Colorectal Cancer

Evidence-Based Chemotherapy Strategies

Edited by

Leonard B. Saltz, MD

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Colorectal Cancer

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Preface

Management options for patients with colorectal cancer have undergone dramatic changes over the past decade. Whereas at the start of 1996 only one drug, 5-Fluorouracil, was available for the treatment of this disease, a mere 10 yr later, six drugs are licensed for use in colorectal cancer, and others are in the late phases of clinical development. Likewise, surgical and ablative options, as well as an array of supportive medications, have shown substantial progress and undergone a dramatic proliferation over the past decade.

With the increased number of therapeutic options from which to choose, the clinician is better able to offer effective therapy to the patient with colorectal cancer. The clinician is challenged, however, to keep up with the rapidly changing landscape and the rapidly emerging data that shape the options for treatment today and tomorrow. In this text, leaders in the management of colorectal cancer review the current literature that has led us to where we are today. Critical evaluations of the data are offered, and evidence-based recommendations are made.

The initial chapters update the current thinking on the biology of colorectal cancer, and methods of possible prevention, both from the points of view of chemoprevention and screening. The state of the art for use of both cytotoxic chemotherapy and the incorporation of the newer biological therapies are then reviewed. Practical chapters on radiological evaluation of colorectal cancer treatment, and nursing issues related to supporting the patient through chemotherapy are then presented. An additional chapter focuses on the specifics of pain management in colorectal cancer patients. Finally, a forward-looking chapter explores possible new paradigms under development for colorectal cancer treatment in the future.

The goal of *Colorectal Cancer: Evidence-Based Chemotherapy Strategies* is to offer the practitioner a concise, authoritative reference, so that the knowledge gained over recent years can be disseminated, digested, and rapidly applied to clinical practice. Clinicians who treat colorectal cancer are all too cognizant of the extensive work that remains to be done in terms of developing definitive treatments for this disease. While recognizing the long way that we have to go, I hope that *Colorectal Cancer: Evidence-Based Chemotherapy Strategies* will help practitioners appreciate the strengths, as well as the limitations, of the data that have recently emerged, thereby helping to allow all patients to benefit from the progress that has been made thus far.

Leonard B. Saltz, MD

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1 Molecular Biology of Colon Cancer

William M. Grady, MD

Summary

Colorectal cancer affects approx 140,000 people in the United States each year, resulting in more than 55,000 deaths. Colorectal cancer develops as the result of the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colon adenocarcinoma. The loss of genomic stability is a key molecular and pathophysiological step in this process and serves to create a permissive environment for the occurrence of alterations in tumor suppressor genes and oncogenes. Alterations in these genes, which include *APC, CTNNB1, KRAS2, BRAF, MADH4*/*SMAD4*, *TP53*, *PIK3CA*, and *TGFBR2*, appear to promote colon tumorigenesis by perturbing the function of signaling pathways, such as the transforming growth factor-β and PI3K signaling pathways, or by affecting genes that regulate genomic stability, such as the mutation mismatch repair genes.

Key Words: Colon cancer; mutation; oncogene; tumor suppressor gene; DNA methylation.

1. INTRODUCTION

Colorectal cancer (CRC) arises as the consequence of the progressive accumulation of genetic and epigenetic alterations that drive the evolution of normal colonic epithelial cells to colon adenocarcinoma cells. This process of colon carcinogenesis, which has been termed the polyp-carcinoma sequence, is believed to typically take place over 10–15 yr and involves concurrent histological and molecular changes. The subsequent effect of these genetic and epigenetic alterations on the cell and molecular biology of the cancer cells in which they occur is the acquisition of key biological characteristics that are central to the malignant phenotype. From the analysis of the molecular genetics of colon cancer, it has become clear that the formation of colon cancer involves a multistage process, which is currently characterized at the molecular level by the underlying form of genomic instability (i.e., the loss of the ability to maintain the wild-type DNA coding sequence and repair DNA mutations) present in the

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cancers. In this background of genomic instability, genetic and epigenetic alterations accumulate and cooperate with each other to drive the initiation and progression of colon cancer *(1–3)*.

Colon cancer appears to be most commonly initiated by alterations that affect the Wingless/Wnt signaling pathway. The initiated colon cancer then progresses as the result of the accumulation of sequential genetic or epigenetic events that either activate oncogenes or deactivate tumor suppressor genes that are involved in other signaling pathways, such as the RAS-RAF-MAPK pathway, transforming growth factor (TGF)-β pathway, and the phosphatidylinositol 3 kinase (PI3K)-AKT pathway *(4,5)*. Some of the alterations that have been convincingly shown to promote colon carcinogenesis affect *KRAS2*, *TP53*, the gene for p53, and elements of the TGF-β signaling pathway, such as *TGFBR2* and *MADH4*/*SMAD4*. The identification of these alterations has provided potential targets for the development of new therapies for the prevention and/or treatment of colon tumors (Fig. 1).

2. POLYP-CARCINOMA SEQUENCE

The evolution of normal epithelial cells to adenocarcinoma usually follows a predictable progression of histological changes and concurrent genetic and epigenetic changes. These gene mutations and epigenetic alterations provide a growth advantage to these mutant cells and lead to the clonal expansion of these altered cells. This process leads to the progression of adenomas to adenocarcinomas by the serial acquisition of genetic and epigenetic alterations that produce clonal heterogeneity followed by Darwinian evolution at the cellular level. Until recently, it was believed that only adenomatous polyps had the potential to undergo malignant transformation; however, it now also appears that a subset of hyperplastic polyps may have the potential to transform through a hyperplastic polyp-serrated adenoma-adenocarcinoma progression sequence *(6)*. Colon cancers arising through a hyperplastic polyp-serrated adenoma-colon cancer pathway appear to have a unique molecular as well as histological pathway through which they arise.

3. GENOMIC INSTABILITY

Genomic instability, which is the loss of the ability of the cell to maintain the fidelity of the DNA, is a fundamental aspect of the tumorigenesis process. At least three forms of genomic instability have been identified in colon cancer: (1) microsatellite instability (MSI), (2) chromosome instability (CIN; i.e., aneusomy, gains and losses of chromosomal regions), and (3) chromosomal translocations *(7)*. The etiology of CIN has only been identified in a small subset of colon cancers; however, MSI is known to result from inactivating mutations or the aberrant methylation of genes in the DNA mutation mismatch repair (MMR) family, which repairs DNA base-pair mismatches that arise during DNA

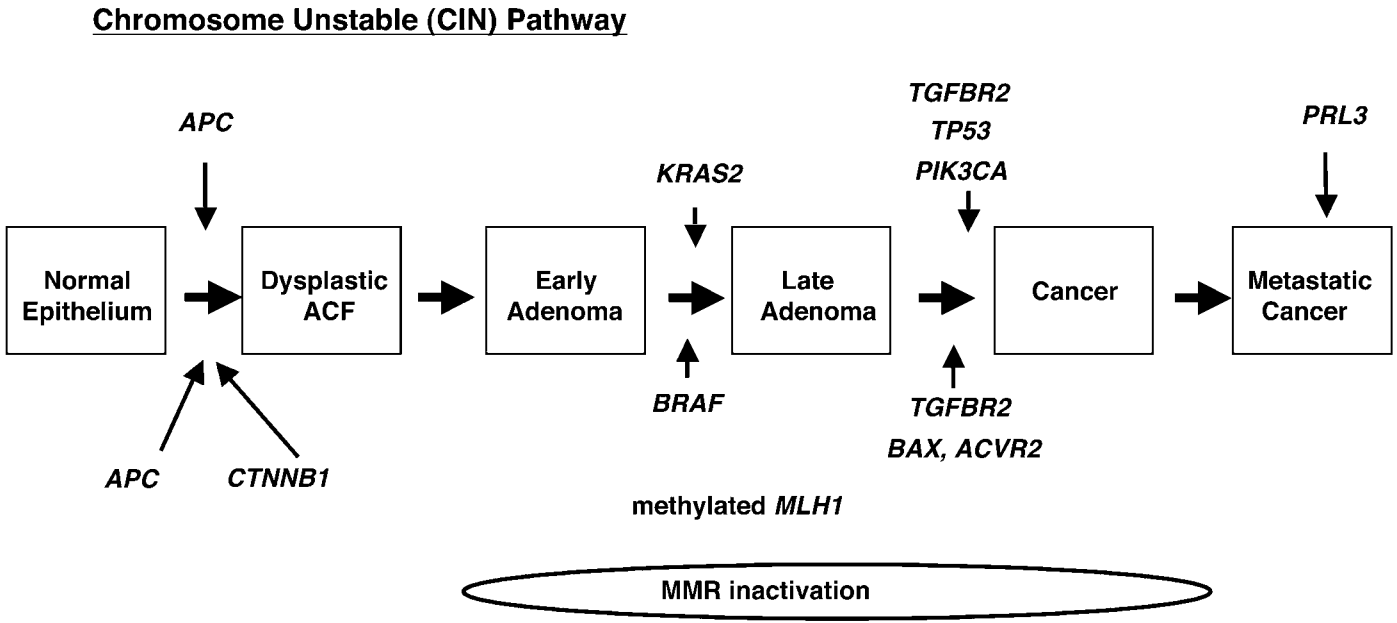

Fig. 1. Schematic representation of polyp-carcinoma progression sequence.

replication. Genomic instability contributes to the accumulation of mutations in tumor suppressor genes and oncogenes that drive the polyp-cancer progression sequence. The timing of the loss of genomic stability, either CIN or MSI, appears to be after adenoma formation but before progression to frank malignancy. In fact, both CIN and MSI can be detected in colon adenomas *(8–14)*. Shih et al. demonstrated that more than 90% of early adenomas (1–3 mm in size) exhibited allelic imbalance (also known as loss of heterozygosity [LOH]) of at least one of four chromosomes tested *(8)*. Ried et al. detected a stepwise increase in the average number of copy alterations using comparative genomic hybridization as adenomas progressed from low- to high-grade and then finally to carcinoma *(13)*. Despite the accumulation of data demonstrating the presence of genomic instability in early colon tumors, the causative role of genomic instability in cancer remains a source of considerable controversy *(2,7)*. Nonetheless, genomic instability is an attractive target for anticancer therapies because it is nearly ubiquitous in colon cancer and is a unique characteristic of cancer cells that is not present in normal epithelial cells. The feasibility of targeting genomic instability for anticancer treatments has been shown in in vitro systems *(15)*.

3.1. DNA Mismatch Repair Pathway/Inactivation of MMR Genes

Genomic instability arises because of inactivation of the normal mechanisms used by the cell to maintain its DNA fidelity. Defects in two of the systems that regulate DNA fidelity, the MMR system and Base Excision Repair (BER), have been identified in independent subsets of colon cancer. The DNA mismatch repair system (also known as the MMR system) consists of a complex of proteins that recognize and repair base-pair mismatches that occur during DNA replication. Inactivation of the MMR system occurs in 1–2% of CRCs owing to germline mutations in members of the MMR system, *MLH1, MSH2, PMS2*, and *MSH6*, and is the cause of the colon cancer family syndrome, hereditary nonpolyposis colon cancer (HNPCC) *(16,17)*. In addition to HNPCC-related colon cancers, approx 15% of sporadic colon cancers have inactivated MMR systems owing to the aberrant methylation of MLH1 (*see* p. 8) *(18)*. MSI occurs as the consequence of inactivation of the MMR system and is recognized by frameshift mutations in microsatellite repeats located throughout the genome. Because many colon cancers demonstrate frameshift mutations at a small percentage of microsatellite repeats, the designation of a colon adenocarcinoma as showing MSI depends on the detection of at least two unstable loci out of five from a panel of loci that were selected at a National Cancer Institute consensus conference *(19)*.

