these cases, as the dense contrast material may obscure subtle abnormalities in the adjacent bowel wall, and streak artifact may preclude accurate identification of small mesorectal lymph nodes (17,21). Given that MDCT is almost never utilized for local tumor staging, rectal contrast administration is not a common component of these imaging protocols, and no attempt is usually made to

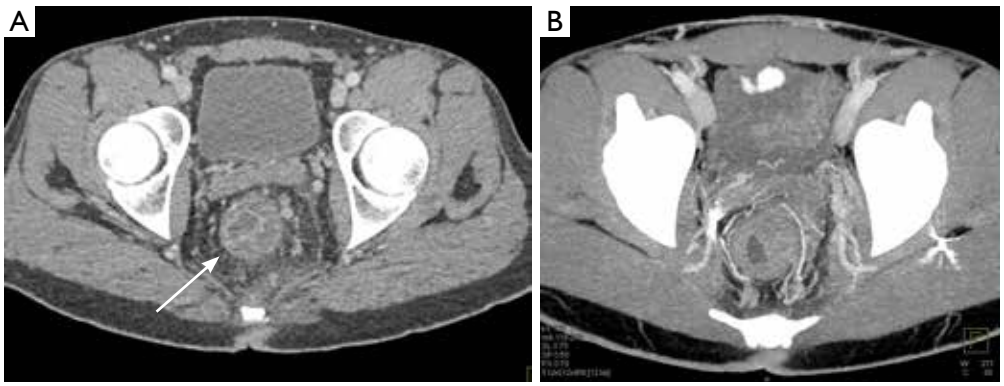

Figure 7 Rectal cancer on MDCT. Axial (A) contrast-enhanced and axial volume rendered (B) images demonstrate severe circumferential wall thickening of the rectum, with neovascularity nicely illustrated on the volume rendered 3-D image. While there is stranding and edema in the mesorectal fat, it is not possible to distinguish tumor invasion into the mesorectum from edema and inflammation. MDCT, multidetector computed tomography.

distend the rectum with contrast material.

The latest generation of MDCT scanners allows the acquisition of thin-section isotropic images, with identical resolutions in the x, y, and z-axes, at 0.5-0.75 mm collimation. These images are reconstructed to 3 mm for routine axial image review, as well as to 0.75 mm for the further generation of multiplanar reformations (coronal and sagittal) and 3-D images. The 3-D reconstructions typically include maximum intensity projection (MIP) images, which highlight the highest attenuation voxels in a dataset and project them into a 2-dimensional (2-D) representation, and volume rendered (VR) images, which utilize a complex computer algorithm to assign colors and transparencies to each voxel in a study based on its attenuation and relationship to other nearby voxels, thus creating a 3-D representation of the data set. These two 3-D techniques can be of great value in allowing the identification of lesions that might otherwise not be visualized on the standard 2-D images, as well as potentially highlight lesion features that might allow a more specific diagnosis (17,21-24).

Local staging

The MDCT appearance of rectal tumors can vary, including circumferential wall thickening, focal mural wall thickening, or a discrete polyploid mass (*Figure* 7). The conspicuity of these tumors can vary significantly depending on rectal distension, and the degree of enhancement can also vary widely. Even with the last generation of MDCT scanners, which have offered dramatic improvements in both spatial and temporal resolution compared to prior generations of technology, the layers of the rectal wall cannot be clearly differentiated in any phase of imaging (whether arterial, venous, or delayed). As such, like MRI, it is impossible to differentiate T0, T1, or T2 tumors. However, the mesorectal fat surrounding a tumor can be clearly visualized on CT, and in those cases where the tumor is seen to directly extend into the perirectal fat, a T3 tumor can be diagnosed. However, this is often confounded by the fact that perirectal fat stranding or induration secondary to rectal inflammation or peritumoral fibrosis cannot be definitively differentiated from tumor extension. Unfortunately, diagnosis of T4 tumors can be difficult in some cases as a result of MDCT's general lack of soft tissue resolution in the pelvis, and it can be quite difficult in the more subtle cases to clearly delineate tumoral involvement of adjacent organs, the pelvic sidewalls, or the adjacent vasculature. Diagnosis in these cases is contingent on loss of fat planes between a tumor and the adjacent organ or structure (*Figures 8*,*9*).

Unfortunately, despite multiple studies over the last 15 years seeking to establish MDCT as a tool for local rectal cancer staging, the results have been mixed (1). In a study by Juchems *et al.* in 2009 MDCT was unable to correctly differentiate lesions requiring neoadjuvant therapy from those lesions that could directly undergo surgical resection (25). Another study by Vliegen *et al.* in 2007 found that MDCT had a relatively poor accuracy in determining tumor involvement of the mesorectal fascia (26). However, in a study by Kanamoto *et al.* in 2007 the sensitivity/specificity for T1 and T2 tumors was 93.9%/94.3%, while the sensitivity/specificity for T3 tumors was 93.8%/94.3%,

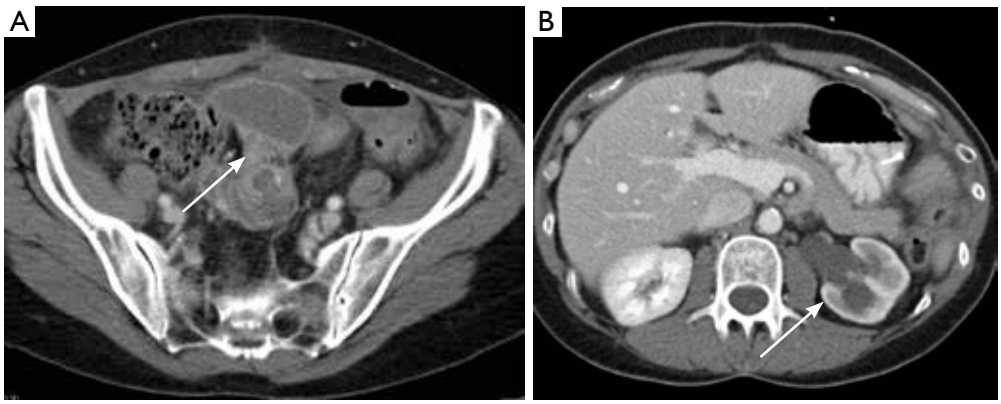

Figure 8 T4 rectal cancer on MDCT. In this case, a high rectal cancer (arrow in A) directly invades the bladder, resulting in severe left-sided hydronephrosis (arrow in B). The loss of fat plane between the bladder and rectum, as well as an appearance suggesting direct invasion, allow the diagnosis of a T4 tumor. MDCT, multidetector computed tomography.

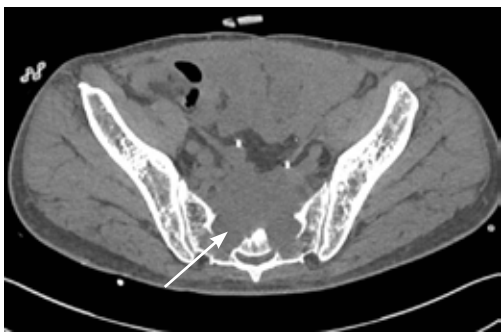

Figure 9 T4 rectal cancer with destruction of the sacrum on MDCT. A large bulky mass directly invades, and destroys, the adjacent sacrum. MDCT, multidetector computed tomography.

while another study by Taylor *et al.* in 2007 found that MDCT and MRI were relatively similar in their accuracies for CRM involvement (27,28). Overall, while individual studies dating back over several years have shown variable results, with some studies demonstrating T-staging and CRM involvement accuracies that are acceptable, a large meta-analysis by Kwok *et al.* examining close to 500 patients found that MDCT had a sensitivity of only 78% for extension of tumor through the rectal wall (with an accuracy of only 73%), as well as a sensitivity and specificity for mesorectal lymph node metastasis of only 52% and 78% respectively (29-31). Overall, there is little doubt that MDCT should not be utilized as a 1st line modality for the local staging of rectal cancer, particularly with regard to T-staging and assessment of the CRM (32). However, in those cases with clear tumor extension outside the rectum, the radiologist should not hesitate to make the diagnosis of a T3 or T4 tumor, even given the limitations of MDCT.

Distant staging

The American College of Radiology recommends that all patients with colorectal cancer undergo a preoperative staging MDCT not only because of its proven efficacy in the identification of metastatic disease, but also because of its ability to identify complications that might alter a patient's management (perforation, obstruction, abscess, pulmonary embolus, etc.) (2).

The most common site of distant metastases for colorectal cancer patients as a whole is the liver. These metastatic lesions tend to be most conspicuous on venous phase images, and will typically appear as hypoenhancing solid nodules that are easily juxtaposed against the avidly enhancing surrounding liver parenchyma (*Figure 10*). In some cases, the arterial phase images may be of benefit, as small liver metastases may demonstrate a rim of surrounding hyperemia, prominent peripheral enhancement or a surrounding perfusion abnormality that might increase lesion conspicuity. There is a wealth of data in the literature supporting the efficacy of MDCT in identifying colorectal cancer liver metastases: The overall sensitivity of MDCT for liver metastases is very good, with sensitivities ranging from 77-94% (33-35). Particularly with larger lesions (i.e., lesions measuring over 1 cm), MDCT is relatively specific as well, as most lesions measuring over 1 cm in size can be reliably differentiated from benign liver lesions (such as cysts or hemangiomas). However, while MDCT

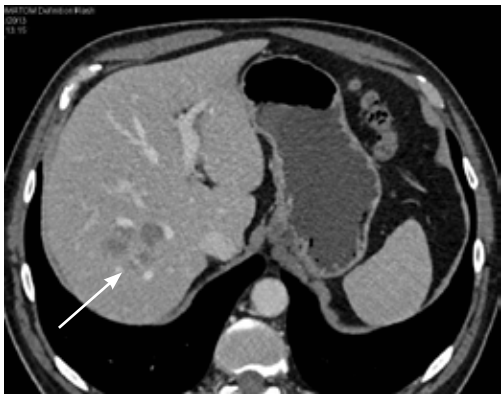

Figure 10 Typical MDCT appearance of colon cancer metastases to the liver. Axial contrast-enhanced MDCT image demonstrates small, ill-defined hypodense lesions (arrow) in the right hepatic lobe. MDCT, multidetector computed tomography.

is excellent in identifying larger metastases, it struggles with smaller lesions measuring under 1 cm in size, with reported sensitivities dropping to as low as 41.9% (18). The specificity of MDCT is also suboptimal for lesions under 1 cm, as it can be difficult to differentiate a tiny cyst or hemangioma from an early liver metastasis with confidence. Unfortunately, this can be quite problematic, as these small, nonspecific hypodensities measuring <1 cm (also known as 'too small to characterize' hypodensities) are very common, perhaps present in as many as 17% of all patients (36). Nevertheless, in the vast majority of cases, even in those patients with a known underlying malignancy, these small hypodensities in the liver are overwhelmingly likely to be benign (~90%), and can be safely followed over time. As a result, the relative lack of specificity of MDCT for smaller lesion is not clinically important in the vast majority of cases. It should be noted that many of these studies judging the efficacy of MDCT in identifying and characterizing liver metastases were performed on older generation scanners with inferior spatial and temporal resolutions to the last generation of technology. Accordingly, it is quite likely that these studies underestimate the efficacy of MDCT, which is likely to be substantially higher than the numbers reported in these studies.

Evaluation of lung metastases is also an important component of MDCT distant staging, and it is important that a chest CT be included when a patient undergoes their initial staging examination. In a study by Kirke *et al.*, 17.9% of patients with rectal cancer had evidence of at least one pulmonary metastasis on MDCT, with an increasing risk of pulmonary metastasis with rising tumor grade (37). Just as importantly, rectal cancers seem more likely than other colon cancers to present with pulmonary metastases without liver metastases, likely reflecting the unique systemic venous drainage of the rectum compared to the remainder of the colon (2). Accordingly, the ACR guidelines recommend that a patient's initial staging MDCT include images through the chest (2).

Unfortunately, as with MRI, MDCT has significant limitations in establishing a patient's nodal status, largely because the diagnosis of a malignant lymph node is contingent on enlargement and size criteria. This is particularly a problem when evaluating mesorectal lymph nodes, where 95% of all malignant lymph nodes measure under 5 mm, and 50% of all malignant lymph nodes measure under 3 mm, making any size cut-off inaccurate (38). Although at least one study has suggested utilizing a size cut-off of 4.5 mm in the mesorectum, such a cut-off would clearly miss a sizeable number of positive lymph nodes (38). Not surprisingly, a study by Ju *et al.* found that MDCT had an accuracy of only 61.5% when evaluating perirectal lymph nodes (39).

Positron emission tomography (PET)

Technique

PET is a nuclear medicine examination utilizing ^{18}F-fluoro-2-deoxy-D-glucose (FDG) as a primary tracer. This tracer, which acts glucose analog in the body, is transported into cells, phosphorylated, and subsequently accumulated, without entering the glycolytic cycle. Accordingly, given that many tumors demonstrate increased metabolism of glucose, FDG-PET utilizes the degree of FDG uptake as a surrogate measure of a tumor's metabolic activity, and this uptake can be assessed both qualitatively (via visual examination of the degree of uptake of a tumor relative to other tissues) and quantitatively (via a SUV value). Not only is FDG taken up by tumors, but also there is also some degree of physiologic uptake by normal tissues and organs, including the bowel, renal collecting systems, muscle, fat, and brain. This places great importance on proper patient preparation prior to a study, as a patient's blood glucose level, activity levels, ambient temperature, medications (particularly G-CSF), and food ingestion can all have a dramatic impact on the degree of uptake of FDG by not only by the tumor itself, but normal physiologic uptake as well. While PET was traditionally performed as a stand-

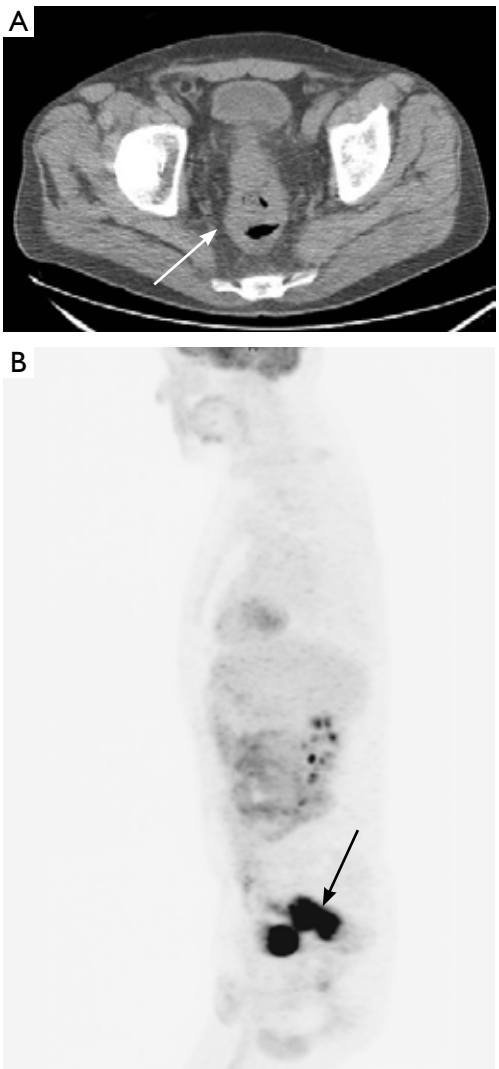

Figure 11 Axial non-contrast, non-diagnostic CT image (A) acquired as part of a PET-CT examination demonstrates severe mass-like thickening (arrow) of the rectum, corresponding to the patient's known rectal cancer. PET image (B) demonstrates marked FDG uptake associated with the mass (arrow). Notably, the spatial resolution of PET does not allow local T staging of the lesion.

alone examination, these studies are now almost always performed in conjunction with a CT (in dedicated PET-CT scanners), with acquisition of either a non-diagnostic non-contrast CT intended only for accurate localization of lesions or abnormalities seen on the PET portion of the study, or alternatively, a dedicated diagnostic quality intravenous contrast-enhanced CT meant to both serve both as a localizer for abnormalities on the PET, as well as a stand-alone diagnostic-quality MDCT examination (40,41).

Local staging

PET has a relatively low spatial resolution of only 5 mm, and as a result, is highly limited in its ability to locally stage tumors (*Figure 11*). Specifically, T-staging is not possible with PET-CT, as it has neither the anatomic detail (in terms of the layers of the rectal wall) or the spatial resolution to accurately judge the degree to which a tumor extends through the rectal wall (42). Moreover, PET is not particularly useful for evaluating locoregional lymph nodes in the mesorectum, as many of these perirectal or mesorectal lymph nodes measure 5 mm or less (below the resolution of PET), and moreover, 'blooming' (i.e., significant radiotracer uptake in a lesion artifactually appearing to extend into the adjacent soft tissues) from the primary lesion in the rectum can obscure uptake in small mesorectal lymph nodes (42). Nevertheless, while PET may not be of value in traditional TNM staging, it may have some value in terms of establishing a tumor's ultimate prognosis based on examinations performed before and during a patient's preoperative chemoradiation, although the data is certainly not conclusive. In a study by Lee *et al.*, a formula utilizing the total lesion glycolysis (TLG) (a PET parameter) of the primary tumor was found to be predictive of a patient's survival after neoadjuvant chemoradiation, a finding also confirmed elsewhere (43-47). Similarly, a meta-analysis by de Geus-Oei *et al.* suggested that PET-CT performed before and during a patient's chemoradiation regimen was able to predict which patients would respond to the treatment (48). In addition, as some groups have begun to advocate for a "watch and wait" approach after chemoradiation for rectal cancer, choosing to defer surgery in those patients who have a clinical complete response (cCR) based on imaging, it is conceivable that pre- and post-therapy PET might offer a better correlation with "true pathologic response" compared to digital rectal examination, sigmoidoscopy, or other imaging studies (CT, MRI), although this will certainly require far more rigorous study if this treatment algorithm becomes more widely utilized (49).

Distant staging

PET-CT serves as a very important modality in the distant staging of patients with colorectal cancer, potentially identifying 30% more distant metastases compared to MDCT (*Figure 12*) (42). In a study by Llamas-Elvira *et al.* PET showed an excellent diagnostic accuracy of 92%

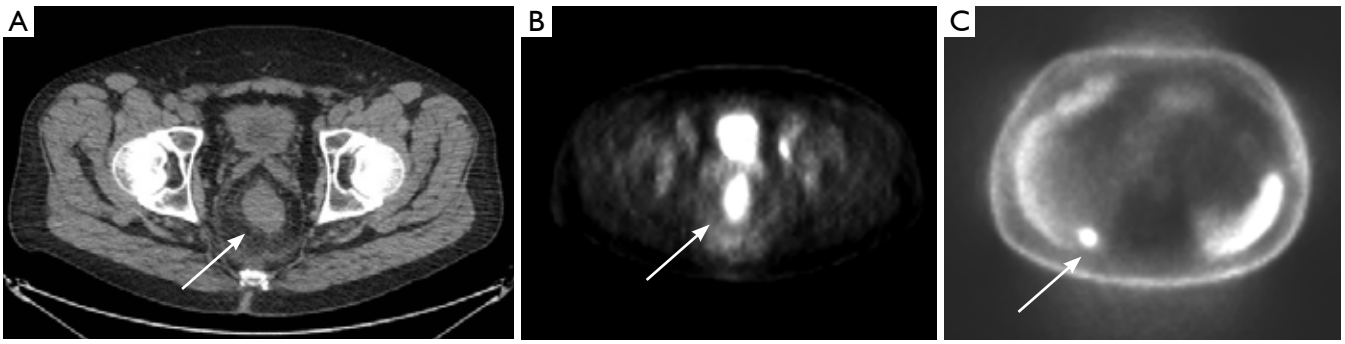

Figure 12 Axial non-contrast, non-diagnostic CT image (A) demonstrates mass-like thickening (arrow) of the rectum, corresponding to the patient's known rectal cancer. PET image (B) at the same level demonstrates marked FDG uptake associated with the mass. PET image (C) though the liver demonstrates an occult metastasis (arrow), which was not identifiable on the patient's formal contrast-enhanced MDCT. MDCT, multidetector computed tomography.

(as opposed to 87% for MDCT), changed the patient's stage in 13.5% of cases, identified previously unknown disease in 19.2% of cases, changed the patient's planned surgery in 11.5% of cases, and changed the patient's therapy in 17.8% of cases (50). Another study by Abdel-Nabi *et al.* found PET-CT to be superior to MDCT in identifying liver metastases (51), while a study by Gearhart *et al.* found that PET-CT upstaged 50% of patients, downstaged 21% of patients, and changed the patient's treatment plan in 27% of patients (52). This study noted that PET-CT was particularly likely to identify 'discordant' findings (i.e., findings not identified on MDCT) in patients with low rectal cancers due to the propensity of this group of lesions to metastasize to local lymph nodes in the pelvis (particularly nodes in the inguinal, femoral, or iliac chains), as PET-CT identified metastatic lymphadenopathy in 13.5% of patients in this study which were not diagnosed on MDCT (52).

Conclusions

MRI, MDCT, and PET are complementary imaging modalities in the preoperative staging of patients with rectal cancer, and each offers their own individual strengths and weaknesses. MRI is clearly the best available radiologic modality for the local staging of patients with rectal cancer, and has the potential to play an important role in accurately distinguishing which patients should receive preoperative chemoradiation prior to total mesorectal excision. Alternatively, while MDCT and PET are both quite limited in local staging, both should be considered primary modalities when performing preoperative distant staging. In particular, every patient with a newly diagnosed rectal cancer should undergo a preoperative staging MDCT which includes the chest, abdomen, and pelvis, as MDCT can not only accurately stage distant metastatic disease, but it can also identify acute complications which may change a patient's treatment algorithm. Alternatively, PET may offer a valuable diagnostic adjunct for identifying distant metastatic disease, changing a patient's management in a sizeable percentage of cases.

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None.

Footnote

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

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The bacteria-hypothesis of colorectal cancer: pathogenetic and therapeutic implications

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Abstract: It is estimated that up to 20% of malignancies worldwide can be attributed to infections. The most convincing evidence, in this context, is the link between *Helicobacter pylori* and both gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. A growing body of evidence in the last years has raised up the question of the putative causal role of gut microbiota in the carcinogenetic process. Bacteria are an important component of the human body. The human intestine contains >500 different types of microorganisms, usually referred to as the commensal intestinal microbiota. A chronic alteration of the intestinal microbiota homeostasis or "dysbiosis" underlies many diseases, including cancer. The main mechanisms by which bacteria may induce carcinogenesis include chronic inflammation, immune evasion and immune suppression. If the microbiota is involved in cancer development, being the colon the site where the microbiota reaches its highest concentration, it is expected to be its major site of action. Numerous data from experimental, animal model and human studies support the gut-bacteria hypothesis of colorectal cancer (CRC). Germ-free rats, compared with conventionally reared animals, develop fewer and smaller tumors both spontaneously and after chemically-induced CRC. The absence of the physiological inflammation caused by the commensal microbiota may explain the capability of the germ-free rats to develop a more efficacious anti-cancer immune response. Several microorganisms, including *Streptococcus bovis*, *Bacteroides fragilis* and *Escherichia coli* have been implicated in the pathogenesis of CRC. The emerging relationship between gut microbiota and cancer prompts new ways of thinking about cancer prevention and leads to the development of innovative treatments such as probiotics. However, although *in vitro* and animal model studies suggest a protective anticancer effect of probiotics, the results of human epidemiological studies are still controversial and very few data are available from interventional studies.

Keywords: Gut microbiota; colorectal cancer (CRC); probiotics

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Introduction

Back to 1863 Rudolf Virchow, a German pathologist, affirmed that cancer may be considered the end result of a chronic inflammatory process triggered by an adverse toxic environment, including infections. The concept that bacterial infections could lead to cancer was first proposed in the late 19th century, following the pioneering work of Robert Koch and Louis Pasteur, based on the discovery of bacteria at the sites of tumors. Nowadays up to 20% of malignancies worldwide can be attributed to infections with a global total of 1.2 million cases per year (1). The most convincing evidence, in this context, is the link between *Helicobacter pylori* and both gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. The hypothesis of the infectious origin of cancer is corroborated by the association of *Salmonella typhi* with gallbladder cancer, *Chlamydia pneumoniae* with lung cancer, and *Streptococcus bovis (S. bovis)* with colorectal cancer (CRC).

Based on these historical perspectives a growing body of

evidence in the last years has raised up the putative causal role of gut microbiota in the carcinogenetic process (2). If the microbiota is involved in cancer development, being the colon the site where the microbiota reaches its highest concentration, it is expected to be its major site of action.

Colorectal cancer is the third most common cause of cancer-related death in woman and the fourth leading cause of cancer mortality in males. Over 140,000 new cases of CRC are estimated for the U.S. in 2012 with disease-specific mortality of up to 60,000 reported in 2011 (3). Colorectal cancer is classified as inherited (due to genetic instability), inflammatory (associated to inflammatory bowel disease) or sporadic, which accounts for more than 80% of all CRCs. Sporadic CRC, is the focus of both tremendous epidemiological research efforts, with the goal to determine potential causative and risk factors associated with the disease, and continuous basic research, aimed to clarify the pathogenetic mechanisms of the disease. Several potential risk factors have been identified, such as high-fat diet, red meat consumption, alcohol intake, and obesity, but the list continues to evolve, and in the past few decades has expanded to include infectious agents, and in particular alterations of the gut microecology.

Here, we will address the link between gut microbiota and CRC focusing on pathogenetic and therapeutic implications.

Gut microbiota and carcinogensis

Our gut harbors the majority of mammalian-associated microbes. The fetal intestine is sterile but, following delivery, the colonization of the intestine by a variety of microorganisms begins. Gastrointestinal colonization involves a succession of bacterial populations varying as the diet changes and the host develops. This assemblage of bacteria inhabiting the gut is usually referred to as the commensal intestinal microbiota. Each human adult harbors approximately 10^{14} bacteria in the gut, which is about 10 times the number of cells making up the human body (4). There are at least 500 different bacterial species and these species can again be divided into different strains, highlighting the enormous complexity of this ecosystem. The bacteria in the gut interact with their human host and, although some bacteria are potentially pathogenic and can become a source of disease, this host-bacterial interaction is mainly symbiotic and health-conferring. The result of this interaction may lead to a "physiological inflammation" that regulates the presence of the resident gut microbiota or, to a "pathological inflammation", the degree of which depends on the number and virulence of the invading pathogens (5). Physiological inflammation maintains a dynamic yet fragile homeostatic balance; however, persistent inflammation may be the link between gut bacteria and carcinogenesis process. Chronic inflammation can profoundly alter local immune response and lead to the release of reactive oxygen species (ROS) and nitric oxide (NO) that in turn may induce DNA damage and consequently alter tissue homeostasis (6). Nevertheless, cytokines and chemokines can act as tumor growth and survival factors and may induce tumor development by promoting angiogenesis and suppressing immune-surveillance. Cancer-promoting cytokines include tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-1. By contrast, IL-10 and transforming growth factor beta (TGF-β) inhibit carcinogenesis (6). In summary, chronic inflammation, immune evasion and immune suppression are the mechanisms by which bacteria may induce carcinogenesis.

The gut microbiota elicits both innate and adaptive immune mechanisms that cooperate to protect the host and maintain intestinal homeostasis. Activation of innate host defense depends on specific pattern recognition receptors (PRRs) that recognize highly conserved microbial signature molecules called "pathogen-associated molecular patterns" (PAMPs). The PRRs include the family of toll-like receptors (TLRs), which scan the extracellular space, and Nod-like receptors (NLRs), which guard the intracellular cytoplasmatic compartment (7). Different TLRs recognize different classes of PAMPs, characterizing different pathogens. After PAMP ligation, TLRs dimerize and transmit intracellular signals through four adaptor proteins: myeloid differentiation primary response gene 88 (*MyD88*), toll/interleukin-1-receptordomain-containing adaptor inducing interferon-β (*TRIF*), toll/interleukin-1-receptor-domain-containing adaptor protein (*TIRAP*), and TRIF-related adaptor molecule (*TRAM*), that have an important role in inflammation and tissue regeneration (8). Therefore, TLRs are likely candidates to mediate the effects of the innate immune response on tumorigenesis. Mice that lack either TLR4 or its MyD88 adaptor exhibit decreased epithelial cell proliferation and increased apoptosis in response to chemical-induced injury (9,10). Finally, the blockade of the TLR4 receptor in mice with CRC xenografts decreases the growth of colon tumors.

TLR4 has been associated with the process of tumor progression via the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway

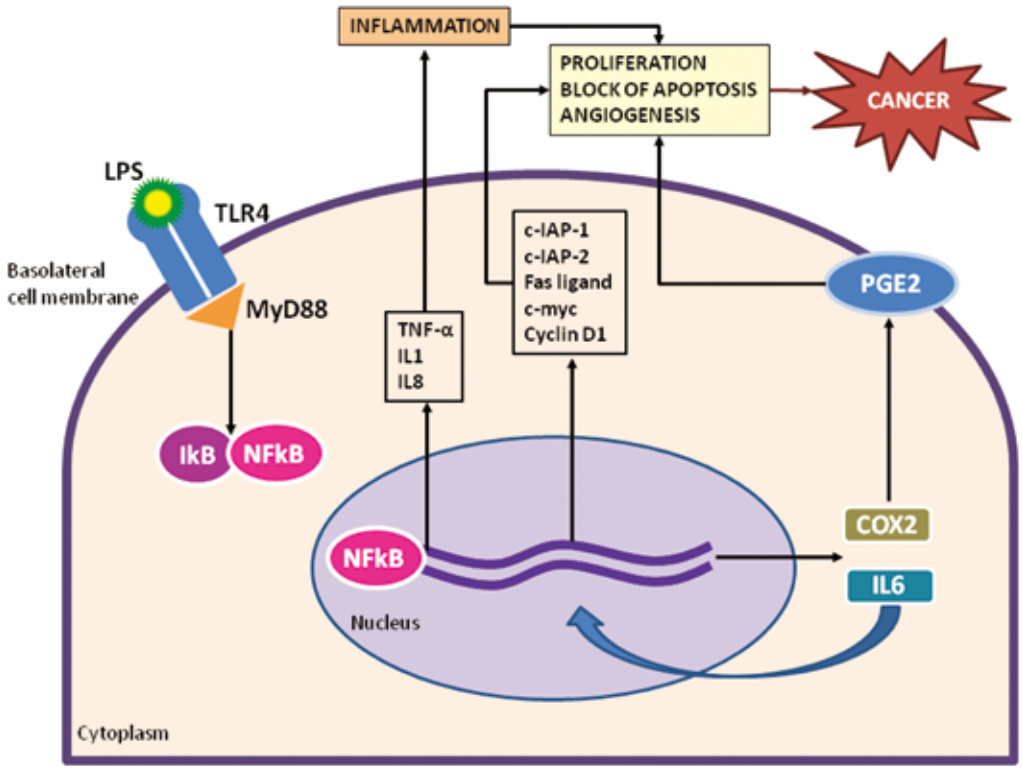

Figure 1 Toll like receptor signaling in colorectal cancer. LPS, lipopolysaccharide; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IκB, inhibitor of NF-κB; IAP, inhibitor of apoptosis; TNF-α, tumor necrosis factor alpha; IL, interleukin 6; COX2, cycloxigenase 2; PGE2, prostaglandin E2.

resulting in the transcription of inflammatory cytokines, chemokines and antimicrobial genes. How NF-κB-induced inflammatory process drives carcinogenesis is unclear, although IL-6 seems to have a pivotal role. IL-6 induces the procarcinogenic signal transducer and activator of transcription (Stat)3 pathway and transcriptionally activates proliferative, antiapoptotic and proangiogenic genes involved in cancer growth, such as c-IAP-1 and c-IAP-2, Fas ligand, c-myc, p53, and cyclin D1 (*Figure 1*) (11).

Findings from animal models of CRC are corroborated by human studies. The TLR4/MyD88 co-receptor complex is over-expressed in CRCs compared to the normal and adenomatous colonic epithelium, confirming that this signaling pathway is important in human sporadic CRC (12). Specific polymorphisms of toll receptors are also associated with an increased CRC risk and influence prognosis (13). In both murine models and human samples, TLR4 and IL-6 expression in the tumor microenvironment are associated with the presence of adenocarcinoma, and higher levels of TLR4 expression in the tumor stroma are noted with disease progression (14). TLR4 expression in the stroma of patients with stage 3 CRCs correlates with early relapse, suggesting the importance of this marker in predicting prognosis or as a therapeutic target (15).

The gut-mucosal arm of the adaptive immune system, localized predominantly in the small bowel, provides humoral and cell-mediated immunity against ingested antigens and luminal organisms. Effector lymphocytes are diffusely distributed in the lamina propria as isolated lymphoid follicles or are organized into structures termed "Peyer's patches". Locally recruited cells of the adaptive immune system may have either pro- or anti-tumorigenic roles. T cells, for instance, are required for inflammation, cancer development, and tumor progression (*Figure 2*), as well as for anticancer immunity (16). In sporadic CRC, there seems to be a well-defined balance between immunosurveillance (executed by $CD8^+$ T cells, NK cells, and $CD4^+$ T cells) and tumor-promoting inflammation (executed by innate immune cells, B cells, and various subtypes of T cells) (8).

Three effector pathways of T helper (Th) cell differentiation have been characterized: Th1, Th2 and

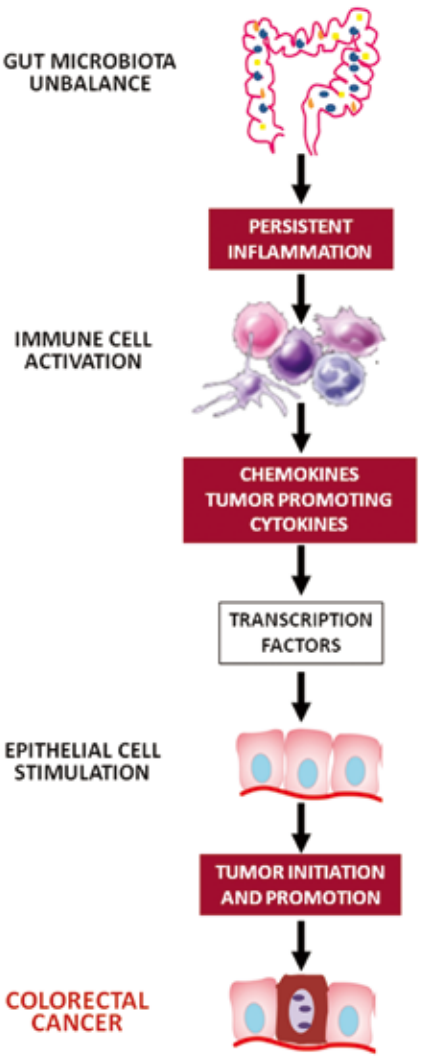

Figure 2 The role of immune cells in the gut microbiota-related colorectal carcinogenesis.

Th17 responses. While the Th1 response is typically anticarcinogenic, the contribution of Th2 or Th17 responses to cancer remains to be defined (17). Microbiota-induced Th17 cytokines in the lamina propria are crucial for protection against intestinal pathogens but, they can also contribute to inflammation. Indeed, IL-23-responsive innate lymphoid cells in the lamina propria contribute to colitis in $Rag^{-/-}$ mice by producing IL-17 and interferon gamma (IFN-γ) (18). Whether the highly inflammatory nature of Th17 cells is sufficient to cause or contribute to carcinogenesis is still debated. Experimental evidence shows that Th17 cells progressively increase in the tumor microenvironment during tumor development and that IL-17 up-regulates the expression of pro-inflammatory cytokines and pro-angiogenic factors. On the other hand, a number of reports have described tumor-inhibitory effects of IL-23 and IL-17 in mouse models genetically engineered to overexpress IL-23 or IL-17. Therefore, the activation of the IL-23/IL-17 pathway may promote tumorigenesis by inducing local inflammatory response, or inhibit it by stimulating anti-tumor immunity (19). More recently, a T regulatory response (TReg), driven by IL-10 and TGF-β has been shown to counterbalance the pro-inflammatory effect of the Th17 response. The induction of TReg cells by commensal microorganisms and the occurrence of intestinal inflammation in their absence indicate that TReg cells regulate the equilibrium between non-inflammatory homeostasis and intestinal inflammation. However, experimental and clinical findings have demonstrated that TReg cells, by suppressing the innate and adaptive immune responses, are a major factor contributing to the immunosuppressive tumor microenvironment, thus fostering tumor progression (20). Strategies that deplete or inhibit Treg cells and promote a competent immune response in the tumor microenvironment could be the goal in future immunotherapeutic studies in cancer patients.

Gut microbiota and colorectal cancer

In 1975 Reddy *et al.*, firstly linked the gut microbiota to CRC development. They found that only 20% of germ-free rats develop chemically induced CRC; in contrast, the tumor incidence in conventional rats was 93% and the neoplasms were multiple (21). This data has been recently confirmed by Vannucci *et al.* who found that germ-free rats, compared to conventionally reared animals, develop fewer and smaller tumors both spontaneously and after chemically-induced carcinogenesis (22). In addition, germ-free mice has also shown less oncogenic mutations and a decreased tumor formation in both colitis-associated cancer and *Apc*-related CRC (23).The absence of the physiological inflammation caused by the commensal microbiota may explain the capability of the germ-free rats to develop a more efficacious anti-cancer immune response.

Many bacterial species have been found in CRC samples and in tissue adjacent to tumors, namely, *S. bovis*, *Bacteroides fragilis* (*B. fragilis*), *Escherichia coli* (*E. coli*), etc (*Table 1*).

The best known association is that between *S. bovis bacteremia* and CRC, recognized since 1951, when McCoy and Mason first reported a case of enterococcal endocarditis, likely from *S. bovis*, associated with a carcinoma of the

Table 1 Bacteria and related pathogenetic mechanisms linked to colorectal cancer

Microbe	Pathogenetic mechanism
Bacteroides fragilis, enterotoxigenic	Activation of STAT3
	Induction of Th-17 immune response
	Production of IL-1
	Cleavage of E-cadherin
	Activation of b-catenin signaling
Bacteroides vulgates	Activation of MyD88-dependent signalling
	NF-κB activation
Bifidobacterium longum	Increased bacterial presence
Clostridium butyricium	-
Mitsuokella multiacida	-
Escherichia coli, invasive	Intracellular colonization
Enterococcus faecalis	ROS production and DNA damage
Streptococcus bovis	Production of IL-8
	Aberrant crypt formation
	Increased proliferation

ROS, reactive oxygen species; Stat, signal transducer and activator of transcription; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor κB.

cecum. Since then, the connection between *S. bovis* septicemia and colonic neoplasia has been confirmed by several other case reports and case-control studies. About 25-80% of patients with *S. bovis bacteremia* exhibit a CRC; in addition, a significantly higher fecal carriage of *S. bovis* has been reported in patients with CRC compared with control subjects (24). The mechanisms underlying this association are not known. Ellmerich *et al.* reported that *S. bovis* enhanced the expression of the proliferation markers and polyamines, and induced the formation of colonic adenoma in 50% of rats, as well as a higher number of aberrant colonic crypts. The authors also found that *S. bovis* and its wall antigens are able to increase the production of IL-8 in the colonic mucosa (25). IL-8 induces the formation of NO and ROS that contribute to the neoplastic process by altering cell DNA. On the basis of these data, several authors have suggested that all patients with *S. bovis* bacteremia should undergo a complete endoscopic evaluation of the colon.

B. fragilis strains comprise approximately 0.1% of the normal colonic flora and are found in the colonic flora in up to 80% of children and adults. The "enterotoxigenic *B. fragilis*" (ETBF), producing *fragilisyn*, has been associated with CRC. The toxin cleaves the extracellular domain of the E-cadherin, which is the principal structural component of the zonula adherens and is responsible for cell-to-cell adhesion (26). Treatment of HT29/C1 cells with *B. fragilis* toxin triggered the nuclear localization of β-catenin, which in turn, after binding with T-cell factor-dependent transcriptional activators, induced c-myc and cyclin D1 transcription and translation, resulting in persistent cellular proliferation (27). Activation of β-catenin signaling via mutations in one or more of the APC complex proteins, contributes to the development of inherited and sporadic forms of CRC and possibly other cancers. Toprak *et al.*, by investigating the prevalence of ETBF in stool specimens from 73 CRC patients and 59 controls found the enterotoxin gene in 38% of the isolates from CRC patients compared with 12% of the isolates from the control group (26). More recently Wu *et al.* (27) showed that ETBF strongly induces CRC in multiple intestinal neoplasia (Min) mice, by activating Stat3 and a selective TH17 response. The authors also demonstrated that the antibody-mediated blockade of IL-17 as well as that of the receptor for IL-23, a key cytokine amplifying TH17 responses, inhibits ETBF-induced tumor formation (28).

E. coli is a normal inhabitant of the human gut. The colonic mucosa of patients with adenomas and carcinomas has shown an increased intracellular mucosal carriage of *E. coli* compared to healthy controls (29). Whether this

increased carriage had a causal or incidental origin is currently not known. *E. coli* strains of the phylogenetic group B2 harbor a genomic island called "pks" that codes for the production of a polyketide-peptide genotoxin, colibactin. The *in vivo* infection with *E. coli* harboring the Pks Island, but not with a pks isogenic mutant, induced the formation of phosphorylated H2AX foci in mouse enterocytes, contributing to the development of sporadic CRC (30).

Until now the relation between gut microbiota and CRC was based on culture *ex vivo* methods. However, 60-80% of the gut bacteria are uncharacterized because they cannot be cultivated *ex vivo*. Recent advances in molecular methods, based on the highly conserved bacterial 16S ribosomal RNA (rRNA) gene have enhanced our ability to study and characterize both luminal and adherent bacteria communities in the gut. By using these approaches, only a few studies have investigated changes in the microbiota composition during CRC. Nevertheless, these studies indicate that the altered colonic environment in CRC could have implications for the composition of the microbiota in the lumen and on mucosal surfaces. Gueimonde *et al.*, by qRT-PCR, analyzed samples of colonic mucosa from 34 patients (21 CRCs, 9 divertiulitis and 4 inflammatory bowel diseases) and found that patients with CRC had significantly lower levels of both *Bifidobacterium longum* and *bifidum* than patients with diveritulitis and inflammatory bowel disease (31). Similarly, Shen *et al.*, by evaluating adherent bacteria in 21 adenoma and 23 non-adenoma subjects by a sophisticated molecular approach, sequenced and processed for phylogenetic and taxonomic analysis a total of 335 clones and found higher *Proteobacteria* and lower *Bacteroidetes* numbers in tumor cases compared with control subjects (32). Sobhani *et al.* using pyrosequencing of stool bacterial DNA and subsequent Principal Component Analysis (PCA) demonstrated a composition change in the microbiota of CRC patients; in particular *Bacteroides/Prevotella* species were more numerous in cancer patients (n.60) than in control subjects (n.119). In addition, IL-17 immunoreactive cells were expressed at significantly higher levels in cancer patients than in those with normal colonoscopy (33). Very recently Marchesi *et al.* compared differences in healthy and cancerous tissue within cancer patients and found that species of the genera *Coriobacteridae*, *Roseburia*, *Fusobacterium* and *Faecalibacterium* were over-represented in tumor tissue; these are generally regarded as gut commensals with probiotic features. Further, this study found decreased colonization by members of *Enterobacteriaceae*, such as *Citrobacter*, *Shigella*, *Cronobacter*, and *Salmonella* in CRC tissue from the investigated patients (34). Finally, Scanlan *et al.* investigated the diversity and presence of methanogens in healthy, polyp and cancer patients and found significant differences in bacterial stability over time. Specifically, the diversity of the *Clostridium leptum* and coccoides subgroups was increased compared to healthy controls. Importantly, metabonomic faecal water analysis was able to distinguish CRC and polyp groups from healthy controls, indicative of an altered metabolic activity of the intestinal microbiota in these patients (35).

Taken together, these data show that the gut microbiota may play a major role in CRC development at both quantitative and qualitative level.

Probiotics and colorectal cancer

The emerging relationship between the gut microbiota and cancer opens the door to new ways of thinking about cancer prevention. Probiotics are defined as viable microorganisms that, when administered in adequate amounts, confer a health benefit to the host. They may positively affect the gut microbiota and have a beneficial effect in the prevention and treatment of specific pathological conditions. There are many mechanisms by means of which probiotics positively affect the gut microbiota and liver health, i.e., inhibition of intestinal bacterial enzymes, stimulation of host immunity, competition for limited nutrients, inhibition of bacteria mucosal adherence and epithelial invasion, protection of intestinal permeability and control of bacterial translocation from the gut to the bloodstream. The biological activity of probiotics depends prevalently on delivering anti-inflammatory mediators that down-regulate pro-inflammatory cytokines, including IFN-γ and TNF-α, via the NF-κB pathway. The mechanisms through which probiotics may exert beneficial effects include macrophage activation, cytocrome P450 blocking, reduction of carcinogen generation, down-regulation of Ras-p21 expression, increase of cell differentiation, inhibition of COX-2 up-regulation, inhibition of NO synthase, increase of short chain fatty acid production, and reduction of intestinal pH with lessening of putrefactive bacteria (36,37).

The anticarcinogenic effects of probiotic microorganisms *in vitro* and in animal studies are well documented. In a very recent study, Bassaganya-Riera *et al.* investigated the ability of VSL#3 bacteria to modulate mucosal immune responses and thereby ameliorate colonic carcinogenesis in mouse models of inflammation driven CRC. In mice

treated with VSL#3, adenoma and adenocarcinoma formation was diminished by both treatments (38). Chang *et al.* demonstrated that the oral administration of *Lactobacillus acidophilus (L. acidophilus)* KFRI342 to rats with 1,2-Dimethylhydrazine (DMH)-induced CRC inhibited the development of preneoplastic lesions and lowered the microbiota populations of both *E. coli* and aerobic bacteria, which have been associated with carcinogenesis (39). The possibility that probiotics modulates immunity may inhibit colon carcinogenesis has been also investigated. Foo *et al.* by evaluating the effect of long term (24 weeks) treatment with *B. longum* and *Lactobacillus gasseri (L. gasseri)* on the development of DMH-induced colonic precancerous lesions and tumors in 70 male mice showed that both probiotics significantly inhibited DMH-induced aberrant crypt foci formation, as well as decreased tumor multiplicity and the size (40). Several studies have shown that the intake of probiotics can influence enzyme activities and can be linked with the risk of colon carcinogenesis. *Lactobacillus casei (L. casei)* treatment of mucosa samples from duodenum, jejunum, ileum, cecum, and colon of 45 male Wistar rats was able to monitor the expression of selected cytochromes P450, testing the hypothesis that the L. casei probiotic might contribute to preventing CRC by decreasing levels of certain forms of xenobiotic-metabolizing enzymes (41). Finally probiotics may retard colon carcinogenesis by stimulating tumor cell apoptosis. Preinoculation with the probiotic L. acidophilus NCFM for 14 days in BALB/cByJ mice in which orthotopic CRCs were implanted, reduced the severity of colonic carcinogenesis caused by CT-26 cells (42), such as the level of colonic involvement and structural abnormality of epithelial/crypt damage (43). A significant down-regulation of the CXCR4 mRNA expression, associated with reduced apoptosis, was observed (44).

Data from human studies are still controversial. An epidemiological study performed in Finland demonstrated that, despite a high fat intake, CRC incidence is lower than in other countries because of the high consumption of milk, yoghurt and other dairy products (44). In two population-based case-control studies of CRC, an inverse association was observed for yoghurt and cultured milk, adjusted for potential confounding factors (45,46). An inverse relationship has been demonstrated between the frequency of consumption of yoghurt and other fermented milk products and breast cancer in women. On the other hand, two American prospective studies, the Nurses' Health Study and the Health Professionals study, did not provide evidence that intake of dairy products is associated with a decreased risk of CRC (47). In a cohort study in the Netherlands, it was shown that the intake of fermented dairy products was not significantly associated with CRC risk in an elderly population with a relatively wide variation in dairy product consumption, although a weak non-significant inverse association with CRC was observed (48). The contrasting results may be related to study designs, population examined, follow-up, bacterial strains used, endpoints, dietary habit and so on. An intervention study in humans in which both probiotics and prebiotics were used was recently performed among 17 patients with FAP. In this single-center human study on patients with FAP, a 4-week intervention with (I) sulindac; (II) inulin/VSL#3; and (III) sulindac/inulin/VSL#3 was performed. Cell proliferation was lower after treatment with sulindac or VSL#3/inulin; the combination of sulindac/inulin/VSL#3 showed the opposite effect. Glutathione S-transferase activity increased after treatment with sulindac or VSL#3/inulin; the combination treatment showed the opposite effect (49). However, FAP is a rare disorder, so the main weakness of this study is the small number of patients included in a single-center fashion.

In 2006 Capurso *et al.* produced a systematic review of data from basic science (animal and *in vitro* models) and human (epidemiological and interventional) studies, addressing the risk of CRC and the use of probiotics (50). The *in vitro* studies, confirm the ability of probiotics to dialogue with intestinal cells. Overall, 26/29 animal model studies suggested that probiotics had a protective anticancer effect; however, given the different study designs and treatments, the results are difficult to compare. Finally, the epidemiological human studies are difficult to interpret given their extreme heterogeneity (50). Further experimental studies in animal models and clinical trials in humans are needed to quantify the effect and elucidate the mode of action of probiotics in prophylaxis and treatment of CRC.

Conclusions

Over the years, it has become apparent that the gut microbiota is not a bystander in the complex biological events regulating intestinal homeostasis, but it may lead to beneficial or detrimental effects to the host. Multiple lines of evidence support the notion that gut microbiota can contribute to colorectal carcinogenesis. Various bacteria have been linked with experimental carcinogenesis in animal models or correlated with CRC in human observational

studies and multiple microbiota-based studies suggest differences in mucosa associated and luminal bacteria in subjects with CRC.

Therefore, a beneficial modulation of the composition and metabolic activity of the gut microbiota might represent an interesting approach to reducing the risk of CRC development. Even though the mechanisms by which probiotics may inhibit CRC are not fully elucidated, certain potential mechanisms have been disclosed, such as the alteration of the composition and the metabolic activities of the intestinal microbiota, the changing physicochemical conditions in the colon, the binding of dietary carcinogens, the production of short chain fatty acids, the protection of the colonic mucosa and enhancement the immune system. In the near future, high quality mechanicistic experimental studies and interventional human studies might provide the scientific premises for the clinical use of probiotic in the prevention of CRC.

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None.

Footnote

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

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The evolution of colorectal cancer genetics—Part 1: from discovery to practice

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Abstract: Colorectal cancer (CRC) is an increasing burden on our society. Identifying those who are at the greatest risk and improving triage for treatment will have the greatest impact on healthcare. CRC is a prime paradigm for cancer genetics: the majority of disease results from stages of progression lending itself to prevention by early detection of the pre-disease (neoplastic) state. Approximately 10% represent well defined hereditary cancer syndromes. Hereditary CRC has the added benefit that many are slow growing and family members are armed with the knowledge of potential risk of associated carcinomas and empowerment to reduce the disease burden. This knowledge provides the indication for early endoscopic and/or surgical intervention for prevention or treatment of an entire family cohort. The molecular basis of CRC allows enhanced characterization of carcinomas, leading to targeted therapies.

Keywords: Colorectal cancer (CRC); genetics; prevention; CRC syndromes; Lynch syndrome (LS)

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Introduction

The role of genetics in colorectal cancer (CRC) has become critical to the mission of disease prevention, early detection and effective treatment. Over the last century, CRC genetics has emerged from an unrecognized to a specialized field, encompassing all aspects of cancer care. CRC is a preventable disease. The natural history of CRC differs in individuals with a hereditary predisposition: with an abbreviated length of tumorigenesis, often presenting at an earlier age. The incorporation of cancer risk assessment (CRA) and presymptomatic genetic testing results in effective stratification. Identification of high-risk individuals/families leads to more appropriate screening, options of prophylactic surgery for primary prevention and knowledge of potential associated cancers. Moreover, given the autosomal dominant inheritance of most CRC syndromes, 50% of a family cohort will be spared increased surveillance and anxiety associated with a positive family history.

Background

As infectious diseases have waned, and healthcare has improved, we are faced with diseases that occur at ages not previously attained. CRC is the third most common cause of cancer death in the world and is estimated to have an incidence of over one million cases per year (1). Research has led to micro diagnosis and improved systemic treatment however, despite advances in detection and care, morbidity and mortality from CRC continues to be high. Incorporating medical genetics dramatically improves outcomes at the public health level.

This review is a tribute to the pioneers who possess the ingenuity, perseverance, and collaborative nature to painstakingly (often with no support) collect data across generations, and laboriously isolate and analyze DNA. Throughout the last 100 years, as their discoveries blossom, we are awarded with the fruits of their labor.

History

The sentinel account of a hereditary colorectal family was by Dr. Aldred Warthin, who first suspected the disorder in the family of an affected woman (who subsequently died of endometrial cancer) over 100 years ago. He began studying her family (Family G) in 1895 and published his first report on it in 1913 documenting a pattern of gynecological cancer—specifically endometrial cancer—and gastrointestinal cancers, particularly gastric and colon (2). In 1971, updated studies of Family G by Lynch and Krush showed it to be consonant with what became known as Lynch syndrome (LS) (3). A marked 70-80% percent excess of proximal colon cancers was observed in patients with LS (4). Cutaneous manifestations of the Muir-Torre syndrome, such as sebaceous adenomas and sebaceous carcinomas also were found to be associated with the disorder (5). CRCs are the most frequent cancers associated with LS; endometrial cancers have been identified as the second-leading cancer associated with the syndrome. The MutS, E. Coli, Homolog of, 2 mutation was subsequently identified in Family G in 2000 (6). With current detection and treatment options, it is felt that no one with LS should die of CRC, assuming that the patient at increased risk has been identified, has a knowledgeable physician, and has been referred to a gastroenterologist or surgeon who prescribes frequent (annual) screening colonoscopies initiated at age 25.

Knudson's two hit hypothesis provided the basis of our understanding of how tumor suppressor genes could explain the younger ages of onset in familial cancers as well as variable penetrance. Although susceptibility is increased, a second mutation is required to produce a tumor (7,8). Fearon & Vogelstein showed us that in some cancers, the adenomatous polyposis coli (APC) gene is mutated as the initial step in the carcinogenic pathway (9). Mutations in the adenomatous polyposis coli gene are responsible for the syndrome originally recognized in the 1930's as autosomal dominant familial severe polyposis, currently known as familial adenomatous polyposis (FAP) (10-12).

Once some of the putative genes for colon cancer were identified, the value of a detailed family history became apparent. The expanded histories often led to the characterization of hereditary cancer syndromes and a better understanding of the natural history. For the first time, phenotypes could be predicted from the genotype providing valuable information towards prevention. The locations of mutations in the APC gene were shown to be associated with extracolonic manifestations as well as the severity and age of onset of polyposis (13). Shortly thereafter the extracolonic cancers in LS were confirmed (14-16). Identification of the familial mutation allowed pre-symptomatic genetic testing of family members opening the possibility of prevention and early detection of related cancers. Equally important is the sparing of those who are mutation negative thus reducing the psychological ramifications of the unknown.

In 1990 Congress awarded $3 billion to the Human Genome Project (HGP) which was completed with an international consortium in 2003. The hopes of genomic information raised the possibility of unforeseen consequences. For example, the Ethical, Social and Legal Implications (ELSI) committee was established to deal with the non-technical impact of this knowledge. A new branch of the National Institutes of Health, the National Human Genome Research Institute (NHGRI) is the result of the HGP and dedicates 5% of the budget towards ELSI which continues to guide us through this exciting social transformation.

Technological advances provided a boost towards new genetic discoveries launching the arena for high throughput analysis. Large amounts of data are now available in a short amount of time with small amounts of DNA. Our understanding of CRC continues to grow, and it is now estimated that up to 10% of the population has a known hereditary CRC syndrome. More importantly, there are 20-30% of CRC cases with evidence of a familial component, but without an identified hereditary gene mutation (1,17,18). Genetics has increased our understanding of the somatic events of tumorigenesis. The molecular pathology of the tumor describes two pathways to carcinogenesis mismatch repair and serrated polyposis (19,20). More recently, we have come to appreciate how cancer can be caused by the epigenetic modification of cancer genes, both heritable and acquired.

Genetic counseling (cancer risk assessment, CRA)

Genetic counseling is the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease. This process integrates: (I) interpretation of family and

medical histories to assess the chance of disease occurrence or recurrence; and (II) education about inheritance, testing, management, prevention, resources and research, and counseling to promote informed choices and adaptation to the risk or condition (21).

CRA is a specialized area of genetic counseling and is an integral component of cancer care and prevention in a modern healthcare system. CRA is the process of obtaining a family history, detailed medical and surgical history, psychosocial assessment, risk counseling, education regarding preventative measures, and natural history of disease, discussion of genetic testing and informed consent. Guidelines for offering CRA are documented with position statements by leading healthcare organizations such as The National Comprehensive Cancer Network (NCCN), American Society of Clinical Oncology (ASCO), American College of Gastroenterology, National Society of Genetic Counselors (NSGC) and Collaborative Group of the Americas on Inherited Colorectal Cancer (CGA-ICC) (22,23). The NCCN 2014 clinical practice guidelines provide guidance for the management of high-risk patients with a hereditary cancer predisposition. In addition: "all individuals with CRC should be considered for a risk assessment with collection of family history" (24). Screening and predisposition genetic testing has introduced new opportunities along with fear of developing disease. Due the complex nature of cancer genetic testing, pre and post-test genetic counseling is recommended by NCCN, ASCO, American College of Physicians, and American College of Medical Genetics.

Qureshi *et al*. note that family history is a fundamental component of health information and therein all primary care physicians should have as a core skill the ability to take an adequate and accurate family history, even though few questionnaires have been developed for, and evaluated in, primary care settings (25). Further, few questionnaires "… have been compared with either gold standard (genetic interview) or current primary care 'standard practice' (family history as recorded in charts)…". The limited evidence, which depends on extrapolation from studies in settings other than primary care, suggests that systematic questionnaires may significantly improve the family health information gathered under current primary care practice.

While the above is essential for data gathering in the quest for a presumptive diagnosis, patients at high risk will profit immensely by being evaluated by a knowledgeable physician, genetic counselor, and/or center of genetic expertise. Hampel *et al*. discuss decision making for cancer genetics consultation, based in part on criteria from consensus statements such as those from the NCCN as well as other publications whenever guidelines have been defined (26). In the case of the LS, for example, they suggest any of the following as high risk: (I) three first-degree or second-degree relatives (SDRs) affected with any LS associated cancers, wherein all cases can occur in one generation with no age restriction; (II) one first-degree relative (FDR) or SDR with two or more LS associated cancers; (III) one FDR with CRC earlier than 50 years of age. They suggest the following as moderate risk: (I) one FDR with CRC diagnosed at age 50 or later and one SDR with CRC at any age; (II) two FDRs with CRC diagnosed at any age, including age 50 years and later. They concluded that these criteria should improve the ease of referral and add to the promotion of consistency among hereditary cancer specialty centers when evaluating patients for referral to such specialists.

The aim of Rubin *et al*. was to determine whether patients with CRC are aware of the risk to their family members and to investigate an educational intervention (27). Two hundred fifty-three CRC patients agreed to participate in the study, but only 120 (47.4%) were aware that their FDRs were at increased risk for CRC. An educational survey instrument was developed to assess patients' understanding of family risk of CRC, coupled with the importance of early surveillance, which served to educate them about CRC screening guidelines. An educational and assessment brochure was provided for patient reference as a targeted intervention. They were then contacted by telephone and requested to complete a similar survey. In primary analysis of its effectiveness, it was found that less than half acknowledged that they understood their increased risk when compared with general population expectations. In addition, 34.8% believed that their FDRs possessed the same risk of CRC as the general population. Of further interest was the finding that 14.2% believed that their FDRs were at lower risk than the general population. Among those patients who understood that their FDRs were at increased risk, "…91.7% reported that they have warned their family members about their increased CRC risk, but only 56.7% could state the correct recommended age for screening within five years".

Nearly half (45.8%) of all patients surveyed mentioned that their doctor was their source for knowledge about CRC risk with primary care physicians and gastroenterologists being the most commonly identified, followed by oncologists and surgeons. After doctors, magazines were identified as the second most likely source of information

regarding colon cancer risk (15.8%). Finally, with respect to the post-educational intervention, it was found that this did not increase patient understanding of familial CRC risk, even among those who reported reading it. This study is believed to be the first to evaluate the communication of CRC risk from a patient to an at-risk family member. Of particular significance was the finding that more than half of these patients were found to have an inadequate understanding of familial risk, coupled with the fact that a mailed educational intervention was unsuccessful in educating these patients. These findings stress that family information services, using direct patient contact, is more effective than mailed or telephoned pursuits. More research clearly is needed in this vital and potentially life-saving communication process, especially where it involves communication between family members.

Domanska *et al.* call attention to the need to identify and adequately manage patients at risk for LS, since this knowledge could be effectively translated into surveillance programs in the interest of reducing morbidity and mortality (28). These authors used a questionnaire that was answered by 67 mutation carriers and 102 physicians from a health care region in Switzerland. Both groups answered questions pertaining to CRC risk, surveillance, and genetic testing, but, unfortunately, answers about inheritance and risk for LS-associated cancers were less accurate. Unfortunately, only half of the family members and one-third of the physicians correctly estimated the risk to inherit a LS-predisposing mutation. These findings reflect the challenge to physicians in keeping up-to-date on hereditary cancer.

Wong *et al.* utilized an informatics program enabling them to link data from a prospective CRC database from four hospitals in Melbourne, Australia, wherein they were able to determine the number of patients who, on the basis of at least one risk factor for hereditary CRC, could then be considered for Familial Cancer Clinics (FCC) which enable counseling of patients and families about risk reduction strategies followed by genetic testing when appropriate (29). Their findings showed that "Of the 829 new diagnoses of CRC 228 (27.5%) would potentially have benefited from FCC referral. Of these, 50 persons (21.9%) were referred and 32 (14.0%) attended. The highest referral rates were in young, early-stage CRC patients with a family history and the lowest in late-stage and multiple-polyp patients. Patient sex, language and insurance status did not influence referral or attendance." These findings suggest that appropriate FCC referral is low and that "…certain subgroups are at particular risk of non-referral and that many referred patients do not ultimately attend. Interventions that increase referral rates and encourage attendance need to be considered."

Sweet *et al.* compared the extent to which a detailed family history was present in the physician's medical record in the setting of a touch-screen family history computer program (30). The study comprised 362 patients who were evaluated at a comprehensive cancer center ambulatory clinic over a one-year period and who voluntarily used the computer program. The computer entry was then evaluated by genetic staff and compared with the medical record for corroboration of family history findings, followed by appropriate physician assessment. Family history findings from the medical record were identifiable for comparison to the computer entry in 69% of the 362 computer entries; only 101 were assigned to a high-risk category. Yet, evidence from the records was able to confirm only 69 high-risk individuals. Furthermore, "… Documentation of physician risk assessment (i.e., notation of significant family cancer history) was found in only 14 of the high-risk charts. Only seven high-risk individuals (6.9%) had evidence of referral for genetic consultation." These findings clearly demonstrate the necessity for, and failure, for the sufficiently detailed collection of family history on all new and established patients so that an adequate CRA can be achieved.

Tyler and Snyder reviewed ambulatory records of 734 patients relevant to CRA and characterized them as suggestive of average, moderate, or high genetic risk for cancer (31). Those patients with a family history of CRC, modification of CRC screening were assessed to reflect degree of cancer risk wherein the frequency of cancer genetic referral in such high-risk patients was noted. While the family history was documented in 97.8% of the medical records, there nevertheless were insufficient findings "… to adequately assess risk in 69.5% of the charts. Detail of family cancer documentation was associated with personal history of cancer ($P<0.01$), patient age ($P<0.01$), and physician training status ($P=0.04$), but not with patient or physician gender, duration of care, or completion of a pedigree. For persons with a family history of CRC, compliance with cancer screening individualized to degree of risk was achieved in 50% of patients. Ten patients met criteria for moderate or high genetic risk for cancer. None had been offered cancer genetics consultation." The authors concluded that, while all records documented the presence or absence of a family history of cancer, nevertheless, "…in those with a positive family history, the detail of information was insufficient to permit risk assessment in over two thirds of individuals; risk-stratified colon cancer screening was

not achieved in half of the patients with a positive family history of CRC; individuals at moderate or high cancer risk were not identified as such; and those at high risk were not offered cancer genetics referral…". Clearly, family physicians must adopt explicit risk assessment criteria to enable assessment criteria that could lead to optimal care for those patients at increased hereditary cancer risk.

Ait Ouakrim *et al*. note that patients with a family history of CRC may show a substantial benefit from most kinds of screening and therein such screening could be cost effective (32,33). Specifically, CRC screening guidelines are generally more aggressive among persons with an established cancer-prone family history when compared with those who are at general population risk (34). However, in reviewing the literature, these investigators found that there is only limited information that depicts the level of screening uptake coupled with screening practices and/or the level of adherence to recommended screening guidelines. They quote the work of Rees *et al*. who comprised a review of 14 studies on the screening participation of FDRs of persons with CRC, and therein findings disclosed that only a few investigations had specifically studied screening uptake among those at increased risk through family history (35). In addition, many of these investigations were unable to provide details of the family history sufficient enough to determine if the screening was based upon risk-appropriate recommended screening intervals. Ait Ouakrim *et al*. concluded that there was a paucity of information relevant to those factors which best influence screening behavior among individuals with a strong family history of CRC (32).

Given these limitations in knowledge about screening behavior, Ait Ouakrim *et al*. used a population-based family study approach in order to estimate the CRC screening practices among unaffected Australians who were at increased familial risk (32). This enabled them to examine the association between self-reported screening behavior and socio-demographic factors. Their study involved 1,236 participants at moderately increased risk of CRC, wherein 70 (6%) "…reported having undergone guideline-defined 'appropriate' screening, 251 (20%) reported some, but less than appropriate screening, and 915 (74%) reported never having had any CRC screening test. Of the 392 participants at potentially high risk of CRC, 3 (1%) reported appropriate screening, 140 (36%) reported some, but less than appropriate screening, and 249 (64%) reported never having had any CRC screening test…". Factors associated with compliance were patients of middle age who were more highly educated and who had resided in Australia for a longer period of time. It was concluded that guidelines for CRC screening were simply not being implemented in the population and there is a dire need to implement more effective strategies for population screening.

Ait Ouakrim *et al*. report the first population-based study incorporating risk-category-specific estimates of CRC (32). The level of screening uptake was found to be low in both moderate and high-risk categories. Specifically, "…Of 1,236 participants considered at increased risk for CRC, only about one in four reported ever having a screening colonoscopy and only one in 15 screened according to published guidelines. Participation in colonoscopy screening was slight for participants at potentially high risk of CRC for whom one in three had some screening, but only about one in 130 had appropriate screening." The main strength of the Ait Ouakrim *et al*. study was their ability to examine screening participation in accordance with specific CRC risk levels as defined by family history of cancer. Attention was called to the findings of Dove-Edwin *et al*. who showed that screening is known to reduce CRC risk for persons with a positive family history (36). Furthermore, Ait Ouakrim *et al*. have shown that the majority of such persons undergo inappropriate screening or no screening at all, thereby demonstrating the loss of a potentially preventable CRC occurrence in their Australian population which, incidentally, has one of the highest incidence of CRC in the world, with more than 13,500 cases diagnosed each year and an adjusted incidence rate of 38.7 per 100,000 persons (32,37,38). Attention was called to the fact that "Medical practitioners are often not familiar with CRC screening guidelines or not proactive in implementing them (39). Given that patients' compliance with guidelines is unlikely without their doctor's influence and encouragement, we speculate that our findings remain relevant to the current Australian context, as no major or specific initiative to increase screening participation by people above average-risk of CRC has been implemented during the last decade…" (40,41).

Inherited colorectal cancer (CRC)

From a genetic perspective CRC can be grouped into three categories: sporadic (75% of cases), familial (20% of cases) and hereditary (10% of cases). Sporadic cases have no apparent indications of a hereditary component. Familial cases have a family history of CRC that suggests multifactorial hereditary factors or common exposures to non-genetic risk factors or both. Inherited highly penetrant single gene genetic mutations account for about

5% of cancer cases. This review focuses on the genetics of hereditary cancers particularly LS but also FAP and MUTYH-associated polyposis (MAP) (1).

Lynch syndrome (LS)

LS, also referred to as hereditary nonpolyposis colon cancer (HNPCC), is the most common autosomal dominant cancer predisposition syndrome responsible for about 3% of all cancer cases. Two variant forms of LS are recognized: Muir-Torre syndrome (LS and sebaceous adenomas) and Turcot syndrome (LS and glioblastoma). Patients with LS have an 80% lifetime risk of CRC and women have a 60% risk of endometrial cancer. In addition they have an elevated risk of other cancers including stomach, biliary, ovarian and urogenital cancers. Rare individuals who inherit biallelic mis-match repair gene mutations have severe disease often presenting in childhood with hematologic cancers, brain tumors and early onset colon cancer, a condition referred to as constitutional MMR deficiency syndrome (42,43).

LS has the following cardinal features (44,45):

- Early age of cancer onset;
- Proximal colon involvement of CRC;
- Increased incidence of synchronous and metachronous CRCs;
- Autosomal dominant inheritance pattern and MMR germline mutation, most common of which are *MSH2*, *MutL*, *E. Coli*, *Homolog of*, *1*, and *MutS*, *E. Coli*, *Homolog of*, *6*;
- An excess of extracolonic adenocarcinomas;
- Frequent occurrence of distinctive pathologic features;
- Increased survival from CRCs (33);
- Accelerated carcinogenesis and interval CRC.

The LS is characterized by a defect in the mis-match repair process. This is a specific type of DNA repair involving the identification and repair of mis-incorporation of bases, largely due to replication and recombination, but also some forms of DNA damage (46,47). This specific DNA repair defect offers a very exact screening method for inherited colon cancer, namely "microsatellite instability", or MSI. Testing involves comparing tumor and non-tumor tissue for changes in size of stretches of poly-nucleotides, which are particularly prone to the same type of insertion/deletion mutations that result from aberrant DNA replication. While all forms of LS include defects in mismatch repair the reverse is not true, as this repair pathway may be impaired through somatic (non-inherited) mutations, such as aberrant methylation, aberrant expression of other genes in the MMR pathway, degradation of mRNA via targeted microRNA overexpression (48). Inherited mutations are detected by sequencing the entire set of genes: (I) MLH1 located on chromosome 3p21.3 accounts for 50% of cases; (II) MSH2 located on chromosome 2p22 accounts for 40% of cases; (III) MSH6 located on chromosome 2p16 accounts for 7% of cases and PMS2 located on chromosome 7p22 accounts for less than 5% of cases; (IV) epithelial cellular adhesion molecule gene (also called tumor-associated calcium signal transducer 1) located upstream of MSH2 on chromosome 2p21 accounts for 1-3% of cases and can lead to inherited epigenetic silencing of MSH2 (43,49,50). For this reason, it is suggested that sequencing also include a substantial portion of neighboring DNA. Somatic (i.e., non-inherited) inactivation of the MLH1 gene can occur by methylation and often results in an absence of this protein that can be detected through immunohistochemistry but is not as reliable a test when compared to MSI as the protein may be present but is non-functional. An abnormal MSI or IHC result is used to determine the appropriate next set of tests, either germline testing or BRAF and MLH1 promoter methylation analysis (43,51).

About 15% of sporadic CRCs will manifest MSI along with absent MLH1 and PMS2 expression on IHC. In sporadic tumors the loss of MLH1 results from methylation of the MLH1 promoter in the somatic cells of the tumor only and not in the patient's normal cells. The absent PMS2 expression results because PMS2 normally forms a stable complex with MLH1 and in the absence of MLH1 PMS2 is unstable and degraded. Also, over half of sporadic tumors with loss of MLH1 expression have a mutation in the BRAF gene (p.V600E), a mutation that is not found in patients with LS associated cancers (43,49,51).

For germline mutation analysis Sanger sequencing, as opposed to "next-generation" sequencing, of all coding exons and flanking intronic regions of all MMR genes is the gold standard for mutation detection. In the case of PMS2 the presence of multiple highly homologous pseudogenes is problematic and necessitates the need for locus-specific, long range PCR amplification. Special methods are used for detection of large gene rearrangements (such as deletions or duplications of entire exons) as these lie beyond the limits of sequencing technologies. Such rearrangements are not uncommon especially in the MSH2 gene. The multiplex ligation-dependent probe amplification (MLPA) test is often used to detect large rearrangements. If only one exon is deleted by MLPA analysis using a second confirmatory test (for example, real-time PCR) is often used to confirm

the result. Comparative genomic hybridization (CGH) with gene-targeted arrays can also be used for detection of exon deletions or duplications (43,49,51).

A deletion of the 3'-end of the EpCAM gene located upstream of MSH2 causes transcriptional read through from the EpCAM gene and resultant silencing of the MSH2 gene by promoter methylation. EpCAM 3' exon deletions are readily detected by MLPA analysis. EpCAM deletion carriers have a risk of CRC similar to that of MSH2 mutation carriers. The risk of endometrial cancer in female EpCAM carriers is lower but if the deletion extends close to the MSH2 gene the risk of endometrial is much increased. The EpCAM gene encodes the epithelial cell adhesion molecule expressed exclusively in epithelial tissues. Since EpCAM is expressed only in epithelial tissues there is considerable mosaic expression of MSH2 hypermethylation in EpCAM deletion carriers (52). This can lead to complications in evaluating IHC results.

Mutations identified in MMR genes are classified as pathogenic (deleterious), benign or as a variant of uncertain significance (VUS). These variants are usually single nucleotide substitutions causing a missense mutation or a single nucleotide variant located near a splice consensus sequence. Factors such as the frequency of the variant in the normal population, family segregation studies, the nature of the missense substitution (for example, a nonconservative substitution involving an evolutionarily conserved amino acid), and in silico tools (such as SIFT or Polyphen) may be helpful in classifying a VUS. RNA analysis can be useful in determining the significance of splice variants as well as in silico software to predict the effects of variants on RNA splicing (SpliceSite Finder) (53).

Epigenetics and Lynch syndrome (LS)

Epigenetics refers to heritable changes in gene expression that occur independently of changes in the DNA sequence (54). Epigenetic mechanisms often involve DNA methylation and chromatin remodeling through histone modifications and non-coding RNAs (such as microRNAs). A constitutional epimutation is an epigenetic aberration, found in all cells that usually involve promoter hypermethylation that leads to silencing of the gene. The identification of epimutations in MLH1 and MSH2 in LS families has brought to light the important role of epigenetic mechanisms in cancer development. Epimutations may be primary or secondary. Primary epimutations have been identified in MLH1 and they show unpredictable, non-Mendelian inheritance patterns varying from apparent heritability to reversion to the normal state in successive generations. Secondary epimutations result from indirect genetic alterations that activate epigenetic factors to cause gene methylation and silencing. A classic example of a secondary epimutation is the EpCAM deletion, which results in a read-through transcript that induces hypermethylation of the MSH2 gene. Secondary epimutations have also been identified in the MLH1 gene. In contrast to primary epimutations, secondary epimutations in MLH1 and MSH2 show Mendelian autosomal dominant inheritance because they result from genetic alterations. Future challenges involve understanding the basic mechanisms involved in primary (or reversible) and secondary (or dominant) epimutations. Until the mechanisms are more clearly defined, family members of individuals with epimutations should be offered methylation testing to determine their carrier status (55,56).

There currently are no methods of detecting carriers of LS, short of searching for specific mutations once an affected family member has been tested. Universal LS screening propels genetics into the primary care arena, by identifying individuals with a hereditary predisposition towards LS. NCCN 2014 endorses Universal Lynch screening either by testing all tumors or all <70 plus those who meet the Bethesda criteria since guidelines such as the Amsterdam and Bethesda criteria fail to identify 50% of individuals with LS (24). The cost effectiveness of Universal LS Screening is further realized with the expansion of cancer risks for family members (57).

Familial adenomatous polyposis (FAP)

FAP is an autosomal dominant disease caused by mutations in the APC gene located on chromosome 5p22.2 and characterized by large numbers of adenomatous polyps (hundreds to thousands) throughout the colon. A variant of FAP called attenuated FAP (AFAP) is characterized by less than 100 colon polyps and the onset of polyposis and cancer occurs later than in FAP. The APC gene encodes a large protein (2,843 amino acids) with multiple cellular functions including its role in the wnt-signaling pathway, a role in intercellular adhesion and in microtubule assembly and stabilization. A number of variable features may be associated with FAP including congenital hypertrophy of the retinal pigment epithelium (CHRPE, 60% of families), upper gastrointestinal tumors (especially periampullary carcinoma), epidermoid cysts, osteomas and desmoids tumors (58,59).

Over 1,500 mutations leading to FAP have been identified in the APC gene, the majority being nonsense (28%) or frameshift (small deletions, 46% or insertions, 10%) mutations producing a truncated and defective protein. Gross deletions or duplications of the APC gene account for about 10-15% of mutations. In addition, the new mutation rate for APC is reported to be about 20%. In about 30% of FAP cases mutations involve codon 1061 and codon 1309; germline mutations rarely occur beyond codon 1600. However, mutations associated with AFAP often occur in the 5'-part of the gene (exons 1-4) and in the 3' part of exon 15 (49). Standard APC gene testing involves full sequence analysis; if no mutation is identified testing for gross gene deletions or duplications is done by MLPA analysis. A mutation is detected in about 80% of classic FAP patients by sequencing and in an additional 10-15% of patients a mutation is detected by MLPA analysis. The penetrance of APC mutations is almost 100% (49).

The significance of missense variants (VUS) in the pathogenesis of FAP is unclear. One particular missense variant found in about 6% of those of Ashkenazi Jewish ancestry is associated with a several-fold higher risk for development of colon adenomas and CRC. Testing for this variant is appropriate only for people of Ashkenazi Jewish ancestry and early screening is recommended for those who test positive (49,59).

MUTYH-associated polyposis (MAP)

Some patients who present with a low number of polyps without affected parents may have the syndrome of MAP. MAP shows autosomal recessive inheritance and results from biallelic mutation of the MutY, E. Coli, Homolog of (also referred to as MutY, E. Coli, Homolog of) gene which functions to remove adenine residues mispaired with 8-hydroxyguanine in DNA (49). The majority of the mutations detected (over 100 to date) are point mutations (nonsense, missense, or small insertions or deletions). Two common missense mutations (p.Y165C and p.G382D) account for about 70% of the mutant alleles in a Northern European population. About 1-2% of the general population is thought to carry a MUTYH mutation (49).

Chemoprevention

In addition to endoscopic surveillance, chemoprevention of CRC appears promising. Non-steroidal anti-inflammatory agents were shown to reduce the occurrence of adenomas in FAP (60,61). Recent reports of ongoing studies show the promise of Cox-1 inhibitors (Asprin) in CRC prevention (62,63). The Pharmacogenomics of aspirin metabolism shows promising results (64).

Summary

The successful incorporation of genetics in CRC prevention and treatment has the potential to greatly reduce the burden of disease. Ideally, healthcare providers must include detailed extended family histories, and discuss all the technical information currently available. A team approach involving clear communication between the healthcare specialists is optimal. Technological advances help to improve personalized care through triage and stratification but risk alienating patients' understanding due to the increased use of scientific jargon. The goals of genetic counseling are to educate the individual and their family regarding the natural history of the disease and hereditary predisposition, reduce anxiety related to that risk, and provide the tools aimed at prevention. It is our hope that this two-part manuscript will enable more providers to become a partner in CRC prevention.

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Footnote

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

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The evolution of colorectal cancer genetics—Part 2: clinical implications and applications

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Abstract: The genetic understanding of colorectal cancer (CRC) continues to grow, and it is now estimated that 10% of the population has a known hereditary CRC syndrome. This article will examine the evolving surgical and medical management of hereditary CRC syndromes, and the impact of tumor genetics on therapy. This review will focus on the most common hereditary CRC-prone diseases seen in clinical practice, which include Lynch syndrome (LS), familial adenomatous polyposis (FAP) & attenuated FAP (AFAP), MutYH-associated polyposis (MAP), and serrated polyposis syndrome (SPS). Each section will review the current recommendations in the evaluation and treatment of these syndromes, as well as review surgical management and operative planning. A highly detailed multigeneration cancer family history with verified genealogy and pathology documentation whenever possible, coupled with germline mutation testing when indicated, is critically important to management decisions. Although caring for patients with these syndromes remains complex, the application of this knowledge facilitates better treatment of both individuals and their affected family members for generations to come.

Keywords: Surgical oncologic management; medical oncologic management; hereditary colorectal cancer (hereditary CRC)

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Introduction

A historical review and summary of the molecular basis of hereditary colorectal cancer (CRC) has been previously discussed in part 1 of this volume. This article will examine the evolving surgical and medical management of hereditary CRC syndromes, and the potential impact of tumor genetics on therapy. CRC is the third most common cause of cancer death in the world with an estimated incidence of over one million cases per year (1). Advancements in colonoscopy have reduced the incidence of CRC by 45-77% and have recently been reported to have reduced mortality by greater than 30% since 1990 (2). The genetic understanding of CRC also continues to grow, and it is now estimated that 2-10% of the population has a known hereditary CRC syndrome. In addition, there are 20-30% of CRC cases with evidence of a familial component, but without a clear hereditary disease identified (1,3,4). Prior to identifying genetic mutations, the diagnosis of a familial cancer syndrome was based on highly-targeted clinical and family history alone (5,6). Now that surgical and medical management of this disease can often be based on pathological variants in the patient's DNA, the physician's suspicion for a hereditary component of CRC in high-risk patients should be greater (6). This knowledge provides the

indication for early endoscopic and/or surgical intervention, and plays a role in guiding adjuvant chemotherapy. This approach may not only prevent or treat CRC for the individual, but also allows for the care of the entire family (7).

This review will discuss the indications and recommendations for the surgical and medical oncologic management of hereditary colorectal cancer syndromes. It will emphasize how this knowledge can be used in formulating an operative plan, and decision making for adjuvant therapy. We will focus upon the most common hereditary diseases seen in clinical practice, which include Lynch syndrome (LS), familial adenomatous polyposis (FAP) and attenuated FAP (AFAP), MutYH-associated polyposis (MAP), and serrated polyposis syndrome (SPS).

Lynch syndrome (LS)

LS is an autosomal dominant condition that results from a defect in one of the mismatch repair (MMR) genes and in the past was also referred to as Hereditary Nonpolyposis Colorectal Cancer (HNPCC). It is the most common hereditary CRC syndrome, and is characterized by the early presentation of colorectal, endometrial, and various other cancers (8). The MMR genes clearly involved in the development of LS are *MLH1*, *MSH2*, *MSH6* and *PMS2*, with other gene candidates such as *EPCAM* and epimutations being evaluated. The surgical options for CRC in the setting of LS previously ranged from segmental colectomy, to subtotal colectomy, and even total proctocolectomy (TPC). Presently, our preferred option is total colectomy for a colonic cancer or endoscopically unresectable advanced adenoma, and TPC for patients with the less frequent presentation of a primary rectal neoplasm.

Prior to the early 1990's when pathologic variants were discovered, some of which were distinctive of LS, the diagnosis and management of LS was based on a clinical assessment using the Amsterdam criteria, which have been modified over time (8,9). However, identifying patients preoperatively who are not parts of a known LS family is often challenging. These patients are typically young, often present with locally advanced tumors, with associated bleeding, or obstruction, and not infrequently harbor multiple primary extracolonic cancers, particularly endometrial cancer. These patients frequently require timely operative intervention without the ability to wait for genetic testing (10,11). LS associated cancer has been reported in 15% of CRC patients less than 50 years old (11). Baiocchi *et al.* used immunohistochemical (IHC) testing to retrospectively compare patients with CRC both above and below the age of 50 (10). They analyzed previous specimens for *MLH1*, *MSH2* and *MSH6* expression and determined that 51% of CRC patients did not express at least one of these MMR proteins, and therefore were a likely LS carrier. Furthermore, after establishing a diagnosis of LS by IHC, retrospectively, only 31% of the LS patients met the Amsterdam II criteria, and only 50% met the Bethesda criteria, findings which demonstrate the importance of molecular analysis. This study may even underestimate the incidence of LS due to not analyzing PMS2 and microsatellite instability (MSI). However, an abnormal test for *MLH1* should be interpreted with caution. Based on a false negative rate of 5-10% by using IHC, the current National Comprehensive Cancer Network Guidelines recommend that abnormal *MLH1* IHC should be followed by testing the tumor for BRAF V600E or hypermethylation of the *MLH1* promoter, as this pattern has been identified in spontaneous colon tumors (12,13). Germline genetic analysis should always be performed; however, IHC is a simple, cheap, and rapid way of determining LS in a patient who may not have the time to wait for the germline testing to be completed (10).

CRC surgical implications

There are three main groups of patients who would require a colectomy: (I) newly diagnosed colon cancer patients with or without a known personal or family history of LS; (II) patients with a LS affected family member; and (III) LS patients considering prophylactic colectomy, particularly those harboring MMR deleterious mutations and who decline recommended colonoscopy. The complex decision making process for the surgical options in LS must consider the risk of a synchronous and/or metachronous CRC, the risk of the operation, and the expected alteration of the patient's quality of life (QOL) particularly with a rectal primary cancer.

The risk of synchronous CRC in LS has been reported to be approximately 6-18%. The risks of metachronous tumors are reported to be 16% at 10 years, 41% at 20 years and 62% at 30 years after segmental resection (14,15). More recently Cirillo *et al.* identified a risk of at least one metachronous CRC after a median of 6 years. This was broken down into colon and rectal cancers with a risk of a metachronous tumor of 22.2% and 27.7% respectively. A proportional hazards model in the development of a metachronous CRC showed a 6-fold increase in the risk of death, with the metachronous cancer at a greater risk of being diagnosed at either stage III or IV (16).

Colon cancer is identified more commonly than rectal cancer in LS. However, rectal cancer occurs approximately 11-35% of the time, when one includes both synchronous and metachronous lesions (16,17). It is our opinion that a newly diagnosed CRC is best managed by a total abdominal colectomy. However, in the setting of significant metastatic disease a segmental colectomy may be offered. A low anterior resection, abdominoperineal resection or ileal pouch-anal anastomosis can be performed for rectal cancer, but the operation should be tailored to the patient (14,18). Total abdominal colectomy can be considered both therapeutic and prophylactic, given the high rate of metachronous CRC (16,18). Furthermore, removing the entire colon allows for easier outpatient intensive surveillance of the rectum. Parry and colleagues demonstrated that with every 10 cm of bowel removed in a LS patient, there is a reduction in their risk of metachronous CRC by 31% (15). Although a survival benefit has yet to be shown, multiple studies now advocate for an extended resection for patients with LS (10,15,16,19,20).

To further support the indication for extended resection, Natarajan and colleagues compared prophylactic colectomy, or extended resection, at the time of an initial CRC diagnosis, with a segmental resection (19). There was a longer time period to develop a second CRC after extended resection compared to segmental resection, (16-175 *vs.* 6-160 months respectively). In addition, the segmental resection group required a second operation sooner, (4-195 *vs.* 7-275 months). Subsequent operations were due to complications from the initial operation, treatment of a second primary or metachronous lesion, or endometrial/ovarian cancer. Although the risk of a metachronous lesion was less with subtotal colectomy, this study also did not demonstrate a survival difference (19).

The QOL following subtotal colectomy versus segmental resection is also a significant patient concern. Haanstra *et al.* in 2012 evaluated the effect of extended resection on functional outcomes and QOL. This study excluded cases of rectal cancer, end ileostomy, and ileal pouch-anal anastomosis. Patients were evaluated with QOL questionnaires. To assess generic QOL the Short Form-36 health survey was used; to evaluate disease-specific QOL the European Organization for Research and Treatment of Cancer Colorectal Cancer-specific Quality of Life Questionnaire Module was used; and to determine functional QOL the Colorectal Functional Outcome questionnaire was used. Subtotal colectomy patients had a significantly greater frequency of bowel movements as well as a worse functional outcome; however there was no difference in QOL impacted by multiple bowel movements. This study supports the use of an extended resection for a LS associated colon cancer (21).

The management of postoperative patients who received a segmental colectomy for a Lynch related colon cancer is often encountered in situations where LS was not suspected pre-operatively, or when a resection was the patient's preference. It is critical to counsel the patients on their risk of metachronous tumors, with the current options being frequent colonoscopic surveillance or completion colectomy (14,22). For patients who do not receive a completion colectomy, postoperative endoscopic surveillance is critical to survival, and close interval follow up is important since the transition from adenoma to carcinoma in LS is faster (3 *vs.* 8-15 years in sporadic CRC) (18). A subsequent study demonstrated that the median time from the diagnosis of CRC and the most recent colonoscopy was 11.3 months; therefore, this data supports surveillance at least once a year with a full clearing colonoscopy (23). It is our practice to add narrow band imaging to the surveillance colonoscopy, as it has been shown to increase the detection of additional adenomas by 27%, and to improve the detection rate of flat adenomas from 12% to 45% when compared to standard colonoscopy (24).

The risk of CRC in LS is approximately 60-85% depending on which MMR gene is involved. Patients with *MLH1* and *MSH2* mutations have a higher risk of cancer, with diagnosis at a younger age, compared to *MSH6* and *PMS2* mutations (25,26). *MLH1* mutation carriers have a higher risk of CRC, while *MSH2* carriers have a higher rate of multiple primary extracolonic cancers, to include brain (glioblastoma), ovarian, stomach, hepatobiliary, urinary tract, breast, and prostate cancers (27-32). Colonoscopy screening decreases the risk of a second CRC by 62% when patients have routine surveillance (33). It is rare for colonoscopy to miss a polyp >10 mm. However, for polyps between 1-5 mm, up to 35% can be overlooked (34). With this knowledge, prophylactic colectomy may be ideal for some patients, requiring only a subsequent yearly rectal surveillance. Prophylactic colectomy before the age of 25 has been associated with the greatest increase in life expectancy when compared to older patients and those where surgery was performed after a CRC diagnosis (35). It is still widely debated about recommendations for a prophylactic colectomy. It is important to evaluate the patient for both emotional and physical perspectives, understand his or her MMR mutation status, and ensure that a genetic counselor is actively engaged with the decision making process. In women who present with uterine cancer,

prophylactic colectomy can be considered in addition to the surgical treatment of gynecologic diseases, if the patient is being managed in a comprehensive manner (36). Prophylactic hysterectomy and bilateral salpingo-oophorectomy is a prudent option given limited endometrial and extremely poor ovarian cancer screening (36).

Familial adenomatous polyposis (FAP)

FAP is an autosomal dominant syndrome found in less than 1% of all CRCs, but will progress on to colon cancer nearly 100% of the time (26). FAP results from a mutation of the adenomatous polyposis coli (*APC*) gene located on chromosome 5q21-22 (37). The genotypic understanding of the APC mutation is clinically relevant to the phenotypic presentation. Classic FAP occurs when there are mutations between codons 168-1,580 with severe disease between codons 1250-1464 (38). Classical FAP is defined clinically if there are 100 or more adenomatous colon and rectal polyps, and typically occurs in patients younger than age 40. AFAP is generally defined in individuals with 10-99 colonic adenomatous polyps, or those with 100 or more colonic polyps occurring at an older age, or those with a history of CRC before age 60 and a family history of multiple adenomatous polyps (39,40). The latter group of patients will usually have rectal sparing, have right-sided colonic adenomas, and lack extra colonic manifestations (37). Because of the greater genotypic and phenotypic variability in AFAP, a high clinical suspicion and thorough family history is critical for the diagnosis, as these patients frequently, if not always, progress on to a colon cancer (37). Although there have been multiple studies describing the sequence and the location of the APC gene, the significance of a single amino acid missense variants in the APC gene is difficult to interpret (37). With the advent of genetic testing, it has become important to characterize these variants in order to properly counsel and treat FAP patients, particularly those patients with AFAP.

The treatment for FAP is surgical removal of the colon and rectum. The options for surgery include abdominal colectomy with ileorectal anastomosis (IRA), restorative proctocolectomy with either ileal J-pouch anal anastomosis (IPAA), and TPC and ileostomy (14,41). Patients are recommended to undergo this procedure during their teens or early twenties. Initial screening colonoscopy has been recommended to begin at age 10 years, but Kennedy and colleagues recommends that the ideal time at 7 years old. In their series the average age for colectomy was at 15.4 years old with the youngest patient being four. The majority of operations performed in this group were IPAA, with 88% having a hand sewn anal anastomosis with a mucosectomy. This series had no recurrences following IPAA and routine surveillance pouchoscopy was recommended (42).

If there is limited rectal polyposis, then IRA is a feasible option. This is also recommended in woman of child-bearing age, as this operation can be converted to an IPAA once child bearing has been completed (43). The risk of developing rectal cancer in a patient undergoing IRA increases from 4% at 5 years to up to 25% at 20 years. This data has even lead to recommendations of patients having their IRA converted to IPAA before the age of 50 (44). The number of rectal polyps and the presence of rectal cancer should be the main factor in the determination of whether or not to perform a proctectomy. As with LS, the functional outcomes following IPAA are of a significant concern for patients when making these operative decisions. In a meta-analysis performed by Aziz *et al.*, no difference was found in postoperative compilations, though IRA required a significantly lower rate of reoperations within 30 days (44). IPAA demonstrated superior results in cancer reduction: 0% *vs.* 5.5% following IRA. There was a decrease in the long-term need for reoperation in IPAA group. There was no difference in dietary restrictions or sexual dysfunction, although patients with IRA had a lower incidence of social restrictions compared to IPAA, 4% *vs.* 14%. Furthermore the frequency of daily stools, need for night defecation, and incontinence over 24 hours, was greater in IPAA (44). Recent studies advocate for a laparoscopic approach, and have demonstrated an association with fewer postoperative complications, better overall outcomes, and shorter length of stay. However, this operation requires technical expertise, and a large volume of cases to maintain this skill. Future studies are needed to further elucidate these findings (45-48).

MutYH-associated polyposis (MAP)

MAP was discovered in 2002 when studying patients who appeared to meet clinical criteria for FAP but tested negative for a defect in the *APC* gene. Further testing identified a biallelic mutation in the *MYH* gene, which produced an autosomal recessive polyposis syndrome. *MYH* mutations can vary with ethnicity, and phenotypically this disease can mimic FAP. It has been shown that 7.5% of classical FAP that was negative for an APC mutation had a biallelic *MYH* mutation (49). Biochemically, the MYH gene repairs DNA mutations damaged by reactive oxygen species. It typically presents as FAP with multiple colon adenomas, though it can also result in a MMR gene mutation and present

similar to LS. The diagnostic confusion makes the surgical recommendations challenging (50). Due to the rarity and complexity of the diagnosis of this disease, referral to a genetic counselor is recommended for the optimal care of these patients.

Leite *et al.* evaluated the incidence of germline *MYH* mutations in 19 APC-mutation negative patients. They found 69% of patients registered as classical FAP and 17% registered as AFAP to actually have a *MYH* mutation. All ten patients in this series with a *MYH* mutation had surgical resection, which included: five total colectomies, four restorative proctocolectomies and one left partial colectomy. The patient with a partial colectomy eventually underwent a completion colectomy. Two patients had a prophylactic colectomy prior to the diagnosis of cancer. Ten patients had a diagnosis of CRC and three of these patients also had a synchronous or metachronous lesion. The mean age to the development of CRC cancer was 50.6 years, which is about 10 years later than classical FAP. Although the number of patients identified in this study is low, the data suggests that screening alone with polypectomy is not sufficient for these patients, and they should be treated as AFAP including consideration for a prophylactic colectomy. The timing of prophylactic colectomy, however, may be later than AFAP based on this study (51).

Serrated polyposis syndrome (SPS)

Serrated polyps, previously called hyperplasic polyps, are characterized by the serrated appearance of the crypt epithelium on histology. These lesions were previously thought to be benign, but recent data shows a 15-20% risk of CRC arising through this serrated neoplasia pathway. Gene inactivation through hypermethylation of a promoter region, BRAF mutations, or MSI is thought to be the molecular etiology of this syndrome (52). SPS is characterized by: (I) ≥5 serrated polyps proximal to the sigmoid colon with at least 2 greater than 10 mm; (II) a least one serrated polyp proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis; (III) >20 serrated polyps of any size but distributed throughout the colon (53,54).

Jasperson *et al.* analyzed 51 patients with SPS. The average age of diagnosis was 49 years old. The average number of serrated polyps identified was 35, and 71% had at least one greater than 10 mm. Adenomas were identified in 82% of patients, and CRC in 16%, with the earliest age of onset at 22 years. This study advocates for full colonoscopy every 1-2 years for surveillance and endoscopic treatment as needed due to the development of cancer. Surgical management is considered when polyps cannot be endoscopically controlled, or if there are numerous large serrated lesions in the proximal aspect of the colon (52,53).

Hazewinkel *et al.* also supports annual surveillance colonoscopy for serrated polyposis. Recurrence rates were 80% if the serrated polyp was ≥3 mm. It took, on average, two procedures to completely clear the colon. Advanced adenomas were detected in 9% of patients, with a median interval of 13 months between detection and the previous clearing colonoscopy. Prophylactic resection was performed in 8% of SPS patients after clearing colonoscopy. These patients received a subtotal colectomy with IRA, which is the recommended resection (52,53,55,56).

Familial CRC-type X

Familial CRC-type X or "syndrome X" involves patients who clinically meet all criteria for LS, but have neither MSI nor an expression of a MMR mutation, and therefore are not genetically defined LS (3,6,25). These patients have a lower incidence of CRC than LS patients, but a greater incidence than the general population (25). The mean age of diagnosis is later than in LS patients, but approximately 10 years younger than in spontaneous cases (57,58). Tumors are found mainly in the left colon or rectum, and there is a lower association of tumors with mucinous features (3). There are no current reports of extracolonic neoplasia in these patients (5). Without more knowledge of the molecular nature of this disease, there are no genetically based current guidelines or recommendations for surgical management.

Chemotherapy implications

CRC genetics has the potential to influence screening, prevention, treatment and survivorship. At this juncture, the current knowledge of CRC genetics has an impact on the therapy of both adjuvant and metastatic disease. While multiple molecular markers and gene expression assays have been studied, only MSI has prognostic value. A survival benefit has been demonstrated when comparing hereditary CRC, to include FAP and LS to sporadic cases, and this benefit is more pronounced for patients with LS. This benefit was previously thought to be due to selection bias, younger age and/or more aggressive screening (59-62). Bertario *et al.* demonstrated in a study group of over two thousand patients no survival difference in LS and FAP compared to sporadic cases (62). This study further analyzed

patients under 60 years old, which again showed comparable outcomes between the groups. To counter these findings, Stigliano and colleagues retrospectively compared survival between LS and sporadic CRC. This study demonstrated an improved five year survival with LS CRC compared to spontaneous cases (94.2% *vs.* 75.3%; P>0.01). Interestingly this study was able to show that all tumors that demonstrated MSI had a 100% survival, suggesting that MSI plays a critical role in the prognosis of colon cancer (63).

MSI may result from germline mutations in MMR genes *MLH1*, *MSH2*, *MSH 6* or *PMS2* and EpCam. Somatic mutations in these genes may occur in up to 20% of sporadic CRC patients and hypermethylation of *MLH1* results in gene inactivation in 50% of cases. These mutations will result in LS and these cases are classified as either MSI-H or MSI-L (high or low).

MSI-H tumors are more common in stage II patients (20%) and proximal colon (29%). Evidence suggests that MSI-H stage II colon cancer patients do not benefit for adjuvant chemotherapy with a fluoropyrimidine, and tumors treated with these agents may even have a worse outcome (64-66). Stage II MSI-H patients with adverse clinicopathologic features such as obstruction and perforation should be counseled regarding the benefit of adjuvant chemotherapy. However, a recent analysis of the Mosaic Trial demonstrated a benefit to the combination of oxaliplatin and infusional 5-FU/leucovorin in stage III MSI-H patients (67).

Five gene expression profiling assays are marketed in the US. Of these the 12 gene recurrence score (oncotype-DX Colon Cancer Assay) has the most data and validation (68). This assay has prognostic but not predictive value, and unlike MSI-L is not endorsed by the NCCN for routine decision making in stage II patients. This assay may, however, be useful in the counseling of MSI-L patient who have other risk factors.

Genetic based treatment has been established and widely accepted in patients with metastatic CRC. Epidermal growth factor receptor (EGFR) overexpression is seen in more than 50% of patients. This however, does not correlate with response to treatment with targeted inhibitors against a downstream EGFR signaling pathway (69). Tumors that are KRAS exon 2 wild types have a higher response rate to the EGFR inhibitors cetuximab and panitumumab. KRAS activating mutations are associated with resistance to these agents and are seen in approximately 40% of patients with metastatic CRC (70,71). Due to these findings, the American Society of Clinical Oncology issued a provisional clinical opinion that testing for KRAS gene mutations in patients with metastatic colorectal carcinoma should be performed to predict response to anti-EGFR monoclonal antibody therapy. Furthermore, metastatic CRC patients with KRAS exon 2 codon 12 or 13 mutations should not receive an EGFR inhibitor as part of their treatment (72).

Even though wild type KRAS is necessary for a response to anti-EGFR agents, it may not be sufficient in up to 20-40% of cases. In the PRIME study, 17% of patients without KRAS exon 2 mutations had mutations in KRAS exons 3 and 4 or exons 2.3 and 4 on NRAS. Panitumumab based treatment had an inferior progression free and overall survival in combination with FOLFOX versus the chemotherapy arm alone. A recent meta-analysis looked at 22 studies that included 2,395 patients. It concluded that further examination of downstream mutations in KRAS exons 3 and 4, NRAS, BRAF and PIK3CA and non-functional PTEN are able to demonstrate resistance to anti-EGFR therapies. This study suggests that biomarker analysis beyond KRAS exon 2 should be implemented for prediction of a clinical benefit of anti-EGFR antibodies in metastatic CRC (73). NCCN currently recommends performing genotyping for RAS mutations to include the exon 2 and non-exon 2 for KRAS, and NRAS. The guidelines further state that there was insufficient information in the use of BRAF V600E mutation, which is downstream of KRAS, as a status to guide anti-EGFR therapy. BRAF can be prognostic for adverse overall progression free and overall survival in the adjuvant setting but are less predictive for response to treatment (74).

Conclusions

Advances made in screening, diagnosing and treating CRC, progressively increases our understanding of CRC tumors with respect to their genome, biome, and proteome, and ultimately clinical outcomes. With further study and subsequent elucidation of the molecular basis and biologic mechanisms of CRC, the application of this knowledge holds the promise to better treat not just a general disease, but each individual disease. In essence, a molecular based prescription for optimal care. Furthermore, the application of a multidisciplinary approach to the evaluation and management of these syndromes has fundamentally changed the best practices used to help not just a single individual, but entire families for generations to come.

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Footnote

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

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Genomic approach to translational studies in colorectal cancer

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Abstract: Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. The pathogenesis of CRC is complex with molecular subtypes defined by pathways of sequential (epi-) genetic alterations and different forms of genomic instability. The management of CRC has evolved considerably over the past decade, consisting of surgery and treatment with radiation, chemotherapy or molecularly targeted agents. Population screening is progressively being introduced for early detection of disease. Decisions to use a particular treatment are principally related to primary site, TNM stage and comorbidity of the patient. Advances in the development of microarray and next-generation sequencing (NGS) technologies have enabled the possibility of personalized medicine for CRC, revolutionizing our knowledge of tumor biology, identifying prognostic and predictive biomarkers, and contributing new tools for diagnosis and surveillance. Genomic studies have identified new cancer driver genes and druggable targets, revealed substantial inter- and intra-tumor (epi-) genetic heterogeneity, and highlighted the importance of cell-of-origin, differentiation hierarchy, phenotypic plasticity and stromal contribution for tumor clinical behavior. Here, we review results of recent translational studies of the CRC genome, transcriptome, methylome and miRNAome, with a focus on tumor classification, diagnostic, prognostic and predictive findings.

Keywords: Colorectal cancer (CRC); genomic; methylation; microRNA; transcriptome

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Introduction

Colorectal cancer (CRC) is the third most common malignant neoplasm worldwide and a leading cause of cancer-related morbidity and mortality (1). Metastatic CRC (also called stage IV or advanced CRC) is the principal cause of death, but if cancer is detected at early stages curative treatment is often possible. Surgery is the primary form of treatment and results in cure for ~60% of patients with localized (stage I-III) disease (2,3). However, recurrence following surgery remains a major problem, and patients with lymph-node positive stage III and high-risk stage II disease are offered fluoropyrimidine-based adjuvant chemotherapy (5-fluorouracil, capecitabine) with or without oxaliplatin. Rectal cancers may also receive pre-operative chemoradiation. Recurrent tumor can develop in the bowel or at distant sites including the liver, lung, peritoneum, brain and bone (2,3). Guidelines for post-surgery surveillance recommend a combination of clinical assessment, serum carcinoembryonic antigen (CEA) testing, colonoscopy and computed tomography (CT) scanning (4-6). In current practice, many CRC patients receive adjuvant therapy unnecessarily, either because they were cured by surgery alone, or because they will relapse despite treatment. Conversely, some stage II patients with low-risk clinicopathological features who are currently not considered for adjuvant therapy do relapse and might benefit from therapy.

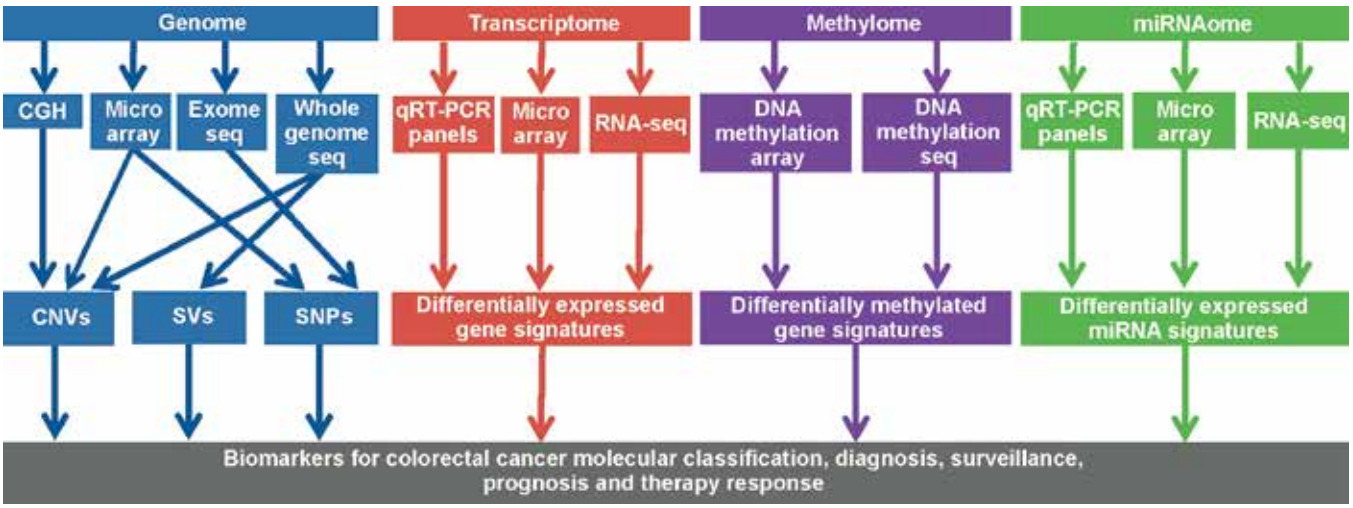

Figure 1 Genomic technologies used to uncover biomarkers for colorectal cancer molecular classification, diagnosis, prognosis, surveillance and therapy response. CGH, comparative genomic hybridisation; CNVs, copy number variations; SVs, structural variations; SNPs, single nucleotide polymorphisms; qRT-PCR, quantitative real-time PCR; miRNA, microRNA.

Outcomes from metastatic CRC remain poor, with a 5-year survival rate of less than 20% (7). Curative surgery is only rarely possible in these patients, but an increase in therapeutic options has resulted in an improvement of median overall survival to ~24 months. Approved agents include standard chemotherapeutics (5-fluorouracil, capecitabine, oxaliplatin, irinotecan) and targeted therapies directed against the epidermal growth factor receptor (EGFR) (cetuximab, panitumumab) or angiogenesis (bevacizumab, aflibercept, regorafenib). Although these treatments have prolonged the lives of patients with metastatic CRC, clinical responses are limited to a subset of individuals and are generally short-lived with most tumors developing resistance within a few months. Significant side effects and costs are associated with these treatments, and identification of individuals who are likely to derive the greatest benefit remains a major challenge.