Study of the biochemistry of the MMR proteins has revealed that recognition of the base–base mismatches and insertion/deletion loops is performed by a heterodimer of either MSH2 and MSH6 or MSH2 and MSH3. Of interest, the MSH2–MSH3 heterodimer preferentially recognizes insertion/deletion loops and thus cannot compensate for loss of hMSH6. Consequently, cancers arising

with a loss of MSH6 function display MSI predominating in mononucleotide repeats and may display an attenuated form of MSI called MSI-low *(20)*. The MLH1, PMS2, and PMS1 proteins appear to operate primarily in performing the repair of the base–base mismatches and insertion/deletion loops. A heterodimer of MLH1–PMS2 operates as a "molecular matchmaker" and is involved in executing the repair of the mismatches in conjunction with DNA-polymerase ∂ and the replication factors proliferating cell nuclear antigen (PCNA), replication protein A, and replication factor C, as well as the 5′→3′ exo/endonucleases EXO1 and FEN1 and other unidentified 3′→5′ exonucleases and helicases *(20,21)*.

The MSI that results from loss of MMR activity affects mono-, di-, and trinucleotide tracts predominantly. However, cell lines from these tumors also show up to a 1000-fold increased mutation rate at expressed gene sequences, and in particular show instability of short sequence repeats with expressed sequences *(22)*. Genes that possess such "microsatellite-like" repeats in their coding regions appear to be the targets relevant to carcinogenesis. This pathway to tumor formation appears to be distinct from that seen in colon cancers that are microsatellite stable (MSS) *(23)*. The most frequently targeted gene for mutation in this pathway is the TGF-β receptor type II tumor suppressor (*TGFBR2*) gene, which is discussed in greater detail in Section 6.7. Other, less frequently targeted genes include the *IGF2* receptor; *BAX* and *CASPASE 5,* proteins which regulate apoptosis; *ACVR2,* a receptor for activin; *MSH3* and *MSH6*, DNA mismatch repair proteins; *RIZ*, the retinoblastoma protein-interacting zinc finger gene; and *CDX2*, an intestinal homeobox factor *(23–28)*. Importantly, MSI and the subsequent target gene mutations appear to occur throughout the adenoma-to-carcinoma progression. The timing of many of these events during tumor formation remains to be mapped, but preliminary studies have shown they occur at distinct phases of tumor progression *(10)*. Thus, MSI creates a favorable state for accumulating mutations in vulnerable genes that promote tumorigenesis, and these alterations ultimately lead to the generation of colon cancers.

The relationship between the MSI pathway and other genetic alterations frequently found in colon cancer is only partially understood. Alteration of the Wnt/Wingless pathway can be observed in tumors irrespective of MSI status *(29)*. Mutations in *APC* and *CTNNB1* can be found in 21 and 43% of MSI tumors, respectively *(30,31)*. In addition, the incidence of *KRAS2* mutations appears to be as high as 22–31%, which is similar to the incidence observed in MSS colon cancers *(32,33)*. Mutations in *TP53* are less frequent in MSI cancers than in MSS cancers. The mutation incidence in MSI colon cancers ranges between 0 and 40%, whereas the incidence in MSS tumors is between 31 and 67% *(30,32,34,35)*. Of interest, monoallelic and biallelic *BAX* mutations are found frequently in MSI colon cancers and may serve to replace the role of mutant *TP53* in colon carcinogenesis. Thus, the microsatellite mutator pathway appears to be initiated through

changes in the Wnt/Wingless pathway and to share some alterations with the MSS colon cancer pathway. However, other events, such as *TP53* and *TGFBR2* mutations, occur at different frequencies in the MSI vs the MSS pathway.

The impact of MSI on the clinical behavior of CRCs has been intensely investigated, but remains only partly understood to date. Several retrospective studies have shown mixed results regarding the effect of MSI on prognosis. Watanabe et al. found that 18qLOH correlated with a reduction in 5-yr survival from 74 to 50% in stage III CRC patients and that *TGFBR2 BAT-RII* mutations correlated with improved 5-yr survival in tumors with MSI, 74 vs 46% *(36)*. In addition, a systematic review of MSI and CRC prognosis revealed that there was a combined hazard ratio estimate for overall survival associated with MSI of 0.65 (95% confidence interval [CI], 0.59 to 0.71) *(37)*. Finally, at present, no definite conclusions regarding the effect of MSI on CRC treated with adjuvant therapy can be made.

4. BER DEFECTS AND COLON CANCER

Inactivation of a second "DNA caretaker" mechanism, the BER system, has been found in a subset of colon cancer cell lines and is a cause of an autosomal recessive form of adenomatous polyposis, called the MYH adenomatous polyposis syndrome *(38)*. Germline mutations in *MYH*, which encodes for a protein involved in BER, is the cause of adenomatous polyposis in up to 5–10% of individuals who have an adenomatous polyposis syndrome. *MYH* germline mutations were discovered as a cause of adenomatous polyposis when investigators identified an excessive number of somatic G:C →A:T mutations in neoplasms of people with adenomatous polyposis but no detectable germline mutations in *APC (39–41)*. This type of mutation is commonly a consequence of oxidative damage to DNA that results in 8-oxo-7,8-dihydro2′deoxyguanosine (8-oxodG), which is one of the most stable deleterious products of oxidative DNA damage *(38,42)*. The BER system is responsible for repairing this form of DNA damage, which led these investigators to assess candidate genes involved in this process, *OGG1, MTHF1*, and *MYH* (Fig. 2). This assessment revealed biallelic germline mutations in a subset of people with adenomatous polyposis, but who did not have germline mutations in *APC*. The most common mutations are Tyr165Cys and Gly382Asp, which account for 82% of the mutant alleles detected to date *(41)*. Somatic *MYH* mutations do not appear to be common in sporadic colon cancer. A study of 1042 unselected patients with CRC in Finland revealed no somatic *MYH* mutations *(38,43)*. Of interest, the tumors arising in the setting of biallelic *MYH* germline mutations do not show differences in the frequency of *TP53, SMAD4,* or *TGFBR2* mutations but do show an absence of MSI or CIN, suggesting that they have a unique molecular pathogenesis *(44)*. The discovery of *MYH* germline mutations in people with a hereditary colon cancer syndrome provides more evidence for the importance of genomic instability in cancer formation.

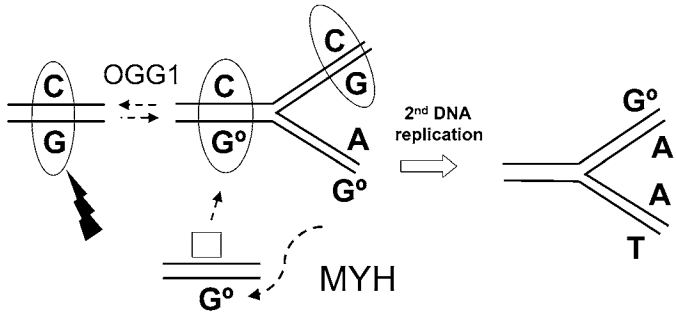

Fig. 2. Schematic representation of base-excision repair system.

5. EPIGENTIC ALTERATIONS

Heritable phenomenon that regulate gene expression without involving changes of the DNA base-pair code are defined as epigenetic. Recently, epigenetic alterations have been increasingly recognized as being common and likely pathogenic in a variety of cancers. DNA methylation, the most commonly studied epigenetic phenomenon that appears to be altered in cancer, is normally present throughout the majority of the genome and is maintained in relatively stable patterns, which are established during development *(45)*. In humans, approx 70% of CpG dinucleotides are methylated. However, there are regions that contain high proportions of CpG dinucleotides, called CpG islands, which are present in the 5′ region of approx 50–60% of genes and are normally maintained in an unmethylated state. In cancers, many of these CpG islands become aberrantly methylated, and this aberrant methylation can be accompanied by transcriptional repression *(46,47)*. An ever-increasing number of genes have been shown to be aberrantly methylated in CRCs, including *CDKN2A, HLTF, MGMT, p14, TIMP3, TSP1*, and others.

The significance of these epigenetic alterations has been a point of substantial controversy. For instance, whether aberrant methylation is generally a cause or an effect of cancer formation remains unresolved because the mechanism responsible for aberrant DNA methylation has yet to be identified *(48,49)*. Nonetheless, there is substantial data that the aberrant methylation of at least some genes, such as *MLH1*, is pathogenetic in cancer *(18,50,51)*. Inactivation of *MLH1*, a member of the MMR system, presumably plays an initiating role in the pathogenesis of colon cancers. Thus, the demonstration of aberrant methylation of *MLH1* in sporadic MSI colon cancers, and the restoration of *MLH1* expression by demethylating the *MLH1* promoter in MSI colon cancer cell lines, strongly suggests that such aberrant methylation could be a cause rather than a consequence of colon carcinogenesis *(18,50,51)*. Moreover, it is likely that the aberrant hypermethylation of 5′ CpG dinucleotides that has been demonstrated to silence a variety of

known tumor suppressor genes in colon cancer, including *CDKN2A/p16*, *MGMT,* and $p14^{ARF}$, may be similarly pathogenetic in colon cancer *(46,50–54)*. Of specific note, methylation of *CDKN2A/p16,* a canonical tumor suppressor gene, is detected in 40% of colon cancers *(53)* and has been found not only in colon cancer but also in colon adenomas, as have other aberrantly methylated genes *(55,56)*. This observation demonstrates that aberrant promoter methylation is occurring early in the adenoma sequence, although it does not confirm that the aberrant *CDKN2A/p16* methylation is a primary rather than a secondary event in the tumorigenesis process. More broadly, early work has suggested that colon cancers that hypermethylate *MLH1* and/or *CDKN2A/p16* may belong to a distinct subclass of colon cancers, termed the CpG island methylator phenotype (CIMP), that demonstrate genome-wide aberrant methylation of gene promoters and that may arise by a distinct and unique mechanism *(53,54,57)*.

Also of note is recent progress in our understanding of mechanisms through which DNA methylation may affect transcription. DNA methylation may impair transcription by direct inhibition between methylated promoters and transcription factors, such as AP-2, CREB, E2F, and NF-κB *(45)*. CpG island methylation also can mediate transcriptional silencing by recruiting methyl-binding proteins, MeCP2, MBD2, and MBD3, that recognize methylated sequence and recruit histone deacetylases (HDACs). The HDACs then induce changes in chromatin structure that impede the access of transcription factors to the promoter *(46)*. The relationship between DNA methylation and posttranslational modification of histones appears to be complex, as other studies have shown that changes in the methylation state of H3-lysine 9 and H3-lysine 4 precede changes in DNA methylation, suggesting that the histone modification state and chromatin structure may cause the DNA methylation changes *(45)*. There is considerable interest in targeting these histone changes and methylation for anticancer therapies, using drugs such as histone deacetylases inhibitors.

6. GENETIC ALTERATIONS

6.1. The Wingless/Wnt Signaling Pathway

6.1.1. Adenomatous Polyposis Coli

The role of genetic alterations in colon cancer formation was initially suggested by the colon cancer family syndrome, familial adenomatous polyposis (FAP). FAP is a hereditary colon cancer predisposition syndrome that is characterized by the development of hundreds of intestinal adenomatous polyps. The gene responsible for this syndrome, adenomatous polyposis coli (*APC*), was identified as the result of the discovery of an interstitial deletion on chromosome 5q in a patient affected with FAP and from classical linkage analysis of families affected by FAP *(58–60)*. The *APC* gene has 15 exons and encodes a large protein (310 kDa, 2843 amino acids) that possesses multiple functional domains that mediate oligomerization as

well as binding to a variety of intracellular proteins, including β-catenin, γ-catenin, glycogen synthase kinase (GSK)-3β, axin, tubulin, end-binding protein 1 (EB1), and homologue of discs large (hDLG) *(3)*. Germline mutations in *APC* result in FAP or one of its variants: Gardner's syndrome, attenuated FAP, Turcott's syndrome, or the flat adenoma syndrome *(61–64)*.

APC is mutated in up to 70% of all sporadic colon adenocarcinomas, and these mutations are present beginning in the earliest stages of colon cancer formation and precede the other alterations observed during colon cancer formation *(31,65–68)*. In fact, dysplastic aberrant crypt foci, presumptive precursor lesions to colon cancer, have been found by some investigators to harbor *APC* mutations *(69,70)*. The mutations observed in sporadic colon cancer are observed most frequently in the 5′ end of exon 15, between amino acid residues 1280 and 1500 *(71)*. Mutations in this region can affect the domains between amino acid residues 1020–1169 and 1324–2075, which have been implicated in β-catenin interactions. These mutations can also affect the SAMP (Ser-Ala-Met-Pro) domains located between amino acids 1324–2075 and thus disrupt *APC*'s interaction with axin *(72–74)*. The vast majority of *APC* mutations (>90%) result in premature stop codons and truncated gene products *(75)*. As mentioned previously, these mutations are often accompanied by chromosomal deletion of the residual wild-type allele, but biallelic inactivation of *APC* can also occur by second somatic mutations *(76)*.

One of the central tumor promoting effects of these mutations is to lead inappropriate activation of the Wingless/Wnt signaling pathway with the subsequent expression of genes that favor cell growth (Fig. 3). The disruption of the association of APC with β-catenin leads to over-activation of the Wnt signaling pathway, which leads to the transcription of genes that favor tumor formation, such as *c-MYC* or *MATRILYSIN (65,77)*. Normally, GSK-3β forms a complex with APC, β-catenin, and axin, and phosphorylates these proteins. The phosphorylation of β-catenin targets it for ubiquitin-mediated proteasomal degradation. Truncating *APC* mutations prevent this process from happening and cause an increase in the amount of cytoplasmic β-catenin, which can then translocate to the nucleus and interact with other transcription factors like T-cell factor/lymphoid-enhancing factor (TCF/LEF). TCF-4 is the predominant TCF family member expressed in colonic epithelium. Consistent with the concept that increased Wnt-β-catenin pathway activity is a central tumor-promoting effect of *APC* mutations, oncogenic mutations in the β-catenin gene (*CTNNB1*) have been observed in some CRCs, as has methylation of *SFRP2* and *SFRP4*, members of a family of secreted Wnt antagonists called secretory frizzled related proteins *(78–80)*.

The clinical effects of *APC* mutations are best understood in the context of FAP, in which the location of the mutations associates with the severity of the phenotype and the occurrence of extraintestinal tumors, such as desmoid

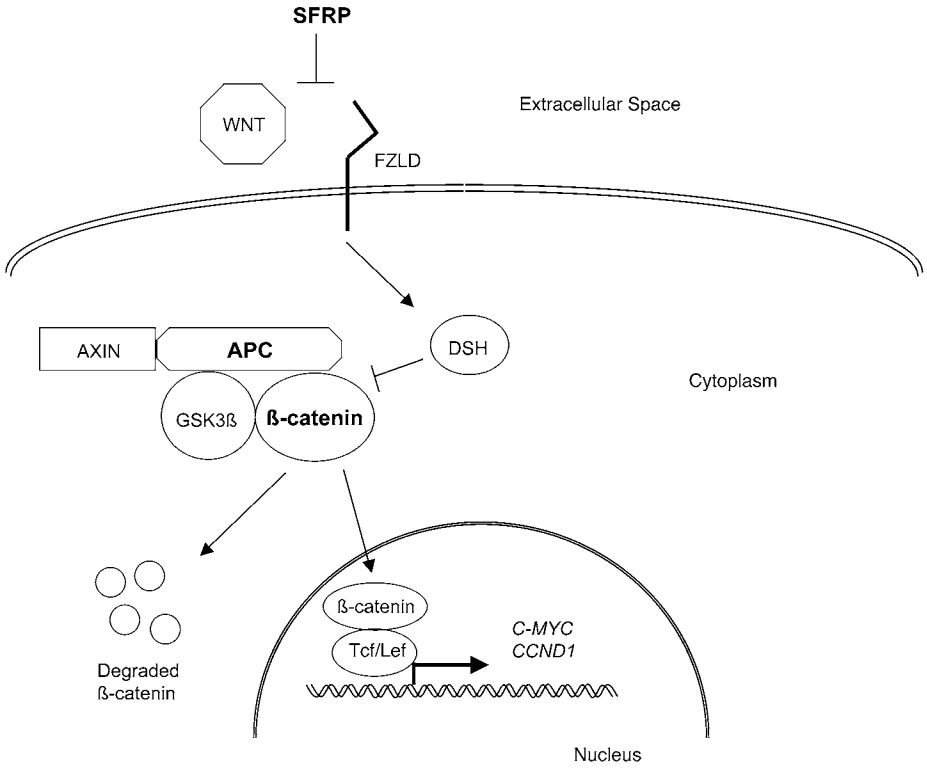

Fig. 3. Wnt signaling pathway diagram.

tumors *(33,81–83)*. Polymorphisms in the *APC* gene that associate with a slight increased risk of CRC have also been identified and include *I1307K* and *E1317Q* polymorphisms. *APC I1307K* occurs exclusively in people of Ashkenazi Jewish descent and results in a twofold increased risk of colonic adenomas and adenocarcinomas compared to the general population *(84,85)*. The *I1307K* polymorphism results from a transition from T to A at nucleotide 3920 in the *APC* gene and appears to create a region of hypermutability.

6.1.2. β-Catenin (CTNNB1)

β-Catenin is a member of the APC/β-catenin/TCF-LEF pathway that plays a role in the formation of a subset of colon cancers. β-Catenin is a homolog of armadillo, and its expression is increased by activation of the Wnt signaling pathway *(86–88)*. APC interacts with β-catenin and forms a macromolecular complex with it and GSK-3β. β-Catenin is consequently directed toward degradation as a result of phosphorylation by GSK-3β *(89–91)*. Mutations of *CTNNB1* or *APC* often render β-catenin insensitive to APC/β-catenin/GSK-3β-mediated degradation *(92,93)*. One of the functions of β-catenin is to bind members

of the TCF family of transcription factors and activate gene transcription. Accordingly, cancers with *APC* or *CTNNB1* mutations have increased β-catenin/TCF-mediated transcription, which leads to the over-expression of genes such as *CYCLIN D1* and *c-MYC (94,95)*. The majority of these mutations are in a portion of exon 3 encoding for the GSK-3β phosphorylation consensus region of β-catenin. These mutations are often missense mutations in the highly conserved aspartic acid 32 and presumably impair the ability of GSK-3β to phosphorylate β-catenin *(96)*. Caca et al. found *CTNNB1* mutations in the NH2-terminal phosphorylation sites of β-catenin and found increased TCF/LEF transcriptional activity in association with this mutation *(97)*. Mutations that abolish β-catenin binding with E-cadherin have also been identified and have been shown to impair cell adhesion *(98,99)*. Like *APC* mutations, *CTNNB1* mutations have a role in early colon tumor formation. Mouse models with conditional alleles that lead to the stabilization of *Catnb1* in the intestinal tract, resulting in an FAP phenotype, have provided functional evidence that *CTNNB1* mutations lead to the formation of adenomas *(100)*. Interestingly, the incidence of *CTNNB1* mutations decreases from 12.5% in benign adenomas to 1.4% in invasive cancers, suggesting that *CTNNB1* mutations do not favor the progression of adenomas to adenocarcinomas *(101)*. Frameshift mutations in a polyadenine tract in *TCF-4* have also been identified in microsatellite unstable tumors, although their functional significance is unknown *(102)*.

6.2. KRAS2, BRAF, *and* RAS-RAF-MAPK *Signaling Pathway*

One of the most prominent proto-oncogenes in colon carcinogenesis is a member of the *RAS* family of genes, *KRAS2*. The *RAS* oncogenes, which include *HRAS, NRAS*, and *KRAS2*, were initially discovered as the transforming genes of the Harvey and Kirsten murine sarcoma viruses (Ha-MSV, Ki-MSV) *(103,104)*. *KRAS2* is the most commonly mutated *RAS* family member in colon cancer, although *NRAS* mutations are also observed in a small percentage of colon cancers *(105)*.

The *RAS* family genes encode a highly conserved family of 21-kDa proteins, which are involved in signal transduction. One major function of the ras protein family is to couple growth factors to the Raf-mitogen-activated protein kinase kinase→MAP kinase signal transduction pathway, which leads to the expression of early response genes *(106)*. *KRAS2* consists of four exons that produce either a 188- or 189-amino acid peptide, depending on whether the fourth exon is alternatively spliced *(107)*. The protein encoded by *KRAS2* has three domains that either (1) bind guanosine triphosphate or diphosphate (GTP/GDP); (2) attach the protein to the inner side of the plasma membrane after posttranslational modification (isoprenylation) of the carboxy terminus; or (3) interact with cellular targets. Inactive KRAS2 binds GDP, and upon its activation GDP is exchanged for GTP. The activated KRAS2 then interacts with downstream signaling molecules to propagate cell proliferation. The activated KRAS2 is

normally immediately deactivated by intrinsic GTP hydrolysis. Oncogenic mutations of *KRAS2* disrupt the GTPase activity of KRAS2 and allow it to remain in an activated state *(107)*. In fact, the most common mutations observed in human cancers involve codons 12, 13, and 61, which correspond to areas in the GTP-/GDP-binding domains in the KRAS2 protein. The consequence of these mutations is that approx 30% of the KRAS2 protein is in the GTP-bound state as compared to less than 0.3% in cells with wild-type *KRAS2 (108)*. The increased fraction of activated KRAS2 leads to activation of the RAS-RAF-MAPK signaling pathway, which promotes cell proliferation and increased survival, as well as other protumorigenic effects (Fig. 4).

Mutation of *KRAS2* and *KRAS2* amplification has been observed in a large percentage of gastrointestinal tract tumors. As in other tumors, the *KRAS2* mutations observed in colon cancer almost always affect codons 12, 13, and 61. *KRAS2* mutations can be detected in 37–41% of colon cancers, and codon 12 is the most commonly mutated in CRC, usually undergoing a missense mutation *(68,109–111)*. The *KRAS2* mutations appear to follow *APC* mutations and are associated with advanced adenomatous lesions *(68)*. Evidence for this model comes from the observation that small adenomas with *APC* mutations carry *KRAS2* mutations in approx 20% of the tumors; whereas approx 50% of more advanced adenomas have been found to have *KRAS2* mutations *(66,112)*. Thus, alterations of *KRAS2* appear to promote colon cancer formation early in the adenoma-carcinoma sequence by mediating adenoma growth. Of interest, however, they do not appear necessary for the malignant conversion of adenomas to adenocarcinomas.

More recently, mutations in *BRAF*, which is a kinase in the RAS-RAF signaling pathway, have also been recognized. *BRAF* mutations can be found in 27–31% of MSI colon cancers and 5% of MSS colon cancers and can be detected in ACFs, adenomas, and adenocarcinomas *(113–115)*. Of all the mutations, 80% are V600E mutations, which are predominantly found in MSI cancers, and which lead to activation of the ERK and NF-κB pathways *(116)*. *BRAF* mutations appear to be mutually exclusive from *KRAS2* mutations, suggesting that mutations in either gene affect tumor formation by activating the RAS-RAF-MAPK pathway. *BRAF* mutations also appear to occur rarely in MSI colon cancers that occur in the setting of HNPCC and instead are tightly associated with CIMP colon cancers, suggesting that there may be two distinct molecular pathways for the formation of MSI colon cancers *(57,117–119)*.