Many patients with CRC will remain asymptomatic until the development of late-stage disease, where symptoms may include abdominal pain, changes in bowel habit and the presence of blood in stool. The principal method adopted by national CRC screening programs for early disease detection is the fecal occult blood test (FOBT), targeted at high-risk age groups with follow-up by colonoscopy (8,9). FOBT screening is cost effective, but tests suffer from limited sensitivity and specificity. A further challenge is population participation for stool-based diagnostics.

Advances in the development of microarray and next-generation sequencing (NGS) technologies have enabled global studies of CRC genomes, methylomes, as well as coding and non-coding transcriptomes (*Figure 1*). Integrated omics data have led to the identification of new cancer genes and pathways, and have improved our understanding of tumor biology and molecular subtypes. Translational genomics studies have revealed clinically relevant biomarkers for improving CRC diagnosis, surveillance, prediction of prognosis and therapy response. In addition, such studies have identified new druggable targets, opening up novel therapeutic opportunities. Here, we summarize pertinent results of CRC genomics studies to date, with an emphasis on tumor classification, diagnosis, prognostication and prediction of therapy benefit.

The CRC genome

Comparative genomic hybridization (CGH) arrays, single nucleotide polymorphism (SNP) arrays and more recently NGS approaches have provided fundamental insights into the complex landscapes of CRC mutations, DNA copy number alterations and chromosomal rearrangements. Sjöblom *et al.* and Wood *et al.* first used classic PCR-based Sanger sequencing for exome-wide profiling of CRC mutations, identifying well-known, high-frequency mutated genes such as *APC*, *KRAS*, *PIK3CA*, *SMAD4*, *TP53* and *FBXW7* as 'gene mountains', and describing a large number of 'gene hills' that were mutated at low frequency (10,11).

These pioneering studies were followed by integrated whole-exome NGS and DNA copy-number studies by The Cancer Genome Atlas (TCGA) Network, presenting a detailed survey of the genomic profiles on over 270 sporadic CRCs (12). Approximately 15% of CRCs were found to exhibit hypermutation with two distinct mutation patterns: microsatellite instability (MSI) in three-quarters of cases, characterized by increased insertions, deletions and single nucleotide substitutions, usually with hypermethylation and MLH1 silencing, and a nucleotide substitution hypermutator phenotype (NSHP) in one-quarter of cases, associated with mutations in polymerase ε (POLE). Twenty-four genes were highlighted as significantly mutated, targeting the WNT, RTK/RAS, PI3K, TGF-β and TP53 pathways in both non-hypermutated and hypermutated tumors, but with different genetic alterations between these CRC subtypes. Non-hypermutated tumors showed common mutations in *APC*, *TP53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, *TCF7L2*, *NRAS*, *CTNNB1*, *SMAD2*, *FAM123B*, *SOX9*, *ATM* and *ARID1A*, while hypermutated tumors showed frequent alterations in *ACVR2A*, *APC*, *TGFBR2*, *BRAF*, *MSH3*, *MSH6*, *SLC9A9* and *TCF7L2*. At the chromosomal level, non-hypermutated tumors tended to be aneuploid, while hypermutated tumors tended to be near-diploid. Consistent with previous CGH and SNP array studies (13-18), the most commonly deleted chromosome arms were 8p, 15q, 17p (including *TP53*) and 18q (including *SMAD4*), and the most commonly gained regions were chromosome 7, 8q (including *MYC*), 13 and 20q. Recurrent copy-number alterations included potentially drug-targetable amplifications of *ERBB2* and *IGF2*. Low prevalence chromosomal translocations were detected between *NAV2* and the WNT pathway member *TCF7L1* using whole-genome sequencing on a subset of samples. A similar genomic study on 74 primary colon tumors reported highly concordant results, and also identified recurrent fusion transcripts involving R-spondin family members (*EIF3E-RSPO2* and *PTPRK-RSPO3*) that were shown to contribute to activation of oncogenic WNT/β-catenin signaling (19,20). Additional low prevalence translocations identified by whole-genome or targeted NGS studies in CRC include *C2orf44-ALK*, *VTI1A-TCF7L2* and *LACTB2-NCOA2* (19,21,22). Recent NGS studies have provided additional details on the mutation spectra of colorectal adenomas, MSI and microsatellite stable (MSS) carcinomas (23-29). Mutational heterogeneity has been investigated between primary cancers and matched metastases indicating high genomic concordance, with a thick common trunk and smaller genomic branches (30-33). Some evidence exists for intra-tumor mutational heterogeneity, but data on this are still emerging (33). CGH array studies have proposed a refined classification of non-hypermutated CRCs into chromosomally stable (CSS) and chromosomal instability (CIN) groups (17,34). However, these groups have not as yet been systematically investigated for specific mutation signatures.

Non-invasive analysis of circulating tumor DNA (ctDNA) is an emerging genomics-tool that is actively being developed to improve CRC diagnosis and post-surgery surveillance. It is based on the detection of tumor specific single-base substitutions or larger somatic structural variations (SSVs) in DNA fragments that are released by tumors into plasma. Assays are typically designed against either point mutations in hotspot genes or patient-specific SSVs (35-40). Hotspot mutations can be utilized in both the diagnostic and surveillance setting, but these may only identify a subset of patients and have limited specificity. Application of patient-specific SSVs is restricted to the surveillance setting, requiring low coverage whole-genome sequencing and/or microarray analysis of resected tumor for assay design, yet highly-specific tests can in principle be produced for all individuals. Several reports have shown that assays against point mutations in hotspot mutated genes like *KRAS*, *BRAF* and *PIK3CA* can identify ctDNA fragments in plasma and serum in ~70% of patients with CRC (38,39). Recently, a clinical pipeline for identification of patient-specific SSVs for post-surgery CRC surveillance has been presented, demonstrating sensitive temporal assessment of disease status, response to surgical and oncological intervention, and early detection of recurrence (40). Recommending the use of at least three SSVs per patient to counter observed primary-metastasis genetic heterogeneity, this approach achieved sensitivity and specificity of 100% for detecting relapse, with a 2-15 (mean 10) months lead time compared to conventional follow-up.

Stool-based diagnostic tests have also been successfully tested for detection of mutations in high-frequency mutated CRC genes, including *APC*, *KRAS* and *TP53* (41-48) such as the clinically used PreGen-Plus™ kit (49). Additionally, studies have evaluated long fragment DNA from exfoliated cancer cells in stool as diagnostic marker, with modest sensitivity and specificity (50-52).

Genomic instability phenotypes of CRC, MSI and CIN, have been demonstrated to be predictive of good and poor prognosis, respectively (53,54). The extent of CIN may provide additional prognostic value (55,56). Several CGH

array studies have attempted to define particular regions of chromosomal gain or loss related to tumor progression and outcome (14,16,57-68). Perhaps the strongest data exist for loss of chromosome arms 4q and 18q and inferior survival, but whether these relationships are independent of global CIN status remains uncertain (68,69). Recently, different types of CIN, such as genome-doubling and chromothripsis, have been suggested to be adversely related with patient outcome (70,71).

Targeted gene sequencing studies to develop integrated mutation signatures for CRC prognostication are only beginning to emerge. A recent study evaluating 187 recurrent and pathway-related genes in 160 patients with stage I-IV CRCs, has proposed a five-gene-signature (*CDH10*, *COL6A3*, *SMAD4*, *TMEM132D*, *VCAN*) for stratifying patients by outcome independent of TNM status (72).

Genomic approaches are gradually being applied for identification of molecular markers of therapy benefit. To date, unbiased exome mutation and DNA copy number studies have focused on cancer cell lines in the context of high-throughput drug screens. However, only small numbers of CRC cell lines have been included in such screens thus limiting the power of these studies to identify robust biomarker-drug response associations (73-75). In patients with metastatic CRC, several targeted gene mutation and copy-number analyses have investigated resistance to treatment with monoclonal antibodies targeting EGFR. These studies have largely considered "rational" candidate genes indicated by previous focused studies. For example, Peeters *et al*. evaluated cancer resistance to panitumumab using massively parallel multigene tumor sequencing of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *PTEN*, *TP53*, *EGFR*, *AKT1* and *CTNNB1*. As found in other reports (76,77), wild-type *KRAS*, *NRAS* and *BRAF* status were associated with longer progression-free survival (78). Ciardiello *et al*. reported a similar targeted NGS study interrogating 22 genes in patients treated with FOLFIRI plus cetuximab, reporting worse outcome in cases with *KRAS*, *NRAS*, *BRAF*, or *PIK3CA* mutations (79). The potential of ctDNA analysis for monitoring intrinsic and acquired resistance to anti-EGFR antibody therapy has been successfully demonstrated, applying both targeted mutation and SSV analysis (38,80-82). Limited data suggest that tumor DNA copy number profiles may correlate with outcome in advanced CRC patients treated with fluoropyrimidine-based regimens. In particular, chromosomal losses of 18q, 17p11.2-p13.2 and gains of 20p13-q13.3 have been associated with response to the FU + irinotecan (FOLFIRI) and capecitabine + irinotecan (CAPIRI) (83,84).

Limited data exist for rectal cancer response to preoperative chemoradiation. A study by Chen *et al*. highlighted loss of chromosome 4 as associated with the risk of lymph node metastasis (85). Similarly, Grade *et al*. suggested that pre-therapeutic evaluation of gains of chromosomal regions 7q32-q36 and 7q11-q31, and amplifications of 20q11-q13 may predict responsiveness to chemoradiotherapy (86).

The CRC transcriptome

Analysis of the protein-coding CRC transcriptome using microarray platforms has provided a framework for classification of CRC subtypes and prediction of cancer outcomes and therapy benefit. These signatures are generally derived from the analysis of resected tumor specimens with limited micro-dissection and capture neoplastic, stromal and immune components.

Several classification schemes for CRC have been proposed based on unsupervised clustering of tumor gene expression data (87-91). Approaches to tumor categorization have included hierarchical clustering, non-negative matrix factorization and the clustering of meta-genes (medians of groups of genes with correlated expression). Although these classification schemes differ in the number and detail of the subtypes proposed, ranging from three to six groups, major themes are separation into classes differentiated by MSI and CIN status, tumor location, and expression of epithelial versus mesenchymal markers (*Table 1*). One study has aligned their classification with different types of precursor lesions, classic versus serrated adenoma (87), while another has connected their classes with the cell types in colorectal crypts, stem cell, transit-amplifying cell, goblet cell and enterocyte (88). Recently, two studies have demonstrated major contributions of stromal cells in tumor groups with increased mesenchymal marker expression, rather than tumor cells undergoing epithelial-to-mesenchymal transition as was originally proposed (92,93).

mRNA extracted from blood and stool have been considered as biomarker analysis for diagnosis of CRC (94-96). Several groups have used expression microarrays on blood from patients with CRC and healthy controls to identify an initial set of candidate diagnostic mRNAs followed by further refinement of candidates using RT-PCR (96,97). Other groups have screened normal and tumour tissue to find differentially expressed candidate genes, from which a refined set was obtained upon

Table 1 Comparison of gene expression-based classification schemes for colorectal cancer from De Sousa *et al.* (87), Sadanandam *et al.* (88), Marisa *et al.* (89) and Budinska *et al.* (90) Roepman *et al.* (91)

Classification scheme	Class	MSI, CIN	CIMP, BRAF	Prognosis (RFS, OS)	Molecular phenotype	Similarity to precursor polyp	Site
MSS/conventional							
De Sousa E Melo	CCS1-CIN	MSS, CIN	CIMP0, *BRAFwt*	Intermediate	Epithelial	Tubular	Left
Roepman	B-type	MSS	*BRAFwt*	Intermediate	Epithelial		Left ↑
Sadanandam	Transit-amplifying		*BRAFwt*	Mixed	Epithelial		Both
Marisa	C1	MSS, CIN	CIMP0, *BRAFwt*	Intermediate	Epithelial	Not serrated	Left ↑
Marisa	C5	MSS, CIN	CIMP0, *BRAFwt*	Intermediate		Not serrated	Left ↑
Budinska	B	MSS, CIN	CIMPH ↑*, *BRAFwt*	Good	Epithelial		Left ↑
Sadanandam	Enterocyte	MSI, MSS	*BRAFwt*	Intermediate	Epithelial		Left ↑
Budinska	E (mixed)	MSS, CIN	CIMP0 ↑*, *BRAFwt*	Poor	Epithelial, inflammatory		Left
Budinska	A	MSS, CSS	CIMP0 ↑*, *BRAFwt*	Good	Epithelial		Both
MSI-like							
De Sousa E Melo	CCS2-MSI	MSI	CIMPH, *BRAFmt* ↑	Good	Inflammatory		Right
Sadanandam	Inflammatory	MSI	*BRAFmt* ↑	Intermediate	Inflammatory		Right ↑
Marisa	C2	MSI ↑, CSS ↑	CIMPH ↑, *BRAFmt* ↑	Intermediate		Serrated	Right ↑
Budinska	C	MSI, CSS	CIMPH ↑*, *BRAFmt* ↑	Intermediate	Inflammatory		Right ↑
Roepman	A-type	MSI ↑	*BRAFmt* ↑	Good	Epithelial		Right ↑
Sadanandam	Goblet-like	MSI	*BRAFmt* ↑	Good	Epithelial		Right ↑
Marisa	C3	CSS ↑	CIMPH ↑, *BRAFwt*	Intermediate		Serrated	Right ↑
MSS/serrated							
De Sousa E Melo	CCS3-serrated	MSS ↑		Poor	Mesenchymal	Serrated	Both
Roepman	C-type	MSS/MSI	*BRAFmt* ↑	Poor	Mesenchymal		Left ↑
Sadanandam	Stem-like	MSS	*BRAFmt* ↑	Poor	Mesenchymal		Left ↑
Marisa	C4	MSS, CIN ↑	CIMPH ↑, *BRAFwt* ↑	Poor	Mesenchymal	Serrated	Both
Marisa	C6	MSS, CIN	CIMP0, *BRAFwt*	Poor	Mesenchymal	Serrated	Left ↑
Budinska	D	MSI ↑, CIN	CIMP0* ↑, *BRAFmt* ↑	Poor	Mesenchymal		Left ↑

*CIMP status assigned using a microarray expression signature rather than a panel of methylation markers.

follow-up using mRNA extracted from stool-derived colonocytes (98) or blood (99). A related approach has been to examine previously described candidate genes with reported high levels of expression in tumour or patient blood (e.g., *COX2*, *MMP7* and CEA) (100-102). One commercial blood-based diagnostic test for CRC, ColonSentry®, which uses a 7-gene mRNA signature is currently available (103,104) (*Table 2*). Validation in two cohorts yielded sensitivity and specificity of 72-82% and 64-70%, respectively (103,111). However, research into using mRNA for CRC diagnosis appears to be waning relative to approaches which utilise aberrant DNA methylation or miRNAs discussed below.

Multiple studies (126-139) have searched for gene expression signatures for predicting risk of tumor recurrence following surgical resection of the primary tumor (112,120,125,140-155). Early studies often had modest sample sizes and relied on cross-validation to assess performance of their signatures, while later studies evaluated larger sample sizes and included independent patient cohorts for signature assessment. A survey of 31 gene signatures demonstrated little overlap in the component genes (156), and only modest prognostic performance when assessed in independent datasets (156). Recognized reasons for these

Table 2 Diagnostic and prognostic tests for colorectal cancer in which genomic methods were used as part of development and/or implementation of clinical test

Name	Description	Assay(s)	Source	Reference(s)
Diagnosis				
ColoVantage® Plasma	Methylation status of *SEPT9* DNA promoter region	RT-PCR	blood plasma (cell-free DNA)	(105)
Epi proColon®	Methylation status of *SEPT9* DNA promoter region	RT-PCR	blood plasma (cell-free DNA)	(106-108)
RealTime mS9™	Methylation status of *SEPT9* DNA promoter region	RT-PCR	blood plasma (cell-free DNA)	(109)
ColoSure™	Methylation of *VIM* from DNA	RT-PCR	Stool	(110)
PreGen-Plus™	21 point mutations in *KRAS*, *APC*, *TP53*; a MSI marker (BAT-26) and a DNA integrity marker	Capillary electrophoretograms	Stool	(49)
ColoGuard®	*KRAS* mutations, *VIM*, *NDRG4* and *BMP3* methylation (plus ACTB reference), plus presence of haemoglobin	RT-qPCR	Stool	(48)
ColonSentry®	Expression levels of seven gene biomarkers	RT-PCR	Blood plasma (mRNA)	(103,111)
Prognostic				
Oncotype DX® Colon Cancer Assay	Expression levels of 12 genes (7 cancer related genes and 5 reference genes)	RT-qPCR	FFPE primary tumour	(112-119)
ColoPrint®	Expression levels of 18 genes	Microarray	Fresh-frozen primary tumour	(120-122)
OncoDefender™	Expression levels of 5 genes	RT-PCR	FFPE primary tumour	(123,124)
GeneFx Colon® (formerly ColDx)	Expression levels of 634 genes	Microarray	FFPE primary tumour	(125)

RT-PCR, quantitative real-time PCR.

findings are technical differences in sample preparation and microarray processing, cohort heterogeneity and gene selection methods. These challenges can be overcome using rigorously controlled assay conditions. Accordingly, four prognostic gene expression signatures have been translated into clinical use following extensive validation on external cohorts: Oncotype DX® (Colon), a 12-gene RT-PCR based assay (112-119,157), ColoPrint®, an 18 gene microarray-based assay (120-122), OncoDefender™, a 5 gene RT-PCR based assay (123,124), and GeneFx Colon®, a 634 gene microarray-based assay (125) (*Table 2*). Gene expression based CRC classification schemes may also have prognostic potential, with MSI-associated classes showing good prognosis and serrated/mesenchymal classes exhibiting poor prognosis (87-91).

Transcriptome analyses have also been attempted for predicting response to chemotherapy and radiotherapy for CRC. Perhaps the most studied scenario has been that of pre-operative chemoradiation in rectal cancer patients, utilizing pre-treatment biopsies (158-165). These transcriptomic studies typically involve smaller training sets (n<100) than those for prognosis signatures and generally lack external validation. Classifier genes show little overlap, and an evaluation of three reported signatures found poor performance in an external dataset (166). One recent review concluded that an optimal gene signature for prediction of chemoradiotherapy in rectal cancer patients has not yet been found (167). A small number of studies have used transcriptomic data to generate models of 5-FU-based chemotherapy benefit in patients with advanced CRC (168-171). These studies are limited by small numbers of patients and a lack of validation in large patient cohorts. In general, gene signatures developed for predicting risk of tumor recurrence following surgical resection of the primary tumor have not been shown to exhibit predictive value for 5-FU based adjuvant chemotherapy

benefit, although in two studies benefit was suggested to be limited to the poor prognosis groups (148,149). The application of transcriptome approaches to targeted biological therapies is an emerging field (172-176). Several of the CRC classification schemes have been suggested to have predictive value for 5-FU-based chemotherapy or radiotherapy, but with apparently conflicting results (*Table 1*). For example, the mesenchymal "stem cell-like" class of Sadanandam *et al*. (88) was found to be sensitive to FOLFIRI and radiotherapy, while the mesenchymal "C-type" class of Roepman was associated with 5FU-resistance (91).

The CRC methylome

DNA methylation of cytosines in the context of CpG dinucleotides is a central mechanism of epigenetic control, with essential roles in the maintenance of genome integrity, genomic imprinting, transcriptional regulation, and developmental processes. Multiple approaches for genome-wide studies of DNA methylation patterns have been developed, generally combining DNA analysis by microarrays or NGS with one of three techniques to convert DNA methylation patterns into DNA sequence information or library enrichment: endonuclease digestion, affinity enrichment and bisulphite conversion (177,178).

Genome-wide methylome analyses have highlighted extensive disruption of DNA methylation in CRC. Tumors are typically characterized by global loss of methylation (hypomethylation), predominantly in repetitive sequences, and focal gain in methylation (hypermethylation) in CpG islands, the latter often occurring simultaneously within defined megabase regions (179-181). Hypermethylation within CpG islands is associated with transcriptional silencing of tumor suppressor genes, whilst hypomethylation within gene bodies can affect transcriptional elongation or alternative promoter usage and cause aberrant transcription of oncogenes (182-196). Global loss of methylation may trigger cancer genomic instability and activation of transposons and genes within regions of repetitive sequence (186-188). Both hypo- and hypermethylation occur early in tumorigenesis (189-196), and the average CRC genome carries thousands of methylation changes with marked impact on the cellular transcriptional program (197,198).

Studies have identified a subset of CRCs that exhibit particularly widespread promoter hypermethylation, referred to as the CpG island methylator phenotype (CIMP) (199,200). CIMP is observed in ~30% of CRCs, and presence and extent of CIMP have been used to classify CRC into three major subgroups, CIMP high (CIMP-H), CIMP low (CIMP-L) and non-CIMP (CIMP-0), with distinct clinical and molecular features (201,202). CIMP-H is associated with proximal tumor location, female gender, $BRAF^{V600E}$ mutation, *MLH1* methylation and MSI; CIMP-L is characterized by proximal tumor location and *KRAS* mutation, while CIMP-0 is associated with distal tumor location, *TP53* mutation and CIN (202-205).

Aberrant DNA methylation patterns are attractive tumor biomarkers because of their high frequency in neoplasms, and the detection of methylation in DNA isolated from stool and/or blood has emerged as a promising approach for early diagnosis and surveillance of CRC (206,207). Microarray based studies of hypermethylated CpG sites in CRC and benign adenomas have revealed a large number of tumor-specific candidate detection markers (195,208-210). Translation of these candidates into blood- or stool-based diagnostic tests is actively being pursued by academia and industry, involving method development, validation of specificity against normal tissues and other pathologies, and evaluation of performance against routine clinical assays (FOBT, CEA). A recent study evaluating circulating DNA detection of *HLTF* and *HPP1* hypermethylation in addition to CEA serum measurements showed that combination of all three markers outperformed each assay on its own (211). In a related study, Lange *et al*. suggested that blood-based detection of *THBD* and *C9orf50* hypermethylation outperformed CEA (212). Two diagnostic tests have already been introduced into clinical practice, including a blood-based PCR test for methylated septin-9 (Epi proColon®, ColoVantage® Plasma, RealTime mS9™ kit) (105-109,213-215), and a stool-based test for methylated vimentin (ColoGuard® assay, ColoSure™ assay) (48,110) (*Table 2*).

The association between CIMP and risk of CRC recurrence has been analyzed extensively, but results remain inconclusive. Several studies indicate CIMP-H as a poor prognostic factor in MSS but not MSI tumors (216,217), while CIMP-L has been suggested to be an indicator of poor outcome regardless of MSI (216,218,219). An association between CIMP and shortened survival was also reported in advanced CRC patients, among whom the contribution of MSI is relatively limited (220). However, there is evidence that the adverse effects associated with CIMP status may be attributable to *BRAF* mutation (205,221,222). Global hypomethylation as measured by analysis of LINE-1 elements has also been associated with poor outcomes, but

data are limited (223,224). Several studies have investigated small numbers of methylated candidate loci not included in CIMP marker panels identifying some evidence for prognostic associations (210,225-228), but no genome-wide methylome studies have been reported.

Epigenetic signatures are increasingly being considered in the context of response to chemotherapeutic and target agents. Recently, Ha *et al*. correlated genome-wide methylation array data with histopathological rectal tumor regression grade, highlighting hypomethylation of *KLHL34* as a candidate predictive marker for sensitivity to preoperative chemoradiation therapy (229). Miyaki *et al*. related *DEX1* hypermethylation and transcriptional silencing, identified by genome-wide methylation sensitive amplified fragment length polymorphism (MS-AFLP) analysis, to resistance of camptothecin 11 (CPT-11) based chemotherapy via inhibition of apoptosis (230). Integrating gene expression microarray analysis and methylation-specific PCR, Tan *et al*. identified *PPP2R2B* hypermethylation and transcriptional silencing as a modulator of PDK1-directed Myc signaling and rapamycin sensitivity in CRC (231). CIMP status has been assessed in the context of 5-FU-based adjuvant chemotherapy, but results have not been conclusive. Some investigators have found that 5-FU treatment increases survival in patients with CIMP-H CRC (205,232,233), but others have not replicated this finding (234).

The CRC miRNAome

MicroRNAs (miRNAs) are short (19 to 25 nucleotides), double-stranded, non-protein coding RNAs, that regulate expression of complementary mRNAs at the post-transcriptional level by inducing mRNA degradation or blocking translation into protein. Abnormal miRNA expression profiles are related to clinical and biological behavior of tumors and, given their high stability, have been investigated as robust diagnostic, surveillance, prognostic and predictive biomarkers in cancer tissues and body fluids from cancer patients (235,236). Genomic approaches have mainly utilized qRT-PCR and microarray technologies.

To date, multiple studies have reported unsupervised principle component or cluster analyses of miRNA expression data to classify CRC. Oberg *et al*. analyzed 315 normal colonic mucosa, tubulovillous adenoma, MSS/proficient mismatch repair (pMMR) sporadic carcinoma, and MSI/deficient mismatch repair (dMMR) sporadic and inherited carcinoma samples using microarrays (237). Unsupervised analysis demonstrated that normal colon tissue, adenomas, MSS/pMMR carcinomas and MSI/dMMR carcinomas were clearly discernible. Consistent with these data, several other studies analyzing MSS/pMMR and MSI/dMMR cancers also found miRNA expression differences between these tumor groups (238-241). One report suggested that Lynch syndrome tumors may display a different miRNA profile as compared to sporadic MSI tumors (242), but this was not noted by Oberg *et al*. (237). However, overlap between MSI/dMMR associated genes identified across studies is limited. One supervised analysis has suggested miRNA expression differences by CRC location, CIMP, *KRAS* and *TP53* status although this has not been replicated (241). A recent microarray study on 1,141 CRC cases, analyzing 121 miRNAs previously reported with advanced tumor stage and/or survival, verified stage associations for five miRNAs (hsa-miR-145-5p, hsa-miR-31-5p, hsa-miR-200b-3p, hsa-miR-215 and hsa-miR-451a) (243).

miRNA signatures in the blood or stool of CRC patients have been evaluated as an alternative to FOBT testing for CRC diagnosis. Multiple studies have used separate discovery and validation cohorts to derive diagnostic miRNA blood/stool profiles using qRT-PCR panels or microarrays (244-255). Proposed classifiers comprise 1 to 21 miRNAs, with sensitivities of 34-85% and specificities of 68-97% reported across studies. In particular, up-regulation of miRNAs miR21 and miRNA92/miRNA92a have been highlighted in several blood- and stool-based studies for CRC diagnosis (236). To date, signatures have not been validated in independent follow-up reports or been rigorously compared against FOBT testing. Despite these caveats, miRNA signatures show promise as non-invasive CRC biomarkers.

Tumor miRNA signatures have been studied to predict prognosis using qRT-PCR, microarrays and NGS approaches. Several prognostic miRNA signatures for stage I-IV CRC patients have been proposed with little overlap between classifiers (243,256,257). One international study identified a 2-miRNAs classifier for predicting recurrence risk in MSS stage II-III CRC using NGS (258). The most comprehensive discovery and validation study to date has been reported by Zhang *et al*. (259). Using microarrays, a panel of 35 miRNAs was identified as differentially expressed between 40 paired stage II colon cancer tumors and adjacent normal tissues, and validated in independent samples from 138 patients. Based on these candidate genes, a six-miRNA prognostic classifier (miR-21-5p, miR-20a-

5p, miR-103a-3p, miR-106b-5p, miR-143-5p, and miR-215) was built using LASSO Cox regression and validated in an external cohort of 460 stage II patients. Notably, miR-21-5p has been linked to prognosis and advanced tumor stage in multiple other studies (256,260-262). Similar investigations in stage II CRC patients using smaller sample sizes have proposed classifiers with 1-4 miRNAs, and some of these have been validated using additional sample data (239,263,264). None of the proposed prognostic miRNA signatures for stage II CRC overlap. Recently, a study compared the miRNA expression profile in primary cancers and matched liver metastasis using NanoString screening, identifying both primary CRC and serum miRNA signatures with metastasis predictive potential (265).

Multiple studies have investigated the relationship between CRC miRNA signatures and therapy response. A recent microarray-based investigation proposed miRNA-17-5p expression as a predictive marker for 5-FU-based neoadjuvant chemoradiation and adjuvant chemotherapy (266). In metastatic CRC, three studies have identified miRNA signatures associated with the added benefit of oxaliplatin or bevacizumab to 5-FU or capecitabine (267-269), and one study examined the benefit of 5-FU (270). In rectal cancer, miRNA signatures have been proposed to predict response to pre-operative chemoradiotherapy (266,271-275). Interestingly, several studies have suggested that their prognostic classifiers could also predict patients benefit from adjuvant 5-FU based chemotherapy or irinotecan-cetuximab combination therapy (256,257,259,276,277). For example, the 6-miRNA prognostic classifier identified by Zhang *et al*. also predicted 5-FU treatment response for stage II CRC patients (259,277), while high miR-345 expression identified by Schou *et al*. was also associated with lack of response for patients to treatment with cetuximab and irinotecan (259,277). Recently, a serum miRNA signature has been proposed as a non-invasive predictor of response to chemotherapy for CRC patients (278).

Conclusions and perspectives

Ongoing global genome characterization efforts are transforming our understanding of CRC biology and pathogenesis. Knowledge of the molecular aberrations driving cancer development—including genome, transcriptome, methylome and miRNAome alterations—can be applied, in principle, to develop integrated approaches for personalized cancer treatment. Recent genome-wide DNA sequencing and copy number studies in CRC have validated established genetic pathways of tumorigenesis and mutator phenotypes, while highlighting extensive mutational heterogeneity and identifying novel cancer gene candidates. Gene expression and DNA methylation data have demonstrated widespread deregulation of the CRC epigenome and indicate the importance of the cell-of-origin, retention of differentiation hierarchy and tumor stroma for CRC molecular classification (87,92,279,280).

Advances in genomics have begun to contribute new tools for clinical diagnosis and management of CRC. Blood- and stool-based tumor DNA sequencing, miRNA detection and DNA methylation assays are being developed for improved population screening, to facilitate surveillance of tumor recurrence and for dynamic monitoring of cancer response to therapy (207,281,282). Direct genomic and transcriptomic analyses of patient tumors are being pursued to provide prognostic and predictive information about the course of disease and benefit of treatment, with the current standard of care already involving assessment of *KRAS* mutation prior to treatment of metastatic CRC with anti-EGFR antibody therapy (283). Germline pharmacogenomic variation, which we did not consider in this review, further has the potential to predict patient treatment tolerance in order to avoid deleterious side effects (284).

Challenges for translation of genomic-based CRC biomarkers include the need for well-defined clinically characterized cohorts and for standardization regarding specimen collection, handling, and storage. Biomarker translation may further be improved through integration with functional genomics approaches to establish mechanistic rather than correlative links with tumor biology (285). Besides inter-tumor molecular heterogeneity, intra-tumor molecular heterogeneity poses a major hurdle to the translation of genomics findings and remains to be fully elucidated. Efforts focusing on molecular profiling of tumor regional heterogeneity and (epi-) genomic variation between metastatic deposits are ongoing. Besides clonal heterogeneity, hierarchical organization and phenotypic plasticity may play clinically important roles and will be subject of future genomic studies (286,287).

The application of genomic approaches, in particular whole exome sequencing, presents issues beyond the assessment of molecular alterations related to the patient's original presentation of CRC. Given the comprehensive nature of these tests, incidental findings on clinically relevant variants in genes with no relationship to the primary diagnosis may be made. This raises questions as to

whether such findings should be reported back to patients, what method of reporting should be used, and when to disclose these results (288-290).

The revolutionary advances in genomic technologies are enabling the possibility of personalized medicine for CRC. Evolving platforms such as NGS and high-density microarrays are starting to bring precision genomic profiling to the clinic at a reasonable cost. Ongoing innovations in existing applications and clinical informatics algorithms, as well as the many emerging technologies, will continue to advance translational cancer genomics and ultimately contribute to improving patient outcomes.

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Footnote

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Extended RAS testing in metastatic colorectal cancer—Refining the predictive molecular biomarkers

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Abstract: Mutations of exon 2 of *Kirsten rat sarcoma viral oncogene homologue (KRAS)* (exon 2 codons 12/13) lead to constitutive activation of the EGFR (epidermal growth factor receptor) mediated signal transduction pathway and been shown to be a negative predictive biomarker for EGFR-directed monoclonal antibodies among patients with colorectal cancer (CRC). As selection of patients is very important for administration of anti-EGFR therapy, this lone biomarker has proved to be insufficient for selecting the appropriate patients as more patients lacking exon 2 *KRAS* mutation were resistant to anti-EGFR therapy. The results of various randomized clinical trials have confirmed the presence of other *RAS* mutation including additional *RAS* mutations (*KRAS* exons 3/4 and *NRAS* exon 1/2/3/4). Extended *RAS* analysis should be considered before initiating anti-EGFR therapy to patients of metastatic CRC. This can help in proper selection of patients leading to tailored individualistic treatment, decreasing cost of treatment and the adverse effects related to use of monoclonal antibody therapy. The new evidence is supporting the need to make 'Extended *RAS*' analysis essential before start of treatment with anti-EGFR monoclonal antibody therapy. Prior to this the Extended *RAS* testing should be standardized.

Keywords: Extended *RAS* analysis; metastatic colorectal cancer (CRC); monoclonal antibody therapy

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Introduction

According to the American Cancer Society, the latest records of year 2012 showed that colorectal cancer is the third most commonly diagnosed cancer and the third leading cause of cancer death among both men and women in USA (1). The management of this widely prevalent cancer has also been evolving from being non-specific to being patient and target specific in the recent past. As a step towards targeted treatment, epidermal growth factor receptor (EGFR) was validated as a therapeutic target for chemotherapeutic agents (2).

Various randomized controlled trials (RCTs) proved the beneficial effects of anti-EGFR monoclonal antibodies as monotherapy as well as in combination therapy among patients with metastatic colorectal cancer (mCRC) in the last decade (3-7). Two anti-EGFR monoclonal antibodies (mAbs), cetuximab and panitumumab, were approved for use alone or with standard chemotherapy among patients with advanced CRC (8,9). As the mAbs are expensive and can be potentially toxic drugs, there was a need for proper selection of patients eligible for administration of the antibody based therapy. EGFR expression level was the first biomarker to be studied among patients likely to be prescribed anti-EGFR mAbs. But, no correlation could be established between the response to anti-EGFR mAbs and the EGFR expression levels (6,10). Later, an association between the occurrence of mutation of *KRAS* gene and the poor response with anti-EGFR mAbs was established (11). This was followed by the recommendation of testing of

mutational status of *KRAS* gene before initiation of therapy with anti-EGFR mAbs among patients of mCRC (12,13).

EGFR and RAS signaling pathway

EGFR, a tyrosine kinase receptor involved in signal transduction mechanism, is one of the important molecular targets for drug therapy (14). Binding of EGF or any other ligand to EGFR activates signal transduction via various pathways. These include the RAS-RAF-BRAF-MAPK (mitogen activated protein kinase) pathway or phosphatidylinositol 3-kinase (PI3K)-Akt or phospholipase Cγ pathway (15). *RAS* is the most important superfamily of proteins, which includes mainly *KRAS* and *NRAS* proteins. *KRAS* is a guanosine triphosphate cleaving enzyme (GTPase). The signaling through KRAS-RAF-BRAF-MAPK pathway controls gene transcription, cell proliferation, apoptosis, angiogenesis, invasion and migration (16-18).

Although EGFR is a molecular target for anti-EGFR mAbs and is also over expressed among approximately 80% of CRCs, it could not be established as a predictive biomarker in the management of CRC (16,19). Positive EGFR protein expression proved to be a poor biomarker for response with anti-EGFR mAbs (18). Thus, other effectors in the downstream signal transduction pathway were evaluated for their predictive value. It was observed that mutation in *KRAS*, *NRAS*, *BRAF or PI3KCA* genes result in constitutive activation of signaling pathway. Approximately 30-50% CRCs carry a mutation at codon 12 or 13 of exon 2 of the *KRAS* gene, followed by mutations of *NRAS*, *PI3KCA* and *BRAF* (20,21). These mutations are responsible for constitutive activation of EGFR downstream pathways which disrupt the normal signaling pathway independent of EGFR (15,18). Mutations in *BRAF* lead to uncontrolled *BRAF* activation independent of EGFR and *RAS* (17).

KRAS mutant status as a predictive biomarker

After the approval of cetuximab and panitumumab for use among patients with mCRC, various studies demonstrated that these drugs were effective among patients with *KRAS* exon 2 wild type tumors only and not among those with *KRAS* exon 2 mutant tumors (22,23). The median progression free survival (PFS) and overall survival (OS) significantly improved among the *KRAS* exon 2 wild type group with anti-EGFR antibody therapy when used either in monotherapy or combination therapy as compared to the basic support care group or standard chemotherapy regimen respectively (22,23). On the other hand, the *KRAS* exon 2 mutant group did not show any difference in efficacy with the addition of anti-EGFR mAbs as compared to the standard chemotherapy regimen (22-25). In addition, somewhat unexpected detrimental effects were observed in the mutant *KRAS* groups in the PRIME (panitumumab randomized trial in combination with chemotherapy for metastatic colorectal cancer to determine efficacy) and OPUS (oxaliplatin and cetuximab in first-line treatment of mCRC) studies (26,27). Both prospective and retrospective analysis of the clinical studies concluded that mutation of codon 12 or 13 of exon 2 of *KRAS* is a negative predictive biomarker for therapy with anti-EGFR antibody therapy (11,22-27).

This led to the recommendation for routine *KRAS* exon 2 mutational testing. The American Society of Clinical Oncology and National Comprehensive Cancer Network (NCCN) recommended that all patients with mCRC who are candidates for anti-EGFR antibody therapy should have their tumor tested for *KRAS* mutations. If *KRAS* mutation in codon 12 or 13 is detected, then patients with mCRC should not receive anti-EGFR antibody therapy as part of their treatment due to the predicted lack of response (12,13,28). This recommendation restricted the use of anti-EGFR mAbs to about 60% of all patients with *KRAS* wild type tumors (20). A meta-analysis of 45 clinical studies (29) concluded that *KRAS* mutations are predictive of survival, disease progression, and treatment failure in patients with advanced colorectal cancer treated with anti-EGFR antibodies. The benefits of anti-EGFR therapy were largely limited to *KRAS* wild type patients (29).

Unfortunately, not all patients with *KRAS* wild type status respond to anti-EGFR mAbs. The presence of *KRAS* mutations has low sensitivity and relatively high negative likelihood for determining non-responsiveness among the patients (30). One hypothesis to explain this could be the simultaneous or isolated presence of genetic aberrations of genes encoding the other downstream effectors of the EGFR mediated signal transduction pathway (31-34). This hypothesis was proven by the results of the following clinical studies which show that additional *RAS* mutation (*KRAS* exons 3 and 4 and *NRAS* exon 1, 2, 3, 4) analysis can help in further refining the treatment modalities.

Table 1 PRIME study, primary end points of (PFS and OS) efficacy results according to *RAS* mutation status

Variable	FOLFOX4 + panitumumab (months)	FOLFOX4 (months)	P value
PFS			
Extended *RAS* wild	10.1	7.9	0.004
No *K-RAS* exon 2 (12+13) mutation	9.6	8	0.02
Non *K-RAS* exon 2 (12+13) mutation but other *RAS* mutation present	7.3	8	0.040
Extended *RAS* mutation	7.3	8.7	0.001
OS			
Extended *RAS* wild	25.8	20.2	0.009
No *K-RAS* exon 2 (12+13) mutation	23.8	19.4	0.03
Non *K-RAS* exon 2 (12+13) mutation	17.1	17.8	0.01
Extended *RAS* mutation	15.3	18.7	0.001

Abbreviations: PFS, progression free survival; OS, overall survival.

Clinical evidence of presence of other genetic mutations in patients resistant to anti-EGFR therapy

In the era of personalized medicine various retrospective and prospective analyses are being conducted to search for more predictive biomarkers in the treatment protocol for various malignancies specially mCRC. As *KRAS* wild type status was not sufficient to ensure response to anti-EGFR mAbs, other predictive biomarkers (*KRAS*, *NRAS*, *BRAF* mutations, *PIK3CA* mutations and PTEN loss) from the signaling pathway were analyzed. Although the results are favorable for the predictive strength of some other genomic biomarkers, till now no recommendation has been made for extensive genotypic analysis before initiation of anti-EGFR antibody therapy (34-36).

A systematic review and meta-analysis by Yang *et al.* explored the association of *BRAF*, *PIK3CA* mutations and/or loss of PTEN expression with PFS, OS and objective response rate (ORR) among patients with *KRAS* wild type tumors treated with anti-EGFR mAbs were included. The authors concluded that *BRAF* mutations, *PIK3CA* mutations and loss of PTEN are promising biomarkers and can help in identifying the appropriate patients (37). In contrast, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (EWG) discouraged the testing of *BRAF*, *NRAS* or *PIK3CA*, and/or loss of expression of PTEN or AKT proteins for taking decisions regarding the administration of anti-EGFR antibody therapy among patients with mCRC (38).

These contradictory statements could not help in establishing the status of other biomarkers in the algorithm of mCRC management. Later, the retrospective analysis of PRIME study by Douillard et al initiated the concept of Extended *RAS* analysis (39). The prospective-retrospective analysis of PRIME study assessed the efficacy and safety of panitumumab plus FOLFOX4 (oxaliplatin, fluorouracil and leucovorin) as compared with FOLFOX4 alone, according to *RAS* (*KRAS* or *NRAS*) or *BRAF* mutation status. Of the study population, 48% patients had tumors with non mutated *RAS* (no *KRAS* or *NRAS* mutations in exons 2, 3, or 4) and rest had mutations in *RAS* (any *KRAS* or *NRAS* mutations in exon 2, 3, or 4). The administration of panitumumab–FOLFOX4 led to a significant improvement in PFS and OS (*Table 1*). In the subgroup of patients without *RAS* mutations, there was a significant improvement in PFS (P=0.004) and OS (P=0.04) with panitumumab–FOLFOX4, as compared with FOLFOX4 alone (39). Another subset (17%), consisting of those patients with wild type *KRAS* tumors, but with mutations in other *RAS* exons [non *K-RAS* exon 2 codon (12 and 13) mutation, *KRAS* exon 3 (at codon 61) and exon 4 (at codons 117 and 146); *NRAS* exon 2 (at codons 12 and 13), exon 3 (at codon 61), and exon 4 (at codons 117 and 146); and *BRAF* exon 15 (at codon 600)], showed a non-significantly shorter PFS and OS in the panitumumab–FOLFOX4 group than in the FOLFOX4-alone group (*Figure 1*). These results were similar to those observed in the subgroup of patients with *KRAS* mutations in exon 2 in tumors (*Table 1*). Another important observation of the study was that the treatment effects were different between the subgroups of patients without *RAS*

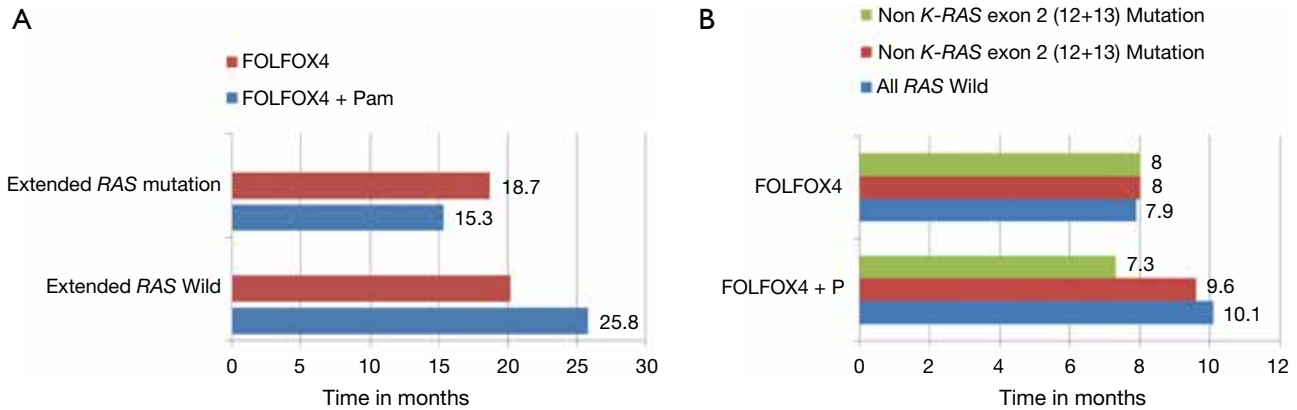

Figure 1 (A) PRIME study, in the wild K-RAS exon 2 group FOLFOX + P improved progression free survival (PFS) when compared with FOLFOX only group (9.6 *vs.* 8 months respectively), the absolute magnitude of improvement of PFS is more pronounced when extended RAS analysis is used to determine the RAS status with PFS 10.1 m in FOLFOX + P compared with 7.9 m in FOLFOX only group (P=0.004). The absolute improvement in PFS has increased from 1.6 months with wild K-RAS exon 2 analysis to 2.2 months when extended K-RAS analysis is utilized; (B) PRIME study, clinically and statistically significant improvement in survival in FOLFOX and panitumumab in extended RAS wild group when compared to FOLFOX alone, the presence of RAS mutation (any *KRAS* or *NRAS* mutations in exon 2, 3, or 4) in this population had detrimental effect on survival and did not drive any survival benefit from the addition of panitumumab in contrast to extended RAS wild population where significant improvement in survival with FOLFOX and panitumumab comparing with FOLFOX only population (25.8 *vs.* 20.2 months respectively) (P=0.009).

mutations and those without *KRAS* mutations in exon 2 but with other *RAS* (*KRAS* or *NRAS* mutations in exons 2, 3, 4) mutations. This might suggest that *RAS* mutations, in addition to *KRAS* mutations in exon 2 codon (12 and 13), were negative predictive factors. The results suggest that presence of *RAS* mutations was a negative predictive factor. Further analysis showed that in the nonmutated *RAS* and nonmutated *BRAF* subgroup, panitumumab–FOLFOX4 was associated with a 1.6-month improvement in PFS and a 7.4-month improvement in OS, as compared with FOLFOX4 alone. Analysis of the prognostic effect of *BRAF* mutations showed that *BRAF* mutations were associated with reduced OS among patients without *KRAS* mutations in exon 2 and among those with *NRAS* mutations in exon 3. The safety profile for patients with *RAS* mutations was similar to that reported for patients with *KRAS* mutations in exon 2 (39).

Similarly, Soeda *et al.* while studying the response with cetuximab among irinotecan- and oxaliplatin-refractory Japanese patients with mCRC, found that the *KRAS*, *BRAF*, and *PIK3CA* wild type group had a better response rate and PFS than did the wild-type *KRAS* exon 2 subgroup (40). In the GERCOR efficacy, tolerance and translational molecular study, Andre *et al.* also studied *BRAF*, *NRAS* mutations and EGFR copy number in addition to the *KRAS* mutant status. Patients with *BRAF* mutations had a poorer prognosis and lower response rates to anti-EGFR antibody therapy as compared to other groups. Evidence for rare *KRAS*, *NRAS* and *PIK3CA* mutations was poor because of small number of patients in these groups. The response was highly dependent on the mutant status of the patients and thus recommended an extended genotyping including rare *KRAS* and *NRAS* mutants (41).

The PEAK [panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6] study also assessed the treatment effect with an extended *RAS* analysis including exons 2, 3, 4 of both *KRAS* and *NRAS* among patients with previously untreated, unresectable, wild type *KRAS* exon 2 mCRC. Patients with wild type *RAS* tumors had better PFS (P=0.029) and median OS (P=0.058) with anti-EGFR therapy. PFS was similar and OS was better in the panitumumab group among the patients with wild type *KRAS* exon 2 tumors (42).

New evidence was presented at the American Society of Clinical Oncology 2014 and European Cancer Congress 2013 (25,43). Peeters *et al.* assessed the effect of second line treatment of panitumumab plus FOLFIRI (continuous infusion fluorouracil, oxaliplatin, and irinotecan) *vs.* FOLFIRI based on *RAS* mutation status in the population of the earlier study conducted in 2010. Mutations detected included *KRAS* exon 3, 4 and *NRAS* exons 2, 3, 4 in patients

Table 2 FIRE 3 study, primary end points of (PFS and OS) efficacy results according to RAS mutation status

Variable	FOLFIRI + cetuximab (months)	FOLFIRI + bevacizumab (months)	P value
PFS			
K-RAS exon 2 (12+13) wild	10.0	10.3	0.55
Extended *RAS* wild-type [Excluding all non *K-RAS* exon 2 (12+13) mutation]*	10.4	10.2	0.54
OS			
K-RAS exon 2 (12+13) wild	28.7	25	0.017
Extended *RAS* wild-type [Excluding all non *K-RAS* exon 2 (12+13) mutation]*	33.1	25.6	0.011

*exon 3 (codon 61), and exon 4 (codon 146), and *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146). Abbreviations: PFS, progression free survival; OS, overall survival.

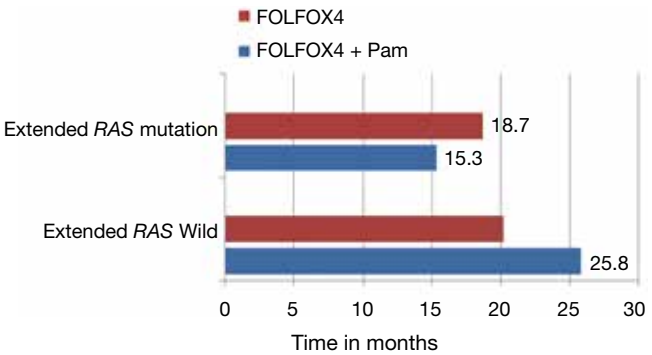

Figure 2 FIRE 3 study, clinically and statistically significant improvement in survival in FOLFIRI and cetuximab K-RAS wild group in the preplanned analysis when compared to FOLFIRI and bevacizumab, the overall survival was more pronounced among extended RAS wild type patients with the addition to cetuximab to FOLFIRI regimen as compared to addition of bevacizumab to FOLFIRI group.

with known *KRAS* wild type exon 2 mCRC. About 18% of the wild type *KRAS* patients had additional *RAS* mutations. The PFS and OS were better in the *RAS* wild type group as compared to *RAS* mutant group. Bokemeyer *et al.* studied *KRAS* exon 2 wild type patients from the OPUS study for 26 mutations (referred as *new RAS*) and additional *KRAS*, *NRAS* codons. New *RAS* mutations were present among 26% of patients. The patients from *RAS* wild type group showed significant improvement with addition of cetuximab to FOLFOX4 therapy. The distinctive observation of this study is that there was a trend towards worse outcome among patients with *RAS* mutation with the addition of cetuximab (26,44). Tejpar *et al.* (45) presented another set of results from the OPUS study about the patients which were tested for *KRAS* exons 3 and 4 and *NRAS* exons 2, 3 and 4. The tumor status was available for 31% of patients and there was benefit among *RAS* wild type population with addition of cetuximab to FOLFOX4. There was a less favorable outcome and no benefit among *RAS* mutant population with addition of cetuximab (45). Ciardiello *et al.* studied the *new RAS* mutations among *KRAS* wild type exon 2 tumors from CRYSTAL study patients and *RAS* mutations were present in 15% of the patients. There was a significant benefit in all end points among *RAS* wild type patients with the addition of cetuximab to FOLFIRI regimen. Also, there was no benefit among the *RAS* mutant group with the addition of cetuximab (46). Stintzing *et al.* evaluated the effect of mutations in exon 3 (codon 61), and exon 4 (codon 146), and *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) on the ORR, PFS and OS among the *KRAS* (exon 2) codon 12/13 wild type patients. The ORR and OS were increased among *RAS* wild type patients with the addition to cetuximab to FOLFIRI regimen as compared to addition of bevacizumab to FOLFIRI regimen (*Table 2*, *Figure 2*) (47).

A recent systematic review and meta-analysis on alterations in *KRAS* exons 3 and 4, *NRAS*, *BRAF* and *PIK3CA* and PTEN and the outcome with anti-EGFR antibody therapy suggests that mutations in *KRAS* exons 3 and 4 and *NRAS* predict resistance to anti-EGFR mAbs. The ORR was significantly poor among those with *KRAS* mutation in exon 3 and 4 (odds ratio 0.26). The PFS was also significantly shorter due to mutations in *KRAS* exons 3 and 4 and *NRAS* (48). Sorich *et al.* have included all the above mentioned clinical trials assessing the role of

anti-EGFR mAbs for tumors harboring *RAS* mutations. They divided the patients from various RCTs into three subgroups. First group was the "*KRAS* exon 2" mutant group; second consisted of "*new RAS* mutant" (wild-type for *KRAS* exon 2, but with a *KRAS* mutation in exons 3 or 4 and/or a *NRAS* mutation in exons 2, 3 or 4) patients and third consisted of "Extended *RAS* wild type" patients. Tumors without any *RAS* mutations (either *KRAS* exon 2 or *new RAS* mutations) had significantly superior response [PFS (P<0.001) and OS (P=0.008)] with anti-EGFR mAb treatment as compared to tumors with any of the *new RAS* mutations. There was no PFS and OS benefit with anti-EGFR mAbs for tumors with any *RAS* mutations (P>0.05) (49).

Discussion

Although in the initial years of use of anti-EGFR mAbs for mCRC, testing of *KRAS* exon 2 mutation helped in individualizing the treatment with anti-EGFR mAbs, yet, even after this analysis, a subset population of *KRAS* exon 2 wild type patients showed continues resistance to anti-EGFR agents. Since the isolation of *KRAS* mutant status as a lone negative predictor marker few years back to the present day scenario each and every step has been corroborated by evidence from clinical studies. The results of the above mentioned RCTs, systematic reviews and meta-analysis show that patients with tumors that are *KRAS* exon 2 wild-type (which includes both the "Extended *RAS* wild-type" and "*new RAS* mutant" subgroups) should not be considered to represent a single homogenous group for efficacy or resistance to anti-EGFR mAbs. The "Extended *RAS* wild-type" subgroup is distinct and has a significantly better response to anti-EGFR mAbs as compared to other patients. The response is indistinguishable among the *KRAS* exon 2 mutant patients and those with newly identified *RAS* mutations which include *KRAS* mutation in exons 3 or 4 and/or a *NRAS* mutation in exons 2, 3 or 4. Although the beneficial effects of anti-EGFR mAbs are explicit in the Extended *RAS RAS* wild type group, results are still limited regarding the detrimental effects of anti-EGFR mAbs among *RAS* mutant groups (39,44). A broader analysis of mutant status can help in tailoring patient specific regimen and achieving maximum benefit. Thus based on the emerging benefit Extended *RAS* analysis, beyond *KRAS* exon 2, should be utilized in practice for predicting the benefit from the anti-EGFR mAbs among patients with mCRC.

Conclusions

The additional analysis of *KRAS* and *NRAS* genes as predictive markers can allow more accurate selection of patients who are more likely to benefit from anti-EGFR antibody therapy. Treatment with anti-EGFR mAbs should only be initiated after screening tumors for mutations in exon 2, 3 and 4 of both *KRAS* and *NRAS* genes. This will help in preventing unnecessary drug toxicity and associated expenses. Prior to the implantation of such recommendation there is a need to establish a standardized acceptable expanded *RAS* mutant status testing.

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Footnote

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

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Current surgical considerations for colorectal cancer

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Background

Colorectal cancer is the second leading cause of cancer-related deaths in the United States each year. Among men and women, it is the third most common cancer following lung cancer, prostate and breast cancers, respectively. In recent years, It has been estimated that in 2012 there were more than 100,000 new cases of colon cancer and more than 40,000 cases of rectal cancer (1,2). Fortunately, both the incidence and mortality of colorectal cancer have declined steadily in the past three decades. This has been largely attributed to more effective screening programs and improvements in treatment modalities (1,2). Surgical resection offers the best chance of achieving cure, but the management of colorectal cancer often requires a multidisciplinary approach, which has been pivotal in achieving better patient satisfaction and outcomes.

Surgery for colon cancer

Overview

The diagnosis of an invasive colon cancer requires a complete staging work up that includes endoscopic evaluation of the entire colon, baseline imaging of the abdomen and chest to rule out distant spread, and routine labs including a baseline carcinoembryonic antigen (CEA) level (1). Colectomy should be offered to those patients with resectable tumors that have no evidence of distant metastasis. The extent of the colectomy is primarily determined by the location of the tumor and the blood supply to that segment of bowel. Adequate margins (≥5 cm) should be gained proximal and distal to the primary tumor and should include the associated mesentery containing regional lymph nodes. Tumors that are adherent to adjacent structures should be resected *en bloc* to ensure complete removal of the cancer. Adjuvant chemotherapy is offered to patients with evidence of lymph node metastasis.

Laparoscopy for colon cancer resections

Traditionally, colectomies have been approached via a laparotomy with good clinical outcomes. However, the advent of laparoscopy has revolutionized surgery and, in appropriate patients, is now a popular alternative for the surgical management of colorectal disorders. This has been primarily due to the substantial short-term benefits, which include less postoperative pain, earlier return of bowel function, and shorter hospital stays (3,4). While laparoscopy has been shown to be consistently safe and feasible for a variety of gastrointestinal pathology, initial enthusiasm about employing a minimally invasive approach for colorectal cancer was tempered by a steep learning curve as well as reports of wound and trocar site recurrences (4). Therefore, the steady implementation of this approach has required balance of the potential short-terms benefits with preservation of oncologic outcomes. These criticisms were addressed with initial data reported in retrospective studies and later confirmed by larger, randomized clinical trials, which demonstrated that laparoscopy does not compromise oncologic outcomes or increase perioperative complications (3,5-8).

The Barcelona trial was among the first randomized, prospective, single-institution trials, which compared laparoscopic colectomy to the conventional open approach. From 1993 to 1998, 206 patients were enrolled (105 patients in the laparoscopic arm) with cancer-related survival as the primary endpoint. The authors found that laparoscopy was more effective than open surgery with respect to morbidity,

hospital stay, tumor recurrence, and cancer-related survival. A follow up to this study with longer follow up data (median 95 months) comparing laparoscopic and open colectomies demonstrated that the overall survival and recurrence rates favored the laparoscopic group, but did not reach statistical significance (5,6).

A larger prospective, randomized, multicenter trial by the Clinical Outcomes of Surgical Therapy (COST) Study Group showed similar long-term results. Between 1994 and 2001, 872 patients (435 patients in the laparoscopic arm) were randomized. The median follow-up time was 52 months and the primary endpoint was time to tumor recurrence. Analysis at three years demonstrated similar recurrence rates in the laparoscopic and open groups, 16% and 18%, respectively. Additionally, there was no difference in overall survival (86% in the laparoscopic group *vs.* 85% in the open group). The authors have also recently published 5-year data from this original cohort demonstrating that overall and disease-free survival were similar between the two treatment groups. Additionally, overall recurrence rates remain similar (19.4% laparoscopic group; 21.8% open group) (7,9). These survival data have been confirmed in the slightly larger European multicenter Colon cancer Laparoscopic or Open Resection (COLOR) trial that was designed to evaluate disease-free survival and overall survival 3 years after laparoscopic or open resection for colon cancer. For all stages, the 3-year overall and disease-free survival rates were not significantly different between groups. Local and distant recurrence rates were also similar (8). It should be noted that lymph node harvest is also similar between open and laparoscopic groups. The main criticisms of these trials center on the probability of selection bias when offering a laparoscopic approach to those with cancer.

In fact, those with smaller tumors (amenable to smaller incisions) and those with tumors that involve only the colon (T3 and below) are most likely still the best candidates for laparoscopy.

Postoperative complications and quality of life (QOL)

While the short-term benefits of laparoscopy have been well documented and reproducible across practices, many also postulate that laparoscopy also facilitates fewer complications than traditional open surgery. While the primary endpoints of the aforementioned clinical trials were tumor recurrence and survival, these initial data also offer some information on intraoperative and perioperative complications. The Barcelona Trial found that the patients in the laparoscopic group had significantly less intraoperative blood loss and postoperative morbidity (5). However, the COST study and the COLOR trial did not demonstrate any significant difference in postoperative morbidity or 30-day mortality. The rates of intraoperative complications, rates or severity of postoperative complications, rates of readmission, and the rates of reoperation were similar between groups (7,8). Tjandra *et al.* recently published a systematic review of 17 randomized trails of laparoscopic resections for colon cancer, which analyzed 4,013 patients. The authors found that there were no significant differences in the overall complication rate. However, laparoscopic surgery had significantly lower perioperative mortality as well as lower wound complications (infection and dehiscence) (10).

Overall quality of life parameters after colorectal cancer resection have also been fertile ground for study and there is significant data to suggest that patients undergoing laparoscopic colectomies have modest improvements in these parameters. Analysis of the responders from the COST study (428 patients) showed short-term benefits according to the global rating scale score at 2 weeks after surgery. No difference was found between the groups using the other instruments or at other time points (2 days and 2 months) (11). Long-term follow up of the patients in this study found that at 18 months after surgery, patients who underwent laparoscopic resections had significantly greater improvement from baseline in the global QOL rating and total QOL index (QLI) (12).

The role of surgery in metastatic colon cancer

Up to 25% of patients with colon cancer will present with synchronous colorectal cancer metastasis and of these, only approximately 10-20% will have lesions that are ultimately resectable (1,13). More commonly, patients will develop metastasis in the interval after resection of the primary colon tumor with the liver being the most commonly involved organ.

Patients with colorectal liver metastasis (CLM) should have a complete evaluation with the coordinated care of a multidisciplinary team—including oncologists, radiologists, colorectal and hepatobiliary surgeons in order to assess resectability. Surgical resection of these metastatic lesions should only be considered in medically fit patients with good performance status, if obtaining negative margins is feasible and adequate functional liver reserve (>20%) can be maintained. While surgery is the gold standard

for resectable disease, other potential treatment adjuncts, including radiofrequency ablation (RFA) and hepatic artery infusion (HAI) of chemotherapy, have been employed. Neither of these other modalities alone has been shown to be as effective as chemotherapy and surgical resection, which have reported 5-year survival rates up to 40% (1,14-16).

While the benefit of surgery and chemotherapy are clear, considerable controversy still remains in the optimal sequence of these treatments. Proponents for a surgery-first approach cite the potential for progression of disease and chemotherapy-associated liver injury as reasons to forego neoadjuvant chemotherapy; however, there is limited data that supports that this approach confers an advantage in overall survival (17). Contradictory data has been presented in the EORTC 40983 trial, which compared perioperative chemotherapy (pre- and postoperative) with surgery alone. The authors found that there was an 8.1% improvement in the 3-year progression-free survival with perioperative chemotherapy. However, postoperative complications were more frequent in the chemotherapy group (18).

The management of patients with synchronous, resectable CLM has also been subject to controversy. The traditional approach has been resection of the primary colon tumor followed by adjuvant chemotherapy and staged hepatic resection; however, more recent studies have shown that simultaneous colon and liver resections are safe in specialized centers and appropriately selected patients (19). This combined approach is advantageous in sparing the patient the morbidity of additional surgery and eliminating potential progression of liver disease during recovery from primary colorectal surgery. More recently, a reverse strategy, or liver-first approach, has been proposed for early management of metastatic liver disease, which proponents assert optimizes the potential for cure (20). While the data related to this approach is not as robust, the greater body of study on the management of synchronous CLM suggests that the approach should be individualized. The patient's functional status and burden of disease must be assessed in order to balance surgical risk and oncological benefit (21).

In patients with asymptomatic primary colon tumors and unresectable minimally symptomatic metastatic disease, chemotherapy is the mainstay of treatment. The available data supports that there is little benefit in resection of the primary tumor. Doing so risks delaying necessary chemotherapy and offers no survival advantage. In 2009, Poultsides *et al*. reported a series of 233 patients with unresected primary tumors and synchronous metastasis receiving chemotherapy. They found that 93% of patients did not require any surgical palliation of their primary tumor (22). Clearly, if the patient is exhibiting signs and symptoms of obstruction, which cannot be controlled with dietary changes alone, then palliation with resection is required. This seems to be the minority of cases.

Surgery for rectal cancer

Overview

The surgical decision-making process for rectal cancer is complex and often requires a multidisciplinary approach. While the pathophysiology of rectal cancers is believed to be identical to that of colon cancers, the anatomic location within the bony pelvis offers unique surgical challenges. Over the past century, an improved understanding of the histopathology as well as patterns of recurrence has afforded significant strides in the treatment of rectal cancer (23).

The initial management of rectal cancer requires complete evaluation of the local extension as well as distant spread. Unlike colon cancers, rectal tumors are more easily accessible by physical examination, which can provide added information on size, the degree of fixation, and location (2). Ultimately, the choice of treatment hinges primarily on the location of the tumor in the rectum and the depth of local invasion. Therefore, modalities such as endorectal ultrasound (ERUS) and pelvic MRI are often used for local staging of tumor depth and nodal involvement (24,25). Patients with evidence of locally advanced cancers in the distal and mid rectum (defined as Stage IIA and beyond) are now routinely referred for neoadjuvant chemoradiation, which has been shown to decrease rates of local recurrence (23,26). This paradigm has been challenged and the Alliance for Clinical Trials in Oncology is currently accruing patients for a phase II/III trial of neoadjuvant chemotherapy with the selective use of radiation in locally advanced rectal cancer. Treatment of upper rectal cancers (those above the peritoneal reflection or at the rectosigmoid junction are more controversial. Data suggests that a more individualized approach may be needed for these patients, with bulky large tumors getting neoadjuvant and smaller ones getting treated primarily with surgery.

Total mesorectal excision

Historically, local and radical resections for rectal cancers have been plagued by significant patient morbidity and high local failure rates (25). In 1982, Heald *et al*. named the concept of total mesorectal excision (TME), which has

drastically changed the surgical approach to proctectomy. An appropriate TME requires sharp dissection in the areolar, presacral plane between the mesorectal envelope (fascia propria) and the adjacent pelvic structures (27). For distal rectal cancers, TME is performed circumferentially down to the pelvic floor muscles incorporating the entire mesorectum. This allows complete removal of the rectal tumor and the regional lymph nodes while ensuring a negative radial margin and preserving the autonomic nerves (23,24,27). This has been shown to be an integral part of achieving lower local recurrence. A prospective, randomized trial, organized by the Dutch Colorectal Cancer Group, which was among the first to include surgical quality control for TME, reported a local recurrence rate of 8.2% at 2 years (10.9% at 6-year follow-up) in patients who underwent complete rectal cancer resection alone (28,29). Proximal rectal tumors, as mentioned, often do not require a total mesorectal excision since lymphatic spread is generally limited to within a few centimeters of the tumor. In these cases a partial mesorectal excision can be performed after ensuring an adequate distal margin. Bulky large proximal tumors may, however, benefit from preoperative chemotherapy and radiation in selected patients.

Radial and distal margins

Achieving the appropriate distal and radial margins is often not problematic in segmental colon cancer resection, but these are critical concepts in the surgical management of rectal cancer. A high-quality TME has improved our ability to achieve negative radial or circumferential resection margins (CRM), which has been shown to be an important predictor of local recurrence, distant metastasis, and survival (27,30,31). A positive CRM is defined as tumor extension to within 1 mm of the radial tissue edge and can occur due to direct tumor extension, mesorectal tumor deposits, involved mesorectal lymph nodes, or inadequate surgical dissection. In 2002, Wibe *et al*. reported a series of 686 patients who underwent proctectomy without adjuvant radiation, which underscored the significance of the circumferential margin. After a median follow up of 29 months, they found that the overall local recurrence rate for those with a positive CRM was 22% as compared to 5% for those with a negative margin (>1 mm). The CRM was also an independent risk factor for distant metastasis (hazard ratio 4.7) and mortality (hazard ratio 3.7) (32).

The ideal distal margin in rectal cancer surgery remains relatively controversial, especially in this era of sphincter-preserving procedures. A 5-cm distal margin had been previously advocated; however, this has been largely refuted based on pathology data demonstrating limited intramural spread of low rectal cancers (33,34). The degree of intramural and extramural spread is crucial in determining the ideal distal resection margin. In one of the larger retrospective review on the subject, Shirouzu *et al*. reported a series of 610 patients who underwent rectal cancer resections and found that only 10% had distal intramural spread. Moreover, the majority of these cases were within 2 cm of the distal border of the primary tumor. As a result, the authors postulated that a distal margin of 1 cm would be appropriate for most rectal cancers (34). Based on the available data, current recommendations suggest that a 2-cm distal margin is adequate for most rectal cancers. Smaller tumors that are low in the rectum may be resected with an acceptable margin of 1 cm (35,36).

Sphincter-preserving surgical procedures for rectal cancer

The extent of surgical resection for rectal cancer largely depends on the location of the mass in the rectum, the degree of local invasion, and the patient's baseline sphincter function and medical co-morbidities (23,26,35). For tumors in the mid and upper rectum a low anterior resection (LAR) is generally the ideal approach. During the procedure, a TME dissection is carried out after the sigmoid colon and upper rectum are dissected free from the peritoneal attachments. The inferior mesenteric artery, which is the principal feeding vessel, is ligated and divided proximally. The distal rectum is left in place after ensuring a margin 4-5 cm distal to the inferior edge of the tumor. A colorectal anastomosis is then created using a circular stapler; however, a hand-sewn anastomosis is also possible. Tumors in the lower rectum can also be considered for LAR as long as a 1-2 cm distal margin can be obtained adequately. Intestinal continuity is then restored with a stapled or hand-sewn coloanal anastomosis. The potential for pelvic sepsis due to anastomosis leak can be mitigated by a temporary loop ileostomy in those patients with low pelvic anastomoses and those that have required preoperative radiation.

Many patients experience disordered bowel function after LAR, typically characterized by increased stool frequency, bowel fragmentation, fecal urgency, and incontinence, which has been termed "low anterior resection syndrome" (37). The incidence is variable, as there are no validated tools for diagnosis, and the etiology is likely multifactorial. Reported rates range from 20-50% and possible causes include sphincter

injury, decreased rectal compliance, or neuropathy (37). Alternative reconstructive techniques to the straight end-to-end anastomosis following TME with coloanal anastomosis including colonic J-pouch and transverse coloplasty have been explored in attempt to improve postoperative function. In these cases, randomized trials have shown that the colonic J-pouch results in superior postoperative bowel function for at least 18 months after surgery, after which function becomes similar to the end-to-end anastomosis (38). The ability to do this from a technical standpoint, however, is quite dependent upon the patient's body habitus with a narrow pelvis often precluding the safe formation of a colonic pouch.

Abdominoperineal resection

Patients with pre-existing fecal incontinence or with very low rectal cancers will ultimately require an abdominoperineal resection (APR). During the abdominal phase of the procedure, the TME dissection is carried out down to the pelvic floor muscles and a permanent colostomy is created using the descending colon. During the perineal dissection, the anus and the sphincter complex are excised widely in continuity with the proximal specimen. High rates of bowel perforation, positive circumferential margins, and subsequently local recurrence have been reported with conventional APR (39-41). Therefore, much emphasis has been placed recently on achieving a cylindrical resection, which avoids narrowing of the resected specimen at the level of the levator ani muscles. This approach has been shown to reduce the risk of local recurrence without increasing local complications (42).

The primary closure of the perineal wound has been plagued with significant complications, especially in the setting of preoperative radiation. Infection and wound dehiscence are among the most frequent complications with incidences that range from 10-40% in the existing literature (43). As a result, efforts to mitigate these complications with the routine use of rotational myocutaneous flaps have been proposed with variable success (43,44). Currently, there is no standard recommendation for the use of myocutaneous flaps in the reconstruction of the perineal wound. Individualizing treatment is required—those at higher risk of perineal wound complications (obese, diabetic, malnourished) may be selective candidates for flap closure.

Minimally invasive surgery for rectal cancer resections

Laparoscopy for rectal cancer resection has been approached with as much enthusiasm as initial studies for colon cancer; however, the available data is not as mature. While a minimally invasive approach to proctectomy with laparoscopy, or even robotically, is more challenging and costly, the available technology offers the added benefit of better visualization and more precision than traditional open surgery. Initial nonrandomized studies demonstrated that laparoscopic proctectomy was safe and feasible with similar short-term benefits and oncologic outcomes (45). This has been confirmed in subsequent small, randomized trials; however, sufficient long-term data is lacking. The American College of Surgeons Oncology Group (ACOSOG) is nearing completion of a large phase III prospective randomized trial comparing laparoscopic-assisted resection versus open resection for rectal cancer which should further illuminate this subject. However, recent meta-analyses of the available randomized clinical trials comparing laparoscopic to open rectal cancer resections conclude that laparoscopy is associated with significantly lower rates of intraoperative bleeding and postoperative blood transfusion, quicker return of bowel function and shorter hospital admission (46,47). Additionally, when compared with open TME, there is no difference in the number of lymph nodes harvested, involvement of CRM, local recurrence, 3-year overall survival, and disease-free survival for rectal cancer (48). The results of larger multicenter, randomized clinical trials are pending. Complicating adoption of this technology is the large learning curve needed to implement these techniques in practice. Often "hybrid" open/laparoscopic approaches are utilized with some success to keep incision sizes small and mimic the advantages of a total laparoscopic approach in less time.

Local excision for early rectal cancers

In carefully selected patients, local excision has generally been considered as an acceptable treatment option for small, early (T1 and T2) cancers in the mid to distal rectum that have favorable histologic features (well-differentiated, absence of lymphovascular invasion, superficial submucosal invasion) (49,50). It has also been proposed in patients that are unsuitable for radical surgery as the resection of these lesions with traditional transanal surgery, or transanal endoscopic microsurgery (TEM) for more proximal tumors, is associated with lower patient morbidity.

Traditional transanal excision (TAE) is reserved for small tumors within 8 cm of the anal verge that are readily accessible. A full-thickness resection through the bowel wall into the perirectal fat is carried out with a minimum of

1-cm margins. In some cases, prominent lymph nodes can be resected but generally a thorough lymphadenectomy is not feasible, which is a major concern in more advanced tumors; therefore, preoperative patient selection and accurate staging is critical. The mucosal defect is then closed primarily. More proximal tumors can be accessed using TEM, which was introduced in the early 1980s as a minimally invasive alternative. The operating platform consists of an operating proctoscope and specialized microsurgical instruments that allow dissection in the upper rectum for lesions that previously could only managed with abdominal surgery (50).

The initial studies of local excision for early rectal cancers demonstrated that this procedure was associated with high local failure rates (17% for T1 tumors and up to 46% for T2 tumors) (51,52). In 2000, Mellgren *et al.* reported a retrospective study comparing 108 patients T1 and T2 rectal cancers excised locally with 153 patients who underwent radical resection. They found that local recurrence was significantly higher after local excision for both T1 and T2 cancers as compared with standard resection (T1: 18% *vs.* 0%, T2: 47% *vs.* 6%). Additionally, overall 5-year survival decreased significantly after local excision of T2 cancers as compared with standard resection (81% *vs.* 65%) (51). These findings were confirmed in a larger, retrospective study using the National Cancer Database. In this report, local recurrence after local excision was 12.5% for T1 cancers and 22.1% for T2 cancers. These were both statistically higher than rates for standard resection. Interestingly, despite these data, the authors also found that the use of local excision had increased significantly from 1989 to 2003 (53).

Salvage surgery may be possible for local recurrence after local excision but often not without significant morbidity. It often involves multimodality treatment including preoperative chemoradiation and extensive surgery (multivisceral resection or pelvic exenteration). Sphincter preservation is not always possible and overall 5-year survival is relatively poor (54).

These data suggest that in appropriately selected patients with T1 rectal cancers, local excision has similar acceptable overall survival rates as compared with standard resection. However, patients should be counseled that the reduced short-term morbidity of local excision is also associated with significantly higher rates of local and overall recurrence. Local excision of T2 rectal cancers has not been routinely recommended outside of clinical trials. The preliminary results of the ACOSOG Z6041 trial of neoadjuvant chemoradiation followed by local excision of T2 cancers have just been reported. The authors found that this strategy resulted in high rates of complete response (44%) and 64% of patients had their tumors downstaged. Negative resection margins were achieved in 99% of the included patients; however, the chemoradiation toxicity and postoperative complications were not insignificant. Sixty-two patients (72%) were able to complete chemoradiation per protocol and 39% of patients developed grade 3 adverse events or higher. Perioperative complications occurred in 58% of study patients and the most common grade 3 adverse events included rectal pain, bleeding, infection, urinary retention, and anal incontinence (55).

Management of locally recurrent rectal cancer

Despite the advances in chemoradiation therapy and surgical technique, local recurrence occurs in up to 10% of cases (56,57). The prognosis is generally poor and is only slightly improved with additional adjuvant treatment alone; therefore, radical surgical resection offers the only possibility for cure. The patterns of local recurrence are variable but may occur at the anastomosis or within the pelvis with attachments to the pelvic sidewall(s), bony structures, or adjacent pelvic organs. There is currently no accepted universal classification to define local rectal cancer recurrence; however, important features include patient symptoms, anatomic location, and the degree of fixation (57).

Patients who are suspected to have locally recurrent disease require a thorough endoscopic and radiographic evaluation to rule out distant metastasis and to define the degree of local involvement. Suspicious lesions should be biopsied with the help of useful diagnostic modalities including pelvic MRI, CT scan, or PET scan. Urologic and gynecologic exams should be performed as indicated.

Surgical resection is often complex and requires careful preoperative planning incorporating a multidisciplinary team (colorectal surgery, urology, gynecology, orthopaedics, and oncology). Patients that have not previously received chemoradiation should have neoadjuvant treatment followed by the anticipated resection, while those that have had previous radiation should proceed to surgery, if medically fit. Intraoperative radiation therapy (IORT) or brachytherapy may be indicated based on the degree of residual disease after resection. Extended resection should be performed *en bloc* with any contiguous organ to ensure no residual disease remains (57).

A recent series of 304 patients with locally recurrent rectal cancer undergoing subsequent curative resection found an overall 5-year survival rate of 25%. Preoperative external beam radiation was given in 244 patients (80%) and IORT in 131 patients (43%). Negative resection margins were achieved in only 138 patients and 5-year survival was significantly improved in these patients as compared with those that had residual gross or microscopic disease (32% *vs.* 16%). Extended resections (involving at least one surrounding organ) were performed in 130 patients and were associated with a higher complication rate; however, survival was not significantly different from those that underwent limited resections. Symptomatic pain and fixation in more than one location were associated with a poor prognosis (58).

Conclusions

Colorectal cancer remains a significant cause of morbidity and mortality worldwide. Surgery is the mainstay of treatment for cure in these patients but the overall management of these cancers often requires a multidisciplinary approach. The advent of laparoscopy, robotic and other surgical technology, as well as an increased awareness of the importance of operative technique, have revolutionized the surgical management of this disease. Likewise, innovation in newer chemotherapy regimens and radiation therapy have increased median survival and decreased local recurrence in advanced disease. Despite these advances, there is ample room for further improvement.

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Footnote

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Local excision for early rectal cancer: transanal endoscopic microsurgery and beyond

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Abstract: The goal of treatment for early stage rectal cancer is to optimize oncologic control while minimizing the long-term impact of treatment on quality of life. The standard of care treatment for most stage I and II rectal cancers is radical surgery alone, specifically total mesorectal excision (TME). For early rectal cancers, this procedure is usually curative but can have a substantial impact on quality of life, including the possibility of permanent colostomy and the potential for short and long-term bowel, bladder, and sexual dysfunction. Given the morbidity associated with radical surgery, alternative approaches to management of early rectal cancer have been explored, including local excision (LE) via transanal excision (TAE) or transanal endoscopic microsurgery (TEM) and transanal minimally invasive surgery (TAMIS). Compared to the gold standard of radical surgery, local procedures for strictly selected early rectal cancers should lead to identical oncological results and even better outcomes regarding morbidity, mortality, and quality of life.

Keywords: Rectal cancer; local excision (LE); transanal excision (TAE); transanal endoscopic microsurgery (TEM); transanal minimally invasive surgery (TAMIS)

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Introduction

Colorectal cancer (CRC) is the third most common cancer diagnosis and the third leading cause of cancer death in the United States (US). With the increase in population screening, the overall incidence of CRC in the US has decreased (1). Furthermore, there has been an increase in the detection of early stage CRC. In 2013, the American Cancer Society reported data from the National Cancer Institute indicating that approximately 40% of all CRC are early stage cancers (1). Early stage cancer is associated with higher (~90%) 5-year survival. Early stage CRC is defined as lesions limited to the bowel wall with no disease extension beyond the submucosa (T1) or the muscularis mucosa (T2). Furthermore, there is no evidence of lymph node spread (N0).

The management of early stage CRC, in particular rectal cancer, can be challenging. Traditionally, treatment has involved major radical abdominal surgery known as the total mesorectal excision (TME) with the potential for a temporary or permanent stoma. The aim of this procedure is to achieve adequate tumor clearance through the removal of the primary tumor including the mesorectum with the associated regional lymph nodes (2-4). TME or radical surgery is the primary surgery that offers excellent rates of local control and therefore, excellent long-term survival. Patients who undergo radical surgery for stage I and II rectal cancer can expect excellent long-term results which approach 4.5% 5-year local recurrence rates and 90% 5-year disease free survival (DFS) rates (5). However, the morbidity is high (30-68%) with a mortality that approaches 7% (2,5-7). Radical surgery is often followed by significant complications including anastomotic leakage, sepsis, permanent or temporary stoma, perineal wound complications, and urinary, sexual and bowel dysfunction that may diminish quality of life (2,3,5-9).

Given these significant complications, there has been increased interest in the locoregional treatment of early

Table 1 Suggested criteria for LE
Physical examination
Tumor <3 cm
Tumor <30% of bowel circumference
Tumor within 15 cm of dentate line
Tumor freely mobile
Imaging (ERUS/MRI)
Tumor limited to submucosa (T1)
No lymph node involvement (N0)
Histology
Well to moderately differentiated
Absence of LVI or PNI
No mucinous or signet cell component

LE, local excision; ERUS, endorectal ultrasound; MRI, magnetic resonance imaging; LVI, lymphovascular invasion; PNI, perineural invasion.

rectal cancer, as some patients may be cured by avoidance of radical surgery and its concomitant disadvantages (10,11). Local excision (LE) of early rectal cancer is an attractive alternative to radical surgery for several reasons. First, the surgery is less invasive and associated with less postoperative pain and a shorter length of stay. The surgery preserves normal bowel function without the use of a stoma. There is less associated perioperative morbidity. Furthermore, newer methods known as transanal endoscopic microsurgery (TEM) or transanal minimally invasive surgery (TAMIS) have been introduced that provide better visualization of tumors in the mid and upper rectum. The aim of this review is to guide the reader in the understanding of the current debates in the management of early stage rectal cancer. This review will include a discussion of patient selection, surgical techniques, and expected oncological outcomes following treatment.

Patient selection

Strict patient selection for LE, together with full-thickness and margin-free excision is crucial for patient outcomes (12). In carefully selected patients local recurrence rates have been reported to be <4% and LE can be curative, with similar oncological outcomes to radical surgery (10). There are several variables that must be evaluated when considering a patient for LE. The key variables include the following characteristics of the tumor: differentiation, the presence of lymphovascular invasion (LVI), the location in the rectum, the size, and the clinical stage. Other key variables that are important to consider prior to performing surgery for rectal cancer are the characteristics of the patient that may put him or her at a higher surgical risk.

To properly select the patients that will benefit from LE, first, digital rectal exam is performed which may determine the mobility of the tumor, the distance from anal verge, and the strength of the anal sphincter. Further, proctoscopy will help in examining more proximal tumors for size and distance from the anal verge. In general, LE can be technically performed for tumors that occupy no more than 30% of the bowel circumference, are no larger than 3 cm in size, and are mobile.

The best method for clinical staging of rectal cancer remains a controversial topic among health care providers. Preoperative identification of tumor depth of invasion (T stage) in the rectal wall and lymph nodes (N stage) can be a challenge. Both modern imaging modalities of endorectal ultrasound (ERUS) and magnetic resonance imaging (MRI) have been used to detect depth of tumor invasion and lymph nodes metastases in rectal cancer (3,10). The reported sensitivity and specificity of ERUS for depth of tumor invasion, perirectal tissue invasion and lymph node involvement is 94%, 90% and 67%, and 86%, 75% and 78%, respectively (13). The major disadvantage of ERUS is the variability in the interpretation of the study due to its dependence on one individual to perform and read the study accurately. MRI has a sensitivity and specificity for T staging ranging from 85% to 100% and from 91% to 98%, respectively (14,15). MRI is also superior at mesorectal lymph node staging with similar sensitivity and specificity as T staging (16). Both imaging modalities will not determine the absence of occult nodal metastases with complete certainty, and some authors suggest that both modalities can be used in combination to increase the likelihood of accurate local staging (3,17).

Histological evaluation of the initial endoscopic biopsy of a rectal tumor may aid in determining tumors at a higher risk of lymphatic spread. Important histopathological indicators of aggressive tumor behavior include: histological grade, mucinous tumors, signet cell tumors, and the presence of LVI or perineural invasion (PNI) (*Table 1*) (18,19). Though controversial, tumor histologic grade is considered a stage-independent prognostic indicator and poorly differentiated colorectal adenocarcinoma is associated with worse patient survival (20-22). Mucinous adenocarcinoma is defined by the findings of >50% of the tumor volume composed of extracellular mucin.

Table 2 Comparison of techniques for LE

Variables	TAE	TEM	TAMIS
Tumor distance in the rectum (from dentate line)	Up to 8 cm	>4 cm-up to 15 cm	Up to 15 cm
Bowel preparation	Required	Required	Required
Patients position	Tumor dependent	Tumor dependent	Lithotomy
Anesthesia	Spinal or general	General	General
Instrument	Rigid	Rigid	Flexible
Cost	Low cost	Expensive	Low cost
Learning curve	Moderate learning curve	Steep learning curve	Shallow learning curve
View	~180 degree view	220 degree view	360 degree view

LE, local excision; TAE, transanal excision; TEM, transanal endoscopic microsurgery; TAMIS, transanal minimally invasive surgery.

These tumors are frequently associated with hereditary non-polyposis CRC (HNPCC) and have the potential to behave more aggressively especially if the tumor is found to be microsatellite stable (23,24). Signet ring adenocarcinoma occurs in less than 1% of patients with colorectal adenocarcinoma. By definition this tumor is poorly differentiated and carries a worse outcome than conventional adenocarcinoma (24-26). Several authors have identified both PNI and LVI as being poor predictors for survival both in those patients treated with multimodality therapy and those treated with surgery alone. Cienfuegos *et al*. demonstrated a nearly 4-fold risk of recurrence in patient following neoadjuvant therapy for rectal cancer with PNI or LVI. Furthermore PNI and LVI have been shown to be independent predictive variables for poor survival (27). For this reason, many support more radical surgery in this cohort of patients.

Traditionally, only rectal cancer below 10 cm was considered a candidate for LE. This was due to the limitation of the surgeons' ability to reach higher and the lack of proper visualization of the rectal tumor. With advances in technology and instrumentation, tumors that are higher up can be reached with good visualization. Newer methods including TEM and TAMIS may allow access up to 15 cm in the rectum. It is important that the patient is aware that these procedures will most likely result in a perforation of the bowel above the retroperitoneum and into the peritoneal cavity which will require repair. The details of these procedures are discussed further in this review.

Extended indications for LE have been reported. Currently, patients with a clinical stage ≥T2 rectal adenocarcinoma should undergo radical surgery. Patients with a diagnosis of more advanced rectal cancer who are not candidates for radical surgery due to high operative risk or those who refuse to undergo radical surgery may be considered for neoadjuvant therapy followed by LE of residual disease (28). Furthermore, the use of LE in patients with early rectal cancer treated with neoadjuvant therapy has been studied in clinical trials with mixed results (29-31). Currently, there is limited data supporting LE or close observation in those patients with a complete clinical response following neoadjuvant therapy as an alternative to radical surgery (5,7,10).

Surgical methods of local excision (LE)

Transanal excision (TAE)

Tumors that are less than 10 cm from the anal verge can be resected with a TAE. In preparation for surgery, a full bowel prep is prescribed, systemic antibiotics are administered, and all anticoagulant use is discontinued. Positioning in the operating room is dependent on the location of the tumor. The patient is placed in lithotomy position for posterior tumors and in prone jackknife for anterior and lateral tumors. Regional or general anesthesia can be utilized to remove the tumor (*Table 2*). To aid in visualization, the anus is gently dilated and retracted with a Lone Star® (32). The goal of TAE is a full thickness excision of the tumor down to the mesorectal fat with at least 1 cm radial/circumferential margin. In anterior tumors that abut the posterior vaginal wall, this may not be possible and a partial excision is then carried out. Good hemostasis is obtained and the defect in the bowel wall is closed in a transverse manner to avoid narrowing the lumen using interrupted absorbable sutures. The specimen should be oriented by the surgeon for pathological assessment of the margins. Postoperatively, patients experience minimal pain but fever

is not uncommon. Patients can resume regular diet and activity within 24 hours (33). Postoperative complications are infrequent and include rectal bleeding which is the most common (6%), rectal stenosis (5.5%), urinary retention (1.5%), fecal incontinence (0.5%), and rectovaginal fistula (<1%) (34,35). If patients receive radiation prior to resection, rectal pain is the most common complication (8%) (36).

The major disadvantage for TAE is the poorer surgical outcomes. Moore and others have demonstrated that newer procedures such as TEM yields clear margins more frequently than with the traditional TAE (90% *vs.* 71%) and significant less chance of tumor fragmentation, 94% *vs.* 65% respectively (37). Intraoperative suboptimal visualization has been hypothesized as the cause for the increase risk of positive margins and tumor fragmentation following TAE (34).

Transanal endoscopic microsurgery (TEM)

TEM was first introduced in 1980's by Beuss as an alternative to radical surgery for the removal of rectal polyps. The TEM system consists of a dedicated beveled rectoscope with a 4.5 cm diameter and a maximum distance of 200 mm. This scope is placed in the anus forming an airtight seal to allow for insufflation of the rectum and greatly aiding in visualization (11,38,39). The view is magnified and approximately 220 degrees of the rectum can be seen at once. In preparation for surgery, a full bowel prep is prescribed, systemic antibiotics are administered, and all anticoagulant use is discontinued. Anesthesia is provided with either spinal or general and the patient is positioned on the operating room table so the tumor is in the dependent position (*Table 2*) (32,40). The resectoscope allows access to more proximal rectal lesions up to 15 cm. Because the distal rectum will form the seal with the resectoscope, very low tumors (<5 cm from the anal verge) are not visualized adequately with the TEM procedure. The rectum is insufflated with a standard laparoscopic CO_2 insufflator, and then a full thickness excision is performed using laparoscopic instruments to achieve a 1 cm circumferential margin (32,33). The bowel wall defect is closed transversely, and the specimen oriented for pathological review. If the tumor is above the peritoneal reflection, the abdominal cavity may be perforated and this may require a laparotomy to repair (33). Postoperatively, patients are expected to have an overnight hospital stay and quick recovery with early resumption of normal diet and activities (32,33).

The conversion rate from TEM to radical surgery from an abdominal approach has been reported to be 4.3% in one large series of 693 patients (41). The most common complications reported are hemorrhage (27%), urinary tract infection (21%), and suture line dehiscence (14%) (41). Bleeding and perforation can become life threatening especially in multimorbid or elderly patients. They frequently require reoperations and extend hospital stays (42-44). The reported incidence of fecal incontinence developing after insertion of the resectoscope is 1% and this is generally temporary (41).

The major disadvantage to the TEM procedure which has resulted in a slow adoption in the US is the expense of the resectoscope. Although it clearly demonstrates better visualization, it has a very limited clinical role to smaller tumors in the rectum located from 5 to 15 cm. Another disadvantage of TEM is the steep learning curve that is associated with its use. Barendse *et al.* demonstrated by observing four different providers resect 693 lesions with TEM that a significant learning curve was associated with lowering conversion rates, peritoneal entrance, and procedure time (41). This same study also demonstrated that in patients undergoing TEM after the surgeon had performed at least 35 procedures, the risk of recurrence for malignant lesions declined by 10% as compared to those individuals undergoing surgery in the first 1-35 procedures (41).

Transanal minimally invasive surgery (TAMIS)

TAMIS was first described in 2009 as an alternative to the more expensive system for TEM. The "Tamis platform" uses any of the several available single incision laparoscopy surgery (SILS) ports. By using this port, conventional laparoscopic instrumentation including the camera can be used to perform the procedure. In preparation for surgery, a full bowel prep is prescribed, systemic antibiotics are administered, and all anticoagulant use is discontinued. Anesthesia is provided with either spinal or general and the patient is placed in the dorsal lithotomy position (*Table 2*). A SILS port is first lubricated and introduced into the anal canal and pneumorectum is established with a standard laparoscopic CO_2 insufflator (45,46). Laparoscopic camera lens (preferably using a 5-mm 30 degree or 45 degree lens) and instruments such as graspers, thermal energy devices, and needle drives are introduced through the SILS port to assist the operator in performing a full-thickness resection of the neoplasm with 1 cm margins. The remaining rectal defect is closed in the transverse direction and the specimen oriented for pathological review (46). If the tumor is above the peritoneal reflection, the abdominal

cavity may be perforated and this may require laparotomy to repair (33). Postoperatively, patients are expected to have an overnight hospital stay and quick recovery with early resumption of normal diet and activities. Several investigators are designing the TAMIS platform so that the procedure can be performed with the assistance of the Da Vinci® robot.

Complications following the TAMIS procedure are infrequent with an overall rate of 7.4% (45). The conversion rate in 390 cases performed for both benign and malignant lesions was 2.3% (45). Inadvertent peritoneal entry during TAMIS was reported in 1% of cases and in some cases, the closure of the rectum was successful transanally (45). In malignant polyps, the rate of positive margins was 4.4% and the rate of tumor fragmentation was 4.1% (45).

Oncological outcomes from LE

The advances in the management of rectal cancer have risen from a desire by those who take care of these patients to improve oncological outcomes while maintaining good quality of life. This desire has been the leading force for the development of newer surgical methods which are less invasive. Colorectal surgery is one of the leading specialties in minimally invasive and robotic surgery techniques and the desire to expand the role of LE follows naturally. Early results from studies examining LE for rectal cancer have been mixed (*Table 3*). For this reason, TAE became a procedure reserved for benign lesions. Presently, only clinically staged T1 rectal tumors with favorable histopathology are considered eligible for LE alone without multimodality therapy (54-58).

Interest in developing newer procedures for LE of rectal tumors was driven by the findings of high recurrence rates seen after transanal resection of benign and malignant lesions. Pigot *et al.* demonstrated that in large rectal tumor up to 6 cm, the risk or recurrence of benign polyps was 10% (34). If a malignancy was identified, the risk of recurrence was 20%. Others have reported local recurrence rates up to 39% (59-63). Pigot further speculated that the results from TAE can be explained by inadequate intraoperative exposure and suggested that the newer and improved techniques of LE may improve outcomes (34).

Several single series have been published demonstrating superiority of new procedures such as TEM or TAMIS over TAE with regards to margin of resection and tumor fragmentation. Baatrup *et al.* examined his series of 143 consecutive TEM resections for rectal cancer. Of the patients that were pathological stage T1 tumors, the local recurrence rate was 12% (64). He also found that the significant predictors for survival in his group of patients were tumor size and patient age. He strongly urged that tumors greater than 3 cm should not be removed by LE. In a similar study by Lezoche *et al.*, 135 patients were followed who underwent TEM (65). There were no local recurrences noted in patients with pathological stage T1 tumors and the overall survival rate was 86% at 193 months. Moore *et al.* in 2007 reported a retrospective comparison of TEM to TAE for rectal cancer (37). In this study, 171 patients (82 with TEM) were analyzed. This study included equal number of patients in each group with T2 and T3 tumors. Patients undergoing TEM had an overall lower recurrence rate (8%) when compared to patients undergoing TAE (24%) but this did not reach statistical significance.

When comparing the results of LE to radical surgery, local recurrence rates tend to be higher for both T1 (8.2-23%) and T2 adenocarcinomas (13-30%) undergoing LE when compared to radical surgery for T1-T2 disease (3-7.2%) (36,49,53,66). However, in the studies evaluating LE there has not been a significant difference in DFS when compared to radical surgery. In patients undergoing LE for T1-T2 disease the DFS at 5 years following LE was 55-93% (36,53). This was comparable to patients undergoing radical surgery whose DFS at 5 years was 77-97% (48,49). The inability to demonstrate improved survival following radial surgery may be due to the retrospective analysis that occurred in many of these studies and the lack of adequate follow up. Only recently has there been an emphasis on appropriate follow up following LE. In addition, Nash *et al.* emphasizes from his review of this topic that when he analyzed the patients he followed after LE, there was a survival difference seen between LE and radical surgery and this difference was the result of longer follow up (50). He noted a significantly increased rate of cancer-related death at 4-8 years following LE when compared to radical surgery. He recommend that all patients undergoing LE be committed to long term follow-up.

Whether LE compromises the oncological outcome with the risk of recurrence and local failure remains unknown. Lymph node metastasis occurs in 0-12% in T1 and 10-22% in T2 rectal cancer, however, as local lymph nodes are not sampled using TEM, it is reliant on preoperative staging and histopathological features of the tumor to direct further adjuvant treatment (3,67,68). Comparing different LE techniques; the negative margin is most likely achieved with TEM compared to TAE (64,65). Furthermore, the local

Table 3 Summary of the studies comparing LE *vs*. radical surgery for early rectal cancer

Study	T stage	Patients (n)	Excision technique	FU (mos)	5-year local recurrence (%)	5-year distant recurrence (%)	5-year disease-free survival (%)	5-year overall survival (%)	Negative margin (%)	Intact specimen (%)	Post op complications (%)
TAE *vs*. TME											
Nascimbeni *et al*. (47), 2004	T1	70	TAE	54	6.6	14.2	66.6	72.4	–	–	–
		74	TME	–	2.8	6.9	83.6	90.4	–	–	–
Endreseth *et al*. (48), 2005	T1	35	TAE	60	12.0	0	64.0	70.0	46	–	–
		256	TME	60	6.0	7.0	77.0	80.0	100	–	–
Bentrem *et al*. (49), 2005	T1	151	TAE	48	15.0	12.0	93.0	89.0	–	–	–
		168	TME	58	3.0	3.0	97.0	93.0	–	–	–
Nash *et al*. (50), 2009	T1	137	TAE	59	13.0	–	83.0	–	–	–	–
		145	TME	77	2.7	–	96.0	–	–	–	–
TEM *vs*. TME											
De Graaf *et al*. (51), 2009	T1	80	TEM	42	24.0	0	90.0	75.0	–	–	5.8
		75	TME	84	0	8.0	87.0	77.0	–	–	64
Palma *et al*. (11), 2009	–	34	TEM	86.5	5.9	5.9	82.4	88.2	–	–	2.9
		17	TME	93	0	0	82.4	82.4	–	–	23.5
TAE *vs*. TEM *vs*. TME											
Ptok *et al*. (52), 2007	T1	85	TAE	44	6.0 (combined LE)	4.0 (combined LE)	91.4 (combined LE)	83.6	–	–	9.4
		35	TEM	–	–	–	–	–	–	–	2.9
		359	TME	–	2.0	4.0	92.3	91.5	–	–	25.1
LE *vs*. TME											
You *et al*. (53), 2007	T1	601	LE	60	8.2	3.6	93.2	77.4	–	–	LE (T1 + T2)
		493	TME	–	4.3	2.6	97.2	81.7	–	–	5.6
	T2	164	LE	–	12.6	5.0	90.2	67.6	–	–	TME (T1 + T2)
		866	TME	–	7.2	7.7	91.7	76.6	–	–	14.6
TAE *vs*. TEM											
Moore *et al*. (37), 2008	T1, T2	89	TAE	53	24.0	4.0	–	–	71	65	17
	T3	82	TEM	20	4.0	1.0	–	–	90	94	15

LE, local excision; TAE, transanal excision; TME, total mesorectal excision; TEM, transanal endoscopic microsurgery.

recurrence rate is lower with TEM compared to TAE (37). This is likely the direct result of improved visibility that is achieved with TEM (69) Whether or not these differences ultimately affect DFS is yet to be determined.

Radical resection immediately after LE

Due to the variability in the sensitivity and specificity of the preoperative staging modalities, it is not uncommon for a preoperatively staged T1N0 rectal cancer to have a final pathological stage of T2 or T3. Moreover; a positive margin following LE carries a high risk of recurrence (68). One method of managing unfavorable pathology is to offer the patient immediate radical surgery. Hahnloser *et al.* reported his experience at Mayo clinic with immediate radical resection after LE of rectal cancer (70). In this series, 52 patients underwent radical surgery within 30 days after LE were matched with 90 patients with a T2-3N0-1 primary as a radical surgery control group. The indications for radical re-resection were: cancerous polyp, positive margins, LVI, advanced stage, nodal disease and residual cancer. The five-year overall survival for the study cases *vs.* the control case was (79% *vs.* 91%), respectively and the ten-year survival was (65% *vs.* 78%), respectively with no statistical significant.

Several studies have reported that the oncologic outcomes in patients treated by immediate radical surgery after LE for unfavorable histologic findings are comparable to that of radical surgery performed as a primary treatment (2,10,33,70). However, there is no consensus on the timing of radical surgery or on the use of radiotherapy before radical surgery (9).

LE following neoadjuvant therapy

Excellent response to neoadjuvant therapy for rectal cancer has been observed with complete tumor regression even for advance clinical stages in 10 % to 30% of patients (10,71,72). These finding have translated into a significant reduction in local recurrence rates from 12% to 4% (73). In patients with pathological complete response (pCR), the risk of lymph node involvement is 1.8% compared to 24-52% in those who didn't have pCR (9). Furthermore, patients with a pCR tend to have favorable long-term outcomes, including better overall survival and lower recurrence rates (9,74,75). This had led some clinician to question the need for radical surgery with its associated morbidity in those who have a clinically complete response (cCR) confirmed by endoscopic exam.

Habr-Gama *et al.* compared the long term outcomes between patients who were found to have incomplete clinical response (iCR) and underwent radical surgery with patients who had cCR and underwent a "watch and wait" approach (30). In this series, a total of 265 patients with T2-4 rectal adenocarcinoma received neoadjuvant chemoradiotherapy (CRT). A total of 71 (26.8%) had cCR and underwent watch and wait approach and 194 (73.2%) had iCR and underwent radical resection. At resection, 22 (8.3%) were found to have pCR on the resection specimen. The five-year overall and DFS was 100% and 92% in the watch and wait group and 88% and 83% in the radical resection group respectively. In addition, Perez *et al.* reported on 15 patients with clinical stage T2N0 rectal cancer who underwent neoadjuvant therapy (31). Therapy was followed by "watch and wait" if a cCR occurred, TEM was performed for a partial response with minimal residual disease, and radical surgery was performed for non-responders. The findings from this study demonstrated that for T2N0 tumors, if a cCR to neoadjuvant therapy does not occur, this appears to be a poor prognostic indicator for unfavorable pathological features as nearly 70% of these patients had ypT2 or ypT3 features and those patients are not ideal candidates for LE.

Currently, the standard of care for T2 rectal adenocarcinoma is radical surgery to ensure accurate staging and decrease the risk of local recurrence but with the promising results of pCR; extended indications for LE have been considered as a middle ground between radical surgery and observation in good responders. The American College of Surgeons Oncology Group (ACOSOG) completed a prospective phase II trial that examined the efficacy and safety of neoadjuvant chemoradiotherapy and LE for T2N0 rectal cancer (76). A total of 77 patients who underwent neoadjuvant therapy and LE were included in the analysis. The pCR rate was 44% and tumor downstaging occurred in 64% of patients. The rate of margin positivity at the time of resection approached 0%. However, 39% of patients developed CRT-related grade ≥3 complications and the trial was closed early. Therefore, long-term survival data is not available, presently. Belluco *et al.* compared patients with T3N0-1M0 mid and distal rectal adenocarcinoma who underwent TME or LE and were found to have a pCR (74). A total of 139 patients were included and 110 (93%) underwent TME and 29 (17%) underwent LE, 42 (30.2%) were found to have a pCR. In follow up of 55.4 months, there was no difference in the local recurrence between

radical surgery *vs.* LE. Currently, although neoadjuvant therapy may benefit some patients with early stage rectal cancer, indiscriminate use is not recommended in this population owing to the overtreatment of the majority (36).

Adjuvant therapy following LE

In an attempts to improve the oncological outcome and decrease recurrence; adjuvant therapy has been given following LE. To examine the efficacy of this approach, the Cancer and Leukemia Group B (CALGB) has performed a prospective, multi-institutional study on patients with T1 and T2 distal rectal cancer treated with LE with and without adjuvant therapy (77). In this study, 59 patients with T1 tumor were treated with LE alone and 51 patients with T2 tumor were treated with LE followed by adjuvant CRT. The median follow up was 7 years. The ten-year overall survival and DFS were 84% and 75% for T1, and 66% and 64% for T2 respectively. The local recurrence and distant failure rates for T1 tumors were 8% and 5%, while T2 tumors were 18% and 12% respectively. This results show that T2 tumors had a higher rate of recurrence and shorter overall and DFS even with the administration of adjuvant CRT when compared to T1 or historic radical resection. Therefore, adjuvant CRT following LE maybe reserved for patients with high risk pathology who are unfit to undergo radical resection.

Surveillance following LE

Surveillance guidelines published by the National Comprehensive Cancer Network (NCCN) following LE for T1 rectal cancer include the following: (I) a complete history and physical exam every 3-6 months for 2 years, then every 6 months for a total of 5 years; (II) CEA every 3-6 months for 2 years; (III) chest, abdomen, and pelvic computerized tomogram annually for 3 years; (IV) colonoscopy at one year and thereafter depending on findings; (V) proctoscopy every 6 months for 5 years (78). However, as stated early, others have demonstrated a benefit in follow up for up to 9 years following LE (67).

Conclusions

Historically, oncological outcomes from the use of LE for the treatment of early rectal cancer have been disappointing. However, in carefully selected patients with early (T1) rectal cancer, LE by means of the newer methods of TEM and TAMIS is a promising alternative to radical surgery with minimal morbidity and acceptable oncological outcomes. Currently, there are minimal studies evaluating combined use of neoadjuvant therapy and LE for ≥ T2 lesions which limits its generalizability. Furthermore, several authors are supporting no surgery with a "watch and wait" approach for patients with a cCR because the oncological outcomes are no different than radical surgery. Further prospective clinical trials are needed to determine the most promising roles for LE in the management of rectal cancer.

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Footnote

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A critical review of the role of local excision in the treatment of early (T1 and T2) rectal tumors

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Abstract: The optimal treatment of early (T1 and T2) rectal adenocarcinomas remains controversial. Local excision and radical resection with total mesorectal excision are the two surgical techniques for excising early rectal cancer. Each has their respective benefits, with local excision allowing for decreased operative morbidity and mortality while radical resection provides an oncologically complete treatment through lymphadenectomy. Local excision can be accomplished via transanal endoscopic microsurgery or transanal excision. There is no significant difference in the recurrence rates (21% *vs.* 33%) or overall survival (80% *vs.* 66%) between the two local excision modalities; however, transanal endoscopic microsurgery does allow for a higher rate of R0 resection. Current selection criteria for local excision include well to moderately differentiated tumors without high-risk features such as lymphovascular invasion, perineural invasion, or mucinous components. In addition, tumors should ideally be <3 cm in size, excised with a clear margin, occupy less than 1/3 of the circumference of the bowel and be mobile/nonfixed. Despite these stringent inclusion criteria, local excision continues to be plagued with a high recurrence rate in both T1 and T2 tumors due to a significant rate of occult locoregional metastases (20% to 33%). For both tumor groups, the recurrence rate in the local excision group is more than double compared to radical resection. However, the overall survival is not significantly different between those with and without metastases. With intense postoperative surveillance, these recurrences can be identified early while they are confined to the pelvis allowing for salvage surgical options. Recently, neoadjuvant therapy followed by local excision has shown favorable short and long-term oncological outcomes to radical resection in the treatment of T2 rectal cancer. Ultimately, the management of early rectal cancer must be individualized to each patient's expectations of quality and quantity of life. With informed consent, patients may be willing to accept a higher failure rate and an increased post-operative surveillance regimen to preserve a perceived increased quality of life.