6.3. p53 (TP53)

The p53 protein was initially identified as a protein forming a stable complex with the SV40 large T-antigen, and was originally suspected to be an oncogene *(120)*. Subsequent studies demonstrated that *TP53* is located at 17p13.1 and is mutated in 50% of primary human tumors, including tumors of the gastrointestinal

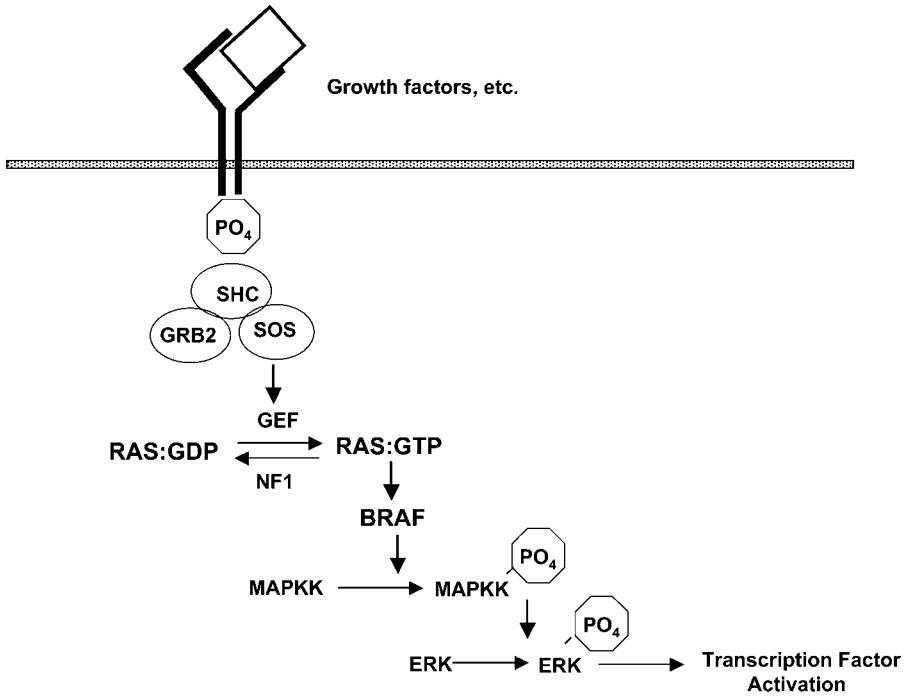

Fig. 4. Ras-Raf-MAPK signaling pathway diagram.

tract *(121)*. p53 is currently appreciated to be a transcription factor that is involved in maintaining genomic stability through the control of cell cycle progression and apoptosis in response to genotoxic stress *(121)*. The protein encoded by p53 has been structurally divided into four domains: (1) an acidic amino-terminal domain (codons 1–43) required for transcriptional activation; (2) a central core sequence-specific DNA-binding domain (codons 100–300); (3) a tetramerization domain (codons 324–355); and (4) a C-terminal regulatory domain (codons 363–393), rich in basic amino acids and believed to regulate the core DNA-binding domain *(121)*. The spectrum of mutations in *TP53* seen in colon cancer appears similar to that seen in other tumors with mutations of *TP53* clustering at four hot spots in highly conserved regions (domains II–V). *TP53* is mutated in more than 50% of colon adenocarcinomas and the mutations localize primarily to exons 5–8 *(68,122)*. The mutations found to occur commonly in colon carcinoma are G:C to A:T transitions at CpG dinucleotide repeats, and in general interfere with the DNA-binding activity of the protein *(123,124)*. The mutation of *TP53* in colon cancer is commonly accompanied by allelic loss at 17p consistent with its role as a tumor suppressor gene *(125)*. In colon cancers, *TP53* mutations have not been observed in colon adenomas but

rather appear to be late events in the colon adenoma-carcinoma sequence that may mediate the transition from adenoma to carcinoma *(68)*. Furthermore, mutation of *TP53* coupled with LOH of the wild-type allele was found to coincide with the appearance of carcinoma in an adenoma, providing further evidence of its role in the transition to malignancy *(125–128)*.

p53 normally serves to regulate cell growth and division in the context of genotoxic stress. It is expressed at very low levels in cells until it is activated, by poorly understood mechanisms, by DNA damage resulting from γ-irradiation, ultraviolet irradiation, or chemotherapeutic agents *(129)*. Its activation results in the transcription of genes that directly regulate cell cycle progression and apoptosis. These genes include $p21^{WAF1/CIP1}$, *GADD45*, *MDM2, 14-3-3-σ*, *BAX, B99, TSP1, KILLER/DR5, FAS/APO1, CYCLIN G,* and others *(121)*. Expression of many of these genes effectively halts DNA replication and induces DNA repair *(130–133)*. This function of p53 to recognize DNA damage and induce cell cycle arrest and DNA repair or apoptosis has led to p53 being called the "guardian of the genome" *(129)*. Thus, *TP53* normally acts as a tumor suppressor gene by inducing genes that can cause cell cycle arrest or apoptosis and also by inhibiting angiogenesis through the induction of *TSP1 (134,135)*. Mutant p53 protein can block these functions through forming oligomers with wild-type p53, causing diminished DNA-binding specificity *(136)*. Furthermore, the majority of p53 mutations occur in the sequence-specific DNA-binding region and serve to interfere with binding to the consensus sequence, 5′-PuPuPuC(A/T)-3′ *(137)*.

With regards to *TP53* mutation status as a prognostic or predictive marker for CRC response to treatment, there are conflicting results in the literature. *TP53* mutations are common in CRC and are believed to play a fundamental role in deregulating the cell cycle and inducing resistance to apoptosis in CRC. The over-expression of p53 by immunohistochemistry has been interpreted to indicate the presence of mutant p53 protein because the mutant forms of p53 have prolonged protein half-lives. Using this method or DNA mutation analysis for assessing *TP53* mutations, p53 has not consistently shown any prognostic or predictive value in colorectal cancer *(138,139)*. It is possible that the prognostic value of *TP53* mutations will only be appreciated when specific *TP53* mutations are correlated with clinical outcomes.

6.4. The Phosphatidylinositol 3-Kinase (PI3K) Pathway

The PI3Ks are a family of lipid kinases that regulate the activity of kinases such as AKT and p70S6K, which ultimately regulate cell proliferation, apoptosis, and cell motility, hallmark biological functions that are commonly deregulated in cancer *(140)*. Multiple isoforms of PI3K can be identified in mammalian cells and can be divided into three classes, including notably the class I PI3Ks, which are composed of a p110 catalytic subunit and a regulatory adapter subunit.

The class I PI3K members share homologous domains that include the lipid kinase domain, the helical domain, the C2 domain, a Ras-binding domain (RBD), and a NH2-terminal domain that interacts with the regulatory subunit *(141)*. Recently, large-scale mutational analysis studies of members of the PI3K signaling pathway have identified mutations that activate this pathway in a large proportion of colon cancers *(4,142)*. Gain-of-function mutations in *PIK3CA*, the p110α catalytic subunit of PI3K, have been found in 32% of colon cancers *(142)*. Of the *PIK3CA* mutations, 75% occur in two small clusters in the regions encoding the helical and kinase domains of the protein, which are highly evolutionarily conserved. One of the most common mutations, H1074R, has been shown to increase lipid kinase activity in in vitro studies, and a broader screen of other mutation hot spots identified in colon cancers, including E542K, E454K, and five other PIK3CA mutations, revealed that all of these mutations increased lipid kinase activity of PIK3CA *(142,143)*. Analysis of 76 colon adenomas and 199 colon cancers detected *PIK3CA* mutations only in advanced adenomas or CRCs, suggesting that these mutations influence the transition of the adenomas to adenocarcinomas *(142)*. In addition to mutations in *PIK3CA*, mutations in other members of the PI3K pathway have been detected in a series of 180 colorectal cancers, including mitogen activated protein-kinase kinase-4 (*MKK4*/*JNKK1*), myosin light-chain kinase-2 (*MYLK2*), phosphoinositide-dependent protein kinase-1 (*PDK1*), p21-activated kinase 4 (*PAK4*), v-akt murine thymoma viral oncogene homolog-2 kinase (*AKT2*), MAP/microtubule affinity-regulating kinase 3 (*MARK3*), cell division cycle-7 kinase (*CDC7*), a hypothetical casein kinase (*PDIK1L*), insulin related receptor (*INSRR*), and v-Erb-B erythroblastic leukemia viral oncogene homolg *(ERBB4) (4)*. Amplification of insulin-receptor substrate *IRS2* was also detected in a subset of colon cancers. In addition, inactivating mutations in *PTEN*, a lipid dual-specificity phosphatase, and in *PIK3R1*, the p85α regulatory subunit of PI3K, have been demonstrated in 5 and 2% of colon cancers, respectively *(140,144)*. Remarkably, mutations that affect the PI3K pathway can be detected in nearly 40% of CRCs and these mutations are nearly mutually exclusive, suggesting that they have equivalent tumorigenic effects through the activation of the PI3K pathway. These results suggest the PI3K pathway is an attractive pathway for targeted therapies *(4)*.

6.5. TGF-β Superfamily and Signaling Pathways

TGF-β is a multifunctional cytokine that can induce growth inhibition, apoptosis, and differentiation in intestinal epithelial cells *(145,146)*. Evidence of TGF-β's role in colon cancer formation first came from studies that demonstrated colon cancer cell lines were resistant to the normal growth inhibitory effects of TGF-β *(147)*. Furthermore, this pathway is deregulated in approx 75% of colon cancer cell lines, suggesting it is an important tumor suppressor pathway in colon cancer *(148)*.

TGF-β mediates its effects on cells through a heteromeric receptor complex that consists of type I (TGFBR1) and type II (TGFBR2) components. TGFBR1 and TGFBR2 are serine-threonine kinases that phosphorylate downstream, signaling proteins upon activation *(149)*. The receptor complex is activated by TGF-β binding to the TGFBR2 component of the receptor complex, causing formation of the heteromeric R1–R2 receptor complex. The activated TGFBR2 component then phosphorylates the TGFBR1 component in the GS box of TGFBR1, a glycine-serine-rich region of the receptor. TGFBR1 then propagates the signal from the receptor through the phosphorylation of downstream proteins, including the Smad proteins, Smad2 and Smad3, and non-Smad proteins, such as PI3K, p38MAPK, and RhoA *(145,150)*. The Smad pathway is the most extensively characterized post-TGF-β receptor pathway. Upon activation, Smad2 and Smad3 form a hetero-oligomeric complex, which can also include Smad4, and translocate to the nucleus *(149,151)*. In the nucleus, they modulate transcription of specific genes through *cis*-regulatory Smad-binding sequences and through binding with other transcription factors such as p300/CBP, TFE3, Ski, and c-jun *(65,152,153)* (Fig. 5).

The downstream transcriptional targets of the TGF-β signaling pathway are involved in the regulation of cell proliferation, extracellular matrix production, immune surveillance, and so on. These functions not only are an integral part of tissue homeostasis but also are logical targets for dysregulation in colon carcinogenesis. Elements involved in growth regulation that have been clearly shown to be controlled in part by TGF-β include the cyclin-associated proteins cyclin D1, cdk4, p21, p27, p15, and Rb *(154–159)*. C-myc is also a downstream target of TGF-β and has been shown to be transcriptionally repressed in MvLu1 cells after treatment with TGF-β1 *(158,160)*. In addition to the cyclin-associated proteins, the extracellular matrix proteins and regulators of extracellular matrix proteins, fibronectin, tenascin, and plasminogen activator inhibitor 1, also appear to be regulated by TGF-β *(161,162)*.