Keywords: Transanal excision; early rectal tumors; local excision

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Introduction

Concurrent with the widespread use of population based screening programs, there are more than 42,000 newly diagnosed cases of rectal cancer each year (1). Radical surgical resection of both the primary tumor and the draining lymph node basin (by either low anterior or abdominoperineal resection) remains the corner stone of curative therapy in rectal cancer of all stages. However, the staging accuracy of endorectal ultrasound (ERUS) and pelvic magnetic resonance imaging (MRI) has led some to question the necessity of a major surgical resection for early T-stage cancers when the entire tumor burden could (theoretically) be resected complete by transanal excision. The optimal treatment of early rectal adenocarcinoma remains debatable, although most surgeons recommend

Table 1 Appropriate tumor selection for local excision

Characteristics	Favorable	Unfavorable
1	Well differentiated	Poorly differentiated
2	Moderately differentiated	
3	No lymphovascular invasion	Lymphovascular invasion
4	No perineural invasion	Perineural invasion
5	No mucinous components	Evidence of mucin production
6	Invasion to level sm1 and sm2	Invasion to level sm3
	<3 cm in size	>3 cm in size
	<1/3 the circumference of the rectal wall	>1/3 the circumference of the rectal wall
	<10 cm from the anal verge	>10 cm from the anal verge
7	Mobile lesion	Fixed lesion

sm, submucosa; cm, centimeter.

radical resection for T2 lesions.

The surgical approach to rectal cancer has evolved continually over the last 100 years. The trend towards less invasive surgical procedures is clear: from the initial attempts at trans-sacral resection, to the popularization of universal abdominoperineal resection (the Miles procedure), followed by acceptance of low anterior resection (greatly facilitated by surgical stapling technology). Transanal, local excision of early cancers is the logical extension of this trend. At present, radical resection with total mesorectal excision (TME) is the surgical standard of care for rectal cancer. This approach completely removes the primary tumor and draining lymph node basin, allowing accurate and complete pathological staging. Radical resection with TME is also fully curative in patients with node-negative and early T-stage cancers. However, radical resection carries a 2-3% perioperative mortality rate and 20-30% overall complication rate (2). Additionally, long-term complications such as sexual impotence, decreased fecundity in women, alterations in bowel function (e.g., the anterior resection syndrome), and the potential for a permanent ostomy all adversely affect quality of life (2-5).

In contrast, local excision avoids the common complications associated with a major operation allowing for decreased anesthesia, minimal fluid shifts and blood loss in combination with a shorter hospital stay and quicker recovery. But the decreased invasiveness comes at the expense of an oncologically incomplete surgery. Advocates for local excision assert that failure due to occult mesorectal lymph node metastases is potentially treatable with salvage total mesorectal excision. Although current imaging modalities have improved, some patients will not be accurately staged. Only after presenting with a local failure will they receive appropriate adjuvant therapy. For this reason, local excision mandates a strict adherence to an intense post-operative surveillance schedule extending beyond 5 years to detect any recurrence.

Appropriate patient and tumor selection remain a major obstacle to transanal excision of rectal cancer, although advances in understanding tumor biology may improve this process. There are no widely accepted guidelines for utilizing local excision. In general, it is reserved for tumors isolated to the submucosa (T1) that are well to moderately differentiated with low-risk histopathological features. Lymphovascular invasion, perineural invasion, and mucinous components are considered high-risk characteristics, and local excision should be avoided due to an increased rate of lymph node metastasis. In addition, tumors should ideally be <3 cm in size with a clear margin, occupy less than 1/3 of the circumference of the bowel and be mobile/nonfixed (6). Despite these stringent inclusion criteria, local excision continues to be plagued with a high recurrence rate (*Table 1*). The goal for the treatment of early (T1 and T2) rectal cancer is to optimize oncologic control while minimizing the long-term impact of treatment on quality of life (7). This paper will review the data for both T1 and T2 adenocarcinomas, as well some of the promising surgical and combined modalities for treating these early cancers.

Transanal excision for T1 adenocarcinoma

Cancer biology differs substantially throughout the lower gastrointestinal tract, with a predisposition for early lymph node spread in the rectum compared to the proximal

Table 2 Outcomes after local excision *vs.* radical surgery for T1 adenocarcinoma of the rectum

Series	Surgery performed	N	High grade (%)	LR (%)	DR (%)	OS (%)	DFS (%)	Median F/U (mo)
Local excision								
Garcia-Aguilar *et al*., 1999	TAE	55	0	16.0	4.0	82	77	52
Paty *et al*., 2002	TAE	74	15.0	17.0	–	74	–	120
Gopaul *et al*., 2004	TAE	32	–	13.0	–	–	–	37
Bentrem *et al*., 2005	TAE	151	9.0	15.0	12.0	89	93	60
Endreseth *et al*., 2005	TAE	35	1.0	12.0	0	70	64	60
Madbouly *et al*., 2005	TAE	52	0	23.0	12.0	75	70	55
You *et al*., 2007	LE-ANS	601	5.3	8.2	3.6	77	93	60
Nash *et al*., 2009	TAE	137	52.0	19.0	19.0	69	83	59
Doornebosch *et al*., 2010	TEM	88	6.0	21.0	8.0	–	–	36
Radical resection								
Bentrem *et al*., 2005	RR-NOS	168	10.0	3.0	3.0	93	97	60
Endreseth *et al*., 2005	LAR/APR	256	15.0	6.0	7.0	80	77	60
You *et al*., 2007	RR-NOS	493	7.5	4.3	2.6	82	97	60
Nash *et al*., 2009	LAR/APR	145	–	–	–	85	94	77

TAE, transanal excision; TEM, transanal endoscopic microsurgery; LE-ANS, local excision, approach not specified; LAR, low anterior resection; APR, abdominoperineal resection; RR-NOS, radical resection, not otherwise specified; LR, local recurrence; DR, distant recurrence; OS, overall survival; DFS, disease free survival; F/U, follow-up; mo, months.

colon (4). Although limited to the bowel submucosa, T1 rectal cancer has a 13-25% rate of occult lymph node spread compared to only 3-8% in the colon (4,8). While it's not uncommon to retrieve 1 or 2 lymph nodes along with a full-thickness transanal resection, the nodal basin is not sufficiently staged by local excision alone. Despite lingering concerns about the adequacy of a transanal excision, a paradoxical increase in the use of local excision for T1 tumors occurred in the United States between 1989-2003 (9). Subsequently, several authors have cautioned against local excision, citing excessively high local recurrence rates and worse oncological outcomes (see below), possibly due to lack of rigorous patient and tumor selection.

Most neoplasms less than 10 cm from the anal verge can be resected transanally. Local excision results in a full-thickness specimen including some mesorectal fat. At least 1 cm circumferential mucosal margins should be obtained. The specimen is usually pinned to corkboard or sponge by the surgeon to avoid confusion over orientation and specimen contraction from soaking in Formalin. The defect in the bowel wall is subsequently closed, typically in a transverse manner to prevent restriction of the rectal lumen. Patients perform a full mechanical bowel preparation prior to the surgery, but recovery postoperatively is rapid, with early resumption of regular diet and activity and minimal discomfort.

ERUS and/or pelvic MRI are mandatory for the preoperative staging of rectal cancers. ERUS is more sensitive in distinguishing early bowel invasion of the primary, while MRI is superior at evaluating mesorectal lymph nodes and the circumferential resection margin. The utility of combining ERUS and MRI to direct surgical therapy has also been explored by various investigators (10). Recently though, several studies have reported a significantly lower sensitivity rate (48-54%) of ERUS for detecting early T1 cancer as compared to higher staged lesions (11,12). The success of transanal excision relies on the accuracy of preoperative clinical staging as it fails to address possible occult lymph node metastasis. Presumably the higher regional recurrence rate following local excision is at least in part explained by a failure of preoperative imaging modalities to detect micro-metastatic disease within mesorectal lymph nodes.

The literature on local recurrence rates after transanal excision for T1 rectal cancer is comprised mostly of retrospective studies containing a heterogeneous population of high and low risk lesions (*Table 2*). Despite the differences between the series, the type of surgery (transanal excision

vs. radical resection) remains a constant predictor of local recurrence, with radical resection always maintaining a lower local recurrence rate. The gap between the treatment modalities does narrow though when stratifying tumors by both clinical and pathologic criteria. Blumberg and colleagues demonstrated that excluding high-risk factors (lymphovascular invasion, mucin production, poor differentiation) and applying strict clinical factors (distance from anal verge and size) could decrease the lymph node metastases rate from 16% to 7% (2.3 fold) (5). Kikuchi *et al.* showed that not all T1 tumors behave in the same manner, and their invasiveness stems from the level of submucosal infiltration. For tumors only slightly invading the submucosa (sm1) there were no nodal metastases observed, as opposed to tumors invading the deepest one-third of the submucosa (sm3) that had a 25% rate of metastases (13). Sm3 depth of invasion has been confirmed by other authors as a contraindication for local excision (8). Greenberg *et al.* provided long-term follow-up on the prospective CALGB 8984 study of local excision of T1 rectal cancer (14). The authors found a local recurrence rate of only 8% at 7.1 years median follow-up using stringent selection criteria. Others have confirmed that oncological outcomes in prospective series seem improved relative to the larger retrospective reports, reinforcing the importance of strict attention to patient and tumor selection (2).

Although there is an increased local recurrence rate between the surgical modalities, this has failed to translate into a survival benefit. After 5 years, there is an overall survival rate of 70-89% *vs.* 77-97% and disease free survival rate of 64-93% *vs.* 80-93% in the transanal excision and radical resection groups, respectively (2,4). Conversely, the similar survival rates may reflect an inadequate follow-up time. During 10 years of follow-up by Nash *et al.*, the authors found a similar overall survival in the first 4 years after diagnosis but an increased rate of cancer-related death between 4-8 years (peak period of cancer recurrence) in the transanal excision group. Only after 9 years did death from other causes dominate in the transanal excision group (12). Patients undergoing local excision must be committed to a long-term follow-up schedule to detect recurrences.

If high-risk features are identified in the original pathologic specimen, an immediate radical resection should be performed. This does not compromise outcomes and has a 94% disease free survival rate at 5 years (15). In this manner, the transanal excision may be viewed as a "large biopsy", the results of which may direct further immediate surgery. However, the aggressive use of salvage surgery after identifying a local recurrence can still allow for an R0 resection to be accomplished in a majority of cases (77%) (4). With routine post-operative surveillance, the detection rate is up to 88% with proctoscopy and ERUS alone, although most centers also utilize either computed tomography (CT) or MRI (16). Salvage surgery, though, comes with the cost of increased morbidity compared to an initial radical resection and may require multivisceral resection and an ostomy in up to 43% (4). After salvage surgery, the 5-year overall survival is significantly decreased to 43-56.2% compared to those without a recurrence (3,4). The relatively poor outcomes following salvage surgery emphasize the importance of the appropriate initial treatment of early rectal cancer.

Transanal excision for T2 adenocarcinoma

Similar to T1 tumors, there was almost a fifty percent increase across the US between 1989-2003 in the use of local excision to treat T2 rectal cancer (12% to 21%) (7). While local excision is now generally an acceptable treatment of T1 tumors, there is a growing concern about extending its application to T2. Transanal excision of T2 tumors carries a nearly double local recurrence rate compared to T1 lesions, ranging from 13-30% for the more advanced primary lesions (*Table 3*). The higher local recurrence rate is likely due to the increased occult nodal metastasis rate of 28-38% (17). Conversely, radical resection has only a slightly increased rate of local recurrence at 7.2% compared to that for T1 tumors (9). This finding emphasizes both the staging and therapeutic benefits of total mesorectal excision.

The increased invasiveness and locoregional metastatic potential of T2 tumors is also reflected in the decreased overall survival, and the difference is increased for patients undergoing local excision as compared to radical resection. In the nationwide cohort study by You *et al.*, there was a significant difference in overall survival (68% *vs.* 77%, P=0.01) between local excision and radical resection (9). This was strongly impacted, though, by nononcologic factors related to the patient [age (>75) and multiple comorbidities (>2)] rather than the type of surgery. The disease-free survival did not differ (90% *vs.* 92%, P=0.95) at 5 years, likely due to early death by other non-cancer related causes (9). Given the advanced age and poor health of this study population, they may not have been candidates for a radical resection. Nevertheless, the 90% disease free survival in the radical resection group demonstrates the effectiveness of the procedure in providing a cure.

Table 3 Outcomes after local excision *vs.* radical surgery for T2 adenocarcinoma of the rectum

Series	Surgery performed	N	High grade (%)	LR (%)	DR (%)	OS (%)	DFS (%)	Median F/U (mo)
Local excision								
Garcia-Aguilar *et al*., 1999	TAE	27	0	30.0	7.0	63	55	58
Paty *et al*., 2002	TAE	51	–	28.0	–	75	–	120
Gopaul *et al*., 2004	TAE	25	–	24.0	–	–	–	37
You *et al*., 2007	LE-ANS	164	13.4	13.0	5.0	68	90	60
Radical resection								
You *et al*., 2007	RR-NOS	866	7.9	7.2	7.7	77	92	60

TAE, transanal excision; TEM, transanal endoscopic microsurgery; LE-ANS, local excision, approach not specified; RR-NOS, radical resection, not otherwise specified; LR, local recurrence; DR, distant recurrence; OS, overall survival; DFS, disease free survival; F/U, follow-up; mo, months.

Table 4 Outcomes after neoadjuvant chemoradiation therapy for T2 adenocarcinoma of the low rectum

Series	Surgery performed	N	Chemo *vs*. radiation	LR (%)	DR (%)	OS (%)	DFS (%)	Median F/U (mo)
Local excision								
Nair *et al*., 2008	TAE	10	Both	10	10	81	–	60
Lezoche *et al*., 2012	TEM	50	Both	8	4	72	89	115
Perez *et al*., 2013	TEM	18	Both	14	19	85	68	15
Radical resection								
Lezoche *et al*., 2012	Lap LAR	50	Both	6	4	80	94	115

TAE, transanal excision; TEM, transanal endoscopic microsurgery; Lap LAR, laparoscopic low anterior resection; LR, local recurrence; DR, distant recurrence; OS, overall survival; DFS, disease free survival; F/U, follow-up; mo, months.

At present, it seems imprudent to locally excise T2 rectal cancers in fit patients (11). Local excision offers a moderate chance of cure and is reasonable for patients in whom major surgery is contraindicated due to medical comorbidities.

Transanal excision after neoadjuvant chemoradiation

Neoadjuvant chemoradiation has consistently demonstrated the ability to reduce local recurrence rates and downstage primary tumors in select patients with rectal cancers (18,19). This has sparked interest in its application in early rectal cancer (20). Most of the neoadjuvant and adjuvant scheduled in the literature used a similar of radiation dose (50.4-54 Gy), and all chemotherapy regimens are 5-fluoruracil (5-FU) based. As shown in *Table 4*, tumor downstaging and downsizing has been demonstrated in 51-64% and 26-100% of T2 rectal cancers, respectively (20,21). It is important to note that complete clinical response only translates to a 30-60% pathologically complete response for which there is minimal disease recurrence (20-22). Lezoche *et al*. reported that overall recurrences occurred primarily in the low response and non-responder groups, at rates of 12% after local excision and 10% after radical resection (21). A more aggressive surgical approach is indicated for these patients, as an incomplete response likewise may exist in the regional lymph nodes (22). Using a neoadjuvant regimen consisting of 4,500 cGy in 25 fractions of radiation over three fields with a boost of 540 cGy to the tumor in conjunction with a continuous infusion of 300 mg m^{-2} day^{-1} of 5-FU on days of radiation over a 5-week course, Nair *et al*. noted that the overall survival was not significantly different between the local excision group and radical resection group (72% *vs*. 80%, P=0.61) (22). In the transanal excision of T2 rectal cancer, neoadjuvant therapy has shown favorable short-term and similar long-term oncological outcomes to radical resection.

The improved oncologic benefits of combined chemoradiation therapy do not come without a price. Chemo-radiation increases the rate of post-operative

complications; however, most of these are minor complications (91%) that can be managed without additional surgery (20). The most common side effects were gastrointestinal, dermatologic, and hematologic.

The use of neoadjuvant therapy for T2 rectal cancer should not be over utilized, though, as radical surgery alone provides an adequate treatment for T2 N0 disease. It's role may be to downsize and downstage borderline T2-T3 tumors. Local excision may then be utilized to determine the pathological response to the chemoradiation. If there is only a partial response and tumor still remains, immediate radical resection should be performed. It is the authors' current practice to determine surgical treatment prior to initiating neoadjuvant therapy.

Transanal endoscopic microsurgery for T1 and T2 adenocarcinoma

Transanal endoscopic microsurgery (TEM) for local excision of rectal adenomas was originally described by Dr. Buess of Germany in 1983. Although the technique and instruments have undergone refinement over the past 30 years, the surgical principles have remained the same. The patient is positioned on the table (lithotomy, prone jackknife, lateral decubitus) such that the tumor is in the posterior position. A specialized set of instruments including a 40 mm rectoscope and laparoscopic style tools are required, although newer minimally invasive equipment can be adapted for transanal use (e.g., transanal minimally invasive surgery). After appropriate insufflation of the rectum, the tumor is visualized and a 1 cm circumferential margin marked with electrocautery. A full thickness excision is then performed, the specimen oriented on the back table and the resulting defect closed transversely with absorbable sutures.

TEM is similar to transanal excision in that patients can expect a short (1-2 day) hospital stay, decreased complications and quicker recovery. The complication rate after TEM is <5% and includes bleeding, rectovaginal fistula, transient incontinence to gas and stool, and transient urinary retention (23). There are several key differences, though, between the two operations. TEM often requires a general anesthetic to perform the procedure, which may be contraindicated in patients with severe cardiopulmonary disease, opposed to spinal or local anesthesia for traditional transanal excision. The superior visualization and instrumentation afforded by TEM relative to traditional transanal excision often permits en bloc specimen removal, thus avoiding piecemeal resection. This allows for an increased rate of R0 resection and a more accurate histological evaluation of the circumferential and deep margin.

TEM is the gold-standard operation for the resection of rectal adenomas, but its use as a curative option for rectal carcinoma is debatable. Despite having a significantly decreased rate of R1 resection between TEM and traditional transanal excision (2% *vs.* 16%) (24), achieving an R0 resection did not prevent local recurrence (16). Even when stratifying to low-risk T1 tumors, there is still a 17% local recurrence rate after TEM (16). There was no significant difference in the 5-year recurrence rate between T1 and T2 tumors removed by either local excision technique (21% *vs.* 33%, P=0.07) (24). Due to the high rate of local recurrence in low-risk patients with even an R0 resection, improving criteria for tumor resection by TEM is of major importance.

TEM suffers from the same shortcomings as traditional transanal excision in being unable to adequately stage the pelvis. Using the same post-operative surveillance schedule as transanal excision, most recurrences can be detected early enough to allow for salvage surgery. Short term follow-up after salvage surgery shows a cancer-related survival of 79% at 1 year and 58% at 3 years, which is comparable to transanal excision (16). Between the two local excision modalities, the 5-year disease free survival (85% *vs.* 70%, P=0.146) and overall survival (80% *vs.* 66%, P=0.119) were similar across both T1 and T2 lesions (24).

In summary, TEM provides better visualization of the tumor allowing for a more proficient operation to be performed. However, this has not translated to improved local recurrence or overall survival compared to traditional transanal excision. While some authors advocate for TEM as the treatment of choice for local excision, patient and tumor-specific features remain paramount regardless of the surgical approach. Further studies are needed examining the relative effectiveness of TEM compared to traditional transanal excision.

Surveillance following local excision

Following local excision, a long-term surveillance schedule is mandatory to identify recurrences that are potentially resectable and metachronous lesions. Although centers vary slightly in their follow-up regimen, each consists of at least a semiannual history and physical exam, carcinoembryonic antigen (CEA), and proctoscopy in

conjunction with annual imaging (CT or MRI) (4,6,11). There has been an increased trend in the combined use of CT/MRI with ERUS postoperatively to increase the sensitivity in detecting locoregional recurrences. It is the authors' current practice to perform a history and physical examination every 3-6 months for the first 2 years and then annually after. A baseline CEA is obtained prior to surgery and then followed at every appointment. To detect mucosal recurrences, a digital rectal exam and proctoscopy or flexible sigmoidoscopy are performed every 3-6 months for 2 years and then yearly after. This is alternated with ERUS every 6 months to evaluate for lymph node metastases. Finally, a CT or MRI is obtained annually to detect local or distant recurrences. Most surveillance schedules only extend out to five years, but given the propensity for late recurrences, long-term follow-up after local excision should be pursued.

Conclusions

The management of early (T1 and T2) rectal cancer must be individualized to each patient's expectations of quality and quantity of life. Even in the lowest risk patients, transanal excision is inferior to radical resection from an oncologic standpoint due to inadequate local control and staging of the pelvis leading to an increased local recurrence rate. However, with informed consent, patients may be willing to accept a higher failure rate and an increased post-operative surveillance regimen to preserve a perceived increased quality of life. Accurate and appropriate patient selection for local excision hinges on preoperative imaging techniques and sound histopathology. Local excision remains an acceptable option in well-to-moderately differentiated T1 rectal cancers with favorable histological features, provided the surgeon can obtain clear margins. Future investigations to improve preoperative clinical and pathological staging may improve patient selection and decrease local recurrence.

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Complete mesocolic excision with central vascular ligation: is this the approach to improve colon cancer surgery oncological outcomes?

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The concept of total mesorectal excision (TME) as proposed by Heald *et al.* (1) in the early 80's resulted in significantly better oncological outcomes in rectal cancer surgery (1-3). TME also raised the issue of better outcomes in colon cancer surgery, which, until then, was not standardized and the reports in the literature displayed a great deal of heterogeneity and high recurrence rates (4-7). In 2009 and in parallel to the TME concept, came the first report and description of the complete mesocolic excision (CME) with central vascular ligation (CVL) from the Erlangen group of Hohenberger (8), with quite impressive oncological outcomes and an overall 5-year survival reaching up to 70% for stage III colon cancer patients. They also showed that it is a safe and feasible technique which bears at least the same morbidity and mortality as the "so called" standard technique (8).

CME with CVL consists of two main components. Firstly, it aims at the preservation of intact fasciae of the mesocolon between which relevant lymph nodes are contained. Secondly, the vessels that supply the tumor colon site must be ligated at their origin, namely (I) at the level of the superior mesenteric vein for right sided lesions, and (II) at the level of the take-off of the inferior mesenteric artery for left sided lesions. In this way the nerves of the celiac plexus, which run along the superior mesenteric artery, and the hypogastric plexus, which runs on the aorta, respectively, are protected, and the removal of the complete mesocolon and the maximum lymph node yield is achieved.

The concept of CME with CVL has been strongly criticized. At the beginning the criticism was on the novelty of the technique. Many supported that it is a concept already implemented by the majority of colorectal surgeons, in particular for tumors of the left colon (9,10). In addition, the issue of reproducibility of the technique due to the high level of expertise necessary was raised. Many experienced and skillful colorectal surgeons claimed that some steps of the CME with CVL, as described by Hohenberger *et al.* (8), were very technically demanding, some were unnecessary, and because of that the technique was rendered very difficult to teach and to reproduce (11-14). Furthermore, reports from Japan also demonstrated that perhaps the main element of the technique is the CVL, because they showed similar results not by removing longer specimens but by removing more radically the supplying vessels (15).

The establishment of CME with CVL in the mind of many, at first skeptical, colorectal surgeons came when the leading group of pathologists from Leeds, which is involved in almost all the techniques used by pathologists to assess and measure surgical quality in rectal surgery, published the first morphometric criteria of macroscopical and microscopical assessment of colon cancers specimen (16). In this way, the superiority of the CME with CVL, as far as the quality of the surgical specimen is concerned, was proven (16).

Thereafter, reports by many groups followed comparing the CME with CVL with retrospective cohorts of conventional colectomies and contemporarily the first reports concerning laparoscopic CME appeared in the literature (12,17-30). The results from all these studies were rather conflicting, probably due to the fact that conventional colectomy is not at all standardized and that surgeons

participating in the studies may not have any specific CME training offered by an established training course.

One of the first reports, which compared the standardized CME with CVL with a cohort of conventional colectomy in a defined population after the special training of the surgeons on CME with CVL, came from a group in Denmark (31). The authors studied all patients who underwent elective colectomy for colon cancer from the Danish Colorectal Cancer Group national registry for a specific region from June 2008 until December 2011. The CME with CVL group consisted of patients operated in a specific hospital at which a previous special training program on CME with CVL was implemented and surgeons were trained. The conventional colectomy group consisted of patients operated in three other hospitals of the same region. The medical records from all patients were cross-checked by the participating surgeons ensuring the highest possible validity of data analyzed.

The authors found that the implementation of CME with CVL is a significant independent predictive factor for higher disease-free survival for all patients, irrespective of the stage of the disease. As expected they also showed a significantly higher lymph node yield for the CME with CVL group as well as a significantly higher rate of mesocolic-graded resections. They also report a higher number of invaded lymph nodes in the CME with CVL group. On the contrary, they showed that overall survival was not effected by CME with CVL. The authors speculate that this is probably due to advances in surgery of the metastatic disease and chemotherapy and to the short period of follow-up.

Despite the fact that this study is very well designed and uses a quite meticulous statistical methodology using complex multivariable modeling and propensity score matching to reduce the bias due to confounding factors, it has some methodological flaws many of which could be anticipated. First of all, a very important issue that appears is the quality of the pathology reports. The surgeons performing CME with CVL were trained but similar training is required for the pathologists who grade the macroscopic and microscopic quality of the resected specimens for both groups in order for the pathological data to be equally valid. The pathologist for this kind of study is even more important than the surgeon because he is the one who searches for and determines the biomarkers that are important for the final oncological outcome both prognostic and predictive. If the quality of the pathology is poor or even worse, has a huge variability then the dataset for analysis is biased by definition.

Secondly, a methodological flaw is that the authors chose to exclude R2 resections. This creates bias in favor of the standard colectomy group, because, since all operations were performed on an intention-to-treat basis, a R2 resection in the standard group may be a technical disadvantage of the technique itself as compared to the CME with CVL group where a R2 resection is a real limit of oncological radicality. R2 resections and the exact site of positive margin should have been included in detail in order to identify any advantage in resectability of the CME with CVL over the standard technique.

Thirdly, the variability in the type, the time-intervals and the duration of follow-up among the participating centers is a potential source of bias affecting the timing of identification of possible recurrences. This is discussed also by the authors in the discussion section of the manuscript.

Fourthly, the dataset of the study itself and the type of analysis bear some possible sources of bias the majority of which are also discussed and accounted for in the final analysis. Pathological features of the tumors resected in the CME with CVL group displayed some differences that could have confounded the data in favor of the conventional colectomy group such as higher serosal invasion rates, higher rates of extramural venous invasion and higher rates of signet-ring cell and undifferentiated carcinomas. All these confounders were picked up by the authors and by the use of propensity score matching their effect on the outcomes was correctly adjusted.

Also, a matter for discussion and overall criticism for CME with CVL is the lack of effect in stage III patients in whom theoretically the maximum effect is anticipated. This comparative study is the only that identified a positive effect in this subgroup despite the almost equally use of adjuvant therapy in both groups. In parallel, the importance of accurate staging is stressed because the effect of false down-staging could be an important source of bias. In the same sense, the quality of the pathology handling of the specimen during lymph node identification (especially identification and status of apical lymph nodes) is of utmost importance for the determination of all relevant lymph node status and the correct staging. In their study, the methylene-blue injection technique was used by the pathologists in the middle of the study period only in the CME with CVL group and this fact might be an important source of bias. They conclude that patients' staging has a very low chance of being inaccurate because data do not differ from that of the whole country.

An additional argument that is discussed is the effect of CME with CVL on stage II patients. In this dataset, a significantly higher proportion of stage II patients received adjuvant chemotherapy possibly due to worse pathological features of the tumors (serosal invasion, extramural venous invasion) and this is the fact that one could attribute the positive effect of CME with CVL. The authors put these variables in the modeling for the multivariable analysis and none of them proved to be an independent prognostic factor causing bias.

In our opinion, the most important omission in the analysis of the data is the absence of a subgroup analysis on the basis of tumor location. This is of great importance given the fact that conventional colectomy has a great variability in the definition of the term and this becomes more complicated when the tumor site changes from left to right. In detail, left-sided conventional resections are closer and sometimes coincide with the concept of CME with CVL. On the other hand, right-sided and transverse tumors constitute a different group of tumors, and the CME with CVL technique differs hugely from the conventional one. In this sense, tumor site may be a significant confounder when all cases are being analyzed together and the only way to account for this is the subgroup analysis of the data based on tumor location. CME with CVL is expected to have a greater effect for tumors located in the right and transverse colon. A hint towards the above is given by the authors when discussing the lower rate of laparoscopy in the CME with CVL group and attribute it to the fact that right-sided and lesions of the transverse colon are not preferred to be operated laparoscopically due to limitations of the approach to the radicality of the technique.

In conclusion, the study, despite several methological limitations, points towards the correct direction. The setup of a randomized study that compares the conventional to the CME with CVL colectomies for colon cancer seems impossible, and this is because conventional colectomy cannot be standardized at all. Therefore, large series of patients been prospectively subjected to CME with CVL must be accumulated and be compared to conventional surgery cases deriving form large archive data-bases. A prerequisite for more reliable and less biased results and conclusions are the adequate training of both surgical teams and pathologists, and the subgroup analysis that must take into account several parameters, such as tumor location, type of surgical approach, quality of surgery, histo-pathological characteristics including stage of the disease and adjuvant treatment.

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Footnote

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Complete mesocolic excision (CME) with central vessel ligation (CVL): a new standard in colon cancer surgery

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Abstract: The new surgical technique of complete mesocolic excision (CME) with central vessel ligation (CVL) has been reported to lead to improvements in oncological outcomes for patients with colon cancer. Here we discuss a recent large-scale retrospective report by Bertelsen *et al*. that provides compelling evidence to support previously smaller and/or less well designed studies and confirms CME surgery to be oncologically superior to the traditional approach. This Danish study importantly demonstrates an approximately 10% improvement in four-year disease free survival with CME surgery for all patients with stage I-III colon cancer. These data are the first to also show that relatively small surgical advances can still lead to major improvements in cancer survival.

Keywords: Colon cancer; surgery; complete mesocolic excision (CME)

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The seminal work of Richard 'Bill' Heald in the 1980s paved the way for improvements in both local recurrence and cancer-free survival rates for patients with non-metastatic rectal cancer from 20-45% (1) to <3% (2). The approach Heald used was fundamentally anatomical in origin. Rather than performing a close rectal dissection he removed en bloc the draining lymph nodes from the rectum by performing a total mesorectal excision (TME). Although oncological improvements have been seen for patients with colon cancer these have been far less dramatic than with rectal cancer and it is difficult to attribute these to better quality colonic surgery, rather these appear to be as a consequence of improvements in adjuvant therapies. Indeed, the recent focus in colonic cancer surgery outcomes has been driven in the main by the introduction of laparoscopic resections together with its associated improvements in morbidity and length of stay. More recently, several groups have asked whether improvements in colon cancer resection technique can lead to similar improvements in patient survival and local recurrence as were seen with the introduction of TME. These new approaches have, in the main, utilised a more radical approach with meticulous dissection along anatomical planes, an extensive lymphadenectomy and ligation of feeding vessels at their origins. Small volume reports inspired by data from the pioneers of the technique in Erlangen, Germany (3) have demonstrated that these operations, coined complete mesocolic excision (CME) with central vessel ligation (CVL), can be performed safely and appear to lead to improvements in patient disease-free survival and local recurrence (4,5). To-date a large scale randomised control trial comparing CME with CVL to traditional surgery has yet to be performed and is argued by some as unfeasible not least as a consequence of surgical equipoise. At the end of last year a significant report was published by Claus Anders Bertelsen in the December issue of *Lancet Oncology* detailing a large retrospective study on outcomes following CME and CVL in comparison to standard surgery (6).

Bertelsen's work is a further study that convincingly demonstrates that CME, in European hands, improves survival for all non-metastatic stages of colon cancer (I-III). The study performed between 2008 and 2011 involved

four centres. One centre performed CME and the three others non-CME. Data was collected retrospectively for stage I-III, colonic resections involving 364 patients in the experimental arm and 1,031 in the control group. For all stages of colon cancer operated on, 4-year disease-free survival was improved by approximately 10% in patients undergoing CME. These improvements were most marked for stage I and II disease. On regression analysis CME was found to be an independent predictive factor for disease free survival for all stages analysed.

From a demographic perspective the two groups were generally well matched. The CME group had a larger proportion of extended right hemicolectomies performed than non-CME and predictably there were a larger proportion of open operations in the CME group although still almost 50% of CME resections were laparoscopic. Although larger numbers of lymph nodes were identified in the CME resections there was no evidence of stage migration. A larger proportion of patients in the CME group with stage II disease received chemotherapy; however this was not found to be an independent predictive factor for disease free survival on regression analysis. It does however remain to be determined whether the results presented are entirely attributable to the CME technique or related to institutional differences. No historical data, for example, are provided showing equivalence in outcomes in a pre-CME era between the four centres. The authors acknowledge a further potential minor confounder in relation to the use of methylene blue injection to improve pathological yields of lymph nodes in the CME group. The fact that stage migration was not observed suggests that this minor confounder, even if present, plays only a minor role. No data are also provided in the paper as to complication rates although it has been shown before in other studies that CME is likely as safe as traditional surgery (3). Mortality rates were comparable between the two groups.

This Danish study is important not only for its size and convincing collection of data but also for the questions it raises in relation to the aetiology of the oncological improvements seen. It has been argued by some that CME with CVL is no different from good quality colon cancer surgery (7). In the Far East, although not having used the same nomenclature, similar approaches described as D3 lymphadenectomies, have been used for some time as standard of care in stage II and III disease (8). Furthermore, comparing oncological outcomes following an eastern style D3 lymphadenectomy and CME are essentially equivalent (9). It is also unclear through which mechanism CME achieves its benefit i.e., Halsted or Cady-Fisher like mechanisms (10). It appears that stage migration is unlikely to be a predominant mechanism for the apparent benefits with CME and therefore the role of the super-high pedicle ligation also remains uncertain. The importance of sharp dissection, paying particular attention to not disturbing peritoneal planes as with TME appears to be of utmost importance. Many clinical and scientific questions are raised by the data surrounding CME and with time the various components will likely become stratified according to their relative importance.

Evidently, and based on this important study, CME-type surgery provides an important advance for improving outcomes for patients with stage I-III colon cancer. In perspective, the oncological improvements seen with CME surgery exceed those shown to be attributable to adjuvant chemotherapy. Although these data presented are of a lower evidence level than a formal RCT the authors are quick to point that currently a RCT would be near impossible to perform. There have been several other large retrospective and prospective non-randomised studies looking at CME-type surgery. In addition there have been two recent systematic reviews similarly concluding that CME surgery is likely oncologically superior to standard surgery and doesn't appear to carry an increased morbidity (11,12). There is therefore a reasonable weight of evidence in support of accepting CME as standard of care despite a RCT having not taken place. Several important questions however remain in relation to its application, driven in part by the lack of understanding of the mechanism of the apparent effect. These include the necessity of CVL with CME, complication rates compared to standard surgery and whether a laparoscopic approach is as good as open when applying CME principles. Some of these questions could be addressed by a suitably organised RCT. It appears that CME-type surgery is here to stay and it follows that standardisation, training and nomenclature need to be internationally agreed upon. With time we may find that this radical form of surgery is not necessary for all patients but at present we feel that the principles of CME should be embraced by the surgical community.

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Is lymph node metastasis the only concern in high-risk submucosal colorectal cancer following endoscopic resection?

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Endoscopic resection represents a curative therapy for Tis colorectal cancer (carcinoma *in situ*; intraepithelial or invasion of the lamina propria) as it has no risk of lymph node metastasis (1-3). However, lymph node metastasis occurs in 7-15% of T1 colorectal cancers (invasion of submucosa) (4-10). In order to achieve curative resection of submucosal colorectal cancer, predictors for lymph node metastasis have been evaluated in many studies (7,9-14) and found to be depth of submucosal invasion (1,000 and 3,000 μm for nonpedunculated and pedunculated submucosal colorectal cancers, respectively), lymphovascular invasion, and poorly-differentiated adenocarcinoma (11,15,16). In cases of submucosal colorectal cancer with no risk factors for lymph node metastasis, no further treatments such as surgical resection appear to be necessary following complete endoscopic resection. Conversely, additional surgery has been recommended for high-risk submucosal colorectal cancer (11).

Some patients with high-risk submucosal colorectal cancer, however, hesitate to undergo surgery due to surgery-associated morbidity and mortality. In certain circumstances, endoscopists also struggle with whether to offer surgery as the majority of patients with risk-factors for lymph node metastasis actually have no metastatic spread. Such scenarios seem to be more frequent in rectal cancers compared to colon cancers. Abdominoperineal resection—the standard treatment for low rectal cancer—can leave some patients with permanent stomas (17). Therefore, when taken together with the rate of lymph node metastasis of approximately 10%, careful observation can also be an alternative treatment option in select patients.

Until now, risk of lymph node metastasis has only been a concern in patients with high-risk submucosal colorectal cancer following endoscopic resection. Because rates of lymph node metastasis do not differ between submucosal colon cancer and submucosal rectal cancer (8,11,15), tumor location does not appear to be an important variable in evaluating high-risk submucosal colorectal cancer. However, Ikematsu *et al.*'s recent study (18) in the *Gastroenterology* demonstrated that the risk for local cancer recurrence was significantly higher in patients with high-risk submucosal rectal cancer than in patients with high-risk submucosal colon cancer when treated with endoscopic resection alone. That study reviewed data from 573 patients with submucosal colon cancer and 214 patients with submucosal rectal cancer and who underwent endoscopic or surgical resection at six institutions. This dataset constituted the largest retrospective study population for patients with submucosal colorectal cancer. In total, the number of patients treated with endoscopic resection was 327 and 101 for submucosal colon cancer and submucosal rectal cancer, respectively. Of those patients, 218 and 84 were high-risk for lymph node metastasis, respectively. Patients that refused additional surgery, designated as group B, were 31.7% of high-risk submucosal colon cancer (69 of 218) and 44.0% of high-risk submucosal rectal cancer (37 of 84). The results from this study suggest that patients with high-risk submucosal rectal cancer decline additional surgery more frequently than patients with high-risk submucosal colon cancer (P=0.043, Chi-square test). In group B, rate of local recurrence was higher in submucosal rectal cancer than in submucosal colon cancer (10.8% *vs.* 1.4%, respectively, P<0.01). This serves as an interesting finding as there was no difference in local recurrence rates for patients who underwent surgery or in patients with low-risk submucosal colorectal cancer treated with endoscopic resection alone. This study further demonstrated that disease-free survival for patients with high-risk submucosal rectal cancer was inferior to patients with

high-risk submucosal colon cancer (5-year disease-free survival rates: 77.7% *vs.* 96.5%, respectively, P<0.01). The authors proposed that recurrence rates greater than 10% might be expected when no additional surgery was pursued due to the increased possibility for micrometastasis. Based on these collective findings, it appears important to consider not only risk of lymph node metastasis but also risk of local recurrence when evaluating treatment options for patients with high-risk submucosal rectal cancer following endoscopic resection.

In this study, long-term disease-free survival of patients with low-risk submucosal colorectal cancer following endoscopic resection alone was excellent. All 104 patients with low-risk submucosal colon cancer did not exhibit recurrence during the defined follow-up period (mean: 55.2 months). In the low-risk submucosal rectal cancer group, only one patient (6.3%) had distant metastases—this patient had originally been classed as low-risk for lymph node metastasis, but upon reexamination of the original pathology specimen, additional slices exhibited lymphovascular invasion. Therefore, this patient was actually high-risk for lymph node metastasis, and additional surgery should have been recommended. Evaluation of this patient raises important considerations including: (I) further demonstration that long-term outcomes of low-risk submucosal colorectal cancer are excellent, and (II) presence and impact of pathologic error. A prior retrospective study demonstrated that pathologic errors in cancer diagnosis occur in up to 11.8% of cases (19). Such data underscore the importance of careful evaluation of cancer recurrence following endoscopic resection even in patients with low-risk of lymph node metastasis. In addition, other reports have proposed further risk factors for lymph node metastasis including tumor budding and background adenoma beyond the classic criteria mentioned earlier (7,13,20). Although further research may be necessary, we believe that additional pathologic assessment for tumor budding and background adenoma in patients with low-risk submucosal colorectal cancers may help to better assess risk for lymph node metastasis. In contrast to patients with low-risk submucosal colorectal cancer, seven patients (6.6%) with high-risk submucosal colorectal cancer who underwent endoscopic treatment had recurrence. In addition, 14 patients with high-risk submucosal colorectal cancer (2.6%) had recurrence despite undergoing surgery. Lymph node metastasis was identified in 12.4% of patients (66 of 532) with high-risk submucosal colorectal cancer and who underwent surgery, findings consistent with previous reports (11,15).

In spite of extraordinary conclusion, results of the study should be interpreted with caution given study limitations. First, the en-bloc resection rate was not reported despite including of patients who underwent endoscopic piecemeal mucosal resection. Local recurrence of colorectal tumor occurs more frequently after piecemeal resection than with en-bloc resection (21,22). Second, multivariate analysis for disease-free survival may not have been appropriate, although univariate analysis showed that disease-free survival rate was lower in patients with high-risk submucosal rectal cancer than in patients with high-risk submucosal colon cancer. Tumor location was an independent risk factor for disease-free survival according to the proposed Cox regression hazard model (HR of rectum =6.73, 95% CI, 1.04-43.43). This model included tumor depth (≥2,000 or <2,000 μm), lymphatic invasion, vascular invasion, and tumor differentiation (well-differentiated or moderately-differentiated). However, based on the established risk factors for disease-free survival, tumor depth (≥1,000 or <1,000 μm) and tumor differentiation (well- to moderately-differentiated or poorly-differentiated) should be included in the model. We speculate that the differences in proposed models might be due to fewer patients having either poorly-differentiated adenocarcinoma or submucosal cancer within 1,000 μm of tumor invasion. Third, disease-free survival in this study appears to be analyzed incorrectly. The 3rd table of the article demonstrated no recurrence in patients with low-risk submucosal colon cancer—however, Kaplan-Meier curves for disease-free survival showed that some lesions (perhaps three) had recurrence. In addition, Kaplan-Meier curves for disease-free survival were similar to overall survival curves. It seems, then, that disease-free survival of patients without recurrence of colorectal cancer and who died from other causes were considered as uncensored data. However, in disease-free survival analyses, such patients should be classified as censored data. Therefore, upon reclassification of the data, 5-year disease-free survival of patients with low-risk submucosal colon cancer was 100.0% and not 95.9%. A similar error was also found in Kaplan-Meier curves for disease-free survival in patients from the high-risk endoscopic resection group. Although such errors may not alter the ultimate conclusions, they do question study reliability.

Despite these limitations, this was a strong study that revealed that risk of local recurrence following endoscopic resection was significantly higher in patients with high-risk submucosal rectal cancer than in patients with high-risk submucosal colon cancer. Why local recurrence occurs more frequently in high-risk submucosal rectal cancer as

compared to high-risk submucosal colon cancer remains unanswered, although micrometastasis was suggested as a plausible theory. Whether more extensive cancer excision with sufficient lateral margins improves disease-free survival in high-risk submucosal rectal cancer also remains unclear. Future studies should address these questions. At present, if an endoscopically-resected submucosal rectal cancer has been proven to be a high-risk lesion for lymph node metastasis, additional surgery should be considered to reduce not only distant metastasis but also local recurrence.

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The emerging role of neoadjuvant chemotherapy for rectal cancer

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Abstract: Locally advanced rectal cancer remains a substantial public health problem. Historically, the disease has been plagued by high rates of both distant and local recurrences. The standardization of pre-operative chemoradiation and transmesorectal excision (TME) have greatly lowered the rates of local recurrence. Efforts to improve treatment through use of more effective radiosensitizing therapies have proven unsuccessful in rectal cancer. Presently, due to improved local therapies, distal recurrences represent the dominant problem in this disease. Adjuvant chemotherapy is currently of established benefit in colorectal cancer. As such, adjuvant chemotherapy, consisting of fluoropyrimidine and oxaliplatin, represent the standard of care for many patients. However, after pre-operative chemoradiotherapy and rectal surgery, the administration of highly effective chemotherapy regimens has proven difficult. For this reason, novel neoadjuvant approaches represent appealing avenues for investigation. Strategies of neoadjuvant chemotherapy alone, neoadjuvant chemotherapy followed by chemoradiation and neoadjuvant chemoradiation followed by chemotherapy are under investigation. Initial encouraging results have been noted, though definitive phase III data is lacking.

Keywords: Rectal cancer; neoadjuvant; adjuvant; chemotherapy; chemoradiation

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Introduction

In 2014 it is estimated that there will be more than 136,000 new cases of colorectal cancer diagnosed as well as greater than 50,000 colorectal cancer associated deaths in the United States. Approximately 40,000 patients will be diagnosed with rectal cancer (1). National uptake of screening via colonoscopy has markedly increased in the last decade, with a corresponding decrease in the incidence of colorectal cancer over this time. In contrast, among individuals under the age of 50, a slight rise in the rates of distal colon and rectal cancers has been observed in the US, as recently reported in Norway (2). Over the last three decades, outcomes of patients with rectal cancer have substantially improved stage for stage, likely attributable to improvements in therapy (3). Prior to the standard use of radiotherapy, systemic therapy, and transmesorectal excision (TME) surgery, both local and distant recurrences represented major problems in the treatment of rectal cancer. Unacceptably high rates of devastating local recurrences prompted multiple efforts to improve local control. In the ensuing years, the benefit of peri-operative radiotherapy, specifically 5-FU based chemoradiation, was established to improve outcomes in patients with rectal cancer (4-7). The primary benefit seen is in reduced local recurrence rates, with a less consistent impact on disease free and overall survival. Moreover, this benefit is demonstrated to be greater with the use of pre-operative rather than post-operative chemoradiation (4). This has led to the incorporation of neoadjuvant 5-FU-based chemoradiation into the standard treatment paradigm for locally advanced rectal cancer.

Notably, since the initial trials of chemoradiation, surgical approaches for rectal cancer evolved significantly, with TME becoming the standard of care. This technique involves *en bloc* removal of the mesorectum, including the primary tumor and the associated perirectal lymph nodes via meticulous dissection so as not to disrupt the mesorectal plane. The advent of TME brought single-institution reports of local recurrence rates as low as 4-9%, compared with

rates of 32-35% through use of conventional surgery (8). Of course, these vast surgically mediated improvements in local control brought into question by some the necessity of pre-operative radiotherapy; as noted, the benefit most consistently observed with chemoradiation has been the reduction in local recurrence rates. For the most part, the pivotal trials evaluating the benefit of adding of radiotherapy to surgery incorporated a suboptimal, but formerly standard non-TME surgical approach. However, the Dutch neoadjuvant trial of short course pre-operative radiotherapy (5×5 Gy) utilized the modern surgical approach, TME, and yet demonstrated a consistent benefit of improved local control (9). Outcomes appear comparable with the two techniques: short course pre-operative radiotherapy and 5-FU based neoadjuvant chemoradiation, though the former has not been widely adopted in the US to date (10,11). Given the bulk of the data supporting pre-operative chemoradiation, as well as demonstration of improved outcomes with TME, utilization of both modalities is currently the standard approach for locally advanced rectal cancer (T3, T4 or node positive disease). Most guidelines also support the addition of post-operative adjuvant chemotherapy, which is administered for the majority of patients (12).

While the data for adjuvant chemotherapy in rectal cancer treated via multimodality therapy is less robust, it is generally accepted that adjuvant chemotherapy is a necessary part of therapy. GITSG protocol 7175 closed early following interim analysis and demonstrated improvements in recurrence rates and disease free survival (DFS) with the use of adjuvant chemotherapy, with or without radiotherapy (13). A survival benefit was not established here. However, the subsequently published NSABP R-01 study, utilizing adjuvant 5-FU based chemotherapy (5-FU, semustine, and vincristine), and the NCCTG study which added 5-FU and methyl-CCNU to radiotherapy both demonstrated that post-operative chemotherapy improves survival (14,15). Of course, refinements in these regimens followed. These chemotherapy choices do not represent the standard for colorectal cancer today. Through investigation, the options of infusional 5-FU or bolus 5-FU and leucovorin were established as the optimal regimens (16,17). The non-inferiority of capecitabine was subsequently confirmed (18). Further building upon this, the MOSAIC trial and NSABP C-07 demonstrated an additional improvement in DFS with the addition of oxaliplatin to 5-FU based adjuvant therapy for colon cancer (19,20). This has led to the routine offering of 5-FU based chemotherapy, typically FOLFOX to stage III and high risk stage II colon cancer patients. A Cochrane meta-analysis of 21 randomized controlled trials supports this practice in rectal cancer, demonstrating a 25% reduction in risk of recurrence for rectal cancer patients treated with adjuvant 5-FU based regimens (21).

On the other hand, long term results of EORTC 22921 were recently reported (22). This trial employed a 2×2 factorial design to assess the value of adding chemotherapy (5-FU and leucovorin) to preoperative radiotherapy concurrently, post-operatively or in both settings. The addition of chemotherapy, either concurrently with radiotherapy or post-operatively, clearly increased local control rates. However, there was no apparent impact of adjuvant chemotherapy on disease-free or overall survival (22). While these results are in some ways disappointing, it is important to note the very poor rates of adherence to chemotherapy: 82% pre-operatively and just 42.9% post-operatively (5). Both the poor compliance rates and the lack of use of a now standard oxaliplatin-based regimen have caused many to view these negative trial results with skepticism. Regardless, conclusive data is lacking, leaving room for debate as to the optimal incorporation of chemotherapy in rectal cancer.

Multiple investigations have been carried out to improve upon the gains described above, including the incorporation of additional radiosensitizing agents to 5-FU. Though irinotecan, oxaliplatin, bevacizumab, and anti-EGFR therapies have improved survival in the metastatic setting, none have yet proved superior as a radiosensitizer when compared to 5-FU-based chemoradiation (23-25). In addition, apart from oxaliplatin, none of these has conclusively improved outcomes in the adjuvant setting for early stage colorectal cancer (26). The testing of new agents in the adjuvant setting and the development of improved radiosensitizing agents may yet provide gains. However, toxicity appears to be greater with post-operative chemotherapy as well as post-operative chemoradiation, leading to delays in therapy as well as premature discontinuation, undermining its potential benefit. The CAO/ARO/AIO-94 trial demonstrated that post-operative as compared to pre-operative chemoradiation increased rates of grade 3/4 acute (40% *vs.* 27%) and long term adverse events (24% *vs.* 14%) (27). Full dose radiation and chemotherapy were administered in just 54% and 50% of post-operatively treated patients as opposed to 92% and 89% of pre-operatively treated patients (27).

Of importance, as highlighted by the results of EORTC 22921, tolerance and compliance with post-

operative chemotherapy is consistently dismal, possibly accounting for its inability to demonstrate benefit (5). In fact, greater than one in three patients do not receive post-operative chemotherapy, for a variety of reasons, as recently reported (28). Even in those who ultimately receive chemotherapy, post-operative complications are linked to delays in the initiation of adjuvant chemotherapy and linked to worsened survival (29). Given the lesser toxicity and improved compliance with therapy in the pre-operative setting, there is a growing interest in developing further neoadjuvant treatment strategies for locally advanced rectal cancer. The remainder of this paper will focus on review of recent data and ongoing neoadjuvant therapy efforts. The three major strategies of focus include neoadjuvant chemoradiation followed by chemotherapy, induction chemotherapy followed by chemoradiation, and neoadjuvant chemotherapy alone.

Neoadjuvant chemotherapy alone

As current surgical techniques achieve very good local control rates and the majority of recurrences represent distant metastatic disease, there is a strong argument to be made for turning our focus to improving the delivery of systemic therapy. The current treatment paradigm utilizes nearly 6 weeks of neoadjuvant chemoradiation, 6-8 weeks of recovery prior to surgery, and another 4 weeks of recovery prior to consideration of adjuvant therapy. As such, the standard approach delays the time to initiation of full dose systemic therapy for 4 months, at a minimum. Beginning chemotherapy sooner provides the theoretical advantage of treating micro-metastatic disease earlier, in hope of reducing the incidence of distant recurrence. In addition, as radiotherapy has not improved survival in the vast majority of the studies published, it is possible the added toxicities of this modality may be obviated through use of chemotherapy alone. Radiation related toxicities are not insignificant; there is a substantial incidence of fecal incontinence and sexual dysfunction which tend to be worse with chemoradiation as opposed to radiation alone (30). Patients treated with chemoradiation as compared with surgery alone note worsening of altered bowel habits: more frequent bowel movements per day, more frequent nighttime movements, and a greater incidence of occasional or frequent incontinence necessitating a pad (31).

However, radiotherapy has an established role in this disease. In addition, the MRC CR07/NCIC-CTG C016 comparing pre-operative short course radiotherapy with selective post-operative chemoradiotherapy demonstrated inferior local recurrence rates and DFS with the selective use of chemoradiation, suggesting that we may not be able to pick and choose the patients in whom to administer radiotherapy (32). In subset analysis, the benefit of radiation was maintained in those patients who underwent TME, but TME was not standard in this trial. Also, less than 50% of patients received any chemotherapy. Both of these factors limit the applicability of these results to the current patient population (32,33). Potentially further alleviating this concern, recent updated results of the MERCURY study suggest that pre-operative magnetic resonance imaging (MRI) assessment of the circumferential margin may be very helpful in predicting those patients who will have clear circumferential margins, with a 94% negative predictive value (34). Such assessments may aid in tailoring therapy, limiting the potential harms of withholding any valuable components.

The experience with neoadjuvant chemotherapy as the sole modality is very limited when compared to other approaches. However, initial results are encouraging. A single institutional study of neoadjuvant IFL (weekly irinotecan, 5-FU and leucovorin) was carried out in the early 2000's in Stage II & III rectal cancers. After 2 months of therapy, 15 of 26 (58%) patients achieved tumor downstaging with one (4%) pathologic complete response (pCR) achieved. A 5-year DFS of 75% was achieved, though there were three pelvic recurrences (35). Importantly, irinotecan is not of proven benefit in adjuvant therapy, and the majority of other efforts focused on oxaliplatin-based therapies. A recent multi-institutional Japanese study evaluated the use of four cycles of neoadjuvant CAPOX (capecitabine + oxaliplatin) and bevacizumab in high risk rectal cancer prior to surgery (T4 in 59.4%, <5 cm from anal verge in 50%). In this 32-patient study, the scheduled chemotherapy was completed by 91% of patients with an R0 resection rate of 90%. pCR was noted in 13% of patients with a total of 37% experiencing good tumor regression (36). A second effort was recently reported from a different group in Japan also utilizing CAPOX and bevacizumab in high risk patients: those with T4 or node positive rectal cancers. Twenty five patients were evaluated, though seven discontinued therapy after 2-3 cycles. One patient (4%) achieved a pCR, and the vast majority were downstaged. Ninety-two percent of patients underwent resection, all with R0 resections. However, post-operative complications were observed in 26% of patients, and at a median follow-up of 31 months, there have been five

Table 1 Studies of neoadjuvant chemotherapy alone in rectal cancer

Study	Key inclusion criteria	#pts	Treatment	pCR rate	Outcomes
Ishii, *et al.* (35)	T3 or T4	26	Irinotecan, 5-FU, Leucovorin ×8 weeks	3.8%	5-year DFS—74% 5-year OS—84%
Uehara, *et al.* (36)	MRI-defined poor risk: T4, N2, CRM ≤1 mm, extramural invasion >5 mm	32	CAPOX, bevacizumab ×12 weeks	13%	R0 resection rate—90%
Hasegawa, *et al.* (37)	T4 or N+	25	CAPOX, bevacizumab ×12 weeks	4%	R0 resection rate—92% DFS at 31 months—68%
Cercek, *et al.* (38)	No radiation, resected primary	20	FOLFOX +/– bevacizumab	35%	N/A
Schrag, *et al.* (39)	T3	32	FOLFOX + bevacizumab ×8 weeks	25%	R0 resection rate—100% 4-year LR—0% 4-year DFS—84%

pCR, pathologic complete response; DFS, disease free survival; OS, overall survival; CRM, circumferential resection margin; LR, local recurrence.

distant recurrences, including one with accompanying local recurrence (37). While neoadjuvant chemotherapy may be beneficial for high risk rectal cancer, the small numbers and poorer prognosis limit interpretation of the outcomes achieved. There is good reason to proceed with caution in eliminating local therapies for those patients at highest risk of local recurrence.

Average risk patients have also been evaluated through such an approach. A review from Memorial Sloan Kettering of 20 patients with colorectal cancer who were treated initially with FOLFOX +/– bevacizumab demonstrated an impressive pCR rate of 35% (38). Similar results were noted by the same group in a prospective evaluation of rectal cancer patients with standard risk (T3 or N+) tumors >5 cm from the anal verge and without bulky nodes. T4 tumors were not permitted. Thirty two patients were treated with 6 cycles of FOLFOX and bevacizumab followed by TME. Radiation was to be utilized for those without response. In this study, all patients demonstrated tumor regression with a 100% R0 resection rate and a 25% pCR rate. At a mean 53 months follow-up, the local recurrence rate is 0% with a 4-year DFS of 84% (39). While these results are encouraging, the small number of patients significantly hampers our ability to estimate the true benefit of this approach. A summary of select neoadjuvant chemotherapy studies is available in *Table 1*.

Appropriately, these encouraging results have prompted a prospective randomized trial evaluating this approach: the PROSPECT trial (NCT01515787). The PROSPECT trial is a phase II/III trial from the Alliance for Clinical Trials in Oncology, "The Alliance", examining the efficacy of 6 cycles of preoperative FOLFOX with the selective use of chemoradiation in patients with non-bulky Stage II/III rectal cancer. Patients are being randomized to pre-operative FOLFOX versus pre-operative chemoradiation, with post-operative treatment left to the discretion of the individual investigator. In the chemotherapy only arm, the use of chemoradiation will be limited to the pre-operative setting in those having less than a 20% reduction in their rectal tumor and the post-operative setting for those patients with positive circumferential margins. MRI will be utilized to guide therapy, with a primary end-point of DFS (*Figure 1*).

Similar studies evaluating pre-operative chemotherapy are ongoing on overseas. The BACCHUS trial is a medium sized phase II trial evaluating the efficacy and toxicity of 6 cycles of FOLFOX + bevacizumab versus 6 cycles of FOLFOXIRI (5-FU, leucovorin, oxaliplatin, irinotecan), with bevacizumab held in the final cycle for both (NCT01650428). Chemoradiation will only be selectively utilized and the primary outcome is pCR rate. There is also an ongoing 3-arm, randomized phase II trial in China evaluating 4 cycles of pre-operative FOLFOX versus FOLFOX followed by FOLFOX-based chemoradiation versus chemoradiation with 5-FU alone (NCT01211210). The primary end-point is 3-year DFS.

The results from the aforementioned trials will be important in the coming years in shaping the face of rectal cancer therapy, though at present neoadjuvant chemotherapy remains investigational given the limited

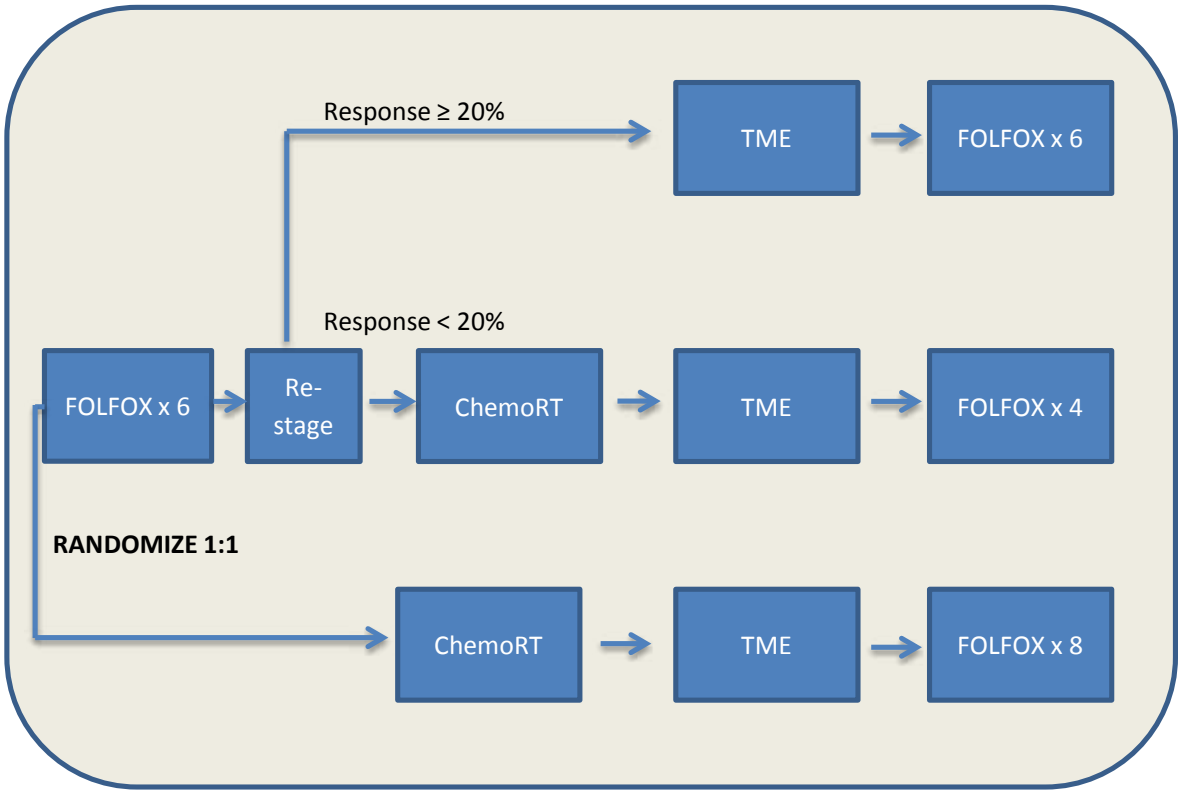

Figure 1 PROSPECT schema. ChemoRT, chemoradiation with 5-FU or capecitabine. Post-operative chemotherapy regimens are suggested, but left to the discretion of the investigator.

experience, coupled with the lack of data to predict which locally advanced patients may forgo radiotherapy.

Neoadjuvant chemotherapy followed by chemoradiation

Perhaps the most frequently explored tactic, induction chemotherapy followed by chemoradiation represents an attractive approach. With recognition that distant metastases largely remain the major risk, early systemic therapy is maintained. Still, a positive circumferential margin places patients at greatest risk for local recurrence and a using a combined approach may provide even greater benefit for those patients at elevated risk (distal tumors, >5 mm extramural spread, T4, or bulky nodal disease). As demonstrated in advanced disease, combination chemotherapy with FOLFOX or FOLFIRI induces response in 50-60% of patients with colorectal cancer (40). In sum, induction chemotherapy may allow for early treatment of micrometastatic disease and initial downstaging of the primary tumor. In turn, by following this immediately with chemoradiation, optimal local control may be attained, with the hope of increased complete response rates. It should be noted that this approach, however, has not shown benefit to date in other tumors, such as anal cancer, lung cancer or head and neck cancer. In addition, there is a theoretical risk of selecting for radio-resistant clones by the administration of chemotherapy prior to radiotherapy.

There have been reports on the results of induction chemotherapy followed by chemoradiation in several sizeable trials to date. The EXPERT and GCR-3 studies both examined 12 weeks of induction CAPOX (capecitabine + oxaliplatin) followed by chemoradiation (41,42).The EXPERT trial enrolled 104 patients who were treated with this approach as well as 12 weeks of adjuvant capecitabine. Ninety seven patients underwent resection and 20% of all patients were noted to have a pCR. In this high risk group, 3-year progression free survival (PFS) was 68%, with a 74% 3-year relapse free rate in those patients who underwent resection (41). The Spanish GCR-3 study randomized 108 locally advanced patients to induction CAPOX followed by chemoradiation versus a strategy of chemoradiation

followed by post-surgical adjuvant CAPOX. This was also a high risk population. Patients were deemed locally advanced on the basis of MRI; inclusion criteria included involvement of or threated circumferential resection margin (CRM), tumor ≤6 cm from anal verge, resectable cT4 tumors and node positivity. Outcomes between the two arms were comparable, with a pCR rate of 13% *vs.* 14% (42). Recently with updated follow-up, there is comparable 5-year DFS (60.7% *vs.* 64.3%) without a significant difference in local relapse (7.1% *vs.* 1.9%, P=0.36) (43). It is notable that acute grade 3/4 toxicity was observed in 19% of patients who received pre-operative chemotherapy versus 54% of post-operatively treated patients. Not surprisingly, the proportion of patients who completed all 4 cycles of chemotherapy was much improved when administered preoperatively: 94% *vs.* 57% (42). While not clearly improving outcomes, this supports the notion that a strategy of pre-operative as opposed to post-operative chemotherapy may decrease acute toxicity.

More protracted as well as abridged courses of neoadjuvant therapy have been examined, producing similar results. The CONTRE trial utilized a longer course of 8 cycles of FOLFOX prior to chemoradiation. In a preliminary report, an impressive pCR rate of 33% was demonstrated, albeit in a cohort of just 30 patients (44). Two cycles of CAPOX prior to chemoradiation was evaluated by a Danish Group, producing encouraging results in a phase II study of 85 patients with poor risk rectal cancer. A pCR rate of 25% was obtained, with 5-year for DFS and overall survival (OS) of 63% and 67%, respectively (45). Additionally, a randomized phase II trial utilizing 2 cycles of FOLFOX followed by chemoradiation with chemoradiation alone was also conducted in Belgium. After 57 patients had been enrolled, the trial was closed early for futility based on identical rates of major downstaging (34.5% and 32.1% achieving ypT0-1). Greater grade 3/4 toxicity was seen with induction chemotherapy (46). Finally, utilization of 1 cycle of CAPOX prior to chemoradiation with CAPOX has produced similarly encouraging tumor downstaging rates, pCR rates (23%), and R0 resection rates (98%) (47). Again, it remains difficult to compare merit of the various approaches given substantial issues with patient selection and small numbers.

Additional studies have evaluated the benefit of adding targeted therapies to this treatment paradigm, most notably the EXPERT-C and AVACROSS trials. The EXPERT-C trial compared treatment with four cycles of neoadjuvant CAPOX followed by chemoradiation with or without the addition of cetuximab to the entire pre-operative course. One hundred and sixty five patients with MRI-defined high risk rectal cancer were enrolled. After conception, data emerged supporting cetuximab use only in KRAS wild-type patients. As such, the primary endpoint of complete response was analyzed for the 90 KRAS wild-type patients. Cetuximab increased response rate (95% *vs.* 73% post-chemoradiation), but complete response rates were similar with or without cetuximab (11% *vs.* 9%), and there was no difference observed in PFS (48). In a recent follow-up, after a median follow-up of 63.8 months, an exploratory analysis including expanded RAS testing (KRAS non-exon 2 and NRAS) revealed no significant differences in outcomes. However, there was a hint of activity with trends toward improved complete response (15.8% *vs.* 7.5%, P=0.31), 5-year PFS (78.4% *vs.* 67.5%, P=0.17) and 5-year OS (83.8% *vs.* 70%, P=0.20) with cetuximab (49). The AVACROSS trial, demonstrated encouraging results in a poor risk patient population. CAPOX and bevacizumab were used as induction therapy and afterwards radiosensitizers through a multimodality neoadjuvant approach. Though almost all 47 patients (98%) underwent R0 resections and demonstrated a pCR rate of 36%, post-operative complications were abundant. Eleven (24%) patients required repeat surgical interventions (50). Similarly high complication rates have been reported by other groups utilizing neoadjuvant bevacizumab in this manner (24). A summary of select studies utilizing neoadjuvant chemoradiation followed by chemotherapy is available in *Table 2*.

The verdict is out on whether there is any true improvement in pathologic response rates and more importantly, long term outcomes. As described, the current data comes largely from small phase II studies with great heterogeneity in the proportion of patient with T4 tumors, the dose of radiotherapy administered and timing of surgery. All of these factors may have a substantial impact on pCR rates. The conduct of randomized phase III studies is needed to definitively evaluate this approach. Fortunately, this is an area of active research. The French phase III randomized PRODIGE 23 trial is evaluating a strategy of neoadjuvant FOLFIRINOX prior to chemoradiation versus standard chemoradiation in locally advanced rectal cancer, with plans to enroll 460 patients (NCT01804790). In addition, the ongoing UK COPERNICUS trial is evaluating the feasibility of administering 4 cycles of neoadjuvant FOLFOX prior to short course radiotherapy, followed immediately by surgery (NCT01263171).

Table 2 Studies of neoadjuvant chemotherapy followed by chemoradiation

Study	Key inclusion criteria	#pts	Treatment	pCR rate	Outcomes
EXPERT (41)	MRI-defined poor risk: T4, T3 at or below levators, N2, CRM ≤1 mm, extramural invasion >5 mm	77	CAPOX ×12 weeks → chemoRT with capecitabine → adjuvant capecitabine ×12 weeks	24% (16/67)	R0 resection rate—99% ORR—97% 1 year DFS—87% 1 year OS—93%
GCR-3 (42)	Tumor within 2 mm of CRM, T3 ≤6 cm from anal verge, T3N+, resectable T4	108	ChemoRT with capecitabine and oxaliplatin → surgery → adjuvant CAPOX	13%	R0 resection—87% Downstaging—58% 18 months DFS—82% 18 months OS—89%
			CAPOX → chemoRT with capecitabine and oxaliplatin → surgery	14%	R0 resection—86% Downstaging—43% 18 months DFS—76% 18 months OS—91%
CONTRE (44)	T3, T4 or N+	36	FOLFOX ×16 weeks → chemoRT with capecitabine or 5-FU	29% (6/21)	R0 resection—100%
Maréchal, *et al.* (46)	T2-T4N+	57	Chemoradiation with 5-FU	28%	ypT0-1—34.5% Downstaging—72% CRM + (≤1 mm)—14%
			FOLFOX ×4 weeks → Chemoradiation with 5-FU	25%	ypT0-1—32.1% Downstaging—61% CRM + (≤1 mm)—4%
EXPERT-C (48)	T3 at or below levators, T4, CRM ≤1 mm, extramural extension ≥5 mm, extramural venous invasion	165	CAPOX + cetuximab × 12 weeks → chemoRT with capecitabine + cetuximab	11%*	R0 resection—92%* Response rate—84% (93%*)
			CAPOX ×12 weeks → chemoRT with capecitabine	9%*	R0 resection—92%* Response rate—76% (75%*)
AVACROSS (50)	T3 low rectal, mid rectum with CRM ≤2 mm, N+ with CRM ≤2 mm, operable T4, T3N+	47	CAPOX + bevacizumab × 12 weeks → chemoRT with capecitabine + bevacizumab	35% (16/45)	R0 resection—98% DFS at 32 months—84%

*, results for analysis of KRAS wild-type population; pCR, pathologic complete response; CRM, circumferential resection margin; ORR, objective response rate; DFS, disease free survival; OS, overall survival.

Neoadjuvant chemoradiation followed by chemotherapy

A strong argument can be made for the approach of initial chemoradiation followed by chemotherapy, though this has been the least fully explored to this point. Chemoradiation remains the standard neoadjuvant treatment with established benefit. Initial utilization of this modality minimizes risk of interruption due to complications induced by other modalities. As this may be definitive treatment, itself, any detrimental effect that initial chemotherapy may induce is avoided. Moreover, as interest grows in the potential of non-surgical management of rectal cancer, data have suggested that an increased interval between the completion of chemoradiation and surgical evaluation may allow for improved response, namely increased pCR rates, as seen in anal cancer (51). Further validation is needed, and there is potential for worsened fibrosis and more a difficult surgical intervention with prolonged delays between radiotherapy and surgery. Arguing against this approach, the delivery of pelvic radiation may hamper the subsequent ability to deliver full dose chemotherapy, potentially lessening its impact. Further, the response to chemotherapy may not be fully appreciated when chemoradiation is first administered.

Studies of long course chemoradiation followed by pre-operative chemotherapy for locally advanced rectal cancer have been conducted by several groups. Two groups have conducted studies evaluating initial chemoradiation with capecitabine followed by an addition 2-4 weeks of capecitabine prior to surgery. These demonstrated feasibility, without marked increase in acute toxicity or post-operative complications (52,53). At this point, the pCR rates are comparable to other techniques and long term outcome data has not matured. A trial from Italy which used chemoradiation followed by two 3-week cycles of capecitabine (1,250 mg/m^2 bid) revealed more encouraging long term follow-up. The pathologic response rate was 18%, with a 5-year DFS of 85.4%. For those patients with tumors ≤6 cm from the anal verge, sphincter preservation rate was 62%. There was a low prevalence of T4 tumors or other high risk features in this study, perhaps accounting for the favorable long term outcomes (54).

As with other approaches, fluoropyrimidine and oxaliplatin based combinations have also been attempted. In a recent study of high risk locally advanced rectal cancer patients, 1 cycle of CAPOX was administered following chemoradiation with CAPOX. pCR was observed in 13 (36.1%) of the 36 patients enrolled (55). An intriguing Dutch report of 50 patients with metastatic, but resectable rectal cancer evaluated a strategy of short course radiotherapy (5×5 Gy) followed by 6 cycles of CAPOX + bevacizumab, which was initiated within 2 weeks of radiotherapy completion. Radical surgical resection was ultimately possible for 72% of all patients treated. The primary rectal tumor was resected in 43 (90%) patients, though a suboptimal R1 resection was achieved in four. In those undergoing primary resection, downstaging was evident in 47% with a pCR rate of 26%. Local recurrence after R0 resection was noted in just 2 (6%) patients (56). Thus, in the metastatic setting, this appears to be a viable approach. At times, short course radiotherapy is not embraced due to the perceived lesser rates of down-staging. The strategy of short course radiotherapy followed immediately by full-dose systemic therapy may allow for optimal downstaging with use of the 5×5 schema, and only minimally delay systemic therapy.

A larger experience has been reported utilizing long-course chemoradiation. In a non-randomized multicenter US study, 144 patients with stage II and III rectal cancer were assigned to one of two study groups. Both received initial 5-FU based chemoradiation. The first group had surgery within 6-8 weeks of completion. The second group was reassessed at 4 weeks and if with evidence of clinical response, patients were treated with two cycles of FOLFOX, followed by surgery 3-5 weeks after completion. Overall pathologic response rates were improved in the group with additional chemotherapy (and delayed surgical intervention), though differences in the pCR rate did not reach significance: 18% *vs.* 25%, respectively. Importantly, while there was a slight increase in pelvic fibrosis seen, the complication rates were not different between the two groups (51). From this same data set, preliminary results which include a third group of 48 patients have also been reported. In group 3, where two further cycles of FOLFOX were delivered, delaying surgery 4 further weeks, pCR rates increased to 31%, without increased complication rates (57). Thus initial chemoradiation followed by pre-operative chemotherapy appears at least as promising as the other strategies described. A summary of selected studies utilizing this approach is available in *Table 3*.

Multiple trials are ongoing with this approach. The Polish Colorectal Cancer Study Group is conducting a phase III study comparing short-course preoperative radiotherapy followed by three cycles of FOLFOX with conventional chemoradiation to 50.4 Gy with concurrent 5-FU (NCT00833131). The accrual goal is

Table 3 Studies of neoadjuvant chemoradiation followed by chemotherapy

Study	Key inclusion criteria	# pts	Treatment	pCR rate	Outcomes
Zampino, *et al.* (54)	T3, T4 or N+	51	ChemoRT with capecitabine → capecitabine ×6 weeks	18% (9/50)	R0 resection—100% 5-year DFS—85.4%
Gao, *et al.* (55)	T4, bulky (>5 cm), <6 cm from anal verge, N+, elevated CEA	36	ChemoRT with CAPOX → CAPOX ×3 weeks	36%	R0 resection—100% Downstaged—81%
van DijK, *et al.* (56)	Metastatic rectal cancer	50	Short course radiation → CAPOX + bevacizumab for up to 18 weeks	26% (11/43)	R0 resection of primary—91% (39/43) 2-year OS—80% LR rate after R0 resection—6% (2/36)
Garcia-Aguilar, *et al.* (51)	T3, T4 or N+	144	Chemoradiation with 5-FU	18%	R0 resection—97%
			Chemoradiation with 5-FU → FOLFOX ×4 weeks	25%	R0 resection—96%

pCR, pathologic complete response; DFS, disease free survival; CEA, carcino-embryonic antigen; LR, local recurrence.

540 patients and positive results could be practice changing for both radiation and medical oncologists in the United States. An interim analysis revealed no major differences in acute toxicity or local efficacy, with a trend toward improved pCR rates in the short-course radiotherapy group: 21% *vs.* 9% (58). Equally important is the phase III RAPIDO study which is very similar in design, though goes further in moving the entire current treatment regimen to the pre-operative setting (NCT01558921). Only patients who are deemed high risk by MRI are to be included. In this study, a strategy of short course chemoradiation followed by 6 cycles of CAPOX will be compared with long course chemoradiation. Post-operative adjuvant chemotherapy is left up to the individual investigator.

Conclusions

Outcomes continue to improve in colorectal cancer as affected patients are discovered earlier in the disease process, largely attributable to increased screening efforts. Improved surgical technique, incorporation of pre-operative radiotherapy and the use of adjuvant chemotherapy all appear to confer additional benefit for a large portion of patients. Recent efforts to build upon 5-FU based chemoradiation regimens have yielded negative results. In the meantime, adjuvant colorectal cancer chemotherapy has not progressed further beyond the fluoropyrimidine and oxaliplatin based combination. In rectal cancer, neoadjuvant treatment offers a unique opportunity to improve the current paradigm. There is opportunity to both improve disease free and overall survival outcomes through the differential layering of therapy, as well as to reduce toxicity through the selective use of therapeutic modalities. Selection of the optimal patient population for each paradigm may prove critical in affecting the results and applicability of ongoing studies. Beyond clinical criteria, further biomarker validation may allow for the additional tailoring of therapy moving forward. As always, the support of clinical investigation remains paramount in improving future outcomes for our patients.

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Footnote

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Hyperthermic intraperitoneal chemotherapic perfusion in colorectal cancer

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Introduction

The term peritoneal carcinomatosis (PC) includes all tumoral dissemination, either local or massive, to the peritoneal serosa and neighbouring anatomical structures. The term PC was first used by Simpson in 1931 to describe the peritoneal dissemination of an advanced ovaric cancer (1).

Traditionally, the PC is considered a stage IV tumour indistinguishable from other metastatic sites (2).

The PC may manifest very differently, since few millimetric implants adjacent to the primary tumour to the occupation of the entire abdomen and pelvis of bulky tumour masses. Most patients with PC progress to intestinal obstruction, ascites formation, tumour cachexia or combination of them all. The term PC is associated with very advanced tumours without therapeutic possibilities. Patients often suffer a significant deterioration in their quality of life before death (3-5).

The incidence of PC is difficult to establish with certainty due to the diagnostic limitations of image-based media and current biological measurement. The ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) are sensitive to diagnose visceral recurrences, retroperitoneal, and some indirect signs of PC, but miss infracentimétric peritoneal disease (6).

Laparoscopy seems to be an effective method for diagnosis, establishing the location extension of peritoneal disease and to determine tumour histology, but has technical limitations, and involves a risk of peritoneal extent of spread (7).

Over 400,000 new patients/year are diagnosed of colorectal cancer in Europe, wherein PC is detected to coincide with the diagnosis of primary tumour in 10% of the patients (8). Recurrence is only at peritoneum in 10-35% of the patients who relapse after treatment of the colorectal tumour (3-5,9,10).

The usual treatment of the PC is palliative and therefore with limited survival. A prospective, multicenter study included patients with PC from colorectal cancer showed a survival of only 5.2 months (11). In other reports published before 2002, including large series of patients with PC of colorectal origin, the mean survivals were referred from 5 to 9 months (12). Current chemotherapy protocols that include new systemic drugs such as oxaliplatin or irinotecan alone or in combination with biologic agents get to prolong survival of these patients from 21.5 to 24 months. These studies have been conducted in patients with colorectal cancer who had any kind of metastatic disease (13-19). It is known that the natural history and response to systemic chemotherapy of the peritoneal disease are significantly worse than in other metastatic sites, such as liver or lung (13). To date, there are no published studies that have evaluated the response of patients with peritoneal metastatic disease exclusive to these new lines of chemotherapy. Surgery as sole treatment in the PC is associated, to a new peritoneal recurrence (14,20,21). It is rare that a patient diagnosed with PC treated with any type of palliative treatment, remains alive at 5 years.

In recent years, interest in the peritoneal dissemination of tumours has increased due to better clinical outcomes achieved with multimodal treatments and recent knowledge on the development and peritoneal tumour growth, which allowed considering the PC as a locorregional disease (22).

PC may benefit from intensificated regional therapy as successfully as metastatic liver disease.

In late 1980, Sugarbaker laid the foundations of a multidisciplinary approach that combines the PC radical surgery and immediate administration of intraperitoneal chemotherapy with or without hyperthermia, designed to eradicate microscopic residual tumour. This treatment has been quite favourable in the treatment of low-grade tumours, especially in the peritoneal pseudomyxomas from appendiceal origin and in some peritoneal mesotheliomas. In recent years, several working groups specialised in many centres in America and Europe are applying multidisciplinary treatment in the PC, and indications have been extended to other types of malignant tumours of the peritoneum, due to the good results published.

Controlled prospective studies are conditioned by the difficulties in recruiting patients with rare tumours with highly variable clinical presentations, the complexity of homogenisation of each of the elements of a complex treatment, especially surgery, and the patients agreement to be assigned to a palliative treatment arm versus the possibility of potentially curative treatment (23).

Pathophysiology of peritoneal carcinomatosis

The peritoneum is an organ that covers the three-dimensional structures contained in the abdominopelvic cavity. It cvomprises a single layer of mesothelial cells on a basal membrane and five layers of tissue with a total thickness of 90 microns. The layers of tissue includes interstitial cells and a matrix of collagen, hyaluronic acid and proteoglycans (24). The known functions of the peritoneum are the production of a lubricating substance to facilitate contact between the elements of the abdominal cavity to act as an important organ of defense against intra-abdominal infections. It is now recognised another function of the peritoneum in the development of neoplasms, acting as a first line of defense against the introduction and tumour development (25). Any injury or wound the peritoneum acts as a facilitator of tumour cell implantation into the abdominal cavity and is involved, along with other elements in tumour proliferation (26).

Neoplasms of the digestive, gynaecological and other sources often use the coelomic route for the tumour spreading.

Tumour cells can be released into the abdominal cavity from the serosal surface of the organ infiltrated by the tumour (27). Surgery can contribute very significantly to the exfoliation of tumour cells into the abdomen. It has been shown that during the extensive removal of primary tumours and/or lymph node involvement, a significant number of tumour cells are released into the abdominal cavity (28-30).

The meaning of free tumour cells in the abdominal cavity is still unknown. The number of tumour cells that are required to effectively implant in the peritoneum is much lower than those necessary for the development of other types of metastasizing tumour. This phenomenon is known as “metastatic inefficiency” and was corroborated by animal studies that demonstrated the greatest tumour tropism of some strains by peritoneum (31,32).

Free tumour cells in the abdominal cavity have to evade the immune system and develop a network of vascular substitution to meet their metabolic needs in order to survive. Due to the complexity of these processes, many tumour cells cannot become metastatic tumour deposits.

Tumour cells that remain viable are moved into the abdominal cavity by hydrodynamic movements associated with breathing and following predictable routes, which would explain the predominance of tumour implants on the surface of the right hemidiaphragm. The presence of ascites and resorption areas with high phagocytic capacity, as the omentum and epiploic appendices, justify the very large tumour accumulations, known as omental cake. Intestinal peristalsis, together with the effect of gravity, facilitate the distribution of the tumor in most areas slopes, such as Douglas sac, the parietocolic gutters, retrohepatic fossa and those fixed anatomical structures such as the ileocecal region and the first jejunal portion (33).

In women, tumour cells very often affect the ovaries, especially at points of follicular rupture. Tumour cells have high affinity for the intercellular matrix of the injured peritoneum or bloody areas caused by the surgery. The tumoral entrapment process is especially fast and can occur in minutes facilitated by the effect of integrins, cell adhesion molecules, and production of growth factors such as growth factor for fibroblasts (fibroblast growth factor, FGF), epidermal growth factor (epidermal growth factor, EGF) and transforming growth factor beta (transforming growth factor beta, TGF-) (34). All these molecules appear during the physiological mechanisms of inflammation and tissue healing. The binding of tumour cells with the intercellular matrix of tissues is also very strong and impossible to avoid using washing/stripping solutions commonly used during conventional surgery. After surgery, the implantation of tumour cells in the intercellular matrix is usually immediate and once they are coated with fibrin and other products in

the processes of tissue repair, they become "sanctuaries" where cells can proliferate protected from the external environment. Tissue adhesions formed early after surgery avoids the cytotoxic effect of intraperitoneal chemotherapy and the absence of a neovascular network prevents the access of systemic chemotherapy.

Multimodality treatment - Therapeutic basis

The approach and development of multidisciplinary treatment of the PC (radical surgery plus intraperitoneal chemotherapy +/- hyperthermia), also known as regional treatment of malignant diseases of the peritoneal surface or Sugarbaker's technique, is related to the current understanding of the pathophysiology of the peritoneum and the mechanisms for implementation and growth of tumours in the abdominal cavity.

In 1989, Sugarbaker defined PC as a locoregional manifestation of neoplastic nature. He proposed a treatment of "regional therapeutic enhancement" for the PC, based on a radical surgery, designed to remove the entire macroscopic tumour of the abdominal cavity, followed by immediate administration of intraperitoneal chemotherapy, with or without the use of hyperthermia (35,36).

The more widespread use of multidisciplinary treatment has advanced the definition and practice of the radical surgery, the type and timing of intraperitoneal chemotherapy, the adaptation of the techniques of hyperthermia, the protocols of care and postoperative controls and, particularly, in the appropriate selection of patients. Biannually since 1998, meetings of experts from the Peritoneal Surface Oncology Group International (PSOGI) are being held, and experiences are addressed and discussed on the treatment of these diseases. The 5th Workshop Meeting, held in Milan, was particularly relevant, since it addressed controversial issues of each part of the therapy and established consensus on issues as important as the methodology of the radical surgery, intraperitoneal chemotherapy and hyperthermia, the role of the various specialties involved in the management of these patients and, especially, the criteria for patient selection and multidisciplinary treatment indications. The most important conclusions of this meeting in Milan were published in a special issue of the Journal of Clinical Oncology (37).

Radical surgery

The prognosis of patients with PC undergoing multidisciplinary treatment is directly related to the extension of the disease and surgical radicality (38). The aim of radical surgery is to remove the abdominal tumour without leaving any visible macroscopic residual disease. The extent and distribution of the PC must be fully established before starting the process. The highest concentration of tumour is usually located in the retrovesical space, the pouch of Douglas, the parietocolic gutters, the right subhepatic space and more posterior subdiaphragmatic areas. Very often, the omental transcavity, the retrogastric compartment, the splenic hilum and the mesentery of intestinal segments, more fixed and less mobile (duodenojejunal angle, distal ileum and sigmoid colon) are affected. The postsurgical adhesions and structures with low venous return (hernia sacs) present special predisposition to tumor development. All anatomical regions of the abdomen and pelvis may be affected by tumour seeding and should be explored carefully. An important step of this operation corresponds to the identification of all tumour foci present in the abdominal cavity. The correct characterization and quantification of PC allows determining the technical and clinical benefits of the radical surgery. Sugarbaker described the peritonectomy procedures which are a key therapeutic element in the multidisciplinary treatment of PC (39). Peritonectomy procedures can eliminate the gross tumour present in the peritoneal serous as well as the removal of the viscera and surrounding structures deeply infiltrated by the tumour.

The removal of the implants with diffuse and extensive distribution in the peritoneal surface requires the stripping of the entire peritoneum of the corresponding anatomical region. Few isolated implants of visceral or parietal peritoneum that infiltrate can be completely removed or electrovaporised by high voltage electric scalpel.

Bulky implants invading deeply into an organ or anatomical structure may obly to associate an excision of it. In the extensive or limited but high volume PC may require multivisceral resections and/or large bowel resections, sometimes multisegmental, followed by digestive anastomosis. Tumour involvement of a significant portion of the small intestine may limit or prevent any radical surgery. When the length of residual intestine does not ensure an adequate supply, surgery should be avoided. In addition to the extensive involvement and/or multisegmental bowel, other operative findings that impair or limit the complete cytoreduction in patients with CP, is the gross involvement of the hepatobiliary hilum, full retraction of the mesentery and/or massive retroperitoneal nodal involvement (40). The

use of electrocautery provides hemostasis while a bed of sterilized dissection plane of tumour cells

Intraperitoneal chemotherapy

Chemotherapy administered regionally aims to achieve high concentrations of a cytotoxic agent in tumours located at a particular point of the body. Administered intraperitoneally, enables a very intensive treatment of tumours located in the abdominal cavity in relation to the dose of drug used. Dedrick showed that in various chemotherapeutic drugs, hydrophilic peritoneal permeability was considerably less than its plasma clearance, resulting in proportionally much higher concentrations of intra-abdominal chemotherapy (41).

The primary objective of intraperitoneal chemotherapy is to achieve high concentrations of drug in the site of the tumour, minimizing the systemic side effects.

The first use of intraperitoneal chemotherapy correspond to Spratt, who used the intraperitoneal thiotepa in a patient with peritoneal pseudomyxoma, Speyer used 5-fluorouracil (5-FU) and methotrexate. Koga then associated intraperitoneal chemotherapy with hyperthermia in the treatment of gastric carcinomatosis (42).

The molecular weight of the drug, its lipid solubility and capillary permeability determines its passage into the systemic circulation. Other requirements that must be taken into account in the choice of intraperitoneal chemotherapy are the time of removal from the systemic circulation, the ability to pass the portal system and the empowerment of their effects by hyperthermia. Cell cycle-nonspecific drugs are a priority for the intraperitoneal use (43,44).

Several studies have established a maximum of 2-3 mm penetration of chemotherapeutic agents in tumour tissue. This ability to penetrate tissue explains that the ideal limit set of residual disease after radical surgery considered is equal to or less than 2.5 mm (45,46). Peritonectomy procedures do not affect the pharmacokinetics of intraperitoneal drugs (47,48). The molecules used are 5-FU, mitomycin C, oxaliplatin and irinotecan. Drugs can be administered alone or in combination (49).

The dose of chemotherapeutic agents administered in HIPEC is calculated from the body surface that correlates with drug metabolism and systemic toxicity. Nevertheless some authors propose to dosify based on drug concentration (mgr/L) (50).

The procedures for intraperitoneal administration of chemotherapy vary according to time and how to apply them in the abdominal cavity. The maximum benefit is achieved when used immediately after surgery, before the "entrapment" of tumour cells by fibrin and the partitioning of the abdominal cavity for surgical adhesions.

When chemotherapy is administered intraperitoneally from days 0 and 5 of immediate postoperative period is called early postoperative intraperitoneal chemotherapy (EPIC). The EPIC was initiated after tumour removal, allowing fibrin and microscopic cellular remnants removal from the abdominal cavity, which is then bathed with the chemotherapic solution. The solution is stored for 23 hours and removed daily through catheters (51). Several cycles of intraperitoneal chemotherapy are given to increase the chances of exposure of chemotherapy to tumour cells, but has the disadvantage that produces greater systemic adverse effects and allows the partitioning and sequestration of chemotherapeutic agents located favouring infection (52,53).

Hyperthermia

The association of heat to intraperitoneal chemotherapy enhances the therapeutic effect of some chemotherapic drugs and creates a "toxic shock" directly on tumour cells. At a meeting of the international medical community held in Madrid in 2004, it was agreed that this technique should be referred to as HIPEC (54).

Some animal studies show that chemohyperthermia offers a greater therapeutic benefit above that of hyperthermia or chemotherapy administered intraperitoneally alone (55). Hyperthermia destroys tumour cells when temperature reaches 43 ℃. Normal cells are heat resistant up to 45 ℃ (47). Cellular metabolism increases with temperature until a point at which irreversible damage occurs. The critical point of human cells is 43.5 ℃, while *in vitro* temperature of 42.5 ℃ produces a high cytotoxic effect by acting on the interstitial pressure in tumour tissue, favouring the penetration of drugs such as mitomycin C, cisplatin, oxaliplatin and irinotecan, or acting directly on the cell itself and its molecular composition. It has been described effects on the cytoskeleton, such as changes in the stability and fluidity of cell membrane alterations in cell shape, decreased intercellular transport mechanisms, alterations in membrane and induction of apoptosis. Also, alterations in protein synthesis, protein denaturation, aggregation of nuclear matrix proteins and induction of synthesis of heat shock proteins (HSP) have been demonstrated in the intracellular proteins. Heat has also shown effects on nucleic acids, decreased synthesis of RNA/DNA, inhibition DNA repair enzymes and alteration

of the latter. Hyperthermia influence cellular function by affecting the metabolism of several intracellular substrates expression of the genes and signal transduction. Other effects are related to the cellular immune response with the induction of those already mentioned HSP involved in antigen expression and tumoral immunity.

Hyperthermia has shown clinical efficacy in several randomized studies, either as direct mechanism or due to the enhancing effect on radiation therapy and chemotherapy. Clinically, the major tumoricidal effects of hyperthermia are achieved between 41 and 43 ℃ (56).

There are two ways to settle the perfusion. The technique described by Sugarbaker, called open technique or coliseum, is the most widespread. It involves the administration of HIPEC leaving the abdomen open.

The other mode, called the closed technique is applied with a temporarily closing of the abdomen for the administration of chemohyperthermia. This type of HIPEC is supposed to increase the drug penetration in the tumour by an increased abdominal pressure. There are no studies to demonstrate which mode provides greater clinical benefit to patients. The technical feasibility of HIPEC has been established in recent years by several authors (57,58).

The optimum temperature of the HIPEC is a very important parameter. Most chemotherapeutic agents used are chemically stable to 50 ℃. Studies *in vitro* and in cell culture show that the cytotoxicity is more effective at 45 ℃ than at 41 or 42 ℃, so it would be reasonable to use the maximum temperature within the limits of clinical tolerance checked, which, as we mentioned above, is marked by tolerance of the small intestine and corresponds to 43 ℃ (59,60).

Other parameters

The carrier solution used in intraperitoneal chemotherapy can modify the exposure time of chemotherapeutic agents in the abdomen. With the aim of increasing the exposure time, various types of solutions have been used. A high molecular weight creating ascitis maintains a higher availability of the drug. The selection of the solution is particularly relevant in the EPIC (61,62). In HIPEC, with a dwell time relatively short, one might expect that a hypotonic solution increases the uptake. But Elias demonstrated that dextrose solution of 100 and 150 mOsm/L, which not only does not increase tumour penetration, but also is associated with a high rate of serious complications (50%) and peritoneal bleeding and thrombocytopenia, so this author contraindicated hypotonic solutions as transport solution for HIPEC (63).

The duration of HIPEC is an issue still debated. The safety of hyperthermia has only been demonstrated in established empirically based schemes: temperature of 41 ℃ for 90 minutes or 43 ℃ for 30-40 minutes. In clinical practice, the duration of administration of HIPEC is set between 30 and 90 minutes, and varies according to the pharmacokinetic characteristics, the total dose of chemotherapy and the protocols. The intra-abdominal pressure during HIPEC directly influences the diffusion and penetration into the tissues and, consequently, a greater cytotoxic effect of chemotherapy.

Multidisciplinary treatment indications

The multidisciplinary treatment is widely recommended for the PC secondary to colorrectal tumours (33,34,37). Current indications were recently updated in the Journée Nationale du Traitement par des Carcinoses Peritoneal Chirurgie et Chimiothérapie Intrapéritoneale (Paris, May 2008).

These Indications were previously discussed at the Fourth International Workshop on Peritoneal Surface Malignancy (Madrid, December 2004) and Peritoneal Surface Malignancy in the Workshop-Consensus Statement (Milan, November 2006). Data from the United States calculates an incidence of 130,000 new cases per year, in colorectal cancer, of which between 10-15% will start with peritoneal involvement.

In Europe, annual incidence data of the PC are even higher: 25,000 to 37,500 new cases annually of PC of colorectal origin. An analysis by the French groups dedicated to the treatment of PC, estimated that approximately 10% of patients with CP can benefit from a multidisciplinary treatment applied with curative criteria (64).

Patient slection

Preoperative assessment

The indication of the multidisciplinary treatment of PC has to be done from a strict selection of patients. The highest survival rates described with this treatment correspond to those patients who were able to perform a complete tumour debulking. The incomplete cytoreduction was associated with a mean survival about 6 months (65,66). The distribution and especially the extension of the PC are the main determinants to achieve complete cytoreduction, so it is essential to establish preoperatively the characteristics of

the PC to define the indications.

There are several techniques to help identify patients likely to undergo multidisciplinary treatment: CT, MRI, PET, laparoscopy and tumour markers.

There is consensus on the need to perform a colonoscopy in all patients. The CT has great value in the detection of primary lesions or recurrences affecting solid organs and retroperitoneum, but has limitations in identifying small peritoneal implants, particularly those located in the small intestine, and mesenteric leaves. When CT fails to detect this type of implants, the disease is usually advanced and we consider a limiting data to achieve a complete cytoreduction. The CT findings of small bowel obstruction in several segments or the presence of tumour greater than 5 cm located outside the terminal ileum are associated with 88% chance of incomplete surgical resection. Contrary, the absence of these two radiologic findings, achieves a 92% complete cytoreduction. Helical CT was compared with operative findings and the sensitivity obtained was 25% to 37% with a negative predictive value ranging from 47% to 51% (67).

MRI is an exploration that provides a sensitivity and specificity of intestinal tumour involvement in the PC of 73% and 77%, respectively (68). Other studies provide a sensitivity of 84-100% for detecting peritoneal metastases with this test (69). In patients undergoing surgery, chemotherapy or prior radiotherapy and/or associated inflammatory diseases, the specific diagnosis of peritoneal involvement is difficult to determine by MRI.

The PET scan has a low sensitivity in small tumours (<1 cm), poor specificity and limitation in low-grade tumours. It also presents difficulties in the interpretation of lesions in the diaphragm, lung bases and top of the liver due to breathing artephacts. Any of the current means of imaging has limitations to establish the extent and exact location of the peritoneal tumour disease. The use of CT, MRI, PET and/or laparoscopy should be individualised and considered as part of a diagnostic-therapeutic approach of patients with PC, which may depend on the availability, cost and experience of the radiologist. The result of the consensus of Milan was to consider CT as the imaging technique essential to investigate the indications of multidisciplinary treatment.

Some centres use laparoscopy to determine the possibilities of multidisciplinary treatment, as it has the advantage of providing direct visualisation, allows detection of small lesions and practice biopsies. The disadvantages of this technique are its relative invasiveness, technical difficulties due to adhesions, limitations on access to the retroperitoneal compartment, the risk of implantation at trocar sites and the increased cost to the overall therapeutic process. There is a study evaluating the role of exploratory laparoscopy in the selection of patients with PC candidates for complete cytoreduction. In this study laparoscopy could be performed in all patients with a mean operative time of 38 minutes (range, 23-75 minutes) was well tolerated in all patients, it achieved a very accurate set of the real characteristics of the peritoneal disease and adequately identified patients for complete cytoreduction (70).

Another study, involving 97 patients with PC undergoing laparoscopy for peritoneal staging, concluded that laparoscopy allowed establishing the extent of PC in 96 of the 97 patients and only two were classified in a lower stage. It shown a good correlation between the findings of laparoscopic exploration and open surgery. Laparoscopy showed no mortality in this group of patients and observed no tumour implantation at port sites. In patients with inadequate or contradictory information on the extent of the PC, laparoscopy is a useful technique to establish the extent and distribution of the PC, to visualise the small bowel involvement and determine the possibility of a complete debulking more accurately (71).

Intraoperative assessment

The importance of establishing with certainty the distribution and extent of peritoneal disease to determine the applicability of multidisciplinary treatment has forced to design intraoperative quantification of the extent of the PC.

Currently we have three staging systems to assess the intraoperative peritoneal spread of the disease, none of which has been shown to have prognostic value for all types of PC. Gilly *et al*. (72,73) described a system for intraoperative measurement of the PC and it has shown to correlate with the patient outcomes in certain types of PC. Zoetmulder *et al*. established a simplified system of Sugarbaker's classification (74). Simplified Peritoneal Cancer Index (SPCI) demonstrated the validity in peritoneal pseudomyxoma and PC of colorectal origin. This system is also a predictor of complications and acts as a guideline in selecting patients for multidisciplinary treatment. The most universally used system of quantification is the Peritoneal Carcinomatosis Index (PCI) described by Jacques and Sugarbaker (75). It describes 13 anatomical regions, dividing the abdomen into 9 regions and the small intestine in 4. It rates each region from 0 to 3 depending on the size of the tumour lesion: 0 point, no macroscopic lesion;

1 point, tumour exceeding 0.5 cm; 2 points, a tumour of 0.5 to 5 cm and 3 points, greater than 5 cm or tumour confluence, resulting in a maximum score of 39 points. The PCI ranks of the PC extension, determines the possibilities of radical surgery and helps to establish the prognosis of patients. It also has proven to be predictive in survival of patients with PC of colorectal origin being PCI 20 the cutting point (76). This system of intraoperative tumour quantification was considered in the consensus meeting of Peritoneal Surface International Workshop Malignancy, in Milan, as the most useful, reliable and reproducible in the multidisciplinary treatment of the PC (77).

Intraoperative determination of the intensity of the radical surgery has the same importance as determining the extent of the PC. There is a direct relationship between the size of residual disease after surgery and the survival of patients undergoing multidisciplinary treatment. We have several systems that classify the size of residual disease after debulking. Most of these classifications belong to the R residual tumour classification and correspond to changes in the American Joint Committee on Cancer (78): Lyon (79) classification, Netherland's classification (80) and Winston-Salem's (81). The classification used is the Completeness of Cytoreduction Score (CC) (82), which rates residual disease after surgery in: CC-0 in the absence of gross residual disease, CC-1 if the residue tumour is equal to or less than 2.5 mm, CC-2 if the residue is 2.5 to 25 mm and CC-3 when the residue is above 25 mm or confluent persists after tumour surgery. This system does not provide the definition of microscopic residual disease in PC. The rationale for setting between 0 and 2.5 mm size limit of residual disease and appropriate to establish the concept of complete cytoreduction is due to the ability of a chemotherapeutic intraperitoneal to penetrate the tumour tissue.

But the definition of complete cytoreduction currently most accepted corresponds to the CC-0 and CC-1 cytoreduction and incomplete, CC-2 and CC-3. The CC has been associated with patient survival in carcinomatosis of colorectal origin (74,83-85).

In the future the use of more active chemotherapeutic agents can modulate the effort of the cytoreductive and the definition of radical surgery matches other criteria of residual tumour volume.

The type of previous surgery performed on the primary tumour has also been associated with chances of achieving a complete cytoreduction and the prognosis of patients who undergo multidisciplinary treatment. Sugarbaker introduced the Prior Surgical Score (PSS) (83). The PSS determines the number of regions dissected during surgery prior to the multidisciplinary treatment, and has been shown to correlate with survival.

Inclusion and exclusion of patients

The multidisciplinary approach provides a significantly higher survival rates than conventional palliative treatments, but is associated with significant morbidity and mortality. The identification of factors associated with the outcome of multidisciplinary treatment application and the patient selection is important to establish the treatment indications and maximise the clinical benefit (86).

Currently the parameters considered most useful are the following:

- Performance status (Eastern Cooperative Oncology Group): 2.
- Absence of extra-abdominal tumoral disease.
- Less than three hepatic lesions which are technically resectable.
- Absence of biliary obstruction.
- Absence of ureteral obstruction.
- Unique location intestinal obstruction.
- Absence of intense involvement of the small intestine disease.
- Little bulky disease in the gastrohepatic ligament.

ECOG patients with 2 to 3 have a median survival of 9.5 months, while patients classified from 0 to 1 is significantly higher, 21.7 months. Patients with bowel obstruction or malignant ascites and subsequent malnutrition have a worse survival than those without these complications, 6.3 and 23 months, respectively (87). Even so, in patients with malignant ascites multidisciplinary treatment prevented the recurrence of ascites in 75% of patients,being HIPEC recommended in these clinical circumstances (88). Regarding the extension of PC, Sugarbaker refers to the prognostic value of PCI in patients with PC of colorectal origin. A PCI below 10 was associated with 50% survival at 5 years, while survival was 0% in those cases with a PCI greater than 20 ($P<0.0001$). This author considers this treatment contraindicated in patients with PCI over 20, while others raise the PCI to values of 26. Verwaal used as a criterion for extension of PC the level of affectation of the different regions. Of the total of seven, more than five affected regions are associated with lower survival benefit and high morbidity rates (65). There is consensus among experts that the best long-term clinical benefits with the multidisciplinary treatment are achieved

in patients with limited extent of the peritoneal disease (89). In the evaluation of preoperative CT, patients with PC of colorectal origin class III presenting involvement of the small intestine or the mesentery (as classified by Yan), bulky retroperitoneal lymph node involvement and/or radiological PCI over 20 should be excluded for multidisciplinary treatment.

There are other useful recommendations on patient selection and indication of the multidisciplinary treatment of colorectal origin with PC that are based on primary tumour staging (90):

- T4 N0 M1 tumours (in the form of limited peritoneal disease): upfront multidisciplinary treatment.
- T4 N2 M1 (with limited peritoneal disease): treatment with chemotherapy for 3 months followed by multidisciplinary treatment and best systemic chemotherapy.
- Clinically asymptomatic patient with resectable extensive disease, ascites and small bowel involvement: multidisciplinary treatment followed by the best systemic chemotherapy.
- The multidisciplinary treatment should be scheduled at least 1 month after the last administration of systemic chemotherapy.

The type and degree of histological differentiation of the tumour causing the PC have also shown to impact on survival.

The most suitable application of multidisciplinary treatment corresponds to: "young" patients with good general condition, no previous treatments, localised PC caused by tumours of low mitotic activity and completely resectable. The short-term clinical outcomes (morbidity and mortality) and long-term (survival and quality of life) of the multidisciplinary treatment are closely related to the proper application of these criteria in the selection of patients.

Exclusion criteria accepted by most of the groups are:

- Patients who have a PC judged unresectable by clinical or paraclinical: mesenteric retraction evident on CT, infiltration/retraction bladder by endoscopy.
- Extrabdominal metastases or unresectable liver metastases or requiring major hepatectomy conditioning a limited hepatic reserve.
- The presence of other malignant disease.
- Multisegmental complete bowel obstruction.
- Active infection or other condition that prevents or incapacitate the patient to receive the proposed treatment per protocol.

Results of multidisciplinary treatment

Morbidity

Complications can arise directly from surgery, chemotherapy, hyperthermia or the sum of these. Radical surgery in the treatment of CP is usually the most important cause of complications and the main reason to alter the therapeutic process. Elias recently described a specific classification system for complications related to the multidisciplinary treatment of PC (91). This author considers 6 degrees of complications, defined as grade 0: no complications, grade 1: complications that do not require action or minor treatment as oral antibiotics, basic controls···, grade 2: complications requiring moderate actions, as intravenous medication, parenteral nutrition, prolonged nasogastric tube, pleural drainage, grade 3: complications requiring hospital readmission, reoperation or interventional radiology, grade 4: chronic complications, removal of organs or digestive derivations, and grade 5: complications leading to death of the patient. At the consensus meeting in Milan was agreed to use the new Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 as a system of classification of complications. This is an extensive guide which includes types of complications in 28 categories, based on the anatomy and pathophysiology (92). The complication rate grade III-IV is around 30 to 65%. Specific surgical morbidity is 30% and relates mainly to digestive sutures dehiscence, perforation, intestinal fistulas, collections, intra-abdominal abscesses and postoperative bleeding. Around 10% of patients require one or several surgical operations (93-95). A multivariate analysis fulfilled by the group from Washington Hospital Centre determined that the rate of postoperative complications is related to the extension of the PC (PCI), duration of surgery and the number of digestive anastomosis performed (96). Although the morbidity described in this complex treatment is not higher than that referenced in the gastrointestinal major surgery extreme care is required, especially in the immediate postoperative period. Systemic complications correspond to those of any major surgery but may be covert or increased by the effects of systemic toxicity, gastrointestinal or haematological of HIPEC. Patients undergoing peritonectomy have an altered inflammatory response caused by surgical removal of the peritoneum and the effect of intraperitoneal chemotherapy, which often affect an evident decrease in peritoneal-abdominal pain that hinders the clinician to early diagnose postoperative abdominal complications. The immediate follow-up of these patients should be performed in a unit of critically ill patients with specific clinical protocols and expert staff.

Mortality

The reported mortality in the multidisciplinary treatment of PC ranges from 0 to 14%. Mortality rate of 2-6% are the most frequent in most published studies. Mortality is related with the intensity of surgical invasiveness, reflected in the number of peritonectomy procedures performed, the PCI, the number of digestive anastomosis and volume of perioperative blood transfused (97).

The causes of mortality referenced in the literature are related to intestinal perforations, bone marrow suppression, respiratory failure, pulmonary embolism and infection by methicillin-resistant Staphylococcus aureus. There are several factors that predict mortality in the multidisciplinary treatment of PC, as the presence of abundant ascites, bad general status and bowel obstruction (87). Both the morbidity and mortality in the multidisciplinary treatment are directly related to the surgical team's experience and proven the importance of the learning curve in this treatment. The series providing 100 or more patients usually have a lower rate of complications, and these are less severe (98,99).

Quality of life

Studies addressing the quality of life of patients undergoing HIPEC conclude that it is a complex and invasive therapy but generally well tolerated (100,101). Usually patients can be with a similar activity pattern to its previous one at 3 months after surgery. Almost half of the survivors at 3 years return to work with the same intensity as before treatment. The groups of patients who benefit the most, according to the quality of life scales applied, were those with ascites before surgery. These results were similar to those published by the National Cancer Institute (Bethesda) about a group of patients assessed at 3, 6 and 9 months after surgery (102). The interpretation of the evidence of the published studies on quality of life in the multidisciplinary treatment is difficult to establish by several factors(103-107): the clinical heterogeneity given by the variation in the type of underlying disease, degree of surgical cytoreduction and the mode to administer intraperitoneal chemotherapy, the methodological heterogeneity between studies and variations in the scales used to measure the quality of life and the lack of a control group using the assessment of patients with the same condition subject to other treatments. The clinical significance of these variations is difficult to establish.

Failure of multidisciplinary treatment

Peritoneal recurrence occurs in 70% of patients (108-109).

Patterns of recurrence following multidisciplinary treatment can help to detect the cause of treatment failure and to modify it. A localized form of peritoneal recurrence could correspond to a failure of the surgery for "forgetting" a tumour foci between the adhesions and scar tissue where intraperitoneal chemotherapy is less effective against free tumour cells. Peritoneal recurrence detected in the intestinal wall may be due to a failure of the electrofulguration, while the diffuse peritoneal recurrence may be due to failure of intraperitoneal chemotherapy to eradicate minimal residual tumour disease after surgery.

It is important to determine the characteristics of the multidisciplinary treatment failures in order to advance in its development and to establish which patients may benefit from a new therapeutic approach. Another type of multidisciplinary treatment failure, is the spread of peritoneal carcinomatosis in the pleural cavity or the lung parenchyma, which occurs mainly in low-grade mucinous tumours associated with peritoneal pseudomyxoma. Sugarbaker considered the most likely mechanisms for the extension of the disease to extra-abdominal compartment were: (I) presence of congenital diaphragmatic hiatus holes or, (II) laceration of diaphragm muscle fibres caused by surgery, (III) communication openness and surgical abdominal and pleural cavities, and (IV) pulmonary tumour emboli.

It is very important to avoid aperture, and if it occurred, should be left the peritoneal-pleural communication open during the HIPEC phase to allow removal of the tumour cells migrated to the thorax by chemohyperthermia.

Summary

As occurred in the past with metastatic liver disease from colorectal cancer, peritoneal dissemination in colorectal cancer is still considered a widespread condition and treated with palliative procedures. For years, the locoregional treatment of liver metastases by the combination of liver surgery and chemotherapy has modified previous therapeutic concepts and criteria and has provided significant benefits on the survival in these patients. Currently the PC of colorectal origin is also considered a locoregional tumour manifestation confined to the abdomen.

Evidence in the different studies regarding the efficacy of

HIPEC for the PC from colorectal origin show that the survival after treatment varies between 22 and 60.1 months, and that survival rates at 5 years are between 11% and 48.5%, with a disease free survival of 34% for the same time period (66).

The 2-year survival of these patients is higher than that observed with the treatment without surgical cytoreduction and intraperitoneal chemotherapy, as evidenced by a properly randomized study (65). Patients in which it was possible to achieve a complete cytoreduction had better results. The results of a phase III trial demonstrated the clinical benefits of the multidisciplinary treatment compared with systemic chemotherapy and palliative surgery, and was first published survival rates of 5 years in the treatment of colorectal PC (11).

Elias presented 5-year survivals of 48.5% of patients with 34% of patients free of disease in this same period and a median survival time of 60.1 months using the open technique and a bidirectional chemotherapy consisting of application, 1 hour before HIPEC, a dose of 5-FU + folinic acid systemically. The intraperitoneal chemotherapy used was oxaliplatin at a dose of 460 mg/m^2 administered over 30 minutes at 43 ℃. Patients followed adjuvant chemotherapy. The risk for this clinical benefit was a 27% chance of developing complications grade III or higher toxicity (91).

In the past 10 years a large number of specialized centres have incorporated this therapeutic modality in the treatment of malignant diseases of the peritoneum, with improvements in therapeutic procedures, criteria for patient selection in the adjuvant chemotherapy and subsequent monitoring for the detection of early peritoneal recurrence and radical rescue surgery. The standardization of the entire therapeutic process has been reflected in better survival rates at 3 and 5 years and declines in the figures relating to morbidity and mortality, particularly evident in those studies involving over 100 patients in their series. It is considered that 130 patients treated by the same team, are the appropriate number of patients to complete the learning curve with this type of treatment.

Most important groups consider appropriate selection of patients according to their general, the extension of the PC (five or least affected regions or ICP <25) and the absence of multiple interventions and/or lines of chemotherapy failed. The feasibility of a complete cytoreduction (CC0-CC1) is crucial as an inclusion criterion (110).

It has been shown that the survival of the patients with PC of colorectal origin undergoing multidisciplinary treatment depends basically on the extent of PC at the time of surgery and the completion of surgical debulking. Almost all studies agree on the impact of the debulking with no macroscopic residual tumour in terms of survival. The patients who achieved a complete cytoreduction had a survival rate nearly twice that those patients in whom it was not possible to perform (111).

The risks are that between 25-50% of major complications (surgical or medical), although they do not significantly differ from those referred for patients undergoing major digestive surgery.

The multidisciplinary treatment is associated with risk of death by 5-12%.

Although there are two randomized controlled trials, only one could conclude as planned, while the other had closed prematurely due to difficulties in recruiting patients (66). So most of this evidence is level 3 (case series, most of them retrospective), and part was summarized as intermediate quality in a systematic review of the literature (112).

It has been shown that the survival of colorectal origin of PC patients undergoing multidisciplinary treatment depends largely on the extent of the PC at the time of surgery (PCI) and the completion of the surgical cytoreduction (CC). Almost all studies agree on the important impact that involves debulking with no macroscopic residual tumour (CC0) on survival.

Most groups consider important the proper selection of patients according to their status, the PCI <26 (<10 according to Sugarbaker) and the absence of previous surgery and/or lines of chemotherapy failed and the chances of achieving full cytoreduction (CC0-CC1) are crucial to the outcome of these patients.

An ongoing Phase III trial (NCT00769405) addresses this question of how much of the survival benefit is derived from the cytoreduction and how much from hyperthermic intraperitoneal chemotherapy, as patients will be randomly assigned to hyperthermic intraperitoneal chemotherapy or no hyperthermic intraperitoneal chemotherapy after complete cytoreductive surgery.

It is important to conduct controlled clinical trials that redefine the role of HIPEC in the era of new biological molecules and the effect of the best selection of patients using the benefits of recent genomic studies on biopsy material, to establish predictive factors associated with this treatment.

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Footnote

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Preoperative chemotherapy for locally advanced resectable colon cancer - a new treatment paradigm in colon cancer?

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Adjuvant therapy in locally advanced resectable colon cancer

Since 2004, the treatment of locally advanced, resectable colon cancer including high risk stage II or stage III disease is surgery followed by postoperative adjuvant chemotherapy with an oxaliplatin containing regimen. Combination therapy with oxaliplatin and a fluoropyrimidine, including capecitabine, has shown clear superiority to fluoropyrimidine therapy alone (FU/LV) in mitigating risk of recurrence and improving long-term survival (1-6). The results of the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) trial (1,2) and the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07 (4,5), showed that regimens with oxaliplatin (FOLFOX4 or FLOX) compared to FU/LV significantly improved disease-free survival (DFS) as well as overall survival (OS) especially in stage III patients, resulting in a 5-6% absolute improvement in 5-year DFS (73% *vs.* 67% in MOSAIC; 69% *vs.* 64% in NSABP-07), and a 3-4% increment in long-term OS in stage III cancer (73% *vs.* 69% 6-yr OS in MOSAIC; 77% *vs.* 74% 5-yr OS in NSABP C-07).

Current evidence on neoadjuvant therapy in several GI malignancies

Given proven efficacy in the adjuvant setting, the trend has been to test the benefits of *pre*operative or *peri*operative therapy for other GI malignancies including esophageal, gastric and rectal cancers (7-11). A neoadjuvant treatment strategy is attractive with theoretical benefits that could result in eradication of micrometastases and reduction of tumor cell shedding during surgery. Furthermore, patients will likely better tolerate full intensity chemotherapy when administered prior to surgery rather than post-operatively. Neoadjuvant treatment also allows the assessment of initial tumor response and toxicity profile of the same regimen that might be considered for additional systemic therapy given in the adjuvant setting. Use of *pre*operative therapy has resulted in significant downstaging with improved resectability and a better progression-free (PFS) and overall survival (OS) in several GI cancers. The magnitude of such improvement in the case of *esophageal cancer*, as shown by the MRC Working Party study (7), was 6% (60% *vs.* 54%) increase in complete resection rate and 20% improvement of relative risk in 5 year OS with preoperative chemotherapy (two 4-day cycles of cisplatin/continuous infusion 5-FU) compared to surgery alone (HR: 0.79, 95% CI: 0.67-0.93). A greater benefit was reported in the recent CROSS trial (8), where preoperative chemoradiation therapy (weekly carboplatin/paclitaxel for 5 weeks and concurrent radiotherapy) increased the complete resection rate by 23% compared to surgery alone (92% *vs.* 69%). Overall survival was significantly better in the preoperative chemoradiation group (HR: 0.66, 95% CI: 0.50-0.87), leading to a difference in median OS of 25 months (49.4 *vs.* 24 months). An example of perioperative chemotherapy (three 3-week cycles of ECF before and after surgery) as reported by the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial (9) for *gastric*

cancer resulted in a 25% improvement in OS compared to surgery alone (HR: 0.75, 95% CI: 0.60-0.93). The MRC CR07 and NCIC-CTG C016 study (10) established the beneficial role of preoperative radiotherapy compared to initial surgery with selective postoperative chemoradiation in *rectal cancer*. The study noted a 61% reduction in the relative risk of local recurrence for patients receiving preoperative radiotherapy (HR: 0.39, 95% CI: 0.27-0.58), and an absolute difference of 6.2% (95% CI: 5.3-7.1%) at 3 years. There was a 24% improvement in DFS associated with preoperative radiotherapy (HR 0.76, 95% CI: 0.62-0.94); an absolute difference of 6.0% (95% CI: 5.3-6.8%) at 3 years (77.5% *vs*. 71.5%). Notably, overall survival did not differ between the two groups (HR: 0.91, 95% CI: 0.73-1.13). Neoadjuvant chemotherapy regimens combined with radiation as multimodality treatment in locally advanced rectal cancer have been reported. All include a fluorouracil based regimen with or without oxaliplatin. The NSABP R-04 trial (11) compared 4 neoadjuvant regimens (infusional 5-FU or capecitabine, each with or without oxaliplatin) with concurrent preoperative radiation. The result showed similar efficacy overall for complete pathologic response (pCR) (~20%), sphincter-saving surgery (~60%) and surgical downstaging (~20%). Incremental benefit of adding oxaliplatin in this setting was minimal with added toxicity, results which are similar to other reports.

Evidence on preoperative chemotherapy in colon cancer with potentially resectable liver metastasis

The standard use of perioperative chemotherapy for patients with resectable liver metastasis remains controversial (12-15). It should be noted that only a minority of patients with liver metastases are technically resectable at diagnosis. Patients with initially unresectable liver tumors are first treated with chemotherapy and some of them can be converted to resectability with 5-year survival comparable to those who were initially resectable (16). A representative study supporting the perioperative chemotherapy for resectable liver only metastases is the EORTC intergroup phase III study 40983 (17-19), which compared perioperative FOLFOX4 chemotherapy (6 cycles pre- and post-surgery) to surgery alone in selected patients. Among 364 (1:1) randomized patients, the result showed borderline improvement in PFS (HR: 0.79, 95% CI: 0.62-1.02), although no difference in OS (HR: 0.87, 95% CI: 0.66-1.14) over surgery alone in a recent updated report (17).

Preoperative chemotherapy in locally advanced, resectable colon cancer

In a recent issue of Lancet Oncology (20), the investigators from the FOxTROT Collaborative Group (Fluoropyrimidine Oxaliplatin and Targeted Receptor Pre-Operative Therapy) reported results from the feasibility phase of a randomized study which was the first to examine the value of preoperative therapy in patients with locally advanced operable colon cancer. One hundred fifty high risk stage II and stage III patients, with T3 (≥5 mm invasion beyond the muscularis propria) or T4 cancer were randomized in a 2:1 ratio to 6 weeks [3 cycles of OxMdG, equivalent to FOLFOX6 (21)] preoperative plus 18 weeks (9 cycles) postoperative adjuvant chemotherapy *versus* postoperative chemotherapy only for 24 weeks (12 cycles). Notably, there was a second randomization in each arm to receive anti-EGFR therapy using panitumumab in KRAS wild-type patients (72% of those with known KRAS status) (22,23), of whom 31% were assigned to panitumumab. Although lacking disease progression or survival outcomes, results from this feasibility study showed significant tumor downstaging compared with the postoperative group (P=0.04). There was also less apical node involvement (1% *vs*. 20%, P<0.0001) and fewer positive margins (4% *vs*. 20%, P=0.002). Blinded centrally scored tumor regression grading showed moderate or greater regression of 31% *vs*. 2% (P=0.0001), favoring the preoperative group. The study concluded that preoperative chemotherapy in locally advanced operable primary colon cancer was feasible with acceptable toxicity and perioperative morbidity. The decision was to proceed to a phase 3 study to examine clinical outcomes in correlation with the favorable pathological responses as a result of preoperative therapy including survival.

The FOxTROT trial represents an effort in response to the rising enthusiasm to change the treatment paradigm for patients with resectable and potentially curable colon cancer. The neoadjuvant approach has the potential to improve patient tolerance and acceptance of chemotherapy and would determine if a patient's tumor is "chemo-sensitive". For those who did not respond to neoadjuvant therapy, new therapeutic strategies would be essential, including the development of biologically-driven clinical trials. The ability to access tissue pre- and post-neoadjuvant therapy offers an opportunity to explore biologic targets and

to develop potential agents that would affect these targets and is a strategy under development for neoadjuvant therapy for rectal cancer. For those patients with deficient DNA mismatch repair tumors (dMMR, MSI-H), particularly for stage II colon cancers, survival is excellent and adjuvant chemotherapy has been shown to offer no additional benefit and may in fact be harmful (24). Therefore it may be important to first evaluate patients to determine MSI status prior to neoadjuvant chemotherapy.

In addition, clinical staging prior to neoadjuvant therapy does have limitations compared to pathologic stage; thus, patients who received neoadjuvant therapy may be "over-treated" with neoadjuvant therapy particularly for stage II disease. Thus, there is a concern that inaccurate radiological staging might result in inappropriate chemotherapy for low-risk patients in the preoperative setting. Accuracy of radiological staging was assessed by the authors compared to pathological staging after surgery. CT imaging accurately identified invasion of the muscularis propria in 98% of patients, although was less accurate in discriminating between T3 and T4 stage in half of the evaluated cases. CT was sensitive in detecting nodal spread, yet with a low specificity as a result of overestimation of involved nodes.

The optimal duration of neoadjuvant therapy is also a question and whether 2-3 months of neoadjuvant therapy plus 3 months of postoperative adjuvant therapy is necessary. Advanced disease trials demonstrated that the greatest reduction in tumor size occurs during the first 2-3 months of combination therapy for metastatic colorectal cancer, after which time there is less tumor size decrease and more of a stabilization pattern (25,26). There is a world-wide effort to study 3 months (FOLFOX 6 cycles) *vs.* standard 6 months (FOLFOX 12 cycles) of adjuvant therapy for stage III colon cancer which should help determine the optimal duration of treatment.

Among the current trial subjects who had high T stage colon cancer, the potential risk of tumor growth during the preoperative treatment phase that could lead to bowel obstruction or perforation hence emergency surgery was not demonstrated. One out of the 99 patients assigned to preoperative chemotherapy proceeded directly to surgery due to localized perforation before the start of treatment and there were no cases requiring emergency surgery because of incipient obstruction during the 6-week preoperative treatment. The mean time from randomization to start of chemotherapy was 13 (SD 6) days, and the mean time to surgery from start of chemotherapy of 61 (SD15) days. This included at least a 3 week designated delay to surgery after completion of preoperative chemotherapy. Despite the differences in time course, the safety, tolerance and surgical related complications rates were comparable between the 2 treatment arms. There was also a notable higher chemotherapy completion rate in the pre- plus post-operative therapy group compared to the postoperative chemotherapy group (68% *vs.* 57%).

The use of the anti-EGFR antibody (panitumumab) for KRAS wild-type patients in the neoadjuvant setting was included in the FOxTROT trial because of the increase in response rate when panitumumab or cetuximab has been added to chemotherapy in metastatic colorectal cancer clinical trials (27,28); however, the investigators did not report whether there was any difference in response or resectability rates between the two pre-operative groups (chemotherapy with or without panitumumab). The continuation of panitumumab in the adjuvant setting is of potential concern in FOxTROT since the North American GI intergroup study of adjuvant cetuximab in addition to chemotherapy showed no difference in survival and in fact was detrimental (29).

In summary, the FOxTROT trial was the first randomized study in assessing preoperative chemotherapy in locally advanced operable colon cancer, and has shown promising results from the feasibility phase of the study. The phase III study will determine if neoadjuvant chemotherapy is a viable option for patients and whether the "standard of care" will change. The addition of panitumumab in the trial design is a concern given the previous negative results from a large stage III colon cancer trial comparing adjuvant chemotherapy with or without cetuximab.

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Footnote

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

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Novel radiation techniques for rectal cancer

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Abstract: The concepts for management of rectal cancer have changed drastically over the past few years. Through national bowel cancer screening programmes in the Western countries and the increasing use of endoscopic procedures as diagnostic tool, there has been an increase in detection of rectal cancer in early stages. There is increase in the ageing population worldwide but more so in Western countries. In addition, there is recognition of harm from extirpative surgical procedures which were directed towards managing advanced rectal cancer in the past. The increasing cost of health care has also led investigators to seek alternative treatment options which are effective, safe and economical. There are several modern radiation techniques which fit this bill and we need to be aware of newer radiation techniques to fill this gap.

Keywords: Early rectal cancer; X-ray contact; complete clinical response brachytherapy

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Background

The concept for management of rectal cancer has changed significantly in the past decade. There are several reasons for this. Many western countries have set up national bowel cancer screening programs which have targeted earlier stage rectal tumors compared with the more advanced staged cancers which were only diagnosed when they become symptomatic. Therefore, the surgical techniques that were aimed at treating advanced rectal tumours should not apply to the earlier stage disease. There is also recognition of surgical mortality and morbidity, especially in the elderly cohort (1). Many rectal cancer trials now include a wait and watch approach for those who achieved a complete clinical response. This allows organ preservation which has less detrimental effect on bowel function. Moreover, several clinical trials have shown improved disease free survival for those who achieved a complete response (2). In addition, there is evidence from the population-based statistics of an increase in rectal cancer in the ageing population worldwide with the average age of patients with rectal cancer predicted to rise from 65 to above 75 years within the next decade. The recent economic down turn across the world also has highlighted the financial burden of cancer care on the health care providers and many are seeking alternative strategies to keep the cost down without compromising outcomes. Radiotherapy is cheap compared to other treatment modalities. Novel radiation techniques have been developed which are attractive as alternatives to currently available radiotherapy options especially in treatment of early rectal cancer in the elderly.

Dose escalation to improve outcomes

There is evidence for dose response in rectal tumours and radiotherapy dose escalation could improve local control and other outcomes. However, there is a limit to how much radiation dose can be safely delivered using external beam alone without causing undue toxicity to the normal surrounding tissues. The dose escalation trial from Princess Margaret Hospital has shown that although higher rates of pathological responses can be achieved, the toxicity also increases, which negates the therapeutic ratio (3). The addition of chemotherapy does improve pathological complete response (pCR) rates (*Table 1*) and chemo radiotherapy has now become the standard of care in rectal cancer management (4). Traditionally, 5FU based regimes were used but oral capecitabine, which is much more convenient to use, has

Table 1 Comparative complete pathological response following chemo radiotherapy

	n	Dose (Gy)	pCR (%)
ACCORD	598	44-50	11-25
STAR	720	46	16
NSABP-4	1,608	50	20
CAO-4	1,265	50	13-17
PETTAC-6	1,094	44	11-13

pCR, pathological complete response.

replaced this and has become the standard of care. The addition of oxaliplatin to capecitabine has not kept up with earlier expectations. Both the French ACCORD (5) and the Italian STAR (6) trials have not shown benefit from the addition of oxaliplatin to either capecitabine or 5FU. However, the addition of irinotecan has shown some benefits and there are ongoing trials evaluating the role of irinotecan combined with capecitabine as in the UK lead ARISTOTLE trial.

Brachytherapy in rectal cancer

Over the years investigators have evaluated the role of brachytherapy to assess whether deliver of higher dose of radiation using brachytherapy as a boost improve outcomes. There are three types of brachytherapy:

(I) Contact X-ray brachytherapy (Papillon);
(II) High dose rate (HDR) intra luminal rectal brachytherapy;
(III) Interstitial rectal brachytherapy implant.

Contact X-ray brachytherapy (Papillon)

Low energy (50 KV) X-rays are used to deliver contact X-ray brachytherapy. It has been in clinical use for the past 80 years. However, very few centres around the world have continued to use this technique. There are several reasons for this. Firstly, the numbers of cases suitable for this type of treatment are small. There is development of newer competing surgical techniques e.g., Trans anal Endoscopic Micro Surgery (TEMS), Trans anal Endoscopic Operation (TEO) and Trans Anal Minimally Invasive Surgery (TAMIS) which are currently being used more for patients with early small rectal cancers. Only very few patients who are not fit for general anesthesia are referred for brachytherapy. Secondly, there were no replacement machines for the obsolete Philips 50 KV machines, which have been out of production since the mid 70's. Recently, there has been a revival of interest in contact X-rays brachytherapy and there are at least two companies Ariane (Derby, UK) and Xsoft (Axxend, CA) which have manufactured modern machines to produce 50 KV X-rays for use in contact X-ray brachytherapy.

The principle of contact X-ray brachytherapy consists of delivering high dose (30 Gy) of low energy (50 KV) X-rays applied straight on to the tumour under direct vision. This minimizes the chance of geographic miss. The dose falls off rapidly. The 100% dose is prescribed at the surface and the dose falls to 60% at 5 mm depth. Tumour size <3 cm can be offered X-ray contact radiotherapy initially. The treatment is given every 2 weeks which allows recovery of normal tissues in between treatments. As it is an orthovoltage radiation, the biological equivalent dose (EQD) is high at 1.4-1.6. Therefore, the total radiation dose delivered is above 40 Gy given in just over a minute instead of the usual protracted small doses of radiation given over 4-5 weeks. The applicator size use depends on the size of the tumour ranging from 30-22 mm. The patient is usually treated in knee chest position traditionally but can be treated in lithotomy position depending on the location of the tumour. The treatment can be delivered as an out-patient without the need for general anesthesia.

Assessment after two treatments is crucial to differentiate the good responders from the poor responders. If the response is favorable, further X-ray contact brachytherapy is offered to a total of four treatments (*Figure 1*). The radiation dose is usually 90-110 Gy in three to four fractions given every 2 weeks. For tumors which are initially staged as T2 or early T3, the risk of lymph node spread is high (20-30%). External beam chemo radiotherapy 45 Gy or its radiobiological equivalent should be offered to sterilize the lymph nodes. For bulky tumors >3 cm the treatment starts with external beam chemo radiotherapy. The response is assessed within 2-3 weeks after the completion of treatment. For good responders (tumour regresses >80%), this can be followed by X-ray contact radiotherapy to improve local control and increase the chance of a complete clinical response (7). This assumption will be evaluated in a clinicaltrial (OPERA) in which patients will be randomised to standard chemoradiotherapy against standard CRT and contact X-ray radiotherapy boost. This trial is planned to start early next year. If the response is poor (<80% regression) then patients are advised to accept immediate salvage surgery, provided the patient is fit and agreeable for surgery that involves a stoma.

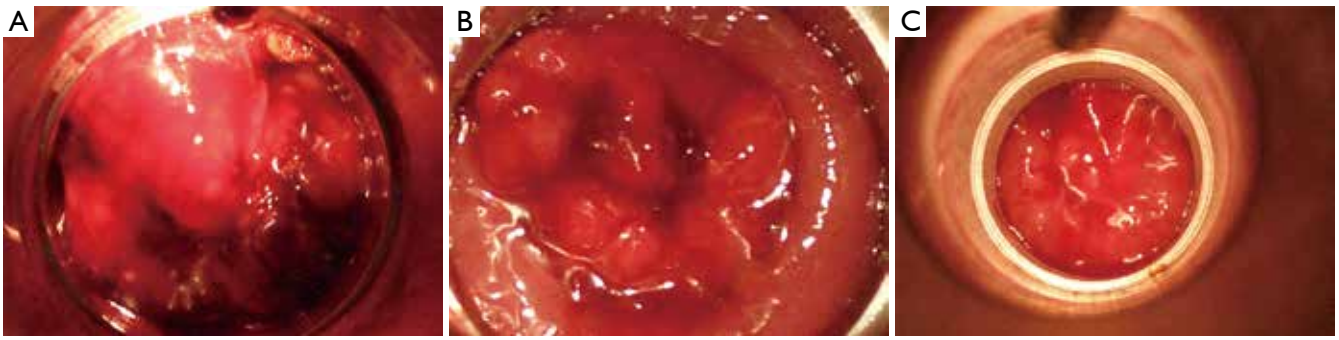

Figure 1 (A) Showing polypoidal tumour pre-treatment (day 1); (B) showing regression of tumour after one fraction (day 14); (C) showing complete regression of tumour after two fractions (day 28). Example of a good responder.

HDR rectal brachytherapy

HDR intra luminal rectal brachytherapy uses either Ir^{192} or Co^{60}. There are several commercially available remote after loaders. A number of different rectal applicators can be used depending on the system selected:

(I) Multiple channel rectal applicator (OncoSmart®, Elekta);
(II) Rectal/vagina rigid single line applicator Elekta/Eckert & Ziegler (Bebig);
(III) Rectal/vagina rigid single line applicator with variable central shielding Elekta/Eckert & Ziegler (Bebig);
(IV) Single line flexible endo-bronchial source (Elekta).

Multi channel rectal brachytherapy applicator

This rectal applicator has the advantage of using the channels close to where the tumour is situated and thus spare the contra lateral rectal mucosa (*Figure 2*). A balloon can be used to push the normal rectal mucosa away from the treatment source. Central shielding to minimize the dose to contra later rectal mucosa has also been investigated. It is suitable for any height of rectal tumour either low, mid or upper. It is a flexible applicator and more comfortable for the patient. It can be applied without general anesthesia (8) (*Figure 3*).

Rectal/vaginal rigid single line brachytherapy applicator

This type of applicator is suitable for low rectal tumors which occupy more than 50% of the rectal wall. It is not suitable for mid to high rectal tumors. There are different diameter applicators and stenosing tumors may need a defunctioning stoma before brachytherapy. This applicator is much easier to use.

Rectal/vagina rigid single line applicator with variable central shielding

This type of rectal applicator is suitable for smaller low rectal tumors which occupy less than half the circumference. Central shielding can be used to protect the contra lateral uninvolved rectal mucosa (9).

Rectal brachytherapy procedure

Endoscopy is carried out initially to assess the position and length of the rectal tumour. Marker seeds are inserted at the lower end of the tumour to locate it on the radiographs. The rectal brachytherapy applicator is inserted via the anus into the rectum either under general or local anesthesia. The position of the rectal applicator is checked on the fluoroscopy and adjusted as necessary. Once the position is satisfactory it is secured in place by clamps or strings tight to the corset. The patient treatment position is shown in *Figure 3*. The patient is then scanned on the CT simulator. The tumour position is outlined based on the information from the digital examination (lower rectal tumour), endoscopy and MRI. The dose is prescribed to cover the PTV (CTV + margin). The dose given depends on whether brachytherapy is given as monotherapy or as a boost after external beam chemo radiotherapy (10). Although the dose for monotherapy (26 Gy given over 4 daily treatments) is now fairly standard based on extensive experience from McGill University (8) much work is still needed to be done to determine dose for the brachytherapy boost.

Interstitial rectal implant using rectal template

For rectal tumors which extend into the anal canal, none of the above brachytherapy techniques are suitable. However, an interstitial implant using a rectal/anal jig can

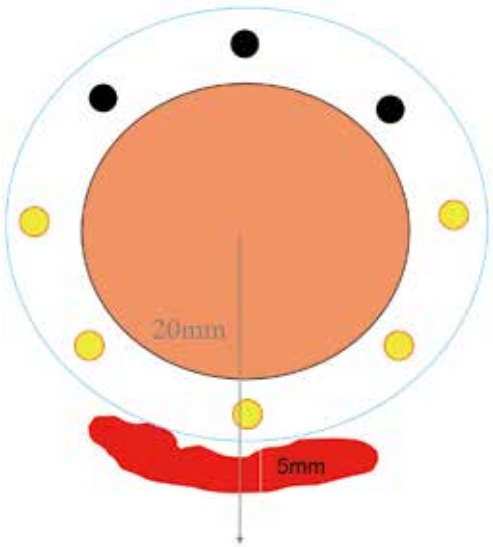

Figure 2 Showing multiple channels in flexible rectal applicator. Treatment is loaded towards residual tumour thus sparing the contra lateral uninvolved rectal mucosa.

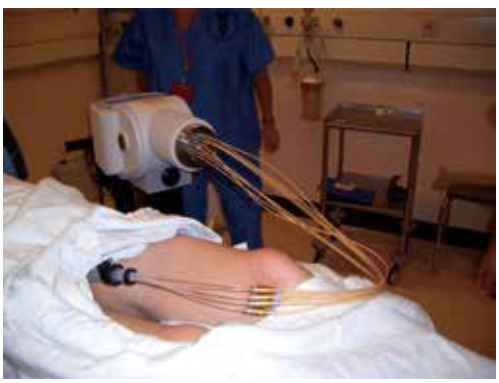

Figure 3 Showing treatment position for high dose rate (HDR) rectal brachytherapy.

be performed if there is residual tumour following external beam chemo radiotherapy. Most centres use a template with needles which are implanted through the perineum and into the tissues outside the wall of rectum. The iridium wires which were formerly used have now been replaced by fractionated HDR brachytherapy. The dose given varies but the usual schedule is 5-7 Gy in 3 fractions over 24 hours.

Selection of type of brachytherapy

Whether we should use contact X-ray brachytherapy or HDR isotope brachytherapy is determine by the morphology and the stage of rectal cancer. Exophytic usually sessile rectal cancers confined to the bowel wall are best treated by X-ray brachytherapy as the maximum dose of radiation is applied on to the surface of the rectal wall.

There is very little penetration and it is not useful for a tumor that penetrates much beyond the rectal wall. Therefore, tumors that infiltrate beyond the rectal wall are not suitable for contact X-ray brachytherapy. The exophytic component that protrudes from the rectal wall into the lumen gets a much higher dose due to the inverse square law. The tumour is shaved off layer by layer with each application of the contact X-ray brachytherapy until it regressed down to the surface of the rectal mucosa. The shrinkage is centripetal and the tumor regresses back to the site of origin in the case of a small rectal tumor. At the end of treatment, there may be a small superficial ulcer with smooth edge or a supple mucosa with no indurations beneath its base. This usually heals within 3-6 months if there is no residual tumour. However, those with residual tumour (if viable) can grow back within this period. Contact X-ray brachytherapy is therefore only suitable for T1/early T2 tumors that have not penetrated much into the muscularis propria. However, it is often very difficult to differentiate between T1 and early T2 tumors with the currently available radiological techniques.

HDR isotope brachytherapy is used when the tumour penetrates beyond the rectal wall (T3). This penetration can be readily seen on the MRI and EUS. It can be used as monotherapy or as a boost after external beam chemo radiotherapy when the residual tumour extends beyond the rectal wall. The radiation dose required to sterilize and kill off the residual tumour after external beam chemo radiotherapy is still under investigation and is not yet fully established. The dose currently in use is either 5-10 Gy in single fraction or 7-10 Gy per fraction in 3 weekly fractions. The volume irradiated is slightly larger, resulting in greater mucosal toxicity compare to contact X-ray brachytherapy.

Side effects

There is no reported mortality associated with rectal brachytherapy. No perforation or uncontrolled bleeding has been reported immediately following brachytherapy in experienced hands. The late toxicity is mainly bleeding which occurs in 26% of cases but usually resolves after 6-12 months. However, bleeding can be troublesome in 5% of patients

who are on anti-platelet medications e.g., warfarin or clopidrogel. Argon plasma coagulation is necessary in about 5% of patients if bleeding is troublesome (11). Endoluminal stricturing occurs in about 1%, usually in cases following surgical resection. Stricturing can also occur if there is residual tumour growing extra luminally. MRI can be difficult to interpret when attempting to differentiate the two processes. Surgical intervention may be necessary to establish the underlying pathology.

Discussion

The standard of care is surgery even for early rectal cancers, resulting in a permanent stoma for about a third of patients. The population is ageing and it is predicted that the majority of patients with rectal cancer will be above 75 years in the next decade. The mortality and morbidity is high for elderly patients and it is best to reserve surgery for those with advanced disease. Increased use of endoscopy to investigate bowel symptoms and screening programmes for asymptomatic patients have led to an increase in the diagnosis of early stage rectal cancer. These should be treated differently from advanced stage disease. There are now a number of alternative treatment options available to manage early rectal cancer.

Many novel radiation techniques in brachytherapy are now available and these may be more suitable for patients with early stage disease. All cases should be discussed in a multidisciplinary team meeting following diagnosis so that the optimal plan of management can be offered to the patients for best possible outcome. Difficult cases should be referred to centres of excellence and experience so that optimal treatments including brachytherapy can be offered as appropriate without compromising their chance of cure. Many centres have HDR brachytherapy facility for gynecological malignancies and these centres should look into setting up a rectal brachytherapy facility. Those centres with surgical expertise offering TEMS, TEO or TAMIS should consider introducing contact X-ray brachytherapy to compliment their services as not all patients referred will be fit for general anesthesia. Team work is important for successful outcomes and centres with expert multidisciplinary teams should consider expanding their services to include rectal brachytherapy facilities with both contact X-ray and HDR brachytherapy to improve the range of options they could offer for properly selected patients in the management of their rectal cancer.

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Footnote

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

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Evidence behind use of orthovolt intraoperative radiotherapy and other techniques of IORT in recurrent colorectal cancer treatment

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Abstract: There are only scarce literature data on the use of with orthovolt intraoperative radiotherapy (IORT) in treatment of colorectal cancer recurrence. Previous experience with IORT using low-energy photons highlights the need for better strategies of combined therapy in referral centres where multidisciplinary treatment options are widely available. There is an absolute need for large randomized studies that will clearly assess the value of different treatment options in colorectal cancer (CRC) recurrences, and thus create uniform rules to be observed in this disease.

Keywords: Colorectal cancer (CRC); recurrence; orthovolt intraoperative radiotherapy (orthovolt IORT); surgery; survival

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Introduction

There are only scarce literature data on the treatment of colorectal cancer (CRC) recurrence with the use of orthovolt intraoperative radiotherapy (IORT). IORT is a type of radiation treatment delivers a concentrated beam of radiation to tumor or tumour bed, as they are located during surgery. Depending on the type of radiation source following types of IORT are distinguished: intraoperative electron radiotherapy (IOERT) (delivering electron beams of high energies), high-dose rate intraoperative brachytherapy (HDR-IORT) (using high-dose rate source and remote afterloading technique) and orthovolt IORT (with low voltage X-ray beams). IORT, used as a component of combined therapy, allows to increase survival rates by about 15% (1). IORT is a safe and effective method of irradiation, significantly decreasing the risk of "geographical error". IORT allows the administration of a single, high dose of radiation, applied during surgery under direct vision (2,3). The principle for the use of IORT is to eliminate the microscopic tumour foci by maximizing the radiobiological effects of a single dose of radiation and to optimize the treatment duration (4,5). At the time when the application of a sufficiently high dose of external beam radiation therapy (EBRT) is limited by tolerance of organs at risk (OAR), IORT is an excellent alternative allowing for safe dose escalation to cover the tumour while dose-limiting structures, such as the bowel, bladder or ureters, are safely shielded (1,6,7). Moreover during surgery, it is possible to release adhesions, moving normal tissues beyond the irradiation field, thereby protecting them while giving an appropriate dose to the precisely defined surgical bed area with a safe margin (8,9). IORT enables extra "sterilization" of surgical margins, otherwise the only chance to perform radical treatment is to extend the resection which is not always possible (2,3,10-12). According to Williams *et al.*, IORT allows to limit the extent of the mutilating surgery, such as sacrectomy or exenteration (13). Most studies report a significant increase in survival rates in patients with recurrent CRC treated with IORT (5,14,15). The theoretical assumptions are promising, but the literature data on IORT in recurrent CRC come only from retrospective, single-institution studies.

IORT in recurrent colorectal cancer

Location of recurrent CRC in the pelvis is associated with a high risk of infiltration of the surrounding bony structures,

Table 1 Long-term survival results in treatment of recurrent CRC depending on IORT technique

Author (Reference)	Year	IORT Technique	IORT N	Overall survival (%)				
				1-year	2-year	3-year	5-year	10-year
Hashiguchi (15)	1999	IOERT	27			43	21	
Nag (19)	1999	IOERT	28			12		
Lindel (25)	2001	IOERT	49				27	
Mannaerts (26)	2001	IOERT	33			60		
Wiig (27)	2002	IOERT	59				30	
Pezner (28)	2002	IOERT	15			29		
Hahnloser (9)	2003	IOERT	131				21	
Haddock (29)	2011	IOERT	583				30	16
Roeder (6)	2012	IOERT	97				30	
Suzuki (14)	1995	IOERT/HDR-IORT	42			42	19	
Rutten (30)	2000	IOERT/HDR-IORT	62			49	33	
Vermaas (31)	2008	IOERT/HDR-IORT	11	77		51		
Alektiar (7)	2000	HDR-IORT	74		75		23	
Nuyttens (32)	2004	HDR-IORT	19			34		
Turley (33)	2013	HDR-IORT	21			60		
Guo (10)	2012	IOXRT	32				43	
Daly (34)	2012	IOXRT	55			59		

IOERT, intraoperative electron radiotherapy; HDR-IORT, high-dose-rate intraoperative brachytherapy; IOXRT, orthovolt radiotherapy.

which drastically reduces the chances of radical resection and usually involves the extended resections of multiple organs (16,17). The infiltration of side walls is associated with worse outcomes and obtaining radical resection is then significantly reduced (18,19). Surgical resection of recurrent CRC is frequently associated with a high risk of residual tumour tissue in the tumour bed and may have a negative impact on overall survival (OS) (20-22). The literature data also confirm better local control, with a lower risk of relapse at higher doses of radiation in the context of combination therapy with IORT (8,11). Improved local control and survival rates have been reported when IORT was used after neoadjuvant chemoradiotherapy for locally recurrent disease in radiation-naïve patients (23). Meta-analysis by Mirnezami *et al.* concerned the application of IORT in the treatment of recurrent CRC. The authors analyzed 29 studies, both prospective and retrospective, covering a total of 3,003 patients, of which 1,211 had recurrent CRC. IORT was used in patients with narrow or involved margins. The use of IORT was found to be associated with a significantly higher rate of wound healing complications without affecting the overall rate of complications and improved 5-year OS rates (P=0.001) (24). To date there are only two studies describing the use of orthovolt IORT in recurrent CRC, both published in 2012, by Guo *et al.* and Daly *et al.* (*Table 1*) (10,34). However, each of these publications describes the use of different systems for orthovolt IORT. In the study by *Guo et al.*, the INTRABEAM® PRS 500 system was used whereas Day *et al.* used Phillips RT -250. Daly *et al.* analyzed a total of 61 cases, including 41 patients with recurrent colon (n=16) and rectal (n=25) cancer. All patients were treated with IORT. The 2-year survival rate was 52%. The median survival time was 22 months in all patients without distinguishing between primary CRC and recurrence (34). The study team from Cleveland analyzed a group of 42 patients, of whom 32 (76%) had recurrence of CRC. The median survival time in this subgroup was 32 months and the 3-year survival rate was 43% (10). Distribution of R0 and R1 resection rates was 52% and 45%, respectively. R2 resection was performed only in 2.4% of cases (10). Hashiguchi *et al.* analyzed 51 patients with recurrent rectal and sigmoid cancer, 27 patients were treated with IOERT at a dose of 15-30 Gy. The authors found significant effects of IOERT (P=0.0007) and a small volume of tumour tissue left on higher survival rates (P=0.0022). In this analysis, the use of EBRT had no effect

on the late results (15). IOERT was an important prognostic factor, irrespective of the presence of distant metastases or radicality of resection. The median survival time in the IOERT group was 27 months. In the group without IOERT, 3- and 5-year survival rates were 5% and 0%, respectively. The authors have questioned the validity of the research in the arm without the IOERT scheme, proving the superiority of IOERT in terms of survival rates (15). Suzuki *et al.* found a significant difference in the 3- and 5-year survival rates between the IORT (+) and IORT (–) groups, 42%, 19% and 18%, 7%, respectively. None of the patients had distant metastases. All patients underwent non-radical resection and EBRT was performed in 41 of the 42 patients treated with IORT. In the IORT (+) group, patients with bulky, residual disease attained the 3-year survival rate of 44%. Moreover, the use of IORT improved local control and reduced the risk of re-recurrence. The 3-year re-recurrence rate in the IORT (+) group was 40% compared to 93% in the IORT (–) group (14). The literature is dominated mainly by studies describing the application of the IOERT technique for the treatment of CRC recurrence. The study by Nuyttens *et al.* analyzed 37 cases of patients with rectal cancer, including 19 who had local recurrence. HDR-IORT was used only in patients undergoing non-radical resection with a resection margin less than or equal to 2 mm. All patients had preoperative EBRT performed. The 3-year local re-recurrence rate was 57% (32). Summary of results depending on the IORT technique is presented in *Table 1*.

IORT treatment times and doses

In the study by Guo *et al.* the radiation dose administered was 5 Gy, at a distance of 1 cm from the applicator surface. The range of doses was 13.4-23.1 Gy with median 14.4 Gy (10). A dose in IOERT is given to the depth of 0.5-1 cm and reported on the surface (35). However, there is no uniformity in terms of the dose delivery reporting process. Generally, a dose is calculated to the surface or at a certain distance chosen by the study team (32). Lindel *et al.* differentiated the prescribed radiation dose ranges, depending on the radicality of resection. In the group of non-radical resection, the doses given ranged from 15 to 20 Gy, patients after radical resection received doses from 10 to 15 Gy (25). The median dose of IORT in the analysis by Eble *et al.* amounted to 13.6 Gy; however, in the R2 resection group the dose was higher –16 Gy (4). Haddock *et al.* determined the dose rate depending on two criteria: history of prior external field irradiation and radicality of resection. The patients previously irradiated received a dose of 12.5 Gy and those non-irradiated –17.5 Gy. Depending on the type of resection, the prescribed doses were as follows R0 –12.5 Gy, R1 –15 Gy and R2 –20 Gy. In this analysis, 98% of patients received a dose of not less than 20 Gy and the range of doses prescribed was 7.5-30 Gy (29). A similar IORT dose range was used by Hashiguchi *et al.* (15-30 Gy) and the median dose was 23 Gy (15). The dose of IORT is very important, because it allows in a measurable way to increase the total dose used in the treatment in order to eradicate the tumour. Doses exceeding 50 Gy allow to provide better control of symptoms; however, in cases of R1 resection the radiation doses should exceed 60 Gy to achieve a satisfactory treatment outcome (29,30). In some patients due to insufficient volume of a single field, it is essential to use the multiple radiation field technique (15). It is not the standard practice, but allows to cover a larger area, and in the case of IOERT it does not extend a total time of the entire treatment. The duration of IOERT irradiation is short (3-5 minutes) but preparations for the procedure with treatment usually take 30-45 minutes (19). In HDR-IORT, the operative time is extended by about 90 minutes due to the time of preparation and treatment (33). The multiple field technique in orthovolt IORT could significantly prolong the duration of surgery. IORT time prolongs with the increasing size of the applicator. The need to combine the radiation fields is often associated with the extensive areas of invasion, which means connecting the largest applicators, and thus the longest irradiation times. In Cleveland analysis, the median duration of IORT was 35 min (range, 14-39 min) (10). The size of applicators was selected based on the volume of recurrent tumour, so the surface of the applicator would adhere as closely as possible to the walls of the surgical bed. The median size of the applicator in Guo *et al.* analysis was 5 cm (10). Precise adherence between the tumour lodge and the applicator surface is extremely important. If the applicator is not fitted closely, the dose delivered to the lodge surface may vary markedly, resulting in the areas with insufficient dose coverage.

Re-irradiation for recurrent colorectal cancer

Dresden *et al.* analyzed 147 patients with non-metastatic relapse of rectal cancer. Their study confirmed an important role of combined therapy with the use of IORT in the treatment of local recurrence. The median OS time was 28 months. The 5-year OS rate was 31.5%. Patients re-irradiated or those who received a full course of radiotherapy before resection of recurrence had increased

survival rates (P=0.043), longer times to local re-recurrence (P=0.038) and to distant metastases (P<0.001). The median time to re-recurrence was 13 months, and to disclose distant metastases 18 months (3). Safety of the second line of radiation is kept, when the dose does not exceed 30-40 Gy, the period between both lines of radiation is longer than 6 months and radiosensitive organs (i.e., small intestine) are moved from the irradiation field (3). The analysis by Koom *et al.* evaluated the toxicity profile of second line preoperative radiotherapy in patients with recurrent rectal cancer. The studied group consisted of 22 patients. Resection of recurrence was performed in 23% of patients. In the study acute and late toxicity rates, above the third degree, were 9% and 36%, respectively. Toxicity was mostly related to the digestive and urinary systems. The median dose of re-irradiation was 50 Gy (range, 30-66 Gy). The increased toxicity rate was significantly related to correlate with the central or anterior location of recurrence and the resection of tumour after re-irradiation (36). The doses prescribed in that study were higher than those conventionally used in the second line irradiation (30 Gy). Rutten *et al.* analyzed 62 patients with local recurrence of rectal cancer without distant metastases. The study proved that the total dose of radiation and R0 resection were significantly associated with improved survival. The study analyzed the use of two systems for IORT: IOERT and HDR-IORT in two major cancer centres in the Netherlands: Daniel den Hoed Cancer Center (DHCC), and Catharina Hospital Eindhoven. The basic assumption of the use of IORT was to supplement EBRT to achieve the highest possible dose of irradiation. The R0 resection rate was 48%. Forty-three percent of patients received prior EBRT, all patients previously non-irradiated and 10 of the 27 previously irradiated patients received the second line radiation to a dose 30 Gy or to a full dose. The survival results were found to be worse in patients who did not receive radiation before resection of recurrent tumour (P<0.05). The risk of death in patients not irradiated before resection of recurrence was 3-fold higher compared to patients undergoing radiation therapy (30). Vermaas *et al.* analyzed a small group of 11 patients with recurrent rectal cancer in whom re-irradiation with EBRT to a dose 30 Gy and resection with IORT were performed. IORT was performed using the IOERT or HDR- IORT technique. All patients were qualified as Tr4/5 according to the Wanebo classification and no cases of R0 resection were reported. Despite poor prognosis, the results achieved with this treatment schedule were similar to those obtained in other studies (*Table 1*). The median survival time without pain was 5 months (31). A group of researchers from Memorial Sloan-Kettering Cancer Center analyzed 100 patients who underwent resection with HDR-IORT for non-metastatic recurrent rectal cancer. Absence of angioinvasion and radical resection of the tumour were found to be independent predictors of longer survival (P<0.01 and P<0.05). The rate of re-recurrences after IORT was 60%, and the median time to re-recurrence was 15 months (37). Alektiar *et al.* analyzed 74 patients with recurrent rectal cancer undergoing surgery with HDR-IORT. The results confirmed the effectiveness of association of EBRT and IORT to prolong OS (P=0.04) (*Table 1*) (7). The analysis by Mannaerts *et al.* evaluated patients treated for recurrent CRC using three protocols. Patients were radiotherapy-naive, and had no evidence of distant metastases. The first group included patients who underwent only EBRT, the second one EBRT with resection and the third group EBRT with resection and IORT. The median dose of EBRT in each group was 50 Gy. R0 resection was performed in 37% and 64% cases, respectively. In group I (EBRT) the 3- and 5-year OS rates were 14% and 10%, respectively and the median survival time was 18 months. In group II (EBRT + resection) the 3-year survival was 11% (depending on the radicality of resection: R0, 29%; R1/2, 0%) and the median survival time was 19 months. In group III (EBRT + resection + IORT) the 3-year survival rate was 60% (R0, 63%; R1/2, 52%). The analysis demonstrated significantly higher survival rates in group III compared to group II after radical resection (26).

Radiation toxicity after IORT

Radiation-induced toxicity is extremely difficult to distinguish from surgical complications or symptoms of disease progression (38). Depending on the IORT technique, and thus the energy applied, the effects, both early and late, on the surrounding tissues are different. The main issue of research on the use of IORT using electrons is a high rate of complications (19). The incidence of postoperative complications varies from 15% to 68% (39). The most common types of early complications after treatment with IOERT are wound healing disturbances 3-46%, small bowel obstructions (14%) and formation of pelvic abscesses (12%), while in the group of patients who underwent surgical resection alone—pelvic abscesses (15%) (9,14,15,24,27,40). The percentage of serious postoperative complications ranges from 27% to 81% (15,19,41,42). Turley *et al.* reported 45% of postoperative complications

without postoperative mortality (33). Wiig *et al*. and Hashiguchi *et al*. found no difference in the incidence of complications depending on the application of IORT (15,27). In the analysis by Williams *et al*., the most common acute complications associated with resection and IOERT were urinary tract infections, urinary incontinence and bladder dysfunction –13% (13). Dutch analysis by Dresen *et al.* reported 24% of acute complications of the urinary tract (3). Roeder *et al*. in the analysis of 97 patients with recurrent CRC, reported 59% rate of complications, including wound healing disorders, formation of abscesses, fistulas and disorders of micturition (6). An increased risk of complications in wound healing occurs in patients after preoperative radio and/or chemotherapy (4). In the analysis of the use of orthovolt IORT by Guo *et al*., the most frequent type of complications was hydronephrosis that occurred in 10 (24%) patients (10). Hydronephrosis and stricture of ureters in the case of high energy IOERT occur in about 2-12% of cases, however, it is difficult to compare these data with different types of IORT. Analysis by Daly *et al*. revealed 17 (31%) cases above the third degree of toxicity, including two cases of postoperative deaths. The most frequently reported complications were pelvic abscesses, small bowel obstruction, fistulas formation, ureteral stricture, and anastomotic leakage (34). The basic issue in the analysis of both Guo *et al.* and Daly *et al.* is that the incidence of complications was assessed in all patients, both in cases of recurrences and primary, advanced CRC. The toxicity profile in these groups of patients can vary greatly, mainly because patients with recurrent tumours have already undergone resection and radiation. Acute complications can also be caused by immobilization of bowel loops due to adhesions after primary treatment (14). The use of combined therapy (preoperative chemoradiotherapy, resection with IORT) in the analysis of researchers from the Mayo Clinic resulted in significantly higher rates of complications in patients with the grade of tumour immobilization above F2, according to the Suzuki-Gunderson classification (9). The most common complications were pelvic abscesses, intestinal obstruction and fistulas (9). Analysis of studies reporting complications after HDR-IORT in CRC recurrences shows that the types and rates of complications are similar to those reported for IOERT. Alektiar *et al.* reported that in patients with recurrent CRC undergoing surgery with HDR-IORT (followed by EBRT or otherwise) the rate of peripheral neuropathy was 16% and was similar to the data from other studies (7,32,37). Turley *et al.* found that surgical resection and HDR-IORT was associated with a high rate of both early (45%) and late (38%) complications. The most common early complications were postoperative wound infections (28%) and formation of intra-abdominal abscesses (14%). The prevalence of late complications such as neuropathy is reported to be 2% to 22% and is directly proportional to a dose of radiation (3,6,13,24,29,30). In the analysis by Nuyttens *et al*. abnormal wound healing occurred in 46% of patients, intra-abdominal abscesses in 16% and intestinal anastomotic leakage in 5% (32). To avoid the most common complications, it is necessary to perform the IORT procedure in sterile conditions and to shield the surrounding, healthy tissues, especially the ureters and the pelvic nerves. In cases at risk of ureter exposure to radiation, implantation of ureteral catheters or stents should be considered. The incidence of ureteral stricture requiring implantation of the catheter to prevent the development of hydronephrosis is as high as 23% (7,37). Despite the relatively high complication rates in patients undergoing resection with IORT, this treatment method is not less safe than surgery. In addition, IORT implementation can benefit in increased local control. Postoperative mortality depends, inter alia, on the scope of combination therapy, and the selection of patients for a particular treatment. Daly *et al.* reported two (3.6%), postoperative deaths unrelated to the use of IORT, whereas Guo *et al.* reported no deaths (10,34). Hahnloser *et al.* from the Mayo Clinic reported only one (0.3%) case of postoperative death (9). In the literature the 3-month postoperative mortality rate ranges from 0% to 8% (3,6,24,27,30,33). IORT is a technique that does not seem to increase the rates of complications or mortality (27). In the study by Guo *et al*., the median duration of postoperative hospital stay was 7 days (range, 2-59 days) (10). Some authors provide information about the duration of the entire hospital stay (8-19 days), which does not allow to make a meaningful comparison of results (3,9,27,31,33). The time of hospitalization in one of the studies, evaluating the use of IOERT in recurrences of rectal cancer, was shorter (13 days) in the group IOERT (+), compared to the IOERT (–) one (16 days) (27).

Conclusions

Analyzing the results of research on orthovolt IORT in recurrent CRC in the context of available literature, a number of limitations should be noted. Reports describing the use of orthovolt IORT in CRC recurrence do not constitute sufficient evidence, nor do they allow us to

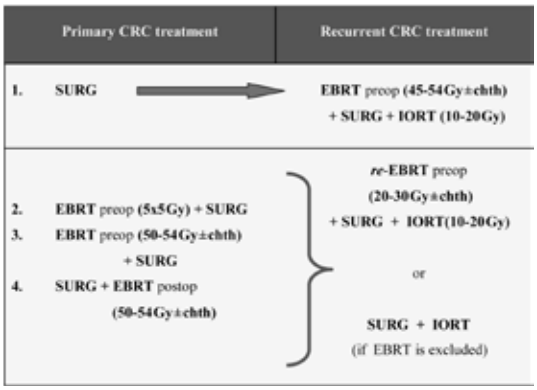

Figure 1 Algorithm for treatment of recurrent CRC. SURG, surgery; EBRT, external beam radiation therapy; EBRT preop, preoperative EBRT; EBRT postop, postoperative EBRT; re-EBRT, EBRT reirradiation of recurrent CRC; chth, chemotherapy.

draw uniform conclusions, as these are single-centre studies. In both studies, a relatively small number of cases limit the possibility of statistical comparisons of certain parameters. The conclusions from these studies should be formulated with caution in relation to the known limitations of retrospective analyses in general, and in particular the possibility of selection bias. Unsatisfactory outcomes in patients treated with IORT arise mostly from inability to obtain a free resection margin (38). Particular emphasis should be placed on early detection of recurrence. Previous experience with IORT using low-energy photons highlights the need for better strategies of combined therapy and multidisciplinary care of patients with recurrent CRC. At the same time, the treatment of recurrence should be performed in referral centres where multidisciplinary treatment options are widely available. There is an absolute need for large randomized studies that will clearly assess the value of different treatment options in CRC recurrences, and thus create uniform rules to be observed in this disease. Based on the literature data available, following algorithms seems to be optimal to take full advantage of oncological treatment in patients with recurrent CRC (*Figure 1*).

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Footnote

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Aspirin for colorectal cancer with *PIK3CA* mutations: the rising of the oldest targeted therapy?

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A recent report in *The New England Journal of Medicine* by Liao and colleagues highlights the benefit of aspirin use in a molecular defined subgroup of patients affected by metastatic colorectal cancer (CRC). Authors concluded that it is very likely that the regular use of aspirin after CRC diagnosis is associated with longer survival among patients with mutated-*PIK3CA* tumors. In contrast, aspirin has no effect on cancer-specific survival in patients with wild-type *PIK3CA* CRC (1). The phosphatidylinositol 3-kinase (PI3K) signaling pathway plays an important role in carcinogenesis of CRC. Activating mutations in *PIK3CA* occur in two "hotspots" located in exon 9 (E542K, E545K) and exon 20 (H1047R) in approximately 15% of CRCs (2). *PIK3CA* encodes for a lipid kinase that regulates signaling pathways downstream of the Epidermal Growth Factor Receptor (EGFR), and its mutations hamper sensitivity to the anti-EGFR monoclonal antibodies cetuximab or panitumumab registered for metastatic CRC treatment (3,4). Activation of PI3K enhances PTGS2 (prostaglandin-endoperoxide synthase 2, also known as cyclooxygenase-2) activity and prostaglandin E2 synthesis, inhibiting apoptosis of CRC cells. The long standing knowledge of the inhibitory effect of aspirin on PTGS2 may therefore suppress cancer-cell growth and induce apoptosis by blocking the PI3K pathway (1).

Several studies demonstrated that aspirin reduces the incidence of colon polyps, and its preventive effect is detectable even when given at low doses (75 mg daily). After CRC diagnosis, aspirin use reduces recurrence of adenomas (relative risk 0.65 *vs.* placebo) (5). Recently, a randomized trial in patients affected by Lynch syndrome showed that two years of aspirin 600 mg/die improved CRC-specific outcome (HR 0.63 for CRC incidence) (6). The observation that aspirin reduces the formation of colonic polyps provides the rationale for the use of this drug in the cancer prevention setting. Among participants in a large study of cardiovascular prevention, after long-term follow-up, any dose of daily aspirin displayed cancer preventive effect by lowering by 24% the risk of CRC at 10 years (7). In a pooled analysis of 35,535 patients from 6 randomized trials, aspirin use reduced the risk of developing metastatic CRC and the risk of death from CRC, and this effect was maintained with low aspirin dose (8). However, use of aspirin for primary CRC prevention faces an increased risk of bleeding (9), so a selection of patients who are likely to benefit from this drug is warranted. On the other hand, in the secondary prevention of CRC, Chan and colleagues reported that, after surgical removal of primary tumor, aspirin use reduces the risk of CRC overexpressing COX-2 among patients from two large cohorts (Nurses' Health Study and Health Professionals Follow-up Study, started in 1976 and 1986, respectively) (10). These results were confirmed in a subsequent analysis of the same cohorts with a 29% CRC mortality risk reduction in the same subgroup of patients whose cancers over-express the enzyme COX-2 (11).

In their recent pivotal study, Liao *et al.* provided clinical hints for a bridging between molecular bases of CRC progression and pharmacogenomic of aspirin. In particular, they identified a subgroup of patients in whom the mutation of *PIK3CA* appears to be associated with reduced risk of mortality from CRC. This is the first study demonstrating the association between a specific genetic alteration which is relevant for cancer progression and a reduction in CRC mortality with the use of aspirin. Authors reported indeed a remarkable improvement in CRC-specific mortality and overall survival in a small subgroup of patients (mutated *PIK3CA* who used aspirin regularly after CRC diagnosis *vs.*

non-users), with a multivariate HR for CRC death of 0.18 (95% CI: 0.06-0.61, P<0.001) and 0.54 (95% CI: 0.31-0.94, P=0.01) for death from any cause. Conversely, tumors with wild-type *PIK3CA* did not benefit from aspirin use.

Although the hypothesized mechanism of action and data shown are compelling, caution is needed prior considering these results as practice-changing. Firstly, as acknowledged by authors, the statistical sample is limited (the patients with tumor harboring *PIK3CA* mutations who received aspirin were 62), and the use of multiple statistical tests on the same sample may increase the probability of making observations due to chance. Secondly, interpretation of results is hampered by the lack of detailed follow-up data including subsequent cancer treatments (i.e., adjuvant chemotherapy) which could impact on survival. Thirdly, it cannot be excluded that the use of self-prescribed aspirin, which was reported in many cases by patients for analgesic purposes, may have been associated with wider use of diagnostic investigations and, at least in symptomatic patients, with an anticipation in the detection of relapse, thus leading to an overall better CRC-related outcome.

In conclusion, the study of Liao *et al.* is providing compelling evidence toward a rationale use of aspirin in molecularly defined subgroup of CRC, but requires validation in independent cohorts of patients. Such studies should have mortality from CRC as primary endpoint and specific follow-up including cancer treatments. In Asia, the randomized ASCOLT trial (12) is undergoing to evaluate the efficacy of aspirin 200 mg daily for stage III and high-risk stage II CRC. It would be of great interest to assess whether the predictive role of *PIK3CA* mutation will be confirmed in this cohort of patients.

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Footnote

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Oral tyrosine kinase inhibitors targeting VEGF-receptors in patients with metastatic colorectal cancer

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Our understanding of cancer genomics and proteomics associated with normal and malignant cell growth and angiogenesis has increased exponentially in recent years and has resulted in the identification of several critical molecular events that are fundamentally involved in carcinogenesis and tumor progression. Targeting these key ligands, receptors and molecular pathways offers survival benefit in several cancers such as breast cancer, colon cancer and lung cancer.

It is a decade ago since the first targeted drugs proved their efficacy in the treatment of patients with metastatic colorectal cancer (mCRC) (1) and since then Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved a limited number of targeted drugs (cetuximab, panitumumab, bevacizumab, aflibercept, regorafenib) for clinical use in patients with mCRC and a much larger number are in various phases of clinical development.

The anti-epidermal growth factor receptor (EGFR) antibodies—cetuximab and panitumumab—were first successfully implemented in the later line of therapies, and then moved forward into first line therapy. In contrast, the anti-angiogenic antibody bevazicumab was directly introduced in the first line setting and subsequently showed its efficacy in later lines.

In the pivotal BOND study (2), the combination of cetuximab with irinotecan (CetIri) significantly increased response rate (RR) and prolonged progression free survival (PFS) and based on these data CetIri was approved for patients with irinotecan-resistant disease in US and Europe in 2004. Soon after, the benefit of cetuximab and panitumumab as monotherapy was confirmed in patients with chemo-resistant mCRC (1) and as second line in combination with chemotherapy (3,4). Ligand-induced activation of EGFR achieves most of its effect via the RAS-RAF-MAPK pathways, which promote proliferation, invasion, migration and neovascularisation. KRAS mutation in exon 2, found in approximately 40% of mCRC patients, is now an established predictive marker of resistance to anti–EGFR therapy (5,6), but in addition patients with KRAS mutations may even experience inferior outcome if combined with oxaliplatin-containing regimens (7,8). Based on data from a number of phase III studies, cetuximab and panitumumab was subsequently approved in the first line treatment of mCRC patients with KRAS wild-type tumors, in combination with chemotherapy (9,10).

The advantage of anti-EGFR and anti-angiogenic therapy led to hope for additional progress, and it was obvious to test if multi-blockade with a combination of anti-angiogenic and anti-EGFR therapy could further improve survival.

This "add-on principle" was supported by promising data from preclinical models suggesting that increased angiogenic potential may be involved in the resistance to anti-EGFR antibodies (7). Clinical data supported the hypothesis of an increased efficacy of combined therapy as a randomized phase II study (8) comparing the combination of irinotecan, cetuximab and bevazicumab to cetuximab and bevazicumab in patients with pre-treated mCRC showed a higher RR and longer PFS compared to historical data on cetuximab and irinotecan in the BOND study (2,8).

However, despite the above-mentioned promising results on double-blockade in preclinical models and from early clinical data, two large phase III studies—

the CAIRO2 and the PACCE studies—failed to confirm this and both trials actually showed that addition of bevazicumab to an anti-EGFR antibody and chemotherapy in chemo-naïve patients was associated with an inferior outcome compared to an anti-EGFR antibody and chemotherapy (11,12).

Another way of achieving multi-blockade is by the use of oral multi-targeted receptor tyrosine kinase (TKI) inhibitors including sunitinib, sorafenib, regorafenib, valatinib, axatinib, cediranib, and brivanib (13-22).

Sunitinib is an inhibitor of several TKI receptors including platelet-derived growth factor receptors (PDGF-R), the vascular endothelial growth factors receptors (VEGFRs), c-KIT, RET and FLT3. Saltz *et al*. published a phase II trial with sunitinib as mono-therapy in 82 patients with chemo-resistent mCRC (23). One patient achieved a partial response. Median PFS in the prior bevacizumab and bevacizumab-naive cohorts was 2.2 and 2.5 months, respectively, whereas median overall survival (OS) was 7.1 and 10.2 months, respectively. The authors concluded that sunitinib did not demonstrate a meaningful single-agent activity, but the mechanisms of action, the relative mild safety profile and easy administration warranted further study in combination with standard chemotherapy regimens for mCRC.

In a phase I study, the maximum tolerated dose (MTD) of sunitinib combined with FOLFIRI for untreated mCRC was 37.5 mg/day when administered 4 weeks on and 2 weeks off (24). The predominant dose limiting toxicity (DLT) was neutropenia. The authors concluded that the combination had acceptable tolerability and showed preliminary antitumor activity and based on these promising data a large phase III study comparing FOLFIRI plus placebo or FOLFIRI and sunitinib was initiated—without a phase II study—to confirm the activity of sunitinib in mCRC. The primary aim was to prolong PFS from 8.0 months to 10.8 months (35% improvement), which would require 568 events (16).

Two interim analyses were planned at 25% and 60% of the 568 PFS events, and the stopping boundary for futility at the second interim analysis was a hazard ratio (HR) of ≥0.88. A final analysis was planned after inclusion of 720 patients.

Enrolment began in July 2007. At the second interim analysis in June 2009, after enrolment was complete and 367 PFS events had occurred, the HR for PFS was 1.095 in favour of the placebo arm. There were also increased toxic events (including neutropenia and diarrhoea and numerically a larger number of toxic deaths) in patients receiving sunitinib plus FOLFIRI.

As mentioned, two interim analyses were planned. The authors do not disclose the result of the first interim analysis, and they do not explain why 48 supplementary patients were included. Shortening of the time to approval of new drugs is crucial, however it is important that interim analyses can terminate a trial before inclusion of the planned number of patients—especially if a phase III study is built directly upon a phase I study.

As shown in *Table 1*, sunitinib is not the only oral multi-TKI inhibitor that has failed to improve OS in mCRC patients. So far the only randomized phase III study in which an oral multi-TKI inhibitor has prolonged PFS and OS is the CORRECT trial (21), in which regorafenib monotherapy prolonged PFS from 1.7 to 1.9 months (HR, 0.49) and OS from 5.0 to 6.4 months (HR, 0.77).

One of the most important advances in recent years in the treatment of patients with mCRC is the translational studies discovering the impact of the KRAS mutational status on efficacy of anti-EGFR therapy as described above. Recently, retrospective analyses of prospective randomized studies have demonstrated that additional mutations in KRAS and NRAS predict a lack of efficacy to anti-EGFR therapy. Therefore the European label for panitumumab (10) and cetuximab (25) was recently modified to require testing for KRAS and NRAS mutations and in addition a meta-analysis suggests that mutation in BRAF and PIK3CA and a non-functional PTEN also predict resistance to anti-EGFR therapies (26). Some of the multi-TKI inhibitors have improved PFS; however without translation into improvements in OS (17,19,22) and thus may have efficacy in subgroups of patients.

It is therefore very important that clinical studies—also in late lines of therapy—are combined with translational studies in order to improve our knowledge of the biology of mCRC and the identification of new predictive markers. However, it is important that these marker studies do not solely focus on the targeted agents but as well aim to identify predictive markers for the "classic cytostatics" in order to further improve outcome for patients with mCRC.

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None.

Table 1 Randomized studies evaluating TKIs targeting VEGFR in mCRC

Randomized studies	Author, year	Regimen	Phase	No.	RR (%)	Median PFS (months)	Median OS (months)
First line therapy	Tabernero *et al.*, *CCR* 2013, RESPECT	FOLFOX + Pl	II	101	59	8.7	18.1
		FOLFOX + Sor		97	44	9.1	17.6
	Hecht *et al.*, *JCO* 2011, CONFIRM 1	FOLFOX + Pl	III	583	-	7.6	20.5
		FOLFOX + Val		585	-	7.7	21.4
	Infante *et al.*, *Cancer* 2013	FOLFOX + Ax	II	42	29	11.0	18.1
		FOLFOX + Bev		43	49	15.9	21.6
		FOLFOX + Ax + Bev		41	39	12.5	19.7
	Carrato *et al.*, *JCO* 2013	FOLFIRI + Pl	III	382	34	8.4	19.8
		FOLFIRI + Sun		386	32	7.8	20.3
	Hoff *et al.*, *JCO* 2012, HORIZON II	FOLFOX/XelOx + Pl	III	358	50	8.3	18.9
		FOLFOX/XelOx + Ced 20		502	51	8.6*	19.7
		FOLFOX/XelOx + Ced		216	Terminated, 20 mg sufficient		
	Schmoll *et al.*, *JCO* 2012, HORIZON III	FOLFOX + Bev	III	713	47	10.3	21.3
		FOLFOX + Ced 20		709	46	9.9	22.8
Second line therapy	Cutsem *et al.*, *JCO* 2011, CONFIRM 2	FOLFOX + Pl	III	429	-	4.2	11.9
		FOLFOX + Val		426	-	5.6*	13.1
	Cunningham *et al.*, *BJC* 2013, HORIZON I	FOLFOX + Bev	II	66	27	7.8	19.6
		FOLFOX + Ced 20		71	18	5.8	14.3
		FOLFOX + Ced 30		73	20	7.2	16.8
Third line therapy	Grothey *et al.*, *Lancet* 2013, CORRECT	BSC	III	255	0	1.7	5.0
		Rego		505	1	1.9*	6.4*
	Siu *et al.*, *JCO* 2013, C0.20	Cet + Pl (KRASwt)	III	374	7	3.4	8.1
		Cet + Briv (KRASwt)		376	14*	5.0*	8.8

*, Significant difference; Pl, placebo; Val, valatinib, oral TKI that targets VEGFR1-3; Sor, sorafenib, oral TKI, against VEGFR, PDGFR, KIT; Ax, axatinib, oral TKI that targets VEGFR1-3; Sun, sunitinib, oral TKI against VEGFR, PDGFR, KIT; Ced, cediranib, oral TKI that targets VEGFR1-3; Briv, brivanib, oral TKI that targets VEGFR and FGFR; Rego, regorafenib, oral TKI that targets VEGFR, PDGFR and FGFR; Cet, cetuximab; Bev, bevacizumab; BSC, best supportive care; TKI, tyrosine kinase; VEGFR, vascular endothelial growth factors receptor; mCRC, metastatic colorectal cancer; RR, response rate; PFS, progression free survival; OS, overall survival; *CCR*, *Clinical Cancer Research*; *JCO*, *Journal of Clinical Oncology*; *BJC*, *British Journal of Cancer*.

Footnote

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Targeted therapies in colorectal cancer: surgical considerations

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Abstract: Colorectal cancer (CRC) is a leading worldwide health concern that is responsible for thousands of deaths each year. The primary source of mortality for patients with CRC is the development and subsequent progression of metastatic disease. The most common site for distant metastatic disease is the liver. Although patients with metastatic disease to the liver have several effective treatment options, the only one for cure remains surgical resection of the liver metastases. Historically, most patients with liver metastases have had unresectable disease, and only a small percentage of patients have undergone complete curative resection. However, improved systemic therapies have led to an evolution in strategies to treat metastatic CRC to the liver. Under most conditions the management of these patients remains complex; and as chemotherapy options and new targeted therapies continue to improve outcomes, it is clear that a multidisciplinary approach must be the foundation on which advanced surgical and medical techniques are employed. Here, in this review, we highlight the role of targeted therapies in the surgical management of patients with metastatic CRC to the liver.

Keywords: Colorectal cancer (CRC); metastatic colorectal cancer (mCRC); liver; targeted therapies; chemotherapy; surgical management

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Background

Colorectal cancer is the 4th deadliest cancer worldwide (1). The liver, followed by the lung, is the most common site of distant metastatic disease. Indeed, for nearly 1/3 of patients with metastatic colorectal cancer (mCRC), the liver is the only affected visceral organ (2). Approximately 15-25% of patients have synchronous liver metastasis at the time of their initial colorectal cancer diagnosis and 10-25% of patients develop metachronous liver metastasis sometime after curative resection of the primary lesion (3-5). Unfortunately, even when metastatic disease remains limited to the liver, the majority of these metastases are unresectable and the reported rates of successful resection have ranged between 20-30% (6,7). These rates of successful curative resection are relevant mostly from a historical perspective and likely underestimate current surgical practice given the recent advances in systemic therapies. Since the selection and timing of therapeutic agents in patients with mCRC is complex, especially in relation to surgical intervention, each component of the multimodality management of patients with mCRC must be carefully planned to provide the best overall outcomes.

Evolution of systemic chemotherapy for metastatic colorectal cancer

Before surgical advances allowed safe resection of colorectal liver metastases (CRLM), patients were treated primarily with systemic therapies. In fact, over two decades have passed since bolus 5-fluorouracil (5-FU) was the standard of care for patients with mCRC (8-10). Variations in the administration of 5-FU and combinations with agents to modulate its activity [levamisole and leucovorin (LV)] produced incremental improvements in patient outcomes; however, median overall survival (OS) largely remained near 12 months (11-14). A major advance in systemic therapies for mCRC was reported in 2000 when two phase III trials showed that the addition of irinotecan (CPT-11), a DNA topoisomerase I inhibitor, to 5-FU/LV significantly increased overall response rates (ORR), progression-free

survival (PFS), and OS (15-17). In the report by Saltz *et al.*, weekly treatment consisted of irinotecan (125 mg/m^2), bolus 5-FU (500 mg/m^2), and LV (20 mg/m^2) (IFL) (15). In the 2nd trial, Douillard *et al.*, observed improved outcomes using bi-weekly FOLFIRI (irinotecan, 180 mg/m^2; LV, 200 mg/m^2; and bolus 5-FU, 400 mg/m^2 followed by 22 h infusional 5-FU, 600 mg/m^2) (16). These positive studies led to the acceptance of the combination of irinotecan with 5-FU/LV for first-line therapy of mCRC.

During the same period of time that improvements with irinotecan were observed, oxaliplatin, a platinum-based agent that blocks DNA replication, was also tested in combination with 5-FU/LV (FOLFOX) for patients with mCRC (18). In a phase III study reported by de Gramont *et al.*, patients who were administered FOLFOX4 (LV, 200 mg/m^2; 5-FU, 400 mg/m^2 bolus followed by 22 h infusion of 600 mg/m^2; and oxaliplatin, 85 mg/m^2) had improved ORR and prolonged PFS, although increases in OS did not reach statistical significance (19). This study led to the acceptance of FOLFOX as another option for first-line treatment of patients with mCRC.

More recently, the combination of oxaliplatin and irinotecan has also been explored. In a randomized phase III study by Falcone *et al.*, patients received either 48-h infusional 5-FU (3,200 mg/m^2), LV (200 mg/m^2), oxaliplatin (85 mg/m^2), and irinotecan (165 mg/m^2) (FOLFOXIRI) *vs.* FOLFIRI (20). The FOLFOXIRI regimen was associated with significantly increased ORR (66% *vs.* 41%, respectively), PFS (9.8 *vs.* 6.9 months, respectively), and OS (median, 22.6 *vs.* 16.7 months, respectively). Even though FOLFOXIRI was also associated with higher levels of Grade 2/3 toxicities, the FOLFOXIRI regimen has been accepted as another first-line therapeutic option for patients with mCRC.

Emergence of targeted therapies for metastatic colorectal cancer

Although outcomes have improved with advances in systemic chemotherapy for mCRC, potent small molecules and antibodies targeting specific proteins have also been developed over the past decade and have further improved the efficacy of standard chemotherapy regimens. The first of these aptly named "targeted agents" to show benefit as first-line therapy for patients with mCRC was bevacizumab, a recombinant humanized monoclonal IgG_1 antibody targeting vascular endothelial growth factor (VEGF). Hurwitz *et al.* showed that patients with mCRC who received bevacizumab + IFL had significantly better ORR (44.8% *vs.* 34.8%, respectively), PFS (10.6 *vs.* 6.2 months, respectively), and OS (median, 20.3 *vs.* 15.6 months, respectively) compared to IFL alone (21). By virtue of its mechanism of action as an anti-angiogenesis agent, bevacizumab must be used with caution in both medical and surgical patients because of known adverse events including gastrointestinal perforation, hemorrhage, and impaired wound healing (22,23).

The second well-established molecular target in mCRC is epidermal growth factor receptor (EGFR), which is overexpressed in nearly 85% of colorectal cancers (24,25). Cetuximab, a chimeric IgG_1 monoclonal antibody directed against the external surface of EGFR, was first evaluated in combination with chemotherapy in patients who were refractory to irinotecan and also as a single agent in patients intolerant to standard chemotherapy (26-29). These randomized, phase II and phase III trials showed improved PFS without differences in OS (29). More recently, Van Cutsem *et al.*, demonstrated an OS benefit with cetuximab when the cohort was limited to patients with wild-type *KRAS* in their cancers (30). A 2nd EGFR-targeted antibody, panitumumab is a fully humanized IgG_2 monoclonal antibody that was initially approved by the FDA as a third-line treatment for mCRC in 2007 (31). The PRIME trial utilized a combination of panitumumab + FOLFOX4 in patients with wild-type *KRAS* that revealed improved PFS but a non-significant increase in OS compared to FOLFOX4 alone. Currently, panitumumab is FDA-approved for use in patients with refractory mCRC (32). A summary of the major trials demonstrating benefit with standard and targeted agents in mCRC is listed in *Table 1*.

Paradigm shift in surgical resection of colorectal liver metastases

Although contemporary therapeutic regimens have increased the longevity of patients with CRLM, the only option for cure remains complete resection of the metastatic disease. Fortunately, the improvements in medical therapies for mCRC have been concomitant with refinements in surgical and critical care techniques and technologies. Routinely, patients who undergo hepatic resection for CRLM now have 5-year survival rates nearing 40% or higher (35-38). In the past only a fraction of the one-quarter of patients with mCRC limited to the liver were considered for curative surgical options. Much has changed with the advent of more powerful chemotherapy regimens and effective targeted agents. The response rates have increased and patients who in the past would have been considered

Table 1 Phase III trials that have established the benefits of chemotherapy and targeted therapies in metastatic colorectal cancer

Trial	Regimen	Number of patients (N)	Response rate (%)	Median PFS (months)	Median OS (months)
Standard agents					
Petrelli *et al.* [1989] (14)	5-FU *vs.* 5-FU/LV	343	12.1 *vs.* 30.3; P<0.01	--	46 *vs.* 55; P=NS
Saltz *et al.* [2000] (15)	IFL *vs.* 5-FU/LV	683	39 *vs.* 21; P<0.001	7.0 *vs.* 4.3; P=0.004	14.8 *vs.* 12.6; P=0.04
Douillard *et al.* [2000] (16)	FOLFIRI *vs.* 5-FU/LV	387	35 *vs.* 22; P<0.005	6.7 *vs.* 4.4; P<0.001	17.4 *vs.* 14.1; P=0.031
de Gramont *et al.* [2000] (19)	FOLFOX4 *vs.* 5-FU/LV	420	50.7 *vs.* 22.3; P<0.0001	9.0 *vs.* 6.2; P=0.0003	16.2 *vs.* 14.7; P=NS
Falcone *et al.* [2007] (20)	FOLFOXIRI *vs.* FOLFIRI	244	66 *vs.* 41; P<0.0002	9.8 *vs.* 6.9; P=0.0006	22.6 *vs.* 16.7; P=0.032
Targeted agents					
Hurwitz *et al.* [2004] (21)	IFL-bevacizumab *vs.* IFL-placebo	813	44.8 *vs.* 34.8; P=0.004	10.6 *vs.* 6.2; HR=0.54; P<0.001	20.3 *vs.* 15.6; HR=0.66; P<0.001
Van Cutsem *et al.* [2011] (30)	Cetuximab-FOLFIRI *vs.* FOLFIRI	666	57.3 *vs.* 39.7; P<0.001	9.9 *vs.* 8.4; HR=0.696; P=0.0012	23.5 *vs.* 20.0; HR=0.796; P=0.0093
PRIME [2009] (32)	Panitumumab-FOLFOX4 *vs.* FOLFOX4	1183	--	9.6 *vs.* 8.0; HR=.80; P=0.02	23.9 *vs.* 19.7; HR=.83; P=NS
VELOUR [2012] (33)	FOLFIRI-Ziv-aflibercept *vs.* FOLFIRI-placebo	1226	19.8 *vs.* 11.1; P<0.0001	6.9 *vs.* 4.7; HR=0.758; P<0.0001	13.5 *vs.* 12.1; HR=0.817; P=0.0032
CORRECT [2013] (34)	Regorafenib *vs.* placebo	760	--	--	6.4 *vs.* 5.0; HR=0.77; P=0.0052

never resectable are now approached with treatment plans with intent for cure. Since surgical resection represents the only curative option for CRLM, the definition of resectability, the timing of hepatic metastasectomy, the role of maximizing treatment response, and the effect of chemotherapy and targeted agents on surgical outcomes are all key issues that must be addressed.

Consideration of surgery for CRLM mandates a clear and reproducible definition of resectable liver disease. Although the relative criteria for resectability may vary among institutions, the absolute criteria are generally the same. First, the designation that CRLM is resectable must indicate that complete microscopic negative margin resection (i.e., R0) can be achieved with adequate future liver remnant (FLR). Second, absolute contraindications to hepatic resection include current or expected hepatic failure, the presence of unresectable extrahepatic disease, and medical co-morbidities precluding safe surgical intervention. Prior randomized trials have used the following criteria to define unresectable disease: >4 metastases, tumor size >5 cm, bilobar involvement, and involvement of major vascular structures (39,40). However, these outdated criteria have been largely replaced by the goal for R0 resection with appropriate FLR, generally more than 20% in normal livers and >30% in livers with impaired function (41-43). The emphasis on R0 resection is important, because positive resection margins predict an unfavorable prognosis (37). Although a 1-cm margin was traditionally defined as an adequate margin, more recent studies suggest that any negative margin is acceptable (35,44).

The timing of hepatic metastasectomy in patients presenting with primary colorectal cancers and synchronous CRLM is another dilemma. Simultaneous colorectal resection and hepatic metastasectomy may be considered to limit the risks of morbidity and mortality with the 2nd operative procedure. De Haas *et al.*, reported fewer overall complications with simultaneous colorectal resection and liver metastasectomy (11% *vs.* 24%, respectively); but mortality rates were similar when compared to staged resections (45). Other studies have reported similar rates for both morbidity

and mortality with simultaneous resection compared to staged resections (46-48). Despite these results, some centers still support a staged resection, with initial colorectal resection followed by future interval/delayed hepatic resection (35,49,50). The management of metachronous CRLM disease is generally straightforward and involves initial colorectal resection and later resection of CRLM.

Treatment algorithms for patients with CRLM have evolved because of improved response rates with the addition of targeted agents to treatment regimens. Multiple trials have been shown to significantly increase response rates when adding bevacizumab or cetuximab to irinotecan or oxaliplatin backbone regimens (51-54). For example, cetuximab was evaluated in the phase II multi-center CELIM trial. Patients with unresectable CRLM were randomized to receive cetuximab with either FOLFOX6 or FOLFIRI (52). The ORR was 68% in the FOLFOX6 arm and 57% in the FOLFIRI arm (52). R0 liver resection was subsequently performed in 20 of 53 (38%) patients in the cetuximab/FOLFOX6 group and in 16 of 53 (30%) patients in the cetuximab/FOLFIRI group. The increases in ORRs have ranged between 10-30% with corresponding increased rates of hepatic resection of 5-20% when cetuximab was combined with chemotherapy across most studies (29,52,55). Improvements in ORRs and subsequent rates of surgical resection have also been observed with bevacizumab. In the First Bevacizumab Expanded Access Trial (First BEAT), bevacizumab was added to the investigator's choice of fluoropyrimidine-based chemotherapy for patients with CRLM (54). Of 1,914 patients, 225 were able to undergo surgery with curative intent (11.8%). Resection rates were higher in patients receiving oxaliplatin-based chemotherapy (16.1%) than in those receiving irinotecan-based chemotherapy (9.7%). Finally, Falcone *et al.* reported a 66% ORR with FOLFOXIRI alone, whereas response rates with single backbone chemotherapy regimens in most trials were much lower and ORRs have generally increased with the addition of bevacizumab or cetuximab (20,21,51).

Despite great improvements in response rates and resectability with standard and targeted agents, chemotherapy has the potential for liver damage and toxic side-effects that can affect surgical outcomes. Significant decreases in liver function have been described with 5-FU, oxaliplatin, and irinotecan and can contribute to increased perioperative morbidity (43,56). Steatohepatitis, the accumulation of lipids in hepatocytes leading to inflammation and fibrosis, has been associated with irinotecan, while oxaliplatin can cause sinusoidal dilation, perisinusoidal fibrosis, and occlusion of venules (56-58). To offset the effects of chemotherapy-associated liver injury, a delay period from the last dose of chemotherapy to resection of CRLM is required. The National Comprehensive Cancer Network (NCCN) recommends waiting one month from the last dose of chemotherapy to surgery (59). A time interval of at least 4-6 weeks after the last dose of chemotherapy is also supported by major trials (52,54,60). Interestingly, while sinusoidal injury resulting in the "blue liver" syndrome has been attributed to oxaliplatin, bevacizumab may have a protective effect by decreasing the severity of sinusoidal obstruction and damage (61). Bevacizumab has also been associated with non-liver adverse effects such as impaired wound healing and increased risk of intestinal perforation due to its anti-angiogenesis properties (23,62,63). For surgical patients who have received bevacizumab, the NCCN recommends wait-times of approximately 4-6 weeks after the last bevacizumab dose before surgery (59). For the anti-EGFR agents cetuximab and panitumumab, no specific liver toxicity, wound healing, or other adverse effect which impact surgical care has been reported; hence, the necessary wait period is similar to that for non-targeted agents (64,65).

Preoperative treatment strategies

Patients with CRLM may present in a number of different manners. Common presentations include: (I) unresectable disease; (II) borderline resectable disease; and (III) resectable disease. The role of systemic agents and targeted therapies may be different in each of these conditions (see *Figure 1*). For patients with CRLM who are initially declared unresectable, therapies may be given to optimize shrinkage of the tumor to convert initially unresectable to resectable disease. This so called "conversion" therapy may be similar to standard chemotherapy regimens when patients are considered never resectable. For patients undergoing treatment for initially unresectable CRLM, the close involvement of the surgical team is essential. Patients should be reevaluated for possible surgical resection after two months of therapy and every two months thereafter if treatment is continued.

Neoadjuvant therapy is the administration of therapy to patients who have CRLM that is considered resectable at time of diagnosis. Advantages to neoadjuvant chemotherapy include decreasing the size of the CRLM to allow less extensive liver resection and greater likelihood of margin negative resection and evaluating disease biology during treatment. Furthermore, chemosensitivity and responsiveness can be determined by evaluating treatment response. Perioperative therapy (i.e., preoperative and

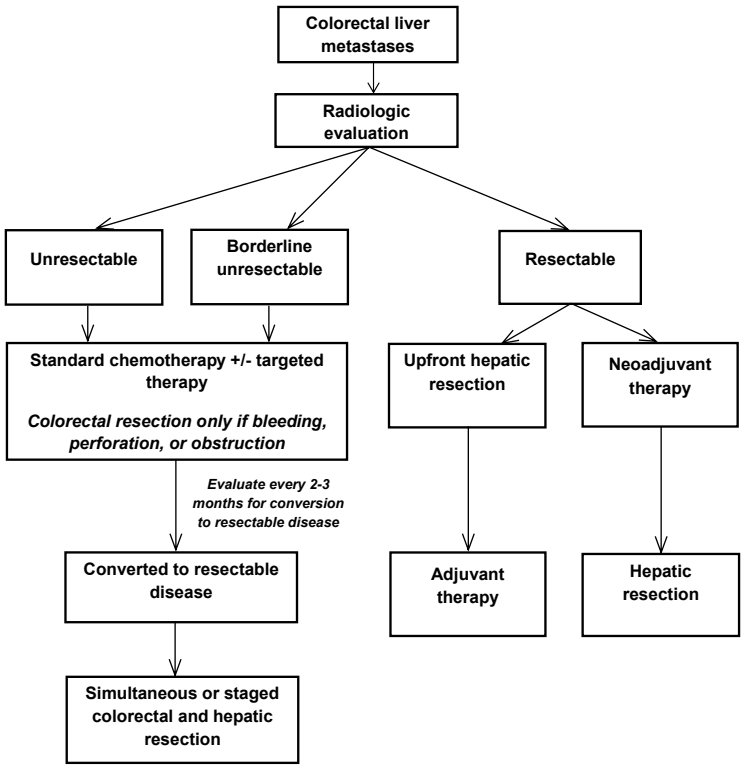

Figure 1 Summary of treatment recommendations in potential surgical patients with metastatic colorectal cancer

postoperative) with standard regimens was tested in the EORTC 40983 trial, which evaluated the role of chemotherapy in patients with resectable CRLM. Increased PFS was observed in the perioperative FOLFOX4 arm compared to surgery alone (66), however, follow-up survival analysis did not demonstrate significant differences in OS between the two treatment arms (67).

Adjuvant chemotherapy and targeted agents

After resection of liver metastases, up to 70% of patients may develop recurrence of disease either in the liver or in extra-hepatic locations, thus providing rationale for postoperative or adjuvant chemotherapy (68). However, data for systemic therapies in this setting is severely lacking. If data from patients with stage III disease were extrapolated to stage IV patients, then chemotherapy regimens would be recommended since recurrence was lower and OS was higher with adjuvant chemotherapy. However, neither bevacizumab nor cetuximab in the adjuvant setting provided survival benefits when combined with chemotherapy in stage III trials (69,70). Regardless, it may not be reasonable to compare complete resection of disease in stage III patients who have locoregional disease with stage IV patients who have distant metastatic disease. Currently, no Level 1 recommendation based on a randomized trial can be made regarding adjuvant targeted therapy after resection of CRLM. Nevertheless, most patients will receive some form of adjuvant therapy given the improved outcomes with standard and targeted therapies in patients with mCRC.

Management of the primary tumor

The management of the primary tumor is a topic of controversy in patients with unresectable mCRC. The current strategy is to leave the primary cancer in place unless there are complications that include bleeding, obstruction, or perforation. This strategy is based upon the observation that patients receiving chemotherapy or targeted agents do not have increased rates of complications or emergent resections (NSABP C-10) (71). However, a recent retrospective analysis suggested a potential survival benefit with resection of the primary tumor when mCRC was unresectable (72). More work is needed to clarify the most appropriate management of the primary tumor in patients with unresectable mCRC.

The future is now: novel targeted agents

Ziv-aflibercept and regorafenib are two newly approved targeted agents for mCRC. Ziv-aflibercept is a soluble recombinant protein that acts as a "trap" for multiple angiogenic factors (73). This protein interferes with angiogenesis by binding to VEGF-A, VEGF-B, and placental growth factor (PlGF), thus "trapping" these growth factors and preventing binding to and activation of VEGF receptors, thereby interfering with angiogenesis. In the phase III randomized, double-blind, multi-national VELOUR trial, patients with mCRC previously treated with oxaliplatin were randomized to receive ziv-aflibercept or placebo every two weeks in combination with FOLFIRI (33) with the primary endpoint of OS. At a median follow-up time of 22.3 months, patients receiving ziv-aflibercept had significant increases in both OS (median, 13.5 *vs.* 12.1 mos, respectively) and ORR (19.8% *vs.* 11.1%, respectively) when compared to placebo. Thus, ziv-aflibercept is now FDA approved for second-line use in combination with FOLFIRI or irinotecan in patients with disease progression on oxaliplatin. There are no studies in surgical patients as of yet.

Another oral agent, regorafenib, has also been investigated in the treatment of mCRC. Regorafenib inhibits multiple tyrosine kinases and possesses anti-angiogenic properties, specifically targeting VEGFR1-3, the angiopoietin receptor TIE2, RAF, PDGFR, fibroblast growth factor receptor (FGFR), as well as KIT and RET (74,75). In the multi-national phase III CORRECT trial, patients with mCRC who had progressed on standard therapy were randomized to regorafenib or best supportive therapy with a primary endpoint of OS. Patients who received regorafenib had improved OS (median, 6.4 *vs.* 5 mos, respectively) (34). Therefore, regorafenib is now indicated as a single agent in patients with mCRC refractory to chemotherapy. Currently there is no data in surgical patients; therefore, retrospective reports and prospective trials will help determine the role and safety of these agents in surgical patients with CRLM.

Summary

Great advances have been made in the management of patients with mCRC in the past three decades. Without treatment, patients with CRLM had a life expectancy of 4.5-12 months (76,77). The prognosis of patients with metastatic colorectal cancer of the liver has improved significantly over the past decade. Surgical resection of CRLM is still considered the only curative option and advances in surgical techniques and technology have increased the rates of patients with CRLM who may undergo hepatic resection. However, the management of CRLM mandates a multi-disciplinary effort because of the complexity of liver surgery and the tremendous advances in targeted therapies.

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Footnote

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Update on antiangiogenic therapy in colorectal cancer: aflibercept and regorafenib

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Abstract: Angiogenesis plays an important role in colorectal carcinogenesis and approaches targeting the vascular growth factor receptor (VEGF) signaling such as bevacizumab yielded significant survival improvement for metastatic colorectal cancer patients. Recent evidence demonstrated the benefit of continuing angiogenic suppression after first-progression following bevacizumab-containing cytotoxic regimen though no benefit was observed with the use of bevacizumab in adjuvant setting. Aflibercept, a soluble fusion protein with high affinity for VEGF-A, -B and PlGF, administered in combination with irinotecan-containing regimen improved the survival of metastatic colorectal cancer patients in second-line setting (VELOUR trial). Regorafenib, a small molecule multikinase inhibitor against various pro-angiogenic and –proliferation targets, improved the survival of metastatic colorectal cancer patients who had progressed on all standard therapy. These developments had renewed enthusiasm in the field and the role of aflibercept and regorafenib in other treatment settings will continue to be defined by on-going and future clinical trials. As other anti-angiogenic approaches are being tested clinically, other novel non-angiogenic targets deserve to be evaluated in our effort to improve the outcome of colorectal cancer patients.

Keywords: Antiangiogenic therapy; colorectal cancer; aflibercept; regorafenib; vascular growth factor receptor (VEGF)

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Background

Colorectal cancer is a major cause of morbidity and mortality throughout the world. It is the third most common cancer diagnosis worldwide and affects men and women equally (1). In the United States, colorectal cancer accounted for 9% of all cancer mortality in 2012 (2). The survival of patients with metastatic colorectal cancer (mCRC) has markedly improved since the 1990s when 5-fluorouracil (5FU) based chemotherapy achieved an overall survival (OS) of 12 months. The addition of oxaliplatin and Irinotecan increased the OS to approximately 18 months (3-6). The survival was further augmented with anti-angiogenic agents and bevacizumab, in combination with chemotherapy, was the first of the drug class to receive regulatory approval for use in mCRC therapy (7,8). Recently, 2 other anti-angiogenic drugs, aflibercept and regorafenib, were found to improve the survival of mCRC patients in randomized trials which further reiterates the importance of targeting angiogenesis in CRC therapy (9,10). This article will review the development of aflibercept and regorafenib and their current role in the treatment of colorectal cancer (*Table 1*).

Tumor angiogenesis and VEGF signaling pathway

Angiogenesis refers to a multi-step process leading to the formation of new blood vessels to supply nutrients and oxygen to the tissues (11). The process begins with vasodilatation, increased vessel permeability, stromal degradation and endothelial cell proliferation and migration, resulting in the formation of a new or extended capillary (12). Whilst angiogenesis is ordered and occur only during wound repair, tissue remodeling or inflammation under normal physiologic conditions, the process is chaotic in neoplasms resulting in leaky, tortuous

Table 1 Compare bevacizumab, afibercept and regorafenib

	Bevacizumab	Aflibercept	Regorafenib
Classification	Recombinant humanized Monoclonal antibody	Soluble fusion protein contains domains from VEGFR-1 and VEGFR-2	Small molecule multikinase inhibitor
Targets	VEGF-A	VEGF-A, VEGF-B and PIGF	VEGFR-1, -3, RAF, TIE-2, and mutant oncogenic kinases KIT, RET and BRAF
Molecular weight	149 kD	115 kD	500.83 D
Doses in colorectal cancer	5-10 mg/m^2 IV every 2 weeks in combination with FOLFOX and FOLFIRI	4 mg/kg IV every 2 weeks in combination with FOLFIRI	160 mg oral daily for 21 days of a every 28 days cycle
Common and clinically significant side effects	Hypertension, Proteinuria, Thrombosis, Hemorrahge, delay wound healing, GI perforation (rare)	Hypertension, Proteinuria, GI perforation (rare), delay wound healing, Hemorrhage	Hypertension, Fatigue, Hand-foot syndrome, Hepatotoxicity, GI perforation (rare), Hemorrhage, Reversible Posterior leukoencephlopathy syndrome

and inefficient vessels (13-15).

The VEGF/VEGFR signaling is a well studied pro-angiogenic pathway and the ligands include VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF) that interact with membrane bound tyrosine kinase receptors VEGFR-1 (FLT-1), VEGFR-2 (FLK-1/KDR) and VEGFR-3 (FLT4); and other co-receptors include neurophilin (NRP)-1 and NRP-2 (16-18). The binding of VEGF-A (or VEGF) to VEGFR-2 had been found to be key mediator of angiogenesis (17). VEGF-A (commonly known as VEGF) is expressed in many human cancers and binding with VEGFR-2 in tumor microenvironment triggers a number of intracellular signaling cascades in endothelial cells leading to formation and enhancement of tumor microvasculature (18,19).

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biologic activity of VEGF by preventing its binding to VEGFR-1 and VEGFR-2 (*Figure 1*). The therapeutic role of bevacizumab in treating metastatic CRC patients is well established and supported by well-conducted randomized trials (7,8,20-22). These topics had been well reviewed in the literature and we refer readers to those articles (23,24). Recently, the benefit of continuing angiogenetic suppression beyond first disease progression in mCRC patients was confirmed recently by the ML18147 study. In this randomized phase III trial, bevacizumab beyond disease progression while switching the cytotoxic chemotherapy improved the PFS (5.7 *vs.* 4.1 months) and OS (11.2 *vs.* 9.8 months) in the group that continued bevacizumab compared to those who didn't (25).

Despite benefit in metastatic setting, the addition of bevacizumab had not improved clinical outcome in adjuvant setting in CRC (26,27). The AVANT trial randomized curatively resected stage III or high risk stage II colon cancer to 3 arms: FOLFOX4 for 12 cycles, bevacizumab 5 mg/kg plus FOLFOX4 for 12 cycles or bevacizumab 7.5 mg/kg plus oxaliplatin and capecitabine (XELOX); both bevacizumab arm will receive additional bevacizumab 7.5 mg/kg monotherapy every 3 weeks for eight cycles after completing combination therapy. The hazard ratio (HR) for disease-free survival (DFS) and OS for bevacizumab-FOLFOX4 versus FOLFOX4 were 1.17 (95% CI: 0.98-1.39; P=0.07) and 1.27 (95% CI: 1.03-1.57; P=0.02) respectively; and for bevacizumab-XELOX versus FOLFOX4 was 1.07 (95% CI: 0.9-1.28; P=0.44) and 1.15 (95% CI: 0.93-1.42; P=0.21) respectively (27). In summary, in the AVANT trial, the addition of bevacizumab did not improve DFS including subset analysis according to baseline VEGF-A or VEGFR-1 or 2 levels. Interestingly, the data suggested potential detrimental effect in the bevacizumab-containing arms from more relapses and deaths due to disease progression (27). One hypothesis proposed to explain the failure of bevacizumab in adjuvant setting was that established CRC metastatic tumors were more dependent on angiogenesis than micrometastases, which were more

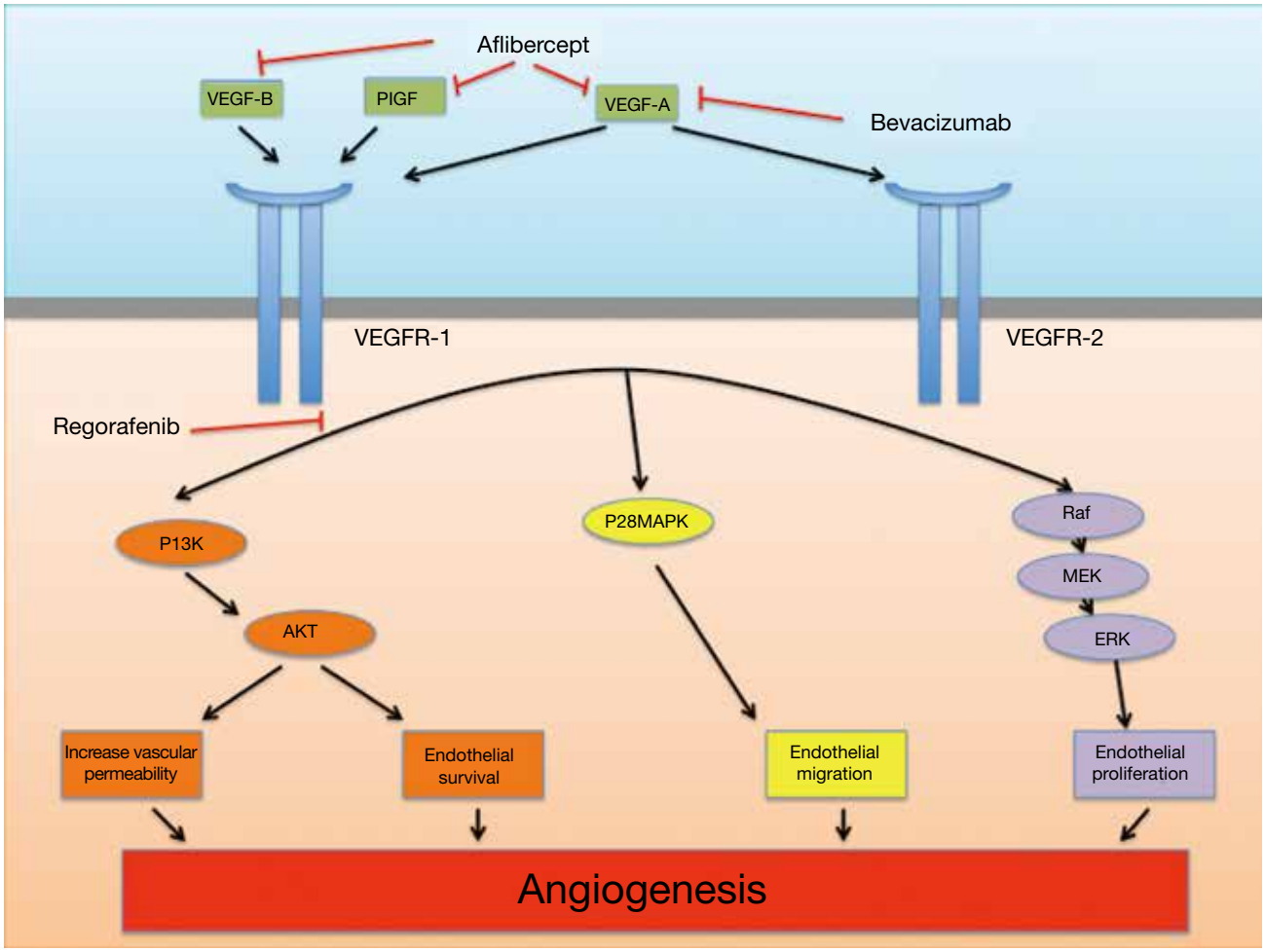

Figure 1 Pro-angiogenic targets of bevacizumab, aflibercept and regorafenib. Bevacizumab binds to VEGF-A and interrupts the interaction with VEGFR-1 and -2. In addition to VEGF-1, aflibercept binds to and interrupts the function of VEGF-B and PlGF. Regorafenib is a small molecule multi-kinase inhibitor which targets include VEGFR-1, -3, RAF, TIE-2, and mutant oncogenic kinases KIT, RET and BRAF

sensitive to cytotoxic chemotherapy (28,29).

Aflibercept

Aflibercept (or VEGF Trap) is a recombinant fusion protein consisting of the extracellular domains of human VEGFR-1 and 2 fused to the Fc portion of human IgG1 (30). The decoy protein binds to VEGF-A, VEGF-B and PIGF and prevents the activation of VEGFR-1 and VEGFR-2 by these ligands, in contrast to bevacizumab in which binds VEGF-A only (*Figure 1*). VEGF-A is a key regulator of tumor angiogenesis and most human malignancies express high VEGF-A level (14,17). PIGF also plays an important role in angiogenesis by enhancing VEGF-A expression (31). Furthermore, patients with metastatic renal cell cancer previously treated with anti-VEGF therapy had increased PIGF level suggesting that PIGF may play a role in resistance to anti-VEGF treatment (32,33). In addition, compared to bevacizumab, aflibercept has a higher affinity for VEGF-A and its native receptor (34). Preclinically, aflibercept inhibited tumor growth, angiogenesis, metastases and improved the survival of tumor-bearing mice for various cancer types including pancreas, ovarian and renal cell carcinoma (30). Aflibercept in combination with cytotoxic drugs (Irinotecan, 5FU, paclitaxel, docetaxel), transtuzumab or radiotherapy exerted greater inhibition of tumor vasculature and growth than aflibercept alone in tumor xenograft models (35-40).

In the phase I trial, 47 patients with refractory solid tumors or non-Hodgkin's lymphoma were enrolled to receive aflibercept intravenously every 2 weeks at doses ranging from 0.3 to 7.0 mg/kg (41). Dose-limiting toxicities

(DLT) were rectal ulceration and proteinuria at 7.0 mg/kg dose. Aflibercept was also evaluated in combination with various chemotherapeutic agents including FOLFOX4 (42,43), irinotecan with 5FU and leucoverin (44), docetaxel (45) alone and with cisplatin (46), and gemcitabine (47) in advanced solid tumors patients. In combination with FOLFOX4, aflibercept doses 2, 4 and 5 mg/kg were explored in patients with advanced solid tumors and no DLT was encountered in the phase I trial (42). Grade 3 or worse toxicities included neutropenia, thrombocytopenia, hypertension, proteinuria, hemorrhagic events (include 1 Grade 5 hemorrhagic stroke at 4 mg/kg), febrile neutropenia and deep vein thrombosis. In subset of mCRC, partial response was observed.