The disruption of the normal extracellular matrix production may play a role in tumor invasion. In support of this concept, *TGFBR2* mutations in MSI colon adenomas are only detected in areas of high-grade dysplasia or in adenomas with concurrent adenocarcinoma, suggesting that TGFBR2 inactivation promotes the malignant transition of colon adenomas to adenocarcinomas *(10)*. Furthermore, analysis of neoplasms that form in an in vivo mouse model that is knocked out for *Tgfbr2* in the colon (*Fabp*$^{4xat\text{-}132}$ Cre;*Tgfbr2*$^{flx/flx}$) suggest TGFBR2 inactivation promotes the progression of adenomas to adenocarcinomas *(7)*.

6.5.1. *TGFBR2*

A common mechanism through which colon cancers acquire TGF-β resistance is through genetic alterations of the *TGFBR2* gene. Functionally significant alterations of *TGFBR2* have been identified in up to 30% of colon cancers

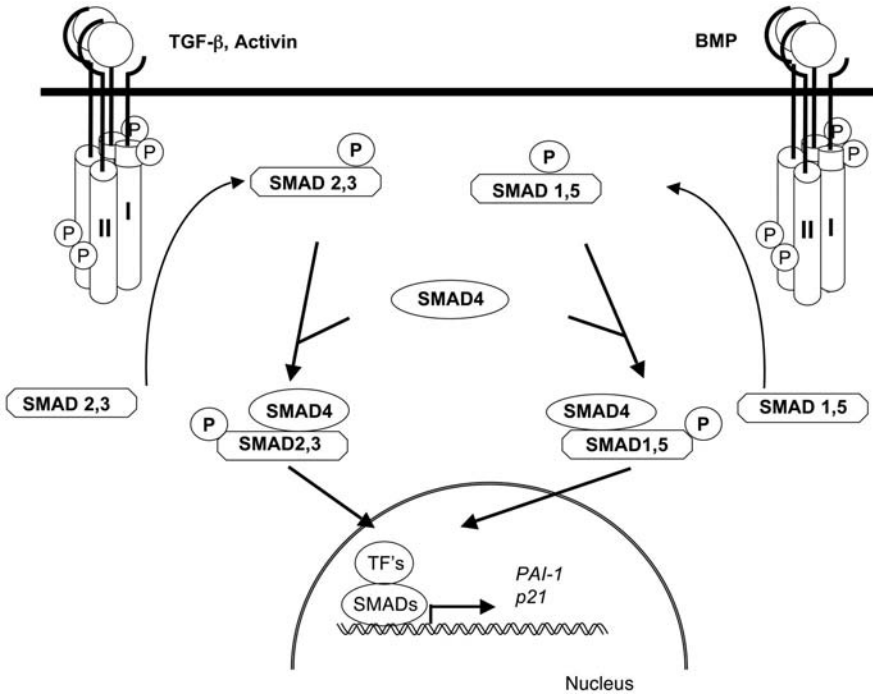

Fig. 5. TGF-β-SMAD signaling pathway diagram.

and are the most common mechanism identified to date for inactivating the TGF-β signaling pathway *(24,148)*. No alterations in *TGFBR1* or the type III TGF-β receptor (*TGFBR3)* have been observed in studies of TGF-β-resistant colon cancer cell lines, suggesting mutational inactivation of *TGFBR2* is a particularly favorable event that leads to tumor formation. Markowitz et al. have demonstrated that mutational inactivation of *TGFBR2* is an extremely common event in MSI colon cancers because *TGFBR2* has a microsatellite-like region in exon 3 that consists of a 10-base-pair polyadenine tract, making it particularly susceptible to mutation in the setting of MSI *(24,163,164)*. The mutations in this region, which has been named *BAT-RII* (Big Adenine Tract in TGF-β Receptor type II), are frameshift mutations that result in the insertion or deletion of one or two adenines between nucleotides 709 and 718, introducing nonsense mutations that encode a truncated TGFBR2 protein lacking the intracellular serine-threonine kinase domain *(24)*. In a series of 110 MSI colon cancers, 100 were found to carry *BAT-RII* mutations, and in almost all of these cases the mutations were biallelic consistent with the tumor suppressor function of TGFBR2 *(163)*. *TGFBR2*'s role as a tumor suppressor gene in colon cancer has been further elucidated by studies showing that reconstitution of wild-type *TGFBR2* in colon cancer cell lines with mutant *TGFBR2* suppresses the tumor

phenotype of the cell line *(148,165)*. Further support for *TGFBR2*'s role as a tumor suppressor gene in colon cancer in general was provided by the demonstration of *TGFBR2* mutations in colon cancer cell lines that are MSS. *TGFBR2* mutations have been found in 15% (n = 3/14) of TGF-β-resistant MSS colon cancer cell lines. These mutations are not frameshift mutations in *BAT-RII* but are inactivating missense in the kinase domain or putative binding domain of *TGFBR2 (148)*. In aggregate, the overall incidence of *TGFBR2* mutation in both MSS and MSI colon cancers appears to be 30% *(148)*. Interestingly, in a study of colon cancer cell lines, the incidence of TGF-β resistance was found to be 55% despite frequently having wild-type *TGFBR1* and *TGFBR2 (148)*. These cancers have presumably inactivated the TGF-β signaling pathway through genetic or epigenetic alterations in post-receptor signaling elements, further underscoring the significance of the TGF-β signaling pathway in colon cancer formation.

6.5.2. *SMAD2* AND *SMAD4*

LOH occurs commonly at 5q, 18q, and 17p in colon cancer and suggests that there are tumor suppressor genes at these loci. LOH of chromosome 18q occurs in approximately 70% of colon adenocarcinomas. The incidence of 18q LOH is approx 10% in early-stage colon adenomas and 30% in later-stage, larger adenomas, demonstrating that the incidence of LOH involving 18q increases through the adenoma-carcinoma sequence *(68,122)*. A region of deletion on 18q that is shared among colon cancers that demonstrate allelic loss involving a contiguous segment of 18q has been observed and is the locus of a number of tumor suppressor genes implicated in colon cancer formation, including *DCC*, *SMAD2*, and *SMAD4*. All of these genes have been shown to be mutated in CRCs *(166–168)*. Other genes that are candidate tumor suppressor genes and map at 18q21-qter include *BCL-2*, gastrin-releasing peptide, and the cellular homolog of *YES-1*; however, none of these have been shown to be altered in CRCs *(169)*.

The most likely tumor suppressor genes that are the targets of 18q LOH are *SMAD2*, *SMAD4*, and *DCC*. The Smad proteins are a family of proteins that serve as intracellular mediators to regulate TGF-β superfamily signaling. The Smad proteins compose an evolutionarily conserved signaling pathway that has been demonstrated *in Caenorhabditis elegans*, *Drosophila melanogaster*, *Xenopus*, and humans. These proteins are characterized by two regions that are homologous to the *Drosophila* ortholog, Mad, and that are located at the N- and C-termini of the protein. These regions are termed the Mad-homology domains MH1 and MH2, respectively, and are connected by a less well-conserved, proline-rich linker domain. Numerous studies have identified three major classes of Smad proteins: (1) the receptor-regulated Smads (R-Smads), which are direct targets of the TGF-β receptor family type I kinases and include Smads1, 2, 3,

and 5; (2) the common Smads (Co-Smads: Smad4), which form heteromeric complexes with the R-Smads and propagate the TGF-β-mediated signal; and (3) the inhibitory Smads (I-Smads: Smad6 and Smad7), which antagonize TGF-β signaling through the Smad pathway. Ligand binding to the TGF-β receptor complex results in TGF-β receptor type I mediated phosphorylation of Smad2 and Smad3 on two serine residues in a conserved –SS(M/V)S motif located at the C-terminus of the R-Smads *(170,171)*. Phosphorylation of these serine residues is required for downstream signaling pathway activation *(172,173)*.

In light of the known tumor suppressor effects of the TGF-β signaling pathway and the role the Smad proteins play in propagating this signal, it is not surprising that alterations of some of the *SMAD* genes have been found in colon cancer. Mutational inactivation of *SMAD2* and *SMAD4* has been observed in a high percentage of pancreatic cancers and in 5–10% of colon cancers *(167,168,174,175)*. *SMAD4* alterations have been found in up to 16% of colon cancers *(167)*. The effect of these mutations on colon carcinogenesis is being investigated in a number of different animal models. One murine model, a compound heterozygote $Smad4^{-/+}/Apc^{\Delta 716}$, develops colon cancer unlike the $Apc^{\Delta 716}$ mouse, which only develops small-intestinal adenomas *(176)*. This model suggests that *SMAD4* inactivation may play a role in the progression of colon cancers. However, in some contexts *SMAD4* mutations also appear to initiate tumor formation and to contribute to tumor initiation while in a state of haploid insufficiency. Old $Smad4^{-/+}$ mice develop gastric and intestinal juvenile polyps and invasive gastric cancer, however, they do not appear to develop colon cancer *(177,178)*. Furthermore, germline mutations in *SMAD4*/*MADH4* have been found in approximately one-third of individuals with Juvenile Polyposis (JPS), an autosomal dominant syndrome characterized by gastrointestinal hamartomatous polyps and an increased risk of gastrointestinal cancer, consistent with the concept that haploid insufficiency of *SMAD4* may contribute to tumor initiation *(179–181)*. Importantly, however, the polyps observed in JPS and the invasive cancers in the $Smad4^{-/+}$ mouse have been shown to have allelic loss of *SMAD4*, supporting the idea that biallelic inactivation of *SMAD4* is needed for cancer formation *(178,182)*. Taken together, these studies suggest that *SMAD4* is a tumor suppressor gene in colon cancer and is one of the targets of 18q LOH. However, given the frequency of 18q LOH vs detected *SMAD4* mutations or deletions, there are likely other tumor suppressor loci on 18q21.

Although also located at 18q21 and presumably a target for inactivation in colon carcinogenesis, mutations in *SMAD2* occur infrequently in colon cancer and have been found in only 0–5% of cancers *(168,175,183)*. The other *SMAD* genes do not appear to be frequently altered in colon cancer, despite the fact that *SMAD3* and *SMAD6* are located on chromosome 15q21–22, which is a frequent site of allelic loss in colon cancer *(175,184,185)*. Interestingly, and in contrast to the studies of human colon cancer, $Smad3^{-/-}$ mice have a high frequency of

invasive colon carcinoma, but Smad2 inactivation does not appear to substantially affect intestinal tumor formation in mouse models *(186,187)*. In conclusion, *SMAD* mutations appear to play a role in tumor formation in a subset of colon cancers, but are not as common as *TGFBR2* mutations. This observation raises the possibility that there are non-Smad TGF-β signaling pathways that play an important role in the tumor suppressor activity of *TGFBR2*.

The effect of 18q LOH, and thus presumably inactivation of the tumor suppressor genes at this locus, on the clinical behavior of colon carcinomas has been subjected to intense scrutiny with inconclusive results to date. Several different groups have assayed for LOH of 18q using microsatellite markers in stage II colon cancer and have found either no association with the clinical behavior of the cancer or an association with more aggressive cancer behavior *(169,188–191)*. The reason for the discrepancy is unclear but may be related to different microsatellite loci assessed in each study and thus the specific region of 18q that was assessed by each investigator. Adding to this confusion, *SMAD4* diploidy and *TGFBR2 BAT-RII* mutations have been shown to associate with improved survival after adjuvant chemotherapy *(36,192)*.