Aflibercept was also evaluated in combination with irinotecan, 5FU and leucovorin in a dose-escalation study. Aflibercept doses 2, 4, 5 and 6 mg/kg doses every 2 weeks were explored and DLTs observed were Grade 3 proteinuria lasting >2 weeks, acute nephrotic syndrome and thrombotic microangiopathy at 4 mg/kg; Grade 3 stomatitis, esophagitis reflux at 5 mg/kg; and, febrile neutropenia, Grade 3 stomatitis and Grade 3 abdominal pain due to intestinal obstruction at 6 mg/kg (44). As such, aflibercept 4 mg/kg dose level was selected as for further development in combination with irinotecan, 5-FU and leucovorin (41,42,44). The pharmacokinetic studies showed that aflibercept's elimination half-life ranged from less than 1-3 days for free aflibercept and was approximately 18 days for VEGF-bound aflibercept (41,48).

The benefit of aflibercept in combination with FOLFIRI was confirmed in the pivotal phase III VELOUR trial. In the study, patients with metastatic CRC previously treated with oxaliplatin-containing regimen, irregardless of prior bevacizumab treatment, were randomly assigned to received aflibercept 4 mg/kg IV every 2 weeks or placebo combination with FOLFIRI. Overall response rate was 19.8% in the aflibercept arm compared to 11.1% in the placebo (P=0.0001). Compared to the control group, the aflibercept-containing arm had better PFS (6.9 *vs.* 4.67 months; HR 0.758; P<0.0001) and OS (13.5 *vs.* 12.06 months; HR 0.817; P=0.0032). Pre-planned subgroup analysis showed that prior bevacizumab use did not influence aflibercept's effect on PFS and OS though the study was not powered to show a treatment difference between arms (9,18). Toxicities related to aflibercept were consistent with those expected from the anti-VEGF drug class (49). When compared to the bevacizumab-related toxicity profile reported in the phase III trial of IFL with or without bevacizumab, the frequency of grade 3 or 4 proteinuria seemed to be higher for aflibercept than bevacizumab (7.5% *vs.* 0.8%) though risks for Grade 3 or 4 bleeding (2.8% *vs.* 3.1%) and hypertension (11% *vs.* 11%) seemed similar (9,21).

Together with the results from ML18147 study, clinicians now have the option of using aflibercept or bevacizumab with FOLFIRI in mCRC patients who progressed following oxaliplatin containing regimen. The benefit achieved by aflibercept and bevacizumab in second-line setting seemed comparable: in ML18147 study, continuing bevacizumab into second-line while switching the cytotoxic chemotherapy achieved a median OS improvement of 1.4 months (HR 0.81, 95% CI: 0.69-0.94; P=0.0062) (25) whilst the addition of aflibercept to FOLFIRI in the VELOUR trial achieved a comparable median OS survival improvement of 1.4 months (HR 0.817, 95.34% CI: 0.713-0.937; P=0.0032) (9). The frequency of vascular-related adverse events seemed to be higher with aflibercept than bevacizumab treatment when comparing across trials. Cost is another consideration: aflibercept treatment costs, in average, $11,063 per month, which is more than twice as high as bevacizumab therapy. As such, aflibercept is not recommended routinely in metastatic CRC patients who progressed on oxaliplatin-containing treatment until more evidence available.

Regorafenib

Regorafenib is structurally related to sorafenib and differ from the latter by the presence of a fluorine atom in the center phenyl ring (50,51). The slight structural difference resulted in higher inhibitory potency against various pro-angiogenic receptors than sorafenib including VEGFR2 (IC50 3 *vs.* 90 nM respectively), FGFR1 (202 *vs.* 580 nM) though IC50s for PDGFRβ were similar (52,53). Other receptor kinases inhibited by regorafenib include VEGFR1, -3, RAF, TIE2, and mutant oncogenic kinases KIT, RET and BRAF (52,54). Interestingly, sorafenib did not demonstrate significant anti-tumor activity in CRC. The effect of sorafenib plus 5-FU in colorectal tumor xenograft strudy was not significantly better than treatment using either drugs alone (55). Two of the 66 refractory mCRC patient who received sorafenib in four phase I had best response as stable disease and no objective response was observed (56). In contrast, regorafenib showed significant anti-cancer efficacy in CRC. In preclinical colorectal tumor xenograft studies, regorafenib treatment reduced tumor microvasculature and inhibited tumor growth in a dose-dependent manner (57). N-Oxide (M-2) and N-Oxide/N-desmethyl metabolite (M-5) are 2 active metabolites of regorafenib with potent pharmacologic activities similar to

but distinct from regorafenib (57).

In the phase I trial, 53 patients with advanced solid tumor received regorafenib at the dose levels from 10 to 220 mg daily, 21 days on followed by 7 days off in repeating cycle. The most frequent adverse events were voice changes, hand-foot skin reaction, mucositis, diarrhea and hypertension. DLTs at 160 mg were skin toxicity and vomiting; skin toxicity, abdominal pain and asthma at 220 mg. On the basis of these observations, 160 mg once daily orally was determined the maximum tolerated dose (MTD) and the recommended dose for future studies. For efficacy, one mCRC patient had partial response at 220 mg but stopped treatment after 5.3 months for treatment-related side effects (58). Pharmacokinetic studies showed that terminal half-life of regorafenib were 20-40 hours, thus supporting once daily dosing schedule. At the 160 mg dose, plasma exposure at steady state of M-2 and M-5 were similar to or slightly greater than parent drug. The terminal half-life of M2 was comparable to regorafenib but the elimination of M-5 was slower with an estimated half-life of 51-64 hours (58,59). The unbound plasma concentration of the pharmacologically active species at the 160 mg dose level exceeded the IC50 of many target kinases, therefore, plausible that M-2 and M-5 may contribute to the clinical activity of regorafenib (58).

In an expanded phase I study specific for relapsed or refractory mCRC patients, 38 patients received regorarefnib dose levels ranging from 60-220 mg daily administered on a "21 days on followed by 7 days off" dosing schedule. Enrolled patients had received a median of 4 previous lines of treatment. The most common adverse event leading to dose reduction was hand-foot skin reaction. Other treatment-related adverse events leading to regorafenib discontinuation included hypertension, fatigue, thrombocytopenia and diarrhea. Among 25 patients treated at 160 mg dose level, 6 patients permanently discontinued due to treatment-related adverse events including hand-foot skin reaction, hypertension, fatigue, thrombocytopenia and duodenal ulcer. In efficacy evaluation, 27 evaluable patients achieved 74% disease control rate with partial response in 1 patient (4%) and stable disease in 19 patients (70%). Overall, regorafenib was well tolerated and adverse events were manageable (59).

The multi-national phase III CORRECT trial enrolled mCRC patients who had received all locally-approved standard therapies and had progressed during or within 3 months after the last standard therapy (10). Patients were randomized in a 2:1 ratio to receive regorafenib or placebo. 500 patients received regorafenib at 160 mg orally 21 days on 7 days off and 253 patients received placebo. Median OS was 6.4 months in the regorafenib group versus 5.0 months in the placebo group (HR 0.77; 95% CI: 0.64-0.94; one-sided P=0.0052). Similar clinical benefit was observed in patient with colon cancer and rectal. The most common treatment-related Grade 3 or worse adverse events were hand-foot skin reaction (17%), fatigue (10%), diarrhea (7%), hypertension (7%), and rash or skin desquamation (6%), consistent with that observed in earlier phase trials. These adverse events were mostly manageable with dose reduction or interruption.

Conclusions

Angiogenesis is now a validated therapeutic target in CRC patients with macroscopic metastases. Recent development added 2 new anti-angiogenic drugs to the CRC treatment armamentarium and confirmed the advantage of continuing angiogenic suppression beyond first progression in metastatic CRC patients (60). Evidence so far supports the use of bevacizumab in both first- and second-line treatment of metastatic CRC patients. In comparison, the role of aflibercept in these settings remains unclear given the comparable efficacy but higher cost compared to bevacizumab. Aflibercept targets a broader set of pro-angiogenic growth factors than bevacizumab, and has the theoretical advantage of more effective angiogenic suppression and overcoming bevacizumab resistance. However, these hypotheses are yet to be confirmed in clinical studies. As the chemotherapeutic options and supportive care improve, more metastatic CRC patients nowadays have good performance status by the time they exhausted all standard therapy. For them, regorafenib is a welcomed option in addition to participation in clinical trials. Looking back, the overall survival of patients with metastatic CRC has increased several folds when compared to decades ago even though, it seemed, each drug achieved only incremental improvement individually. However, it is clear more novel treatment approaches are needed to continue this trend.

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Footnote

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

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Impact of the immune system and immunotherapy in colorectal cancer

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Abstract: The development of cancer is a multi-step process involving the gradual loss of regulation over the growth and functional capabilities of normal cells. Much research has been focused on the numerous cell intrinsic factors that govern this process; however, recent attention has turned to understanding the cell extrinsic factors in the tumor microenvironment that appear equally critical to the progression and treatment of cancer. One critical component of the tumor microenvironment is the immune system and it has become increasingly evident that the immune system plays an integral role in preventing and promoting the development of cancer. Understanding the immune cell types and pathways involved in this process has enabled the development of novel biomarkers for prognosis and accelerated the development of immune-based therapeutics, both of which have the potential to forever change the treatment paradigms for colorectal cancer (CRC). In this review, we discuss the impact of the immune system on the initiation, progression and treatment of cancer, specifically focusing on CRC.

Keywords: Immune system; immunotherapy; inflammation; colorectal cancer (CRC)

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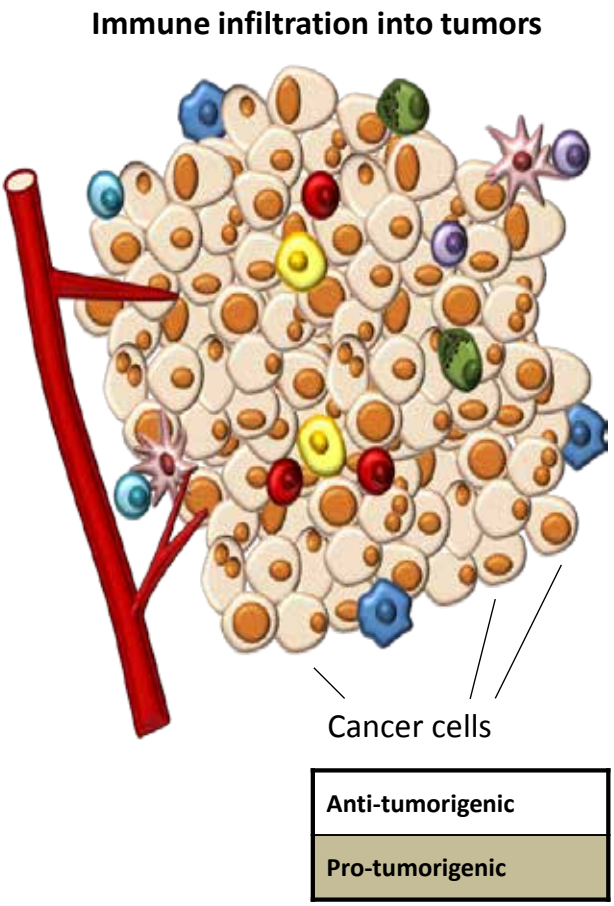
Introduction

The immune system has a complex and multi-faceted role in cancer, affecting all aspects of the disease from tumorigenesis to treatment. Immune cells can act both as suppressors of tumor initiation and progression, as well as promoters of proliferation, infiltration, and metastasis. Within the tumor microenvironment, various immune cells have been described in virtually all tumor types with the exact composition of immune cells depending on the tumor origin, location, and individual characteristics of the patient. Both innate immune cells [macrophages, mast cells, neutrophils, dendritic cells (DCs), myeloid derived suppressor cells, and natural killer (NK) cells] and adaptive immune cells (T and B lymphocytes) are present and interact with the tumor via direct contact or through chemokine and cytokine signaling which shapes the behavior of the tumor and its response to therapy (*Figure 1*). Increased understanding of the immune microenvironment of tumors has allowed for an explosion of the identification of novel immune-based biomarkers and the development of new agents that target immune pathways for therapy. This review is aimed at outlining the numerous roles that the immune system plays in cancer and the treatments that take advantage of our growing understanding of the immune system, with a particular emphasis on colorectal cancer (CRC).

Anti-tumor immune responses

The immune responses to tumors share a number of similarities with the host immune response to infections and foreign antigens. Innate immune cells such as NK cells, macrophages and DCs can respond to both microbe-associated molecular patterns or to inflammatory signals generated by damaged tissues. Recognition by innate immune cells initiates an inflammatory cascade that leads to antigen presentation by DCs and macrophages to T cells, activating an adaptive immune response (1). More specifically, in cancer, the innate immune system recognizes tumor specific antigens on the surface of cancer cells in a manner similar to the recognition of non-self-pathogens.



Immune Cell		Roles in cancer
	Dendritic cell (DC)	Release cytotoxic cytokines Antigen presentation to T cells
		Suppress T cell functions Promote tumor growth and progression
	T cell (CD8+, CD4+)	Directly lyse cancer cells Release cytotoxic cytokines
		Release tumor promoting cytokines
	Treg	Restore homeostasis to reduce chronic inflammation
		Suppress anticancer immune responses Stimulate inflammatory cytokine production
	Macrophage	Release cytotoxic cytokines Antigen presentation to T cells
		Promote angiogenesis, tumor proliferation, chemotaxis, invasiveness, and metastasis
	Myeloid derived suppressor cell (MDSC)	Limited
		Suppress T cell functions Recruit immunosuppressive immune cells
	NK cell	Release cytotoxic cytokines Directly cytotoxic to cancer cells
		Limited

Figure 1 Diagram of key immune cells found in the tumor immune microenvironment. Multiple different immune cells can be found in tumors at different times and locations depending on multiple host and tumor factors. Many immune cells have both anti- and pro-tumor functions based on the context in which they are found.

Innate immune cells, such as NK cells, recognize the lack of MHC-I surface molecules on cancer, engage in active killing of these cells and then recruit other inflammatory cells through their cytokine production (2). Recruited monocytes, namely macrophages and DCs, phagocytose tumor cells and then present tumor-associated antigen on their surface (3) which activates a specific cytolytic T cell response that is directed against the tumor. Then, like a pathogen induced immune response, these specific effector T cells clonally expand and travel to the tumor to eradicate it from the body (4). However, just like microbes that cannot be controlled or are chronic, cancer cells undergo a selection process for cells that have the ability to evade the immune system by acquiring several key properties including decreased immunogenicity, expression of a highly immunosuppressive microenvironment, and the ability to stimulate a supportive immune microenvironment rich in factors that support nutrient acquisition, angiogenesis and matrix remodeling (1). We discuss in more detail these properties of tumors that allow them to evade the anti-tumor immune response in the following sections.

Immunosurveillance

A number of immune cells have the ability to directly and indirectly kill cancer cells. In fact, it has been found that immune cells patrol the body monitoring for the altered cells that become cancer in a process known as

"immunosurveillance". Through immunosurveillance, the body can effectively recognize and eliminate cancerous cells prior to them causing harm (5). Evidence that immunosurveillance plays a critical role controlling the development of cancer comes from patients who have been immunosuppressed such as transplant recipients or patients with advanced HIV infection who have a higher risk of a number of cancers, including colon and pancreatic cancer, compared to normal, uninfected individuals (6,7). One of the key cell types involved in immunosurveillance is NK cells which can cause direct cytotoxicity of cancer cells, which frequently do not express any MHC-I class alleles, making them susceptible to NK killing (8), as well as release cytotoxic granules containing perforin and granzyme B (9). Other immune cells are also involved in the killing of cancer cells, but have more complex roles in which they have also been described to promote tumor growth, depending on the context (10). These other anti-tumor immune cells include but are not limited to, $CD8^+$ T cells with can directly lyse cancer cells and produce cytokines that promote a cytotoxic response such as interferon gamma (IFN-γ) (11), $CD4^+$ Th1 cells which can stimulate production and function of cytotoxic T lymphocytes (CTLs) and produce toxic cytokines including IFN-γ and interleukin 4 (IL-4) (12), $CD4^+$ Th17 cells which activate CTLs (13,14), $CD4^+$ regulatory T (Treg) cells which can suppress chronic inflammation (15), and neutrophils which are involved in direct cytotoxicity and regulation of CTL responses (14,16). Macrophages and DCs can also participate in the production of an anti-tumor immune response through their ability to present tumor antigens to T cells and through their response to danger and stress signals which causes the release of critical cytotoxic cytokines (17-19). These anti-tumor immune cells have been used as both prognostic markers with more anti-tumor immune responses correlating with better outcomes as well as targets for immunotherapy in which groups have sought ways to augment the anti-tumor response.

There is increasing evidence suggesting that immune cells play an important role in regulating the development of tumors in CRC. In one example, a study of 49 fresh CRC tumor samples ranging from stages II to IV found that the higher the number of activated ($CD69^+$) and cytotoxic ($CD107a^+$) $CD8^+$ tumor infiltrating T lymphocytes (TILs), the higher the number of tumor antigen-reactive T cells in the blood and bone marrow. Further, the number of these activated cells inversely correlated with overall stage of the tumor. More specifically, earlier tumor stages showed higher proportions of activated $CD8^+$ TILs. This suggests that early stage CRC may be recognized and undergo surveillance by the immune system (20).

Immunoediting and immune deficiencies in cancer

As immune cells search and destroy pre-cancerous cells, they select for tumor cells that display decreased tumor immunogenicity in a process known as immunoediting. This reciprocal relationship that immune cells have with cancer cells is defined by the "three Es of cancer immunoediting": elimination, equilibrium, and escape (21,22). As a tumor takes root, the immune cells are gradually unable to eliminate all cancer cells but still can prevent expansion and metastasis, keeping the tumor at bay and producing a static phase known as the equilibrium phase. Over time, the dynamic interaction between the tumor and the immune system eventually results in a selection for tumor cells that can now escape the immune system leading to the development of clinically apparent tumors. Evidence for this sequence of events is supported by mouse tumor transplant data. Tumors transferred from immunodeficient mice into wild type mice can be more immunogenic than those arising from wild type mice because the tumor cells are "unedited" and do not undergo a selection process for the less immunogenic cells (23). Additionally, studies have shown that tumors arising in mice with specific immune deficiencies, including IFN-γ (24,25) and NKT cells (26), can be eliminated when transplanted into immune competent mice but grow more aggressively when transplanted into mice with the same immunodeficient genetic background (5). Additional mouse models of various types of cancer have shown that deficiencies in $CD8^+$ CTLs, $CD4^+$ T helper 1 (T_h1) cells, or NK cells all lead to an increase in tumor incidence (27). In CRC, a study of 286 CRC tissue samples revealed that node-negative CRC had an increasing percentage of $CD3^+$ immunoreactive areas which reduced the risk of metachronous tumors. However, in node-positive patients, $CD3^+$ density was no longer predictive, suggesting the importance of immune evasion in CRC (28).

Pro-tumor immune responses

Suppressing the anti-tumor immune response in cancer

In addition to evading recognition by the immune system, recent experimental evidence supports the

notion that tumors establish a microenvironment that actively suppresses an immune response. The first suppression mechanism utilized by tumors is the release of immunosuppressive factors such as TGF-β from cancer cells themselves to prevent CTLs and NK cells from destroying the tumor. The second mechanism involves the recruitment of immunosuppressive immune cells, such as Tregs and $CD11b^+Gr1^+$ myeloid-derived suppressor cells (MDSCs, as defined in mice) by cancer cells to evade lymphocyte-induced death (27). Tregs suppress the proliferation, cytokine expression, and activation of other T cells including $CD4^+$ and $CD8^+$ T cells and larger numbers of intratumoral Tregs has been correlated in numerous tumors types to poorer prognosis (29). MDSCs are a heterogeneous population of myeloid-derived cells defined as $Lin^-HLA^-DR^-CD33^+$ or $CD11b^+CD14^-CD33^+$ in humans and consists of myeloid progenitors, immature macrophages, immature granulocytes, and immature DCs. MDSCs produce factors, such as arginase-1, which are potent suppressors of various T cell functions and thus suppress anti-tumor activities (30).

In colon cancer, a study of 64 CRC patients revealed that CRC patients had markedly increased percentages and absolute numbers of MDSCs [Lin(-/low)HLA-$DR^-CD11b^+CD33^+$] in their peripheral blood when compared with healthy individuals. This increase correlated with clinical cancer stage and tumor metastasis, though not primary tumor size. A similar increase of MDSCs was also seen in the tumor tissue when compared to matched paraneoplastic tissue. Finally, *in vitro* studies revealed that only MDSCs from CRC patients, but not healthy donors, were able to inhibit autologous T cell proliferation (31).

The case of $FoxP3^+$ Tregs is much more complex and varies by tumor type, stage and tissue of origin. Knowing that Tregs suppress an immune response, one would expect that they would be a poor prognostic factor as they would suppress anti-tumor immune responses, which appears to be the case in many situations (29). However, it has also been shown in several studies that Tregs can functionally restore homeostasis during chronic inflammation and reduce risk as well (15,32-34). In some solid tumors, such as ovarian carcinoma, pancreatic ductal carcinoma, and hepatocarcinoma, a large number of $CD3^+CD4^+CD25^+FoxP3^+$ cells correlated with poor prognosis. On the other hand, high numbers of $CD3^+CD25^+FoxP3^+$ has been associated with good prognosis in follicular lymphoma, Hodgkin's lymphoma, and head and neck cancer (35). In CRCs, the complex role of Tregs is only now being elucidated. In ulcerative colitis associated colon cancer, a study found a high frequency of $FoxP3^+IL\text{-}17^+CD4^+$ Tregs in the colitic microenvironment and associated colon carcinoma. These cells were able to not only suppress T cell activation, but the $IL\text{-}17^+Foxp3^+$ Treg cells also contributed to inflammation by stimulating inflammatory cytokine production due to their release of IFN-γ and IL-2 in the colitic tissues (36). In the case of sporadic colon cancer, several studies have shown that increased frequencies of Tregs are associated with poor prognosis and an inability of the immune system to effectively respond to cancer. However, other studies have shown that a large number of intratumoral $FoxP3^+$ Tregs correlates with a positive outcome (32). It is believed that these Tregs inhibit the local inflammatory processes that promote carcinogenesis (37).

Conditioning the tumor microenvironment

Immune cells, especially tumor-associated macrophages, have been shown to promote angiogenesis, cancer cell proliferation, and invasiveness (17,38). Tumor cells require neovasculature to supply nutrients and to clear waste. As the tumor progresses, angiogenesis is required to sustain the environment and new vessels are sprouted from existing vasculature. Before this "angiogenic switch" is turned on, necrotic tumor cell death can occur. Unlike cell death occurring through apoptosis and autophagy which generally results in phagocytosis by neighboring cells and does not elicit an immune response, necrotic cell death results in a spewing of cell contents, triggering proinflammatory signals in the local tissue, causing a recruitment of immune cells (27). These proinflammatory signals, including IL-1 and high-mobility group box 1 (HMGB1), result in both angiogenesis promotion and homing of immune cells that release additional growth factors contributing to the survival of the cancer cells (17). Innate immune cells, including macrophages, neutrophils, mast cells, and myeloid progenitors, help trigger this "angiogenic switch" and stimulate the process of new vasculature formation. The on-going signals from tumor cells, which simulate chronic inflammation, helps maintain the process (27). Immune cells also produce cytokines that work to activate transcription factors, such as NF-κB and STAT3, which promote tumor cell proliferation, growth, and survival (17). Additionally, in order for the cancer cells to continue to grow and metastasize, tumor cells must be able to invade into the peripheral area. Macrophages also contribute to this process by releasing enzymes, including metalloproteinases (MMPs) (39,40) and cysteine cathespin proteases (41), that degrade

the surrounding matrix and allow invasion, eventually leading to metastasis (17,27). Other inflammatory cell types have also been implicated in supporting tumor growth. In particular, neutrophils have been shown to promote the metastatic potential of cancer cells. In one example using a UV-induced melanoma mouse model, Bald *et al.* found the presence of neutrophils stimulated melanoma cells to move towards endothelial cells promoting metastasis to the lung (42). Thus, from the examples above it is evident that while the immune system can protect against cancer development, it can also support the growth and metastasis of tumors through the tumors ability to co-opt the normal repair and wound healing functions of immune cells such as macrophages.

Colon cancer exhibits a number of pro-tumor inflammatory responses. In hepatocellular carcinoma (HCC) and colitis-associated CRC, high levels of IL-6 have been shown to activate STAT3 and have tumor promoting activity (17). In addition, analysis of CRC specimens have been shown to have high levels of macrophage-derived MMP-9. MMP-9 specifically degrades type IV collagen, a major component of the basement membrane, and allows metastasis to occur. The presence of high levels of MMP-9 in CRC tissue was shown to be an independent predictor of metastasis and poor outcome (43).

Chronic inflammation and cancer

In addition to promoting the growth of established tumors, chronic inflammation has been recognized as a contributor to neoplastic formation, as many of the processes such as tissue remodeling and angiogenesis found in chronic inflammatory sites are critical components in tumor development. At least 20% of cancers including pancreatic, gastric, and skin cancers have been directly linked to chronic infections (44,45). The microenvironment that is created during an inflammatory response has also been shown to initiate carcinogenesis through the production of genotoxic compounds that can damage DNA such as reactive oxygen species (ROS) (46). In addition, a number of inflammatory cytokines that can upregulate ROS and reactive nitrogen intermediates (RNI) that lead to deleterious DNA damage or activate pro-survival/proliferation pathways such as STAT3 and NFκB are present and allow damaged cells to survive (47).

As the mechanisms driving carcinogenesis are being elucidated, it has become increasingly clear that chronic inflammation is a carcinogenic process. A few examples of cancer-related chronic inflammatory diseases in the gastrointestinal system include the link of CRC to inflammatory bowel disease, gastric cancer to gastritis and ulcers, pancreatic carcinoma to pancreatitis, HCC to hepatitis and gall bladder cancer to chronic cholecystitis. Other GI malignancies linked to inflammation include anal carcinoma to chronic cervicitis and the link of oral squamous cell carcinoma to gingivitis (35). The cause of chronic inflammation in many of these situations remains unknown. However, in several cancers, specific microbial infections have been revealed to be the underlying etiology of the chronic inflammation. Perhaps the most notable is the gram-negative bacillus *Helicobacter pylori* which is associated with gastric cancer and has been shown both in murine models and humans to cause chronic inflammation that promotes cancer. This observation has been validated in numerous large epidemiologic studies (48). Other infections, such as hepatitis B or C, human papillomaviruses, and *Bacteroides* have been linked to HCC, anal, and colon cancer, respectively (17,49).

IBD-related-CRCs account for less than 2% of all CRC appearing annually. Other high-risk conditions include hereditary diseases, which may account for up to 20% of all cases. However, chronic inflammation of the colon does increase the risk of CRC to varying extents depending on a number of factors that may regulate inflammation including disease severity, duration of the disease, and proper management of the disease (47,50). Interestingly, recent evidence has pointed to intestinal inflammation driven by the microflora. When the intestine becomes overpopulated with "bad" microbes there is thought to be increased barrier disruption with a resultant increase in inflammatory and pro-tumorigenic cytokines from increased exposure to the intestinal microflora. The release of inflammatory cytokines and the ensuing immune reaction result in epigenetic changes, further recruitment of immune cells, and constant tumor-promoting signals that contribute to progression of tumor growth once the cancer is already initiated (47).

Immune cells as prognostic factors

Given the important role of the immune system in the initiation, maintenance, and progression of cancer, it is not surprising that recent studies have revealed a connection between the presence of specific immune cells and disease outcomes. Several groups have developed algorithms that quantify the presence of specific immune cells as prognostic factors. A number of cancers have been shown to have a

favorable prognosis with increased infiltration of certain T cell subsets, particularly those that suggest that an individual already has a pre-existing spontaneous anti-tumor immune response. Memory T cells ($CD3^+CD45RO^+$) of the Th1 and cytotoxic types and $CD8^+$ T cells have been shown to predict for better disease outcomes in esophageal cancer, renal cell carcinoma, and CRC, among others (32). Clinical epidemiology data has shown that patients with colon and ovarian tumors that have large numbers of CTLs and NK cells have a better prognosis than patients with fewer killer lymphocytes (27). In addition to favorable prognosis from the presence of effector cells, a number of studies have shown that the presence of mature antigen-presenting DCs, which theoretically lead to an enhanced immune response, have also been correlated with improved survival. A study of 74 non-small cell lung cancer (NSCLC) found that the presence of DC-$LAMP^+CD83^+$ mature DCs, often in tertiary lymphoid structures with DC-T cell clusters, was highly associated with good prognosis (51). Mature DCs, characterized as $CD83^+$HLA-$DR^+CD40^+CD86^+$, were also found to infiltrate colon cancer. However, the density of these DCs was found to be three times lower than seen in normal colonic mucosa and very rare in metastatic tumor tissue. In patients who had a high number of TNFα-producing TILs, a greater number of mature DCs were also observed. Thus, in many cancers, including CRC, high densities of DCs serve as a positive prognostic factor (52). While mature DCs appear to be favorable, macrophages, which in some settings have similar functions to DCs, appear to be strongly influenced by the tumor microenvironment. They are often alternatively-activated rather than cytotoxic, and produce a number of factors that influence growth and survival of tumor cells, angiogenesis, cell invasion, chemotaxis, or inhibit T cell responses (35). Thus a high number of tumor-associated macrophages is typically considered to be a poor prognostic factor (53,54).

The use of immune cells to predict tumor behavior has been an area of intense research over the past several years and in CRC in particular, several powerful predictive algorithms based on immune cells have emerged. Early studies in CRC found that the presence of $CD8^+$ T cells, $CD27^-CD45RA^-$ effector memory T cells and a Th1 gene signature had improved metastasis free and disease free survival (35,55). As these observations were repeated in large cohort studies, specific immune responses within the tumor site were found to influence clinical outcome at all stages of CRC. In fact, the type, density, and location of immune cells had a prognostic value that surpassed the UICC-TNM classification. Thus, an immune score from 0 to 4 based on the assessment of $CD8^+$ and $CD45RO^+$ cell densities in the center and in the invasive margins of the tumor was created. A higher score, meaning higher density of TH1/cytotoxic memory T lymphocytes in both the center and at the margin, correlates with higher disease free survival and overall survival, as well as low risk of relapse and metastasis, in CRC and is likely applicable to most human tumors, particularly those that are thought to be more sensitive to immune regulation such as melanoma (35,56,57). A worldwide harmonization effort is currently underway to confirm the utility of the immunoscore in CRC and to refine the criteria that will be used for future clinical trials.

Thus, as expected from a system as diverse as the immune system, the role of immune cells in the development, progression and treatment of tumors is very complex and not yet fully understood. The immune system, through NK cells, T cells, macrophages and DCs, helps prevent cancer by detecting and eradicating mutated cells that would become cancerous. This immunosurveillance function has been controlled or subverted by the time tumors have become clinically apparent. The goal of much of immunotherapy, as discussed in the next section, is to reawaken this anti-tumor immune response by attempting to generate *de novo* or more powerful anti-tumor immune responses. However, as the tumor has already managed to prevent the body's normal anti-tumor immune response by developing powerful suppression mechanisms, strategies aimed at inhibiting immune suppressive pathways have also been surprisingly successful. Targeting the cells and pathways used by tumors to accomplish this has produced a number of recent successes that have inspired a new generation of immune-based therapeutic options.

Immunotherapy

Immunotherapy refers to therapeutic approach that harnesses the immune system to eliminate tumors. As we have described above, tumors, including colorectal tumors, employ multiple strategies to evade and suppress the immune system. Immunotherapeutic approaches have aimed at either augmenting the anti-tumor immune response through strategies such as vaccination in combination with immune stimulatory cytokines or preventing the suppression of a response through the use of checkpoint inhibitors such as the anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibody, ipilimumab (*Figure 2*). We review here recent advances in immunotherapy and current progress in

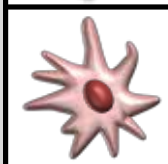
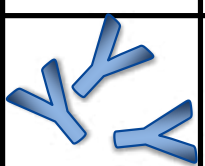
Immunotherapy			Key Features
Vaccines		Whole tumor	✚ Tumor lysates contain all known and unknown tumor antigens ➖ Poorly immunogenic, lots of overlap with normal cells
			Refs 58 - 66
		Peptide	✚ Known specificity for tumor-associated antigen ➖ HLA-restricted, small number of responding T cells
			Refs 67 - 80
		Virus	✚ Known specificity for tumor-associated antigen and naturally immunogenic ➖ Toxicity (cytokine storm)
			Refs 81 - 87
		Dendritic cell (DC)	✚ Known specificity for tumor-associated antigen and can generate its own immune response ➖ Preparation cost and time
			Refs 88 - 95
Cells		Adoptive cell therapy (ACT)	✚ Bypasses need to generate immune response, highly tumor specific ➖ Preparation cost and time, target dependent toxicity (e.g. colitis)
			Refs 96 - 105
Abs		Checkpoint Inhibitors	✚ Targets immunosuppresive pathways allowing for greater anti-tumor immune response ➖ Toxicity (e.g. autoimmune dermatitis, colitis)
			Refs 110 - 115

Figure 2 Overview of different immunotherapeutics. Vaccines, adoptive T cell therapy and checkpoint inhibitors have led the vanguard for a new generation of immune-based therapies. Each therapy has a unique profile of advantages (+) and disadvantages (–). While current immunotherapies have yet to show efficacy in CRC, multiple trials are currently underway to test the potential for emerging immunotherapeutics.

applying immunotherapeutic strategies for the treatment of colorectal malignancies.

Cancer vaccines

A strategy that has been tried in multiple variations over the past two decades, cancer vaccines have had variable degrees of success eliciting an anti-tumor immune response. The concept of cancer vaccination stems from the recognition that the immune system has built in mechanisms to recognize altered self-antigens that are present on the majority of cancer cells. These antigens are often called tumor-associated antigens. Like the vaccination strategies for infectious diseases, the ultimate goal of cancer vaccination is to elicit an anti-tumor immune response that will eliminate a tumor and provide ongoing surveillance to protect against its regrowth. Numerous groups have developed and continue to develop agents that attempt to

generate a productive immune response against tumors. Four major categories of vaccination agents have been explored: whole tumors, peptide antigens, DCs and viral/bacterial vaccinations.

Whole tumor vaccines

Whole tumor vaccines were the earliest of the vaccines because the vaccination material was both readily available and contained all of the known and unknown tumor associated antigens that needed to be eliminated. Thus, while there was no specific antigen identified with this approach, presumably a diverse immune response would occur that would reduce the chance that there would be tumor escape from a more specific vaccine. Typically this approach would require a sample of tumor tissue that would then be lysed or irradiated, mixed with an immune adjuvant such as alum, and then reinjected into patients (58). Autologous whole tumors have been used as cancer vaccines to induced cytotoxic anti-tumor immune responses in several cancer types including renal cell carcinoma (59), melanoma (60) and CRC (61). However, despite initial excitement for whole tumor vaccines, to date even the best trials demonstrate limited efficacy. In CRC, a randomized phase III clinical trial combining autologous whole tumor cell plus BCG vaccine was conducted by the Eastern Cooperative Oncology Group to determine whether surgical resection plus vaccination was more beneficial than resection alone in 412 stage II and III CRC patients but this study showed no significant survival or disease-free survival benefit. However, effective immune responses were associated with improved disease-free and overall survival (61).

One issue with using whole tumor vaccines is that only a small proportion of the proteins in an autologous whole tumor vaccine are specific to cancerous cells, while a vast majority of antigens in the vaccine are shared with normal cells, thus diluting the amount of tumor antigens in a whole tumor vaccine, while simultaneously supplying the antigens for stimulating an autoimmune response. Moreover, whole tumor vaccines are typically poorly immunogenic. Therefore, the immune response generated by whole tumor vaccines is generally insufficient to provide benefit to patients as evidenced by the modest results in clinical trials (62). To improve the immunogenicity of whole tumor vaccines, autologous tumor cells have been genetically modified to secrete immunostimulatory molecules such as GM-CSF and then re-administered to the patient (63). While early trials demonstrated promising results in a wide range of tumors, most of these did not result in survival benefit, though they did augment antitumor immunity (64,65). Another interesting approach to augment the immunogenicity of tumor cell vaccines utilized Newcastle disease virus (NDV)-infected irradiated tumor cells as a vaccine (66). This approach resulted in a 98% 2-year survival rate in patients with resected CRC, compared to 67% when treated with autologous tumor cells combined with BCG, suggesting that the immunogenicity of tumor cells can be altered to make them more immunostimulatory. However, the randomized phase III study of 50 patients with resectable CRC liver metastases vaccinated with NDV-infected tumor cells did not demonstrate improvement in overall survival, disease-free survival, or metastases-free survival (66). The experience with NDV-infected cells supports the notion that the immunogenicity of whole tumor cells needs to be improved for this vaccination strategy to be effective. However, as the randomized trial data demonstrated, further research into which specific agents for killing tumor cells (such as cytotoxic chemotherapeutics, ionizing irradiation, and chemical agents) can generate sufficiently immunogenic whole tumor vaccines to produce an adequate clinical anti-tumor response.

Peptide vaccines

One reason for the limited efficacy of whole tumor vaccines lies in the fact that tumor cells share the bulk of their antigens with normal cells and that the immune system is finely tuned to suppress immune responses against self-antigens. Thus in attempt to address this problem, many groups turned to peptide vaccines in order to develop an immune response against a specific known tumor antigen. Peptide-based vaccines are whole proteins or fragments of proteins typically generated from tumor-specific proteins that are administered with adjuvant. Compared to whole tumor vaccines, peptide vaccines have the potential to generate a more specific anti-tumor response by using antigens that are known to be expressed by tumor cells. Peptide vaccines have been generated for multiple tumor types including breast, prostate and pancreatic cancer (67-69). Thus far, similar to the whole cell vaccines they have shown limited efficacy in the clinical setting with many vaccines eliciting a specific response, but showing no effect on disease progression or survival benefit (62).

In CRC, multiple tumor-associated antigens have been identified and utilized for vaccination with varying success. Typically the peptides employed are designed for MHC Class I, the MHC recognized by $CD8^+$ cytotoxic T cells. These

antigens include carcinoembryonic antigen (CEA) (70), mucin-1 (71), squamous cell carcinoma antigen recognized by T cells 3 (SART3) (72), β-human chorionic gonadotropin (β-hCG) (73), Survivin-2B (74) or p53 (75), all of which have been employed as targets for immunotherapy in CRC, as well as other tumors. Peptide vaccines targeting these tumor-associated antigens have been shown to induce an antigen-specific immune response, which in some cases correlated with improved survival. For example, in one phase II trial, vaccination with the β-hCG peptide induced anti-β-hCG antibody production in 56 of 77 CRC patients (73) and, importantly, anti-β-hCG antibody induction was associated with longer overall survival. However, the majority of trials have not been able to demonstrate a correlation between an immune response and clinical outcomes. In SART3 peptide vaccine therapy, IgE-type anti-peptide antibodies were detected after vaccination; however, immunological responses were limited to patients expressing HLA-A24 (72). The results of the SART3 trial highlight one of the limitations of peptide vaccines: restricted antigen presentation due to the patient's HLA type (76). However, peptide vaccines have other limitations including defective $CD8^+$ CTLs due to the downregulation of certain antigens and MHC class I molecules (77), impaired DC function in patients with advanced cancer (78), and inhibitory tumor microenvironments, where immune suppressive cells such as Tregs and alternatively-activated macrophages exist (79). Given the relatively low efficacy of peptide vaccines, current strategies attempting to improve the response to peptide vaccines have focused on trying to increase the number of T cells that respond to the peptide. One strategy to do this has been to use a larger peptide to increase the number of epitopes and thus the number of T cells that may respond to a given antigen. In a phase I/II trial, 10 CRC patients were vaccinated twice with a set of 10 overlapping p53 synthetic long peptides (75). P53-specific $CD4^+$ and $CD8^+$ T-cell responses were observed in 9 of 10 CRC patients, and 6 of 9 tested patients maintained p53-specific T-cell reactivity for at least 6 months. New trials using peptide vaccines have also focused on administering the peptides with more effective adjuvants such as cytokines. A follow-up phase I/II trial with the p53-specific vaccine combined with interferon-alpha increased the number of interferon-gamma producing cells found in the circulation of patients with CRC (80). Thus, while early trials with peptide vaccines demonstrated low efficacy, new strategies to enhance the immune response show promise.

Viral vector vaccines

The low efficacy of peptide vaccines results from their inability to generate a productive immune response against the peptide. Thus, other groups have taken the idea of a peptide and packaged it such that it would be presented in a more pro-inflammatory way. One such strategy is through the use of a viral or bacterial vector to which the body has already developed multiple pathways to recognize. Using a recombinant virus engineered to express tumor-associated antigens takes advantage of the fact that viruses are naturally immunogenic and typically infect antigen presenting cells (specifically DCs) (81). One of the more promising approaches to augmenting immune activation combines vaccination with tumor antigens plus co-stimulatory molecules in a viral vector. The CEA/TRICOM vaccine which combines radiation, vaccination with CEA with a viral vector that expresses the three co-stimulatory molecules (TRICOM) B7.1 (CD80), intercellular adhesion molecule 1 (ICAM-1), and lymphocyte function-associated antigen 3 (LFA-3) shows excellent efficacy in a murine model of colon cancer and appears to be safe in patients (82,83). In a series of studies, CEA-specific T cell responses were observed and disease stabilization was seen in up to 40% of patients with metastatic cancer (including CRC) (84,85). In a similar strategy, another group published a phase II clinical trial in patients with metastatic CRC that examined the efficacy of chemotherapy in combination with vaccination using a nonreplicating canarypox virus (ALVAC) expressing CEA and the T-cell costimulatory molecule, B7.1 (ALVAC-CEA/B7.1). Anti-CEA-specific T cell responses were produced in 50% of patients undergoing chemotherapy and booster vaccination and objective clinical responses were observed in 40% of the patients (86,87). Current trials are underway attempting to further enhance the response by delivering the virus with tetanus toxoid and results from this strategy are still being accrued. Thus, viral vaccines produce significantly more effective responses compared to peptide vaccines, however clinical success remains elusive and is actively being pursued.

DC vaccines

As detailed knowledge of the mechanism of an immune response has become available over the past decade, it has become apparent that it is critical to provide specific essential signals to the immune system in order to produce an effective immune response against a given antigen.

The three critical steps to activate a T cell are antigen presentation by MHC (signal 1), co-stimulation by an appropriate receptor-ligand pair (signal 2) and expression of key cytokines to direct the ensuing immune response. Peptide and viral vaccines depend on the use of adjuvants that stimulate an immune response themselves or the natural anti-viral immune response to produce their anti-tumor effect. The central cells for this process are DCs, which can provide all three signals for a productive anti-tumor immune response and thus, many groups have attempted to utilize DCs for vaccination (88). Many clinical trials of antigen-pulsed DCs have been completed in patients with various types of tumors, including CRC, and recent trials have begun to bear fruit (89). Several strategies for using DCs to generate an anti-tumor cytotoxic immune response have been developed. DCs have been pulsed with synthetic peptides derived from the known tumor-associated antigens, tumor cell lysates, apoptotic tumor cells or physically fused with whole tumor cells to induce efficient antitumor immune responses (*Figure 2*) (90-93). With respect to CRC, since CEA is a tumor-associated antigen expressed by most CRCs, many DC vaccines for CRC have utilized CEA peptides (94) or CEA-expression vectors (95). In these phase I clinical trials, the majority of vaccinated CRC patients demonstrated the induction of CEA-specific T cell responses. Furthermore, disease progression stabilized in several patients, and the vaccine was safe and well-tolerated. Despite the progress made in other cancers (89), there has not been a DC vaccine in CRC that has improved survival and the search continues for ways to improve the clinical efficacy of DC-based cancer vaccines for CRC.

Adoptive cell transfer (ACT) therapy

With the limited success of cancer vaccines for most tumor types including CRC (62), other strategies have been pursued that eliminate the need to develop a *de novo* immune response and circumvent the tumor-mediated suppression of an anti-tumor immune response. The most successful of these strategies has attempted to restore the cytolytic anti-tumor activity to a patient's own T cells, thus taking advantage of the high specificity and targeting ability of T cells. This method, known as ACT therapy, extracts autologous T cells from the tumors of patients, activates them with cytokines and expands them into large numbers *in vitro*, all in preparation for transfer back into the patient (96). The main source for T cells for ACT comes from lymphocytes found in tumors themselves, known as TILs. It was recognized almost a decade ago that these cells are actually tumor-specific cells that had been actively suppressed by the tumor microenvironment (10,97). In ACT, the *ex vivo* expansion and addition of other co-stimulatory molecules and cytokines is thought to overcome tolerogenic mechanisms by selecting highly reactive T cell populations and activating them sufficiently to overcome the suppressive environment with the tumor (98). This approach has shown early and dramatic success in metastatic melanoma (97,99). However, there are several drawbacks to ACT that should be considered, including the high cost of the procedure, a potential lack of immune memory because the transfer is only activated effector cells, transient survival of the activated effector cells in patients, and the time (1-4 months) required to expand the cells. Additionally, as data from several early trials revealed, there is also a potential risk for severe adverse events (100,101).

Unfortunately, the use of TILs is currently largely limited to patients with melanoma, a reflection of the higher immunogenicity of melanoma in comparison to other cancers which often do not have infiltrating T cells with as high numbers or specificity. To address this, several groups have genetically engineered T cells to express T cell receptors (TCRs) with predetermined affinity to specific antigens to facilitate the targeting of virtually any tumor type. Several groups have shown promising data using T cells engineered to express high avidity TCRs to target tumors of various histological origins (102,103). However, these TCRs are limited to patients with the corresponding MHC haplotype. Thus, other groups have sought to engineer antibody-based chimeric antigen receptors (CARs). These receptors express a single chain variable fragment derived from a tumor antigen-recognizing monoclonal antibody fused to intracellular T cell signaling domains. With specificity provided from the antibody recognition of the antigen, these receptors can be used universally across all patients since CARs target native antigens on the surface of tumors without MHC restriction. This approach has shown early success for acute and chronic lymphoid leukemia (104,105).

Given the low number of TILs in CRC, most of the recent trials have focused on using engineered T cells. Parkhurst *et al.* conducted a phase I trial in colon cancer using human T cells modified to express a high avidity CEA-specific murine TCR (100). Three patients with metastatic colon cancer were treated with these engineered T cells, all of which experienced decreased serum CEA levels and one of which experienced an objective clinical

response. However, all patients developed a severe transient inflammatory colitis. Severe side effects also were seen in one patient treated with Her2-specific CAR T cells for metastatic colon cancer (101). Thus, ACT has failed to demonstrate safety and efficacy in CRC patients and future studies will have to identify mechanisms that allow CAR-expressing T cells to selectively eliminate cancer cells, but leave normal tissues unaffected.

Antibody-based cancer immunotherapy

Monoclonal antibodies (mAbs), which have high specificity, have been clinically effective as cancer therapeutics for decades (106). Antibodies such as cetuximab and panitumumab which both target EGFR and bevacizumab which targets VEGF have been approved and are in current use for the treatment of CRC in the United States. Many other mAbs targeting other pathways are currently being tested in clinical trials (107). These pathways are thought to induce tumor cell death by several mechanisms, including disruption of vital signaling pathways and engaging innate immune effector mechanisms that recognize the Fc portion of the antibody via Fc receptors and induces antibody-dependent cytotoxicity through various cellular mechanisms (108). These targeted therapies are generated to block specific pathways and discussion of their effects on these signaling pathways is reviewed elsewhere (109).

Different from targeting the tumor themselves, a new class of antibodies that target the suppressive mechanisms in the tumor microenvironment has become available and have shown, in some cases, dramatic and unexpected efficacy (110). Known as checkpoint inhibitors, mAbs targeting the inhibitory immune receptors CTLA-4, programmed cell death 1 (PD-1), and PD-1 ligand (PD-L1) have produced successful results in patients with advanced melanoma and NSCLC (111-113). The success of targeting these suppressive pathways has generated tremendous excitement and trials are underway now that have the potential to radically change the concept of how we view the treatment for cancer. However, early data regarding the potential role for checkpoint inhibition in CRC suggests that anti-CTLA-4 may have limited efficacy as a single agent (114). Furthermore, preliminary studies on CRC revealed that CRC has low expression of PD-L1, suggesting that CRC may not respond to PD-1 or PD-L1 inhibition (115). Further study is warranted in the setting of CRC to determine if other therapies in conjunction with checkpoint inhibition would prove more successful.

Combined immunotherapy: key to success?

The modest success of current immunotherapeutic strategies in CRC highlights the relatively resistant nature of CRC to immune-based therapies. The mechanism underlying this resistance may have to do with an underlying connection between the immune factors that are known to drive and determine the behavior of CRC. As a tissue that is in constant contact with antigens from the microbiota, it is not surprising that CRC may have developed strong mechanisms to suppress an immune response, though these factors remain unidentified and appear not to be driven by the PD-1-PD-L1 pathway (115). However, it may be that combinations of immunotherapy or more conventional chemotherapy and radiation with immunotherapy will hold the key to developing an anti-tumor immune response. For example, one strategy might involve stimulating an immune response via vaccines, in combination with blocking inhibitory pathways such as CTLA-4 or PD-1. This would combine the strengths of a vaccine for developing an anti-tumor immune response to override mechanisms that delete anti-tumor immune cells and a checkpoint inhibitor to block inhibition of anti-tumor immune responses to overcome the suppressive microenvironment. Early clinical trials support this notion with growing evidence indicating that combined targeted therapies and simultaneous blockade of multiple immune checkpoints promotes therapeutic synergy and long-term antitumor immunity leading to improved clinical outcome in melanoma patients (116). Further, it has become increasingly evident that the efficacy of radiation and certain chemotherapies depends on the development of an immune response to the cell stress and death caused by these agents (117-119). Combining immunotherapeutics with novel immunostimulatory applications of more traditional cytotoxic agents has also shown early signs of success (120).

Conclusions

Tremendous progress has been made in understanding the role of the immune system in driving the development of cancer, including CRC. This understanding has revealed two trends that have and will continue to influence the treatment of cancer for the foreseeable future: the use of immune cells markers to predict cancer outcomes and targeting various aspects of the immune system to generate an anti-tumor immune response. With respect to the treatment of CRC, the development of the immunoscore is

well underway and will likely emerge as a critical prognostic tool in the clinic. Unfortunately, effective immunotherapies in CRC remain elusive. The complex role of the GI tract, particularly the colon and small intestine, in shaping systemic immune responses is likely to account for some of the difficulties in developing effective immunotherapeutics for CRC. The most promising avenues for therapy going forward will likely be combinations of cytotoxic therapies such as chemotherapy and radiation and multiple immunotherapeutic modalities. Trials that make use of our increasing understanding of the immune system in CRC are currently underway and will no doubt continue to expand our knowledge of where immunotherapeutics fit in our current treatment paradigms.

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Footnote

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Review of systemic therapies for locally advanced and metastatic rectal cancer

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Abstract: Rectal cancer, along with colon cancer, is the second leading cause of cancer-related deaths in the U.S. Up to a quarter of patients have metastatic disease at diagnosis and 40% will develop metastatic disease. The past 10 years have been extremely exciting in the treatment of both locally advanced and metastatic rectal cancer (mRC). With the advent of neoadjuvant chemoradiation, increased numbers of patients with locally advanced rectal cancer (LARC) are surviving longer and some are seeing their tumors shrink to sizes that allow for resection. The advent of biologics and monoclonal antibodies has propelled the treatment of mRC further than many could have hoped. Combined with regimens such as FOLFOX or FOLFIRI, median survival rates have been increased to an average of 23 months. However, the combinations of chemotherapy regimens seem endless for rectal cancer. We will review the major chemotherapies available for locally advanced and mRC as well as regimens currently under investigation such as FOLFOXIRI. We will also review vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) inhibitors as single agents and in combination with traditional chemotherapy regimens.

Keywords: Systemic therapies; locally advanced and metastatic rectal cancer (mRC); chemotherapies

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Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the U.S., with an estimated 142,820 newly diagnosed cases and an estimated 50,830 deaths in 2013. An estimated 40,000 new cases of rectal cancer will be diagnosed in the U.S. in 2014 (1). A total of 20-25% of patients will have metastatic disease at diagnosis and close to 40% will develop metastatic disease (2). Despite the significant proportion of metastatic disease, the 5-year survival for all stages of rectal cancer has significantly improved over the past 4 decades (3). These advances are in large part due to the development of new systemic therapies. Rectal cancer has seen impressive treatment developments over the past 20 years, including neoadjuvant chemoradiotherapy (CRT), novel biologic therapies and second generation chemotherapeutic agents. With these advances, rectal cancer management has evolved into a multidisciplinary approach involving surgery, radiotherapy, chemotherapy, and biologics.

This review will look the current systemic therapeutic options in treating locally advanced and metastatic rectal cancer (mRC). The review will also look at therapies and novel strategies that are currently active areas of research and debate.

Neoadjuvant therapy in locally advanced rectal cancer (LARC)

The last decade has seen a shift toward neoadjuvant therapy for the treatment of LARC. Previously, adjuvant CRT involving 5-fluorouracil (5-FU) following surgical resection was the cornerstone of advanced rectal cancer treatment. The first large scale trials performed in the 1980s, NSABP R-01 and GITSG, revealed that 5-FU based treatments combined with adjuvant radiotherapy following surgery had significant improvements in disease free survival

and local recurrence compared to surgery alone (4,5). Subsequently, the North Central Cancer Treatment Group (NCCTG) performed a trial comparing radiotherapy with and without 5-FU (6). The NCCTG found significantly improved rates of local recurrence, cancer-related deaths, and overall survival (OS) with CRT compared to radiation alone (6). Based on these studies, the National Institutes of Health (NIH) recommended the treatment of LARC be a combination of postoperative chemotherapy with 5-FU and radiation (7,8).

Following the NIH recommendations, shifts in the treatment paradigm for rectal cancer began and trials began looking at the role of neoadjuvant radiotherapy. The Swedish and Dutch rectal cancer trials established the benefit of neoadjuvant radiotherapy in local disease control (9,10). These trials showed that the local recurrence rate of rectal cancer was significantly lower in those that received preoperative radiotherapy followed by surgery compared to surgery alone (9,10). In the landmark German Rectal study (CAO/ARO/AIO-94), neoadjuvant CRT was superior to post-operative therapy (11). In 825 stage II or III patients, Sauer *et al.* compared neoadjuvant CRT with 5-FU followed by surgery with the same regimen in the adjuvant setting. There was significant differences in 5-year cumulative incidence of local relapse (6% *vs.* 13%, respectively), although, there was no significant difference in 5-year survival (76% *vs.* 74%, respectively). These results have persistent at 10-year follow-up and have led to the widespread adoption neoadjuvant CRT in the treatment of LARC (11).

Other studies have looked at preoperative *vs.* postoperative CRT in the treatment of LARC and confirmed the benefit of neoadjuvant therapy. The NSABP R-03 trial was one such trial that showed no significant difference in local relapse but did show a significant difference in 5-year disease free survival with neoadjuvant CRT compared to adjuvant CRT (12). This study was only able to accrue 277 patients out of the 900 originally planned and thus the study could not reach the same power as that of the German Rectal study thus limiting analysis of local recurrence and toxicities (11,12).

Xeloda *vs.* 5-FU in LARC

Fluoropyrimidines are the backbone of both neoadjuvant and adjuvant therapy for LARC. Through inhibition of thymidylate synthetase (TS), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis is impaired (13). 5-FU is the most commonly used drug of this class. It is administered as an infusion in conjunction with leucovorin (LV) which stabilizes the tertiary complex between 5-FU and TS thus enhancing the efficacy of 5-FU (14,15). Given the inconvenience of infusion therapies, capecitabine, the first oral fluoropyrimidine, has been developed as a promising alternative. Capecitabine is a prodrug, which is converted to 5-FU via three enzymatic steps (16). Thymidylate phosphorylase plays a key role in the conversion of capecitabine to its active metabolite and is found in higher concentrations in the malignant tissue (16). Trials looking at the toxicity profile of the drug when compared to 5-FU have suggested an improved side effect profile compared to 5-FU/LV with decreased stomatitis, diarrhea, nausea and neutropenic sepsis (17,18). However, capecitabine did have higher rates of hyperbilirubinemia and hand-foot syndrome (17,18). Considering the potential benefits of an oral pro-drug, the efficacy of capecitabine in comparison to 5-FU as neoadjuvant therapy for rectal cancer was investigated (*Table 1*).

In the first-line monotherapy setting, the two randomized, prospective phase III trials enrolled a total number of 1,207 patients, who were randomized to receive either oral capecitabine (1,250 mg/m^2 bid 2 weeks on/1 week off in 3-week cycles) or the Mayo Clinic regimen (LV 20 mg/m^2 followed by 5-FU 425 mg/m^2 iv bolus on days 1-5 in a 4-week cycle) (18). The results suggested that capecitabine was equally effective with acceptable toxicity. Further retrospective data collected from small trials without treatment protocol standardization suggested that capecitabine had a higher complete response rate in the neoadjuvant setting (24).

The role of capecitabine as neoadjuvant treatment for rectal cancer became widely accepted with Hofheinz *et al.*'s findings in 2012 (25). This phase III non-inferiority trial evaluated capecitabine *vs.* 5-FU in the neoadjuvant and adjuvant settings in LARC (25). The primary endpoint was overall 5-year survival and capecitabine was found to be non-inferior to 5-FU (76% *vs.* 67%, respectively) (25). Post-hoc analysis for superiority showed capecitabine had significantly improved 5-year survival. Capecitabine had a better 5-year survival when compared 5-FU in both the neoadjuvant cohort (66% *vs.* 61%) and adjuvant cohort (81% *vs.* 71%) (25). Local recurrence rate, a secondary endpoint, was not significantly difference between capecitabine and 5-FU (6% *vs.* 7%) (25).

Recently, the NSABP R-04 trial was completed which looked at clinical complete response (cCR), pathologic complete response (pCR) and local-regional relapse in

Table 1 Synopsis of studies comparing capecitabine containing regimens to 5-FU containing regimens. Included are the dosing regimens, PFS statistics, median OS statistics, and toxicities observed in the treatment arms

Study	German AIO group (19)	Spanish group (20)	French study (21)	Tree-1-US study (22)	GOAM-Italian study (23)
Dosing regimen CAPOX/XELOX based treatment arm	CAPOX: oxaliplatin 70 mg/m^2 2-hour infusion days 1 and 8 every 3 weeks, capecitabine 1,000 mg/m^2 bid orally days 1-14 every 3 weeks	XELOX: oral capecitabine 1,000 mg/m^2 bid for 14 days plus oxaliplatin 130 mg/m^2 on day 1 every 3 weeks	XELOX: 2-hour iv of oxaliplatin 130 mg/m^2 on day 1 plus oral capecitabine 1,000 mg/m^2 twice daily on days 1-14 every 3 weeks	CapeOx: oxaliplatin 130 mg/m^2 iv on day 1 and capecitabine 1,000 mg/m^2 orally twice daily on days 1-15 every 3 weeks	XELOX: oxaliplatin as noted below and oral capecitabine at the dose of 1,000 mg/m^2 bid from the 1st to the 14th day
Dosing regimen FU + OX based treatment arm	FUFOX: oxaliplatin 50 mg/m^2 2-hour infusion, folinic acid 500 mg/m^2 2-hour infusion, FU 2,000 mg/m^2 22-hour infusion; days 1, 8, 15, and 22 every 5 weeks	FUOX: FU 2,250 mg/m^2 diluted in saline administered by civ during 48 hours on days 1, 8, 15, 22, 29, and 36, plus oxaliplatin 85 mg/m^2 on days 1, 15, and 29 every 6 weeks	FOLFOX6: 2-hour iv of oxaliplatin 100 mg/m^2 followed by a 2-hour infusion of LV 400 mg/m^2 followed by 5-FU 400 mg/m^2 given as an intravenous bolus injection and then 5-FU 2,400-3,000 mg/m^2 as a 46-hour civ every 2 weeks	mFOLFOX6: oxaliplatin 85 mg/m^2 iv with LV 350 mg iv over 2 hours plus FU 400 mg/m^2 iv bolus and 2,400 mg/m^2 civ over 46 hours every 2 weeks bFOL: oxaliplatin 85 mg/m^2 iv on days 1 and 15 and LV 20 mg/m^2 iv over 10-20 minutes followed by FU 500 mg/m^2 iv push on days 1, 8, and 15 every 4 weeks	PVIFOX: dexamethasone 20 mg in 100 cc of saline by the intravenous (iv) route in 15 min, granisetron 3 mg in 100 cc of saline iv in 15 min, oxaliplatin at the dose of 130 mg/m^2 in 500 cc of 5% glucose solution iv in 2 hours and, at the end, 5-FU at the dose of 250 mg/m^2/daily in civ from the 1st to the 21st day
Number of patients in CAPOX treatment arm	242	171	144	48	62
Number of patients in FU + OX treatment arm	234	171	140	50 (bFOL) & 49 (mFOLFOX)	56
PFS in treatment arms CAPOX/XELOX *vs.* FU + OX (months)	7.1 *vs.* 8.0 (P=0.117)	8.9 *vs.* 9.5 (P=0.153)	8.8 *vs.* 9.3	5.9 (CapeOx) *vs.* 6.9 (bFOL) *vs.* 8.7 (mFOLFOX)	9.0 *vs.* 7.0
OS in treatment arms CAPOX/XELOX *vs.* FU + OX (months)	16.8 *vs.* 18.8 (P=0.26)	18.1 *vs.* 20.8 (P=0.145)	19.9 *vs.* 20.5	17.2 (CapeOx) *vs.* 17.9 (bFOL) *vs.* 17.6 (mFOLFOX)	NA
RR in treatment arms CAPOX/XELOX *vs.* FU + OX	48% *vs.* 54%	37% *vs.* 46%	42% *vs.* 46%	27% (CapeOx) *vs.* 20% (bFOL) *vs.* 41% (mFOLFOX)	43% *vs.* 48%
Toxicity in treatment arms CAPOX/XELOX *vs.* FU + OX	Nausea, vomiting, and diarrhea were similar in both treatment groups. Only HFS grade 2/3 was significantly higher in the CAPOX arm (P=0.028)	Lower rates of grade 3/4 diarrhea (14% *vs.* 24%, P=0.027) and grade 1/2 mucositis (28% *vs.* 43%, P=0.005), with higher rates of grade 1/2 hyperbilirubinemia (37% *vs.* 21%, P=0.001) and grade 1/2 hand-foot syndrome (14% *vs.* 5%, P=0.009) with XELOX arm *vs.* FUOX arm, respectively	XELOX arm had significantly more grade 3/4 thrombocytopenia (12% *vs.* 5%) and diarrhea (14% *vs.* 7%), but significantly less grade 3/4 neutropenia (5% *vs.* 47%), febrile neutropenia (0% *vs.* 6%) and neuropathy (11% *vs.* 26%) than FOLFOX6 patients	Grade 3/4 treatment-related adverse events during the first 12 weeks of treatment were 59%, 36%, and 67% for mFOLFOX6, bFOL, and CapeOx, respectively. CapeOx toxicity included grade 3/4 diarrhea (31%) and dehydration (27%)	Grade 3/4 diarrhea was observed in 14.0% *vs.* 8.2%, grade 3 stomatitis in 3.7% *vs.* 0%, and grade 3 neurotoxicity in 18.5% *vs.* 24.6%, when comparing *vs.* PVIFOX *vs.* XELOX

AIO, Arbeitsgemeinschaft Internistische Onkologie; iv, intravenous infusion; 5-FU, 5-fluorouracil; LV, leucovorin; civ, continuous intravenous infusion; PFS, progression free survival; OS, overall survival; NA, not available; RR, response rate; HFS, hand foot syndrome.

patients who received neoadjuvant capecitabine/radiation *vs.* 5-FU/radiation (26-28). Preliminary data suggests that neoadjuvant capecitabine/radiation compared to 5-FU/radiotherapy have comparable outcomes particularly when looking at pCR, sphincter-saving surgery, and surgical down-staging (26-28). In a preliminary report presented at the 2014 American Society of Clinical Oncology (ASCO) Gastrointestinal Cancer Meeting, patients receiving capecitabine had comparable rates of down-staging surgery and sphincter preservation, similar pCR rates (21% *vs.* 18% for capecitabine and infusional 5-FU), similar rates of locoregional control, the primary endpoint (3-year incidence of any locoregional event 12% *vs.* 11%) and comparable OS (81% *vs.* 80%). Preliminary data has also suggested there are significant differences in overall patient reported outcomes (PROs) and quality of life (QoL) indices favoring capecitabine (26-28). Additionally, the convenience of care noted by patients in the capecitabine treatment arms was also greater (26-28). No major differences were seen in patient reported functional assessment of cancer treatment-colorectal (FACT-C), trial outcome indices (TOI), and ultimately overall PROs (26-28). These data as well as those from the NSABP R-04 and Hofheinz *et al.* strongly support capecitabine as a reasonable alternative to 5-FU in LARC (25).

Oxaliplatin in LARC

Oxaliplatin is a platinum analog which functions as an alkylator (29). Thus, oxaliplatin forms inter- and intra-strand cross-links within DNA preventing replication and transcription (29). Oxaliplatin is highly effective in combination with 5-FU in the treatment of mRC and its efficacy in the neoadjuvant setting has been extensively investigated in several randomized controlled trials (30,31).

The aforementioned NSABP R-04 had two additional treatment arms added oxaliplatin to each of the original treatment regimens (capecitabine ± oxaliplatin and 5-FU ± oxaliplatin) (26). Preliminary data analysis showed no significant differences in cCR, pCR and local-regional relapse when oxaliplatin was added to each treatment arm (26). However, the rate significant toxicity and including neuropathy and diarrhea increased in the arms containing oxaliplatin (26). In addition to the NSABP R-04, four other large trials (ACCORD 12, STAR-01, PETACC-6 and CAO/ARO/AIO-04) have failed to demonstrate a role for oxaliplatin in the neoadjuvant setting for LARC (32-35). Of all these trials, only the CAO/ARO/AIO-04 showed a statistically significant change in pCR with the addition of oxaliplatin (17% *vs.* 13%) (35). There was also a significant incidence grade 3 and 4 neuropathy and diarrhea with the addition of oxaliplatin across all trials except for the CAO/ARO/AIO-04 trial (32-35). However, although 5-FU or capecitabine were included in all trials, dosing strategies and treatment regimens varied (32-35). Additionally, the adjuvant regimens varied with only the CAO/ARO/AIO-04 trial including oxaliplatin in the adjuvant treatment arm (32-35).

An important and relevant clinical outcome after neoadjuvant treatment that was not addressed in detail in these trials was the incidence of distant metastasis after neoadjuvant therapy and prior to surgical intervention (32-35). Overall trend analysis regarding the incidence of distant metastasis indicated a decrease in the rate of distant metastasis at the time of surgery in patients treated with neoadjuvant oxaliplatin (32,33,35). Comparing the incidence of distant metastasis in the neoadjuvant treatment arms containing oxaliplatin *vs.* those without, the ACCORD trial noted 2.8% *vs.* 4.2%, the STAR-01 noted 0.5% *vs.* 2.9% and the CAO/ARO/AIO-04 showed 4% *vs.* 6%, respectively (32,33,35). Both the NSABP R-04 and the PETACC-6 did not comment on distant metastasis (26,34).

More importantly, some of the trials have provided interval analysis on disease free survival and OS. The ACCORD trial at 3 years has noted no significant difference in disease free survival (67.9% *vs.* 72.7%, respectively) between the oxaliplatin and non-oxaliplatin treatment arms (87.6% *vs.* 88.3%, respectively) (32). Preliminary data from NSABP R-04 also has supported these conclusions (26). Outcome and primary end point analysis still remains to be seen regarding the CAO/ARO/AIO-04 and PETACC-6 trials (34,35). With the current data available, consensus among the oncologic community does not support the use of neoadjuvant oxaliplatin for LARC.

Metastatic rectal cancer (mRC)

Fluoropyrimidine based therapy has been the backbone of the systemic approach to CRC over the last 30 years. In the last 2 decades, there have been new classes of chemotherapeutic agents, as well as new biologic agents such as vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) inhibitors approved for the treatment of CRC. These treatments have directly impacted the outcomes of our patients as CRC mortality in the United States has declined 3.0% from 2000 to 2009. This was among the highest rates of decline across all tumor types and likely reflects advances in detection

and the development of improved systemic treatments (1). Our current challenge lies in developing predictive and prognostic markers to enhance the activity of available agents as well as guiding the optimal sequence of treatment.

Oxaliplatin and metastatic CRC (mCRC)

Oxaliplatin is currently an important part of the systemic approach to advanced rectal cancer. It was originally studied in combination with 5-FU/LV in 1998 (36). De Gramont *et al.* subsequently randomized 420 patients to first line 5-FU/LV or FOLFOX4 (30). FOLFOX4 was found to be superior in terms of response rates (51% *vs.* 22%) and progression free survival (PFS) (9 *vs.* 6.2 months) but not in terms of OS (30). This study established FOLFOX's role as a first line therapy for mRC. FOLFOX further established its role in the treatment of mRC in 2004 when the INT 9471 trial was being conducted (31). The trial had to be unblinded early after FOLFOX4 significantly outperformed irinotecan/5-FU/LV (IFL) and irinotecan/oxaliplatin IROX (31). With 785 patients in the initial analysis, FOLFOX4 had improved objective response rates, time to tumor progression (TTP), and most importantly an improved median OS of 19.5 months (31). This is compared to IFL and IROX which had median OS times of 15 and 17.3 months, respectively (31). However, one potential flaw in the INT 9741 trial is that with the IFL regimen, 5-FU/LV are administered via bolus which had already been shown to have worse median survival compared to infusional regimens during initial investigations of 5-FU/LV in mRC (31,37). The FFCD 2000-05 trial followed in 2011 and randomized 410 patients to FOLFOX6 or infusional 5-FU/LV (38). The FOLFOX6 arm showed an improved objective response (58% *vs.* 24%) as well as TTP (7.6 *vs.* 5.3 months) (38). However, median survival was not significantly different between the two arms (38).

Following the success of oxaliplatin in combination with 5-FU, several studies have looked at the efficacy of oxaliplatin and capecitabine in combination. A meta-analysis of trials comparing capecitabine/oxaliplatin (CAPOX) to oxaliplatin/5-FU/LV regimens in the metastatic setting pooled 3,494 patients and found that although CAPOX had a lower response rate, there was no significant difference in median TTP or OS (39). Grade 3 and 4 thrombocytopenia as well as hand and foot syndrome were more common with capecitabine regimens (39). Thus, because of the toxicity profile CAPOX is an option for first line therapy in those who cannot receive or wish to avoid infusional regimens.

Oxaliplatin has also been investigated as second line therapy in advanced rectal cancer. Four multicenter trials have evaluated the efficacy of oxaliplatin after irinotecan failure. Rothenberg *et al.* randomized 463 patients who failed IFL to 5-FU/LV, or single agent oxaliplatin, or FOLFOX4 (40). FOLFOX4 was found to be superior to both 5-FU/LV and single agent oxaliplatin with a median TTP of 4.6 *vs.* 2.7 *vs.* 1.6 months, respectively (40). These findings were duplicated by Kemeny *et al.* when 214 patients were randomized to 5-FU/LV or FOLFOX4 after irinotecan failure (41). Again, FOLFOX4 was superior with a median TTP of 4.8 *vs.* 2.4 months (41). CAPOX has a role in second line therapy and has been found to have similar efficacy to FOLFOX when used as a second line agent after irinotecan failure (42). Rothenberg *et al.* randomly assigned 627 patients to FOLFOX or CAPOX and found that TTP was similar (4.8 *vs.* 4.7 months) as was median OS (12.5 *vs.* 11.9 months) (42). Toxicity profiles were also similar but there was a higher incidence of grade 3-4 diarrhea and hand/foot syndrome but fewer episodes of neutropenia in the CAPOX group (42). Given CAPOX was found to be non-inferior in the second line setting, it is an option for those who have failed irinotecan based regimens but is often deferred to FOLFOX given the side effect profile.

Neuropathy is the dose limiting toxicity of oxaliplatin (30,31,43-47). Oxaliplatin related neuropathy can present in one of two syndromes. The more common being a cumulative sensory neuropathy which begins distally and progresses proximally occurs in 10-15% of patients receiving cumulative oxaliplatin dosages of 850 mg/m^2 (48,49). The cumulative sensory neuropathy is largely reversible as 75% of patients recover roughly 13 weeks after treatment cessation (49). An acute sensory neuropathy can also occur and presents as paresthesias and dysesthesias which more commonly affect the hands, feet, and perioral region (44). This acute neuropathy can also involve jaw tightness and pharyngo-laryngo-dysesthesias (44).

Infusional reactions have been observed in up to 25% of patients receiving oxaliplatin and are characterized by fever, rash, respiratory, and ocular symptoms (50). Respiratory symptoms can be as mild as chest tightness to severe bronchospasm (50). Depending on the severity, oxaliplatin may be continued after the administration of steroids and diphenhydramine (50,51). Infusional reactions can be prevented with pre-medication with steroids and diphenhydramine as well as slowing the oxaliplatin infusion rate (50,51).

Irinotecan and mCRC

Irinotecan, a topoisomerase inhibitor, was first introduced as an active agent for mCRC in 1997 (52). Topoisomerase inhibitors function via preventing the unwinding of DNA via topoisomerase and thus prevent or halt DNA replication and thus prevent cell replication (53). The efficacy of irinotecan as a first line agent was initially defined in combination with 5-FU/LV (54-56). In three studies, irinotecan combined with 5-FU/LV had higher response rates and median TTP compared to 5-FU/LV alone (54-56). The first was performed by Douillard *et al.* where 387 patients were randomized to infusional 5-FU with or without irinotecan administered every 2 weeks (54). TTP (6.7 *vs.* 4.4 months) and median OS (17.4 *vs.* 14.1 months) were significantly improved with irinotecan (54). These results were replicated by Saltz *et al.* where IFL out performed 5-FU/LV and irinotecan as a single agent (56). Köhne *et al.* also showed improved TTP with IFL compared to 5-FU/LV (8.5 *vs.* 6.4 months) but there was only a trend towards improvement in OS in the irinotecan containing arm (20.1 *vs.* 16.9 months) (55). Toxicities were similar in all three trials and included grade 3 and 4 diarrhea and neutropenia, nausea, and mucositis (54-56).

Irinotecan in addition to capecitabine combination regimens have also been explored. A phase II study in 2007 showed promising results with a median OS of 16.8 months in the combination arm of irinotecan 250 mg/m^2 iv on day 1 + capecitabine 1,000 mg/m^2 orally twice daily on days 1 to 14, every 3 weeks (57). However, the phase III BICC-C trial in 2007 did not reflect these findings (58). This trial randomized 430 patients to capecitabine/irinotecan (CapeIRI), IFL, and FOLFIRI with the addition of bevacizumab to all arms during the trial (58). The CapeIRI arm not only had more side effects but also showed a worse PFS and trend towards worse OS compared to the other arms. Median PFS was 7.6 months for FOLFIRI, 5.9 months for irinotecan plus bolus 5-FU/LV (mIFL) (P=0.004 for the comparison with FOLFIRI), and 5.8 months for CapeIRI (58). Thus, it is currently recommended that irinotecan not be used in combination with capecitabine as first line therapy.

Irinotecan also has activity as second line therapy for mRC. Three meta-analyses pooled data on irinotecan use after failure with an oxaliplatin containing regimen (47,59,60). Within these three studies, response rates ranged 4-20% and PFS ranged 2.5-7.1 months (47,59,60). Furthermore, Grothey *et al.* pooled data and found that OS is significantly improved in patients receiving 5-FU/LV, oxaliplatin, and irinotecan at some point along their treatment course (61).

The dose limiting toxicities of irinotecan, especially in combination with 5-FU/LV, are diarrhea and neutropenia. Of important consideration, the pharmacokinetics of irinotecan can vary significantly between patients. Chemotherapies are traditionally dosed using body surface area but the pharmacokinetics of irinotecan poorly correlate with body surface based dosing (62-64). Bilirubin appears to be a better prognosticator of the incidence of neutropenia and diarrhea with irinotecan as is the presence of the UGT1A1*28 polymorphism (65-73). However, given the rarity of this polymorphism, the cost effectiveness of screening individuals for the UGT1A1*28 polymorphism is unknown (72). However, when the patients UGT1A1*28 status is known, it is recommended to dose reduce irinotecan in those that are homozygous for UGT1A1*28 (72).

FOLFOX *vs.* FOLFIRI

FOLFOX and FOLFIRI have been established as first line therapies for mRC and were compared head to head by Tournigand *et al.* in 2004 (47). Two hundred and twenty patients were randomized to FOLFIRI or FOLFOX6 and no difference between TTP (8.5 *vs.* 8.0 months, respectively) (47). At the time of progression, patients in the FOLFIRI arm were switched to FOLFOX6 and vice versa (47). As second line therapies, FOLFIRI and FOLFOX6 showed no significant difference in TTP (14.2 *vs.* 10.9 months) (47). Most importantly, there was no difference in median OS between either arm (21.5 and 20.6 months) (47). Colucci *et al.* also compared FOLFOX4 and FOLFIRI in 2005 when 360 patients were randomized (45). There was no significant difference between FOLFIRI or FOLFOX4 with median times to tumor progression of 7 months for both and a median OS of 14 and 15 months, respectively (45). The major differences between the groups were the toxicities. Gastrointestinal toxicities were more common with FOLFIRI while neuropathy and thrombocytopenia were more common with FOLFOX4 (45).

FOLFOXIRI

Given that Grothey *et al.* found that exposure to 5-FU/LV, oxaliplatin, and irinotecan at some point during the treatment course was key, the question was raised as to whether treating patients with all three agents as first line therapy would be more beneficial (61). Falcone *et al.*

conducted a trial on FOLFOXIRI *vs.* FOLFIRI as first line therapy for mRC in 244 patients (74). The results were promising with FOLFOXIRI being superior in PFS (9.8 *vs.* 6.9 months) and median OS (22.6 *vs.* 16.7 months) (74). FOLFOXIRI did have a less favorable toxicity profile with a higher rate of grade 2 and 3 neuropathy (19% *vs.* 0%) and neutropenia (50% *vs.* 28%) (74). There was no significant difference in febrile neutropenia and patients were able to tolerate the FOLFIRI with only a 9% treatment interruption rate compared to 4% in the FOLFIRI group (74). Recent data on the combination of FOLFOXIRI and bevacizumab, an antibody to the VEGF was presented at the ASCO Annual Conference in 2013. In a randomized study by Falcone *et al.*, 508 patients were randomized to FOLFIRI + bevacizumab *vs.* FOLFOXIRI + bevacizumab. In the primary analysis, FOLFOXIRI/bevacizumab had significantly greater PFS (median 12.1 months) compared with FOLFIRI/bevacizumab [9.7 months; stratified hazard ratio (HR) 0.75; 95% confidence interval (CI), 0.62-0.9; P=0.003]. Median OS for FOLFOXIRI/bevacizumab was 31.0 months compared with 25.8 months in the FOLFIRI/bevacizumab group (stratified HR 0.79; 95% CI, 0.63-1.00; P=0.054) (75). The FOLFOXFIRI/bevacizumab arm had a significantly better response rate measured by response evaluation criteria in solid tumours (RECIST) criteria (65%) compared with the FOLFIRI/bevacizumab arm (53%; P=0.006). With future studies, FOLFOXIRI combined with VEGF or EGFR inhibitors may become the first line therapy of choice in patients with mRC.

VEGF inhibitors: bevacizumab

Bevacizumab is a humanized monoclonal antibody which exerts its effect by inhibiting the effect of VEGF-A thus inhibiting its binding to the VEGF receptor and prevents angiogenesis (76). Thus, as the tumor grows, it is unable to keep up with its oxygen requirements making the tumor tissue exceedingly hypoxic, preventing further growth.

Hurwitz *et al.* demonstrated the impact of adding bevacizumab to irinotecan when they randomized 813 patients to first line IFL with or without bevacizumab (77). Those receiving bevacizumab had improved overall response, TTP, and more importantly improved median OS (20 *vs.* 16 months) (77). The BICC-C trial showed similar results with FOLFIRI combined with bevacizumab with median overall response rates of 28 months when FOLFIRI is combined with bevacizumab compared to 19.2 months with FOLFIRI alone (78). The TREE-2 trial later confirmed the benefits of adding bevacizumab to oxaliplatin containing regimens (22). With 223 patients randomized to one of three oxaliplatin/5-FU/LV regimens with or without bevacizumab, median OS with bevacizumab containing regimens was 23.7 months compared to 18.2 months in regimens without bevacizumab (22). The NO16966 trial again showed improved TTP with bevacizumab combined with XELOX or FOLFOX compared to XELOX or FOLFOX alone but no significant difference in median survival (79). More patients were noted to discontinue bevacizumab secondary to toxicities and thus lack of significant improvement in median OS could be related to patients not completing therapy (79).

Bevacizumab has also been shown to have efficacy with 5-FU/LV in patients that cannot tolerate oxaliplatin or irinotecan secondary to toxicities (80,81). Kabbinavar *et al.* found that of the 209 patients studied, those receiving bevacizumab/5-FU/LV had a median TTP of 9.2 months and OS of 16.6 months compared to 5-FU/LV in which these outcomes were 9.2 and 12.9 months, respectively (80).

Sub-analysis of the BRiTe cohort, the ARIES cohort, and a retrospective analysis of patients from community U.S. oncology practices looked at bevacizumab as a second line agent and demonstrated a survival benefit (82-84). Second line bevacizumab was directly studied in the European ML18147 study in which 820 patients who progressed on bevacizumab containing regimens were randomized to fluoropyrimidine based regimens with or without bevacizumab (85). Those receiving bevacizumab had improved median TTP (5.7 *vs.* 4.1 months) and OS (11.2 *vs.* 9.8 months) compared to those who did not receive bevacizumab (85). Thus, despite failing first line regimens that included bevacizumab, the benefit of bevacizumab was preserved when used in second line therapy. The Food and Drug Administration (FDA) approved the use of bevacizumab in this setting after these data were published.

Although generally well tolerated, side effects of bevacizumab include hypertension, proteinuria/nephrotic syndrome, bleeding, gastrointestinal (GI) tract perforation, and arterial and venous thromboembolic events (86-96). Bleeding most commonly involves epistaxis but rarely includes GI bleed, hematemesis, and intracerebral hemorrhage (89,95,96). Hypertension is the most common side effect and can be managed via regular blood pressure (BP) checks as well as antihypertensives to maintain a goal BP of <140/90 mmHg (97). Ranpura *et al.* performed a meta-analysis on bevacizumab related fatal adverse events which included 10,217 patients (98). Two point five percent

of patients experienced a fatal event related to bevacizumab with the most common being hemorrhage, neutropenia, and GI tract perforation (98).

EGFR inhibitors

Epidermal growth factor (EGF) and its receptor (EGFR) have been shown to play a role in sustaining and controlling CRCs (99,100). Messa *et al.* looked at the EGFR concentrations in 40 colorectal carcinoma specimens and found higher concentrations in tumor tissues especially those from the left side of the colon (100). EGFR has been found to play a key role in progression of cells through the G1 phase of mitosis as well as preventing apoptosis (101). This opened the door for the creation of EGFR inhibitors in the treatment of mRC.

Cetuximab is a mouse/human chimeric monoclonal Ab which is directed against the EGFR (102). Not only does cetuximab prevent binding of the EGF ligand to EGFR via binding the surface portion of the receptor, it also induces internalization of the receptor (102). In addition to direct EGFR inhibition, antibody-dependent cellular cytotoxicity (ADCC) is considered to be an important mechanism of action of cetuximab.

Cetuximab was first studied as a second line agent with one of the earliest studies in mRC in 2007 when 572 patients who failed irinotecan therapy were randomized to cetuximab or best supportive care (103). Cetuximab was found to have improved overall response, PFS, and median OS (6.1 *vs.* 4.6 months) (103). Health related QoL (HR-QoL) was also improved in those receiving cetuximab (103,104).

Cetuximab in combination with irinotecan was first investigated in the BOND trial where 329 patients who failed irinotecan were randomized to cetuximab alone or cetuximab with continued irinotecan (105). TTP was significantly improved with cetuximab/irinotecan combination compared to cetuximab as a single agent (4.1 *vs.* 1.5 months) (105). There was a trend towards improved OS with cetuximab/irinotecan combination (105). The EPIC trial followed with 1,298 patients who had failed oxaliplatin and were randomized to single agent irinotecan with or without cetuximab (106). Patients receiving cetuximab had improved PFS (4.0 *vs.* 2.6 months) and HR-QoL (106). Median OS was similar between the two arms but is likely related to a large volume of patients who were started on cetuximab after the study closed (106).

The CRYSTAL trial opened the door for cetuximab as a first line therapy (107). A total of 1,198 patients were randomized to FOLFIRI with or without cetuximab and the initial analysis showed a significantly improved overall response and PFS with cetuximab (107). Further analysis of the data which looked at wild type (WT) *KRAS* tumors showed cetuximab had improved overall response, PFS (9.9 *vs.* 8.4 months) and median OS (23.5 *vs.* 20.0 months) (108). The European phase II OPUS trial looked at FOLFOX4 with or without cetuximab as first line therapy (109). As with the CRYSTAL trial, FOLFOX4/cetuximab combination showed improved overall response and PFS with a trend towards improved OS even in the *KRAS* wild subgroup analysis (109). The CALGB trial has not published the final data yet but in the initial analysis, those receiving cetuximab with FOLFOX or FOLFIRI have shown improved response rates compared to those receiving FOLFOX or FOLFIRI alone (110). However, the United Kingdom MRC COIN and NORDIC-VII trials failed to show a difference PFS and median OS in oxaliplatin containing regimens with and without cetuximab (111,112). At this time, cetuximab is recommended in those with WT *KRAS* tumors who have failed or cannot tolerate irinotecan. It can be combined with irinotecan containing regimens but its use with oxaliplatin containing regimens has not been fully established. Currently the EXPLORE trial is underway and is comparing FOLFOX4 with and without cetuximab in those who have failed first line irinotecan (113).

Panitumumab is a fully humanized monoclonal antibody that is directed against the extracellular EGFR domain (reference). Van Cutsem *et al.* were the first to perform a phase III study with single agent panitumumab *vs.* best supportive care in 463 patients that failed 5-FU, irinotecan, and oxaliplatin (114). PFS was 13.8 weeks for those receiving cetuximab and 8.5 weeks for those receiving best supportive care (114). After the study closed, a large number of patients in the best supportive care arm were started on panitumumab which is likely why no difference in OS was observed between the two arms (114). The data was re-analyzed with those with WT *KRAS* and those that received panitumumab had improved OS (115). These mutations did predict lack of response to panitumumab.

The PRIME study looked at panitumumab in combination with FOLFOX4 as first line therapy compared to FOLFOX4 for mRC (116). In a subset of 1,183 patients with WT *KRAS*, panitumumab/FOLFOX4 had improved PFS (9.6 *vs.* 8.0 months) but no significant difference in median OS (23.9 *vs.* 19.7 months) (116). Further evaluation revealed that 108 patients that did not have *RAS* mutations at exon 2 actually did have mutations at *KRAS* exons 3 and

4 as well as *NRAS* exons 2, 3, and 4 (117). These mutations did predict a lack of tumor response to panitumumab (117).

The absence or presence of mutations in *KRAS* is extremely important when deciding whether to start EGFR inhibitors. In addition to the findings in subset analysis of the above trials involving cetuximab and panitumumab, a retrospective analysis of 394 tumors for *KRAS* mutations was performed and showed those that were WT *KRAS* had significant responses to EGFR inhibitors while those with mutated *KRAS* did not (118). KRAS is an intracellular protein downstream the EGFR pathway and mutations in the KRAS protein cause it to be turned on permanently. Thus the signal to proliferate and prevent apoptosis is propagated despite inhibition of EGFR.

To date, studies have shown the efficacy of cetuximab and panitumumab in the treatment of mRC and it can be extrapolated that they are equally efficacious. However, only one study has been designed to compare these two EGFR inhibitors head to head, the ASPECCT trial (119). The trial is still ongoing but prelim data was presented in the 4th annual ASCO GI cancer symposium in 2007 and showed that cetuximab and panitumumab are equally efficacious in terms of PFS (4.4 *vs.* 4.1 months) and OS (10.0 *vs.* 10.4 months) (119).

KRAS mutations in exon 2 (codons 12 and 13) are a successful predictive marker for cetuximab efficacy, researchers have identified additional mutations in *KRAS* and in *NRAS*, which is also mutated at a low frequency (<5%) (120,121). Retrospective analyses of tumor samples from the EGFR inhibitor studies have been expanded to include mutations in *KRAS* exon 3 codons 59 and 61 and exon 4 codons 117 and 146, as well as mutations in *NRAS* exons 2, 3, and 4 (116,117). In a retrospective analysis of the PRIME study, 17% of patients were identified too have a mutated *RAS* isoform outside of exon 2 (116,117). Use of the expanded version of *RAS*-mutation further identified a cohort of patients benefiting from EGFR inhibition (116,117). The PRIME study demonstrated improved OS for panitumumab plus FOLFOX4 *vs.* FOLFOX4 alone, specifically in first-line treatment of WT *RAS* patients (median OS, 26.0 *vs.* 20.2 months; HR 0.78; 95% CI, 0.62-0.99; P=0.04) (117).

Improved selection of *RAS* WT patients helped demonstrate a clear benefit of cetuximab in the FIRE-3 trial (122). OS was improved in patients with *RAS* WT tumors who were treated with cetuximab plus FOLFIRI, compared with the bevacizumab plus FOLFIRI arm (33.1 *vs.* 25.6 months, respectively; P=0.011) (122). Patients with *RAS*-mutant tumors showed worsened PFS when cetuximab was added to FOLFIRI (6.1 *vs.* 12.2 months in the bevacizumab arm; P=0.004), and cetuximab was not associated with an OS benefit in these patients (122). These results highlight the importance of providing EGFR inhibitors only to those patients with *RAS* WT tumors and consideration of using expanded criteria to identify *KRAS* mutations and patients not likely to benefit from this approach.

The role of EGFR inhibitors in front-line therapy and the value of expanded *RAS* testing will be validated with the release of data from the upcoming CALGB/SWOG 80405 trial. Like the retrospective analyses described above, this study will also review efficacy (bevacizumab plus FOLFOX or FOLFIRI *vs.* cetuximab plus FOLFOX or FOLFIRI) in light of the expanded mutational analysis.

Common EGFR inhibitor side effects include weakness, malaise, nausea, electrolyte abnormalities, and acneiform rashes. Infusion reactions occur in 25% of patients treated with cetuximab (123). These reactions are often severe, most common with the first infusion and within the first 3 hours of infusion (123).

Combined bevacizumab with EGFR inhibitors

Given the success of bevacizumab, EGFR inhibitors, and combination therapy in improving OS, combining the EGFR and VEGF inhibition has been studied. This question was addressed in the BOND-2, PACCE, and CAIRO2 trials (124-126). The BOND-2 trial, cetuximab and bevacizumab were combined with the addition of irinotecan to one of the arms in patients that failed oxaliplatin (124). The initial data was promising and showed significantly improved PFS (7.3 *vs.* 4.9 months, respectively) and OS (15.4 *vs.* 14.4 months, respectively) with cetuximab/bevacizumab/irinotecan compared to cetuximab/bevacizumab (124). However, the PACCE and CAIRO2 studies were larger and looked at the combination of EGFR inhibitors with bevacizumab as first line therapies (125,126). The PACCE trial compared bevacizumab with either oxaliplatin or irinotecan containing regimens with or without panitumumab (125). Hecht *et al.* had to close the study early after those receiving panitumumab with bevacizumab had worsened OS compared to those not receiving panitumumab (19.4 *vs.* 24.5 months respectively) (125). A significant increase in skin toxicities, diarrhea, infections, and pulmonary embolisms were also noted in those receiving panitumumab/bevacizumab/

oxaliplatin (125). The CAIRO2 study looked at combination XELOX and bevacizumab with and without cetuximab and had similar findings to the PACCE trial (126). PFS was significantly decreased with the cetuximab arm (9.4 *vs.* 10.7 months) and the toxicity profile was worse with cetuximab (126). Thus, given the lack of survival benefit and increased incidence of grade 3 and 4 toxicities, combination bevacizumab and EGFR inhibitors is not recommended.

Bevacizumab *vs.* EGFR inhibitors

The FIRE-3 trial presented at ASCO 2013 introduced data to challenge the use of bevacizumab over EGFR inhibitors in the first line metastatic setting (127). Five hundred and ninety-two patients with WT *KRAS* were randomized to FOLFIRI with either bevacizumab or cetuximab (127). The first analysis showed no difference in response rates or PFS between the two arms (127). However, the cetuximab arm had a significantly improved OS compared to bevacizumab (28.8 *vs.* 25.0 months, respectively) (127). Updated data were presented later in 2013 at the annual European Cancer Congress (ECC) forum and excluded patients with mutations in *KRAS* exon 2, but also those with mutations in *KRAS* exons 3 and 4 as well as *NRAS* exons 3 and 4 (122). With these exclusions, the difference in median OS was more pronounced with 33.1 months for the cetuximab arm compared to 25.9 months for bevacizumab (122). Although the trial has not published its final data, it has suggested that EGFR inhibitors may be appropriate for first line use. Both the final data from the FIRE-3 trial and the currently ongoing U.S. intergroup trial C80405 will help answer this question once the final data is published.

Summary

Since the introduction of 5-FU over 40 years ago there have been major advances in the treatment of locally advanced and mRC. The addition of neoadjuvant CRT has improved outcomes and QoL for our patients. This approach is now widely accepted and the standard of care throughout the world. Adding second-generation chemotherapeutics to the neoadjuvant setting has not improved outcomes to date, however, new approaches are under investigation in locally advanced disease.

Advances in treatment regimens for mRC have been extensive. Combination regimens with infusional 5-FU, such as FOLFOX and FOLFIRI, have significantly extended life. Currently the triplet combination FOLFOXIRI is showing additional promise but further studies are needed. The advent of EGFR and VEGF inhibitors has significantly improved outcomes in patients with advanced disease. These agents have demonstrated activity and reasonable toxicity profiles. Their addition to chemotherapy backbones has led to improved PFS and OS. Further development and expansion of our understanding of *KRAS* mutations and additional predictive and prognostic markers will continue to lead to improved outcomes. The future appears promising.

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Footnote

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Therapeutic approaches in the management of locally advanced rectal cancer

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Abstract: Combined-modality therapy, using radiotherapy and chemotherapy with surgery, has been the traditional therapeutic algorithm for locally advanced rectal cancer. Standard of care in the United States has evolved to include neoadjuvant concurrent chemotherapy and radiotherapy followed by surgical excision and adjuvant chemotherapy. This approach has led to a significant improvement in local recurrences (LR), to the point where distant sites are the more common site of failure. Further improvements in local control have failed to improve overall survival. This article reviews historical trials that shifted the treatment paradigm to the current standard of care, as well as recent research trials, which have sought to incorporate new treatment methodologies, and treatment agents to improve outcomes. Finally this article describes ongoing studies and their potential impact on the future of therapeutic management of locally-advanced rectal cancer.

Keywords: Rectal cancer; neoadjuvant therapy; combined modality therapy; radiation therapy

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Combined-modality therapy

Surgical resection has been the mainstay of definitive therapy for rectal cancer. Historically, recurrence rates with surgery alone were upwards of 50% (1-3). Adjuvant therapy in the form of combined post-operative radiotherapy and 5-fluorouracil (5-FU)—based chemotherapy was shown to improve local control and provide an overall survival benefit over surgery alone or surgery plus irradiation (4,5). As such, postoperative chemoradiotherapy (CRT) was recommended as the standard of care in patients with stage II (T3-T4) or stage III (node positive) rectal cancer by a National Institute of Health consensus conference in 1990 (6).

Total mesorectal excision (TME)

In addition to the incorporation of CRT, the now-widespread use of TME as pioneered by Heald *et al.* (7) significantly improved local recurrence (LR) rates when compared to rates using standard surgical technique. LR rates at 5 years in surgery-only arms of large randomized trials that did not mandate TME use were typically in excess of 25% (8,9), compared to 11% for surgery-only arms in trials that mandated TME use (10). When radiotherapy was added to surgical resection with standard technique, local control was improved by over 50% (local relapse rate of 11% with RT, 27% with surgery alone), and it also improved overall survival (9). Once TME was incorporated, radiotherapy had the same relative improvement in local relapse rates, but with less absolute benefit (5% with RT, 11% with TME alone) (11). Radiotherapy, when combined with TME, had a lesser absolute local control benefit, and thus failed to further increase overall survival.

Neoadjuvant chemoradiation

The current standard of care in the United States for stage II and stage III rectal cancer is neoadjuvant chemoradiation followed by surgical resection using a TME technique. The paradigm shift from postoperative to neoadjuvant therapy was largely a result of the German Rectal Cancer Study. The study randomized 823 patients with clinical

stage T3-4 or node positive rectal cancer to surgery with TME followed by postoperative CRT or preoperative CRT followed by TME 6 weeks later. The preoperative regimen consisted of 50.4 Gy delivered using either a 3- or 4-field box technique with continuous-infusion 5-FU (1,000 mg/m^2) on days 1-5 of weeks 1 and 5. The postoperative regimen was identical, except for a 5.4 Gy boost (55.8 Gy total) to the postoperative tumor bed. In both arms, an additional 4 cycles of bolus 5-FU (500 mg/m^2 every 4 weeks) was given, starting either 4 weeks after surgery (in the preoperative group), or 4 weeks after chemoradiation (in the postoperative group).

At 5 years, there was a statistically significant lower number of LRs in the preoperative CRT arm (6% *vs.* 13%, P=0.006). However, there were no significant differences in the rates of distant metastases, disease-free survival, or overall survival. After preoperative CRT, there was evidence of tumor downstaging, with 8% of patients demonstrating histopathological complete response (pCR). Twenty five percent of patients receiving preoperative CRT had positive lymph nodes (compared to 40% who had surgery first in the postoperative CRT arm). Prior to randomization, every patient was evaluated by a surgeon for the need to perform an abdominoperineal resection (APR), resulting in permanent colostomy. In the group of patients deemed to require APR, preoperative CRT resulted in a higher rate of sphincter-preserving surgeries (39% *vs.* 19%, P=0.004) actually performed. There were fewer grade 3 or 4 acute (27% *vs.* 40%, P=0.001) and late toxicities (14% *vs.* 24%, P=0.001) in the preoperative CRT group (12). After 11 years of follow up, the significant LR benefit persisted (10-year cumulative incidence of 7.1% *vs.* 10.1%, P=0.048). There were also no significant differences in the 10-year cumulative incidence of distant metastases, disease-free survival and overall survival (13).

The findings of the German rectal trial were further supported by that of the National Surgical Adjuvant Breast and Bowel Project (NSABP) R-03 trial, which also compared preoperative and postoperative CRT. The radiation (45 Gy plus a 5.4 Gy boost) and chemotherapy (5-FU plus leucovorin) were identical in both arms. Surgery (TME was not mandated) followed CRT after 8 weeks in the preoperative group. The trial closed early secondary to poor accrual. Despite enrolling only 267 of a planned 900 patients, the trial demonstrated a 5-year disease-free survival improvement (64.7% *vs.* 53.4%) favoring preoperatively-treated patients. A pCR was achieved in 15% of the preoperative patients (14).

Shortly after publication of the landmark German study, the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology for locally advanced resectable rectal cancer included neoadjuvant RT with concurrent 5-FU infusion, followed by TME and an adjuvant course of consolidative chemotherapy. This approach has been widely adopted across the United States (15).

Neoadjuvant short-course radiotherapy

In some European countries, instead of preoperative CRT, a short-course of preoperative radiotherapy alone (SC-RT) is used. The Swedish Rectal Cancer Trial randomized 1,168 patients to receive 25 Gy in 5 fractions followed by surgery within 1 week, or surgery alone. TME was not mandated in this trial. At 5 years, radiotherapy reduced LRs (11% *vs.* 27%, P<0.001), and improved overall survival (58% *vs.* 48%, P<0.004) compared to surgery alone (9). After 13 years, these benefits persisted (8). The Dutch TME trial randomized 1,805 patients to be treated with or without SC-RT followed by TME. At 5 years, a LR benefit was seen (5.6% *vs.* 10.9%, P<0.001); however no improvement in overall survival was demonstrated. Additionally, the LR benefit was limited to those patients with negative circumferential resection margins (CRM) (10). After 12 years of follow up, the effect of SC-RT on LR persisted. In an unplanned subgroup analysis, in patients with a negative CRM, SC-RT was found to improve cancer-specific survival (50% *vs.* 40%, P=0.03) (11).

The Medical Research Council (MRC) in the United Kingdom and the National Cancer Institute of Canada (NCIC) randomized 1,350 patients in four countries to preoperative radiotherapy (25 Gy) or to surgery with selective postoperative CRT (45 Gy in 25 fractions with concurrent infusion 5-FU). CRT was given only to patients with positive CRM (57 of 606 patients). TME was not mandated but was performed in 92% of patients. With a median follow up of 4 years, LR was 4.4% in the preoperative SC-RT group, versus 10.6% in the selective postoperative CRT group (P<0.0001). Also noted was an improvement in disease-free survival (77.5% *vs.* 71.5%, P=0.013) without an overall survival benefit (16).

Neoadjuvant short-course radiotherapy versus long-course CRT

Both approaches to neoadjuvant therapy described above have shown benefits over no additional therapy and adjuvant

chemoradiation. However, due to differences in eligibility criteria, efficacy comparisons between trials using different approaches are problematic. Trials that used SC-RT enrolled patients with 'resectable' rectal cancer (cT1-3Nx), where the CRT trials allowed only Stage II (T3-4) or Stage III (node positive) disease.

Bujko *et al.* were the first to conduct a randomized trial between the two neoadjuvant therapies. A total of 316 patients with clinically staged T3 or T4 rectal cancers were randomized between neoadjuvant short-course radiotherapy (25 Gy in 5 fractions) followed by TME within 7 days or "long-course" CRT (LC-CRT, 50.4 Gy in 28 fractions with concurrent 5-FU and leucovorin) with TME to follow at 4-6 weeks. Postoperative chemotherapy was allowed as indicated. This trial was powered to show a difference of 15% or greater in sphincter preservation (17,18).

After 4 years of follow up, the authors reported no significant difference in sphincter-sparing, LR (9% *vs.* 14% in short course and long course, respectively), or survival. Acute toxicity was higher in the CRT group (18%, compared to 3% in the radiotherapy-alone group, P<0.001). However, there was no difference in late toxicity or severe late toxicities (17).

More recently, Ngan *et al.* reported the outcomes of the Trans-Tasmanian Radiation Oncology Group (TROG) trial 01.04. A total of 326 patients with ultrasound or MRI-staged T3N0-2 rectal cancers were randomized between short-course preoperative radiotherapy (25 Gy in 5 fractions) followed by surgery within 1 week or long-course preoperative CRT (50.4 Gy in 28 fractions with concurrent 5-FU) followed by surgery within 4-6 weeks. Both groups received adjuvant chemotherapy (six cycles for the short-course group, four cycles for the long-course group). The trial was powered to show a 10% absolute difference in LR (15% short course, 5% long course).

After 3 years of follow up, they reported no significant difference in local relapse (7.5% for short-course, compared to 4.4% for long course, P=0.24). Additionally, no difference was seen in 5-year distant recurrences, relapse-free survival, or overall survival. There was no difference noted in sphincter-sparing. Grade 3 or 4 late toxicity, as reported at 3 years, was not different between the two groups (19).

A third randomized trial of neoadjuvant regimens is the Stockholm III trial, and is only published as an interim analysis. This study randomized 303 patients amongst 3 treatment arms. Two treatment arms used short course RT (25 Gy in 5 fractions) followed by either immediate surgery within 1 week (n=118), or delayed surgery in 4-8 weeks (n=120). Patients in the third treatment arm received long course radiotherapy (50 Gy in 25 fractions) alone, followed by surgery in 4-8 weeks. The significant finding reported in the interim analysis was the rate of postoperative complications in patients randomized to short-course radiotherapy and surgery within a week. Postoperative complications differed according to the timing of surgery relative to the start date of radiotherapy. Significantly more complications were seen in 24 of 37 (65%) patients who underwent surgery 11-17 days after the start of RT, than in 29 of 75 (39%) patients who underwent surgery less than 11 days after the start of RT (P=0.04) (20).

Without any data to-date to suggest significant differences in survival, local control, or sphincter-sparing between neoadjuvant approaches, careful study of the long-term consequence of these treatments is paramount. Quality of life (QoL) data from the Polish study is reported at 1 year after surgery, with patient-reported QoL quantified using the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 questionnaire. Anorectal and sexual function were reported using a separate questionnaire. At a median time from surgery of approximately 1 year, there were no significant differences in global function in symptoms scales for QoL between patients who received SC-RT or LC-CRT prior to surgery. There were also no differences between patient groups in answers to questions regarding anorectal or sexual function (21).

QoL data from TROG 01.04 is reported in abstract form only. Unlike that from the Polish study, 5-year data is reported. The QLQ-C30 questionnaire was used to assess global health status, and the EORTC QLQ-CR38 module was used to measure pelvic function. At 5 years, global health status was not statistically different between arms. There was no clear difference in pelvic functioning or symptoms between the SC-RT and LC-CRT arms. This data has not yet been peer-reviewed (22). Finally, a German cross-sectional study was performed in 225 patients who either underwent SC-RT (29 Gy in 10 fractions) or LC-CRT prior to surgery and were still disease-free. With a median follow-up time of 67 months, QoL analysis was performed using the EORTC QLQ-C30 and QLQ-CR29 questionnaires. Despite a modified SC-RT fractionation, there was no difference in QoL observed between patients who received SC-RT and LC-CRT, except for improved physical functioning in the LC-CRT group (23).

The debate between SC-RT and LC-CRT as the optimal preoperative regimen prior to TME is ongoing. None of

Table 1 Outcomes of four recent trials incorporating oxaliplatin into neoadjuvant chemoradiation prior to surgical resection

Parameters	STAR-01	ACCORD 12	CARO/ARO/AIO-04	NSABP R-04
Number of patients	747	598	1,236	1,608
Preoperative RT (Gy)	50.4	50	50.4	50.4
5-FU based chemotherapy	CI-5-FU 225 mg/m^2 daily	Oral CAPE 1,600 mg/m^2 daily	CI-5-FU 250 mg/m^2 daily (+ OX) *vs.* Bolus 5-FU 1,000 mg/m^2 (week 1 and 5) alone	CI-5-FU 225 mg/m^2 daily OR CAPE 1,600 mg/m^2 daily
OX with *vs.* without	OX 60 mg/m^2 weekly	OX 50 mg/m^2 weekly	OX 50 mg/m^2 weekly	OX 50 mg/m^2 weekly
pCR rate (%)	16 *vs.* 16	19 *vs.* 14	17 *vs.* 13*	21 *vs.* 19
Sphincter-preservation (%)	81 *vs.* 79	78 *vs.* 75	76 *vs.* 75	60 *vs.* 64
Grade 3-4 toxicity (%)	24 *vs.* 8	25 *vs.* 11	23 *vs.* 20	15 *vs.* 7**

*, statistically significant; **, grade 3 and 4 diarrhea only; OX, oxaliplatin.

the data above shows significant differences either in long-term oncologic outcomes or patient-reported QoL.

Concurrent chemotherapy with preoperative radiotherapy

Fluorouracil-based chemotherapy has long been part of adjuvant therapy for rectal cancer. The route of administration (as a continuous or bolus infusion) has been examined in several studies when CRT was given in the adjuvant setting. One intergroup study compared continuous infusion (CI) 5-FU (225 mg/m^2 daily) and bolus 5-FU (500 mg/m^2 daily on days 1-3 and 36-39) during adjuvant radiotherapy. CI 5-FU was associated with reduced distant metastases and improved overall survival (24). In contrast, intergroup study INT-0144 showed that CI 5-FU and bolus 5-FU during adjuvant radiation for rectal cancer resulted in no difference in three-year disease-free survival or overall survival (25).

Capecitabine is an orally-administered prodrug that is enzymatically converted to 5-FU, and was designed to mimic CI 5-FU. In a German phase III trial, 392 patients with stage II/III rectal cancer were randomized to receive either CI 5-FU or capecitabine concurrently with radiotherapy (50.4 Gy) either in the adjuvant (213 patients) or neoadjuvant (161 patients) setting. There was no difference in local relapse or overall survival. However, patients receiving capecitabine had increased rates of tumor downstaging (55% *vs.* 39%) and pathological node-negative rates (71% *vs.* 56%) compared to those receiving CI 5-FU. Patients receiving capecitabine also had significantly more hand-foot skin reactions (31% *vs.* 2%), but less neutropenia (35% *vs.* 25%) overall (26). Results of NSABP R-04 have been reported twice in abstract form so far (27,28). In this phase III trial, patients were randomized between CI 5-FU and oral capecitabine, with or without the addition of oxaliplatin (4 arm study). In both abstract reports, there were no statistical differences between pCR rate, sphincter-preservation, or surgical-downstaging. Taken together, the results of these two trials support oral capecitabine as a substitute for CI 5-FU when given concurrently with preoperative radiotherapy for rectal cancer.

Oxaliplatin, in combination with 5-FU and leucovorin (folinic acid), as part of the FOLFOX chemotherapy regimen, plays an important role in the treatment of colorectal cancer (29). As such, several trials have investigated the addition of oxaliplatin to preoperative 5-FU-based chemoradiation. The results of these trials are shown in *Table 1*. In summary, the addition of oxaliplatin to concurrent preoperative CRT has shown no improvement in tumor response (based on pCR rates), or surgical outcomes (based on sphincter-preservation rates). Its addition does significantly increase the toxicity during preoperative treatment. Thus, its addition cannot be justified based on these results.

Adjuvant chemotherapy

Following neoadjuvant CRT and surgical resection for stage II/III rectal cancer, the NCCN Guidelines recommend adjuvant chemotherapy regardless of the surgical pathology results. Despite limited data demonstrating the efficacy of this approach, adherence to this recommendation is fairly high. A recent study of adjuvant chemotherapy use

at several NCCN institutions between 2005 and 2010 showed that of 1,193 patients who received neoadjuvant therapy, 990 (83%) were also prescribed and initiated further adjuvant chemotherapy (30). Of the remaining patients, the most frequent reason for not recommending chemotherapy was comorbid illness (25 of 50 patients). The most frequent reason that chemotherapy was recommended but not received by the patient was patient refusal (54 of 74 patients).

Most of the evidence for the role adjuvant chemotherapy is from older studies using postoperative therapy alone. EORTC trial 22921 was a four-armed study comparing preoperative radiotherapy (45 Gy in 25 fractions) with or without concurrent chemotherapy (5-FU and leucovorin) and adjuvant chemotherapy (4 or more cycles, every 3 weeks). A total of 1,011 patients were randomized; 787 patients who had an R0 surgical resection with no distant spread before or at surgery were eligible for analysis of outcome by adjuvant treatment. In the initial report, there was no effect of adjuvant chemotherapy on disease-free survival or overall survival for the group as a whole. Adherence to postoperative chemotherapy was poor (43% of patients received at least 95% of the planned fluorouracil without delay) (31). Later, an unplanned subgroup analysis was published, showing a statistically significant survival benefit in patients who underwent tumor downstaging (ypT0-2) from neoadjuvant therapy (32). Long-term results (median follow up of 10.4 years) showed no difference in disease-free survival or overall survival in patients with tumor pathological downstaging, those without, or the group as a whole (33).

Adjuvant chemotherapy, for now, remains as part of recommended therapy in the United States. At several NCCN institutions, the rate of adjuvant chemotherapy prescription and initiation is quite high. However, with increased toxicity, poor adherence to the full prescription course and limited evidence of its benefit, newer clinical trials appear to be shifting further chemotherapy upfront instead of the adjuvant setting.

Neoadjuvant chemotherapy and CRT (or radiotherapy)

The EORTC study above and others (34) have concluded that the addition of chemotherapy to 'long-course' preoperative radiotherapy significantly improved local control. Local control has improved to the point that distant relapses are the more common site of first recurrence. With poor adherence to adjuvant chemotherapy and little evidence of its value, the role of neoadjuvant chemotherapy prior to neoadjuvant CRT is being actively investigated. Potential advantages of upfront chemotherapy include improved compliance, and the early treatment of micrometastases.

One phase II trial (Expert) out of the United Kingdom enrolled patients with high risk disease (based on CRM margin risk, low-lying tumors, T4 and/or node positive tumors) to receive 12 weeks of neoadjuvant capecitabine and oxaliplatin (CAPOX) followed by single-agent (capecitabine) CRT (54 Gy), TME, and four cycles of postoperative adjuvant capecitabine. A total of 105 eligible patients were enrolled. A total of 95 patients underwent TME, of whom 21 had a pCR (20% of eligible patients). Three-year progression-free and overall survival were 68% (95% CI, 59-77) and 83% (95% CI, 76-91), respectively. The authors report acceptable safety despite nine cardiac or thromboembolic events (9%) of which four died, requiring amendment of the protocol for cardiovascular safety (35).

Another randomized, phase II Spanish trial (Grupo Cancer de Recto 3 Study) randomized 108 patients with locally advanced rectal cancer to receive either preoperative CRT (50.4 Gy with concurrent capecitabine and oxaliplatin) followed by TME and postoperative chemotherapy (capecitabine-oxaliplatin), or 'induction' chemotherapy (capecitabine-oxaliplatin) followed by the same CRT and TME (no postoperative chemotherapy). The group of patients that received induction chemotherapy had greater chemotherapy dose exposure than those patients that received adjuvant chemotherapy. However, there was no statistical difference between pCR rate (13.5% and 14.3%), downstaging, tumor regression, or R0 resection. Grade 3 and 4 toxicities were similar in both arms during CRT. Toxicity was compared between the adjuvant chemotherapy window in the first group and the induction chemotherapy window in the second group. Despite a greater chemotherapy exposure for patients who received induction chemotherapy, there was greater grade 3 and 4 toxicity during adjuvant chemotherapy (54% *vs.* 37%, respectively, P=0.0004) (36).

Another approach being investigated in phase III studies, is the use of short-course radiotherapy (25 Gy in 5 fractions), followed by neoadjuvant capecitabine-oxaliplatin chemotherapy and TME. This approach is the experimental arm in both the Rectal Cancer and Preoperative Induction Therapy Followed by Dedicated Operation (RAPIDO) trial, and a Polish Colorectal Cancer Study Group (5-FU,

leucovorin and oxaliplatin chemotherapy) trial. The standard arm in these trials is long-course CRT. It will be imperative for both trials to carefully detail not only differences in outcomes, but also toxicity (acute, late and post-surgical complications) and QoL to definitively differentiate the two approaches.

Neoadjuvant chemotherapy alone

In the TME era, with high-quality MRI and ultrasound staging, the option for omitting preoperative radiotherapy in carefully selected patients has been raised. Preliminary, pilot data out of Memorial Sloan-Kettering Cancer Center treated 32 patients with FOLFOX (5-FU, leucovorin and oxaliplatin) plus bevacizumab alone followed by TME. Pathologic complete response rate was 25% with a 4-year LR rate and disease-free survival of 0% and 84%, respectively (37).

These exciting results have prompted the preoperative radiation or selective preoperative radiation and evaluation before chemotherapy and TME (PROSPECT or N1048) trial. In this multi-institution, phase II/III study, only patients with 'low-risk' Stage II/III rectal cancer [candidates for sphincter-sparing surgeries, CRM not-threatened, non-T4 tumors, clinically node-positive disease must be N1 (1-3 nodes) only] are eligible. Patients are randomized to one of two treatment arms. Group 1 patients receive six cycles of FOLFOX alone followed by restaging. Patients with a greater than 20% tumor regression proceed to surgery with TME. Patients with a less than 20% tumor response undergo CRT followed by TME. Group 2 receives standard-of-care neoadjuvant CRT, followed by TME. Patients in both groups may receive adjuvant chemotherapy.

Targeted therapies

Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor, has been studied in phase I and II trials incorporating it with conventional preoperative 5-FU based CRT. The data so far has shown encouraging pCR rates (16-32%) (38-41), but several studies report increased rates of postoperative wound complications (38-42).

Cetuximab and panitumumab are both humanized monoclonal antibodies against epidermal growth factor receptor approved for use in patients with metastatic colorectal cancer. Phase I/II trials with cetuximab use in preoperative CRT for rectal cancer, as a whole, have shown mixed efficacy with not-insignificant grade 3-4 gastrointestinal toxicity (43). One randomized phase II clinical trial (EXPERT-C) was conducted following a previous trial (EXPERT) looking at neoadjuvant chemotherapy, followed by chemoradiation then surgery. In the EXPERT-C trial, 165 patients received capecitabine-oxaliplatin chemotherapy, followed by capecitabine CRT with or without cetuximab, then TME. In tumors with wild-type k-ras, addition of cetuximab did not improve the primary endpoint of pCR or progression-free survival. Cetuximab did improve response rates and 3-year overall survival (HR 0.27, P=0.034) (44).

The effect of these targeted therapies on long-term outcomes and side-effects requires further study, although the mixed results thus far have been disappointing.

IMRT for rectal cancer

As seen in all of the studies described here, the ability of patients to adhere to treatment schedules and complete full courses of chemotherapy and CRT is a major issue. The most common radiation-induced toxicities are skin and gastrointestinal (diarrhea)-related. Intensity-modulated radiotherapy (IMRT) use in other disease sites within the pelvis, such as prostate, anus and GYN, has been shown to reduce treatment-related morbidities (45-47).

Thus far, evidence for IMRT use in rectal cancer has been building. One dosimetric study has shown that IMRT, when compared to 3D-conformal radiotherapy (3D), reduces the volume of small bowel receiving 15 Gy or higher (V15) (48), a factor shown to be associated with increased rates of Grade 3 diarrhea (49). Another dosimetric study showed that the small bowel V15 is improved, even if the patient is treated in the prone position with a belly board (a device often used to displace small bowel out of the radiation field (50). Clinical data, to-date, consists mostly retrospective series showing reduction in grade 2 or higher GI toxicity and diarrhea (51,52). A recently completed phase II study, RTOG 08-22, examined the role of preoperative radiotherapy using IMRT concurrently with capecitabine and oxaliplatin, and results are pending.

Conclusions

In the treatment of locally advanced rectal cancer, major paradigm shifts such as the TME surgical technique and the use of neoadjuvant therapy instead of adjuvant, have led to significant advances in the local control and overall survival of these patients. In the United States and several European

countries, the standard of care is neoadjuvant CRT followed by surgery with TME and adjuvant chemotherapy. In some countries, short-course radiotherapy, in lieu of CRT, is used. In that case, surgery follows immediately (within 1 week) as opposed to a 4-8 weeks after CRT. Two major phase III trials have compared these two approaches, neither of which found any differences in oncologic or QoL outcomes. A clear theme from several studies included in this review, is that adjuvant therapy adds to patient toxicity. The toxicity of adjuvant chemotherapy has resulted in low adherence to the protocols, and there does not appear to be a clear benefit to this approach. In the modern era of more accurate MRI and/or ultrasound staging, and newer chemotherapeutic drugs and targeted therapies, recent research has attempted to incorporate them into the neoadjuvant setting with mixed success. Current ongoing trials seek to use more aggressive chemotherapy up front, with or without radiotherapy or CRT prior to surgery. Going forward, it will be imperative to balance aggressive therapy to control local relapse and distant metastases with long-term toxicity and effects on patient QoL, as these patients live longer after surviving their disease. It is important to continue to investigate treatments to maximize therapeutic effect (neoadjuvant FOLFOX, targeted drugs), but also to minimize toxicity (IMRT use).

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Footnote

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Multidisciplinary approach and targeted agents increase resectability of liver-limited metastases from colorectal cancer

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The outcome of patients with initially unresectable metastatic colorectal cancer have greatly improved in the past years (1) and at least three important factors have certainly contributed: a multidisciplinary approach, the availability of targeted agents and the knowledge of the molecular pathways of metastatic colorectal cancer. On June 2013, Ye *et al*. (2) published on the *Journal of Clinical Oncology* the results of a single-center randomized trial investigating the effect of the addition of cetuximab to first-line chemotherapy for radical resection rate of liver metastases from colorectal cancer. An editorial by N. Kemeny accompanied the paper (1) and, on December 2013, a correspondence between the authors and other international working groups was published on the same journal (3-5). Overall 138 Chinese patients affected by unresectable synchronous liver-limited metastases (LLM) from KRAS wild-type resected colorectal cancer were enrolled and they were randomized to receive anti-EGFR monoclonal antibody Cetuximab plus first-line fluorouracil-based doublets of chemotherapy (FOLFOX or FOLFIRI) or chemotherapy alone as first-line treatment. The mean age of study population was young, nearly 58 years, with 80% of patients with optimal general condition and ECOG performance status 0. The two arms of treatment (cetuximab plus chemotherapy versus chemotherapy alone) were well balanced regarding the motivation of non-resectability; at the same time, the experimental arm had 22% less patients with features indicating a worse prognosis (1). Nearly 30% of patients received the fluorouracil plus irinotecan combination (FOLFIRI) and a further 20% of patients received the sequence of both irinotecan and oxaliplatin-based doublets. One of the most significant aspects of this trial was that resectability was evaluated, before and after treatment, by a multidisciplinary team involving at least three liver surgeons and one radiologist. After treatment, all of the following issues must be present in order to undergo resection:

(I) Capability to obtain a radical resection;
(II) Preservation of at least two contiguous liver segments;
(III) Preservation of adequate vascularization and biliary drainage;
(IV) Preservation of an adequate hepatic function (at least 20% of healthy liver).

At a median follow-up of 25 months, the radical resection rate (RRR) was respectively 25% and 7% in the cetuximab plus chemotherapy and in the chemotherapy alone arms, with an odds ratio in favor of the experimental arm of 4.37 (primary endpoint). Overall survival in the two groups of resected patients was comparable and about 40 months but, unfortunately, nearly 66% of resected patients recurred. Relatively to the safety, adding cetuximab to chemotherapy increased uniquely the occurrence of severe acneiform rash (12.9% versus 2.9%).

The results of this study confirm the concept of "conversion chemotherapy" in which a marked tumoral shrinkage after first-line chemotherapy can lead to the radical resection of liver metastasis with a relevant prolongation of survival, although often the disease will recur. Even in the setting of unresectable metastases, the addition of targeted agents to standard chemotherapy has improved outcomes (6,7) while, on the contrary, when metastases can be initially resected nor "standard" chemotherapy (8) nor addition of Cetuximab (9) have demonstrated to increase OS. Despite encouraging premises, there aren't at the moment randomized multicentric trials able to confirm if chemotherapy plus cetuximab can be considered the standard of care for patients with LLM from resected, KRAS wild type, colorectal cancer. However the

encouraging results of Ye *et al*. (2) about RRR in LLM can be updated by some recent trials conducted in the setting of "conversion chemotherapy. The recent update of the CELIM phase II trial (CEtuximab in neoadjuvant treatment of unresectable colorectal LIver Metastases), conducted on 114 European patients with unresectable LLM, show RRR data comparable between Cetuximab plus FOLFOX and Cetuximab plus FOLFIRI (10). Median OS and progression-free survival for resected patients were comparable to the trial by Ye *et al.* (2). 53 versus 46 months and 10 versus 10.7 months; overall survival at 5 years was 46% in the CELIM trial (10). In a Japanese trial by Kataoka *et al*. (11), 115 patients with LLM and resected primitive carcinoma were treated with the association of chemotherapy with various targeted agents. A multidisciplinary team evaluated resectability and allocated patients to three groups: resectable, "conversion therapy" and unresectable. An overall 18% resection rate was obtained with a statistically different survival between the "conversion" and the unresectable group. However PFS in the "conversion" group was clearly inferior respect to the "resectable" group (3 versus 16 months), thus confirming that the initial extent of the disease remains the more relevant prognostic factor and that respectability is often not equivalent to cure.

Taken together with recent advances in molecular biology, the results of these trials can ameliorate our clinical practice. First, in patients with LLM, the definition of resectability must be performed by a multidisciplinary team involving both liver surgeons and liver radiologists; particularly, the use of second-level imaging techniques mainly magnetic resonance (MR) or positron emission tomography/computed tomography (PET/CT) scan should be strongly considered (12), owing in mind that the potential benefit of a prolonged survival is realistic. Moreover, when resectability is the aim of treatment, the choice of first-line drugs, particularly in KRAS wild-type patients should comprehend, in fit patients, more than a standard doublet FOLFOX/FOLFIRI. The addition of cetuximab is a valid option (1,10) with a toxicity profile involving mainly the skin: in the CELIM trial grade 3-4 skin toxicity was present in 15-22% of patients (13); in the trial by Ye *et al*. in 13% (2). These data are in accord with available literature, from which it appears that these toxicity is in part preventable (14) and in the majority of cases manageable with dedicated algorithms (15). At the same time, recent evidences showed that a comprehensive analysis of both KRAS and NRAS should be performed before treatment with anti-EGFR monoclonal antibodies (7). Moreover a possible role for the analysis of further genes such as BRAF, PIK3CA and PTEN is under evaluation (16). Facing such complexity, tools able to perform molecular analysis during chemotherapy, like for example, liquid biopsy of circulating tumor DNA, could be in the future fundamental elements in order to personalize the treatment (17).

It is not clear if a chemotherapy with three drugs is better than the association of cetuximab plus FOLFOX/FOLFIRI. The FOLFOXIRI triplet (fluorouracil/irinotecan/oxaliplatin) showed an overall 36% RRR in patients with LLM, superior to those from the trial by Ye *et al*. (2) and the CELIM trial (10), but with a clear increase in toxicities, especially hematological and neurological (18). Recent data from the TRIBE (Combination Chemotherapy and Bevacizumab as First-Line Therapy in Treating Patients With Metastatic Colorectal Cancer) trial showed that the addition of bevacizumab to FOLFOXIRI probably has no effect on resectability (19). Regarding the addition of Cetuximab to FOLFOXIRI, to date only small phase II trials are available, showing the feasibility of these combination with resection rates superior to 30% (20,21). Furthermore, when considering the continuum of care of patients, the use of a doublet, respect as a triplet, has the advantage that the remaining non-cross resistant doublet can be utilized as second-line chemotherapy.

In conclusion, when facing a relatively young and healthy patient affected by LLM from colorectal cancer, RAS (and possibly BRAF) wild-type, only after a multidisciplinary and multi-imaging evaluation of non-resectability with at least CT scan and MR or PET/CT, the treatment with the association of cetuximab with fluorouracil-based doublets should be strongly considered. We can in fact expect the conversion to resectability in up 25% of patients with, in this case, a prolonged survival in 30-50% of patients. Lacking phase III trials in this setting, it is advisable that new multicentric trials will analyze these aspects (22,23) and that new molecular techniques con improve the personalization of treatments in the various subgroups of patients (16,17).

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Footnote

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Predicting complete response: is there a role for non-operative management of rectal cancer?

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Abstract: Pre-operative chemoradiotherapy followed by a total mesorectal excision (TME) is the standard of care for patients with locally advanced (stage II or III) rectal cancer. Approximately 20% of patients may achieve a pathologic complete response after chemoradiation therapy (CRT), which has been shown to be associated with better oncologic outcomes. Whether surgery can be avoided in this population is an area of active investigation. Recent studies demonstrated feasibility and safety of non-operative management in patients with clinical complete response (cCR) after chemoradiotherapy. In this article, we set out to review the current data on non-operative management and to identify areas requiring further investigation, including improvement in imaging for reassessment after CRT and identifying the optimal time frame for restaging. As the field moves forward with non-operative management in select patients with rectal cancer, there continues to be a need to better understand the prognostic factors and biomarkers that may more accurately characterize patients who are qualified for this "wait-and-see" approach and thereby avoid overtreatment, potentially leading to improvements in long-term quality of life.

Keywords: Rectal cancer; non-operative management; chemoradiation therapy (CRT)

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Introduction

Surgery has been the cornerstone in the management of patients with resectable rectal cancer. Selected patients with distal rectal, well-differentiated pT1 lesions can be treated with local excision alone with close follow-up. In patients with pT1 tumors with adverse pathologic features, and patients with pT2 tumors, two prospective trials by Radiation Therapy Oncology Group (RTOG) and Cancer and Leukemia Group B (CALGB) Intergroup demonstrated excellent local control rates and survival with local excision followed by adjuvant chemoradiation therapy (CRT) (1,2). Patients with early rectal cancers treated with pre-operative CRT followed by local excision also resulted in excellent local control. Borschitz *et al*. reported a long-term local recurrence rate of 7% in 237 patients with cT2-3 disease who underwent 5-fluorouracil (5-FU)-based CRT followed by local excision (3). The American College of Surgeons Oncology Group (ACOSOG) single-arm, prospective study of T2N0 rectal cancer patients who received neoadjuvant CRT and local excision demonstrated high rates of treatment response, with 34 (44%) of 77 patients experiencing a pathological complete response (pCR) (4).

In patients with more locally advanced (cT3-4) rectal cancers, pre-operative CRT has been used to downstage tumors before planned resection. The landmark German Rectal Cancer Trial randomized 823 patients with cT3-4N+ rectal cancer to either preoperative or postoperative CRT and demonstrated significantly improved local control with preoperative CRT (local recurrence rate at 5 years of 6% *vs*. 13% with adjuvant CRT). Among patients with low-lying tumors who were to require abdominoperineal resection, those received preoperative CRT were twice as likely to undergo a sphincter-sparing operation (5). Another randomized trial by the National Surgical Adjuvant Breast and Bowel Project (NSABP) investigated the same question but was closed early due to poor accrual. Of the 267 patients

enrolled, preoperative CRT demonstrated a trend toward better disease-free survival (DFS) (6).

These studies demonstrated benefits in preoperative CRT in patients with both early and more advanced rectal cancer. It is effective in inducing tumor regression; in fact, approximately 15-27% of patients who undergo preoperative CRT experience a pCR in which no residual tumor is reported on histologic examination of total mesorectal excision (TME) (7). In a meta-analysis by Maas *et al*. including 3,105 patients, of which 484 patients achieved a pCR after preoperative CRT, it was shown that patients with pCR had significantly increased DFS. The 5-year crude DFS was 83% for patients with pCR and 66% for those without (7). Whether surgery and its risk of complications in these patients could have been avoided is a topic of investigation. Until recently, the only means to detect complete response reliably is through surgical resection and microscopic evaluation of the specimen. There is growing evidence that regimented clinical assessment after CRT can reliably identify patients who achieved clinical complete response (cCR), allowing avoidance of immediate surgery. We will discuss the concept of nonoperative management in patients with rectal cancer who achieved cCR after CRT in this article.

"Wait-and-see"

In 2004, Habr-Gama *et al*. first published their experience with 265 patients with resectable cT2-4N0/N+ rectal adenocarcinoma who underwent preoperative CRT consisting of 5,040 cGy over 6 weeks, leucovorin, and bolus 5-FU administrated intravenously for 3 consecutive days on the first and last 3 days of CRT. At 8 weeks, all patients underwent repeat evaluation, including endoscopy with biopsy. In a later report, fluorodeoxyglucose positron-emission tomography (FDG-PET)/computed tomography (CT) was also reported to be used in post-CRT assessment (8). The presence of any significant residual ulcer or positive biopsies was considered incomplete clinical response and the patient went onto TME. Patients without any abnormalities were considered to have cCR and were referred to monthly physical and digital rectal examination (DRE), proctoscopy, biopsies, and serum carcinoembryonic antigen (CEA) level testing for the first year, every 2 months in the second year, and every 6 months in the third year. Abdominal and pelvic CT scans and chest radiographs were repeated every 6 months for the first year. Of the 265 patients, 71 patients had a cCR 8 weeks after CRT and were enrolled in the wait-and-see cohort. The majority of these patients had T3 disease (69%, T2 =20%, T4 =11%) and did not have radiologic evidence of nodal metastasis (77%, N+ =23%). Among the 71 patients, the 5-year overall survival (OS) was 100% and DFS was 92%, compared with 88% and 83%, respectively, among the patients who did not achieve cCR and went onto immediate TME. Only 2 patients in the wait-and-see group developed local recurrence 56 months after CRT completion; they were salvaged by local excision and brachytherapy. These promising results led the authors to conclude that surgical resection may be safely avoided in patients appropriately identified achieving cCR after CRT (9).

Subsequent to their initial publication, the authors published several updates of their experience with patients treated with preoperative 5-FU-based CRT spanning from 1991 to 2009 (10-13). The largest series was composed of 361 patients with cT2-4 tumors and 99 (27%) achieved a sustained cCR at 12 months. Only 5 patients among the 99 cCR patients developed local recurrence. The 5-year DFS was 85% and OS was 93% (11).

In 2011, Maas *et al*. from the Netherlands published a prospective series of 21 patients with a cCR who were managed nonoperatively with a wait-and-see policy (14). Between 2004 and 2010, 192 patients with cT1-3N0-2 were treated with CRT consisting of 5,040 cGy over 28 fractions with concurrent capecitabine. At 6-8 weeks after CRT, magnetic resonance imaging (MRI) was performed. In addition to standard T2-weighted imaging, diffusion-weighted MRI (DWI) was used to determine the presence of residual tumoral tissue (high signal on DWI) at the primary site, and MRI enhanced with either ultra-small superparamagnetic iron oxide or gadofosveset trisodium was used to evaluate nodal status. If no residual tumor was seen on post-CRT MRI, endoscopy with biopsy was performed. A patient was only determined to achieve a cCR when no residual tumor or nodal disease was seen on MRI, no residual tumor was seen at endoscopy, negative biopsy was achieved after CRT, and there was no palpable tumor on DRE. Among the 21 patients who met this criteria, an intensive follow-up protocol was carried out, which consisted of DRE, MRI, endoscopy with biopsies, CT scan of chest and abdomen, and CEA measurements (*Table 1*). With a mean follow-up of 25 months, only 1 patient developed endoluminal recurrence and underwent surgical salvage. The 2-year OS in this cohort was 100% and DFS was 89%. A control cohort of 20 patients who were found to have pCR had a 2-year OS of 91% and DFS of 93%, similar to patients with cCR, and enrolled in the wait-and-see protocol (14).

At Memorial Sloan Kettering Cancer Center, a

Table 1 Follow-up schedule of patients who achieved cCR who were enrolled on a wait-and-see policy by Maas *et al*. (14)

Year 1
Every 3 months: CEA, DRE, endoscopy, MRI
Every 6 months: CT for distant staging
Year 2-3
Every 3 months: CEA
Every 6 months: DRE, endoscopy, MRI
Every 12 months: CT for distant staging
Year 4-5
Every 6 months: CEA, DRE, endoscopy, MRI
Every 12 months: CT for distant staging

cCR, clinical complete response; CEA, carcinoembryonic antigen; DRE, digital rectal exam; MRI, magnetic resonance imaging; CT, computed tomography.

Table 2 Summary of key studies of patients who achieved cCR after CRT who did not proceed to surgery

Studies	Patients (n)	Follow-up (months)	LRR (%)	OS (%)	DFS (%)
Habr-Gama *et al*. (11)	122	60	6	5-year: 93	5-year: 85
Maas *et al*. (14)	21	25	5	2-year: 100	2-year: 89
Smith *et al*. (15)	32	28	19	2-year: 97	2-year: 88

cCR, clinical complete response; CRT, chemoradiation therapy; LRR, locoregional recurrence; OS, overall survival; DFS, disease-free survival.

retrospective review of patients treated between January 2006 and August 2010 compared outcomes of 32 stage I-III rectal cancer patients with a cCR to CRT who were treated nonoperatively to 57 patients with a pCR after radical rectal resection. With a median follow-up time of 28 months for the nonoperative group, 6 patients developed local recurrence and all were salvaged surgically. Three of these patients also developed distant metastases. The 2-year distant DFS and OS were similar for nonoperative and rectal resection groups (15). These studies show that, with accurate identification of patients who achieved cCR and rigorous follow-up, patients could be safely monitored without undergoing immediate TME and still have excellent oncologic outcomes. *Table 2* provides a summary of the key nonoperative management studies.

Assessment of complete clinical response

Identifying accurately patients who achieved a cCR after CRT is arguably the most important aspect of a nonoperative approach in rectal cancer management. DRE, while an important clinical practice, has been shown to be a poor method for determining cCR when used alone. In 2005, Guillem *et al*. evaluated DRE immediately preceding resection and found that it only identified 21% of pCR patients, thought to be due to local inflammation and fibrosis interpreted as tumor remnant (16). Endoscopy with biopsy can provide additional information to DRE; nevertheless, a negative biopsy could represent a false negative and persistent disease could not be ruled out. In a prospective study of 46 patients who were treated with preoperative CRT for rectal cancer, 22 patients underwent presurgical endoscopic biopsies. While the biopsies were negative in the 6 patients who were found to have pCR on TME, the biopsies were also negative in 11 of 16 cases with residual cancer, yielding a concordance rate of 59% between endoscopic biopsies and surgical specimens (17). Moreover, neither DRE nor endoscopy assesses for regional nodal status after CRT.

Given the limitations of DRE or endoscopy in restaging after CRT, other modalities are needed to assess for residual disease. Endorectal ultrasound (US), while useful in initial staging, has limited benefits after CRT due to the fibrotic tissue. In a large study of 235 patients comparing post-CRT endorectal US staging and pathologic staging, it was reported that endorectal US only correctly matched the T stage in 54% and N stage in 75% of patients (18). Both FDG-PET and CT scans were evaluated prospectively in a recent study by Guillem *et al*. in the identification of complete response after preoperative CRT (19). A total of 121 patients with rectal cancer were prospectively enrolled in the study, and both FDG-PET and CT scans were obtained before and after CRT. While 26 (21%) patients had a pCR after CRT, only 54% of the pCR patients were classified as having a cCR on preoperative PET scan, and only 19% of the patients were classified as having a cCR on preoperative CT scan. Of the pathologic incomplete responders, PET and CT scans were able to identify 66% and 95% of the patients as incomplete responders, respectively. The authors concluded that neither PET nor CT scan alone has adequate predictive value to be clinically useful in identify patients with complete response after CRT.

In 2013, van der Paardt *et al*. reported a meta-analysis

including 33 studies and 1,556 patients on MRI imaging for restaging locally advanced rectal cancer after neoadjuvant treatments (20). For tumor stage, the authors reported a mean sensitivity of 50% and specificity of 91%. In the subgroup analysis, MRI demonstrated 19% sensitivity and 94% specificity for identifying pT0 disease. This is likely due to conventional MRI's inability to distinguish fibrosis and residual tumor accurately. However, after incorporating functional MRI imaging results, such as DWI or dynamic contrast enhanced MRI, significant improvement in sensitivity in detecting complete tumor response after CRT was seen (84%). The specificity was 85%. Dynamic contrast enhanced MRI provides perfusion characteristics of tumor, and some parameters, such as K(trans), differ markedly between patients with cCR and the incomplete responders (21). Serial T2-weighted MRI during CRT also showed promising results in predicting for tumor pCR. Kluza *et al*. showed that CRT induced a significant decrease in T2-weighted signal intensity distribution of 50% in complete responder. The change in T2-weighted signal intensity resulted in high diagnostic performance for identifying complete responders with an accuracy of 92% in the 39-patients study (22). For nodal stage, MRI results in a mean sensitivity of 77% and specificity of 60% (20). With a low prevalence of involved nodes after CRT, the negative predictive value of MRI was 80-90%. Gadofosveset-enhanced MRI, used in the Dutch study, demonstrated 80% sensitivity and 97% specificity in nodal staging with experienced readers (23).

From the above studies, it is appropriate to conclude that determining cCR after CRT requires utilization of multiple methods in restaging and not a single modality alone. As demonstrated by Habr-Gama *et al*. and Maas *et al*., accurate identification of cCR is achievable with a combination of physical examination, endoscopic examination, and imaging, leading to minimal local recurrence rate with nonoperative management. With the emergence of functional MRI imaging, it is hoped there will be further improvements in our accuracy in determining a cCR to therapy.

Timing of assessment

In addition to methods of assessing cCR, another area that requires further investigation is timing of examination after preoperative CRT. The reports from Habr-Gama *et al*. recommended a minimum of 6-8 weeks or longer interval after CRT for assessment of cCR (24). The Dutch series evaluated response at a mean of 6.5 weeks after CRT (14). There is lack of standardization in the timing of examination. As response continues over time, it is possible that more patients with cCR can be captured with longer wait times. A recent meta-analysis of 13 trials including 3,584 patients aimed to answer the question of whether a longer interval between the end of neoadjuvant CRT and surgery is associated with a higher pCR rate. Patients were divided into two groups: patients who underwent TME shorter than 6-8 weeks after CRT *vs*. patients who underwent TME longer than 6-8 weeks after CRT. A longer wait interval, more than the classical 6-8 weeks, from the end of CRT was found to be associated with significantly improved pCR rate (20% *vs*. 14% in patients who waited <6-8 weeks, P<0.001) (25). It has been showed that delaying surgery until after 12 weeks after CRT does not negatively impact oncologic outcomes (8).

Extended chemotherapy

Studies examining new imaging modalities, such as DWI MRI, and determining the optimal clinical assessment time frames are needed. Furthermore, additional chemotherapy after CRT could be another strategy in maximizing clinical response, leading to more patients with cCR qualifying for nonoperative management. Habr-Gamma *et al*. enrolled 70 patients with cT2-4N0-2 rectal cancer prospectively to receive concurrent CRT followed by extended chemotherapy (5-FU/leucovorin for a total of 6 cycles every 21 days). Of the 70 patients, 47 demonstrated clinical response at 10 weeks after CRT and went on to complete extended chemotherapy. Of the 47 patients, 39 demonstrated sustained cCR for 12 months after extended chemotherapy and 4 patients developed local recurrence >12 months after chemotherapy. Overall, 35 (50%) patients never underwent surgery due to sustained cCR (26). The Timing of Rectal Cancer Response to Chemoradiation consortium conducted a prospective, multicenter, Phase II study investigating extending the interval between CRT and surgery and administering additional chemotherapy during waiting period. Sixty patients underwent TME 6 weeks after completion of 5-FU-based CRT, and 67 patients with evidence of clinical response 4 weeks after CRT received 3 additional cycles of modified FOLFOX (5-FU, leucovorin, oxaliplatin) chemotherapy followed by TME 3-5 weeks later. The pCR rate was higher in patients who received additional chemotherapy (25% *vs*. 18% in those who did not receive additional chemotherapy) (27). Cercek *et al*. showed in 2014 that induction chemotherapy, followed by CRT then surgery is another possible approach to maximize cCR. In

this study, FOLFOX chemotherapy was given before CRT. Of the 49 patients who underwent TME after FOLFOX followed by CRT, 47% had tumor response >90%, including 27% of patients achieving a pCR (28).

Conclusions

Nonoperative management is an emerging trend in the treatment of rectal cancer. It has the benefits of reducing surgery-related toxicities. Modern studies with rigorous post-CRT assessments demonstrated that accurately identifying patients with cCR and avoiding/delaying surgery is feasible. Intensive follow-up regimen is needed to ensure lack of clinical progression. Despite the significant progress the field has made in moving toward nonoperative management, it continues to be an area that requires organized investigations. Developing reliable methods for repeat staging after CRT, determining the optimal time frame for maximal response assessment, and understanding the role of additional chemotherapy after CRT can all potentially allow us to capture more patients with cCR that are suitable for the wait-and-see approach, preventing overtreatment in patients with rectal cancer.

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Footnote

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Stage II colon cancer

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Abstract: Colorectal cancer (CRC) is the third commonest cancer in the Western world. Approximately one-quarter of cases are classified as Stage II/Dukes' B, meaning that the disease has breached the bowel wall but not spread to draining lymph nodes or distant sites. Stage II colon cancer is a heterogeneous disease both biologically and in terms of outcome. Although pivotal data have confirmed the benefit of adjuvant 5-fluorouracil (FU) chemotherapy following resection of stage II tumours the absolute reduction in risk of recurrence is small - 3 to 4 percentage points - and so most patients treated fail to gain from therapy. In contrast to stage III disease, the addition of oxaliplatin to FU as adjuvant chemotherapy for stage II disease does not improve outcome. Much attention has focused on the identification of biomarkers that identify patients more or less likely to benefit from treatment. Recent data confirm that patients with T3 primary and tumour microsatellite instability (MSI) have excellent prognosis and do not require adjuvant chemotherapy. For patients with microsatellite-stable disease, a validated recurrence score based on gene expression provides greater prognostic information than conventional clinicopathological features alone and can be used to inform discussion on the benefits of adjuvant chemotherapy.

Keywords: Colon cancer; Stage II; Dukes' B; adjuvant chemotherapy; recurrence score

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Introduction

Colorectal cancer (CRC) is the third commonest cancer in the Western world, with an estimated 142,570 cases diagnosed in the US in 2010 (SEER database: http://seer.cancer.gov). Worldwide approximately 1.23 million new cases are diagnosed each year and 608,000 deaths from CRC occurred in 2008 (1). Overall, one quarter of incident cases are stage II, meaning that the tumour has breached the muscularis (T3) and may invade adjacent organs (T4), but has not spread to draining lymph nodes or distant sites (*Table 1*). However this proportion varies with tumour site, as almost a third of colonic cancers are stage II compared with just over one fifth of rectal cancers (SEER database: http://seer.cancer.gov). Stage II CRC is a heterogeneous disease both clinically and biologically. For instance, the risk of relapse following resection of a microsatellite unstable T3 lesion may less than 10%, while a patient who undergoes surgery for a mismatch repair proficient T4 tumour may have a risk of disease recurrence greater than 50%. The overrepresentation of microsatellite instability in stage II tumours compared to CRC overall also illustrates the variability in CRC biology at differing disease stages. In view of this heterogeneity it is unsurprising that the benefits of adjuvant chemotherapy for stage II CRC vary widely depending on classical histopathological and molecular tumour features.

In this Review, we present an updated summary on the diagnosis and staging, pathological analysis, and therapeutic management of stage II CRC. We limit our discussion to colonic tumours (approximately two thirds of the total), as the management of rectal cancers differs substantially and is reviewed elsewhere in this issue. In addition to providing a précis of stage II colonic cancers we focus particularly on the evolving role of biomarkers in predicting the risk of relapse and guiding decisions on adjuvant therapy.

Table 1 Staging of colorectal cancer

AJCC/Dukes' stage	Anatomical extent of disease	5-year overall survival
I/A	Confined to mucosa (T1) or muscularis propria (T2) No nodal involvement No distant metastases	93.2%
II/B	Tumour penetrates muscularis (T3) or invades adjacent organs or structures (T4) No nodal involvement No distant metastases	82.5%
III/C	Any tumour stage Nodal metastases No distant metastases	59.5%
IV/D	Any tumour stage Any nodal status Distant metastases	8.1%

Diagnosis and staging of CRC

In the absence of screening, CRC is usually diagnosed following symptoms from the primary tumour or metastases. Population analyses have shown that approximately one quarter of all colorectal cancers in an unscreened population are stage II. Interestingly, though it might be hypothesized that the introduction of screening would result in an increase in the proportion of stage II tumours this was not the case in several screening studies (1-3), in which stage migration following implementation of fecal occult blood testing was mainly manifest as an increase in stage I and a reduction in stage IV disease. Consequently, the widespread adoption of screening may not result in a substantial alteration in the frequency of stage II CRC.

While surgical resection of most stage II colonic tumours by open or laparoscopic surgery is straightforward, the management of T4 cancers invading adjacent structures is more challenging. The role of imaging in predicting resectability has evolved substantially in recent years, and in our unit consideration is given to the use of preoperative chemotherapy with aim of facilitating surgery in patients with advanced T4 lesions.

Following resection, accurate pathological assessment is essential to confirm diagnosis of stage II disease, with examination of a minimum 12 lymph nodes recommended by consensus guidelines (4), although evidence suggests that prognosis of stage II disease improves according to the number of nodes analysed - suggesting that a proportion of patients with occult nodal metastases are under-staged by suboptimal pathological evaluation (5-8). The extent of tumour invasion is also essential in informing further management, as is the presence or absence of microsatellite instability (MSI) (discussed below). Other pathological features commonly suggested to be of prognostic import, but in some cases unvalidated are tumour vascular invasion and grade. Though often taken for granted in everyday practice, the pathologist's role in determination of these factors is of pivotal importance in informing subsequent patient management.

Biology of stage II colon cancer

Although there are commonalities with other stages of CRC, there are also notable differences between stage II colon cancer and other disease stages. The most well recognized of these is the high frequency of MSI in stage II colon cancer, present in 15% of cases overall, and around 25% of right sided tumours, in comparison with a frequency of 14 in stage III colon cancer and 4% in metastatic disease (9). Mismatch repair proteins are required for surveillance of the newly synthesized DNA strand following replication, where they serve to recognize mispaired bases, small insertions and deletions incorporated by DNA polymerases (10). Germline mutation of the mismatch repair genes *MLH1*, *MSH2*, *MSH6* or *PMS2* causes Lynch syndrome (also known as hereditary non-polyposis colorectal cancer - HNPCC), associated with early onset colonic and endometrial cancer, in addition to tumours of the ovary, stomach, small bowel, pancreas and other sites (11,12). Defective mismatch repair function in sporadic colonic cancer is commonly due to mutation of *MSH6*, *MSH2* or epigenetic silencing of MLH1 by promoter methylation (12). In both hereditary and sporadic tumours, aberrant mismatch repair function leads to failure to repair defects caused by slippage of DNA polymerases at microsatellites - short tandem DNA repeats - and point mutations, resulting in a characteristic molecular phenotype of microsatellite instability (MSI) and mutation of the tumour suppressors *TGFβR2*, *IGF2R*, *BAX*, and *PTEN*, and the oncogene *BRAF* (12-15). MSI-high tumours are commonly proximal to the splenic flexure, poorly differentiated and demonstrate a prominent lymphocytic infiltrate (12). Confirmation of tumour microsatellite instability can be performed either using PCR - by the demonstration of instability of at least 2 of 5 microsatellite markers examined - or by immunohistochemistry (IHC) for the mismatch repair proteins, as absent staining

demonstrates excellent concordance with MSI-high status (16,17). Testing for MSI in stage II colonic cancer, and particularly in T3 tumours is advised, as it has important prognostic and therapeutic implications, as discussed below.

Adjuvant chemotherapy for stage II colonic cancer

Although the benefits of adjuvant 5-fluorouracil (FU) chemotherapy following resection of stage III disease have been well recognized for over two decades, the role of postoperative chemotherapy for stage II disease remained unclear until the publication of the QUASAR trial. This study randomized 3,239 patients following resection of CRC, 90% of whom had stage II disease, to adjuvant chemotherapy with FU and folinic acid (n=1,622) or to observation (n=1,617). After a median follow-up of 5.5 years, the recurrence rate in the chemotherapy arm was 20% lower than in the observation arm, translating to an absolute reduction in risk of relapse of 3.6% (P=0.04) (18). This unequivocal demonstration of the benefit of adjuvant chemotherapy for stage II colonic cancer is supported by other analyses (19-22) and means that an informed discussion of the risks and benefits of treatment is essential with fit patients following surgery. The MOSAIC study demonstrated that the addition of oxaliplatin to FU improves recurrence-free survival following surgery for stage III disease albeit at the expense of greater toxicity (23). However, subsequent data from this trial indicate that although this translated to a survival benefit at 6 years from combination therapy for stage III disease, no advantage was evident for stage II cancers (24). Consequently, oxaliplatin cannot be routinely recommended for use as adjuvant therapy in stage II colon cancer.

Biomarkers in stage II CRC

In view of the modest overall benefit from adjuvant chemotherapy in stage II colon cancer attempts have been made to restrict its use to patients with high-risk disease, on the premise that such patients are most likely to gain from therapy. The criteria used to identify 'bad prognostic factors' - T4 primary, high grade, lymphovascular invasion etc - have generally been identified by retrospective subgroup analysis, and although the prognostic significance of T4 primary is well recognized (25), most other factors have not been validated prospectively. Indeed, when reflecting on the quality of the underlying data, it is puzzling that some such features have gained traction in clinical practice and been included in treatment guidelines for stage II disease. However, recent high-quality data from the molecular analysis of large prospective clinical trials has clearly demonstrated the prognostic significance of tumour microsatellite instability, and suggested that the analysis of tumour gene expression profiles may aid in treatment decisions in some cases of colon cancer.

Prognostic significance of microsatellite instability (MSI)

Although the prognostic significance of MSI was previously unclear, data from several large randomized clinical trials (RCTs) (9,26-30), and a meta-analysis (31) have conclusively proven that the presence of tumour MSI is associated with favourable outcome. The meta analysis of 7,642 patients, 1,277 of whom had MSI tumours showed a hazard ratio for death of 0.65 (95% CI, 0.59 to 0.71) for patients with MSI tumours compared to those with microsatellite stable (MSS) disease (31). Even disregarding the suggestion that MSI may predict lack of benefit from adjuvant chemotherapy (31), patients with T3 primary and tumour MSI have sufficiently low risk of recurrence to mean that any benefit from post-operative chemotherapy is minimal, and these patients can therefore be spared treatment. Interestingly, the combination of T4 primary and MSI is uncommon - around 2% of cases of stage II colon cancer - and appears to have similar prognosis to that of T3 primary, MSS disease, although there is a large degree of uncertainty in this estimate. Consequently, consideration of adjuvant chemotherapy should be given to patients this group. The mechanisms underlying the favourable outcome of MSI-high cancers is presently unclear, but may be due to and anti-tumour immune response (32) or decreased viability associated with hypermutation in tumours (33). Data regarding the utility of adjuvant chemotherapy for the majority of patients (85%) with MSS tumours, are insufficiently strong to alter the estimated benefit from the QUASAR study (18). A proposed treatment algorithm, accounting for tumour stage and mismatch repair status is shown in *Figure 1*.

Prognostic gene signatures

As an attempt to improve on the utility of conventional clinicopathological features for prognostication in stage II colon cancer, a transatlantic collaboration between QUASAR and NSABP Trials Groups, Cleveland Clinic

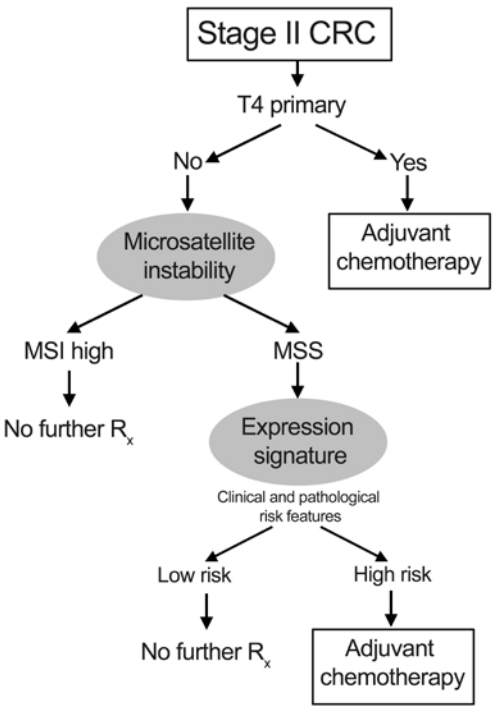

Figure 1 Proposed algorithm for management of stage II colon cancer. The algorithm incorporates conventional and molecular prognostic features to guide management. Patients with T4 primary have high risk of relapse, and should be considered for adjuvant chemotherapy. Patients with T3 primary and tumour microsatellite instability (MSI) have excellent prognosis, and can be spared treatment. Those with microsatellite-stable, T3 primary tumours are candidates for the recurrence score, to predict recurrence risk and likely benefit from chemotherapy

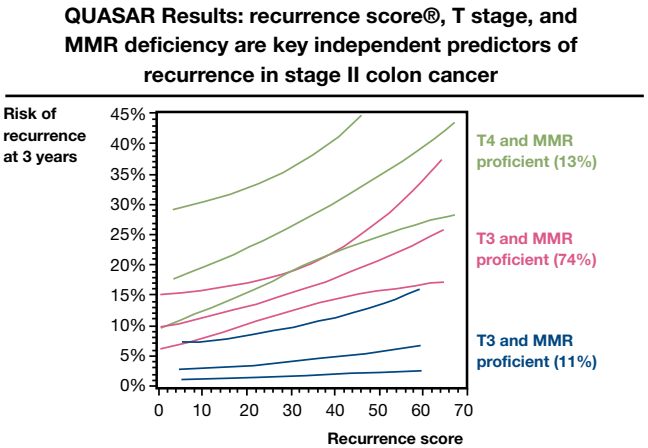

Figure 2 Risk of recurrence of stage II colon cancer in the QUASAR study according to tumour stage and recurrence score. Recurrence score, T stage and tumour MSI are independent predictors of recurrence risk. Cases of T4, MSI-high cancers were uncommon (2% of all patients), and had estimated recurrence risks approximately that of T3, MSS tumours (with large confidence intervals), and are not included in this figure

and Genomic Health, was formed. This collaborative effort sought to examine whether tumour RNA expression levels might serve to improve on conventional parameters for the classification of relapse risk. The developmental study comprised 1,851 patients recruited to NSABP clinical trials C-01/C-02/C-04/C-06 and a cohort of untreated patients from the Cleveland clinic (34). RNA was extracted from formalin-fixed paraffin embedded (FFPE) tumour blocks, and gene expression quantified by RT-PCR. Multivariate analysis of the correlation of expression of 761 candidate genes on recurrence-free survival (RFS), disease-free survival (DFS), and overall survival (OS) adjusted for stage, grade, number of lymph nodes examined and MSI status, yielded 18 informative genes (7 prognostic genes, 6 genes predictive of FU benefit and 5 internal reference genes for normalisation), which were used to generate separate prognostic recurrence score and predictive treatment score signatures. The utility of these gene expression scores was then examined in 1,436 patients with median follow-up of 6.6 years from the QUASAR study (35). In univariate analysis, the recurrence score predicted recurrence risk (hazard ratio/25 units =1.58; 95% CI, 1.15 to 2.15; P=0.004), DFS (P=0.01) and OS (P=0.04). Recurrence risk increased with increasing recurrence score, with 3- year recurrences of 12, 18 and 22% in the predefined low, intermediate and high recurrence risk groups (*Figure 2*). In multivariate analyses, the recurrence score retained prognostic significance (P=0.008) following adjustment for primary tumour stage, number of lymph nodes examined, MSI status, tumour grade, and tumour lymphovascular invasion. However, the treatment score failed to predict chemotherapy benefit (P=0.19) (35). Thus, the continuous recurrence score is able to enhance the assessment of recurrence risk and may be of particular use for the majority (76%) of cases of stage II colon cancer with T3 MSS tumours, as shown in *Figure 2*. In this group, the recurrence score can be used to segregate those into very low risk of relapse for whom the absolute benefits of chemotherapy are too small to recommend its use, from those at greater risk, for whom a 25-30% risk of recurrence is associated with a greater absolute benefit from adjuvant treatment - perhaps 5-6 percentage points. In the group at intermediate risk, a

more informed discussion between the patient and clinician on the likely benefits of adjuvant chemotherapy than is currently possible may be undertaken. The recurrence score has the advantage of using conventional pathologic material, in contrast to alternatives that require frozen tissue - not routinely collected in everyday clinical practice. It is hoped that in the future, an improved predictive score for chemotherapy benefit will provide additional information that can be used to guide treatment decisions in patients with stage II colon cancer.

Conclusions

Approximately one quarter of patients with colorectal cancers have stage II disease, and within this group there is substantial variation in clinicopathological features, molecular biology and outcome between cases. The prognosis varies from the excellent outcome associated with MSI T3 primary to a recurrence risk of >50% for MSS T4 primary presenting with bowel obstruction. Consequently, as we have sought to highlight in this Review, a one-size-fits-all approach cannot be recommended, and treatment decisions must be individualized, informed by tumour stage and MSI status at the very least. In a proportion of cases, the recurrence score may provide further information on the risk of relapse than conventional clinicopathological features alone, and help in decision-making. Ongoing studies should clarify the role of additional molecular markers in assessment of prognosis and likelihood of chemotherapy benefit.

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Footnote

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

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TNF-α in obesity-associated colon cancer

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Abstract: Obesity is leading to an unparalleled increase in the incidence of metabolic diseases and cancers, including colon cancer. Research over the last few decades revealed obesity as a low-grade chronic inflammatory state, which has recently been implicated in colon carcinogenesis. Understanding the molecular links of obesity-associated inflammation with colon carcinogenesis is therefore more critical than ever. In this review we discuss the central role of TNF-α, the prototypical pro-inflammatory cytokine, in the pathophysiology of obesity-associated colon carcinogenesis.

Keywords: Colorectal cancer; obesity; TNF-α

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Introduction

Modern societies are challenged by dramatic changes in the epidemiology of diseases. Scientific and technological advances have resulted in more efficient treatment of acute diseases and changes in human habits contributing to a high increase in the prevalence of chronic inflammatory conditions. In this context, obesity and cancer have emerged as two of the greatest threats to global human health. Here we will examine the evidence that links inflammation as a key mechanism to promote both obesity and cancer. We will extend the discussion to present the pathophysiological mechanisms that implicate obesity-associated inflammation in the development of colorectal cancer, with a special emphasis on the role of TNF-α.

Inflammation: basis for modern diseases

Inflammation is canonically defined as an essential biological response which promotes host repairs of tissue injury and infection (1). In the last decades, striking advances were made in our understanding of the biochemical and cellular mechanisms induced by acute inflammation, whilst the knowledge of the intracellular programs regulated by chronic inflammation advanced at a much slower rate (2). Nevertheless, the spectrum of prevailing inflammatory conditions has shifted from acute to chronic inflammatory states since the end of 20th century, significantly contributing to the pathogenesis of modern diseases such as obesity, type 2 diabetes (3,4), atherosclerosis (5), neurodegenerative diseases (6), and certain cancers (7).

The most obvious signs of inflammation are heat, pain, swelling, and redness, described by Celsius during the time of the Roman Empire. Initially, this inflammatory response was deemed as a biological reaction without deleterious effects, evoked just to protect from infection and normalize homeostasis. This theory influenced the understanding of the field until the 1970s, when it was recognized that inflammation not only preserves the integrity of the body but might also harm host tissues itself (8). Interestingly, recent research brought to light the fact that inflammation-mediated deleterious effects are closely linked to the pathophysiology of chronic multifactorial diseases (9-12). Accordingly, there is increasing interest in the mechanisms involved in the resolution of inflammatory response as much evidence links nonresolving inflammation to the pathophysiology of the ever-growing modern diseases of industrialized societies (10).

There is intense debate about the regulatory mechanisms that control inflammatory response, in part due to its complexity and also because of the multitude of agents

involved in its induction and resolution. However, it is now well recognized that there are two major stimuli that promote acute inflammation: infection and host cell necrosis from sterile tissue injury (13). Intriguingly, the products generated by both processes are recognizable by the same cluster of host molecules, which activate a common inflammatory pathway that eliminates triggering stimuli and repairs the damaged tissue (2). As a result, inflammation is often interrupted by an active and highly regulated process that restores the homeostatic state (2,14,15). One key regulating mechanism of inflammation resolution is the switch from pro-inflammatory prostaglandins and leukotrienes to anti-inflammatory resolution-inducing lipids, such as lipoxins and resolvins (14,16). Specifically, these anti-inflammatory mediators promote the transition from neutrophil to monocyte recruitment (17-19). The subsequent uptake of apoptotic neutrophils orchestrates the production of anti-inflammatory cytokines by monocytes and recruited macrophages, which are responsible for the clearance of dead cells and other debris and initiation of tissue repair at the damaged site (15,20,21). However, if the inflammatory trigger is not eliminated, a chronic state of inflammation is sustained for an undetermined period of time, although signs of acute phase may reappear throughout the course of the disease. This type of chronic inflammation is detected in a myriad of conditions including tuberculosis, unrepaired tissue damage, persistent allergens and undigestible foreign particles and endogenous crystals (10).

Chronic inflammation may also occur in diseases where the initiating trigger is not well defined and does not seem to be related to infection or tissue damage, therefore, without a physiological counterpart (2,9). In these conditions, inflammation appears to be chronic from the outset with infiltration of monocytes, dendritic cells and macrophages into the target tissue. Examples include obesity (22), atherosclerosis (5) and some cancers (23). Notably, in these cases of chronic inflammation there appears to be vicious cycles connecting inflammation and the pathological process it accompanies. Indeed, this reciprocal relationship may be responsible, at least in part, for the chronic nature of these inflammatory conditions and distinguishes them from the first type of chronic inflammation, which is caused by the persistence of the inflammatory inducer.

A causal relationship between chronic inflammation and cancer has long been suspected. It was first detected by Galen and later established in the 19th century by Rudolf Virchow who discovered leukocyte infiltration in malignant tissues. Interestingly, the inflammatory response is similar in many aspects to a wound-healing process and tumors have been considered as wounds that do not heal (24). Research over the last decade in the field of inflammation and cancer pathogenesis has produced abundant evidence of the functionally important tumor-promoting effects that immune cell have on neoplastic progression (7,23,25). Inflammation can contribute to multiple hallmark capabilities by supplying bioactive molecules to the tumor microenvironment, including growth factors that sustain proliferative signaling, survival factors that limit cell death, proangiogenic factors, extracellular matrix-modifying enzymes while enhancing cell proliferation, cell survival, cell migration and angiogenesis (7,23,25). Accordingly, the importance of inflammation for production of the "tumor microenvironment" is now widely recognized as an enabling characteristic of cancer (26).

As a modern epidemic disease, the concept of obesity-induced adipose tissue inflammation is much more recent, about 20 years old (27). Corresponding to Virchow's findings related to cancer tissue, large numbers of macrophages have been observed infiltrating adipose tissue from obese mice and humans (28,29). In obesity, the proinflammatory pathways in adipose tissue macrophages (ATM) are highly activated, leading to the secretion of a variety of cytokines such as TNF-α and interleukin-6 (IL-6) (3,30).

Inflammation is conspicuously associated with certain colon cancers. For instance, colitis-associated cancer (CAC) often arises in patients diagnosed with inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis (31). Moreover, the cumulative incidence of CAC among patients with ulcerative colitis 25 years after diagnosis ranges from 8% to 32%, accounting for one sixth of all deaths in this group (31). Furthermore, Crohn's disease is associated with a pooled estimated relative risk of 2.4 (32). The physiopathology of IBD is multifactorial and involves genetic, mucosal, microbiota and immune system abnormalities (for review see Xavier *et al*. and Danese *et al*.) (33,34). Interestingly, the disrupted communication between the epithelium and the intestinal flora has an important role in activating the immune system and maintaining the inflammatory response (35-40). Therefore, ulcerative colitis and CAC are mainly mediated by the first mentioned mechanism of nonresolving inflammation, whereby the inflammatory trigger is not eliminated and causes an acute inflammatory response to persist for a long period of time.

In addition to IBD, other well-known risk factors for colon cancer are obesity, diets low in fruits and vegetables,

and physical inactivity (41,42). As these habits were initially more prevalent in developed nations, obesity-associated cancer was once a disease primarily observed in longstanding industrialized societies; however nowadays it is a worldwide health burden (43). Specifically, the association between being overweight or obese with colon cancer are positive for both men (RR =1.24) and women (RR =1.09) at an elevation of 5 kg/m^2 in BMI (42). Intriguingly, obesity-associated colon cancer is, at least in part, mediated by the second mentioned mechanism of nonresolving inflammation, in which chronic low-grade inflammation arises without a clear trigger. In the next topics we will further explore these inflammatory features of obesity-associated colon cancer.

Obesity-associated inflammation

In the 1980s and 1990s, the world saw a striking increase in the prevalence of obesity and in the most recent years it trended to levelling out (44). This epidemic had begun in developed countries, but nowadays it is also common in many other regions over the world, such as Asia and Latin America (43,45-47). In conjunction with this epidemic, we faced a dramatic increase in the prevalence of diseases, such as hypertension, dyslipidemia, cardiovascular disease, type 2 diabetes mellitus and certain cancers, making obesity a worldwide public health concern (48).

Obesity-associated tissue inflammation is now recognized as a major driver in the pathogenesis of metabolic diseases (3,4,49,50). Activation of inflammatory pathways has since been observed in classical metabolic tissues, including fat, liver and muscle (27,51,52). At the molecular level, chronic low-grade inflammation induced by obesity leads to activation of protein kinases, such as Jun N-terminal kinase (JNKs) (53) and inhibitor of nuclear factor B kinaseβ (IKKβ) (51,54,55), which phosphorylates serine 307 (Ser307) of IRS-1 (56,57). As a result, the interaction of the PTB domain of IR with the phosphorylated NPEY motif of IRS-1 is inhibited, impairing the interaction of IRS-1 with the insulin receptor and causing insulin resistance (56). Obesity associated inflammation is also associated with increased activity of iNOS, which *S*-nitrosates insulin signaling pathway and promotes insulin resistance (58-61).

A pivotal event in the pathophysiology of obesity-induced inflammation is the recruitment of macrophages into adipose tissue (62). The large accumulation of adipose tissue macrophages (ATMs), representing up to 40% of the cells in obese adipose tissue, determines locally increased levels of pro-inflammatory cytokines, such as TNF-α and IL-6, which sustain insulin resistance in a paracrine manner (28,29,55). In addition, these cytokines may also leak out the adipose tissue and exert systemic effects (28). Congruent with this data, macrophages are recruited to adipose tissue by chemokines secreted by adipocytes, which provide a chemotactic gradient that attracts Ly6C^{hi} monocytes into the adipose tissue, where they differentiate into ATMs (63-66). Once pro-inflammatory ATMs migrate into adipose tissue, they also secrete their own chemokines, attracting additional macrophages and establishing a vicious cycle that stimulates the inflammatory process (55).

Macrophages are dynamic cells that acquire different phenotypes in accordance with the microenvironment that they reside (62). These cells are often classified by their functional inflammatory state and the polarized states are often referred to as classically activated macrophages (CAMs), known as M1, and alternatively activated macrophages (AAMs), known as M2 (67). In adipose tissue these two subpopulations exert opposite immune actions: M1 inflammatory macrophages secrete proinflammatory cytokines whereas AAMs secrete anti-inflammatory ones (22). The majority of ATMs in obesity are M1-like, identified by the specific expression of CD11c, typically negative in M2-like macrophages that reside in lean adipose tissue (55,68). Along this line, macrophage specific JNK deficient mice are protected from insulin resistance induced by high fat diet (69). In contrast, repression of programs that control alternative activation of macrophages is associated with obesity and insulin resistance (70,71). Furthermore, obese animals exposed to a switch from a high-fat diet (HFD) to a chow diet or treated with omega-3-fatty acids or thiazolinediones have macrophages converted from an M1 to M2 phenotype, coincident with increased insulin sensitivity (72,73).

After the observation of the striking switch from AAM to inflammatory macrophages in obese adipose tissue, it was progressively described that not only are macrophages actively mobilized by the obese adipose tissue but also by other innate and adaptive immune cells (22,74). In a simplified way, there is an increase in inflammatory immune cells such as Th1 cells (75), CD8+ T cells (76) and B cells (77), which promote insulin resistance by further activating inflammatory macrophages or directly secreting pro-inflammatory cytokines or antibodies. Meanwhile, this pool of inflammatory cells takes place with resident tolerogenic immune cells, including eosinophils (68), innate lymphoid type 2 cells (ILC2s) (78), regulatory T cells

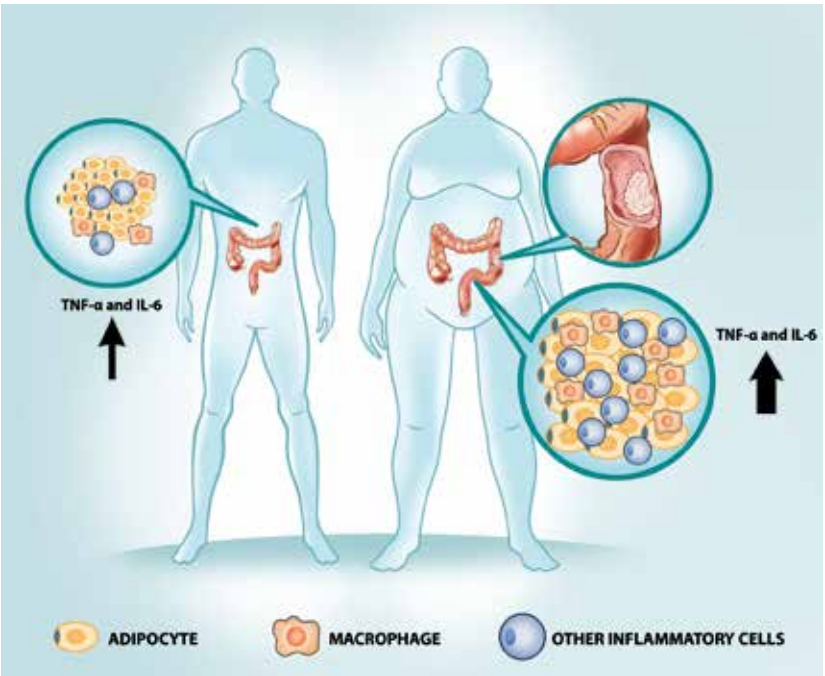

Figure 1 Adipose tissue of obese individuals is highly infiltrated by machophages and other active inflammatory cells. These cells present a pro-inflammatory phenotype characterized by increased levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) secretion, which promote obesity-associated colon cancer by acting through both endocrine and paracrine ways.

(Tregs) (79), invariant natural killer (iNKT) cells (80,81) and Th2 cells (75), which secrete IL-4, IL-5 and/or IL-10 and, therefore, promote direct anti-inflammatory effects or activate the alternative program of resident macrophages to sustain metabolic homeostasis. Despite the debate about the sequence of cells that infiltrate the adipose tissue, obesity assembles a large number of immune cells that promote and amplify the inflammatory response in the adipose tissue.

Another critical mechanism that mediates inflammation in obesity is the interaction with the host-microbiota (82,83). The gut microbiota contains expressive amounts of lipopolysaccharides (LPS) derived from Gram-negative bacteria, which can leak into circulation and may cause inflammation and macrophage recruitment into adipose tissue (84,85). Interestingly, recent studies revealed that obesity changes in microbiota are associated with increased circulating LPS levels (86,87). Accordingly, exercise-induced decreases in LPS circulating levels parallels the increase in insulin sensitivity (88). Mechanistically, LPS binds to TLR-4 Toll-like receptors (TLRs), which exert a central role as a major regulator in microbe-associated molecules recognition and free fatty acids (89). Importantly, TLR4 activation promotes increased JNK and IKK activity and insulin resistance in obesity (76). In addition, TLR4 genetically deficient animals were protected from free fatty acids- and obesity-induced insulin resistance (89,90). Interestingly, gut microbiota modulation by antibiotic treatment decreases LPS and TLR4 activation sustains insulin sensitivity in different animal models (85,87,91).

In aggregate, the studies discussed in this section suggest that obesity is a unique systemic chronic inflammatory disease. Importantly, the interplay between cytokines secreted by inflammatory cells, free fatty acids and gut microbiota products signal through the two prototypical pro-inflammatory receptors, TNF-α and TLR-4 promoting the activation of specific intracellular cascades that include IKK-β, NF-κB and JNK and resulting in the inhibition of insulin signaling and deregulation of metabolic homeostasis. Interestingly, insulin resistance has been suggested to be an adaptive and protective response that properly balances the metabolic homeostasis during the noxious stimulus of overnutrition (3). Since the protective effects of inflammation cannot be dissociated from a cost to homeostasis (92), it is important to better understand how obesity-associated inflammation also promotes human modern diseases including cancer (*Figure 1*).

Obesity-associated inflammation and colon cancer

Besides type 2 diabetes, hyperlipidemia and hypertension, which are classically linked to obesity, other diseases, including cancer, were recently associated with obesity (93). Obesity not only promotes colorectal cancer (CRC) but it is also specifically associated with esophageal, pancreatic, post-menopausal breast, endometrial, thyroid, gallbladder and renal cancers (42). Notably, a meta-analysis of 56 studies, where more than 7 million individuals were evaluated, demonstrated that for each 5 kg/m^2 increment in body mass index BMI there was an increase of 18% in the risk of developing colon cancer (94).

In spite of the prominent epidemiological importance of obesity as a risk factor for colon cancer, the initial evidence that implicates inflammation as a promoter of colon cancer comes from CAC studies. Remarkably, TNF-α production is increased in ulcerative colitis and has been implicated in its pathogenesis (95,96). Although, it is long recognized that TNF-α activates the oncogenic transcription factors NF-κB and AP-1 only recently the importance of inflammatory cytokines in CAC became better understood (97,98). In an elegant study by Greten *et al*., the conditional ablation of IKKβ in epithelial cells resulted in a marked reduction in the development of colonic adenomas, but had little effect on adenoma size (99). Otherwise, lack of NF-κB in myeloid cells, principally lamina propria macrophages, led to a significant reduction in both colonic tumor quantity and size (99). Although IKKβ ablation did not resulted in decreased TNF-α production, it is not clear whether the LysM-Cre deleter used in this study is non-functional in a specific subset of colonic macrophage, or whether TNF-α may be produced by other cell types in CAC, including T cells and epithelial cells (99). Additionally, a very interesting study demonstrated that TNF-α expression is elevated in CAC carcinogenesis and genetic inactivation of the type 1 TNF receptor (TNFR1) or TNF signaling inhibition with a soluble decoy receptor reduced CAC promotion (100). Moreover, the dependence of TNF-α to carcinogenesis in a distinct model of CAC than AOM + DSS, as T-bet deficiency was observed in dendritic cells, reinforces its importance in CAC tumorigenesis (101). Thus, the same prototypical cytokines, TNF-α and IL-6, which are increased in obesity-associated inflammation, have been found to be crucial in promoting colitis induced cancer.

Obesity-associated inflammation is clearly not restricted to adipocytes but disseminated in all metabolic tissues (51,52,102-104). Furthermore, it was recently observed that non-metabolic glandular organs, including colon, also present signs of low-grade inflammation in obesity (105-109). Importantly, TNF-α overexpression was consistently elevated in colons of genetically- or diet-induced obesity rodents (106-109). Congruent with an increased inflammatory response IL-6 and other cytokines are also upregulated in the colons of obese animals (110,111) suggesting that the obese colonic tissue recapitulates the inflammatory timbre constantly observed in metabolic tissues of obese individuals. Accordingly, obese Zucker rats treated with azoxymethane (AOM) manifested higher incidence of tubular adenomas and TNF-α than their lean matched controls (112). Recently, it was observed that leptin deficient and high fat diet fed mice exposed to a combination of AOM + DSS developed higher colonic inflammation than their lean counterparts and increased colonic adenoma numbers in a TNF-α dependent manner (109). Importantly, treatment with infliximab, a monoclonal antibody that neutralizes TNF-α, inhibited the activation of colonic JNK and IKK resulting in the decreased quantity of colonic adenoma and the growth of colon cancer xenografts (109). Interestingly, enhanced production of IL-6 and TNF-α was also observed in a hepatocarcinoma (HCC) mouse model (113). In these animals HFD induced increased expression of TNF-α and ablation of TNFR1 significantly reduced obesity-enhanced HCC development (113). Altogether, these studies suggest that the inflammatory milieu instigated by obesity may be a general mechanism that links obesity to gastrointestinal cancers.

Activation of IKK/NF-κB pathway is consistently associated with both colitis- and obesity-associated carcinogenesis (99,109,113,114). Interestingly, the outcome of TNF mediated NF-κB activation, considering target gene expression, may alternate, depending on the tissue or cell type stimulated. In this context, NF-κB exerts not only intrinsic effects within pre-malignant epithelial cells, but also modulates actions of infiltrating lymphocytes and macrophages (115,116). In normal physiology, NF-κB response is self-limited by the induction of negative feedback loops (117,118). However in chronic inflammation induced by obesity, continuous cytokine release by immune cells of the stromal vascular fraction results in sustained IKK activation, which deregulates NF-κB activity (109).

The pro-oncogenic effects of NF-κB involve other intracellular mechanisms, besides continuous activation of IKK. Transcription factors, including STAT3, may

play a role in NF-κB dependent tumorigenesis (7). In tumors, accumulation of the prototypical NF-κB complex (p50/RelA) in the cellular nucleus is regulated through acetylation by p300 (119,120). It is relevant that STAT3 though p300 mediates RelA acetylation to promote and sustain NF-κB activity (121). Importantly, cytokines and growth factors encoded by NF-κB target genes, especially IL-6, are critical STAT3 activators (122-124). Interestingly, other inflammatory cytokines, such as IL-17, promotes STAT3 activation through NF-κB mediated IL-6 expression (125,126). Congruent with this data, expression of several inflammatory mediators, such as IL-6, COX2, IL-17 and IL-23, is also dependent of STAT3 as a RelA co-transcriptional factor (127-130).

Investigations on the influence of IL-6 in CAC showed that knockout mice for this cytokine developed less and smaller colonic adenomas than controls in a CAC model (123). Moreover, pharmacological inhibition of the common signaling receptor gp130 by a soluble gp130-Fc fusion protein also resulted in decreased tumor number and size in animals exposed to a CAC model (131). In consonance, genetic activation of gp130 in enterocytes of mice in a CAC model promoted increased tumor number and growth (132) whereas STAT3 deletion in intestinal epithelial cells markedly decreased the incidence and volume of AOM + DSS induced tumors (123). IL-6 is mostly produced by myeloid cells, primarily by lamina propria macrophages and dendritic cells during tumor initiation and by T cells during tumor progression, in CAC models (123,131,133). This is probably a consequence of the high inflammatory activity of CAC tumors and the continuous injury and death of enterocytes during tumor development (123). In other words, epithelial cells and cancer cells, as well as tumor-associated fibroblasts can also produce IL-6 and may contribute to the total amount of this cytokine, particularly in sporadic colorectal and obesity-associated colorectal cancers.

Taken together, these data provide strike evidence for the involvement of TNF-α by promoting continuous stimulation of IKK/NF-κB pathway in the pathogenesis of obesity-associated colon cancers. Furthermore, interactions between IL-6, STAT3 and NF-κB may have a role in this phenomenon.

TNF-α influence on obesity-associated colon carcinogenesis phases

Carcinogenesis can be didactically divided into three mechanistic phases: initiation (which involves stable genomic alterations), promotion (which involves the proliferation of genetically altered cells) and progression (which involves an increase in tumor size, its spreading and acquisition of additional genetic changes) (134). Notably, TNF-α may influence all those stages of tumor development (*Figure 2*).

Initiation

More than six decades ago, Peyton Rous defined initiation phase as a "*subthreshold neoplastic state*", in which "*latent tumor cells*" wait for the promotion stimuli to proliferate (134-136). Since the majority of cancers need at least 4-5 mutations to acquire a neoplastic phenotype (26,137) the initiation phase in current words corresponds to the early mutations observed in premalignant cells. TNF-α modulates the initiation phase by at least three mechanisms. First, TNF-α released by inflammatory cells in the tumor microenvironment may induce reactive oxygen and nitrogen species (RNOS) in adjacent epithelial cells, inducing DNA damage and genomic instability (138,139). Second, colorectal tumors may be initiated by increased activity of Wnt/β-catenin signaling in colon progenitor cells (140-142). Importantly, TNF-α through activation of NF-κB or repression of GSK3β promotes Wnt/β-catenin signaling in gastrointestinal mucosa (143,144). Finally, NF-κB regulates several tumor suppressor pathways; specifically it inhibits p53 activity through competition for the p300 and CBP co-activator proteins (145,146).

In spite of the effects of TNF-α in a number of important molecules involved in tumoral initiation, experimental evidence from obese Zucker rats and high fatty diet fed mice demonstrate that treatment with AOM does not changed the total number of aberrant crypt foci (147,148). Furthermore, recent data showed that obese individuals have an increased risk to develop β-catenin negative colon cancer, but not β-catenin positive (149). Overall, these findings are consistent with minor effects of obesity low-grade inflammation on the colonic tumor initiation.

Promotion

Initiation is an irreversible process, whereas promotion may be modulated by the stimuli intensity and even reversible if the stimuli are removed (134-136). The promotion phase is characterized by increased cell proliferation and reduced cell death. It may be an early or late event in tumor

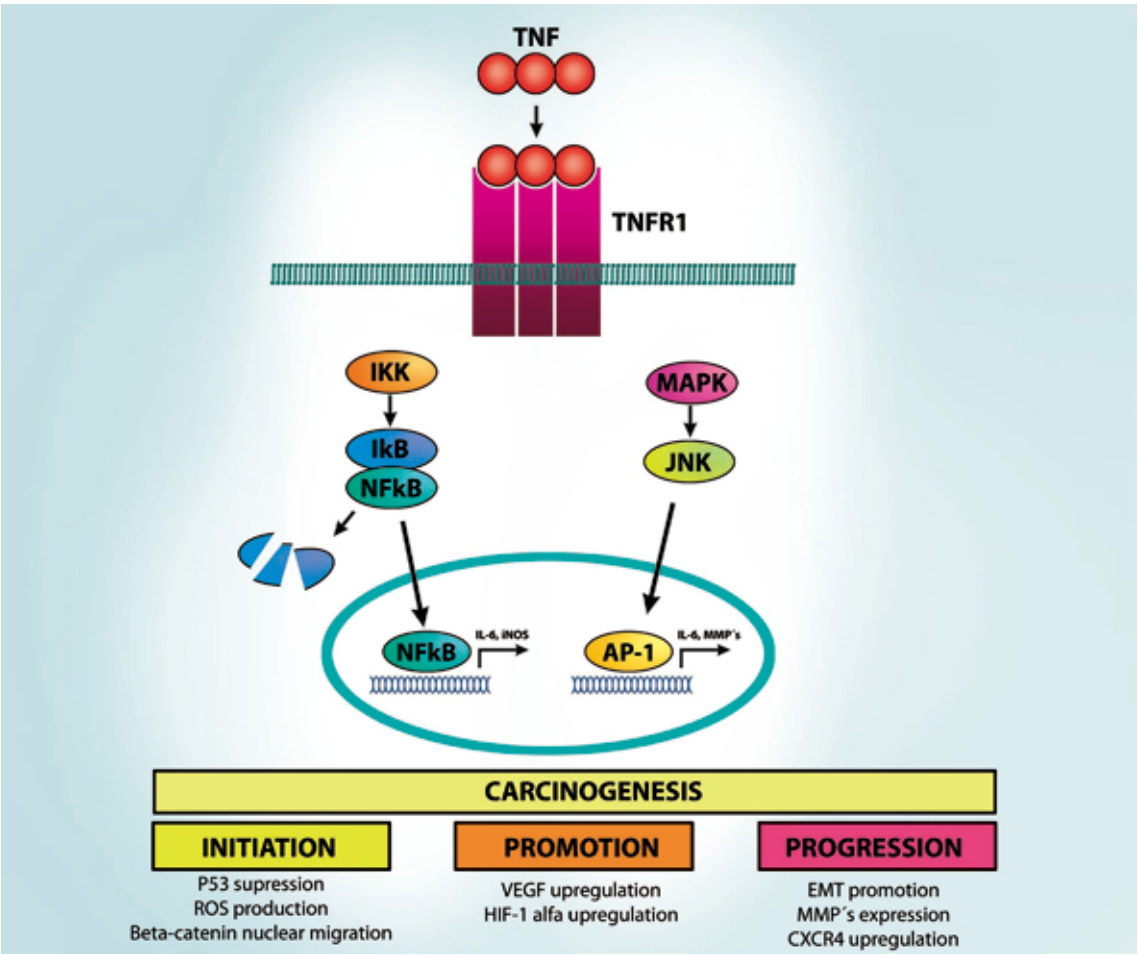

Figure 2 Tumor necrosis factor alpha (TNF-α) sensed by TNF-receptor 1 (TNFR1) phosphorylates inhibitor of nuclear factor kappa B (IKKβ) leading to degradation of inhibitor of kappa B (IκB) and nuclear migration of nuclear factor kappa B (NF-κB). TNF-α also promotes phosphoryation of mitogen-activated protein kinases (MAPK) pathway, resulting in Jun N-terminal kinase (JNK) and the activator protein 1 (AP-1) activity. Sustained activity of both NF-κB and AP-1 mediate important processes in distinct phases of colon carcinogenesis.

development, as late proliferation of dormant malignant lesions may also occur (150). Evidence for TNF-α-mediated colonic adenoma promotion in obesity came from observing elevated numbers and larger tumors size in obese animals compared to their lean controls, which was associated to IKK overexpression in these tumors (109). Accordingly, neutralization of TNF-α reverted the growth rate of colon cancer xenograft implanted in high fat diet fed animals to lean settings (109). Furthermore, obese animals switched from a HFD to regular chow after carcinogen exposure developed more tumors than lean controls, but similar number of aberrant crypt foci, the colonic pre-neoplastic lesion (148).

During tumor promotion, it is necessary to increase tumoral blood supply, mainly by angiogenesis triggered by tumor hypoxia (151). Interestingly, activation of NF-κB, STAT3 and AP-1 in tumoral microenvironment cells, such as tumor-associated macrophages (TAMs) and fibroblasts directly regulate important pro-angiogenic genes, including IL-8, CXCL1, CXCL8, VEGF, and hypoxia inducible factor 1 alpha (HIF1α) (152-154). Inactivation of NF-κB or STAT3, neutralization of CCL2 or CXCL12, or TAM depletion leads to ineffective angiogenesis and reduced tumor growth. Interestingly, the visceral adipose tissue of patients with colon cancer presents concomitantly increases in TNF-α and the pro-angiogenic factors, such as HIF1α and VEGF (155). Altogether, these data indicate that obesity-associated inflammation strongly affects colon

cancer promotion phase.

Progression

Metastatic disease is the most critical feature of cancer in a clinical setting as it is responsible for over 90% of disease mortality (156). The process of invasion and metastasis can be schematically divided into four major steps. First, epithelial-mesenchimal transition (EMT) is required for acquisition of a fibroblastoid phenotype by an epithelial malignant cell, resulting in increased motility and capacity to invade basal membranes and reach blood vessels or lymphatics (157). Second, cancer cells intravasate into blood vessels and lymphatics, with possible involvement of cytokines and inflammatory effectors by promoting increased vascular permeability (158,159). Third, metastatic cells should survive and travel in circulation (158,159). Fourth, circulating cancer cells should adhere and extravasate in a distant site, in which they need to interact with immune, inflammatory, and stromal cells to proliferate (158,159). Some of these cells may already be targeted to a pre-metastatic niche, in which soluble growth factors secreted by the primary tumor prime certain tissues for tumor cell engraftment, known as 'metastatic niche' theory (160-162). Obesity is associated not only with an increased incidence of colon cancer, but also with a more aggressive natural history; the patients are younger, present more metastasis to lymph nodes and the disease free and overall survival are reduced (163). In spite of the lack of direct evidence that obesity-associated inflammation interferes in these endpoints, TNF-α may exert effects in all metastatic phases.

TNF-α may contribute to cell migration-promoting EMT through stabilization of Snail, an inhibitor of E-cadherin expression, a key event in EMT (164-166). Interestingly, TNF-α, through NF-κB signaling, can also induce overexpression of other important regulators of EMT such as Twist, ZEB1 and SLUG, contributing to its induction (165,167-169). Another mechanism by which TNF-α can induce EMT is through synergistic action with transforming growth factor β1 (TGFβ) (170,171). Importantly, in a model of colon cancer, cancer cell invasiveness was associated to extracellular matrix proteolysis, a process that is dependent of matrix metalloproteinases (MMP) release, which may also be regulated by TNF-α induced activation of NF-κB (172,173).

After intravasation in circulation, metastatic cells need to survive in suspension and resist detachment-induced death, named anoikis (174). Notably, TNF-α, and other cytokines can promote survival of circulating metastatic cells, through activation of NF-κB in either inflammatory and cancer cells or by promoting a physical link between cancer cells and TAMs, allowing them to travel together throughout the circulation and evading immunological attacks (175,176). Furthermore, migration of metastatic cells is directed by chemokine gradients that are sensed by many receptors, including CXCR4, which expression is upregulated by TNF-α (177).

In a distant site, circulating metastatic cells are arrested on the endothelium in an integrin-dependent process. Therefore, adhesion between malignant and endothelial cells are important mediators of this process (175). Importantly, bone marrow-derived haematopoietic cells that express vascular endothelial growth factor (VEGF) receptor 1 (VEGFR) migrate and determine the metastatic sites before the arrival of neoplastic cells (160). Interestingly, the pre-metastatic niche is also defined by the tumor-secreted matrix protein versican, which activates TLR2 on host macrophages and promotes release of TNF-α (178). Accordingly, metastasis formation was dramatically reduced, by TLR2 or TNF-α suppression (178). Furthermore, VEGFA, TGFβ, and TNF-α secreted by the primary tumor promoted the expression of inflammatory proteins S100A8 and S100A9, leading to infiltration of lungs, the target site of metastasis, by myeloid cells expressing the cell surface antigens integrin αM (also known as MAC1) or CD11b (161). As a result, treatment with S100A8 and S100A9 antibodies diminished infiltration of MAC1 myeloid cells, resulting in a remarkable reduction in metastasis incidence (161). Specifically in regard to colon cancer, it was observed that targeting VEGF2 and other cytokines involved in the pre-metastatic niche formation reduced liver metastasis formation (179).

Conclusions

Recent clinical and experimental data provide support for the involvement of TNF-α in the pathogenesis of obesity-associated colon cancer. TNF-α promotes colon cancer in obese states through direct effects on premalignant cells and by orchestrating a tumor-promoting microenvironment through actions on several distinct cell types. However, how the cellular component of obese adipose tissue microenvironment promotes a "fertile soil" to carcinogenesis and whether interactions between inflammatory cells and adipocytes contribute to promotion

and progression of cancer is still largely unknown. Since these studies may contribute to a better understanding of carcinogenesis in general and give clues to cancer treatment, it will be critical in the future to systematically evaluate how an obesity-associated inflammatory microenvironment contributes to colon carcinogenesis.

Acknowledgements

None.

Footnote

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

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Management of oligometastatic rectal cancer: is liver first?

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Abstract: Twenty percent of patients with rectal cancer present with synchronous liver metastases at the time of initial diagnosis. These patients can be treated with a curative intent, although the choice and sequence of treatment modalities are not well established and are commonly debated in multi-disciplinary tumor boards. In this article we review clinical evidence for various treatment approaches and attempt to formulate a pathway for clinicians to use in evaluating and managing these patients.

Keywords: Rectal cancer; oligometastatic; radiation therapy; surgery; review

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Introduction

In 2014, an estimated 40,000 new cases of rectal cancer will occur in the United States (1). Approximately 20% of patients with locally advanced rectal cancer will present with synchronous liver metastases at the time of initial diagnosis (2). A recent meta-analysis reported a median survival of 3.6 years after liver resection in metastatic colorectal cancer, and a median 5-year survival of 38% (3). Several retrospective analyses of carefully selected patients with solitary colorectal liver metastases reported 5-year survival rates as high as 70% following liver resection (4-6). This heterogeneous patient population thus presents with the daunting combination of a reasonable curative potential and a high risk of systemic disease progression. The optimal management of this subgroup of patients is not well established and includes surgical resection of primary disease, systemic therapy (including cytotoxic chemotherapy and/or targeted small molecule therapeutics), pelvic radiation therapy and liver-directed therapy. Appropriate use, sequencing and timing of these therapeutic modalities are not supported by randomized clinical trials in patients with synchronous oligometastatic liver disease with primary rectal cancer and are hence open to debate. We will attempt to synthesize a reasonable treatment paradigm based on clinical evidence, realizing that clinical experience and expertise of individual physicians as well as individual patient characteristics and preferences should guide the multidisciplinary team decision. Well-designed clinical trials and novel therapeutic modalities will be expected to either support or reverse our theoretical exercises.

Upfront surgery *vs.* systemic therapy

Upfront surgical resection of all gross disease, whether synchronous or staged, is a common practice at many institutions (7). Two primary arguments for this approach are both the concern for the known hepatic toxicity of prolonged courses of cytotoxic chemotherapy, with irinotecan-based regimens, in particular, contributing to the development of chemotherapy-associated steatohepatitis (CASH) and sinusoidal congestion, which increase the risk of complications at the time of liver resection. Another argument is a potential for liver disease progression on systemic chemotherapy and a possibility of losing a window of opportunity to administer a curative R0 resection for patients expressing a more aggressive malignant phenotype or one unresponsive to standard chemotherapy regimens.

A level 1 data set on this subject, the EORTC Intergroup trial 40983 randomized 364 patients with colorectal cancer and up to four liver metastases to either six cycles of FOLFOX4 before and six cycles after surgery or to surgery

alone. The initial publication (8) with a median follow up of 3.9 years revealed a statistically significant improvement in progression-free survival with the bi-modality approach. Reversible post-operative complications were higher in the chemotherapy group (25% *vs*. 16%, P=0.04), while post-operative death was similar in the two arms (1%), and only 1 out of 182 patients in the chemotherapy arm could not undergo resection due to liver damage. Twelve patients (7%) showed progressive disease on chemotherapy, with only 4 of these 12 becoming unresectable due to progression of liver lesions. The long-term results were published last year (9) and revealed no difference in overall survival (51% *vs*. 48% at 5 years). Two patients in the perioperative chemotherapy group and three in the surgery-only group died from complications of protocol surgery, and one patient in the perioperative chemotherapy group died possibly as a result of toxicity of protocol treatment. The retrospective analysis of EORTC 40983 data suggested a benefit of perioperative chemotherapy in patients with CEA values of >5 ng/mL, good performance score and body mass index <30 (10). While this is certainly a landmark study, it is difficult to draw definitive conclusions from the EORTC data regarding rectal cancer, as only 1/3 in each group had a rectal primary and in the entire cohort only 35% had synchronous disease. It is likely that different considerations should be weighed in those with synchronous disease at presentation. If there is concern for liver damage precluding resection with up-front chemotherapy, strong consideration should be given to proceeding with surgical resection as first-line therapy. Alternatively, in those who may be borderline for resection due to technical considerations, relationship of tumor(s) to critical structures, and size of the future liver remnant, chemotherapy should be the initial choice. Thus, careful planning in the multi-disciplinary setting prior to initiation of therapy is critical.

Response to chemotherapy is recognized as a predictor of outcome after resection (11,12), and patients who are offered metastatectomy in the setting of disease progression on chemotherapy have worse outcomes compared to those with radiographic response based on 5-year survival rates of 8% *vs*. 37%, as published by Adam *et al*. (13). Therefore systemic therapy prior to surgery appears to be safe, effective and can be used to select candidates with a more favorable tumor phenotype for liver metastases resection.

In the setting of oligometastatic rectal cancer, one should also consider the effect of the first treatment modality on the primary disease status. If a curative surgical approach is selected, obtaining local control becomes critical. Consider local recurrence rates of 22% for stage II and 46% for stage III patients treated on the Swedish Rectal Cancer Trial with surgery alone (14). Among patients with synchronous metastatic disease, the rates of advanced primary disease are high—for example, a contemporary series from Johns Hopkins University revealed 86% of patients had T3/T4 primary disease and approximately two-thirds had N+ disease at presentation (15). At the same time 50-60% of patients with stage II and III rectal cancer are down-staged following neoadjuvant therapy, with about 20% of patients showing a pathologic complete response (16-19).

For all the above mentioned reasons upfront surgery should not be considered standard in the setting of oligometastatic rectal cancer. The National Comprehensive Cancer Network (NCCN) have updated their 2014 guidelines version and removed upfront surgery from the standard treatment algorithm (20) for resectable synchronous metastatic rectal cancer.

Neoadjuvant therapy

The current version of NCCN guidelines offer two initial pathways for treating rectal cancer with resectable synchronous metastases—either an oxaliplatin-containing chemotherapy or pelvic radiotherapy with 5-FU-based concurrent chemotherapy. Clearly, the first pathway predominantly focuses on the systemic disease, whereas the second pathway is directed more at the pelvic disease control. The neoadjuvant approach that optimizes the therapeutic ratio should be effective for both local and systemic disease components, and be well tolerated by the patients, who must still have a performance status appropriate for an R0 surgical resection.

A retrospective analysis was carried out on 20 patients (with a total of 41 liver lesions) who underwent preoperative chemo-RT for rectal cancer with synchronous resectable liver metastases (21). All patients received a standard fractionated course of pelvic RT to 45 or 50 Gy over a period of 5 weeks, with operation performed 6 to 8 weeks later. Seven patients received FU-based-chemotherapy and 13 patients received oxaliplatin-based chemotherapy, concurrently with radiation. During oxaliplatin-RT 25 liver lesions showed the following response: 14 showed an objective tumor response, 10 were stable and 1 progressed. Among the 16 liver lesions during 5-FU-RT, 10 lesions were stable and 6 progressed. The absence of concomitant oxaliplatin-based chemotherapy was the sole predictive factor (P=0.002) of liver disease progression on imaging

during chemo-RT. There were no postoperative deaths after either rectal or hepatic surgeries in this series. Three years OS and DFS were 51% and 24%, and 6 out of 13 (46%) patients on oxaliplatin-RT developed disease recurrence *vs.* 6 out of 7 (86%) patients on 5-FU-RT (P=0.157).

Thus, the data suggests that pelvic RT with 5-FU or capecitabine might not be effective enough in controlling liver disease and preventing new distant disease recurrence. Therefore one might argue for either addition of oxaliplatin to pelvic RT or oxaliplatin-based systemic chemotherapy alone with pelvic RT omission. A prospective study enrolled 32 patients with stages II and III rectal cancer and treated with neoadjuvant FOLFOX/bevacizumab without RT. One hundred percent of patients achieved R0 resection, with 25% path CR rate and 100% local control rate at 4 years. The NCCTG phase II/III trial is now recruiting patients with stage II-III rectal cancer to either neoadjuvant FOLFOX or preoperative chemo-RT (clinicaltrials.gov NCT01515787). The results of this randomized trial will reveal whether patients could be spared radiotherapy-related toxicity without jeopardizing local control.

While the addition of oxaliplatin to pelvic RT would seem to be one of the reasonable solutions, prospective clinical trial data suggests otherwise when evaluated in the setting of non-metastatic rectal cancer. The STAR-01 trial randomized patients to 5-FU/oxaliplatin/RT *vs.* 5-FU/RT and revealed no difference in path response rate between the arms, whereas grade 3 and 4 toxicities were higher among patients randomized to oxaliplatin arm (24% *vs.* 8%, P<0.001) (22). Similar results were found in NSABP R-04 (23) and the ACCORD 12/0405-Prodige 2 trial (24). Therefore, addition of oxaliplatin to a 5-FU-based neoadjuvant chemo-RT platform is not justified in non-metastatic setting at this point. However, this might not apply to patients with oligometastatic disease, where systemic disease control is more critical and this approach may be worth the risk of additional treatment toxicity.

Another strategy of combining oxaliplatin with pelvic radiation is currently studied in a Polish Colorectal Study Group randomized Phase III trial. Patients with fixed T3/T4 or locally recurrent rectal cancer without distant metastases are randomized to either short-course RT (5 Gy ×5, given over 1 week) and 3 courses of FOLFOX 4 versus standard 50.4 Gy RT with concurrent 5-FU/leucovorin and oxaliplatin. Surgery in both groups is performed 12 weeks after the beginning of radiation. The interim analysis of the first 100 patients was recently published (25) and revealed a path CR of 21% in short-course RT arm (experimental) *vs.* 8% in the standard RT (control) arm. The experimental arm had 27% rate of post-operative complications and no post-operative mortality.

A small Korean prospective study (26) enrolled 6 patients with oligometastatic rectal cancer on upfront systemic chemotherapy with FOLFOX (with and without biologic agents) and a short-course RT (5 Gy ×5) sandwiched between chemotherapy cycles, prior to surgery. Five patients achieved R0 while all liver metastases had regressed. Prior to surgery, three patients had grade 3 toxicities, controlled by conservative therapy. With a median follow-up of 16 months, there was no locoregional recurrence, one patient developed distant metastases and no patient died. The long-term follow-up report of this experience will be important to confirm the early observations.

At present, it appears that either an oxaliplatin-based systemic therapy alone or with concurrent pelvic RT (either standard fractionated RT or a short-course RT) are reasonable neoadjuvant treatment strategies for patients with *de novo* oligometastatic rectal cancer. Ongoing and future studies that include well-defined cohorts of patients and pre-treatment tumor parameters will help provide clarity as to which strategy yields the optimal therapeutic ratio.

Synchronous (combined) *vs.* staged (sequential) surgical procedures

No randomized studies have ever evaluated the difference between two surgical approaches—synchronous (combined) approach, when liver metastases are resected at the time of TME of rectal tumor, versus a staged approach, when the two surgeries are temporally separated. Consequently, this issue is debated in multidisciplinary tumor boards on a routine basis. Hillingso and Wille-Jorgensen (27) set out to perform a systematic review on the surgical approach for synchronous liver metastases from colorectal cancer in 2007 and found conflicting evidence from available case series. Among the series they have identified, 11 studies showed a tendency towards a shorter hospital stay in the synchronous resection group, 14 studies revealed a lower total perioperative morbidity with this approach, while 15 studies identified a lower perioperative mortality with the staged approach. Eleven studies compared 5-year overall survival, which appeared to be similar in both strategies. Specific factors that have been shown to increase the rate of postoperative complications in the combined procedures were the presence of a diverting stoma, rectal location of

the primary tumor, duration of the surgery, intraoperative blood loss and the need for transfusion. A large multi-institutional retrospective study with over 600 patients revealed similar rates of mortality and severe morbidity after simultaneous colorectal resection and minor hepatectomy compared with isolated minor hepatectomy alone. However, major hepatectomy independently predicted for severe morbidity after simultaneous resections with a hazard ratio of 3.4 (P=0.008). Much debate exists regarding the optimal surgical approach (28). Furthermore, adequately powered studies comparing outcomes for major hepatectomy alone versus in combination with TME are lacking. Another important consideration is the move toward minimally invasive techniques for both the hepatic resection and TME for the primary. Many centers are moving toward these techniques, and the oncologic integrity of these approaches has been validated by several studies and consensus statements (29,30). Currently, laparoscopic techniques tend to yield shorter hospitalizations for major hepatectomy at the expense of increased operative times. Thus, staged operations may confer an overall benefit to the patient in terms of time in the operating room and lower complication rates. Patient and tumor characteristics, surgical experience and patient preference should guide the decision. At the same time, alternatives to these surgeries should also be discussed with patients, when appropriate.

Avoidance of primary rectal tumor resection in complete responders to neoadjuvant therapy

Following the success of neoadjuvant chemo-RT in anal cancer with a shift of treatment paradigm from resection to organ-preservation, led by Nigro over 30 years ago (31), several retrospective studies analyzed the outcomes after observation following complete clinical response to neoadjuvant therapy in patients with localized rectal cancer. One earlier study showed promising results with excellent DFS and OS rates at 5 years (32), but most clinicians remained skeptical of this approach (33). However, a more recent study (34) prospectively selected 21 patients with localized rectal cancer who achieved a clinical CR after chemoradiotherapy, as evaluated by magnetic resonance imaging (MRI) and endoscopy with biopsies, and followed these patients by observation for a mean follow-up of 25 months. Only one patient developed a local recurrence and had a successful salvage surgery, whereas the remaining 20 patients were alive without disease. Because of limited data and concern about the ability of imaging studies to accurately determine a pathologic response (35), the NCCN 2014 panel did not support the observation approach for patients with localized rectal cancer with complete response to neoadjuvant treatment. However, this treatment paradigm, although previously untested, could be considered for patients with known metastatic disease. These patients have a higher likelihood of systemic disease progression than patients with localized rectal cancer, and therefore the tradeoff of a lower primary disease local control for the improved quality of life might be reasonable and worthy of further investigation. Quality of life can be improved in this patient population with surgery reserved for patients with local recurrence in the absence of systemic disease progression or in the event of symptomatic local disease progression. This approach, if used, should incorporate pelvic radiation therapy as part of a neoadjuvant treatment recommendation, as the rate of local recurrence after pathological response to chemotherapy alone has not yet been studied.

Alternatives to liver surgery

It is rare for liver metastases to be permanently eradicated with systemic chemotherapy alone, even in the setting of complete radiographic response. One study revealed an 83% rate of local failure or disease persistence in sites that had initially shown a complete response to systemic chemotherapy by CT imaging (36). These results highlighted the potential pitfalls when interpreting the “disappearing metastasis” as complete response to chemotherapy. Surgery remains the standard of care even when there is a significant or complete radiologic response to up-front chemotherapy for isolated liver metastases, with 5-year overall survival rates up to 70% in selected patients. However, because of tumor size and location, over four-fifths of patients present with unresectable disease (37). Nonsurgical options have emerged and continue to constantly improve.

Radiofrequency ablation (RFA) has recently been shown to offer a 60% rate of local control beyond 12 months (38-40) and should be considered for patients who are technically unresectable or unable to tolerate an open resection. In general, lesions amenable to RFA should be no larger than 3 cm in size, not located near hilar structures, and be treated at centers with expertise in this field. Controversy persists as to whether RFA is equivalent to open or laparoscopic resection for those with appropriately sized lesions and prospective data are sorely needed. In

fact, lack of adequate evidence prompted the American Society of Clinical Oncology (ASCO) to publish a review on this topic, and the data regarding the equivalence or comparative utility of RFA relative to surgical resection was found insufficient to issue a practice guideline (41).

Non-conformal radiation therapy has a very limited role in treatment of hepatic metastases due to the high rates of radiation-induced liver disease (RILD), which develops after large percentage of liver is exposed to the radiation dose, necessary to control the metastatic disease. However, stereotactic body radiation therapy (SBRT) has emerged, which delivers radiation to a target in the body, with sufficient intensity to kill, or at least control, the underlying malignancy, while minimizing the radiation dose to adjacent normal tissues. Effectively and safely accomplishing these conflicting goals requires quantitative visualization and localization of the target lesion, complex radiation plans, continual management of the target position throughout treatment, and robust quality assurance. Detailed review of SBRT technique and clinical data has been expertly reviewed elsewhere (42). The largest series with a long-term follow-up on SBRT in colorectal liver metastases reported on 65 patients with 102 lesions treated at Princess Margaret Hospital, University of Colorado and Stanford University (43). The overall local control rate was 71%, while patients who received biologically equivalent dose (BED) of ≥79 Gy_{10}, 12-, 18- and 24-month local control rates were 86%, 80% and 71%, respectively. On the basis of the best-fit curve, a BED of 117 Gy_{10} would yield a 90% local control rate (which corresponds to a dose schedule of at least 48 Gy given in 3 fractions of 16 Gy, or its equivalent if a different number of fractions is used). In terms of toxicity of this treatment, 17% of patients experienced grade ≥2 acute (defined as within 3 months of SBRT) GI toxicity, 3% did grade ≥3 elevated liver enzymes, but none had symptomatic liver toxicity. Late toxicities were also limited, with 6% of patients experiencing grade ≥2 GI toxicities: two patients had grade 3 gastritis and two patients had grade 2 small bowel ulcers.

Further validation is needed before SBRT can be considered a standard of care for liver metastases from rectal cancer. Currently, phase I trials at University of Pittsburgh (NCT01360606) and the University of Texas (NCT01162278), plus a phase II study at the Massachusetts General Hospital (NCT01239381), are accruing patients. A phase III trial at University of Aarhus is randomizing patients with liver metastases to RFA or SBRT. Whenever possible, patients should be offered a chance to participate in prospective studies. Nevertheless, both RFA and SBRT should be considered for patients who cannot undergo liver resection.

Summary

The heterogeneous group of patients with oligometastatic rectal cancer involving the liver presents with a daunting combination of a reasonable curative potential, yet with a high risk of systemic disease progression. The optimal management of this subgroup of patients is not well established. The 2014 NCCN guidelines have removed upfront surgery as the treatment recommendation for most patients, realizing that systemic and pelvic control take precedence over surgical extirpation of liver and primary disease. As summarized in this review article, oxaliplatin-based chemotherapy with or without pelvic radiation therapy, followed by either resection of primary and liver disease or consideration of non-surgical modalities appear to be the most well-supported treatment approaches in the literature. Multidisciplinary evaluation of each patient is paramount to achieve best outcomes, with taking into account patients' preferences as well the expertise and experience of the multidisciplinary team. Future well-designed studies will shed light on how best manage this heterogeneous group of patients.

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Footnote

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Liver-directed therapies in metastatic colorectal cancer

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Abstract: Colorectal cancer (CRC) is a major health concern in the United States (US) with over 140,000 new cases diagnosed in 2012. The most common site for CRC metastases is the liver. Hepatic resection is the treatment of choice for colorectal liver metastases (CLM), with a 5-year survival rate ranging from 35% to 58%. Unfortunately, only about 20% of patients are eligible for resection. There are a number of options for extending resection to more advanced patients including systemic chemotherapy, portal vein embolization (PVE), two stage hepatectomy, ablation and hepatic artery infusion (HAI). There are few phase III trials comparing these treatment modalities, and choosing the right treatment is patient dependent. Treating hepatic metastases requires a multidisciplinary approach and knowledge of all treatment options as there continues to be advances in management of CLM. If a patient can undergo a treatment modality in order to increase their potential for future resection this should be the primary goal. If the patient is still deemed unresectable then treatments that lengthen disease-free and overall-survival should be pursued. These include chemotherapy, ablation, HAI, chemoembolization, radioembolization (RE) and stereotactic radiotherapy.

Keywords: Colorectal cancer (CRC); liver metastases; hepatectomy; radiofrequency ablation (RFA); portal vein embolization (PVE)

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Introduction

Colorectal cancer (CRC) is a major health concern in the United States (US) with over 140,000 new cases diagnosed in 2012 (1). Worldwide CRC is the second leading cause of cancer deaths (2). However, there has continued to be a decline in the death rates due to increased screening, prevention, and improved treatment options. The 1-year and 5-year survival rates are 83.2% and 64.3% respectively (1). However, once there has been metastasis to distant organs the 5-year survival drops to 11.7% (1). The most common site for CRC metastases is the liver. Approximately 25% of patients have hepatic metastases at their initial presentation, and another 30% develop metastases during the course of their disease (2). Hepatic disease accounts for two-thirds of CRC deaths (2,3), emphasizing the importance of understanding the multidisciplinary and multimodality treatment options for colorectal liver metastases (CLM). Surgical resection remains the gold standard for curative resection with several modalities available to extend the resection criteria and additional modalities to extend survival and provide palliation when the patient is not a resection candidate.

Surgical resection

Hepatic resection is the treatment of choice for CLM, with a 5-year survival rate ranging from 35% to 58% in modern series (4-14). The most common indication for hepatectomy in western populations is CLM (15). The morbidity and mortality rates of hepatic resection in modern series are less than 30% and less than 3% respectively (2,4,13,16-18). There have been multiple risk factors that have been reported to independently predict survival after resection. These factors include age, primary tumor stage, preoperative carcino embryonie antigen (CEA) level, disease-free interval, hepatic

tumor size, number of metastases, margin of resection, and presence of extrahepatic disease (4,12,19). These factors are important to identify the 10-20% of patients with hepatic metastases that are eligible for resection (3,17,20).

In 1999, Fong *et al.* developed a clinical prognostic score, identifying seven factors with a significant impact on survival following resection of colorectal metastases (12). The first two of these factors were positive margin and the presence of extrahepatic disease both of which predicted a risk of death 1.7 times greater than baseline. The authors concluded that those two should be relative contraindications to resection. The other five factors were disease-free for less than 12 months, number of tumors >1, pre-op CEA >200, lymph node-positive primary and size of tumor >5 cm. A scoring system was devised with 1 point for each of the five factors. The 5-year survival rate for patients with 0 points was 60% *vs.* the rate for patients with 5 points was only 14%. They concluded that those with a score of 0-2 have a highly favorable outcome, those with a score of 3-4 have a much more guarded prognosis and resection should be planned only in the context of adjuvant therapies. In patients with a score of 5, resection without effective adjuvant therapy or outside of adjuvant trials would be highly questionable. The prognostic score still remains valid but the 5-year survival of even patients with a score of 5 has improved to 31% in a more recent analysis (21). The improvement is likely related to numerous factors including more effective chemotherapy and adjunct procedures to extend the indications for resection. In the more recent analysis the only patients that derived no benefit from resection were those with ≥8 metastases combined with an inflammatory tumor response (21).

While most studies traditionally looked at clinicopathologic factors like those described above to determine which patients will benefit from liver resection, the focus has now shifted to whether complete intrahepatic and extrahepatic disease resection can be obtained, while maintaining sufficient hepatic reserve (22).

The definition of complete intrahepatic resection has been based on a general consensus that a 1 cm margin should be obtained. More recently the exact definition of an adequate margin has been more closely evaluated. Based on a number of studies it appears that with modern chemotherapy the width of the margin does not impact overall survival (OS) as long as it is negative (4,23-27).