6.6. TGF-β Superfamily Receptors: ACVR2 and BMPR1A

The TGF-β superfamily includes not only TGF-β1, TGF-β2, and TGF-β3, but also the BMPs (bone morphogenetic proteins), activin, nodal, growth and differentiation factors, and inhibin. The identification of germline mutations in signaling elements of the BMP signaling pathway in individuals with JPS, a hereditary colon cancer syndrome, and somatic mutations in the activin receptor in colon cancers has globally implicated deregulation of the TGF-β superfamily in the pathogenesis of colon cancer. Germline mutations in *MADH4*/*SMAD4* and *BMPR1A*, a type I receptor for BMPs, in families with JPS has implicated inactivation of BMP signaling in this subset of hereditary colon cancers. Howe et al. found nonsense and missense germline mutations in *BMPR1A* in four families with JPS, 44-47delTGTT, 715C>T, 812G>A, and 961delC affecting exons 1, 7, 7, and 8, respectively *(193)*. *MADH4*/*SMAD4* germline mutations have been found in 5–56% of families with JPS *(179,194)*.

The BMPs are disulfide-linked dimeric proteins that number at least 15 in total and include BMP-2, BMP-4, and BMP-7 (OP-1). They have a wide range of biological activities, including the regulation of morphogenesis of various tissues and organs during development, as well as the regulation of growth, differentiation, chemotaxis, and apoptosis in monocytes, epithelial cells, mesenchymal cells, and neuronal cells *(195)*. The BMPs transduce their signals through a heteromeric receptor that consists of a type I and type II receptor. BMPR1A is one of two different type I BMP receptors (BMPR1A and BMPR1B). It serves to predominantly bind BMP-4 and BMP-2 as well as other BMPs and transduces their signals when partnered with a BMP type II receptor.

As with the TGF-β receptor, the best understood post-BMP receptor pathway is the Smad pathway. The R-Smads, Smads 1 and 5, partner with Smad4 (Co-Smad) to transduce BMP-mediated signals from the BMP receptors *(195)* (Fig. 5). Thus, the identification of both *BMPR1A* and *MADH4*/*SMAD4* germline mutations in families with JPS strongly implicates BMP signaling disruption in the pathogenesis of this syndrome. Furthermore, mice that over-express Noggin, a soluble antagonist for the BMPs, or a dominant negative *Bmpr1a* in the intestinal epithelium, display ectopic crypt formation and a phenotype reminiscent of JPS *(196,197)*.

With regards to activin, activin is a secreted dimeric ligand, composed of either Activin βA and/or Activin βB, that activates intracellular signaling pathways, including the SMAD2/3-SMAD4 pathway, via a heteromeric receptor that is composed of a type I receptor (ACVRL1, ActRIA, or ActRIB) and a type II receptor (ACVR2 or ACVR2B) *(198)*. Mutations in *ACVR2* have been found to occur in 58–90% of MSI colon cancers as the result of a polyadenine tract in the coding region of the gene *(199,200)*. The identification of mutations that affect activin, TGF-β, and BMP signaling broadly implicate the TGF-β family as a tumor suppressor pathway in colon cancer.

6.7. Genes Associated With Colorectal Metastases

One of the clear challenges in cancer biology is the identification of genes that contribute to the metastatic and lethal cancer phenotype. Intense investigation in this area has led to the identification of promising candidate genes that may influence the metastatic potential of the primary colon cancer. PRL3, a phosphatase, was found overexpressed in 12 of 12 colon cancer liver metastases, but not in matched colon cancer primaries from the same patients *(201)*. Moreover, in 3 of 12 cases, PRL3 overexpression was accompanied by marked *PRL3* gene amplification, suggesting that *PRL3* overexpression is a primary genetic event selected during metastasis. Osteopontin is a protein that also appears to have potential to predict the metastatic potential of CRC. Osteopontin was identified through a global screen using expression arrays and is 15-fold overexpressed in primary colon cancers and 27-fold overexpressed in liver metastases *(202)*. Osteopontin is a phosphoglycoprotein that can bind to several integrins, as well as CD44, and has been shown to contribute to the malignant phenotype in breast cancer *(202,203)*. To date, neither PRL3 or osteopontin has been shown to have the ability to predict the metastatic potential of CRC in a prospective clinical trial.

7. CONCLUSION

Investigation of the molecular pathogenesis of CRC has yielded many insights into the mechanisms driving the tumorigenesis process and to the

identification of many potential therapeutic targets. Key insights from the assessment of the molecular genetics and epigenetics of colon cancer include the multistep nature of carcinogenesis, the central role of tumor suppressor pathways, the role of DNA repair genes and genomic stability in cancer formation, and the role of TGFβ signaling in tumor suppression. Nonetheless, many challenges remain. The molecular genesis of the metastatic phenotype that directly accounts for cancer lethality remains unknown. A mechanistic understanding of the basis of chromosomal instability, aneuploidy, and aberrant methylation of the cancer genome has yet to be achieved. In addition, the translation of molecular genetics to new diagnostic, prognostic, and therapeutic modalities appears promising but has yet to have a major impact on the clinical management of CRC. The promise for the future is that this field of inquiry will yield the important answers to these and other key questions.

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2 Chemoprevention of Colorectal Cancer

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Summary

This article emphasizes current understanding of the multistep process in colon carcinogenesis and discusses the promising strategies of targeting disruption of β-catenin-mediated signaling in colon epithelial cells. The 1,2-dimethylhydrazine (DMH)/azoxymethane (AOM) model of chemically induced colorectal cancer (CRC) and the murine multiple intestinal neoplasia (Min) model have provided useful information about the efficacy of available chemoprevention agents for CRC.

Clinical trials have determined that several classes of agents can reduce polyp incidence and, by extension, may defer the appearance of colon cancer. The most important ones are nonsteroidal anti-inflammatory drugs such as aspirin and selective cyclooxygenase-2 inhibitors such as celecoxib and calcium supplements. Preclinical and epidemiology evidence suggests that statins, eflornithine, ursodeoxycholic acid, selenium, folate, and estrogen may reduce polyps and prevent CRC. Neither increased fiber intake nor antioxidant supplements are associated with reduced polyp/cancer outcomes in carefully controlled clinical trials.

Patients who are predisposed to early onset of CRC may benefit from specific chemoprevention therapy: ulcerative colitis (5-aminosalicylic acid) and familial adenomatous polyposis (celecoxib or sulindac). Several promising agents are under study: curcumin, inulin derivatives, epidermal growth factor inhibitors, statins and nitric oxide-releasing nonsteroidal anti-inflammatory drugs.

Key Words: Chemoprevention; colon cancer; animal models; clinical trials.

1. INTRODUCTION

The colon is an ideal target organ in which to develop chemopreventive interventions for a number of reasons. First, it is estimated that colorectal cancers develop over a period of 10–20 yr, providing a large window of opportunity for

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therapeutic intervention. Second, the relative sequence of genetic events required for tumor formation has been investigated most extensively in the colon *(2,3)*. Third, the established histopathological progression of normal tissue to an intermediate adenoma and, ultimately, invasive cancer presents milestones with respect to where a particular lesion is in the carcinogenic sequence, both in the presence and absence of chemopreventive agent exposure *(4)*. Fourth, the adenomatous polyp serves as a preneoplastic marker of colorectal cancer risk, aiding in the identification of the subpopulation of individuals who would benefit most from chemopreventive therapy. Finally, unique insight can be gained from evaluating chemopreventive response in individuals known to carry germline mutations that predispose them to such familial colorectal syndromes as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer.

Based on our current understanding of the multistep process of colorectal carcinogenesis, we can begin to identify potential points for intervention. Among the earliest known genetic alterations in colorectal cancer development are inactivating mutations in the tumor suppressor gene adenomatous polyposis coli (*APC*) and activating mutations of the *K-ras* oncogene. These alterations are associated with the formation of early and intermediate adenomas, respectively. Alteration of the transcription factors Smad2 and Smad4 appear to mediate the transition to late adenoma. Finally, mutations in the tumor suppressor gene *p53* pave the way for transformation of adenomas into malignant cancers. Additional alterations that occur early in colorectal tumorigenesis include genome-wide DNA hypomethylation and genomic instability, via defects in chromosome segregation or DNA replication fidelity.

Early changes within the colonic epithelium that persist throughout tumorigenesis represent ideal targets for chemopreventive intervention. Additional characteristics that make a potential molecular event an attractive target have been reviewed recently by Hawk and Levin *(5)*. These include (1) differential expression of the marker in neoplastic and normal tissue, (2) knowledge of its functional significance, (3) overexpression of the marker in the neoplastic state such that expression can be downregulated rather than replaced when absent, (4) pharmacologic accessibility, and (5) an established correlation between modulation of the marker and tumor reduction. Finally, appealing targets are typically characterized by overexpression or overactivity because molecular functions are more easily inhibited than replaced *(6)*.

One of the most promising strategies for early chemopreventive intervention in the colorectal carcinogenesis sequence is targeted disruption of β-catenin-mediated signaling. Low levels of cellular β-catenin are maintained in normal cells via the competitive binding of β-catenin to APC and E-cadherin, a calcium-dependent cell adhesion molecule thought to act as an "invasion suppressor." Complexing of β-catenin with the scaffold protein axin and the serine/threonine glycogen synthase kinase 3β (GSK3β) facilitates the phosphorylation of β-catenin by GSK3β

and targets its ubiquitin-mediated proteosomal degradation (Fig. 1). In contrast, inactivation of GSK3β by Wnt signals, mutational activation of β-catenin, and truncation of *APC* lead to the accumulation of β-catenin in the cytoplasm. Once stabilized, β-catenin is translocated to the nucleus where it cooperates with members of the TCF/LEF family of transcription factors *(7,8)* to activate the transcription of downstream target genes including *c-myc (9)*, cyclin D1 *(10)*, peroxisome proliferator-activated receptor (PPAR) delta *(11)*, multidrug resistance protein 1 *(12)*, cyclooxygenase 2 *(13)*, and immunoglobulin transcription factor-2 *(14)*. cDNA array analyses indicate that induction of dominant negative *TCF*-4 in colon carcinoma cells leads to the differential expression of more than 200 genes *(15)*. Based on these data, van de Wetering and colleagues *(15)* have coined the term "master switch" to describe the capability of the β-catenin/TCF-4 complex to effectively regulate the balance between proliferation and differentiation in both non-neoplastic and malignant intestinal epithelial cells.

Although our understanding of the colon carcinogenesis sequence is in general more advanced than that of many other cancer types, the establishment of an efficacious chemopreventive regimen has been severely hindered by the lack of accurate and sensitive biomarkers of chemopreventive response. Because tumor formation cannot be used as an endpoint for clinical investigation, it is essential that intermediate endpoints that can predict with high sensitivity and specificity the future progression and invasive potential of malignant cells be identified. The endpoints most frequently studied in current clinical colorectal chemoprevention trials are polyp number and polyp recurrence, often complemented by correlative measures of cell proliferation and apoptosis. Based upon the generic nature of these biochemical pathways, significant attention is currently focused on invaluable model systems where the molecular events associated with colorectal carcinogenesis and its inhibition can be interrogated extensively under controlled conditions.