The ability to remove all disease from the liver safely with a negative margin is dependent on the future liver remnant (FLR). The FLR should be calculated in a standardized fashion for all patients in whom the expected FLR is ≤40% (28). There is no consensus as to the minimal FLR at which liver surgery can be done safely (28). Suggested guidelines are in a patient without cirrhosis or underlying liver disease, ≥20% of the total liver volume must remain (2,3,29,30). In patients with extensive steatosis or chemotherapy a volume of >30% has been proposed, and patients with cirrhosis should have a FLR of >40% prior to hepatic resection (2,3). Studies looking specifically at extended liver resections show that the complication rate, intensive care unit stay, and hospital stay are all prolonged in patients with an FLR ≤25% (28-30). Another method to assess safety of resection is FLR to body weight ratio rather than percentage of total liver volume. A FLR to body weight ratio of ≤0.5%, puts the patient at considerable risk for hepatic dysfunction and mortality (31).

Extra-hepatic disease

Long-term post-hepatectomy survival is possible in selected patients with extra-hepatic disease (EHD). Multiple studies show long term survival is possible with complete resection of EHD with survival based on the EHD site. Lung metastases with CLM have the best survival, pedicular lymph nodes and peritoneal disease have a somewhat lower OS, and multiple sites and para-aortic or celiac nodes have a dismal prognosis (32,33). OS is significantly lower in the EHD group compared with patients without EHD, but a 5-year OS of 19-38% compares favorably with rates much less than 5% when treated by chemotherapy alone (32-36). A recent review analyzed 22 studies with 1,142 patients with EHD and CLM, morbidity and mortality were 28% and 1% respectively, similar to isolated CLM resection series. The review found a median overall 5-year survival with an R0 resection of 25% (range, 19-36%). As previously noted survival varies by EHD site with a median 5-year OS for lung of 27% (range, 0-33%), porta-caval nodes 17% (range, 0-27%), peritoneal metastases 8% (range, 0-30%), and multiple sites 7% (range, 0-28%) (36). The significantly better survival associated with lung metastases must keep in mind that in these patients, the liver resection and lung resections were likely staged, allowing for potential selection bias as the patients who progressed in the lung were excluded. Looking specifically at CLM and peritoneal disease a recent multi-institutional study of 523 patients with peritoneal disease from CRC, of which 77 had CLM found no that CLM did not impact OS for the entire group but did have a significant impact on the group

that had an R0 resection of the peritoneal disease. Based on this the authors felt that liver metastases could be a relative contraindication if associated with a high peritoneal index (37). In summary, resection of CLM with EHD can result in long term survival in highly selected patients when complete resection of disease is possible.

Synchronous CLM

A subset of EHD is the patient with synchronous presentation of CRC and CLM. Studies are divided on whether synchronous CLM is associated with worse survival than metachronous metastases (38). In resectable patients the decision is whether colon and hepatic resections should occur as a single combined procedure or staged. There are three options including staged resection with colon first, staged with liver first, or simultaneous resection. The concern with simultaneous resection has been increased morbidity and mortality associated with the combined operation. However, recent studies have shown simultaneous resection to be similar in morbidity, and perioperative mortality to staged resection (39-42). A recent multicenter international analysis compared simultaneous resections to staged (colon first and liver first) in over 1,000 patients and found no significant difference in morbidity, mortality or long-term oncologic outcomes between any of the three sequences (39). In addition, a recent meta-analysis confirmed no difference in oncologic outcome between staged and simultaneous resection, and a shorter hospital length of stay and lower morbidity with simultaneous resection (40). Retrospective studies have also shown that complications and mortality are similar between staged and simultaneous procedures even in the setting of major hepatectomy (39-41). Simultaneous resection appears safe in selected patients but most studies addressing staged versus simultaneous resection have a high degree of selection bias given that patients expected to have higher complication rates will generally be offered staged resection. In selected patients the simultaneous resection of the primary colon tumor and hepatic metastasis may be the preferred approach, as it avoids a second surgery, permits earlier completion of surgical therapy, allowing more prompt initiation of adjuvant therapy (41). According to a recent expert consensus the priority in staged resection may be given to colorectal-first or liver-first strategies based on concern for complications related to the primary tumor, such as obstruction, perforation, or bleeding, or the progression of marginally resectable CLM during treatment of the primary (38). The decision to do simultaneous resections is based on the overall complexity of both procedures and the patient's comorbidities (38). The liver-first sequence is most suited to rectal cancers so that the liver metastases are not left untreated during the radiation portion of treatment to the rectum (38). During the simultaneous procedure the liver resection is typically done first so that it may be done with low central venous pressure (38). Whichever order of procedures is used, R0 resections need to be obtained at both sites. If liver metastases are not resectable, resection of the primary tumor does not improve survival (42) and should only be used in patients with symptoms that are not controlled with less invasive techniques.

Adjuncts to improved resectability

When the FLR is anticipated to be marginal there are several options for improving the FLR. These options include systemic chemotherapy, portal vein embolization (PVE), two-stage hepatectomy, and associating liver partition with portal vein ligation (PVL) for staged hepatectomy (ALPPS)/*in situ* split procedure.

Systemic chemotherapy

For patients with unresectable disease, systemic chemotherapy remains the standard first-line therapy. For patients with initially unresectable CLM, systemic chemotherapy offers the possibility of reducing the tumor burden to an extent where resection becomes possible (38). In patients with disease initially determined to be anatomically unresectable, modern preoperative chemotherapy allows complete resection in 12.5-32.5% of patients (43,44). These regimens include FOLFOX (folinic acid, fluorouracil, and oxaliplatin) and FOLFIRI (folinic acid, fluorouracil, and irinotecan) most commonly and more recently the use of the monoclonal antibodies bevacizumab or cetuximab in combination with chemotherapy to increase response rates (45).

Steatosis and steatohepatitis have been associated with the use of fluorouracil and irinotecan. Sinusoidal dilation and congestion can be seen with prolonged use of oxaliplatin. Both steatohepatitis and sinusoidal injury, but not steatosis, have been associated with increased perioperative morbidity with liver resection (45-50). Steatohepatitis has been associated with increased mortality (47). The increase in morbidity appears to be related to duration

of therapy with increased risks with greater than six cycles (45,48). Scoggins *et al.* found no difference in morbidity or mortality with neoadjuvant chemotherapy with a median chemotherapy duration of 4.2 months (51). Steatohepatitis is also more frequently seen in obese patients with neoadjuvant chemotherapy. Bevacizumab does not appear to be increase complication rates when added to standard chemotherapy regimens but studies stop the drug for an average of 6-8 weeks prior to surgery (52,53). There is some data that bevacizumab may be protective when combined with oxaliplatin against development of sinusoidal injury (46). There are no published studies regarding the direct effect on chemotherapy-induced liver injury of the anti-epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab (46).

Because approximately two-thirds of patients have a recurrence following resection of colorectal metastases preoperative systemic chemotherapy has been examined in resectable colorectal metastases as well. The EORTC Intergroup trial 40,983 randomized patients with resectable colorectal metastases to six cycles of perioperative chemotherapy with FOLFOX or surgery and found improved 3-year progression free survival (PFS) for neoadjuvant chemotherapy (49). The study was not powered to adequately assess for a survival benefit but a follow up study showed no difference in OS between the two groups (17). Retrospective studies show variable results based on prognostic factors. Adam *et al.* looked at metachronous solitary lesions and found increased morbidity with no improvement in survival (54). Zhu *et al.* found that patients with more than two poor prognostic factors had a survival advantage with neoadjuvant therapy (55). Malik *et al.* examined more than 600 patients retrospectively and found no difference in disease free survival (DFS) or OS between neoadjuvant versus upfront surgery (56). Reddy *et al.* in a large multi-center retrospective study, examined patients with resectable synchronous colorectal metastases. They found that post-hepatectomy chemotherapy but not preoperative chemotherapy increased OS (57). The variability in these findings has led to differences in expert consensus varying from resection should be performed as soon as feasible, and the duration of neoadjuvant therapy should be carefully considered to most patients regardless of resectability should receive chemotherapy upfront (3,58).

PVE

PVE has been used in pre-operative management of patients with marginal FLR to increase the safety of resection in these patients. The physiologic response is referred to as the atrophy-hypertrophy complex (AHC) and is likely related to increased flow within the portal vein to the non-embolized lobe (59,60). PVE can be performed under conscious sedation by interventional radiology under sonographic and fluoroscopic guidance (30,61). Resection typically occurs 3-6 weeks following embolization. This time frame is based studies showing it takes at least 3 weeks to reach the steady state of liver regeneration (62). The hypertrophy of the FLR reduces the risk of postoperative liver failure and allows potentially curative extended hepatectomy in a group of patients that otherwise would be only marginal candidates for resection based on a small FLR. PVE has been reported to result in a 7-27% increase in the % FLR (30,61,63). With PVE the functional capacity as measured by indocyanine green excretion and 99mTc-GSA scintigraphy appears to improve to a greater extent and sooner than hypertrophy (64,65). PVE is safe, with complication rates less than 10% in most series (61,62,66). PVE results in a greater than 60% resection rate and an R0 resection in greater than 70% of resected patients (30,62,63,67). Liver surgery following PVE can be accomplished safely with morbidity of 19-55% and perioperative mortality of 1-7% (61,63,67-69).

There is a concern that tumors could have increased growth rates following PVE in both the embolized and non-embolized lobes. The hypothesis states that by increasing hepatic artery and portal blood flow there is an increase in local growth factors, leading to tumor growth (70,71). Several studies have indeed demonstrated this in colorectal metastases (15,70-72). The addition of chemotherapy between PVE and resection has shown success in slowing tumor progression, and improving long-term survival for PVE patients (15). Given the proposed etiology of the increased growth rate Bevacizumab has been examined for its potential impact on tumor growth following PVE with a decrease in the tumor growth rate but it did not reach statistical significance (71). Initially it was thought that if a patient continued their neoadjuvant chemotherapy there would be impediment of liver hypertrophy. However, this has more recently been shown to be false, with chemotherapy having no negative effects on the amount of hepatic hypertrophy (73).

The contraindications to PVE are largely relative and include tumor invasion of the portal vein (presumably flow has already been diverted), portal thrombosis, severe portable hypertension, uncorrectable coagulopathy, renal failure, and biliary dilation not amenable to drainage in the FLR (2,3).

Imaging should be performed 3-6 weeks after PVE to assess the amount of hypertrophy, determine the patient's new FLR, and determine if resection for cure is possible.

Two-stage hepatectomy

Two-stage hepatectomy can accomplish complete resection of disease that is initially unresectable, resulting in improved survival over comparative patients treated with chemotherapy only (74). This approach usually begins with 4-6 cycles of systemic chemotherapy. Repeat imaging is obtained and patients with response or stable disease undergo the first-stage resection. The first-stage resection usually involves resection of all metastases from the future FLR in the form of minor resections that avoid hilar dissection or mobilization of the contralateral liver (75). Often PVE is necessary at this stage to increase FLR prior to the second-stage resection. Resecting all disease in the FLR prior to PVE also avoids the increased tumor growth rate seen following PVE (70). After 4-6 weeks, typically with or without chemotherapy, repeat imaging is obtained to assess for liver regeneration and second-stage resection then follows (38). Morbidity following the first procedure is 11-17% with negligible mortality (74,76,77). It is important to minimize morbidity after this first stage to ensure the subsequent resection because there is no benefit of just the first stage for survival (74). The second stage resection is completed in 76-87% of patients who undergo the first stage (74,76,77). The R0 resection rate for the second stage procedure is 58-79% (74,77).The 3-year OS ranges from 50% to 84% for patients completing both stages of resection (74,76,77). This survival is a reflection of both selection of favorable biology and complete resection of metastatic disease (74).

Associating liver partition with PVL for staged hepatectomy (ALPPS)/in situ split procedure

ALPPS or the *in situ* split procedure is an alternative to PVE for increasing the FLR. This is a novel procedure in its developmental stage with promising initial results (78). The first stage is surgical exploration, right PVL, and *in situ* splitting (ISS) of the liver parenchyma to the right of the falciform ligament for proposed extended right hepatectomy or along Cantile's line for right hepatectomy. Computed tomography (CT) volumetry is performed about a week later followed shortly by the second operation performed where completion of the resection of the involved liver is performed (78-83).

The increase in FLR with ALPPS ranges form 63-87% (79-83). The morbidity ranges from 53-71% with a mortality of 0-22% (79-83). The reported mortality after ALPPS is significantly higher in some series than the 4.7-5.6% reported after extended hepatectomy in recent series (79,81,82,84-86). This increased mortality will likely decrease as the technique and indications are further developed (78). A particularly high rate of morbidity and mortality is seen in hilar cholangiocarcinoma patients with preoperative cholestasis and colonized bile, with some authors questioning the indication in these patients (81,82). Given the novel nature of the technique there are no long-term oncologic outcome studies.

The advantage of ALPPS over PVE is the short interval to completion surgery. This short interval may prevent tumor progression. The shorter interval also adds a technical advantage over the more traditional two-stage hepatectomy. There should be fewer adhesions, a faster recovery for the patient, and the ability for the patient to start adjuvant therapy sooner. ALPPS also addresses the most common causes of failure to undergo resection following PVE, disease progression and failure of FLR to hypertrophy (63,87). When compared to PVE the hypertrophy of the FLR generally occurs in less than 10 days compared to over 3 weeks for PVE (29,62,70,78-82). The reason this procedure appears to work much more efficiently than PVE is due to the ISS, allowing complete devascularization of segment IV and preventing formation of collaterals between the left lateral and right lobes (79).

In patients who have insufficient hypertrophy after PVE, ALPPS can still be evaluated as an option in order to convert the patient to resectability. Patients who had insufficient PVE followed by *in situ* liver transection showed rapid growth within 3 days with a mean volume increase of 63% (80).

Unresectable disease

Ablative therapies

Ablative therapies include radiofrequency ablation (RFA), microwave ablation (MWA) and cryoablation. Thermal ablation delivers extreme temperatures to hepatic colorectal metastases causing immediate cell death (38). The advantages of ablation therapies are the ability to spare liver parenchyma; utilization of percutaneous and laparoscopic modalities; it does not limit future therapeutic options; and low morbidity

rates (38,88). The ablative techniques generate and maintain enough temperature change to cause irreversible thermal damage to the tumor and a margin of normal liver tissue in a process called coagulative necrosis (89). RFA is the most common ablation therapy used to treat CLM (89,90). These methods are limited by the size of the lesion in relation to the probe and have largely been used for patients with unresectable disease or significant comorbidities precluding resection.

RFA

During RFA an electrode is placed within the tumor under radiologic guidance. Radiofrequency, or thermal energy, is used to destroy the tumor and a margin of normal surrounding parenchyma. Specifically, high-frequency alternating current causes thermal coagulation and protein denaturation. At 60° Celsius there is immediate cell death, and ablation zones are created in excess of this threshold (38).

RFA can be performed percutaneously, laparoscopically, or during laparotomy. RFA has been most effective for smaller lesions (<3 cm) that are amenable to coverage by a single probe (91-94). For larger lesions it is necessary to apply multiple overlapping RFA probe applications to achieve adequate ablation. Visualizing a sphere and then attempting to cover the surface of that sphere with additional overlapping burns shows the difficulty of this. Open or laparoscopic placement of the probe allows better placement than percutaneous and offers the additional advantage of exploration and intra-operative ultrasound of the liver, which can demonstrate occult peritoneal and hepatic disease (88,89,95). RFA has some limitations to placement within the liver. Placement near major vessels runs the risk of an inadequate ablation secondary to the flow in the vessels conducting the heat energy away from the target. This "heat sink" phenomenon can be overcome by temporary vascular occlusion such as a "Pringle" maneuver (96). RFA should not be performed adjacent to major biliary structures, particularly within 1 to 2 cm of the hepatic hilum due to the risk for bile duct stricture and fistula (13).

The data regarding oncologic outcome of RFA is based on two, phase II trials and a large number of retrospective series. The median survival following RFA for CLM ranges from 24-45.3 months with a 5-year OS of 18-33% (88,93,97-103). This compares to a median survival of 41-80 months and a 5-year OS of 48-71% in resection of CLM (13,97,99,102-104). The local recurrence rate even in the best cases (4-16.1%) is inferior to margin recurrences of 0.9-5% for resected CLM (13,92,93,97-100,105,106). The improved outcome of resection when compared to RFA retrospectively is related to more advanced disease in RFA performed for unresectable CLM and hepatectomy may remove occult parenchymal micrometastases (91).

Three clinical questions remain, is RFA equal to resection in resectable CLM, can RFA extend the pool of patients offered resection for cure, and is there benefit of RFA in addition to chemotherapy for unresectable CLM (91)? The first question is the most difficult to answer. Numerous authors have used retrospective comparison of resected CLM to unresectable CLM treated with RFA as evidence that RFA is inferior regarding local control (13,97,102,103). These are obviously different patient populations (deemed unresectable, failed chemotherapy, and/or are unable to tolerate a liver resection) and comparing retrospective data on RFA versus resection to conclude that RFA is inferior is flawed (93,102,106). However, local recurrence is universally higher for RFA studies and this is associated with decreased survival. This data supports continued use of resection as the "gold standard" for resectable CLM. Some authors have suggested that the increased local recurrence rate can be overcome by repeat applications via a minimally invasive technique in select patients similar to the development of the breast conservation therapy model (88,107). The ultimate role for RFA will be defined by recognizing that RFA and resection have different strengths and weakness inherent, different indications might highlight the advantages of each technique (96).

The question of benefit in using RFA to extend the pool of resectable patients was addressed with a Phase II prospective trial. The EORTC 40004 trial looked at 52 patients with unresectable CLM treated with a combination of RFA and resection. They achieved a 43% 5-year OS (106). Karanicolas *et al.* also recently reviewed their experience with the use of ablation combined with resection in unresectable bilateral CLM with poor prognostic factors and found a 56% 5-year OS (108). This data supports the use of RFA in addition to resection in an attempt at curative resection in otherwise unresectable disease. The use of RFA can potentially obviate the need for a two-stage hepatectomy. This allows sooner recovery, initiation of adjuvant therapy and avoiding the risks of progression between stages.

The question of benefit of the addition of RFA to chemotherapy for the treatment of unresectable CLM was addressed with the CLOCC trial (chemotherapy plus local ablation *vs.* chemotherapy alone). The trial randomized 119 patients to chemotherapy or chemotherapy plus RFA.

The PFS was significantly better at 16.8 months in the patients undergoing RFA when compared to 9.9 months in the chemotherapy alone group (99). The trial was hampered by slow accrual and was not ultimately powered to evaluate OS and so we do not know if the PFS translates into OS.

MWA

MWA has been introduced as a rapid method of delivering high temperatures to a large hepatic area. An electrode is placed into the tumor under ultrasound or CT guidance. The microwave coagulator then generates and transmits microwave energy. Coagulative necrosis causes cellular death and destroys the tissue. MWA induces rapid oscillation in water molecules leading to coagulation necrosis of the tumor, making its effects less dependent on tissue variations (107,109,110). This has some advantages over RFA and could allow safer applications, and potentially resulting in lower local recurrence and complication rates (107). The shorter wavelength of microwave allows more rapid heating and less loss of energy across different densities of tissues. This theoretically addresses two shortcomings of RFA, the heat sink effect near major vessels and the incomplete burn of larger lesions secondary to charring. These benefits have been seen when examining animal models (111-115). MWA offers a potential benefit for patients with lesions >3 cm, because the desiccation and charring seems to be of less importance when using MWA in comparison to RFA (111). However in a recent multi-center trial despite a low local recurrence rate of 6% the greatest impact on recurrence free survival was a lesion ≥3 cm. mirroring findings in RFA studies (116). MWA has not been nearly as well studied, as RFA and the theoretical benefits have not been clearly shown to translate to improved clinical outcome to date.

Cryoablation

Cryoablation involves liquid nitrogen or argon gas being delivered into the liver tumor, guided by ultrasound. Ice crystal formation during rapid freezing causes destruction of cellular structure and kills the tumor cells. Cryoablation has fallen out of favor, because of a higher complication rate and recurrence rate than RFA (117,118). The higher complication rate is marked by the potentially fatal complication of cryoshock manifested by hypothermia, coagulopathy, respiratory failure and renal failure (89).

Hepatic artery infusion

Hepatic artery infusion (HAI) is directed chemotherapy via a pump attached to a catheter which gets implanted through the gastroduodenal artery. The tip of the catheter is positioned at the gastroduodenal-hepatic artery junction. This therapy can be used in combination with systemic chemotherapy, along with resection or RFA if performed via laparotomy or laparoscopy. Chemotherapy given via the hepatic artery decreases toxicity given the knowledge that liver metastases are perfused almost exclusively by the hepatic artery, opposed to normal liver tissue that receives its blood supply predominantly from the portal circulation (119). This directed therapy allows an increased amount of cytotoxic drugs without increasing the systemic side effects. Given the high hepatic extraction rate for FUDR, almost a full dose of systemic chemotherapy can be given concurrently without increasing toxicity (120).

Phase I and II HAI studies show response rates in the liver between 52% and 75% in previously treated patients and even higher in chemotherapy naïve patients (121-123). HAI can be used to convert unresectable CLM to resectable. The combination of HAI and systemic chemotherapy has shown response rates in excess of 90% with 24-47% of patients going on to resection (121,124). The conversion to resectable was even greater at 53-57% in the chemotherapy naïve patients including patients with extensively involved liver (121,124). HAI has been studied in the adjuvant setting in patients with a high risk for recurrence following resection of CLM and increased DFS significantly but not OS (125). Pump complications after catheter placement occur in approximately 20% of patients; however, approximately half can be salvaged and still used for treatment (126). Biliary sclerosis is a long-term complication that can usually be managed by insertion of a biliary stent, without affecting OS.

Chemoembolization

Transarterial chemoembolization (TACE) can be performed in conventional method using either emulsions of ethiodized oil, which are embolic particles, in combination with chemotherapy solution, or as drug-eluting beads loaded with irinotecan (DEBIRI-TACE). There have been no studies comparing the two, so which method to give is usually institutional preference. DEBIRI was first reported in 2006 (127). The toxicity data suggests a more severe post-embolization syndrome compared to radioembolization (RE), with 40% reporting right upper quadrant pain, 80% fever, 27% nausea, and increased transaminases in 70% of patients (128). However, despite these symptoms,

therapeutic response was achieved in 78% of patients, and over 90% of patients report an improvement in their well-being for over 4 months, with a median duration of response lasting 6 months, and a median survival of 25 months (128). A recent prospective study randomizing patients with colorectal metastases who failed standard chemotherapy to DEBIRI versus FOLFIRI chemotherapy. The DEBIRI group had a significantly improved median survival of 22 months compared to 15 months for the FOLFIRI group (129).

RE

RE is the best studied of the embolization techniques for CLM. RE can be performed with microspheres labeled with the β emitter yttrium-90 (^{90}Y). There are two commercially available microspheres, one composed of a biocompatible resin (SIR-Spheres; SIRTex Medical, Ltd., Sydney, Australia) and the other composed of glass (TheraSphere; MDS Nordion, Inc., Ontario, Canada). Portal vein compromise is a contraindication for the SIR-Spheres (130). The most common adverse effect for both is gastrointestinal toxicity (131). The first step in minimizing this toxicity is performing arteriography of the celiac and superior mesenteric arterial distribution and skeletonizing the hepatic arterial vasculature. Gastrointestinal ulceration results from microspheres diverting via extrahepatic arteries supplying the gastrointestinal tract. A technetium 99 ($Tc^{99\,m}$) macroaggregated albumin (MAA) scan is also used in pretreatment evaluation to determine the presence and extent of any arteriovenous shunts and identify non-target organs, such as the gastrointestinal tract, or the lungs. A lung shunt fraction (LSF) is calculated based on imaging and dose reduction needs to be considered if the LSF is between 10-20% (130). Toxicity is usually mild and resolves in 1 to 4 weeks but symptoms include fatigue, abdominal pain, nausea, and anorexia (130). The response rates are 12.9-35.5% with 24-65% achieving stable disease (132-136). The median OS following ^{90}Y is 10.2-12.6 months (132-137). This is achieved in patients who have failed chemotherapy.

External beam

External beam radiation therapy (EBRT) has not been used historically on liver tumors given the small therapeutic window between benefit and liver toxicity (38). Stereotactic radiotherapy, originally developed in neurosurgical practice, allows delivery of highly focused ionizing radiation with extreme precision. The technique is termed stereotactic body radiotherapy (SBRT) (138). The local control rates in the liver at 1 and 2 years for SBRT are 67-100% and 55-92% respectively (139-141). The median survival ranges from 20.5-34 months (139,140). Chang *et al.* also showed that local control for colorectal metastases is dose-dependent, with an 18-month local control of 84% for total doses ≥42 Gy versus 43% for total doses <42 Gy (141). Based on this the authors recommend 3 fractions with a total dose of 42 Gy.

Conclusions

Surgical resection remains the treatment of choice for resectable CLM. There are a number of options for extending resection to more advanced patients including systemic chemotherapy, PVE, two stage hepatectomy, ablation and HAI. There are few phase III trials comparing these treatment modalities, and choosing the right treatment is patient dependent. Treating hepatic metastases requires a multidisciplinary approach and knowledge of all treatment options as there continues to be advances in management of CLM. If a patient can undergo a treatment modality in order to increase their potential for future resection this should be the primary goal. If the patient is still deemed unresectable then treatments that lengthen disease-free and overall-survival should be pursued.

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Footnote

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Non-operative therapies for colorectal liver metastases

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Abstract: Locoregional therapies for colorectal liver metastases complement systemic therapy by providing an opportunity for local control of hepatic spread. The armamentarium for liver-directed therapy includes ablative therapies, embolization, and stereotactic body radiation therapy. At this time, prospective studies comparing these modalities are limited and decision-making relies on a multidisciplinary approach for optimal patient management. Herein, we describe multiple therapeutic non-surgical procedures and an overview of the results of these treatments.

Keywords: Colon cancer; liver metastases; embolization; radiation; ablation

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Introduction

Metastatic disease to the liver is present at the time of diagnosis in 20% of patients with colorectal cancer and develops in an additional 40% over the course of their disease (1). In 30% of patients, the liver is the only site of metastatic disease. Liver resection offers the best chance of cure for patients with liver metastases with 5-year survivals of up to 50%. Unfortunately, only up to 25% of eligible patients undergo resection because of co-morbid conditions (1). First line chemotherapy with oxaliplatin or irinotecan and the addition of a biologic agent have increased median survival time to 18-21 months (2). However, after failing first line chemotherapy, the response rate to second line agents ranges from about 20-35% (3,4). For these reasons loco-regional or liver-directed therapies are significant for treatment of liver-predominant metastatic colorectal cancer (1). Liver-directed therapy may be administered with curative or palliative intent via open surgical, laparoscopic or image-guided percutaneous techniques. This review focuses only on non-operative techniques and their results.

Ablative therapies

Ablation of colorectal hepatic metastases is generally reserved for patients with disease confined to the liver. Ideally, patients should have three or fewer lesions in the liver with each lesion measuring ≤ three centimeters in diameter. Ablative therapies include radiofrequency ablation (RFA), microwave ablation, laser ablation and ultrasound ablation, all of which induce thermal damage to tissues. In contrast, cryoablation freezes tissue at temperatures ranging from –20 to –40 degrees centigrade that leads to cell death. Irreversible electroporation—an emerging technology that uses electrical energy to introduce pores in cellular membranes with resultant cellular destruction-is currently under investigation (5). In addition, percutaneous instillation of ethanol directly into a tumor is often performed in patients with hepatocellular carcinoma, though this technique is not yet frequently used in colorectal carcinoma.

Radiofrequency ablation (RFA)

RFA, the most extensively studied ablative technique for treatment of colorectal liver metastases, is similar in intent to surgical resection. Rapidly alternating electrical current produces ionic oscillations in bipolar water molecules, which then generate frictional heat (*Figure 1*). An electrode

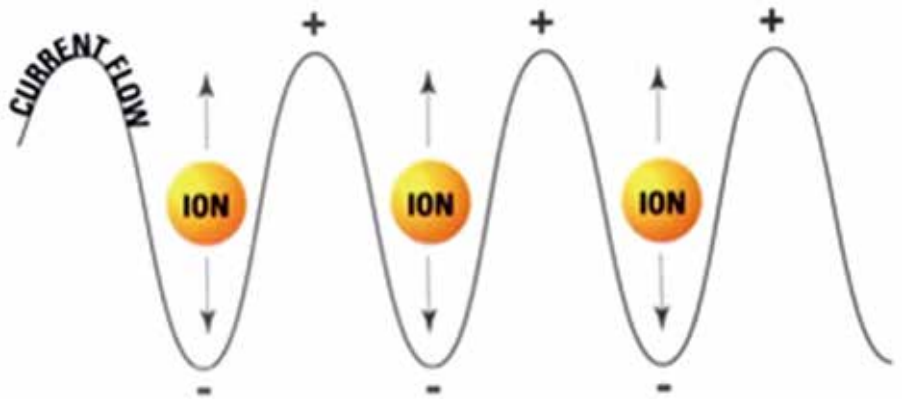

Figure 1 Mechanism of radiofrequency ablation (RFA). Rapidly alternating electrical current produces ionic oscillations in bipolar water molecules, which generate frictional heat.

Figure 2 RFA electrode probe. RFA, radiofrequency ablation.

probe (*Figure 2*) is placed within the metastatic lesion, while grounding pads are placed on the lower extremities. Energy dispersion is greatest in the vicinity of the probe generating temperatures of >100 degrees centigrade. Cell death takes place after several minutes at 50 degrees centigrade and instantaneously at temperatures of >60 degrees centigrade. Current technology can produce lethal burns of >5 cm diameter with a single probe (*Figure 3*). Tissue destruction is dependent on heat conduction generated from the active elements of the probe; therefore, optimal lesions are <3 cm in diameter with incomplete destruction more likely with increasing lesion size. Impediments to effective RFA include lesion location adjacent to critical structures such as bowel or central bile ducts. Flowing blood in vessels >3 mm creates a heat sink effect, drawing heat away from the treated area. Tissue charring in the vicinity of the active elements creates an insulator, which can also limit heat conduction.

For small hepatocellular carcinomas, the results of RFA approach those of surgical resection with respect to recurrence, time to progression, and overall survival. For colorectal liver metastases, multiple retrospective case matched comparisons as well as meta-analyses are available, which compare surgical resection to RFA (6-8). An increased incidence of recurrence in the treated lesion (5-13%), decreased time to progression, and decreased median survival (1-year, –85%; 3-year, –36%, 5-year, –24%) are reported in patients undergoing RFA compared to surgical resection (7). In well-selected patients with 3 or fewer lesions less than 3 cm in diameter, 5-year survivals of up to 33% are reported (9). In patients who are not surgical candidates, RFA remains a valuable tool. It is increasingly used in conjunction with surgical resection to increase the number of surgical candidates. A study by Livraghi in 2003 demonstrated that RFA followed by incomplete ablation or recurrence in the ablative bed did not negatively affect subsequent surgical resection (10). The most important factor for local recurrence after RFA is tumor size, and larger lesions are at higher risk for local failure (*Table 1*).

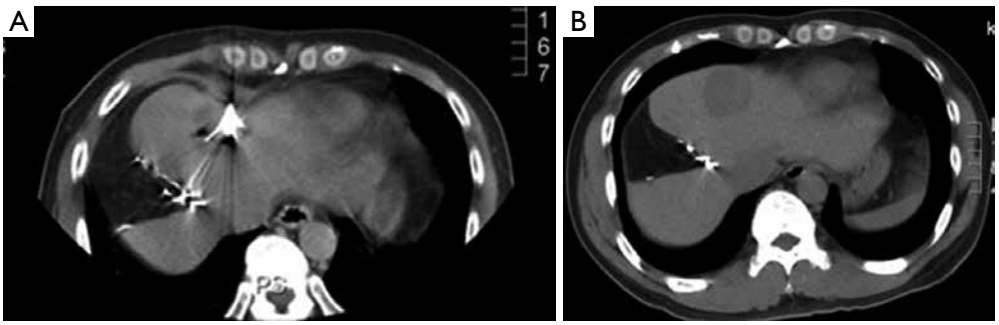

Figure 3 Pre and post-RFA appearance of colorectal hepatic metastasis. (A) Intra-procedural CT demonstrates RFA probe within left lobe hepatic metastasis; (B) post-procedure CT 6 months status post RFA demonstrates left lobe liver ablative lesion with no residual perfusion. RFA, radiofrequency ablation.

Microwave ablation

Microwave ablation relies on generation of electromagnetic radiation in the 900 to 2,450 MHz range from antennae placed within the treated lesion. Similar to RFA, ionic oscillations occur in response to oscillating electrical charge with generation of frictional heat (21). Microwave ablation offers a broader field of power density providing active heating up to 2 cm surrounding the antenna with less dependence on thermal conduction. This differs from RFA, where active generation of heat is only a few millimeters surrounding active elements and has a greater reliance on thermal conduction. Therefore, microwave ablation may lead to more uniform tissue heating with the ability to treat larger lesions using multiple antennae. There may also be less of a heat sink effect from flowing blood and less tissue charring. The elimination of the need for grounding pads, which carry an associated risk of skin burns, as well as shorter procedure times are additional advantages of microwave ablation (21).

Microwave ablation is being used increasingly as an open or laparoscopic procedure but also percutaneously under image guidance (21,22). While there are no randomized trials comparing it to RFA, certain advantages may exist. As with RFA, the incidence of recurrence in the treated lesion, as well as disease-free and overall survival favor surgical resection over microwave ablation. Recurrence rates following microwave ablation reported in observation studies and meta-analyses range from 5-13% with 1-, 3-, and 5-year survival rates of 73%, 30% and 16%, respectively (7). As with RFA, microwave ablation has been described in conjunction with surgical resection to achieve survival rates similar to resection alone (23) (*Table 2*).

Cryoablation

Cryoablation is performed through the use of a probe within the lesion, where argon is infused, dropping tissue temperatures to –40 degrees centigrade and creating an ice ball of various sizes depending on probe configuration. Within the ice ball there are predictable thermal zones ranging from –40 degrees to 0 degrees centigrade. Tissue death occurs at –20 to –40 degrees centigrade. Advantages of cryoablation include ability to visualize the ice ball while using CT guidance and less procedure related pain. There are variable survival rates and high rates of complications (7). Disadvantages include the need for multiple probes and lack of a coagulative effect potentially leading to bleeding complications (28). The high rate of complications and fear of cryoshock has led to this technique falling out of favour as other safer and equally effective techniques have evolved (7). Currently cryoablation has been replaced by RFA and microwave ablation for ablative treatment within the liver (*Table 3*).

Embolization procedures

Locoregional therapies administered through the hepatic artery for the treatment of primary and metastatic hepatic cancer include bland particulate embolization, chemo-infusion, chemoembolization and radioembolization. The dual blood supply to the liver enhances the effectiveness of these techniques. Hepatic malignancies receive 80% of their blood supply from the hepatic artery. In contrast, the normal liver receives 80% of its blood supply from the portal vein with only 20% from the hepatic artery. Therefore, liver directed therapies through the hepatic

Table 1 Radiofrequency ablation (RFA) series—review of survival outcomes and toxicities

Article	No. of patients with colorectal liver metastases	Median No. of hepatic metastases per patient	Tumor diameter (cm^3)	Median follow-up (mo)	Survival	Local recurrence rate (%)	Major complications (%)	Minor complications (%)
Abitabile *et al.* (11)	47	3.1	2	33 months (mean)	Median OS: 39 months; 1 year: 88%; 2 years: 80%; 3 years: 57%; 4 years: 38%; 5 years: 21%	overall: 8.8, tumors <3 cm: 1.6	7	
Gillams and Lees (12)	167	4.1 (mean)	3.9 (mean)	17 (mean)	Median OS: 32 months; 1 year: 91%; 3 years: 40%; 5 years: 17%	14	4	6
Hildebrand *et al.* (13)	56	3.5	3.5	21.2	Median OS: 28 months; 1 year: 92%; 2 years: 67%; 3 years: 42%	17	3.4	5.6
Iannitti *et al.* (14)	52	2.7	5.2 (mean)	20	1 year: 87%; 2 years: 67%; 3 years: 50%		7	
Machi *et al.* (15)	100	3.5 (mean)	3.0 (mean)	24.5	Median OS: 28 months; 1 year: 90%; 3 years: 42%; 5 years: 31%	6.7	4.8	12.3
Solbiati *et al.* [2001] (16)	117	1.6 (mean)	2.6	6-52 (range)	Median OS: 36 months; 1 year: 92%; 2 years: 69%; 3 years: 46%	39.10	1	0
Solbiati *et al.* [2012] (17)	99	2.1	2.3	72	Median OS: 53.2 months; 1 year: 98%; 3 years: 69%; 5 years: 48%; 7 years: 25%; 10 years: 18%	11.90	1.3	
Abdalla *et al.* (18)	158	1	2.5		1 year: 92.5%; 2 years: 60%; 3 years: 37%; 4 years: 22%	Approximately 90		
Wong *et al.* (19)	31		3.1 (mean)	9.5	9 months: 77.5%	15	20	
Schindera *et al.* (20)	14		1.8	18	Median OS: 35 months; 1 year: 72%; 2 years: 60%; 3 years: 60%	14.8	2.1	16.7

Median value unless explicitly noted to be mean value. OS, overall survival; NR, not reported; cm, centimeters; mo, months.

Table 2 Microwave ablation series—review of survival outcomes and toxicities

Article	No. of patients with colorectal liver metastases	Median No. of hepatic metastases per patient	Tumor diameter (cm)	Follow-Up (mo)	Survival	Local recurrence rate (%)	Major complications (%)	Minor complications (%)
Seki *et al.* (24)	15.0	1.0	2.2	18.0	Median OS: 24.2 months	7.0		
Shibata *et al.* (25)	14.0	4.1 (mean)	2.7 (mean)	11.3	Mean OS: 27 months 1 year: 71% 3 years: 57% 5 years: 14%	NR overall recurrence =50		6.7
Martin *et al.* (23)	50.0	2.0	3.0		Median OS: 36 months	6.0	Major + minor =30	
Bhardwaj *et al.* (26)	24.0	2.9 (mean)	2.0 (mean)	48.0	Median OS: 29 months 3 year: 40%	2.0		
Liang *et al.* (27)	21 non-CRC mets: 53	2.0 (mean)	3.0	25.1 (mean)	Median OS: 20.5 months 1 year: 91% 2 years: 60% 3 years: 46% 4 years:29%	14.0	0.0	16.2

Median value unless explicitly noted to be mean value. OS, overall survival; cm, centimeters; mo, months; CRC, colorectal cancer.

Table 3 Cryoablation series—review of survival outcomes and toxicities

Article	No. of patients with colorectal liver metastases	Median No. of hepatic metastases per patient	Tumor diameter (cm)	Follow-up (mo)	Survival	Local recurrence rate (%)	Major complications (%)	Minor complications (%)
Seifert and Morris (29)	116	3.9 (mean)	4.4 (mean)		Median OS: 26 months 1 year: 82% 2 years: 56% 3 years: 32% 5 years: 13 %		31.0	
Joosten *et al.* (30)	30	3.0	2.0	26.0	1 year: 76 % 2 years: 61%	9.0	30.0	
Yan *et al.* (31)	172	4.2 (mean)	3.6 (mean)	23.0	Median OS: 28 months 1 year: 89% 2 years: 65% 3 years: 41% 4 years: 24% 5 years: 19%	39.0	28.0	
Paganini *et al.* (32)	49	5.1 (mean)		39.3	Median OS: 23 months 3 years: 31%		26.0	55.0

Median value unless explicitly noted to be mean value. OS, overall survival; cm, centimeters; mo, months.

artery preferentially target the neoplasm with relative sparing of normal liver parenchyma.

Bland embolization

Bland embolization utilizes inert particles of various sizes and composition to obstruct tumor microvasculature, leading to tumor infarction. While bland embolization is effective in the treatment of hepatocellular carcinoma and neuroendocrine tumors, it not frequently utilized for colorectal metastases.

Chemoinfusion

Chemoinfusion or delivery of chemotherapy directly into the hepatic artery results in high exposure of the liver to the chemotherapeutic agent. It can be delivered through catheters placed percutaneously into the hepatic artery or most commonly through pumps and catheters that are surgically implanted. Since placement of the hepatic artery infusion pump is most commonly placed via a surgical procedure, it will only be briefly discussed in this review. Floxuridine (FUDR) is the most commonly used drug and has a high first pass clearance by the liver, which enhances hepatic exposure and decreases systemic exposure. While it has high response rates (up to 85%), and demonstrates prolonged progression-free survival (up to 31 months), it is not clear that overall survival is improved over current first line chemotherapy. In addition it is plagued by catheter malfunction, arterial occlusion and hepatic toxicity including intra- and extra-hepatic bile duct damage (33,34). Its role after failure of first and second line chemotherapy as well as adjuvant therapy following liver resection also remains to be determined (35).

Chemoembolization

In contrast to simple infusion of chemotherapy through the hepatic artery, chemoembolization combines arterial obstruction with the delivery of chemotherapeutic agents. A commonly employed chemoembolization protocol combines the delivery of chemotherapeutic agents emulsified in ethiodized oil with particulate embolization. The ethiodized oil/chemotherapeutic mixture lodges distally within the hepatic arterioles and portal venules, trapping the agent in the tumor microvasculature. Bland embolization following the delivery of the ethiodized oil/chemotherapeutic mixture leads to stasis and increased contact time within the tumor, which increases local drug delivery while reducing systemic exposure (36). Recently, drug-eluting beads have been developed in which the chemotherapeutic agents such as doxorubicin or irinotecan are ionically bound to particles of various sizes. Following embolization there is a gradual prolonged release of the chemotherapeutic agent within the tumor with greatly reduced systemic release (37).

Hepatic artery chemoembolization is appropriate for patients with liver dominant metastatic disease, ECOG performance status of 0-2, and preserved liver function with serum bilirubin of less than 2 mg/dL. Ideally the portal circulation is preserved and tumor volume is less than 50%. Standard chemoembolization protocols include a variety of agents but often include mitomycin, doxorubicin and cisplatin emulsified in ethiodized oil (38). Treatment is performed in a lobar distribution starting with the more affected lobe and followed by treatment of the contralateral lobe in 15 to 30 days. Bilobar treatment is considered one cycle. Response is assessed with CT or MR imaging at 30-90 days and treatment cycles are repeated as necessary for disease progression. Most often, chemoembolization is considered after failure of first and second line chemotherapy. In a report by Albert *et al.*, using the previously mentioned standard method of chemoembolization with mitomycin C, doxorubicin, and cisplatin mixed with ethiodized oil, the authors reported partial response, stable disease, and disease progression in 2%, 41%, and 57% of patients, respectively. Median time to liver progression was 5 months and overall disease progression was 3 months. Median survival following the first chemoembolization was 9 months (38). These results are similar to other series reporting median survivals of 8-14 months from the time of first chemoembolization (39-41). Complications of chemoembolization are common but most often minor, including the anticipated post embolization syndrome of fever, nausea, vomiting and abdominal pain, which are usually easily controlled and of limited duration. More serious complications including liver failure, renal failure, liver abscess, cholecystitis, myocardial infarction and pulmonary embolus have also been reported

Advances in drug delivery systems have led to further control of the release of chemotherapeutic agents within hepatic metastatic lesions, increasing contact time, while decreasing systemic exposure. These include hydrogels, microspheres, and polymer implants. The most extensively studied of these are non-biodegradable polyvinyl alcohol (PVA) microspheres (beads) (DC Bead, Biocompatables, West Conshohocken, PA, USA). The

beads are modified with the addition of a sulfonic acid containing a moiety resulting in a charge that permits the interaction and binding with oppositely charged drugs, such as doxorubicin and irinotecan (37). Drug eluting beads loaded with irinotecan (DEBIRI) provide an alternative for chemoembolization of colorectal cancer metastatic to the liver. Beads of varying diameter (70-900 microns) are loaded with irinotecan, and based on disease distribution they are delivered in a lobar, segmental or superselective arterial distribution. For lobar administration, a single lobe is initially treated, while the second lobe is treated approximately 14-28 days later, with bilobar treatment considered a single treatment cycle. Cycles can be repeated if disease progression is noted.

In most reports, prior to DEBIRI chemoembolization, patients had undergone previous chemotherapy and failed at least one line of chemotherapy, with some having failed two or three regimens of chemotherapy. In general, patients tolerate DEBIRI well with the most common adverse event being the post-embolization syndrome. Abdominal pain, occasionally severe, is reported in 40-63% of patients. Hypertension is also frequently reported but is most often transient and related to pain. Aliberti reported results in 82 patients who failed initial lines of chemotherapy with response to DEBIRI of 78% at three months, progression free survival of 8 months and median survival of 25 months (42). These results are similar to those reported by Martin with response at 6 and 12 months of 66% and 75%, and progression free and overall survivals of 11 and 19 months, respectively (43). Other reports and meta-analyses report response to treatment in 18-78% of patients with median survival rates of 15-25 months (44). Patients failing only first line chemotherapy exhibit better overall response and survival compared to patients having failed multiple lines of chemotherapy. In a randomized trial comparing DEBIRI to 5-fluorouracil (5-FU)/leucovorin/irinotecan in patients failing first lines of chemotherapy, time to progression and overall survival favored DEBIRI at 7 and 22 versus 4 and 16 months, respectively (44). Toxicity including neutropenia, diarrhea, and mucositis were less frequent in the DEBIRI group (44). Both standard chemoembolization and DEBIRI have been reported to downstage up to 20% of patients to surgical resectability (45) (*Table 4*).

Radioembolization

Radioembolization incorporates Yttrium-90, a radioactive beta emitter (maximum energy 2.27 MeV and mean range of 2.5 mm in liver tissue) embedded in resin (SIRSpheres, SIRTEX) or glass microspheres (Theraspheres, MDS Nordion) for delivery of high dose radiation to the tumor with reduced radiation exposure to the remaining normal liver parenchyma. The radioembolization procedure consists of a femoral artery catheterization approach to the hepatic artery through which Yttrium-90 microspheres are delivered. Once in the tumor's vascular network, these particles occlude the smallest capillaries leaving the majority of the microspheres within the tumor whereby they emit radiation therapy, which is known to be one of the most effective cancer therapies for solid tumors. Traditionally, the concern about radiation delivery to the liver has been the risk of radiation induced liver disease (RILD). Due to the hepatic artery-dominant blood supply for about 80% of liver tumors, the Yttrium-90 microspheres preferentially flow to the tumors. Pathological studies have confirmed the distribution of the microspheres (47). In patients treated with radioembolization, RILD consists of a constellation of icteric ascites, hepatomegaly, and mild transaminitis in relationship to the bilirubin, which is markedly elevated. Patients who have received chemotherapy prior to RE are at higher risk for RILD (48). This technique helps to overcome the radiosensitivity of the liver parenchyma.

As the half-life of Yttrium-90 is 64.8 hours, the particles are radioactive for a period of about 14 days but most of the radioactivity is delivered over five days. Although SIRSpheres and TheraSpheres are used interchangeably, SIRSpheres are FDA-approved for colorectal cancer metastases and Therasphers for hepatocellular carcinoma.

The radioembolization process is conducted in at least two parts. The first session, the mapping portion consists of a Technicium-99 macro-aggregated albumin SPECT scan during which particles mimicking the Yttrium-90 microspheres determine the percentage of lung shunting which may occur with the radioembolization procedure. If greater than 20% lung shunting occurs, then the patient is not eligible for radioembolization. Likewise, the Yttrium-90 dose may be modified based on the percentages of lung shunting. Also during this procedure, occlusion of the gastroduodenal artery or other collateral vessels may be performed to prevent retrograde flow of the microspheres, which can result in gastric and duodenal ulcers (15%). The radiation dose is calculated by the body surface area method or an empiric dose may be administered but is thought to carry higher rates of toxicity (49). The next procedure consists of the administration of the Yttrium-90 microspheres, which may be performed in a whole liver

Table 4 Chemoembolization series—review of methods and outcomes

Article	No. of patients with colorectal liver metastases	Median No. of hepatic metastases per patient	Chemoembolization: chemotherapy	Chemoembolization: embolization medium	Response	Survival (%)	Complications
Albert *et al.* (38)	121	2.0 (mean)	All-mitomycin C (10 mg), doxorubicin (50 mg), cisplatin (100 mg), dissolved in steril contrast (8.5 mL) and water (1.5 mL)	Ethiodized oil (1:1 ratio with chemotherapy solution)	RECIST Criteria: 43% (stable or partial response) Decreased CEA levels: 50%	Median survival since diagnosis: 27 months 1 year: 85% 2 years: 55% 5 years: 6% Median survival since treatment: 9 months	19% patients grade 3 or higher; 2% patients grade 4 or higher
Tellez *et al.* (39)	30	2.23 (mean)	All-cisplatin (10 mg/mL), doxorubicin (3 mg/mL) & mitomycin (3 mg/mL)	Bovine collagen material	Radiologic (CT): 63% Decreased CEA levels: 95%	Median survival since diagnosis: 29 months Median survival since treatment: 8.6 months	Toxicities include GI, anemia, transaminitis, hyperbilirubinemia, RUQP
Sanz-Altamira *et al.* (40)	40		All-5-Fluorouracil (1,000 mg), mitomycin C (10 mg), ethiodized oil (10 mL), followed	Gelfoam	Radiologic (CT): 22.8% partial respones; (additional 40% showed smaller response/reduction in lesions) Decrease CEA levels: 62% patients showed > 50% reduction in CEA	Median survival since treatment: 10 months PS 0-1: 24 months PS 2: 3 months Intrahepatic disease only: 14 months Extrahepatic disease: 3 months Intrahepatic disease & PS 0-1, OS: 1 year: 73%, 2 years: 61%	<2 weeks: abdominal pain, fevers, transaminase elevation 2-4 weeks: fatigue syndrome Other major: worsened ascites (15%), peritonitis (2.5%), sepsis (2.5%), necrotic gallbladder (2.5%)

Table 4 (*continued*)

Table 4 (*continued*)

Article	No. of patients with colorectal liver metastases	Median No. of hepatic metastases per patient	Chemoembolization: chemotherapy	Chemoembolization: embolization medium	Response	Survival (%)	Complications
Vogl *et al.* (41)	463	5.3 (mean)	Arm 1-mitomycin C Arm 2-mitomycin C + gemcitabine Arm 3-mitomycin C + irinotecan	Lipiodol + starch microspheres	Radiologic (MRI): 62.9%	Median survival since diagnosis: 38 months 1 year: 96% 2 years: 80% 3 years: 56% Median survival since treatment: 14 months 1 year: 62% 2 years: 28%	"...a small group of patients had symptoms of abdominal pain, nausea, and vomiting for 2-7 days."
Aliberti *et al.* (42)	82	2.2	All-DEBIRI (drug-eluting bead loaded with irinotecan), 100-200 mg irinotecan preloaded in 2-4 mL DC beads of 100-300/300-500 pm	DC beads (polyvinylalcohol hydrogen microspheres)	Radiologic (CT): 100% exhibited 75-100% reduction in contrast enhancement at 1 month	Median survival since treatment: 25 months	<1 hr: nausea/vomit (27%) <6 hr: RUQP (40%) 24 hr: transaminitis (50%); hyperbilirubinemia (9%)
Martin *et al.* (23)	55	2.0	All-DEBIRI 50 mg/mL irinotecan; 100 mg/vial	DC/LC beads	EASL Criteria: 3 months, 89% (complete, partial or stable response); 6 months, 80%; 18 months: 45% RECIST Criteria: 3 months, 71%; 6 months, 56%; 18 months, 45%	Median overall survival: 28.6 months Disease-free survival: 20.6 months	29% patients reported adverse events. Higher rates in patients receiving >100 mg irinotecan
Eichler *et al.* (46)	11		All-DEBIRI 50 mg/mL irinotecan; 100 mg/vial	DC beads	RECIST Criteria: best overall, 81% (stable or partial response) Decreased CEA levels: 27%		81% patients reported adverse events-61% mild & 39% moderate

Median value unless explicitly noted to be mean value. OS, overall survival; RUQP, right upper quadrant pain; cm, centimeters; mo, months; RECIST, response evaluation criteria in solid tumors; CEA, carcinoembryonic antigen; CT, computed tomography; PS, performance status.

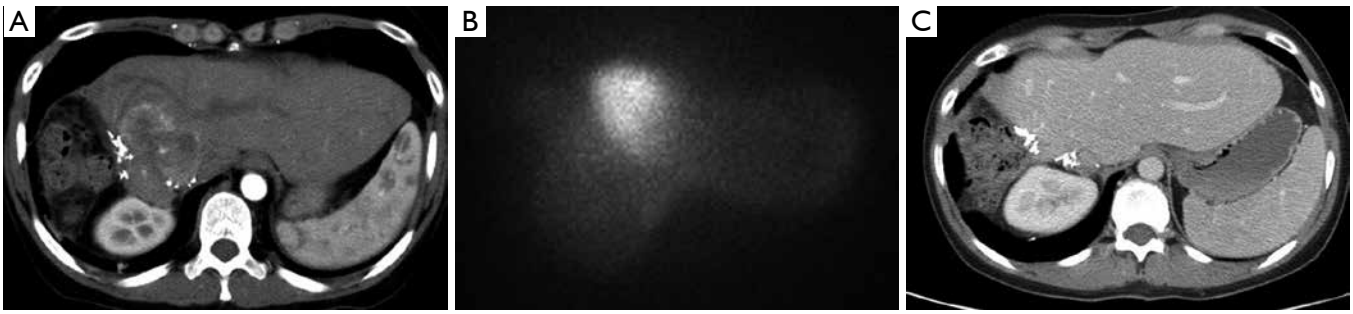

Figure 4 (A) Pre-radioembolization CT demonstrates enhancing colorectal hepatic metastasis; (B) immediate post-radioembolization bremsstrahlung scan demonstrates activity within the hepatic metastasis consistent with selective uptake of radioactive Y90 microspheres by the lesion; (C) 6-month post-treatment CT demonstrates complete response of the lesion.

approach or sequential lobar treatments. The advantage of sequential treatments is to observe the effect of first radioembolization and to assure that sufficient contralateral liver reserve exists. Patients may be then monitored on an every 2-3 month basis after completion of therapy (*Figure 4*).

Appropriate patient selection for this procedure helps to ensure that the maximum benefit of this therapy will be provided. For colorectal cancer patients with liver metastases, it is preferable that the patient has liver-limited confirmed metastatic disease to achieve maximal benefit with RE. Small volume extrahepatic disease is permitted. Relative contraindications include a history of ascites or portal vein thrombosis, both conditions are more likely with hepatocellular carcinoma. Other requirements are a Zubrod performance status of 0-2; bilirubin <2 and ideally <1; creatinine <1.5× upper limit of normal, WBC >1.5×10^9/L; Plt >100×10^9/L; albumin >30 g/L.

The risks of this procedure include constitutional symptoms and abdominal pain, gastric/duodenal ulcer for which prophylactic proton pump inhibitors are initiated, and radiation induced liver injury. Results of radioembolization are shown in *Table 5*.

Several trials combining chemotherapy and radioembolization have demonstrated encouraging outcomes. van Hazel *et al.* (59) evaluated 5-FU and leucovorin with or without radioembolization in a phase II randomized trial in 21 patients. Radioembolization was administered on the 3rd or 4th day of the second cycle of chemotherapy. The response rate, median survival for 11 patients receiving combination therapy was significantly greater than those who received chemotherapy alone. Sharma *et al.* (60) evaluated combined radioembolization with modified FOLFOX4 in a phase I study of 20 patients. Grade 3 abdominal pain occurred in 25% of patients (with a 10% rate of radioembolization-related gastric ulcers), grade 3-4 neutropenia in 60% of patients, and one episode of transient grade 3 hepatotoxicity. Partial responses occurred in 90% of cases. Median progression-free survival was 9.3 months, and median time to progression in the liver was 12.3 months. Hendlisz *et al.* (61) conducted a phase III trial comparing continuous infusion 5-FU alone or with radioembolization for liver-limited metastatic CRC in 46 patients. Median time to tumor progression was 2.1 months for the 5-FU alone group versus 5.4 months in the combination group. Over half of patients (25/44) went on to receive further treatment after progression. Median overall survival was 7.3 months in the 5-FU arm and 10.3 months in the combination arm (P=0.80). Van Hazel also conducted a study of irinotecan concurrently with radioembolization and evaluated three dose levels of irinotecan, and did not reach a maximum tolerated dose, so a dose of 100 mg/m^2 on days 1 and 8 of a 3-week cycle was recommended (62). Based on the available data, radioembolization is most often administered independently of chemotherapy to patients with liver-limited metastases to aid in prolonging survival and time to progression.

Stereotactic body radiotherapy (SBRT)

SBRT also known as stereotactic ablative body radiotherapy (SABR) denotes a precise delivery of high doses of radiation to an extracranial target in a small number (usually up to 6) of fractions (63). Standard fractionation of 1.8-2.0 Gray per day is effective because of the differential response of tumor and normal tissue to radiation therapy, with the repeated fractions allowing repair of normal tissues. In contrast, SBRT is thought to be more effective relative to standard fractionation radiation with apparent improvements

Table 5 Radioembolization series—review of methods and outcomes

Author	Number of patients	Volume of disease (%)	Median prescription dose	Extra-hepatic metastases (%)	Response (%)	Progression-free survival (months)	Median survival from radioembo (months)	Patients with decrease in CEA (%)
Murthy 2005 (50)	12	<25%: 33 25-50%: 25 >50%: 42	396 mCi	NR	CR: 0 PR: 0 SD: 50 PD: 50	NR	4.5	33%
Mulcahy 2009 (51)	72	<25%: 78 25-50%: 19 >50%: 3	118 Gy	40	CR: 3.1 PR: 37.5 SD: 44.5 PD: 14.8	15.4	14.5	NR
Cianni 2009 (52)	41	<25%: 61 25-50%:15 >50%: 24	1.82 GBq	10	CR: 5 PR: 41 SD: 34 PD: 20	9.2	12	100
Chua 2011 (53)	140	<25%: 55 25-50%: 36 >50%: 9	1.8 GBq	36	CR: 1 PR: 31 SD: 31 PD: 37	NR	9	NR
Seidensticker 2011 (54)	29	<25%: 10.3 25-50%: 89.6 >50%: 0	1.76 GBq	48.3	CR: 3.4 PR: 41.4 SD: 17.2 PD: 37.9	5.5	8.3	NR
Martin 2012 (55)	24	NR	1.72 GBq	54	CR: 3.4 PR: 41.4 SD: 17.2 PD: 37.9	3.9	8.9	21
Cosimelli 2010 (56)	50	<25%: 40 25-50%: 60	1.7 GBq	NR	CR: 2.0 PR: 22 SD: 24 PD: 44	3.7	12.6	NR
Stubbs 2006 (57)	100	<25%: 60 25-50%: 21 >50%: 19	2 GBq	25	CR: 1.0 PR: 73 SD: 20 PD: 6	NR	11	96
Kennedy 2006 (58)	208	<25%: NR 25-50%: NR >50%: NR	1.75 GBq	NR	CR: 0 PR: 36 SD: 55 PD: 10	NR	10.5	NR

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NR, not reported.

in tumor cell kill. SBRT is complicated by the need to work with irregularly shaped targets and tumors that are influenced by organ and diaphragm motion. SBRT requires accurate immobilization, a method to manage the respiratory motion of the target, and image guidance to ensure proper alignment and delivery of the radiation dose. Historically SBRT has been used to treat smaller lesions (<6 cm in diameter) in the liver, and it may also be used for larger sized liver metastases as well (63).

SBRT requires careful radiation design with the first step of simulation to create a reproducible position of the patient, using a large rigid pillow conforming to the patient's external contour with a reference coordinate system beneath and around the patient, which allows for a 3-dimensional localization of the patient. After creating of the immobilization device, a high resolution computed tomography (CT scan) with IV and oral contrast generates an image of the patient, target, and immobilization device. Acquisition of a 4-dimensional CT scan provides information about target and respiratory motion. CT images may be obtained with contrast and can be registered to diagnostic images, such as PET scans or MRIs. During simulation, the method of liver immobilization is determined with options based on institutional availability including controlled breath holds, shallow breathing, abdominal compression devices, beam gating timed to the respiratory cycle, or tumor tracking via implanted fiducial markers (64).

Manual delineation of the target and normal organs by the physician is followed by determination of a beam arrangement to meet specific dose constraints. This process may take several days to generate a highly conformal treatment plan via multiple iterations and discussion between the physician and physics team. Radiation beams may be delivered through a multi-field 3-dimensional conformal plan with a combination of coplanar and non-coplanar beams or intensity modulated radiation therapy (IMRT). To achieve an adequate SBRT plan, numerous beam angles must be employed (6 or more) such that each beam is sufficiently weak to spare radiation dose to normal organs, and at the convergence of the beams, the maximal radiation dose is delivered. Similarly, IMRT employs multiple angles, but within each angle, the shields within the linear accelerator (multileaf collimators) dynamically move to spare organs at risk. IMRT is less ideal for SBRT due to the interplay effect.

During each treatment, assessment of patient position is conducted via image guided radiation therapy, with fluoroscopy, megavoltage or kilovoltage X-rays or cone beam CT scans (CBCT) to assure accuracy of liver positioning. Alternatively, tracking may occur after the placement of fiducials. Although this is a relatively new modality for liver directed therapy, the non-invasive nature of therapy makes it particularly appealing.

Candidates for liver SBRT should have a sufficient performance status (ECOG 0-1) and liver function and no extrahepatic disease. The uninvolved liver volume should be 700 mL or greater (64). Based on the volume of disease, patient comorbidities, baseline liver function, and performance status, the multidisciplinary team can begin to make treatment recommendations for liver-directed therapy. For diffuse disease, the embolization procedures may be appropriate therapy. Although outcomes appear similar in chemo- and radio-embolization, it is important to recognize that the volume of disease in the available literature may be inconsistent. Likewise, most SBRT series allow for larger lesions than RFA despite having apparently similar outcomes (*Table 6*).

Summary

Decision making

We provide various cases to demonstrate the decision-making and representative images for each scenario.

Case discussion 1 (*Figure 5*)

A 67-year-old woman with a history of adenocarcinoma of the sigmoid colon status post sigmoidectomy presents with new onset fatigue and a rise in carcinoembryonic antigen (CEA). A CT of the chest, abdomen and pelvis, reveals new liver lesions in segments I and V, and a CT guided biopsy of the segment V reveals adenocarcinoma. As the patient had tolerated any of her chemotherapy with difficulty, medical oncology recommended liver-directed therapy. Interventional radiology reviewed her films but did not this that was an optimal situation for RFA due to possible heat sink of the central lesion and possible gastric injury for the segment V lesion. Given her low volume of disease, chemo- or radio-embolization was not warranted. Therefore, SBRT was proposed and delivered to the patient.

Case discussion 2 (*Figure 6*)

A 45-year-old woman with a diagnosis of diffuse liver metastases due to colon cancer requires a chemotherapy holiday due to neuropathy from therapy. She has been

Table 6 Stereotactic body radiation therapy outcomes

Author, year, prospective/ retrospective	No. of patients/ No. of liver metastases	Organ of origin	Tumor volume	RT dose	Complications	Results
Blomgren 1995 (65), retrospective	14	11 CRC, 1 anal canal, 1 renal, 1 ovarian	3-260 mL	7.7-45 Gy/1-4 fx	Hemorrhagic gastritis (2)	50% response rate
Katz 2007 (66), retrospective	69/174	20 CRC, 16 breast, 9 pancreas, 5 lung	0.11-950 mL	30-55 Gy/5-15 fx	No grade 3 or 4 toxicities	Infield local control at 10 months: 76% at 20 months: 57%
Rusthoven 2009 (67), prospective	47/63	15 CRC, 10 lung, 4 breast, 3 ovarian	0.4-6.8 cm 0.75-97.98 cm^3	36-60 Gy/3 fx	No grade 4 or 5 toxicities	IY LC 95% MS 20.5 months
Lee 2009 (63), prospective	686	CRC 40 Breast Gastric	1.2-3,090 cm^3	27.7-60 Gy/6 fx	gastritis, nausea, thrombocyoenia	1Y local control: 58-95% MS 17.6 months
Chang 2011 (68), retrospective, mult-institutional	65	CRC 102	30 mL (0.66-3,088)	22-60 Gy	No grade 4 toxicities	1Y LC: 62%
Mendez Romero 2008 (69), prospective	17/34	37 CRC, 2 lung, 4 breast, 1 carcinoid	0.5-7.2 cm	5 Gy 5 or 30 Gy ×10	3% duodenal ulcers	Local control at 1 year: 94% and at HCC: 82%
Goodman 2010 (70), prospective	26/32	6 CRC, 5 IHCC, 2 HCC, 27 other primary	0.8-147 cc	18-30 Gy/1 fx	2/31 with duodenal ulcers 2/31: musculoskeletal	Median survival: 28.6 months
Hoyer 2006 (71), prospective	64/44 (lung and other organs also included)		NR	45 Gy/3 fractions	1 hepatic failure, 2 duodenal ulcer, 1 colonic perforation	NR for liver metastases alone

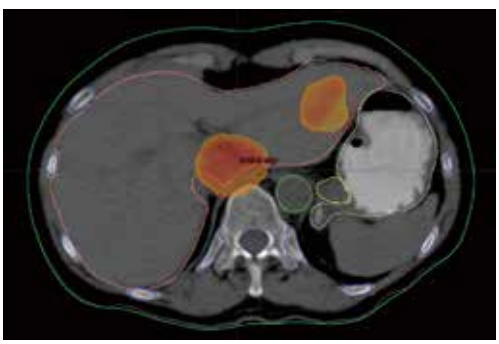

Figure 5 Two colorectal metastases at locations not amenable to RFA were treated with SBRT. RFA, radiofrequency ablation; SBRT, Stereotactic body radiotherapy.

treated with FOLFOX and FOLFIRI. Given the diffuse nature of her liver metastases and clinical situation, Y90 radio-embolizaiton was recommended. Chemoembolization was considered to be an option but available results have shown better outcomes for smaller volume disease.

Case discussion 3 (*Figure* 7)

A 54-year-old man with a remote history of rectal cancer is found to have a new solitary liver metastasis measuring 2 cm. The patient has agreed to receive chemotherapy. RFA was recommended by the multidisciplinary liver tumor board given its long track record and good success in tumors <3 cm.

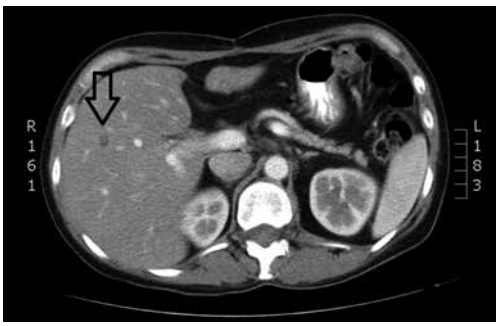

Figure 6 A solitary lesion in a non-surgical candidate, treated with RFA. RFA, radiofrequency ablation.

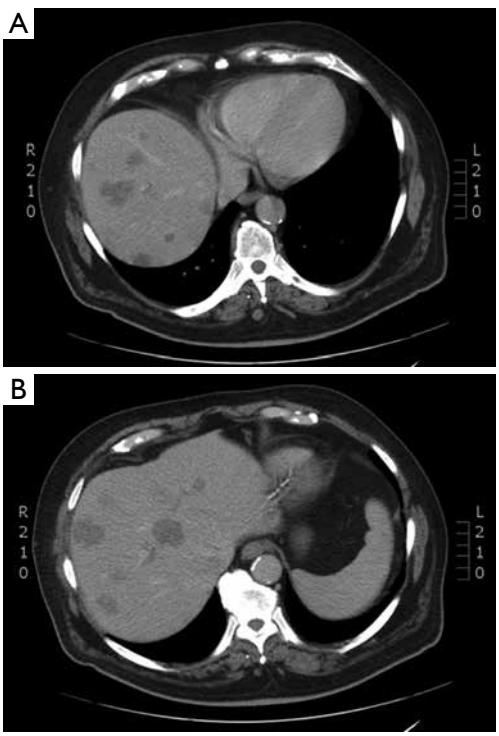

Figure 7 Diffuse liver metastases treated with radioembolization.

Conclusions

While there have been many exciting developments in liver directed therapy, most of our understanding of treatment outcomes is derived from observational studies. This limits our ability to critically compare techniques and to incorporate them into the treatment armamentarium of metastatic colorectal cancer. Randomized control trials of liver-directed therapy are necessary to elucidate the optimal management for patients with hepatic metastases. In the interim, multidisciplinary discussion at tumor boards is necessary to carefully decide on the optimal therapy for each patient.

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None.

Footnote

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

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Regional hepatic therapies: an important component in the management of colorectal cancer liver metastases

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Abstract: The treatment of colorectal cancer liver metastases (CRLM) has evolved significantly in the last 15 years. Currently, complete surgical resection remains the only potentially curative option; unfortunately, approximately 80% of patients with CRLM are not candidates for complete tumor resection. For patients with unresectable CRLM the available treatment options were historically limited; however, the development of regional hepatic therapies (RHT) and improvement of systemic chemotherapeutic regimens have emerged as viable options to improve overall survival and quality of life for this group of patients. The selection, sequence and integration of interventions into a multi-modal approach is a complex and evolving discipline. In this article, the currently available RHT modalities for CRLM are presented as a guide to the options for clinical treatment decisions.

Keywords: Colorectal; liver metastases; tumor ablation

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Introduction

Surgical resection is the only potentially curative treatment option for patients with colorectal cancer liver metastases (CRLM). Unfortunately up to 80% of these patients will present with unresectable metastatic liver disease (1,2). Five year survival for resectable patients is reported to range from 40-58% (3,4) while for unresectable patients the median overall survival is reported to be 15 to 22 months (5). Over the last 15 years, the development of new systemic chemotherapy, targeted biologic agents, as well as regional hepatic therapies (RHT) including ablative technologies and trans-arterial treatments have expanded the management options for patients with CRLM. The use of these RHT has created a paradigm shift in the treatment of CRLM, such that the historical perspective of outcome limited to cure or failure has been replaced by the more dynamic concept of converting cancer to a manageable chronic disease.

A multimodal and multidisciplinary approach is necessary to offer optimal individualized treatment. Defining the appropriate sequence and combination of treatments is challenging and requires both expertise and experience. This article reviews the currently available RHT options for unresectable CRLM and offers management strategies for this group of challenging patients.

Determining CRLM resectability

The definition of resectable CRLM has evolved significantly over the last two decades. The classic resection criteria were based on the number and size of liver lesions. Currently, resectable CRLM are more broadly considered to be any hepatic tumors that can be removed with negative margins while leaving a sufficient volume of functional parenchyma. Patients whom are deemed to be ineligible for CRLM resection at presentation can be considered to be unresectable or potentially resectable. Clearly unresectable patients are those with diffuse liver involvement or multiple extrahepatic sites. Such patients

require systemic chemotherapy and are unlikely to be down-staged to resectable status. The potentially resectable candidates are those that have a reasonable expectation for a treatment response sufficient to enable CRLM resection with or without RHT following systemic treatment. The initial French experience reported CRLM down- staging rates of 13-16% (6). These rates have increased more recently with novel systemic therapy regimens. The Italian study by Masi *et al.* showed that approximately 20% of unresectable patients could be down-staged to resectable status (7) after systemic chemotherapy. Nuzzo *et al.*, using irinotecan-based regimens, found a 35% rate of conversion (8) similar to the 36% found by Falcone *et al.* using FOLFOXIRI (9). The optimal chemotherapy combination for the purpose of CRLM down-staging has not been defined and the response rates vary depending on patient characteristics. Novel therapeutics agents and their combination with targeted therapy promise to improve response rates and conversion to resectability in CRLM.

Neoadjuvant chemotherapy for resectable disease

Given the significant improvement in overall survival with the use of modern systemic agents, interest in defining the role for neoadjuvant chemotherapy in resectable CRLM has emerged. However, the only randomized trial to date is the EORTC Intergroup trial 40983, which demonstrated an increase in recurrence free survival but not overall survival (10). In addition, the rate of complications following surgery was significantly increased in patients that received perioperative chemotherapy. The advantages and disadvantages of using the various neoadjuvant chemotherapy and targeted molecular therapy options for the treatment of CLRM is beyond the scope of this review. However, it is important when considering neoadjuvant chemotherapy for resectable disease to proceed in a multidisciplinary approach with the active involvement of the surgical team. It has been well documented that 4-5% of CRLM will disappear on imaging subsequent to systemic therapies, thus making post-treatment surgical resection planning difficult (11). Moreover, it is well established that a complete clinical response on imaging does not correlate with pathological response, with up to 80% of patients having positive microscopic disease (12). This highlights the importance of early referral to a liver surgeon within the context of a multidisciplinary approach.

Staged hepatectomy

Staged hepatectomy with or without portal vein embolization (PVE) is a therapeutic approach that can be considered for patients with bilateral CRLM. The use of PVE for staged hepatectomy has been demonstrated to have acceptable morbidity and mortality (13). After the initial resection, PVE is performed if necessary. The liver is allowed to hypertrophy for 3-4 weeks and then a second stage resection can be performed. The type of resection done for the first and second stages depends on the distribution and location of the liver metastases and the liver remnant volume. Although this technique has been reported only in highly specialized centers, it is a feasible option for otherwise unresectable disease.

Unresectable CRLM

Unresectable patients can be further divided in two groups: (I) patients that after systemic and/or biological agents alone or in combination can be down-staged to resectable; and (II) the group that after systemic and/or biological agents alone or in combination cannot be down-staged to resectable status. After re-staging, patients in the first group should undergo resection to clear all hepatic disease. For the second group, RHT have emerged as part of the armamentarium to reduce or stabilize disease burden in the liver as stand-alone therapy or in combination with other modalities (*Figure 1*). RHT can be grouped into three broad categories: ablative, arterial and non-arterial modalities. Each of these categories can be further grouped by type of technology use to obtain cancer cells demise (*Figure 2*).

Ablative modalities

As mentioned, ablative options for unresectable CRLM can be divided into thermal and non-thermal modalities. Thermal options can be further divided in "cold" and "hot" ablation modalities. Cold ablation therapies include cryo-ablation and hot ablation modalities include radiofrequency ablation (monopolar and bipolar devices), microwave ablation (2.45 GHz and 915 MHz). Another type of ablation used in the past is chemical ablation, but its use has been abandoned with the emergence of new, more effective, and easier to use modalities. The principal non-thermal option is irreversible electroporation.

Thermal "hot" ablation

Radiofrequency ablation (RFA)

Radiofrequency ablation energy can be delivered by either monopolar or bipolar devices. Monopolar is the most

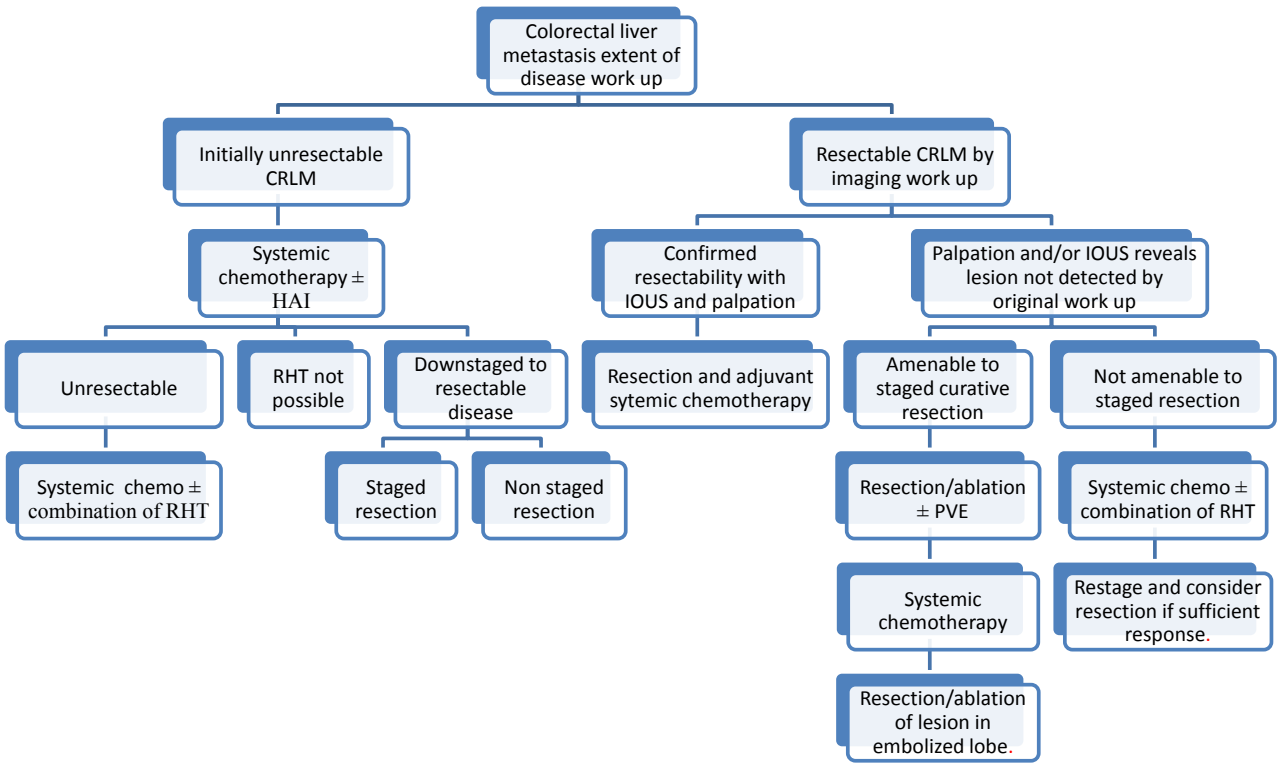

Figure 1 Initial management of patients with CRLM. CRLM, colorectal liver metastasis; RHT, regional hepatic therapies; PVE, portal vein embolization; IOUS, intra-operative ultra sound; FLR, future liver remnant

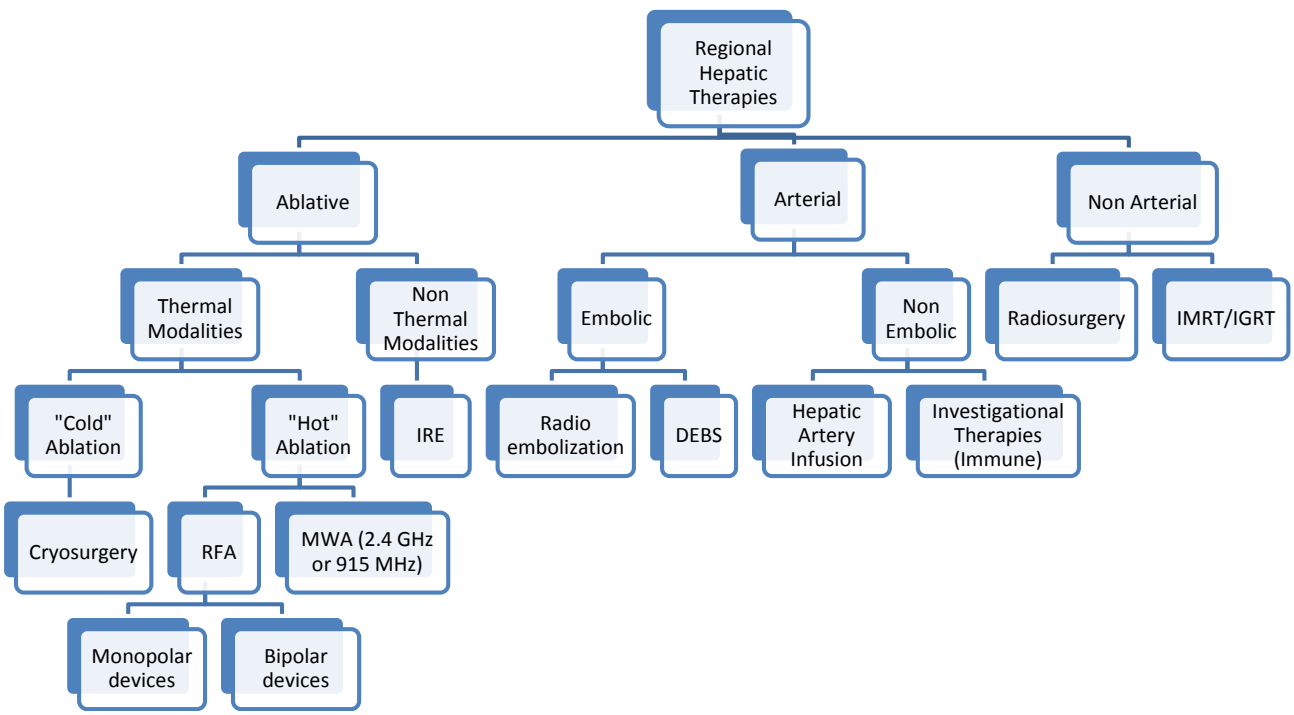

Figure 2 Regional hepatic therapy types. RFA, radiofrequency ablation; MWA, microwave ablation; IRE, irreversible electroporation; SIRT, selective internal radiation therapy; TACE, trans-arterial chemo-embolization; DEBS, drug eluding beads; IMRT, intensity modulated radiation therapy; IGRT, image guided radiation therapy

frequently used, and consist of an electrode that streams energy outwardly in all directions. The radius of tissue necrosis varies and depends in the type and configuration of the electrode used. The other RFA modality is bipolar, which consist of two parallel electrodes facing one another. In bipolar systems, the energy travels between the electrodes and not around them, concentrating energy delivery into the area between the electrodes.

Monopolar RFA

RFA is presently the most common "hot" ablative therapy. RFA induces tumor necrosis by achieving local hyperthermia with temperatures exceeding 58 ℃. The energy for RFA is based on an alternating current of radio frequency waves (500 kHz) that is delivered via a probe into the tissue being treated. The resulting ionic agitation generates frictional heat which extends to adjacent tissue by conduction leading to coagulative necrosis (14). RFA probes may be deployed via open, percutaneous, or laparoscopic approaches. The optimal approach depends on tumor location and the operator preference. Several studies (15-17) have shown lower local recurrence rate with the open approach. Better exposure of the liver, the ability to visually inspect and palpate liver surface lesions, combined with the use of intra-operative ultrasound may explain the superior results of the open approach (18).

In the last decade, radiofrequency ablation has superseded other ablative therapies, due to its low morbidity, low mortality, and technical feasibility (19). However, is very difficult to analyze the available RFA data, in terms of local recurrence, overall survival and progression free survival. Most of the published studies are observational or clinical trials with no randomization, resulting in potential biases that make comparison of groups difficult. Heterogeneity of the treatments approaches further confounds the situation, making it difficult to draw conclusions (19). Despite these difficulties, several studies exist that support the following:

Local recurrence and intrahepatic progression free survival

Progression of intra-hepatic disease and local recurrence has been related to survival of patient with unresectable disease. RFA as standalone therapy or in combination with other modalities is a useful tool to obtain hepatic disease control. Location of the lesion is also associated with an increase in local recurrence after RFA. Lesions close to major hepatic vessels have a higher local recurrence rate due to the decrease in temperature in the ablation area because of blood flow. The local recurrence rates for current RFA technology have been reported as 9-21% (20,21) and this have been associated with tumor size and location of the lesion. With the current RFA probes lesions up to 5 cm are suitable for RFA (22) with higher rates of local recurrence with lesions >5 cm (23).

Overall survival

The 2 year survival rates with classic fluorouracil based regimens have been reported between 22-27% (19). When RFA is used with chemotherapy, the 2, 3 and 5 years survival rates reported are 60%, 34% and 22% respectively, with more recent studies presenting 5 years survival rates between 25-31% (24,25). This data needs to be evaluated carefully because, as mentioned before, most of the studies lack randomization and have high risk of selection bias. While RFA may contribute to improved outcomes in certain situations, increased survival when RFA is added to systemic therapy may be in part due to selection of patients with less extensive disease, amenable to ablation. Randomized clinical trials powered to measure overall survival are required.

Bipolar RFA

Radiofrequency ablation is the most widely accepted and available ablative modality. However, is limited by inconsistent ablation zones, susceptibility to convective heat loss from adjacent high-velocity blood flow or heat sinks (26,27). In an effort to resolve these problems, other RFA ablative configurations have emerged. Bipolar RFA is one such modality, which employs a dual parallel electrode array; the energy wave travels uni-directionally between and not around electrodes. This 'line-of-sight' delivery streams energy between two fixed points and concentrates energy delivery to the area between the probes (27).

The use of two electrodes with very high current density decreases the time required to achieve target temperature in treated tissue in comparison to monopolar devices (27). Convective heat loss seems to be negligible with bipolar RFA. One of the limitations of this technique is that the ablation area is defined by the orientation between the electrodes. If the electrodes are not parallel to each other the ablation area could take unpredictable shapes resulting in unintended thermal injury to noninvolved tissue (26,28). The operator needs to understand the characteristics of the device to obtain the desired ablation shape. The key aspect when planning the ablation area is to define the perimeter

of the ablation target lesion.

In a report published by Baldwin *et al.* (28) 22 patients were treated with bipolar RFA, and after a median follow up of 24 months only one patient showed local recurrence. The time of ablation was 4-7 min with increased ablation time associated with lesion size. Although this is a small series with CRLM and hepatocellular carcinoma (HCC), it demonstrated that bipolar RFA can be used with acceptable results and the laparoscopic approach is not technically challenging. Bipolar RFA is a technology in its infancy and further studies with longer follow-up will be required to establish the long-term oncologic outcomes for this technique. However, the rapid emergence of microwave technology may lead to diminished utilization of RFA, as described below.

RFA in combination with systemic chemotherapy

The use of RFA with systemic chemotherapy has been recently studied in the EORTC 40004 trial. This is currently the only clinically randomized control trial, comparing RFA + systemic chemotherapy to systemic chemotherapy alone for unresectable CRLM (29). This originally planned phase III trial was downgraded to phase II because of slow accrual. The data presented is consistent with previous non-randomized studies, where the combination group (RFA + systemic chemotherapy) had a significantly higher progression free survival at 3 years; 27.6% *vs.* 10% in the systemic chemotherapy only group. Unfortunately, the study was not powered for overall survival. However, a trend towards increased overall survival was seen in the combination group. Appropriately powered studies will be necessary to determine if there is a difference in overall survival (29).

RFA plus resection

The combination of resection and RFA may enable complete intrahepatic tumor clearance under circumstances where resection alone would not leave sufficient remnant liver. Several non-randomized studies (24,30) have shown the use of RFA as a complement to resection may enable complete tumor clearance. No statistically significant difference in overall survival has been observed between RFA + resection and RFA alone (31), suggesting that RFA is a reasonable adjunct to liver resection in selected cases. Ablation therapies appear to be a promising adjunct to resection in patients that otherwise would be rendered free of disease by resection alone.

Microwave ablation

Microwave ablation (MWA) is also dependent on thermal energy. MWA utilizes the region of electromagnetic spectrum between 915 MHz and 2.4 GHz. When the microwaves interact with water molecules, frictional heat is generated, resulting in coagulative necrosis (32). Gravante *et al.* (33) studied pathology specimens after the use of MWA and found no viable tumor cells in 93% of lesions ≤6 cm in diameter.

The use of MWA has been more prevalent in Asia than in the USA, were RFA has been the more commonly used thermal ablation modality. Although most of the data comes from interventions in unresectable HCC patients (34-36), MWA has been demonstrated to be a safe technique with similar morbidity to RFA (35,36). Morita *et al.* (37) reported an experience with 52 patients with CRLM using MWA alone and in combination with resection. For these groups the cumulative 5-year survival rates were similar, at 20% and 24%, respectively. These data are comparable with the long-term survival found after RFA alone or with resection (19). As with RFA, the risk of local recurrence following MWA is higher for lesions larger than 5cm (36,38). A randomized study is necessary to find if any difference in local recurrence and overall survival exist between MWA and RFA.

Irreversible electroporation (IRE)

The known limitations of RFA and MWA such as biliary tract damage, heat-sink effect, and thermal damage to adjacent organs have led to the pursuit of alternative ablation technologies. Irreversible electroporation (IRE) is a novel technology that has been proposed to improve the ablation efficacy around major portal or hepatic vessels. In contrast to RFA and MWA, IRE employs electrical pulses that permeabilize cellular membrane and consequently lead to cell death (39). These electrical impulses create nanopores in both normal and malignant cells. The collagen scaffolding of structures such as vessels and biliary structures do not form nanopores and therefore are not affected by IRE. One of the hypotheses for this "sparing effect" holds that gap junctions in vessels walls allow the electrical impulse to transfer from one cell to the other without affect (40,41). One of the advantages of this modality is the ability to cause cell death in the hepatic parenchyma around major hepatic vessels, avoiding the "sink effect" seen with the use

of RFA or MWA.

IRE has shown to be safe in porcine liver models and recently the first series in humans have been published (42). A recent retrospective study from the Memorial Sloan-Kettering Cancer Center (MSKCC) demonstrated tumor response rates of 98%, which was higher than the 50% rate reported in another studies (43). The MSKCC group utilized an open approach, which may account for the higher response rates. The percutaneous approach has the potential limitation of positioning accuracy of the IRE electrodes, while the open approach facilitates a more accurate positioning of the electrodes aided by palpation. Recurrence rates of 5.7% were seen with 6 months median follow up. Although the study has various limitations including selection bias, short follow-up and tumor type heterogeneity, it demonstrates that IRE can be done safely and with promising results. Larger series with long-term follow up will be required to validate IRE as an effective regional liver therapy modality for liver tumors.

Arterial modalities

Arterial modalities can be divided in embolic and non-embolic. Embolic therapies include selective internal radiation therapy (SIRT), drug eluding beads (DEBS) and trans-arterial chemo-embolization. Non-embolic treatments include hepatic artery infusion of chemotherapy and regional adoptive cellular immunotherapy, which is currently under study at our institution.

Selective internal radiation therapy (SIRT)

Yttrium 90 (Y90) is the most common agent used for SIRT, a new option for patients with unresectable CRLM. This modality can be used as a single therapy for chemotherapy refractory patients or in combination with systemic chemotherapy. Y90 is a pure beta-emitting radioisotope, produced by the bombardment of Y89 with neutrons. Y90 has a high average energy (0.936 MeV), limited tissue penetration (mean 2.5 mm, max 11 mm), and short half-life (64 h), making it an ideal trans-arterial liver-directed agent. After incorporation into glass or resin microspheres, Y90 is selectively injected into the hepatic artery or its branches (44). There are two commercially available forms of Y90 microsphere: SIR-Spheres (Sirtex Medical, Sydney, Australia) and TheraSphere (MDS Nordion, Ontario, Canada). SIR-Spheres are resin-based microsphere, have a diameter of 20-60 μm. SIR-Spheres are used mainly in the treatment of CRLM and received pre-market approval by the FDA in 2002. TheraSphere are made of glass and have a diameter of 20-30 μm, used more frequently in HCC treatment, for which it has a humanitarian device exemption.

Important in the use of Y90 microspheres is the pre-therapy planning. All patients considered Y90 internal radiation must undergo hepatic angiography and a technetium-99 macroaggregated albumin (Tch-99 MAA) nuclear medicine scan. The goal of this assessment is to delineate the hepatic arterial vasculature and quantify the degree of extrahepatic perfusion and hepatopulmonary shunting. Infusion of radioactive microspheres into the gastrointestinal or pulmonary circulation can have devastating consequences. Thus Tch-99 MAA, which has a similar diameter as the microspheres, is used as a surrogate to estimate the distribution of the microspheres in the hepatic circulation prior to therapy. The degree of hepatopulmonary shunting and reflux into the gastrointestinal circulation can be determined. A hepatopulmonary shunt greater than 18% predisposes patients to development of radiation pneumonitis and represents a contraindication to Y90 therapy, unless the shunts can be occluded by embolization. Gastrointestinal arterial reflux that cannot be eliminated by ligation or embolization also precludes patients from undergoing treatment. Severe liver dysfunction or portal vein thrombosis are also contraindications to therapy, although patients with the latter have undergone glass microsphere treatment successfully (45,46).

The treatment response after SIRT can be measured by fluctuations in CEA levels and by imaging. The earliest published data for SIRT in unresectable CRLM examined the combination of SIRT with hepatic artery chemotherapy (HAC) (47). This data showed significantly longer median survival rates in patients receiving SIRT, with 6, 12 and 18 months estimated survival rates of 70%, 46% and 46% respectively. These survival rates were limited by the development of extrahepatic disease. There was no treatment associated mortalities and SIRT was well tolerated. Later studies also from New Zealand and Australia have shown significant difference in tumor response and median survival time in patients who receive SIRT in addition to HAC (48,49) with mean CEA level drop of 50-70% of pre-treatment levels, and greater than 50% reduction in tumor volume. Data gathered form a phase III randomized clinical trial by Gray *et al.* (50) in 2001 showed significantly better tumor response in the

SIRT + HAC group than in the HAC alone group (72% *vs.* 47% respectively). Likewise, time to disease progression was significantly longer (15.9 *vs.* 9.7 months) in the SIRT + HAC group. Van Hazel *et al.* (51) published a RCT in 2004 evaluating SIRT alone or SIRT in combination with systemic 5-FU and leucovorin. In this small RCT the combination group had significantly higher response rates and longer time to disease progression than the chemotherapy alone group (18.6 *vs.* 3.6 months respectively).

Y90 seems to be safe and effective therapy for unresectable CRLM. However, the optimal dose and timing of Y90 therapy remain to be established. The use of doses greater than 225 Gy results in superior response rates and cumulative doses greater than 300 Gy led to a significantly increased survival (52,53). The precise correlation between degree of hepatic dysfunction and tolerance of radiation needs to be characterized and further randomized trials are needed to accurately define the safest and most effective dose and to determine timing between therapies. The principal determinant of survival following SIRT in CRLM is the development of extrahepatic disease. As such, combining SIRT with systemic therapy may prove to be the most rationale approach and pre-SIRT PET may be used to refine patient selection for this modality (49).

Y90 is a novel addition to the RHT for unresectable liver tumors. Sufficient data exist to support it use in unresectable CRLM, with increase in median survival, time to progression of hepatic disease and tumor response rates. The tumor response induced by Y90 radioembolization is a valuable tool for attempted conversion of unresectable to resectable disease. Further studies are needed to assess the optimal Y90dose, indications, and its place alongside the other RHT.

Hepatic arterial chemotherapy

Hepatic arterial infusion chemotherapy is another modality within the RHT used in combination with systemic chemotherapy, to induce greater tumor response and ultimately longer median survival. The rationale behind hepatic artery infusion chemotherapy is based on the principle that CRLM get their blood supply almost exclusively from the hepatic artery, while the normal liver parenchyma receive the majority of its blood supply from the portal vein (54). Thus prolonged drug exposure and higher concentrations in CRLM can be achieved with direct hepatic artery infusion of the chemotherapeutic agents, with limited systemic toxicity.

The use of HAC was first studied alone, and response rates were reported to be between 22% and 62% (55,56). Later the use of HAC in combination with systemic chemotherapy gained more popularity because the tumor response rates were better and better control of extrahepatic disease was possible (57,58). The tumor response seen with the combination of HAC + systemic chemotherapeutics ranged from 35% to 92%. Most of the studies used 5 FU/ Leucovorin as systemic therapy, however higher response rates were seen with Oxaliplatin/Irinotecan regimens (54). HAC + systemic chemotherapy have shown to be a valuable tool to achieved resectability, with rates of resectability in the 50% range when used as first line therapy and 20% after failure with systemic chemotherapy (59).

Complications from HAC have discouraged its use, these are related with the drug itself or technical. Allen *et al.* published his experience at MSKCC with and overall pump complication rate of 22%. Complications such as arterial thrombosis (6%), extrahepatic perfusion (3%), incomplete hepatic perfusion (2%) and hemorrhage (2%) were reported. However, these technical complications improved with increased experience, with significantly lower rates in the second half of the study (60). From the drug related complications the most common and serious is hepatobiliary toxicity. Usually one of the first signs will be elevation of transaminases levels, while elevation of bilirubin and alkaline phosphatase show signs of more significant hepatic damage. Dose-adjusting algorithms have been developed based on changes in the liver function tests to better guide the dosage and avoid toxicity and dexamethasone have also been added to reduce the incidence on biliary toxicity (61).

In the last decade, several studies have provided data about the use of HAC therapy in patients with unresectable CRL, most of them using floxuridine. The combination with modern systemic chemotherapeutics (oxaliplatin/irinotecan based regimens) has further increased the tumor response. Impressive tumor response rates of 92% have been published when HAI is combined with modern systemic chemotherapy, with resection rates of 47-53% when used as first line therapy and median survival of 51 months for chemotherapy-naïve patients and 35 months for previously treated (62). Major complications are associated with HAI; hepatotoxicity and technical problems with the delivery systems limit their use to only a few centers in the world with enough experience to provide this highly specialized treatment.

Drug eluting beads (DEBS)

Drug eluting beads or DEBS have emerged as a tool

to deliver chemotherapeutic agents to a specific area, decreasing the release into non-target regions (63). This facilitates higher doses to tumor cells, limiting the dose to the normal liver parenchyma and extrahepatic sites, decreasing toxicity. The agent is embedded in beads enough to minimize diffusion by embolizing the terminal capillaries. Modern angiographic techniques can deliver these beads directly to the tumor with low complication risk.

Recent reports have shown that DEBS therapy is well tolerated by patients (64,65). Major risks include liver failure and gastric irritation caused by seepage into the gastrointestinal tract; initial studies have demonstrated this technique to be safe in the treatment of CRLM (66). Post embolic syndrome, consisting of nausea, vomiting, dehydration and pain, have been reported in patients receiving multiple treatments with cumulative doses higher than 300 mg (67).

The chemotherapeutic agents used for DEBS have changed with the initial reports describing use of mitomycin C in combination with methylcellulose microcapsules. The more recent studies report Irinotecan (DEBIRI) with doses ranging from 50 to 200 mg per treatment. Data from studies using DEBIRI are difficult to analyze for several reasons: not all the patients received the same chemotherapeutic, the early trials used mitomycin C and the later irinotecan, different number of treatments were used and most of the patients had already failed different systemic treatments or other loco-regional therapies had been used. Martin *et al.* (67) showed tumor response rates with DEBIRI of 73% at 3 months, 56% at 6 months and 40% at 12 months using the RECIST criteria, with a median overall survival of 343 days and median free-survival was 197 days. DEBS is a therapy that is in its infancy and further studies are necessary to better understand the possible benefits and its role in the treatment of CRLM.

Non-arterial modalities

Radiation therapy for colorectal liver metastasis has gained importance in the treatment algorithm in the last few years. The better understanding of liver tolerance to radiation and new techniques to deliver the radiation have played an important role in decreasing toxicity and improved accuracy of radiation therapy.

Pioneer studies combining intensity-modulated radiation therapy (IMRT) with image-guided radiation therapy (IGRT) by megavoltage computed tomography scanning, have shown to be safe and efficient treating CRLM. Grade 2 and 3 toxicity was reported only in 9% and 4% of the patients respectively (68). A phase II trial by Engels *et al.* (69) using the helical tomotherapy (IMRT + IGRT) moderately hypofractionated therapy (10 fractions of 5Gy) was used in 53 patients. Results showed tumor response rate of 55%, with actuarial 1-year local control of 54%, progression free-survival of 14% and overall survival of 78%. The local control rates presented are lower than other reported in the literature with 2-year local control of 67% (70) this is probably because the higher doses used, however to obtain higher local control rates doses >100 Gy needs to be administered and this is only possible with tolerable toxicity in <3 lesion, <4 cm in diameter and far from hollow viscus organs (69).

Radio surgery (Cyber or Gamma knife) technology has emerged as a delivery method capable to deliver high doses of radiation in a very accurate manner, compensating for respiratory movements and with a tracking system to avoid toxicity to adjacent tissues. Up to date we have not found any report studying radiosurgery for CRLM.

Conclusions

The management of unresectable CRLM is constantly evolving, with demonstrated advances in systemic chemotherapy regimens, novel biologic agents, multiple ablation modalities and more accurate radiation delivery systems. With the increasing number of potentially effective therapies and combination therapies the management of this group of patient has become very complex, and requires a well-coordinated multidisciplinary team to achieve optimal outcomes. Selecting the best next therapy for each patient should be individualized and modeled to the different characteristics of each patient and tumor biologic features. In the coming years, randomized clinical trials will potentially offer the information necessary to assess the various RHT options alone and in combination with systemic modalities to better define the choice and sequence for their use in the treatment of the complex CRLM patient. While surgery remains the only curative approach for patients with CRLM, the majority of patients cannot be completely resected and RHT offer an important adjunct to control intrahepatic tumor burden.

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Footnote

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to declare.

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Surgical treatment of colorectal liver metastases

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Abstract: The incidence of colorectal cancer is rising in China. Since nearly 50% of these patients will ultimately develop liver metastases, an understanding of the surgical management of hepatic metastases is important. Surgical strategies for the management of liver metastases have evolved in recent years and now include adjunctive procedures such as portal vein embolization and radiofrequency ablation, which can help increase the number of patients eligible for potentially curative surgical management. In addition, innovations in treatment sequencing, including the use of peri-operative chemotherapy and the liver-first approach to the management of synchronous liver metastases have helped improve outcomes in these patients. Along with such changes in surgical management come new risks, such as chemotherapy-induced liver damage, with which the surgeon must be prepared to contend.

Keywords: Chemotherapy-associated liver injury; radiofrequency ablation; reverse approach to synchronous colorectal liver metastasis; portal vein embolization

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Epidemiology and background

Colorectal cancer is the 3rd most common cancer worldwide (1) and the 5th most common cancer in Eastern Asia (2). The incidence is rising in China (3) and it ranks among the top 5 most common cancers in residents of Shanghai with an incidence of 56 cases per 100,000 residents (4). Approximately 40-50% of patients affected with colorectal cancer will develop liver metastases at some point during the course of their disease, making liver metastases the most common cause of death for these patients (3,5,6). Complete surgical resection offers the only hope of cure and long-term survival for these patients. Using contemporary multimodality therapy, 5-year survival rates of 47-58% have been achieved for the 20-30% of patients who are able to undergo surgical resection (3,7,8).

According the general international classification system, colorectal liver metastases are considered synchronous if they are discovered at the time of initial diagnosis of the primary tumor or within six months of resection of the primary tumor (9). Metastases discovered in the liver more than six months after resection of the primary cancer, on the other hand, are considered metachronous.

Imaging and staging work up

The Chinese Guidelines for the Diagnosis and Comprehensive Treatment of Hepatic Metastasis of Colorectal Cancer recommend that the initial staging work-up for patients with colorectal cancer include measurement of serum AFP, CEA, and CA 19-9 as well as an hepatic ultrasound and abdominal and pelvic computed tomography (CT) scan with contrast to categorize the number and location of liver metastases and exclude additional sites of metastatic disease (9). For patients with suspected liver metastases, the guidelines recommend a liver magnetic resonance imaging (MRI) scan for further evaluation. It should be noted that while MRI has higher sensitivity for detection of tumors within the liver, CT provides superior imaging of extrahepatic disease (10). In addition, the guidelines recommend against routine percutaneous biopsy of suspected liver metastases due to the risks of needle track seeding and false negative

results; however, incisional or excisional biopsy should be performed if any suspicious liver lesions are encountered during resection of the primary tumor.

Following resection of a primary colorectal tumor in a patient without known metastatic disease, the recommended imaging follow up includes liver ultrasound every 3-6 months for the first two years and then every 6 months for 5 years (9). For patients undergoing surveillance after resection for stage II or III disease, the guidelines also recommend annual chest, abdomen, and pelvis CT with contrast with use of liver MRI to confirm any lesions seen on CT that are suspicious for new liver metastases. In patients who have previously undergone resection of liver metastases, the guidelines suggest that CT of the chest, abdomen, and pelvis with contrast be performed every 3 months for 2 years and then every 6-12 months for an additional 5-7 years (9). For each of these patient groups evaluation of the CEA level should be performed every 3-6 months for two years and then every 6 months for an additional 3-5 years.

Positron emission tomography (PET)/CT is not recommended as part of the routine staging work up for colorectal cancer (9). A retrospective British study showed a similar sensitivity and specificity of liver MRI and PET/CT for the detection of liver metastases, with a greater accuracy of MRI for lesions less than a centimeter in size--although it should be noted that this study also found a benefit of PET/CT over contrast-enhanced CT scan for the detection of extrahepatic metastatic disease (11). Similarly, a U.S. study identified the use of PET imaging as an independent predictor of a lower rate of nontherapeutic laparotomy in patients with hepatic colorectal metastases (12). No studies, however, have shown a survival benefit associated with the use of PET/CT. PET/CT is also limited in its detection of tumors less than 1 cm and mucinous tumors. PET-positive lesions are nonspecific, particularly in settings where inflammation may be present. Additionally, prior treatment with chemotherapy may decrease the sensitivity of PET for detection of disease (10).

Although not useful for pre-operative staging, intra-operative ultrasound is an important component of the surgical management of patients with hepatic metastases from colorectal cancer. Intra-operative ultrasound has been shown to detect tumors not seen on helical CT scan in as many as 27% of patients undergoing resection of primary or metastatic liver tumors, with even higher rates of detection of unsuspected lesions in patients with increasing numbers of tumors (13). For this reason, intra-operative ultrasound should be utilized at the time of liver resection for cancer.

Resectability and operability

Operability refers to a patient's ability to tolerate a liver resection (14) and includes factors such as comorbidities and baseline performance status. The resectability of a tumor has do with both technical and oncologic factors (14). Tumors are technically resectable when all metastases can be removed with negative margins with sparing of at least two adjacent segments of liver, and with preservation of adequate blood inflow and outflow, biliary drainage, and remnant parenchyma (generally accepted as at least 20% of estimated total liver volume) (10,15).

Oncologic factors which have previously been considered at least relative contraindications to the surgical treatment of liver metastases include the presence of four or more metastases and the presence of extrahepatic sites of metastases (16,17). Two recent retrospective studies have shown that long-term survival is possible even for patients with four or more metastases if complete resection can be accomplished (18,19). In one of these studies, even though the presence of multiple tumor nodules was independently associated with a lower rate of overall survival, it was not associated with disease-free survival (18). In the other study patients with four or more colorectal liver metastases had a 5-year actuarial disease-free survival rate of 21.5% with an overall survival rate of 50.9% after treatment with multimodality therapy (19). Additionally recent studies have shown favorable survival for patients with liver metastases and limited sites of resectable extrahepatic disease, including lung (20), limited peritoneal disease, and portal lymph nodes (21,22). Patients who develop new liver metastases or new sites of extrahepatic disease while on chemotherapy, however, should not be considered for resection unless a response to other therapy can be demonstrated (14).

Response to therapy

Emerging data suggest that the pathologic response to chemotherapy may represent an important endpoint that is highly correlated with overall survival (23,24). Four to nine percent of patients treated with neoadjuvant oxaliplatin or irinotecan-based chemotherapy may achieve a pathologic complete response (23,24), which has been shown on multivariate analysis to be an independent predictor of improved overall survival, overwhelming other previously established predictors of survival such as disease-free

interval, tumor size, and tumor multiplicity, with a hazard ratio of 4.8 for patients with a major pathologic response (defined as 49% or fewer viable tumor cells) (23). In addition, morphologic response to chemotherapy as seen on CT scan has been shown to correlate with overall survival (25). A study from the M.D. Anderson Cancer Center defined the "optimal" morphologic response as the presence of homogeneous low attenuation lesions with a thin, sharply defined interface between the tumor and the surrounding liver parenchyma and showed that patients treated with bevacizumab were significantly more likely to achieve such a response than those not treated with bevacizumab (47% *vs.* 12%) (25). The patients in the optimal morphologic response group had overall 3- and 5-year survival rates of 82% and 74%, respectively, *vs.* 60% and 45% (P<0.001) for those with a suboptimal response (25).

Synchronous metastases and treatment sequencing

Liver metastases are discovered synchronously with the primary tumor in approximately 25% of patients (26) and can be approached via three different strategies. The Chinese Guidelines for treatment of hepatic metastasis of colorectal cancer recommend either synchronous resection of both the primary and metastatic tumors or two-stage resection with resection of the primary tumor followed by resection of the hepatic metastases either with or without systemic chemotherapy in between the two operations (9). Classically, resection of the primary tumor followed by liver resection for the metastatic disease has been the approach taken to synchronous disease. There are several disadvantages to this approach, however, including the potential for progression of the metastatic disease prior to any systemic therapy, complications from the colorectal resection which may significantly delay or even preclude all together systemic therapy and/or resection of the liver metastases, and a substantial interval between presentation and administration of systemic therapy for stage IV disease. For these reasons, two alternative strategies have also been utilized. The first of these is simultaneous resection of both the primary tumor and the liver metastases. Several studies have shown the feasibility of this approach and have suggested that it can be accomplished without an increase in postoperative morbidity or mortality rates (26-29). Such an approach, however, is typically recommended for patients who either require a low-risk colon resection (e.g., right hemicolectomy) or a limited liver resection (e.g., wedge resection) if a more complex colorectal resection is required (10).

The second alternative strategy for the management of synchronous metastases is the reverse approach, whereby the liver resection is undertaken prior to the colorectal resection. This approach may include administration of neoadjuvant chemotherapy prior to any surgical resection and is feasible when the primary tumor is asymptomatic, without evidence of obstruction or bleeding. The major advantage to this approach is treatment of the metastatic disease prior to progression to an unresectable status (30,31). Progression of the primary tumor during the administration of systemic therapy is rare (32,33), but does require a change in treatment plan, so it is important that surveillance of the primary tumor be performed throughout the period of treatment for the metastatic disease. Once resection of the metastatic disease has been accomplished, focus can be turned to locoregional control of the primary tumor (i.e., resection for a colonic tumor or chemoradiation followed by resection for a locally advanced rectal tumor). In general, the decision regarding operative strategy for management of synchronous colorectal liver metastases should be prioritized based on whether the primary or metastatic tumor is causing symptoms, followed by which of the two sites presents the greatest oncologic risk. Evaluation of these factors is best undertaken by a multidisciplinary team at the outset of therapy.

Cautionary notes on neoadjuvant chemotherapy

Timing of surgery after chemotherapy

A Japanese study reported the results of sequential measurements of 15 minute indocyanine green retention (ICG R15) in patients following neoadjuvant chemotherapy. This study showed a significant improvement in the ICG R15 following the final dose of chemotherapy after a 2-week interval with further nonsignificant improvements at increasing time points up to 8 or more weeks after cessation of chemotherapy (34). Based on this data the authors concluded that resection should be delayed for at least 2-4 weeks following completion of chemotherapy. Another retrospective study of patients undergoing liver resection for colorectal metastases showed that receipt of 5 or fewer cycles of 5-FU-based preoperative chemotherapy was associated with a markedly lower rate of postoperative complications (19% *vs.* >40%) relative to patients receiving greater numbers of cycles (35).

Chemotherapy-induced liver injury

Several studies have described histologic changes in the livers of patients treated with certain chemotherapeutic agents. The first to be described of these was sinusoidal obstruction and veno-occlusive disease [the sinusoidal obstruction syndrome (36)] occurring in up to 78% of patients treated with oxaliplatin (37-40). These histologic changes do not seem to correlate with the total oxaliplatin dose received and may persist for months after chemotherapy (37,38). Although the presence of the sinusoidal obstruction syndrome has not been associated with increased rates of postoperative complications in most studies (38-40), in one French study it was associated with a longer length of hospital stay and a higher morbidity rate (41), and in another it was associated with an increased risk of transfusion (39).

Use of irinotecan has been associated with the development of steatohepatitis in approximately 20% of patients (38,40) and has been associated with higher rates of postoperative mortality (38), and may be correlated with higher rates of postoperative hepatic insufficiency (42). The development of steatohepatitis has also been shown to occur primarily in patients with a high body mass index (43), suggesting that rather than inducing steatohepatitis, irinotecan may cause progression of it (42). Increased rates of postoperative complications have also been correlated with longer durations of preoperative chemotherapy, with the most conservative cutoff occurring after 5 cycles of chemotherapy (35,39,41,44).

The effectiveness of modern chemotherapy regimens has resulted in a phenomenon known as disappearing liver metastases—metastases that become radiologically undetectable during neoadjuvant therapy. A retrospective study of patients treated with liver resection for colorectal metastases who had been treated with preoperative chemotherapy reported that almost 25% of patients had at least one liver metastasis that disappeared during treatment (45). In the patients whose missing tumors were not resected, nearly 60% eventually recurred at that site; however, the overall survival rates were not adversely impacted despite these local recurrences. Another retrospective study of disappearing metastases showed that persistent macroscopic disease was identified intraoperatively in 30% of the lesions, 80% of resected lesions without macroscopic evidence of residual disease had microscopic disease identified, and 74% of unresected lesions without macroscopic evidence of residual disease developed local recurrences with 1 year of surgery (46).

Perioperative chemotherapy

The use of perioperative chemotherapy in patients with resectable colorectal liver metastases was studied in a multicenter randomized trial—the EORTC Intergroup Trial 40983 (5). In this trial oxaliplatin-naïve patients were randomized to either 6 cycles of pre-operative and 6 cycles of post-operative FOLFOX4 or to surgery alone. The trial demonstrated that peri-operative chemotherapy increased the probability of 3-year progression-free survival by 35% (with a 7% absolute risk reduction) (5). Reversible post-operative complications were significantly more common in the peri-operative chemotherapy group (25% *vs.* 16%). A partial or complete response by RECIST criteria was seen in 40% of patients and on average the total tumor diameter decreased by about 25% (5).

A meta-analysis of randomized trials comparing surgery alone with peri-operative chemotherapy plus surgery in patients with stage IV colorectal cancer showed no evidence of a survival benefit for use of hepatic arterial chemotherapy, whereas the survival advantage for patients receiving peri-operative systemic chemotherapy approached significance (HR 0.74, P=0.08) (47). Both hepatic arterial chemotherapy (HR 0.78, P=0.01) and systemic peri-operative chemotherapy (HR 0.75, P=0.003) were associated with a significant recurrence-free survival benefit, however.

Functional liver remnant and portal vein embolization

A Japanese study of liver volumes in living transplant donors showed that in 25% of patients the left liver represents 30% or less of the total liver volume (48). For such patients, an extended right hepatectomy would carry a prohibitive risk of postoperative liver failure due to an inadequate functional liver remnant. The concept of portal vein embolization to induce hypertrophy of the functional liver remnant and thereby decrease the risk of postoperative liver insufficiency was first introduced by Makuuchi in 1990 to allow surgical resection in such patients (49). Since that time, additional studies have clarified the safety of and indications and techniques for the appropriate use of portal vein embolization. Preoperative portal vein embolization is typically recommended for patients with an anticipated functional liver remnant that is less than 20-25% of estimated total liver volume (50,51), with an expected average increase in volume of the remnant liver of 12% of the total liver volume (50). The rate of hypertrophy

has been shown to correlate with the degree of increase in the portal blood flow velocity in the nonembolized segment on postembolization day 1 (52). Portal blood flow in the nonembolized segments remains elevated for at least 14 days after embolization (52), providing the rationale for a 2-4 week waiting period between embolization and resection (50). The rate of hypertrophy after embolization is slower and the degree of hypertrophy is less in patients with cirrhosis (53) and diabetes (54,55). If an interventional radiology suite is unavailable for the performance of percutaneous portal vein embolization, then a transileocolic venous approach for embolization can be undertaken during laparotomy (49).

The technique of right portal vein ligation with in situ splitting (also known as ALPPS-associating liver partition and portal vein ligation staged hepatectomy) has been proposed as an alternate strategy for approaching the treatment of patients with a marginal or inadequate functional liver remnant (56). This technique involves two operations—the first during which the right portal vein is ligated and the hepatic parenchyma is completely (or nearly-completely) transected and a second (occurring after a variable period of delay, but during the same hospital stay) during which the resection is completed. Proponents of this approach feel that the hypertrophy achieved is more rapid and, perhaps, greater than that realized after portal vein embolization (57,58). Critics of the approach, however, feel that the high morbidity rate (68%), in-hospital mortality rate (12%), and lack of data on long-term oncologic outcomes should limit the use of this technique to clinical trials (56,59).

Repeat hepatectomy

Approximately 65-85% of patients who undergo liver resection for colorectal metastases will eventually develop a recurrence, of which 20-30% will be isolated to the liver (60). Repeat hepatic resection for recurrent liver metastases has been shown to have equivalent long-term survival without significant increases in perioperative morbidity or mortality in several studies, provided that a margin negative resection can be obtained (61-64).

(Metachronous metastases) - unresectable with downstaging

Retrospective studies have shown that use of contemporary chemotherapy regimens that include oxaliplatin and irinotecan can convert 12.5-38% of patients with initially unresectable liver metastases into surgical candidates (21,65). While such patients experience a high rate of recurrent disease (approximately 80% of patients will recur), 33-50% of them will be 5-year survivors and 23% of them will be 10-year survivors if an aggressive approach to resection of recurrent disease is used (21,65,66).

Second-line chemotherapy

For patients with marginally resectable or unresectable liver metastases from colorectal cancer who do not respond to first line chemotherapy, a switch to second-line chemotherapy may result in a response to therapy. The question of whether or not liver resection is reasonable in such patients if they respond to second-line chemotherapy has been addressed in a retrospective analysis (67). This study showed that 1-, 3-, and 5-year survival rates of 83%, 41%, and 22%, respectively, with 1- and 3-year disease-free survival rates of 37% and 11%, respectively, can be achieved in this setting with reasonable postoperative morbidity and mortality rates.

Biological agents

Biological agents, such as vascular endothelial growth factor (VEGF) inhibitors and epidermal growth factor receptor (EGFR) inhibitors in combination with cytotoxic chemotherapy frequently have activity in patients with metastatic colorectal cancer. There is emerging evidence from phase II and III randomized clinical trials that chemotherapy regimens that include biological agents may improve the ability to convert unresectable liver metastases into resectable ones (68).

Randomized controlled trials comparing FOLFOX or FOLFIRI with or without the vascular endothelial growth factor inhibitor bevacizumab have shown that the addition of bevacizumab significantly increases the duration of survival, the progression-free survival, and rates of response in both previously treated and previously untreated patients with metastatic colorectal cancer (69,70). The addition of bevacizumab to FOLFOX has been shown in a retrospective study to result in a lower percentage of viable tumor cells, although not a higher complete pathologic response rate, in resected specimens, and a decrease in the frequency and severity of sinusoidal obstruction syndrome was also noted (71). Similar results were obtained in another retrospective study where bevacizumab was

shown to result in decreased severity of the sinusoidal obstruction syndrome, but not to improve the likelihood of response according to RECIST criteria (72). No published randomized controlled trials of bevacizumab have measured rates of resection as a pre-specified endpoint.

Cetuximab is a monoclonal antibody that blocks the EGFR, which is frequently present on colon cancer cells (73). A randomized phase II trial of cetuximab plus either FOLFOX or FOLFIRI in patients with unresectable liver metastases from colorectal cancer showed high rates of partial or complete clinical response by RECIST criteria (68% *vs.* 57%, P=NS) (74). A retrospective analysis of the data from this study showed that partial or complete responses were significantly more likely in patients with KRAS-wide type tumors (70%) *vs.* those with KRAS-mutations (41%), and that chemotherapy with cetuximab increased the baseline resectability rate from 32% to 60% (P<0.0001) (74). A randomized phase III trial of FOLFIRI with and without cetuximab in patients with metastatic colorectal cancer (including, but not limited to patients with liver metastases) showed that the rates of surgery for metastases (7% *vs.* 3.7%) and the rates of R0 resection (4.8% *vs.* 1.7%, P=0.002) were higher in the group receiving cetuximab, although these were not pre-specified endpoints of the study (75). In addition, other EGFR inhibitors, such as panitumumab, have been shown to have activity in patients with metastatic colorectal cancer whose tumors are KRAS-wild type (76), and may eventually show similar rates of conversion to resectability.

Radiofrequency ablation

The EORTC 40004 study, a randomized phase II trial, randomized patients with unresectable liver metastases to either systemic therapy or systemic therapy plus radiofrequency ablation (RFA) (77). This study reported a non-significant improvement in 30-month overall survival and a significantly improved 3-year progression-free survival rate in the patients treated with RFA plus chemotherapy.

A retrospective German study has suggested that RFA may result in equivalent disease-free and overall survival to surgical resection for patients with a small number of metastases <5 cm in diameter (78). The RFA and surgery groups in this study were well-matched except for a significantly larger median tumor diameter in the surgery group (3 *vs.* 5 cm). The incidence of local recurrence was significantly higher and the time to progression was significantly shorter in the group treated with RFA; however, a higher rate of salvage therapy in the RFA group resulted in similar disease-free survival rates (78).

In contrast, another retrospective study concluded that RFA, alone or in combination with hepatectomy, results in significantly poorer overall survival (4-year survival of 22% *vs.* 65%) (7). This study also demonstrated higher rates of local recurrence in the group of patients treated with RFA relative to those treated with resection. While the role of radiofrequency ablation in the management of patients with liver metastases from colorectal cancer is still being defined, it is at the very least a useful adjunctive procedure in certain situations where resection is not technically feasible or would leave a patient with a marginal/inadequate functional liver remnant.

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Footnote

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

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Intraoperative margin re-resection for colorectal cancer liver metastases

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Abstract: One of the basic tenets of surgical oncology is the achievement of margin-negative resection. The importance of surgical margins in hepatic resection for colorectal cancer liver metastases (CRCLM) is reflected in the abundance of literature written about this topic. However, the definition of the ideal surgical margin has evolved in parallel with advances in systemic chemotherapy, biologic therapy and surgical technology. A better understanding of the biology of liver metastasis is of critical importance in the context of surgical strategy for CRCLM. The value of intraoperative margin re-resection to achieve R0 status for CRCLM is addressed, taking into consideration current understandings of cancer biology.

Keywords: Colorectal cancer liver metastasis; intraoperative margins; colorectal cancer; margin re-resection; liver metastasis

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Background

Over 143,000 individuals are estimated to be diagnosed with colorectal cancer (CRC) in the United States in 2012, with nearly 52,000 secondary deaths (1). CRC represents the most common gastrointestinal malignancy globally, and it is believed that of the 1.2 million people afflicted each year, 609,000 will die (2). The liver represents the most common site of initial clinical metastasis and approximately 60% patients develop colorectal cancer liver metastasis (CRCLM) during their primary or recurrent presentation (3). Approximately 20% of these patients will be eligible for hepatic resection with curative intent and with careful selection 5-year overall survival rates up to 25-58% can be reached (4-10). Advances in surgical technique, surgical series defining favorable clinical characteristics, and modern systemic chemotherapies have all contributed to these favorable outcomes. Despite these encouraging numbers, caution should be exercised in interpretation of the data because the benefits of resection are not based on prospectively randomized data but rather on retrospective series showing survival benefits compared to historical controls.

Over the last quarter century, the question of appropriate surgical margins in CRCLM has come to the forefront of debate. Surgical opinion regarding margin management in CRCLM has evolved but has been hindered somewhat by the lack supporting level 1 data. During the 1980s and 1990s, it was claimed that at least a 1-cm margin was required for hepatectomy to minimize disease recurrence and optimize survival (11-15). This led to a substantial period during which a requirement for resectability was the ability to achieve a 1-cm margin. However, this argument was weakened by the retrospective nature of the supporting data, which included studies that were underpowered, had suboptimal patient stratification, and lacked multivariate analysis. Near the turn of the last century, reports that questioned the necessity of 1-cm as the minimal resection margin began to appear. Surgeons from North America, Europe, and elsewhere reported large institutional series showing that outcomes in recurrence and survival depended more closely on the achievement of microscopically negative margins rather than a 1-cm negative margin (16-21). More recently, several groups have begun to question whether negative margins are in fact absolutely necessary in

surgical resections (22-25). These most recent studies have shown that positive microscopic margins may still result in equivalent overall survival and recurrence as patients with negative margins. Given this changing notion of the appropriate surgical margin, the question of whether intraoperative margin re-resection is of benefit in CRCLM becomes even more interesting.

Surgical margins

In 1986 Ekberg and colleagues from Sweden presented their data regarding outcomes after surgical resection for CRCLM (11). In this now-classic retrospective series of 72 patients, they concluded that it is "essential to obtain a margin of resection that is 10 mm or more" because this clinical variable was associated with a favorable overall survival. During this time period, the experience of several other groups was similar and thus the "standard of care" for liver resections in CRCLM was to consider patients for curative resection only if 1-cm margins could be achieved (11-15).

This viewpoint began to change around the turn of the century. The largest retrospective series to question the 1-cm margin paradigm was by Pawlik and colleagues (16). This international, multi-institutional retrospective series comprised of 557 patients stratified margin status by positive margins and negative margins of either 1-4 mm, 5-9 mm and >10 mm. All patients with negative margins had similar overall recurrence rates, but patients with positive margins had a significantly poorer median overall survival (5-year overall survival of 17.1% *vs.* 63.6%, P=0.01) and were more likely to have surgical margin recurrence (38.6% *vs.* 51.1%, P=0.04). Furthermore, patients with positive recurrence margins tended to have more metastatic lesions and a higher preoperative CEA level. This study concluded that subcentimeter, negative surgical margins were sufficient for liver resections. Equally important, it also suggested that a different tumor biology driving metastasis, rather than surgical technique, accounted for a positive margin. Several investigators have also shown that subcentimeter negative margins of resection provide similar clinical outcomes as patients undergoing hepatectomy with greater than 1-cm margins (17-21).

The belief that even microscopically negative margins are absolutely necessary for CRCLM has recently been challenged. De Haas and colleagues reviewed 436 patients undergoing hepatectomy for CRCLM with either an R1 or R0 margin of resection on patients operated between 1990-2006 (22). They showed that patients undergoing R0 and R1 resections had no significant difference in 5-year overall survival (61% *vs.* 57%, P=0.27) and median disease-free survival (P=0.12). Although patients with R1 resections had higher numbers of intrahepatic recurrences, when the investigators looked specifically at surgical margin recurrence, they found both groups to have equivalent surgical margin recurrence. Predictors of poor overall survival were not microscopically positive margins, but rather tumors greater than 3 cm and bilobar distribution. These data also strongly suggest that there are inherent biological differences in tumor behavior in patients undergoing R0 and R1 resections. Interestingly, this difference in tumor biology among positive and negative margins is similar to the conclusions implied in the study by Pawlik *et al.*, which notably drew different conclusions about surgical margins. It is plausible to conceive that when liver resections are performed by experienced hepatopancreatobiliary surgeons, differences in tumor biology rather than surgical technique are responsible for differences in margin status.

It is not a coincidence that evolution of surgical opinion regarding margins has paralleled advances in systemic chemotherapy and biologic therapy in CRC. We have seen substantially improved outcomes in metastatic CRC as more modern systemic therapies have been introduced. In 1993 when systemic chemotherapy with fluorouracil-based therapy was first shown by the Scheithauer and colleagues to improve the overall survival compared to palliative care, therapeutic options were limited (26). This landmark trial reported prolonged median overall survival to 11 months, but it was not until much later that oxaliplatin- and irinotecan-containing regimens were shown in prospective trials to prolong median overall survival to 19 months. Most recently the introduction of biologic agents (i.e., bevacizumab, cetuximab) has further increased median survival data to 24 months (27,28). Not only has survival improved in widely metastatic CRC, but also groups of patients with CRCLM that were initially deemed unresectable have become resectable after systemic chemotherapy, such as demonstrated in a French retrospective series of 701 patients (29). Interestingly, a Dutch group reported a series of 264 patients undergoing hepatectomy for CRCLM and found no differences in clinical outcome in patients receiving neoadjuvant chemotherapy between those with R0 and R1 resections (25). However in patients that did not receive upfront chemotherapy, R1 resection was associated with a worse clinical outcome. Thus, significant advances in systemic therapies have

become part of the multidisciplinary care of CRC patients and will continue to influence the outcome of liver surgery.

As ideas about the importance of margin status have evolved, so too has the role of intraoperative margin re-resection to achieve R0 status during hepatectomy for CRCLM. Unfortunately, the issue of margin re-resection is even less well informed by the surgical literature. When surgeons are confronted with positive intraoperative margins, many will perform re-resection when feasible or ablation with cautery or radiofrequency when re-resection is not feasible, yet these practices are not supported by data (12,16). There is only one study that specifically addressed this topic. Wray and collleagues from the University of Cincinnati reported in 2007 a retrospective single-institution review of 118 surgically resected cases of CRCLM over a 13-year time span (30). Clinical outcomes were compared between patients undergoing intraoperative margin re-resection and patients with resection margins greater or less than 1-cm. Their study showed that patients with >1 cm margins after intraoperative margin re-resection had higher local recurrence rates and worse overall survival than those individuals initially undergoing >1 cm margin resection ($P<0.05$). They also showed that initial margins >1 cm were associated with favorable disease-free survival (39.2 *vs.* 22.9 mo, $P=0.023$).

The results of this study suggest several points. First, and probably most important, tumor biology plays a dominant role in patient outcome. Intraoperative margins requiring re-resection to achieve margins >1 cm resulted in higher local recurrence and lower disease-free survival than individuals with initial margins greater than 1 cm. If margin status were the absolute determining factor for survival, one would expect similar outcomes in both groups. The observation that this was not the case suggests that it is tumor biology and not margin that drives clinical outcome. For example, it is plausible to conceive that a rate-limiting factor precluding an initial R0 resection may be an infiltrative growth pattern near major vascular or biliary structures indicative of aggressive cancer. If one analyzes the recent French and Dutch studies on surgical margins in the context of the University of Cincinnati, the dominant role of tumor biology on clinical outcome is undeniable.

Second, preoperative computed tomography and/or magnetic resonance imaging and intraoperative ultrasonography are critical imaging modalities for the surgeon to utilize in operative planning for hepatectomy. The fact that margin re-resection does not convey the same favorable disease-free survival as an initial negative margin implies that careful preoperative surgical planning and intraoperative ultrasound are important tools for the surgeon to utilize to maximize the chance for an initial margin negative resection. However if intraoperative margin re-resection is performed, the surgeon and medical oncologist must appreciate that the patient is at higher risk for local recurrence and may benefit from additional chemotherapy.

Other points concerning intraoperative margin re-resection relate to surgical technology and specimen interpretation by the pathologist. Surgeons must use caution when interpreting results of intraoperative frozen sections because accurate assessment of surgical margin in liver surgery can be difficult. Intraoperative interpretation of frozen sections may overestimate the true positive margin rate because the commonly used ultrasonic dissector partly aspirates liver parenchyma between tumor and normal tissue. This may decrease the resection margin up to 2-mm, potentially overestimating the proportion of R1, rather than R0, resections. Also the remnant cut section of the liver in contact with the previously removed specimen is commonly treated with argon beam coagulation "sterilizing" another 1 to 2 mm of hepatic tissue. Some surgeons now incorporate radiofrequency energy to coagulate along the margins of the tumor prior to resecting the liver (31). Thus, tumors interpreted as "margin-positive" may incorrectly receive this designation because of failing to take into consideration the false positives secondary to modern surgical technology.

Finally, more effective chemotherapy regimens could reduce the proportion of R1 resections that develop secondary liver metastases, thus minimizing residual micrometastatic disease. It seems that the microscopic margin of resection is less important when effective modern systemic therapy is applied to treat residual occult disease. This concept is supported by recent studies showing R0 resections are not required to achieve optimal outcomes given the efficacy of modern systemic agents (22-25).

The substantial improvements in the effectiveness of newer agents for systemic therapy in metastatic CRC should be taken into account when there is surgical consideration of intraoperative margin re-resection. Re-resection should be performed for an R2 resection since, at minimum, an R1 resection should always be sought for optimal clinical outcomes. However intraoperative margin re-resection is probably of no value in the setting of R1 or sub-centimeter R0 resection. Recent studies show no outcome differences between negative sub-centimeter and >1 cm margins, and between negative and microscopically positive margins. Effective modern chemotherapy, false positives from ultrasonic dissectors, and coagulation necrosis from argon beam coagulators and radiofrequency energy favor this

approach. However if margin re-resection is required clinicians must be wary that this represents a marker for more aggressive cancer and consideration should be made for prolonged systemic therapy.

In summary, definitive surgical resection is critical to the treatment of appropriately selected patients with CRCLM. The definition of what constitutes an ideal margin resection has evolved, with current evidence indicating similar outcomes with R1 or R0 resections with use of modern systemic therapies. Intraoperative margin re-resection should be used selectively and may play less of role in the current practice of liver surgery in light of modern systemic therapies, imaging modalities that allow careful operative planning, and advances in surgical technology. When margin re-resection is undertaken, it should be with the understanding that margin status can be skewed by surgical technique, and that regardless of margin status, margin re-resection is associated with worse clinical outcome. Perhaps the most important point regarding intraoperative margin re-resection is not necessarily whether or not it should be done, but rather that it is an indicator of more aggressive tumor biology and higher rates of local recurrence.

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None.

Footnote

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Potential use of Doppler perfusion index in detection of occult liver metastases from colorectal cancer

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Abstract: Many clinical and preclinical studies demonstrated that measurements of liver hemodynamic [Doppler perfusion index (DPI)] may be used to accurately diagnose and predict liver metastases from primary colorectal cancer in a research setting. However, Doppler measurements have some serious limitations when applied to general population. Ultrasound is very operator-dependent, and requires skilled examiners. Also, many conditions may limit the use of Doppler ultrasound and ultrasound in general, such as the presence of air in digestive tract, cardiac arrhythmias, vascular anomalies, obesity and other conditions. Therefore, in spite of the results from clinical studies, its value may be limited in everyday practice. On the contrary, scientific research of the DPI in detection of liver metastases is of great importance, since current research speaks strongly for the presence of systemic vasoactive substance responsible for observed hemodynamic changes. Identification of such a systemic vasoactive substance may lead to the development of a simple and reproducible laboratory test that may reliably identify the presence of occult liver metastases and therefore increase the success of adjuvant chemotherapy through better selection of patients. Further research in this subject is therefore of great importance.

Keywords: Doppler perfusion index (DPI); liver; colorectal cancer; liver metastases; oncology; surgery; adjuvant chemotherapy; ultrasound; Doppler ultrasound

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Introduction

According to epidemiological research, colorectal cancer is the second most common cancer and the second most common cause of death from malignant disease in Europe, causing approximately 400,000 deaths annually worldwide (1-4). The main cause of death of patients with colorectal cancer is liver metastases (5). It is well known that approximately 25% of patients with colorectal cancer already have liver metastases, and another 25% of patients develop liver metastases during follow up, usually within the first 2 years after the diagnosis of the primary colorectal tumor (6). In rectal cancer, preoperative chemoradiotherapy (CRT) significantly reduces the rate of local recurrence (5.3% *vs*. 14.1%), but patients who were treated with preoperative CRT do not appear to benefit significantly in terms of their long-term prognosis (66.2% *vs*. 67.8%) (7). Also, improvements in surgical technique resulted in less local recurrence (8).

Currently there is no reliable method for detecting small, occult liver metastases. Oncologists use various prognostic factors in deciding on adjuvant treatment. A standard prognostic factor that is used routinely in selecting patients for adjuvant treatment is the Dukes classification of the primary colorectal cancer (9-11). The survival of patients with dukes C stage as well as one part of patients with dukes B stage can be improved by the application of adjuvant chemotherapy after potentially curative surgical resection (12,13). Adjuvant chemotherapy (5-fluorouracil with levamisole or 5-fluorouracil with folinic acid) leads to a 40% reduction in the rate of recurrence and metastases,

and 33% reduction in mortality rates of patients with Dukes C colon cancer (14). Despite that, approximately one third of patients with Dukes C colon cancer will survive 5 years even without adjuvant chemotherapy. On the other hand, approximately one third of patients with Dukes B colon cancer will develop recurrent disease or metastases. However, today there is no clear recommendation for the application of adjuvant chemotherapy in patients with colorectal cancer stage Dukes B (9). Therefore, it is obvious that Dukes classification is insufficient for the selection of patients for the application of adjuvant chemotherapy after potentially curative resection for colorectal cancer (9).

Careful selection of patients is crucial to improve the results of chemotherapy by applying it only to patients with the greatest impact on survival and avoiding harmful effects of chemotherapy in patients with no risk off liver metastasis (15). Therefore, the detection of those patients with micrometastases that are not evident at the time of primary tumor treatment still represents a significant challenge (16).

Current morphological methods for diagnosing liver metastases from colorectal cancer obviously have the limitation in detecting small focal liver lesions less than a few millimeters in diameter (17).

Hepatic perfusion changes in patients with liver metastases

It has been known for a while now, that in patients with liver metastasis there is an alteration of blood flow through the liver (18). Alterations of the hepatic flow in tumors were initially observed using dynamic scintigraphy. In 1983 Parkin *et al.* (19) proved that when malignant tumors are present the arterial hepatic flow is elevated because tumors have a predominantly arterial vascularity. Parkin also proposed the use of the hepatic perfusion index (HPI), which is increased in patients with liver metastases.

Several papers consistently demonstrated that a relative blood flow through the common hepatic artery, expressed as a percentage of total hepatic blood flow is significantly increased in patients with hepatic metastases in comparison to patients without liver metastases (9,20,21).

For a long time it has been considered that the observed change of blood flow through the liver is exclusively the consequence of increased neovascularization within the metastases themselves. Clinical and experimental studies demonstrated that liver metastases from colorectal cancer establish their blood supply mostly through hepatic artery system (22-24).

Although some research demonstrated that liver micrometastases indeed do derive some of their blood flow through the portal system, portal vascularization of liver metastasis is generally considered insignificant in comparison to vascularization derived through hepatic artery, especially considering the fact that the with the growth of metastasis there is also an increase in arterial blood supply compared to a portal blood supply (23).

In spite of the well-known thinking that only metastasis greater than one millimeter in diameter receive their blood supply through newly formed blood vessels (22), it has been demonstrated that even metastasis with only half a millimeter have a defined vascularization derived predominantly through the system of hepatic artery (23,25). Hepatic neovascularization is a complex process during which the relative contribution of arterial and portal blood flow changes during the growth of liver metastases (24,25). In the earliest stage, liver metastasis depend on perfusion from adjacent issue, until they reach a diameter of approximately 150-200 μm (26). Further growth of the metastasis induces new vessel formation derived from those arterial and portal system. As liver metastasis increases to over two millimeters in size, arterial blood flow becomes dominant (27). Angiographic research demonstrated a great variability in the vascularization of liver metastasis. Some metastasis demonstrate minimal accumulation of contrast (hypovascularized metastases), while others are extremely well vascularized with a marked arterial supply of the entire liver lobe (24). These variations in blood supply of liver metastasis play a significant role in the choice and success of local therapeutic procedures such as locoregional arterial chemotherapy, dearterialization and embolisation.

Doppler perfusion index (DPI)

This well known change in liver perfusion in patients with liver metastasis was further investigated by Leen and colleagues using Doppler ultrasound. In a series of papers he demonstrated that a Doppler ultrasound is a simple, non-invasive and reliable method in detecting changes in liver perfusion, especially in patients with colorectal cancer liver metastases (9,28-30).

Some research demonstrated that DPI enables greater precision to determine the likelihood of the existence of occult metastases in the liver. The analysis of preoperative values of DPI on a sample of 120 patients with colorectal cancer confirmed the statistically significant predictive value of DPI the detection of liver metastasis. The sensitivity,

Table 1 Doppler perfusion index—how to do it

Procedure	How to do it
Prepare the patient	Overnight fasting; supine position
Measure the diameter of the common hepatic artery (AHC)	In transverse plane, find the celiac trunk and locate the common hepatic artery. Set the size of the Doppler window to encompass AHC. Set the measurement point so that the Doppler axis is as close as small as possible (should not be greater than 60 degrees to minimize error). Measure the diameter (in centimeters) of the common hepatic artery perpendicular to the longitudinal axis at the selected measurement point
Measure the cross sectional area of the common hepatic artery	Use built-in algorithm or calculate from the diameter of the AHC: cross sectional area (S) = $\left(\frac{d}{2}\right)^2 \pi$
Measure the velocity of blood flow in the common hepatic artery	At the same measurement point, position the Doppler cursor in the middle of the artery and record Doppler waves for at least 3 cardiac cycles (more cycles may be required if patient has arrhythmia). For each cycle determine the peak systolic velocity (PSV), end-diastolic velocity (EDV) and mean velocity (MV) in centimeters per second. Calculate mean PSV, EDV and MV across several cycles
Calculate resistance index (RI)	RI = $\frac{PSV - EDV}{PSV}$
Calculate blood flow in the common hepatic artery (fAHC)	Use built-in algorithm or calculate blood flow in mL/s: fAHC = MV*S
Measure the diameter of the portal vein (PV)	Locate the portal vein before its bifurcation (adjacent to common hepatic duct). Set the size of the Doppler window to encompass PV. Set the measurement point so that the Doppler axis is as close as small as possible (should not be greater than 60 degrees to minimize error). Measure the diameter (in centimeters) of the portal vein perpendicular to the longitudinal axis at the selected measurement point
Measure the cross sectional area of the portal vein	Use built-in algorithm or calculate from the diameter of the PV: cross sectional area (S) = $\left(\frac{d}{2}\right)^2 \pi$
Measure the velocity of blood flow in the portal vein	At the same measurement point, position the Doppler cursor in the middle of the portal vein and record Doppler waves for at least 3 cardiac cycles (more cycles may be required if patient has arrhythmia). For each cycle determine the mean velocity (MV) in centimeters per second. Calculate mean MV across several cycles (modern ultrasound machines will do that automatically)
Calculate blood flow in the portal vein (fPV)	Use built-in algorithm or calculate blood flow in mL/s: fPV = MV × S
Calculate Doppler perfusion index (DPI)	DPI = $\frac{fAHC}{fAHC + fPV}$

specificity, positive and negative predictive values as well as the accuracy of determining DPI for identification of patients in whom liver metastasis will be diagnosed during follow up was found to be 95%, 69%, 73%, 94% and 81% (9).

Five-year follow-up of patients with colorectal cancer demonstrated that the blood flow redistribution through the liver is strongly correlated to the survival (9). In a prospective study, the 5-year follow-up of patients who underwent potentially curative resection of primary colorectal cancer found that patients with normal values of DPI (DPI <30%) had a 5-year rate of disease free survival of 89%, while the 5-year disease free survival was only 22% in patients with elevated DPI (DPI ≥30%). Overall survival of patients with normal values of DPI was as high as 91% and only 29% in patients with abnormally elevated DPI (9).

Measurement of blood flow through blood vessels by color Doppler is extremely dependent on a number of technical parameters, and even small errors in the measurement of the diameter or cross-sectional area of blood vessels or the angle at which the measurements were performed can produce large errors in the calculation of the blood flow through (*Table 1*) (31,32). Therefore, in most

Table 2 The equations that may be used for calculating body surface area in order to standardize blood flow

Author	Formula
Du Bois and Du Bois (40)	$BSA = 0.007184 \times H^{0.725} \times W^{0.425}$
Gehan and George (41)	$BSA = 0.0235 \times H^{0.42246} \times W^{0.51456}$
Haycock (42)	$BSA = 0.024265 \times H^{0.3964} \times W^{0.5378}$
Mosteller (43)	BSA = Square root [(H × W)/3,600]

H, height (cm); W, weight (kg); BSA, body surface area (m^2).

clinical studies where Doppler measurements were used to measure blood flow, mean values of several consecutive measurements of diameters or cross sectional areas of blood vessels were used to reduce the risk of errors in measurements (9). Analyzing the maximum speed of blood flow through the vessel in systole [peak systolic velocity (PSV)], the speed of blood flow at the end of diastole [end-diastolic velocity (EDV)] and calculating the resistance index (RI) can more precisely describe the hemodynamic status of the arteries than the calculation of blood flow volume, because the calculation of the above parameters does not necessitate to knowledge the cross-sectional surface of measured vessels.

Despite the fact that ultrasonic Doppler technique is dependent on the examiner, technically challenging and often requires significant time to perform, it has confirmed good reproducibility and validity of the measurements among multiple examiners (33,34).

Moreover, satisfactory accuracy and reproducibility of measurements of the DPI of the liver was found when the measurements were performed by operators with no classical medical education and after only a few months of training in this specific area (18).

In spite of the standardization of measurements, values of blood flow often demonstrate wide dispersion (6). In order to maximize the extent of comparability and reduce the scattering of value, the flow can be expressed in relation to body surface area.

In histological examinations, the diameter of coronary arteries has been brought in correlation with age and body surface area off young healthy people (35). Body surface area is also used as a method of standardization of the cross sectional area of different arteries (36). Body surface area is routinely used in research of blood flow through different vessels as well as for liver haemodynamics (37). Therefore, utilizing body surface area and expressing the blood flow through different vessels relative to body surface area is a specially suitable to compare vascular parameters between groups with substantial morphological differences (38). Also, body surface area is used to achieve greater comparability of masses of different organs, such as liver, which mass, expressed relatively according to body surface area can be used in different researches (39). There are several formulas that enable the calculation of the body surface area, such as those recommended by Mosteller or DuBois and DuBois (*Table 2*) (40,43,44). The calculation of body surface area is a common procedure in many clinical and scientific branches of medicine (45), but surprisingly in most research this method of standardization was not used (6).

Another possible downfall of determining DPI with the purpose of detection of micrometastasis in the liver in patients with colorectal cancer is the factor that DPI is increased in patients with cirrhotic liver. However, hemodynamic examination of hepatic blood flow demonstrated that in patients with liver cirrhosis there is also an increase in liver congestion index, defined as the ratio of the cross sectional area of the portal vein and portal mean blood flow velocity (30).

However, not all authors were able to prove the clinical usefulness off DPI measurement in the detection of liver metastasis. In a clinical study conducted by Roumen *et al.* (46), 133 patients with different stages of colorectal cancer were examined. Reliable DPI measurements were not possible in 29 patients, mostly due to technical difficulties caused by the presence of air or other contrast media, obesity, scars or other reasons. In their study, they were unable to detect a single cut-off value that could reliably discriminate patients with liver metastases. It has to be noted that in this study no preselection of patients was performed and the focus was placed on the clinical usefulness of Doppler measurements in unselected population of patients. Apart from technical difficulties, Doppler perfusion measurements are characterized by high variability, which has been reported to be as high as 26% (46). Especially important it is the intraobserver variability that is not merely the result of the method or technique but rather a consequence of inherent subject variations.

As the Doppler measurements may well prove not to be

Table 3 Possible etiology of change in flow

Main proposed etiology	Studies
Increased hepatic arterial blood flow	Parkin, 1983 (19); Ridge, 1987 (24); Archer, 1989 (25)
Decreased portal blood flow	Nott, 1991 (48); Carter, 1994 (49); Yarmenitis, 2000 (47)
Decreased portal blood flow with compensatory increase in hepatic arterial blood flow	Kopljar, 2004 (6)

useful in everyday practice, the underlying hypothesis of the existence of a humoral vasoactive substance responsible for hepatic perfusion changes sheds the new light on the clinical and preclinical research directed towards finding an easily and reliably measured systemic factor.

Possibility of a humoral vasoactive factor

Until now, the cause of this redistribution of hepatic blood flow is not clearly explained. According to one hypothesis, this phenomenon is caused by splanchnic vasoconstriction and a consequent reduction of portal blood flow with a simultaneous increase in common hepatic artery flow as a result of hemodynamic compensation (15). Some experimental and clinical research indeed demonstrated that in patients with liver metastases that are too small to be detected by conventional radiologic methods there is already an alteration in the blood flow through the liver that was shown to be highly sensitive in the detection of small, occult liver metastasis (9,18,20,47). This raises the question on the nature of these haemodynamic changes, since these small, occult metastases are unlikely to be the cause of sufficient neovascularization responsible for significant changes in hepatic perfusion (*Table 3*). It was therefore hypothesized that the primary cause of hepatic perfusion changes in patients with colorectal cancer liver metastases is a circulating vazoactive factor causing primarily splanchnic vasoconstriction and subsequent reduction in portal inflow to the liver.

In order to prove the hypothesis of circulating vasoactive factor as a possible cause of liver hemodynamic changes in patients with colorectal cancer and liver metastasis, a group of researchers conducted a research on animal model (49). In this research, isolated intestinal loop of a healthy animal was perfused with blood from another animal with experimentally induced liver sarcoma (HSN sarcoma) (49).

The experiment showed that splanchnic vascular resistance in healthy animals was significantly greater during perfusion with blood from tumor bearing animals [91.6 (SE 21.5), *vs.* 51.7 (SE 7.41), P=0.036]. The results of this experiment suggest that observed hemodynamic changes are at least partly mediated by a circulating agent. Whether this circulating agent is produced by the tumor itself or is an endogenous agent remains unclear (49).

In another animal experiment (47), liver metastases were induced in 30 male Wistar rats by inoculating Walker 256 tumor subcutaneously. Hemodynamic changes were observed and correlated to the liver histology at the time of measurement. By measuring the flow through the hepatic artery and portal vein in this animal model of spontaneous liver metastases, Yarmenitis and colleagues have shown a statistically significant increase in blood flow through the common hepatic artery as early as the fourth day after implantation of the primary tumor, when histological examination of the liver demonstrated only single tumor cells or small clusters in the connective tissue of porta hepatis and periportal interlobular space (47).

DPI values were significantly increased as early as on the fourth day after implantation of the primary tumor, and did not significantly increase until the fifteenth day, when the histological examination showed metastatic tumors in the liver with the largest diameter of 2 mm. Also, the flow through the portal vein was reduced in animals with metastatic tumors in the liver on the fourth day, but the difference was not statistically significant. Therefore, a statistically significant increase in DPI and blood flow through the hepatic artery was observed only four days after implantation of tumor cells, when the histological analysis of the liver in these animals was not able to demonstrate any vascular component of either the hepatic artery or portal vein.

This finding is consistent with the hypothesis of the existence of a humoral vasoactive factor that can lead to hemodynamic changes in the liver in the earliest stages of the development of metastases, and that its effect may manifest both locally, in the liver, as well as in the splanchnic circulation away from metastases (49). Opposite to some other studies (48,49), in the experimental study conducted by Yarmenitis and associates, DPI changes were primarily attributed to a statistically significant increase in

the flow through the hepatic artery and without significant changes in portal flow (47).

In the research conducted by Nott and coworkers (48), the blood flow through the portal vein was significantly reduced in animals with experimentally induced sarcoma of the liver (Walker carcinosarcoma). The results of this study indicate that overt tumor derived from the intraportal inoculation of Walker cells results in an increase in the HPI. The blood supply to the tumor was shown to be derived principally from the hepatic artery. However, hepatic arterial flow did not change in the presence of tumor and the alterations in the HPI were found to be secondary to a reduction in portal venous inflow. Moreover, the presence of overt hepatic tumor was associated with gross derangement of hepatic hemodynamic with a pronounced increase in intrahepatic arteriovenous shunting. It was concluded that hemodynamic changes accompanying the development of overt hepatic tumor are complex and must be taken into account when attempting to potentiate the distribution of cytotoxics to the tumor by regional administration or through manipulation of liver blood flow (48).

Other researchers also demonstrated significant reduction of portal flow in experimental models of liver metastasis, with no changes in arterial hepatic flow (50). An experimental and biomolecular research identified some systemic active factors that might, at least in theory, explain the redistribution of blood flow through the liver in patients with colorectal liver metastasis (51).

One possible causative agent might be endothelin-1 (15), a potent vasoconstrictor with significant influence on the portal blood flow that is regularly produced by the colorectal cancer. Peeters and coworkers measured the serum level of endothelin-1 in 68 patients with colorectal cancer and 20 healthy volunteers without malignant disease. The sera level of endothelin-1 was statistically significantly higher in patients with colorectal cancer compared to healthy participants. Further subgroup analysis was performed and patients were divided into three groups: those with primary colorectal cancer without metastasis, patients with colorectal cancer that developed metastasis during follow up and patients with colorectal cancer and synchronous liver metastasis. All three subgroups had higher concentrations of endothelin-1 compared to healthy participants. However, no statistically significant difference in preoperative concentration of endothelin-1 was found between healthy participants and patients with liver metastasis from previously resected colorectal cancer (15).

Animal models demonstrated that endothelin-1 results in increased blood pressure in the portal vein (52,53). Also, it has been determined that endothelin-1 is synthesized and released from several human epithelial carcinomas. Inagaki and coworkers demonstrated the existence of increased quantities of endothelin-1 in the tissue of primary colorectal carcinoma (54). Furthermore, histochemical methods demonstrated the presence of endothelin-1 in the cytoplasm of colorectal cancer cells metastatic to the liver, as well as in cytoplasm of adjacent myofibroblasts. These results indicate that endothelin-1 is not produced only in tumor cells but also in adjacent cells, thereby influencing the growth of the tumor (51). Unfortunately, survival analysis did not demonstrate prognostic value of pre-operative determination of the concentration of endothelin-1 in patients with colorectal cancer (15).

In one clinical study (6) results showed that the DPI successively increased in patients with colorectal cancer with no signs of liver metastases and in patients with liver metastases. There was a statistically significant difference in the blood flow through the portal vein between patients with colorectal cancer without signs of metastases in the liver and healthy control patients, but no statistically significant differences in the flow through the common hepatic artery (6). However, the flow through the common hepatic artery and portal vein in patients with primary colorectal cancer with no signs of liver metastases and those with liver metastases, as well as between patients with liver metastases and control subjects showed statistically significant differences in the absolute values of flow through common hepatic artery and portal vein as well as the values of the flow through the same blood vessels expressed relative to body surface area (6).

These results suggest that the early hemodynamic changes in patients with liver metastases are associated with a reduction of flow through the portal vein, while the increase in blood flow through the common hepatic artery is associated with the development of tumor neovascularization in larger metastases (6).

Furthermore, clinical research demonstrated no statistically significant differences in the blood flow through the superior mesenteric artery between patients with liver metastases and those without metastases, and the difference in RI was marginally statistically significant (6).

These findings are certainly at least partly influenced by the difficulties in measuring the cross-sectional area of blood vessels and blood flow in general, which is why the measurements of vascular index have the advantage.

The hypothesis of the existence of systematic acting,

circulating humoral vasoactive factor in patients with liver metastases is further supported by the analysis results obtained by measuring the flow rate and RI of the superior mesenteric artery among the three groups of patients (patients with liver metastases, those with primary colorectal cancer and no detectable metastases and patients with no malignancy). If this hypothesis is correct, and if indeed a humoral vasoactive factors can be found in the bloodstream of patients with metastases in the liver, then its actions should be expressed also on remote vessels, outside of the liver.

Indeed, the EDV in the superior mesenteric artery was higher in healthy subjects compared to patients with colorectal cancer and no signs of liver metastases as well as compared to patients with liver metastases, while the difference in end-diastolic velocity in the superior mesenteric artery in patients with colorectal cancer without signs of metastases in the liver and patients with metastases was not statistically significant (6). Statistically significant differences of RI were found among healthy subjects, patients with colorectal cancer without signs of metastases in the liver and patients with metastases (6). These results clearly indicate that even in patients with colorectal cancer without clinical and radiological signs of metastases in the liver, there is a systemic alteration of blood flow, which is reflected in the reduction of the end-diastolic velocity in the superior mesenteric artery.

Conclusions

In the last three decades, many clinical and preclinical studies demonstrated that DPI measurements may be used to accurately diagnose and predict liver metastases from primary colorectal cancer. However, Doppler measurements have some serious limitations when applied to general population. Ultrasound is very operator-dependent, and requires skilled examiners. Also, many conditions may limit the use of Doppler ultrasound and ultrasound in general, such as the presence of air in digestive tract, cardiac arrhythmias (including rather common atrial fibrillation), vascular anomalies (e.g., the origin of right hepatic artery from superior mesenteric artery), obesity and other conditions (6,46). Therefore, in spite of the results from clinical studies, its value may be limited in everyday practice.

On the contrary, scientific research of the DPI in detection of liver metastases is of great importance, since current research speaks strongly for the presence of systemic vasoactive substance responsible for observed hemodynamic changes. Identification of such a systemic vasoactive substance may lead to the development of a simple and reproducible laboratory test that may reliably identify the presence of occult liver metastases and therefore increase the success of adjuvant chemotherapy through better selection of patients. Further research in this subject is therefore of great importance.

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Footnote

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Addressing sexual dysfunction in colorectal cancer survivorship care

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Abstract: Despite the high prevalence of sexual dysfunction in survivors of colorectal cancer, studies have shown that patients and providers rarely discuss how these symptoms may be influencing overall quality of life. The type and severity of symptoms of sexual dysfunction can vary greatly depending on the type of colorectal cancer and treatment, and assessment of sexual dysfunction is key to understanding how patients may be affected by these symptoms. Although patients would like to discuss these issues with their provider, they are often reluctant to ask questions about sexual functioning during appointments. Likewise, health care providers may hesitate to address sexual dysfunction due to time limitations or lack of knowledge regarding treatment of sexual problems. Health care providers can facilitate discussion of sexual dysfunction by (I) assessing sexual functioning throughout treatment; (II) initiating discussions about symptoms of sexual dysfunction at each appointment; and (III) maintaining adequate referral resources for treatment of sexual dysfunction.

Keywords: Colorectal cancer; sexual dysfunction; psychosocial factors; sex counseling

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Introduction

Sexual dysfunction is one of the most common long-term effects of colorectal cancer treatment, yet studies consistently show that this issue is rarely discussed among patients and their providers (1). Colorectal cancer survivorship has increased significantly in recent years due to advances in surgical techniques and adjuvant therapy, and the majority of colorectal patients will become long-term cancer survivors (2). Increasing survival rates in colorectal cancer have shifted the focus of patient health care needs from treating malignancies to addressing survivors' long-term quality of life, which includes sexual functioning (1). When sexual issues are not addressed, it can have a significant negative impact on the quality of life of survivors (3). To improve the quality of survivorship care in colorectal cancer, the sexual health needs of patients require assessment and treatment at all stages of care (4-6).

Sexuality is an important component of quality of life, given that the majority of colorectal cancer survivors will remain sexually active following treatment (7). Changes in sexual functioning in colorectal cancer survivors can affect not only patients, but their partners as well (1). Although colorectal cancer survivors often report that their overall quality of life (QOL) is good, both men and women report significant problems with sexual functioning following treatment (6,8-11).

Prevalence of sexual dysfunction in colorectal cancer survivors

Forty-one percent of all cancer survivors experience a decrease in sexual functioning and 52% experience changes in body image (12). In patients with colorectal cancer, the rates of sexual dysfunction can be even higher given the physiological changes that can result from surgery, chemotherapy, and radiation therapy (*Table 1*). For example, patients who have undergone surgery for rectal cancer are significantly less likely to be sexually active than prior to surgery, and their sexual problems can be complex and multi-factorial (4). The type of surgery can impact sexual function as well. For example, one study found that women

Table 1 Potential impact of colorectal cancer treatments on sexuality

Physiologic changes	Radiation therapy	Chemotherapy	Surgery
Vascular, sensory, and continence	Vascular scarring—decreased genital blood flow (erection dysfunction; decreased vaginal lubrication)	Change in senses—taste bud changes; increased sensitivity to smells; peripheral neuropathy changes sensation of touch	Urinary/fecal incontinence—type of surgery affects risk
Skin changes	Skin changes—texture/color changes can affect body image; can remind partner of patient's diagnosis. Although tattoos are small, they can be a reminder to the patient or partner of diagnosis	Skin sensitivity changes—some chemo causes extreme reaction to cold which affects food that can be eaten on dates; neuropathy affects enjoyment of skin touch; hand/foot (palmar/plantar) syndrome can affect enjoyment of activity with partner/affect ease of touching partner if skin peeling off hands; skin rash can occur; affect color of nails	Surgical scars—body image changes; affect partner's ease in being with patient
Fatigue	Affects social interaction, libido	Affects social interaction, libido	Affects social interaction, libido
Vaginal vault changes	Shortening of vagina; decreased lubrication; risk of dyspareunia; vaginal stenosis	Decreased lubrication; risk of dyspareunia; increased risk of vaginal infection from tiny tears; Mucositis—can affect oral or vaginal cavity	Postoperative adhesions if they occur do not usually affect the vaginal vault unless surgery was done in that specific location
Sexual pattern alterations	If fatigue, may need to change usual positions or time of day for activity; affect spontaneity; if decreased lubrication will need to use artificial lubricant to avoid tears and possible infection; if XRT causes diarrhea, will affect usual pattern if apprehensive re: fecal incontinence	If nausea/vomiting, will decrease desire; affects dating pattern; if taste bud changes, may avoid French kissing/oral stimulation; if fatigue, may need to change usual positions or time of day for activity; affect spontaneity; if decreased lubrication will need to use artificial lubricant to avoid tears and possible infection	If stoma will need to remember to empty appliance prior to sexual activity; perhaps wear cover on appliance to prevent it 'sticking' to body; if patient irrigates, may decide to do prior to activity so can wear smaller 'security pouch'; change in usual position so appliance can lie to the side; if waterplay activity part of sexual pattern may want to irrigate, prior so do not have to wear appliance; avoid 'gassy' food on date or use 'gas filters'; loss of rectal sexual pleasuring if rectum removed
Nerve damage	Skin sensitivity decreased; decreased vaginal lubrication/ erection dysfunction	Skin sensitivity decreased; decreased vaginal lubrication/erection dysfunction	Skin sensitivity decreased; decreased vaginal lubrication/ erection dysfunction
Urethral irritation	Depends on radiation treatment field	Hormonal changes may cause thinning and inflammation of tissues around the vaginal opening; if using spermicidal as birth control, can cause urethral irritation	None

Table 1 (*continued*)

Table 1 (*continued*)

Physiologic changes	Radiation therapy	Chemotherapy	Surgery
Hair pattern	Alopecia—(only of site of radiation treatment) affects body image; daily reminder of treatment/ diagnosis; if loss of pubic hair may be pleasurable OR may be emotionally upsetting to pt or partner if pt feels 'childlike'	Alopecia/hair thinning—affects body image; if single, may affect desire to date; daily reminder of treatment/ diagnosis; if loss of pubic hair may be pleasurable OR may be emotionally upsetting to pt or partner if pt feels 'childlike'	Alopecia/hair thinning—none
Fertility impact	Location/dose affect risk; premature ovarian failure	Type/dose affect risk	Usually not for colorectal cancer; abdominal adhesions can increase risk of female infertility post-tx; pelvic exenteration (hysterectomy); A/P resection = retrograde ejaculation
Fear of recurrence	Impacts libido of patient and/or partner	Impacts libido of patient and/or partner	Impacts libido of patient and/or partner
Delayed complications	Risk of fecal or urinary incontinence due to fibrosis (risk factors for postoperative incontinence included preoperative incontinence, female gender, perioperative blood loss, preoperative bladder emptying difficulties, autonomic nerve damage, and presence of a permanent stoma)	Peripheral neuropathy may be permanent and it can affect sensations/ enjoyment; taste bud changes may be permanent and will affect sexuality	Adhesions can cause pelvic pain during coitus; nerve damage may be permanent and affect sensations

who had abdominoperineal excision (n=73) for rectal cancer were less sexually active, had less frequent coitus, and were less likely to achieve arousal or orgasm than women who had anterior resection (n=222) (13). In males, one study found that total mesorectal surgery (n=49) affected erection (80%) and ejaculation (82%), while another study by Sartori and colleagues found less impact on erection and ejaculation (n=35) (14,15). Other studies have found that stoma creation does not always negatively impact on sexual function. For example, the meta-analysis by Ho, Lee, Stein and Temple found mixed results in terms of the relationship between stomas and sexual dysfunction. Despite the lack of conclusive evidence, the authors recommended that patients be informed that surgery might affect sexual functioning (6).

Sexual dysfunction in colorectal cancer survivors can also be related to medications (e.g., hormonal treatment or psychotropic medications) or changes in body weight during the course of treatment (16-19) (*Table 1*). Prior sexual history, age, partner status, socioeconomic status, cultural beliefs surrounding sexuality, global quality of life and comorbid medical conditions can all have an impact on sexual functioning in survivors (20). Any symptoms of sexual dysfunction can also be exacerbated by symptoms of anxiety, depression, and fatigue which are common among survivors of cancer (21).

Although there is evidence to suggest that high rates of sexual dysfunction are reported among colorectal cancer survivors, very few studies have compared the prevalence of sexual dysfunction in this population with normative control groups. One of the few recent comparative studies of colorectal cancer survivors with a normative sample reported that male survivors of rectal cancer experienced higher rates of erectile dysfunction (54%) than those with colon cancer (25%) or those within the normative sample (27%) (4). Males with rectal cancer also reported higher rates of ejaculation problems (68% rectal versus 47% of colon cancer survivors). Female rectal and colon cancer survivors reported significantly more vaginal dryness (35%

rectal; 28% colon) than the normative population (5%) and pain during intercourse (30% rectal; 9% colon; 0% normative) (4).

Patient-provider communication about sexual dysfunction

Providing education, informational support, and treatment options can help to improve sexual functioning in colorectal survivors (11). Despite the high prevalence of sexual dysfunction reported by colorectal cancer survivors and increasing awareness of the sexual health needs of patients, sexual functioning is often not adequately addressed by health care providers (22,23). Patients often express reluctance to raise sexual issues during appointments, and many report feeling embarrassed or ashamed to ask questions related to sexual health (24). Discussing sexual issues may be a new experience for many cancer patients who may not have felt the need to address this topic with health care providers in the past (25,26). This can result in patients feeling unsure how to broach and describe sexual issues for the first time. Health care providers may also be reluctant to discuss sexual functioning due to time limitations, lack of knowledge regarding treatment for sexual problems, and beliefs about the appropriateness of discussing sexuality within the context of cancer treatment (1).

Recent studies have identified issues related to patient-provider communication for patients with many types of cancer, including colorectal cancer. For example, Flynn and colleagues found that in a survey of 819 patients with cancer and cancer survivors, 78% of participants felt that it was important to discuss how cancer may impact sexual functioning and 64% believed that it was helpful to include partners in discussions with providers about their sex life (5). However, only 29% of participants reported that they had asked their health care provider about problems with their sex life, and 45% reported that they had never received any information from their providers about how cancer or cancer treatment may affect their sexual functioning. Although most patients (59%) who did not ask their providers about sexual problems reported that they did not have any questions, 21% felt that the problems with their sex life were "not bad enough" to discuss, and 9% reported that they felt too shy or embarrassed to bring up the topic. Focus group participants in that study reported that it would be helpful for the oncology provider to initiate discussions about sexual problems (5).

A recent qualitative study of patients (n=21), their partners (n=9), and their health care providers (n=10) assessed sexual health needs for colorectal cancer survivors from their perspective (1). This study sought to identify potential barriers and facilitating factors to communication about sexual functioning through a combination of focus groups and questionnaires. As with the Flynn *et al.* study, participants in this study were not always able to recall if they had received information about sexual functioning after treatment. Patient/partner knowledge about the availability of treatment for sexual problems was also limited (1). The patients and partners noted that having more information about potential sexual problems and heath care options may have facilitated further discussion about sexual functioning with their health care providers. The patients and partners also noted that they felt embarrassed to bring up sexuality with their providers, and many felt that it was inappropriate to discuss sexual problems if the treatment goal was patient survival.

Traa and colleagues reported that health care providers identified a number of barriers to providing adequate sexual health care including knowledge and competence in the area of sexuality, beliefs about sexuality, and attitudes towards discussing sexuality (1). In the Traa *et al.* study, most providers noted that they did not feel sufficiently prepared to have detailed discussions about sexuality or did not consider it to be within the scope of their care. Health care providers echoed some of the same concerns expressed by patients/partners by noting that as providers they felt it was inappropriate to discuss sexuality if the main treatment goal was survival. They also expressed concern that the potential for causing discomfort or embarrassment for the patient and/or their family members might have an adverse effect on overall treatment.

Additionally, sexuality may be considered "irrelevant" for certain patients due to their age, gender, or relationship status (1,10). For example, health care providers may consider elderly or widowed patients as having less sexual health care needs. This is similar to prior studies of communication regarding sexuality in primary care settings that have identified cultural factors (e.g., gender, age, race/ethnicity, or sexual orientation differences) as barriers to openly discussing sexual functioning (22).

Improving communication about sexual dysfunction in survivorship care

Given the high rate of reported sexual dysfunction among colorectal cancer survivors and the limited patient-provider

Table 2 Interviews and self-report questionnaires to assess sexual functioning

Questionnaire	Description
CSFQ	35-item clinician-administered questionnaire for males and females that assesses medication-related changes in sexual function in the following domains: sexual pleasure, sexual desire, arousal, and orgasm
DIFS	25-item clinician-administered questionnaire for males and females that assesses sexual cognition, sexual behavior, orgasm, and sexual drive
SIDI-F	13-item clinician-administered questionnaire for female to assess symptoms of hypoactive sexual desire
IIEF	15-item self-report questionnaire for men to assess erectile function, intercourse satisfaction, orgasmic function, sexual desire and overall satisfaction with sexual functioning
FSFI	19-item self-report questionnaire for women to assess sexual desire, arousal, lubrication, orgasm, satisfaction, and pain

CSFQ, Changes in Sexual Functioning Questionnaire (27); DIFS, Derogatis Interview for Sexual Functioning (28); SIDI-F, Sexual Interest and Desire Inventory (29); IIEF, International Index of Erectile Functioning (30); FSFI, Female Sexual Function Index (31).

communication about sexual functioning in oncology settings, there is a need to address barriers to sexual functioning discussions in order to improve the quality of life aspect of survivorship care. Althof & Parish have identified a number of patient-centered communication skills that may help providers to improve interactions regarding sexual functioning, while taking into consideration the time constraints of appointment time (24). For example, using a combination of clinical interview and questionnaire techniques can help to screen for potential sexual problems, gather information about patients' sexual functioning, and help patients to feel more comfortable addressing questions about sexual functioning with their provider (24).

A number of assessment tools related to sexuality have been developed that can help providers to gather data about sexual functioning quickly prior to meeting with patients (*Table 2*). Although self-report questionnaires are not sufficient to fully evaluate a patient's symptoms of sexual dysfunction, they can provide a useful way to begin conversations about sexuality. Often survivors who have not brought up sexual issue concerns with providers will disclose those concerns on a self-report symptom list (22). Using open-ended questions to help patients to elaborate on their sexual functioning concerns can help to clarify sexual problems and identify potential areas for treatment. Open-ended questions can be particularly helpful in eliciting information about how sexual problems are impacting patient functioning (24).

Overall, sexual dysfunction is prevalent among colorectal cancer survivors and an important aspect of quality of life for health care providers to consider. Despite patients' report of the importance of discussing sexuality with their providers, it is often not addressed during appointments. In order to improve patient-provider communication regarding sexual functioning, the following recommendations may be helpful to consider:

Recommendations for providers:

(I) "*As part of clinical practice, screening and assessment of sexual functioning should be included early in treatment for all patients and continue during all stages of care*" (22). Regardless of age, sexual orientation, or partner status, sexual functioning is an important aspect of the quality of life for all patients that should be made part of clinical practice with assessments being done frequently and continuing during all stages of treatment. As recommended by Althof & Parish, a combination of physical examination, clinical interview, and questionnaires may help to improve assessment, engage patients in a conversation that they may be reluctant to initiate, and, as appropriate, elicit patient concerns (24). Even if patients do not report changes in sexual functioning after treatment has been completed, it is important to re-assess the patients as they may experience delayed onset of sexual problems after treatment or develop new problems over time;

(II) "*Patients may be reluctant to raise the topic of sexual functioning during appointments. Initiating conversations about sexual functioning as part of standard clinical care can help to facilitate discussions about these issues*". Patients consistently state that they feel more comfortable if providers bring up the topic of sexual functioning (5). Asking permission to discuss sexuality may help patients

Table 3 Web based resources on sexuality and cancer
American Association of Sexuality Educators, Counselors, and Therapists: www.aasect.org
National Institutes of Health, National Institute on Aging: www.nia.nih.gov: "Health Information" for Sexuality in Later Life brochure
Wound, Ostomy and Continence Nurses Society: www.wocn.org: locate a certified WOC Nurse near you
American Cancer Society: www.cancer.org: pamphlets about sexuality and cancer
American Cancer Society United Ostomy Association, Inc: www.ostomy.org
Fertile Hope: www.livestrong.org/we-can-help/fertility/risks/ for information re: risk of infertility
Mary-Helen Mautner Project for Lesbians with Cancer: Mautnerproject.org
American Psychosocial Oncology Society: www.apos-society.org

WOC, wound, ostomy and continence.

to feel more comfortable answering questions about their current functioning and provide them with language to help them describe and report their symptoms. Involving sexual partners in these discussions (with patient permission) may also help to facilitate a more open dialogue among patients, partners, and providers throughout treatment;

(III) "*Maintain referral resources and information regarding treatment options for sexual dysfunction for patients and their partners*". Health care providers report that lack of knowledge about treatment options and concerns about treating sexual dysfunction within their scope of practice may limit their ability to discuss these issues with patients (1). In multidisciplinary care settings, it may be possible to consult with another provider with expertise in sexual functioning in the event that a practitioner's knowledge and skill sets are limited in this area (32). If these options for referral are not available, being aware of local external referral sources for treatment of sexual dysfunction can also facilitate further treatment for patients. There are also a number of patient resources that may provider valuable information about sexual dysfunction and help patients to make informed decisions about seeking treatment for sexual problems (*Table 3*).

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Footnote

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

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Diet and supplements and their impact on colorectal cancer

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Background: Colorectal cancer is the third commonest cancer and the third leading cause of cancer death among men and women. It has been proposed that dietary factors are responsible for 70-90% of colorectal cancer and diet optimization may prevent most cases.

Aim: To evaluate the role of dietary components and supplements in colorectal cancer.

Methods: Bibliographical searches were performed in Pubmed for the terms "diet and colorectal cancer", "diet and colon cancer", "diet and rectal cancer", "nutrition and colorectal cancer", "probiotics and colorectal cancer", "prebiotics and colorectal cancer", "alcohol and cancer" and "colorectal cancer epidemiology".

Results: Consumption of processed or red meat, especially when cooked at high temperatures may be associated with increased risk of colorectal cancer. The evidence for dietary fibre is unclear but foods that contain high amounts of fibre are usually rich in polyphenols which have been shown to alter molecular processes that can encourage colorectal carcinogenesis. Meta-analyses provide evidence on the benefits of circulating, diet-derived and supplemented, vitamin D and Calcium. We also found that diets rich in Folate may prevent colorectal carcinoma. The evidence on dietary micronutrients such as Zinc and Selenium in association with colorectal cancer is not conclusive. It has been suggested that there may be a direct association between alcohol intake and colorectal cancer. *In vitro* and *in vivo* studies have highlighted a possible protective role of prebiotics and probiotics.

Conclusions: The lack of randomized trials and the presence of confounding factors including smoking, physical activity, obesity and diabetes may often yield inconclusive results. Carefully designed randomized trials are recommended.

Keywords: Colorectal cancer; nutrition; diet; carcinogenesis

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Introduction

Before the twentieth century, colorectal cancer was relatively uncommon however the incidence has risen dramatically especially in the last fifty years. Several risk factors have been proposed including the adoption of westernized diet, obesity and physical inactivity (1,2). The majority of colorectal cancer continues to occur in industrialized countries. It has been estimated that nutrition could account for more than one third of cancer deaths (3), and that dietary factors are responsible for 70% to 90% of all cases. Therefore, diet optimization could potentially help reduce the incidence of this type of malignancy (4,5). Here we review the key evidence for the role of different dietary components and their effect on colorectal cancer prevention and progression.

Methods

Bibliographical searches were performed in Pubmed for the terms "diet and colorectal cancer", "diet and colon cancer", "diet and rectal cancer", "nutrition and colorectal cancer", "probiotics and colorectal cancer", "prebiotics and colorectal cancer", "alcohol and cancer" and "colorectal cancer epidemiology". The search was performed for the period 1980-2012. As expected, the search yielded an overwhelming abundance of evidence on the association between diet and colorectal cancer. For each type of nutrient/chemical compound we excluded most *in vitro* and animal studies and the remaining results were categorized into different levels of evidence (6) focusing on meta-analyses, systematic reviews and randomized controlled

trials where available. Information on ongoing clinical trials was sourced from the URL: *http://clinicaltrials.gov/*.

Results

Red meat

Red meat might be directly linked to the incidence of colorectal cancer or indirectly because diets high in meat may be deficient of other dietary components such as fibre and polyphenols from fruit and vegetables. Cooking meat at high temperatures may lead to the formation of mutagenic and carcinogenic heterocyclic amines through the interaction of muscle creatinine with amino acids (7) as well as the formation of N-nitroso compounds (8). Frying, grilling, broiling or cooking on coal can potentially induce these changes. Haem in meat can act as a nitrosating agent promoting the formation of N-nitroso compounds. Darker meats are more abundant in haem than white meats and therefore, high consumption of red meat (beef, pork, or lamb) could increase the risk of colorectal cancer (9-13). Haem iron has been positively associated in the literature with the development of colonic polyps (14), adenomas (15) and colorectal cancer (16-18). Other studies including the Nurses' Health Study did not show such association (19-21). Furthermore, colorectal carcinogenesis could involve the secretion of insulin as a response to red and processed meats and thus subsequent activation of insulin and insulin growth factor-1 receptors, may lead to increased cell proliferation and reduced apoptosis (22).

The association of total or red meat cooked at high temperatures and increased risk of colorectal cancer has been shown in some case-control studies (23-25) but not in others (26). High consumption of red meat such as beef, pork, or lamb was associated with increased risk of colorectal cancer in both men and women in cohort studies (27,28). Data from the Health Professionals Follow-up study (HPFS) cohort showed a three-fold increase risk of colon cancer in subjects who consumed red meat more than five times in a week (29). Furthermore, it showed an increased risk of developing distal colon adenoma.

A meta-analysis from 2002 by Norat *et al*. showed a 33% increased risk of colorectal cancer in people consuming higher levels of red and processed meat (30). A systematic review of prospective studies by Sandhu *et al*. determined that an increase of 100 g in daily consumption of all meat or red meat was associated with a 12-17% increase in risk of colorectal cancer (31). However contrary to this, a prospective cohort study of 45,496 women by the National Cancer Institute (32), showed no association between consumption of red meat, processed meat, or well-cooked meat and colorectal cancer risk. Other studies have also been unable to support a role of fresh meat and dietary fat in the etiology of colon cancer (28,33).

In 2007, the research 'Expert Report' of the second world cancer research fund/American research concluded that intake of red and processed meat increases the risk of colorectal cancer (34), however, more recent reviews of prospective epidemiological studies found that there is not enough epidemiological evidence to link red and processed meat with colorectal cancer (35,36). A recent meta-analysis of prospective studies by Chan *et al*. concluded that processed and red meat is associated with increased risk of colorectal cancer, and a linear increase in risk was reported for intake of red and processed meats up to 140 g/day.

Fish and poultry are alternative sources of protein and have been shown to reduce the risk of colon cancer and adenoma (27,28,37-45). Possible mechanisms may involve more efficient methylation due to high methionine content in these foods or the presence of n-3 polyunsaturated fatty acids (PUFA), especially from oily fish.

In summary, performing studies on diet is complex with so many variables and confounding factors. Overall, there is evidence from both case-control and cohort studies that consumption of processed or red meat, especially when cooked at high temperatures by methods such as frying, grilling or broiling, is associated with increased risk of colorectal cancer. The dose-response relationship as well as the gender differences need to be investigated further. A determined diet might suggest limitation or avoidance of red or processed meats and support the consumption of white meat and fish.

Fat

Several case- control studies have demonstrated an increase in the risk of colorectal cancer with increased total energy intake (46-48). Dietary lipids provide a rich source of energy and diets high in lipids, especially animal fat, may increase the risk of colorectal cancer (49,50). In contrast to this, there are large cohort studies that do not support an effect of dietary fat on colon cancer (51,52). Different types of fats may play different roles in colorectal carcinogenesis via different mechanisms such as upregulation of apoptosis, inhibition of interleukin 1 and tumour necrosis factor -α synthesis, COX-2 inhibition and modulation of the redox enviroment in the colonocytes (53,54).

Saturated fat

Saturated fats are principally found in animal products including red meat and dairy products, such as cheese and butter. Coconut oil, coconut milk, palm oil, and cocoa butter are all sources of plant-derived saturated fats. Case-control (55) and prospective cohort (27) studies demonstrated an increase in risk of colorectal cancer in people with higher consumption of saturated fat but confounding factors in the food matrix such as red meat and reduced intake of dietary fibre always pose a challenge for researchers.

A prospective study of 88,751 women confirmed that high intake of animal fat increases the risk of colon cancer and supports substitution of red meat as a source of protein with fish or chicken (27). The results of the Dietary Approaches to Stop Hypertension Diet (DASH) study of 130,000 participants found a 20% relative risk reduction in patients who consumed lower levels of animal fat (56). In a meta-analysis, Alexander *et al*. found no independent association between animal fat intake and the risk for colorectal cancer (33). The Women's Health Initiative Dietary Modification Trial was a randomized controlled trial, which showed that low-fat dietary pattern did not reduce the incidence of invasive colorectal cancer (57).

The advice to reduce intake of saturated fat in order to reduce the risk of colorectal cancer remains only suggestive due to the lack of consistency from clinical studies.

Omega-3 (n-3) PUFA

Epidemiological studies and populations consuming large numbers of polyunsaturated fish oils have been found to have lower rates of colon cancer (58). This has led to the hypothesis that diets high in n-3 fatty acids may reduce the risk of colorectal cancer. An inverse association between n-3 PUFA (omega-3) and colorectal cancer has been shown in case-control (45,59,60) and prospective studies (61,62). On the contrary, Daniel *et al*. reported that one of the major dietary sources of omega-3 fatty acids, alpha-linolenic acid, was associated with increased risk of colorectal cancer in women and that omega-6 intake was inversely related to colorectal cancer risk in men (63). In their cohort, Sasazuki *et al*. found no evidence that omega-6 acids increased the risk.

Fatty fish are an excellent source of omega-3 fatty acids and vitamin D. Butler *et al*. showed that dietary marine n-3 PUFAs were positively associated with advanced colorectal cancer (64) while other studies suggested the opposite (39-42,62,65). A Chinese meta-analysis of prospective studies of nearly half a million individuals did not show any protective properties effect of n-3 fatty acids on colorectal cancer risk (66). A recent meta-analysis of case-control and prospective cohort studies suggested that fish consumption decreased the risk of colorectal cancer by 12%. However, the results showed a less profound effect on colonic as opposed to rectal cancers and highlighted differences between case-control and cohort studies (67). Omega-3 fatty acids may be taken as food supplements however there is very limited data available in association to colorectal cancer. Skeie *et al*. showed that cod-liver oil consumption lowers risk of death in patients with solid tumours without significant results on colorectal cancer risk (68). In fact, a systematic review of 20 prospective cohort studies found that dietary supplementation with omega-3 fatty acids is unlikely to prevent cancer (69).

The evidence to suggest that consumption of diets high in omega-3 PUFAs may prevent colorectal cancer is limited and in many cases contradictory. This includes not only n-3 fatty acids derived from fish but also from other sources such as α-Linolenic acid from food sources including rapeseed, soybeans, walnuts, flaxseed and olive oil. The evidence to suggest supplementation of omega-3 PUFAs with cod-liver oil is non-conclusive.

Dietary fibre, fruit and vegetable

The hypothesis that high fibre consumption may be reducing the risk of colorectal cancer has been postulated following the observation of the low incidence of colorectal cancer in African populations that consume a high-fiber diet (70). Fibre is defined as heterogeneous plant material composed of cellulose, hemicellulose and pectin. It has been proposed to work by reducing faecal transit times, diluting and binding carcinogens, altering the proliferation of gastrointestinal epithelium, maintaining colorectal epithelial cell integrity (71), adsorbing heterocyclic amines (72) affecting bile acid metabolism, and stimulating bacterial anaerobic fermentation to increase the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. SCFAs have been shown to decrease colonic pH and inhibit carcinogenesis (73).

Colorectal adenomas are the precursors of most colorectal cancers. The effect of diet in relation to colorectal adenomas and adenoma recurrence was explored in several studies. Diets high in wheat bran (74), fruit and vegetables (49,75), citrus fruits (19), cruciferous vegetables (76), dark-green vegetables and onions garlic (77) and tomatoes (23) may confer protection against colorectal adenomas and subsequently colorectal carcinoma. Some prospective studies did not show this association (74,75).

Early meta-analyses of case-control studies have generally shown a protective association between fibre

and colorectal cancer (78,79). In one study, high fibre diet was associated with decreased survival (80). Cohort studies yielded mixed results often showing none or a weak inverse association between dietary fiber and risk of colorectal cancer (19,28,37,38). Data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial and the Swedish mammography cohort study showed reduced risk of colorectal cancer and colorectal adenomas among people who consumed the highest amounts of fibre particularly from grains fruits and vegetable (81-85). However, in a meta-analysis of prospective studies, Park *et al*. suggested that high dietary fiber intake was actually not associated with a reduced risk of colorectal cancer (86). In a recent meta-analysis of prospective cohort and nested case-control studies of dietary fibre the authors suggest a 10% reduction in risk of colorectal cancer for each 10 g/day intake of total dietary fibre and cereal fibre (87). Whole grain was also associated inversely (87). Other studies, did not suggest a protective association with specific subtypes of fibre such as fruit, vegetable or cereal (27,88,89).

One can conclude that the evidence for fibre is unclear in terms of benefit in reducing colonic adenoma pathway and colorectal cancer formation. There are discrepancices between case-control and prospective cohort studies for reasons such as recall bias, selection bias and sample size. The general health benefits of fibre which may pertain to a variety of cancers as well as the other benefits to the colon such as diverticulosis and constipation suggests that a high fibre diet including wheat bran, cereal, whole grain, citrus fruits, cruciferous vegetables, dark-green vegetables, onions, garlic and tomatoes may be recommended.

Folic acid/folate (vitamin B9)

These are water-soluble vitamins found in fruits, dark green vegetables and dried beans. Humans are not able to synthesize this vitamin, which has to come from dietary sources. The bioavailability of folic acid is higher than folate because it is non-conjugated and hence more stable. Several mechanisms have been suggested for its role as a preventer of carcinogenesis through molecular mechanisms such as DNA synthesis, repair and methylation (90,91).

The observation that folic acid supplementation was associated with a substantial decrease in colon cancer among patients with ulcerative colitis led researchers to examine the role of folic acid in the prevention of colorectal cancer (92). Observational studies highlighted that deficiency of dietary folate correlates with increased occurrence of colorectal neoplasia (93) but may protect against cancer risk or adenoma formation only in those patients with low folate baseline (94). Examination of the data from the Nurses' Health Study (NHS) and the HPFS, showed that high intake of dietary folate was inversely associated with risk of colorectal adenomas (95). A few years later, using data from the NHS cohort, the same group were able to show a considerably lower risk of colon cancer among women who used multivitamins containing 400 μg of folate (96). This was also confirmed in other populations such as the Cancer Prevention Study II cohort (97). A large scale meta-analysis of prospective studies supported the hypothesis that folate has a small protective effect against colorectal cancer (98). Manson *et al*. showed dietary folate supplementation maybe responsible for reduction of incidence of colorectal cancer in the US and Canada (99), however, Giovanucci *et al*. showed how dietary folate reduced risk of colorectal cancer or adenoma but not when folate came from supplements (100). Giovanucci suggested that folate supplementation could be associated with higher risk of adenoma recurrence and may even be harmful to patients with a previous history of colon cancer (100). A randomized secondary prevention trial reported that folate supplements increased the risk of recurrent advanced adenomas or recurrent adenomas (93).

In conclusion, diets rich in folate may prevent colorectal carcinoma. Further studies are required in order to assess the role of supplemented folate and the reported risks of adenoma recurrence.

Alcohol

The mechanism by which alcohol might be linked to carcinogenesis is unknown but proposed pathways include its ability to reduce folate (101), promote abnormal DNA methylation (102), delay DNA repair, alter the composition of bile salts or induce Cytochrome p450 to activate carcinogens (103).

A large number studies have suggested an association between alcohol intake and colonic adenoma as well as colorectal cancer risk (104-106). Intake of 30 grams of alcohol per day is associated with increased risk of colorectal cancer compared to low intake. Giovannucci *et al*. showed that men in HPFS cohort who drank more than two drinks of alcohol per day had a 2-fold higher risk of colon cancer (107) compared to men who drank fewer than 0.25 drinks per day. Heavy drinkers were found to have a higher risk of colorectal adenoma. Data from the NHS and EPIC cohorts (95,104) showed similar findings. A meta-analysis of five

large cohort studies showed similar results for both men and women (108). This risk may be directly related to alcohol or to the effects of alcohol on folate levels. In fact, women with low serum folate levels who consumed large amounts of alcohol, had a greater risk of colorectal cancer (109). Two other studies found no association of total alcohol consumption with all-cause mortality in colorectal (110) and colon cancer (111) and Zell *et al*. reported a lower risk of all-cause mortality when subjects consumed wine regularly as opposed to infrequently (112). Consumption of red wine can be beneficial but the protective role could be allocated to polyphenols rather than its alcohol content (113).

In conclusion, currently the literature would suggest minimizing alcohol intake as a means to reduce the risk of developing colorectal cancer or colorectal adenoma. A consumption of less than 30 g per day as well as folate supplementation is recommended in people who consume alcohol regularly.

Calcium and vitamin D

Vitamin D is one of the fat-soluble vitamins and more than 90% is synthesized endogenously from skin exposure to UV sunlight (114). The remaining comes from the diet as pro-vitamin cholecalciferol (D3), which is found naturally in oily saltwater fish, egg yolks and livers and from the plant-derived pro-vitamin ergocalciferol (D2) found in foods such as mushrooms. Food fortification may provide an extra source of vitamin D. The active form of vitamin D is 1,25-dihydroxyvitamin D_3 (Calcitriol) which is formed by hydroxylating the pro-vitamins in the liver and kidneys. The use of Calcitriol in experimental studies has been shown to induce differentiation and inhibition of tumour cell proliferation of various types of cancer cells, however, its use is limited due to development of toxic hypercalcaemia. For this reasons, calcitriol analogues are usually used (115,116). Vitamin D and calcium are thought to exert their protective effects by decreasing cell proliferation, inhibiting angiogenesis, stimulating apoptosis and promoting cell differentiation (117). Other proposed mechanisms may involve binding of calcium to bile acids and ionized fatty acids to form insoluble soaps in the lumen of the colon (118,119).

Garland *et al*. proposed that lower levels of vitamin D could account for the increase in mortality from colon cancer in higher latitudes (120) and epidemiological studies showed that deaths from colorectal cancer have been found to be higher in areas with less sunlight (121). Populations consuming higher amounts of fresh fish, shellfish, calcium and vitamin D have lower incidence of colorectal cancer (122) and may even have the lowest incidence of both colon and rectal cancer in Europe and North America (123).

Data from case-control studies are inconsistent. The protective effects of calcium alone were demonstrated in some case-control studies (124) but not in others (125). Case-control studies involving only women showed reduced risk of colorectal cancer (126,127). This was not demonstrated in studies involving both men and women (128). No significant inverse association was observed between calcium and vitamin D levels and the risk of colorectal cancer (125,128). The Women's health initiative study was a randomized controlled trial, which showed that daily supplementation of calcium with vitamin D for seven years, had no effect on the incidence of colorectal cancer among postmenopausal women (129). In terms of Vitamin D levels, a meta-analysis by Garland *et al*. found an inverse association between circulating levels of 25- hydroxyvitamin D_3 and the risk of colorectal cancer (130).

Calcium was found to have protective effect on colorectal cancer risk in some prospective studies (131-133) but not in others (134,135). Data from the HPFS and NHS cohorts showed that total, dietary and supplemented calcium reduced the risk of distal colon but not proximal cancer. Most of the risk reduction was achieved by calcium intake of 700-800 mg/day. A meta analysis of 10 cohort studies showed 22% reduction in the risk of colorectal cancer in those with higher intake of calcium (136).

Regarding colorectal polyps, a three-year intervention study with calcium and antioxidants, found no effect on polyp growth but possibly a protective role against adenoma formation (137). Higher intake of calcium alone (138) or when combined with Vitamin D (139) was found to be protective against adenoma recurrence.

In conclusion, data from case-control studies are inconsistent but cohort studies and meta-analyses provide evidence on the benefits of circulating, diet-derived and supplemented vitamin D and calcium. Further studies are needed to ascertain whether there is any sex predilection. On the basis of current evidence one could suggest intake of vitamin D at a dose of 1,000 IU per day which is regarded as safe, and attaining calcium intakes of 700-800 mg per day. Modest duration of sunlight exposure should be sought to raise levels of 25-hydroxyvitamin D_3. Diets rich in oily fish, shellfish, milk and dairy products contain high amounts of calcium and vitamin D.

Polyphenols

Polyphenols are a class of chemicals known for their

numerous benefits especially their antioxidant effects (113,140,141), inhibition of cellular proliferation (142), induction of cell cycle arrest (143), interaction with apoptotic pathways and antiangiogenic and antimetastatic properties (144). They are divided in five classes; flavonoids, phenolic acids, ligans, stillbenes and others. The most important dietary sources of polyphenols are fruits, vegetables, seeds, and beverages such as fruit juice, green tea, coffee, cocoa drinks, red wine, and beer. The chemoprotective role of polyphenols against cancer has been extensively studied. Evidence from case-control studies (145), cell culture and animal studies have shown a protective role against colorectal malignancy (145,146).

Curcumin

This polyphenol is a curcuminoid found in turmeric spice that has antioxidant, anti-inflammatory and anti-tumour properties (147,148). Curcumin has been shown to work by inhibiting cell invasion (149) and by having anti-inflammatory properties (150). It has been shown to reduce the number and size of ileal and rectal adenomas in patients with familial adenomatous polyposis (151).

Flavonoids

Apigenin is a flavonoid found in parsley and celery and it has been shown to inhibit colonic carcinogenesis by inducing apoptosis in animal models (152). Cyanidin, a flavonoid in strawberries and cherries has been studied *in vitro* and in animal models and has also been shown to inhibit colonic carcninogenesis (153). Other flavonoids with similar properties include Delphinidin which is found in dark fruit (154) and Genistein which is abundant in Soy beans (155). Quercetin from onions, broccoli and apples has been shown to decrease cell growth by interacting with β-catenin (156) and by induction of apoptosis (157). Citrus fruits contain high levels 5-hydroxy-6,7,8,4'-tetramethoxyflavone and Naringenin which has been shown to induce apoptosis and cell-cycle arrest of luminal surface colonocytes (158,159).

Green tea

Green tea is rich in a type of Flavonoids, the Flavonols. Examples include Catechin and Epicatechin. Epigallocatechin-3-gallate (EGCG) is the most abundant Catechin in green tea. The benefits have not only been shown *in vitro* and animal models (113,160-163) but also in large population studies. Consumption of green tea has been associated with a 40% reduction in colorectal cancer risk in a cohort of 69,710 Chinese women (163).

Coffee

Coffee is a complex blend of hundred of chemicals including anti-oxidants, mutagenic, and anti-mutagenic compounds (164). Additionally, it has been shown to affect gastrointestinal physiology such as stimulating a motor response of the distal colon, reducing faecal transit times and reducing the gut's exposure to potentially carcinogenic faecal load (165). Over the last few decades the relationship between coffee and colorectal cancer has been extensively explored (166,167). Outcomes from clinical studies have been inconsistent and no firm guidance has been suggested. Several meta-analyses of cohort and case-control studies found that substantial consumption of coffee is associated with lower risk of colorectal cancer (168-170). Other meta-analyses failed to reconfirm this inverse association (171). Li *et al.* examined the results of 25 case-control studies and 16 cohort studies in the most recent meta-analysis of the literature. Subgroup analysis of case-control results found a significant decrease in cancer risk, especially in Europe and for females. A subgroup analysis of cohort studies, showed a lower risk of colon cancer in Asian women only (172).

There are inconsistencies between case-control and prospective studies as well as noted differences between sex and race. Consumption of coffee maybe protective against colorectal cancer but further studies are required to establish a dose-risk relationship and further clarify whether there is any sex predilection in the risk.

Other phytochemicals

Natural phenols

These molecules are smaller in size than polyphenols. Examples include Resveratol which is found in the skin of grapes and red wine and has been shown to inhibit metastasis by reducing hypoxia inducible factor-1α and MMP-9 expression in colonocytes (173) as well as inhibiting Wnt signalling and β-catenin localisation (174).

Carotenoids

Carotenoids are naturally occuring pigments some of which can be converted by the body into vitamin A. Examples include β-carotene which is found in carrots, red palm oil and pumkin. Lycopene is another example of pigmented phytochemical found in tomatoes, watermelons, papaya, apricots and citrus fruit. They have been found to exhibit anti-oxidant, anti-proliferative and anti-inflammatory properties (175-177).

Isothiocyanates

These are Sulphur-containing phytochemicals found in

abundance in cabbage, turnips, broccoli, kale, cauliflower, watercress, brussel sprouts, mustard seeds and horseradish. They have been found to possess chemopreventative activity (178-180) against colonic cancer.

Overall, diets high in polyphenols and other phytochemicals such as carotenoids, isothiocyanates and natural phenols have been shown to be protective against colorectal cancer. Foods rich in these compounds includes spices such as mustard seeds and tumeric, fruits including strawberries, cherries, apples, citrus fruit, grapes, watermelons, papaya, apricot and vegetables such as onions, brocolli, carrots, red palm oil, pumkin, leafy green vegetables and tomatoes. Consumption of green tea may also be beneficial.

Zinc

Animal models have shown that low zinc levels may be associated with preneoplastic lesions and colonic carcinogenesis (181). *In vitro* studies suggested that Zinc supplementation may positively influence tumour cell response to anticancer drugs by altering colonic cancer cell gene expression (182). In the Iowa Women's Health Study, intake of dietary zinc was associated with a decreased risk of both proximal and distal colon cancer (18). A more recent prospective study by Zhang *et al*. did not find a role for Zinc intake with colorectal cancer risk but the authors highlighted a possible inverse association between dietary zinc and rectal cancer in women (183). Therefore, no substantive evidence is available for dietary Zinc intake however the putative inverse association in women needs to be explored further.

Selenium

An inverse association between Selenium supplementation and the risk of colorectal cancer was observed in several studies (184-189). Selenium supplementation by way of brewer's yeast supplementation was associated with up to 50% reduction in the incidence of colorectal cancer (188,190). Other studies contradict these findings and show no significant associations (191-192). Therefore, studies do not currently provide evidence for Selenium supplementation.

Gut microbiota

The colon contains more bioactive cells than the rest of the body (193). Inulin-type fructants are oligosaccharides obtained through diet and 90% of them are effectively metabolized by endogenous colonic microbiota into gases and organic acids including short chain fatty acids (SCFAs) (194). Animal-model experiments showed that these oligofructants can reduce the numbers of aberrant crypt foci (195) and influence the activity of natural killer cells and production of IL-10 (196). Naturally-occurring oligofructants can be found in foods such as onions, Jerusalem artichokes, garlic, asparagus and chicory. Examples of SCFAs include acetic and butyric acid. SCFAs have been shown to reduce tumourgenesis (197) and proposed mechanisms include promotion of the growth of probiotic *Lactobacilli* species which maintain epithelial health and downregulate the inflammatory response (198). As *Bifidobacteria* and *Lactobacilli* are selectively stimulated to grow, this may happen at the expense of pathogenic bacteria (199). Other benefits of microbiota include synthesis of vitamins such as folate (200). In human trials synbiotics were found to decrease DNA damage in colonic mucosa and lower the level of colonic proliferation (201). Low proliferation is a recognized marker of low colonic cancer risk (202).

Other components in our diet may affect the gut microbiota and influence colorectal oncogenesis. Gut microbiota hydrolyse polyphenols to a great extend affecting the amount of these chemicals being absorbed, thus, ameliorating their protective properties. Excess fat in the diet means that more bile will be produced and more bile acids will escape the enterohepatic circulation. In the colon, these can be metabolized to mutagenic components (203). High butyrate levels are known to protect against the mutagenic effects of bile acids (204). Moreover, *Lactobacilli* have been shown to directly reduce the mutagenic properties in bile acids (205). As mentioned above, meat cooked at high temperatures contains high levels of heterocyclic amines which have been found to be fermented by gut microbiota. The byproducts of this process can damage DNA and increase the risk of colorectal cancer (206).

There is a completed Phase 2 trial assessing the role of probiotics on gut microbiotca and colorectal cancer but the results have not been published yet (207). The role of VSL#3 probiotics in rectal cancer is investigated in a phase 3 clinical trial but results are also awaited (208). Currently there is no strong evidence regarding prebiotics and colorectal cancer risk.

Overall, the role of probiotics and prebiotics is not completely clear but *in vitro* and *in vivo* studies have highlighted a possible protective role of gut microbiota in colorectal carcinogenesis. There appears to be benefit from a diet high in oligofructant-containing foods including

onions, jerusalem artichokes, garlic, asparagus and chicory.

Lifestyle

Apart from alcohol and smoking (38), other lifestyle factors have also been associated with the risk of developing colorectal cancer. Higher levels of physical activity have been reported to reduce risk by up to 40% and several studies have reported adverse outcomes in patients who are obese (209-211), suffer from diabetes (209,212) or use the oral contraceptive pill (213). Non-modifiable factors which may increase the risk include higher body height (38,214), post-menopausal status (213,215) and endogenous oestrogen exposure (215).

Discussion/conclusions

There is an abundance of evidence in the literature on the role of nutrition on colorectal carcinogenesis. Often the evidence may be inconclusive due to the lack of randomized trials and because many studies have been overwhelmed by confounding factors such as smoking status, physical activity, obesity and diabetes. Many studies were influenced by possible recall and selection biases, which make it difficult to draw solid conclusions. In this review, we set out to identify nutritional factors that could play a role in the development of colorectal cancer. Red or processed meats especially when cooked at high temperatures should be limited and can be replaced by the consumption of white meat and fish. Diets high in n-3 fatty acids, dietary fibre, folate, vitamin D, calcium and polyphenols may protect against colorectal cancer and colorectal adenoma formation. The consumption of alcohol is not advocated. The role of probiotics and prebiotics is not completely clear but *in vitro* and *in vivo* studies have highlighted a possible protective role of gut microbiota in colorectal carcinogenesis.

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Footnote

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Psychosocial issues in colorectal cancer survivorship: the top ten questions patients may not be asking

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Abstract: Advances in colorectal cancer screening and treatment have increased survivorship significantly in recent years. This has led to an increased emphasis on the need for continuing patient care long after cancer treatment is completed. Colorectal cancer survivors may face a number of psychosocial issues following treatment, including cancer-related distress, adjustment to physical changes following treatment, and challenges related to returning to work. Although there are many resources available to assist with these challenges, many patients may not seek this information from their providers during follow-up care visits. This article highlights some of the most common patient concerns related to survivorship in colorectal cancer and serves as a reminder to ask about these concerns throughout the course of treatment and follow-up care.

Keywords: Colorectal cancer; psychosocial factors; cancer survivorship

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Introduction

Advances in colorectal cancer screening and treatment have increased survivorship significantly in recent years, with the 5-year survival rates for all colorectal cancer patients estimated to be between 65-66% (1). Cancer survivors face a number of psychosocial challenges including sleep difficulties, pain, changes in sexual functioning, fear of cancer recurrence, financial hardship, and impaired quality of life (QOL) (2-4). While more and more resources are available for colorectal cancer patients to manage psychosocial issues related to survivorship, many patients may not feel comfortable initiating conversations with providers about these concerns. The following questions provide a summary of some of the most common patient concerns related to colorectal cancer survivorship and helpful resources that can help patients and providers manage these psychosocial issues (see *Table 1* for full list of resources).

How long will my cancer-related distress last?

Survivors of cancer have a higher risk of developing anxiety, depression, and post-traumatic stress disorder (PTSD) (5,6). In colorectal cancer survivors, the prevalence of depression and anxiety symptoms appears to be closely related to physical functioning, financial concerns, cognitive functioning, lack of social support, and concerns about cancer recurrence (7,8). Patients who are married or in long-term relationships and those who are physically active tend to report lower levels of anxiety and psychosocial distress (4,9). With these predictors and protective factors in mind, screening for survivors of colorectal cancer is recommended in order to identify patients who are experiencing clinically significant levels of distress, anxiety, or depression. Discussing these symptoms with physicians early in care also increases the likelihood that patients will report anxiety and depression if they occur later in treatment (10). This allows providers to make appropriate referrals for mental health treatment or additional support if needed.

In terms of screening methods, the National Comprehensive Cancer Network (NCCN) has recommended that Distress Thermometers be implemented to assess level of distress and potential problems areas for patients. A cut-off score of 4 is generally recommended to identify patients who may be in

Table 1 Web-based resources on psychosocial issues related to colorectal cancer

American Association of Sexuality Educators, Counselors, and Therapists (www.aasect.org)	Referral resources and information for sexual dysfunction
American Cancer Society (www.cancer.org)	Survivorship plan resources Local support group listings Treatment information
American College of Sports Medicine (www.acsm.org)	Exercise guidelines for cancer patients Exercise prescription information
American Psychosocial Oncology Society (www.apos-society.org)	Distress screening information Information about treatments for patients Referral resources and helpline
American Society of Clinical Oncology (ASCO) (www.asco.org)	Treatment guidelines Survivorship care recommendations
HRSA Health Literacy Resources (www.hrsa.gov/publichealth/healthliteracy/)	Health literacy information Resources for improving patient-provider communication
LIVESTRONG (www.livestrong.org)	Patient resources Information about the LIVESTRONG at the YMCA program (12-week exercise program for adult cancer survivors)
National Cancer Institute (NCI) (www.cancer.gov)	Survivorship plan resources Information about genetic testing
National Comprehensive Cancer Network (NCCN) (www.nccn.org)	Survivor treatment information Distress screening guidelines
National Sleep Foundation (www.sleepfoundation.org)	Sleep disorder and CBT-I information Referral resources for sleep specialists
United Ostomy Association, Inc. (www.ostomy.org)	Information and support group listings Discussion board
U.S. Equal Employment Opportunity Commission (www.eeoc.gov)	ADA act information Questions about cancer in the workplace

ADA, the Americans with Disabilities Act; CBT-I, cognitive behavioral therapy for insomnia.

need of further resources (11). Other questionnaires such as the Hospital Anxiety and Depression Scale (HADS) can also be useful tools for anxiety and depression screening (11,12).

What will it be like going back to work?

Although approximately two-thirds of cancer patients return to work within 1.5 years after their diagnosis, unemployment rates are significantly higher in survivors of cancer (13). Returning to work after treatment may be beneficial for many colorectal cancer survivors as it can help patients to regain a sense of normalcy and routine, re-establish social support networks with co-workers, reduce financial distress, and increase activity levels during the day (7). However, some patients may have concerns about treatment effects on physical functioning and fatigue and whether or not they will be able to return to their previous jobs or continue to work full-time. Patients may also report a decline in cognitive functioning at their job including memory difficulties, concentration impairment, and decreased ability to multitask (13). Changes in bowel functioning, including constipation and diarrhea, are also associated with delays in returning to work for some patients (14).

A recent review of return-to-work interventions showed that multidisciplinary interventions involving physical, psychological, and vocational components have the highest return-to-work rates (15). Other studies have shown that receiving even brief advice or guidance from a health care provider may be very helpful to patients who are considering a return to work (16). Providers may be able to

help patients more accurately assess their readiness to return to work, improve symptom management in the workplace, and provide guidelines for patients to monitor how they are adjusting to work (15,16). Patients may also benefit from information about legal protection through the Americans with Disabilities Act (ADA) and the Family and Medical Leave Act (FMLA). For example, patients may not be aware of reasonable accommodations that their employers can consider as they return to work including restructuring jobs, offering modified work schedules, employee reassignment, or changes that can make the workplace more accessible.

What do genetic testing results mean for me and my family?

While approximately 75% of patients with colorectal cancer have no evidence of an inherited disorder, the remaining 25% of patients have a family history of colorectal cancer that suggests possible hereditary factors [National Cancer Institute (NCI) (17)]. The genetic mutations that have been identified as being linked to hereditary colorectal cancers only account for about 5-6% of colorectal cases currently, although it is likely that more genetic factors will be discovered in the future. For example, 2-4% of individuals diagnosed with colorectal cancer have Lynch syndrome, which predisposes them to colorectal cancer and other malignancies (18).

Studies estimate that 67% of colorectal cancer survivors are interested in screening for genetic factors (19). Patients considering genetic testing may have a number of questions related to how the results may affect their family members, whether or not insurance will cover the testing, and who may have access to their results in the future. Patients with higher levels of psychosocial distress, lower levels of perceived social support, and escape-avoidant coping styles may be less likely to request screening due to concerns about receiving genetic testing results (20). Referring patients to a genetic counselor to discuss testing options, costs, and implications of testing may help patients decide whether or not to pursue genetic testing as a colorectal cancer survivor. The NCI website also has patient materials available that can provide information about the legal, social and ethical concerns related to genetic testing.

Will my cultural background affect my QOL and care in the future?

Cultural factors including race/ethnicity and socioeconomic status are known to be important predictors of survivorship outcomes, with a disproportionate number of cancer-related deaths occurring among minorities (21,22). Patients in minority groups are more likely to report problems with the coordination of their treatment, access to care, and information about their treatment (21,23). Patients from diverse cultural backgrounds may be reluctant to participate in medical treatment that differ from their own beliefs and traditions, may experience fear and mistrust of healthcare institutions, or may have less experience or knowledge in terms of navigating the healthcare system. These differences can create barriers to patient care, misunderstandings between clinicians and patients, and poor adherence to recommendations for long-term treatment (22). Creating a more culturally sensitive treatment environment can involve an evaluation of patients' beliefs and attitudes about cancer during treatment, involving the patient and family members in decision-making and treatment planning, addressing concerns related to health literacy and access to health care services, and providing patient materials in a culturally-tailored language/format (21).

How important is physical activity now that I have survived colorectal cancer?

Physical activity is an important for survivors of colorectal cancer, yet many patients may not feel comfortable engaging in exercise during or after treatment. Zhao and colleagues found that only 56.1% of cancer survivors reported engaging in physical activity at least 150 minutes per week *vs.* 65.7% of adults with no cancer history (4). This is partially due to inaccurate previous recommendations for cancer patients to avoid activity and to rest during treatment (24). Physical activity interventions in cancer survivors have been shown to have positive effects on upper and lower body strength, fatigue, QOL, anxiety, and self-esteem (25).

The American College of Sports Medicine has recommended that cancer survivors adhere to the 2008 Physical Activity Guidelines for Americans which includes 150 minutes of moderate-intensity aerobic activity and muscle strengthening activities at least 2 days per week (24). Although some patients will be able to increase their physical activity levels by following general exercise guidelines, most would benefit from more tailored recommendations that can take into account individual needs (26). This may include providing an exercise "prescription" that specifies type of activity, intensity, and duration. Referring patients to community-based exercise

programs available at the YMCA may help to provide social support and guidance from trained professionals during their exercise program. If needed, a referral to physical therapy or rehabilitation may help patients to address weakness or instability that may be present due to the effects of treatment or deconditioning.

Will my primary care provider (PCP) be able to provide all of my care as a colorectal cancer survivor?

The American Society of Clinical Oncology (ASCO) recommends a model of care that combines the expertise of the oncology team and the PCP to coordinate survivor follow-up (27). However, patients and providers may have different expectations in terms of who will be providing their care after treatment ends (28). For example, some patients expect their oncology team to be more involved with their cancer care follow-up than their PCP. One of the ways to facilitate transfer of care back to the PCP is to create a survivorship plan that details all of the recommendations for the patient's follow-up care and which providers will be responsible for each aspect of treatment. Giving both the patient and their PCP a copy of this survivorship plan helps to create the sense of a warm handoff with clear documentation of needs for additional treatment and monitoring (29). There are many resources available for creating survivorship treatment plans on the ASCO and American Cancer Society websites, including specific guidelines for colorectal cancer follow-up care.

What will it be like having my stoma long-term?

Colorectal cancer patients often have concerns about adjusting to their stoma including: changes in sexual behavior, clothing fit, proper fitting of the appliance, odor or noises related to use of a stoma, and changes in body image (30-32). Despite these concerns, research typically shows that QOL scores remain 'good' when patients are asked to rating living with a stoma (33). Referring patients to an ostomy nurse and providing resources from the United Ostomy Association of America can help to decrease patients concerns as they adjust to their stoma.

How will my sexual life be affected as a survivor?

Although sexual dysfunction is one of the most common long-term effects of colorectal cancer treatment, this issue is rarely discussed among patients and their providers (34). Changes in sexuality can include coital pain, erectile dysfunction, and/or decreased vaginal lubrication (35). Patients may be reluctant to initiate conversations about sexual functioning, so frequent assessment of these symptoms can help to normalize the discussion during follow-up visits. Regardless of age, sexual orientation, or partner status, sexual functioning is an important aspect of the QOL for all patients that should be monitored during survivorship care. In addition to providing patients with resources for sexual dysfunction treatment, a referral to a sex therapist or educator may also be helpful.

What if I continue to have problems sleeping after treatment ends?

It is not uncommon for people receiving treatment for a cancer diagnosis to have changes in sleep patterns including increase sleep onset latency and decreased total sleep (36). These disruptions in the sleep cycle may be associated with reduced tissue growth and repair, fatigue, impaired memory, and decreased QOL (37). When providers do not intervene, patients may self-medicate and potentially choose detrimental remedies, such as alcohol, to help them sleep (38). Although medications for sleep are often considered first-line treatment for insomnia, many patients could benefit from a behavioral approach to treatment which is associated with better long-term outcomes than pharmacological treatment. Cognitive behavioral therapy for insomnia (CBT-I) is a multi-component treatment that is designed to improve sleep through sleep restriction and stimulus control techniques (39). CBT-I can be as effective as medication but without the side effects or potential for patients to rely on medications for sleep. In order to determine whether or not a patient may be a good candidate for CBT-I, providers should do a thorough assessment of their sleep difficulties to determine if a sleep study may be needed to rule out other sleep disorders such as obstructive sleep apnea (OSA).

What will happen if my cancer comes back?

Fear of recurrence is common among cancer survivors (42-70%), and may not decrease over time even when risk of recurrence is low (40,41). It is also associated with poorer QOL, psychological comorbidities, and increased health care costs due to more frequent medical visits. Despite the

negative outcomes associated with fear of cancer recurrence, it is not often discussed during follow-up appointments and patients may feel reluctant to ask questions about their risk of recurrence. Providing patients with a survivorship plan and giving them the NCCN recommendations for follow-up tests and appointments may reduce the uncertainty and apprehensions in the majority of survivors. For some patients, a referral to a behavioral health provider for cognitive behavioral therapy (CBT) or acceptance and commitment therapy (ACT) may be helpful to reduce their fear of recurrence and associated symptoms (40,42). The American Psychosocial Oncology Society has more information and resources for patient referrals.

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Footnote

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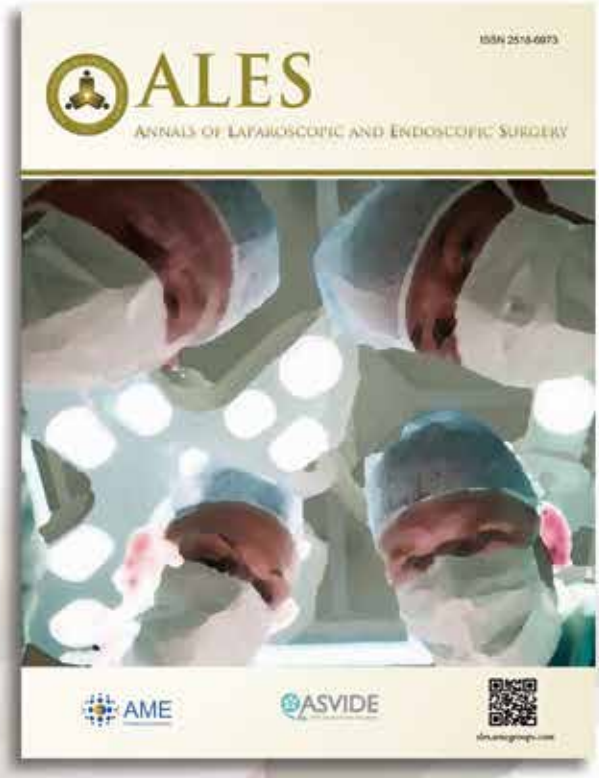

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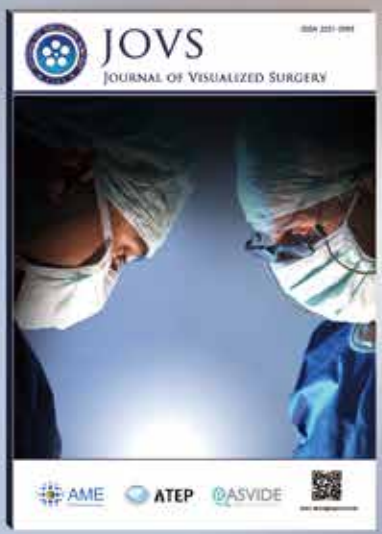

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