2. ANIMAL MODELS OF COLORECTAL CARCINOGENESIS

One of the most widely used animal models for assessing the efficacy of chemopreventive agents against colorectal cancer is the 1,2-dimethylhydrazine (DMH)/azoxymethane (AOM) model of chemically induced colorectal cancer. DMH requires metabolic activation in vivo to AOM, which is then converted to the ultimate carcinogen methylazoxymethanol (MAM). In rats, AOM is injected i.p. once a week for 2 wk *(16)*. One week later, animals are randomized to control and experimental drug treatment groups. Aberrant crypt foci (ACF), a putative cancer precursor, can be identified 14 wk following AOM injection *(17)*. Gross colonic tumors (one per animal) are present 40 wk post-AOM. In mice, female CF1 mice are given MAM i.p. four times in 11 d (low dose) or eight times in 22 d (high dose). Colon tumors are observed within

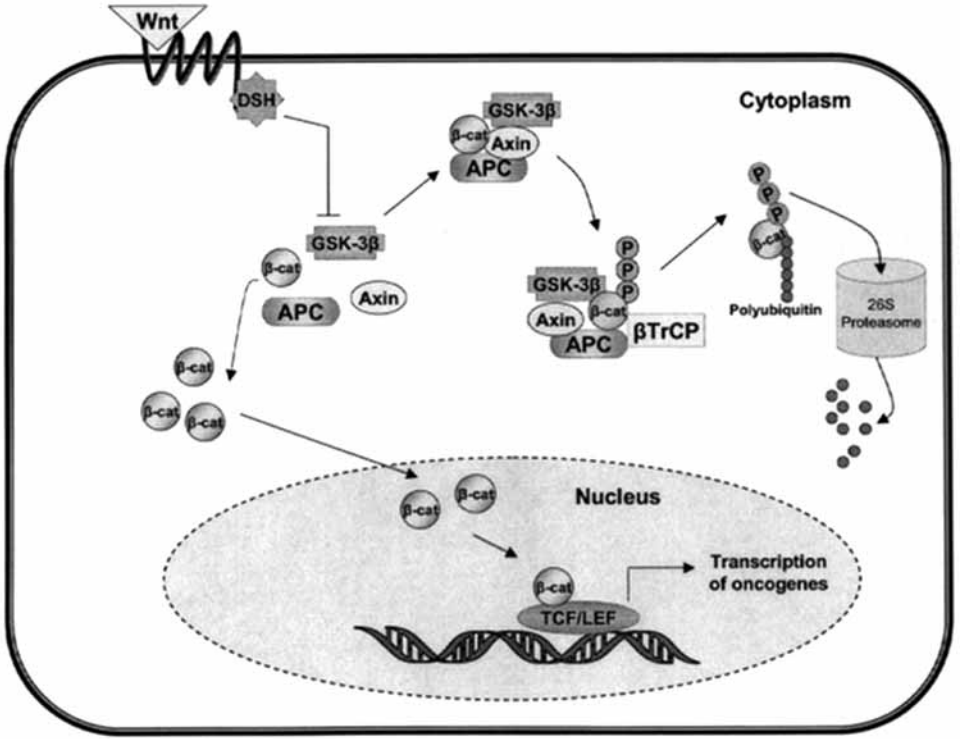

Fig. 1. Role of APC/β-catenin Signaling in Colorectal Carcinogenesis. In the absence of Wnt signals, low levels of cellular β-catenin are maintained via interaction of APC with β-catenin, glycogen synthase kinase-3β (GSK3β) and Axin to facilitate the phosphorylation of β-catenin by GSK3β and lead to the targeted degradation of β-catenin. In contrast, inactivation of GSK3β by Wnt signals, mutational activation of β-catenin and truncation of *APC* lead to the accumulation of β-catenin in the cytoplasm. Once stabilized, β-catenin is translocated to the nucleus where it cooperates with members of the TCF/LEF family of transcription factors to activate the transcription of downstream oncogenes.

38 wk after dosing *(18)*. There are several advantages to using the DMH/AOM model of colon carcinogenesis for chemoprevention studies. First, experimentation to date indicates that the promotional and protective effects of experimental diets can be discriminated in this model *(19,20)*. Second, the evolution of colon tumors in the DMH/AOM model is similar to that in humans, including the progression of ACF to adenomas (often polyps), and ultimately carcinomas. Third, the histopathological features of DMH/AOM-induced colon tumors are similar to those of human tumors. Finally, 30–60% of DMH/AOM-induced colon tumors possess *K-ras* mutations as seen in human colon tumors. The pitfalls of using the DMH/AOM model system to study colorectal carcinogenesis include the fact that both DMH and AOM are carcinogens to which humans are not exposed either environmentally or in their diet. Furthermore, unlike human colon tumors, DMH/AOM-induced tumors seldom exhibit mutations in either *Apc* (approx 8%) or *p53*. However, nuclear localization of β-catenin is observed in AOM-induced colon tumors owing to mutations of β-catenin.

Another animal model used frequently for evaluating the efficacy of chemopreventive agents is the murine model of multiple intestinal neoplasia (*Min*). Conventional *Min* mice carry a germline mutation in the *Apc* gene, which results in a premature translational stop codon at amino acid 850 *(21)*. Since their discovery, *Min* mice have been used widely for chemoprevention studies for several reasons. First, *Min* mice spontaneously develop intestinal tumors at 60–90 d of age. Second, COX-2 and iNOS play an important role in intestinal tumorigenesis in this model as in humans. When the genes for either COX-2 or iNOS were deleted in *Min* mice, few intestinal tumors were observed *(22–24)*. Third, similar to humans, a reduction in DNA methyltransferase activity in *Min* mice suppresses polyp formation *(22,23)*. As with the DMH/AOM model, the *Min* mouse model carries some disadvantages for chemopreventive analyses. The relevance of this model to the study of human colorectal cancer remains uncertain because of the predominance of small intestinal lesions and few, if any, colorectal tumors, as well as no invasive colorectal carcinomas. In addition, mutations in *K-ras* and *p53* are not detected in intestinal tumors from *Min* mice *(25,26)*.

Currently, more than 400 studies have been performed using either the DMH/AOM or *Min* mouse model to assess the chemopreventive activity of synthetic or naturally occurring agents or diets against colorectal cancer (reviewed in *27,28*) Based on these studies, Corpet and colleagues have created a comprehensive database of agents and diets that have been tested to date and have ranked these agents based on their ability to inhibit colorectal cancer (http://www.inra.fr/reseau-nacre/sci-memb/corpet/indexan.html). Agents that afford strong protection against intestinal tumorigenesis in the DMH/AOM rat and/or *Min* mouse models include piroxicam, sulindac, celecoxib, difluoromethylornithine, polyethylene glycol, thiosulfonate, protease inhibitor, sphingomyelin, epidermal growth factor receptor kinase inhibitor, resveratrol, fish oil, curcumin, and calcium.

3. CLINICAL TRIALS FOR THE CHEMOPREVENTION OF COLORECTAL CANCER

Once promising chemopreventive agents have been identified through observational and in vivo efficacy studies or have exhibited evidence of in vitro molecular targeting *(6)*, these agents are then subjected to examination in clinical trials. The long-term goals for the clinical chemoprevention of colorectal cancer include at least one of the following: (1) An additive clinical benefit when combined with colonoscopic colorectal polyp screening/surveillance; (2) an alternative to current colonoscopic screening/surveillance guidelines; (3) improvement in the overall risk profile for the development of serious adverse events or death linked to colonoscopy or polypectomy; and (4) an improvement in colorectal cancer rates in individuals who do not or are unable to comply with standard screening and surveillance recommendations *(6,29)*.

A significant amount of knowledge has been gleaned from the colorectal cancer chemoprevention trials that have been performed to date, data that has proven invaluable in establishing the guidelines for future colon trials. In general, 25 to 35% reduction in adenoma formation represents a reasonable minimum threshold of effect. Positive trials should yield supportive data, including an increase in the number of adenoma-free patients and/or reductions in adenoma size and histopathologic grade. It is essential that trials be well controlled, have adequate compliance, and be 3 to 6 yr in duration so that issues of safety and tumor recurrence upon agent withdrawal can be addressed.

4. CHEMOPREVENTIVE AGENTS TESTED IN HUMANS FOR THEIR EFFICACY AGAINST COLORECTAL CANCER

4.1. Nonsteroidal Anti-Inflammatory Agents

Nonsteroidal anti-inflammatory agents (NSAIDS) are among the most well described chemopreventive agents. Cyclooxygenase (COX)-1 and -2 catalyze the conversion of arachadonic acid to the intermediate PGG2 and then to PGH2. PGH2 is then metabolized to thromboxane and other prostaglandins, which affect various physiologic functions. Whereas COX-1 produces constitutive prostanoids used for normal tissue functions such as platelet aggregation and gastric mucosal protection, COX-2 is inducible, with expression increasing during inflammation and neoplasia *(30)*.

Aspirin, like most nonselective NSAIDs, works to competitively inhibit the active binding site on both COX-1 and COX-2. Side effects of NSAIDs, including renal, gastrointestinal, and antiplatelet effects, are attributed to inhibition of COX-1, whereas their anti-inflammatory activity is a result of their ability to inhibit COX-2 *(31,32)*. Cancers of most organs, including colon, bladder,

breast, liver, and lung express increased levels of COX-2 as compared to the non-neoplastic adjacent tissue, making the COX-2 gene an important target in the study of carcinogenesis *(30)*.

The molecular basis for the activity of NSAIDs in the prevention and treatment of cancer is thought to be pleiotropic. COX-2 overexpression has been shown to increase factors associated with angiogenesis, a mechanism that can be blocked by selective COX-2 inhibitors and some nonselective COX inhibitors. COX-2 overexpression also inhibits apoptosis, a condition that may be reversed by NSAIDs. Animal studies have shown that chemical inhibition or elimination of COX activity results in decreased or slower tumor formation *(30)*.

Four randomized colorectal cancer chemoprevention studies have been published that compare the effect of aspirin administration vs placebo. In the Physicians' Health Study of 22,071 healthy men, aspirin intake did not provide protection against colorectal cancer. *(33)*. However, three smaller studies of aspirin vs placebo in patients with previous adenomas have reported protective effects on subsequent adenoma formation *(34–36)*. Interestingly, the study by Baron et al. *(35)* found that the adenoma prevention occurred in patients taking 81 mg of aspirin, rather than 325 mg. The cause and implication of this inverse dose response is unclear.

4.2. COX-2-Selective Inhibitors

Despite promising results of studies involving nonselective COX inhibitors, concerns about the gastrointestinal and antiplatelet effects of NSAIDs have limited their use as chemopreventive agents. Selective COX-2 inhibitors such as celecoxib and rofecoxib were initially thought to be promising chemopreventive agents based on their favorable gastrointestinal toxicity profile.

The Adenomatous Polyp Prevention on Vioxx (APPROVe) trial randomized 2586 patients with a history of adenomatous polyps to either Vioxx (rofecoxib) (25 mg daily) or placebo in order to determine if rofecoxib would reduce the risk of recurrent neoplastic polyps. However, the study was closed prematurely when the investigators found an increased risk of thrombotic events associated with long-term use of rofecoxib (response rate [RR] 1.92, 95% confidence interval [CI] 1.19–3.11) *(37)*. Based on these data, the Merck Pharmaceutical Company subsequently withdrew rofecoxib from the market.

The results of the APPROVe study prompted a review of the adverse events recorded in a similar study, the Adenoma Prevention With Celecoxib (APC) trial. In this study, 2035 patients with a personal history of previous neoplastic polyps were randomized to receive either celecoxib (200 mg or 400 mg twice daily) or placebo. A dose-dependent increase in cardiovascular disease, myocardial infarction, stroke, or heart failure was observed among the patients who took celecoxib. The attributable risk for death from cardiovascular disease compared to placebo in the 200 mg twice daily group: 2.3 (95% CI 0.9–5.5); 400 mg twice daily group:

3.4 (95% CI 1.4–7.8) *(38)*. As a result, the APC study was also terminated early at the recommendation of the external data safety monitoring board.

The results of these large randomized studies suggest that the increased cardiovascular risk associated with the use of rofecoxib and celecoxib is likely an effect specific to this chemical class of agents, thus limiting the application of these drugs in the chemopreventive setting for average- to moderate-risk individuals.

4.3. Statins

Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase and are widely used in the management of hyperlipidemia. Clinical trials evaluating the relationship between statins and cardiovascular disease have produced conflicting data on the drugs' effects on incidence of cancer. Results from the Molecular Epidemiology of Colorectal Cancer study, a population-based case control study in Northern Israel of 1953 patients with colorectal cancer and 2015 controls, indicate that at least 5 yr of statin use reduces the relative risk of colorectal cancer (odds ratio [OR] 0.5, 95% CI 0.4 to 0.63) *(39)*. This study supports further investigation into the appropriate dose, optimal length of treatment, and most active type of statin. The potential benefit of using this class of compounds, as well as their well-tolerated toxicity profile, make these agents particularly attractive for chemoprevention. However, placebo-controlled trials may be difficult to conduct as the number of patients who are prescribed these medications for treatment for cardiovascular disease continues to increase *(40)*.

4.4. Eflornithine

D,L-alpha-difluoromethylornithine (DFMO or eflornithine) irreversibly inhibits ornithine decarboxylase (ODC), which is the first and rate-limiting step in polyamine synthesis. ODC and polyamines are both elevated in colorectal cancer and adenomatous polyps *(41)*. Earlier studies of eflornithine at high doses (3 gm/[m^2 · d]) did not demonstrate significant clinical benefit, and its administration was also limited by high rates of gastrointestinal and hematological toxicity as well as ototoxicity. However, interest in this drug as a chemopreventive agent prompted phase I studies to determine the lowest effective dose of eflornithine that would still inhibit ODC *(42)*. One study randomized patients with a history of resected colon polyps to three dose levels of eflornithine and found that a dose of 0.2 g/m^2/d suppressed polyamine levels in rectal mucosa at doses with minimal side effects *(43)*. A review by Meyskens et al. *(44)* proposed that future colorectal cancer chemoprevention trials with eflornithine use doses of 0.2–0.4 g/m^2. However, because eflornithine does not completely suppress tumorigenesis, there is ongoing interest in combining it with other agents such as NSAIDs *(44)*.

4.5. Ursodeoxycholic Acid (UDCA)

UDCA is indicated for the treatment of gallstones and primary biliary cirrhosis. A recently published phase III study randomized 1285 patients with personal histories of adenoma removal to either UDCA or placebo for 3 yr or until the time of follow-up colonoscopy. Although the odds ratio of the rate of adenoma recurrence was not statistically significant, a significant reduction in high-grade adenomas was observed in the treatment arm. (adjusted OR 0.61, 95% CI 0.39–0.96) *(45)*. Because high-grade lesions often progress to cancer, the potential therapeutic benefit, along with its favorable side-effect profile, make this an intriguing agent for future chemoprevention research.

4.6. Calcium

Calcium is thought to reduce the risk of colorectal cancer by binding bile acids in the lumen of the bowel and inhibiting their proliferative and carcinogenic effects. A systematic meta-analysis of three randomized trials that evaluated the use of calcium in patients with previous adenomas has been performed. Of the 1279 patients who completed these three trials, a significant reduction in risk of recurrent adenomas was observed in the treatment groups (RR 0.8, CI 0.68–0.93) *(46)*. The largest of these three studies evaluated 930 patients with previous adenomas following treatment with either 3 g of calcium carbonate or placebo daily. Compared to the placebo group, the patients in the treatment group had an adjusted risk ratio for recurrent adenomas of 0.85 (95% CI 0.74–0.98) *(47)*. Given the low cost and low risk associated with calcium supplementation, as well as the potential benefit of osteoporosis prevention, some experts advocate its use as an adjunct to surveillance colonoscopies in patients with previous adenomas *(48)*.

4.7. Selenium

Selenium is a trace element that occurs in meats and grains. Its potential anticancer mechanisms include the induction of apoptosis, protection from oxidative DNA damage, and increased immune system function *(49)*. In a secondary analysis of a randomized study of selenium vs placebo in the prevention of nonmelanoma skin cancer, the treatment group was found to have a statistically significant decrease in colorectal cancer *(50)*, though these differences did not persist after additional years of follow-up *(51)*. A pooled analysis of the results of three randomized studies (the Wheat Bran Fiber Trial, the Polyp Prevention Trial, and the Polyp Prevention Study) revealed an inverse association between higher blood selenium concentration and recurrent adenomas in patients with previous adenomas. This analysis found that the patients with the highest selenium levels had statistically significantly lower odds of developing recurrent adenomas compared to those with the lowest levels *(49)*. These results support further investigation of the use of selenium as a chemopreventive agent.

4.8. Folate

Because folate is necessary for DNA synthesis, it has been hypothesized that a folic acid deficiency may lead to cancer. An inverse relationship between folate and the risk of colon cancer was found in the Nurses Health Study, particularly in women with a first-degree relative with disease *(52,53)*. An analysis of participants in the Wheat Brand Fiber trial also revealed a lower incidence of adenomatous polyp recurrence in patients with a higher self-reported folate intake (OR 0.61, 95% CI 0.42–0.89) and plasma folate concentrations (0.66, 95% CI 0.46–0.97) *(54)*. Folate is under investigation in several ongoing and recently completed studies.

4.9. Estrogen

The chemopreventive activity of postmenopausal hormone replacement therapy (HRT) against colorectal cancer risk is thought to be caused in part by the effect of estrogen on bile acids, estrogen receptors within the intestinal epithelium, and insulin and insulin-like growth factor I *(55)*. The Women's Health Initiative (WHI) found that HRT increased the risk of invasive breast cancer, cardiovascular disease, stroke, and pulmonary embolism in this group of 16,608 healthy postmenopausal women. The analysis also showed a decrease in the risk of colorectal cancer (HR 0.63, 95% CI 0.43–0.92) in the treatment group *(56)*. A separate analysis of the colorectal cancer data from the WHI found that the 43 invasive cancers in the treatment group and 72 cancers in the placebo group (HR 0.56, 95% CI 0.38–0.81) were of similar grade and shared histological characteristics in common. However, a greater number of cases with positive lymph nodes and metastatic disease were present in the treatment group *(55)*. Therefore, although there is a decreased overall risk of colorectal cancer with the use of HRT, it should not be used as a chemopreventive agent, given its risk–benefit profile.

5. AGENTS LACKING CHEMOPREVENTIVE ACTIVITY AGAINST COLORECTAL CANCER

5.1. Fiber

Two large randomized studies failed to find a benefit of a high fiber diet in reducing recurrent polyp formation. The Phoenix Colon Cancer Prevention Physicians' Network randomized 1429 patients with previously resected adenomas to dietary supplementation with either high (>13.5 g/d) or low (2 g/d) wheat bran fiber. No difference in recurrent adenoma formation was observed at a median follow-up time of 34 and 36 mo, respectively *(57)*. In the Polyp Prevention Trial 2079 patients with previous adenomatous polyps were randomized to intensive dietary counseling with a low-fat, high fiber (18 g of dietary fiber/1000 kcal) diet plus fruits and vegetables or observation. Again, there was no difference in the rate of recurrent adenoma

formation, or the number of large or advanced adenomas detected by endoscopy *(58)*. At this point, there is insufficient data to support fiber as a chemopreventive agent.

5.2. Antioxidants

Antioxidant vitamins, such as vitamin C (ascorbic acid), vitamin E (tocopherols), and β-carotene are thought to prevent cancer by neutralizing free radicals, resulting in reduced oxidative damage, as well as stimulation of the immune system to inhibit tumorigenesis *(6)*.

The Polyp Prevention Study used a two-by-two factorial design to randomize 864 patients with previously resected adenomas to placebo, β-carotene alone, vitamins C and E, or β-carotene and vitamins C and E. The incidence of adenomas in patients receiving either β-carotene or vitamins C and E was comparable to that of the placebo control group *(59)*. The Alpha-Tocopherol and Beta-Carotene Cancer Prevention (ATBC) study randomized 50- to 69-yr-old Finnish male cigarette smokers ($N = 29{,}133$) to β-carotene, α-tocopherol, both agents, or placebo. Colorectal cancer incidence in the α-tocopherol arm and in the β-carotene arm were not significantly different *(60)*.

6. CHEMOPREVENTIVE AGENTS FOR PATIENTS AT HIGH RISK OF COLORECTAL CANCER

6.1. 5-Aminosalcylic Acid (5-ASA)

Patients with ulcerative colitis (UC) have an increased risk of colorectal cancer—2% at 10 yr, 8% by 20 yr, and 18% by 30 yr *(61)*. 5-ASA is a derivative of aspirin and is commonly used to treatUC. It has been proposed as a potential chemopreventive agent in this high-risk patient population. A systematic review and meta-analysis of cohort and case control studies examining the relationship between 5-ASA and dysplasia or cancer in 1932 patients with UC showed a protective effect of treatment against cancer (OR 0.51, 95% CI 0.37–0.69), cancer and dysplasia (OR 0.51, 95% CI 0.38–0.69), but not dysplasia alone (OR 1.18, 95% CI 0.41–3.43) *(62)*. Unfortunately, a confirmatory randomized double-blind placebo-controlled study would be difficult to conduct in this population given the ethical difficulties in withholding 5-ASA in one arm, as well as the large sample size and long time frame required to conduct this trial. Because it is unlikely that such a trial will take place, some experts recommend that given the drug's safety and available evidence, it is reasonable to adopt this agent as an adjunct to secondary prevention of surveillance colonoscopy *(63)*.

6.2. Sulindac

Patients with FAP develop innumerable adenomas and virtually all will develop colon cancer in the absence of surgery. In a small randomized double-blind placebo-controlled study of patients with FAP who had not undergone

prior colectomy or had subtotal colectomy, sulindac was found to be effective in reducing both the size and number of polyps when administered for 9 mo. However, 3 mo after the discontinuation of therapy, patients treated with sulindac experienced an increase in both the size and number of polyps *(64)*. In a nonrandomized study, patients with FAP who had total colectomy with ileorectal anastomosis were treated with sulindac to prevent cancer within the rectal stump. The authors concluded that long-term treatment with sulindac was effective in reducing the size and number of polyps in the retained rectal segment *(65)*.

6.3. Celecoxib

In a study of 77 patients with FAP who had previous colectomy, cases were randomized to receive 100 mg celecoxib, 400 mg celecoxib, or placebo twice daily for 6 mo. The patients who were treated with high-dose celecoxib (400 mg twice daily) experienced a significant regression in the number of colorectal polyps *(66)*. Based on these data, the drug is approved by the FDA as an adjunct to endoscopic surveillance in patients with FAP.

7. NEW CHEMOPREVENTIVE AGENTS UNDER INVESTIGATION

7.1. Curcumin

Curcumin (diferuloyl-methane) is a low molecular weight polyphenol that is a major component of the yellow spice tumeric. It is thought to prevent cancer by suppressing COX-2 *(67)*, as well as glutathione S-transferase activity *(68)*. In a Phase I study, it was found to induce regressions of premalignant lesions of the skin, bladder, stomach, cervix, and oral mucosa with an acceptable toxicity profile *(69)*. One pilot study in humans found that oral curcumin was rapidly degraded to metabolites that exhibit less COX-2 inhibitory potential. Interestingly, dose-dependent reduction in COX-2 activity and prostaglandin E2 levels was reported *(67)*. Another study of patients with colorectal cancer showed that although peripheral blood levels may be low, high levels of curcumin glucuronide and curcumin sulfate are found in the rectal mucosa. However, this study did not show an increase in COX-2 levels *(70)*.

7.2. Inulin Derivatives

Inulin stimulates the growth of gut *Bifidobacterium*, which is thought to decrease intestinal genotoxins. When mice were treated with the inulin-like oligofructoses, *Lactobacillus* LGG and *Bifidobacterium* BB12 alone (probiotic), a combination of the two (symbiotic), or control, the prebiotic and symbiotic combinations reduced both DNA damage of the colonic mucosa as well as cancer incidence *(71)*.

Raftilose Synergy-1, an oral compound containing oligofructose and polyfructose chains, was studied in the recently completed Symbiotics and Cancer Prevention in Humans (SYNCAN) trial. This study was a double-blind, randomized, placebo-controlled trial of 80 patients with personal histories of either resected colon cancer or resected adenomatous polyps. Subjects were treated with a 12-wk course of a food supplement containing Synergy-1 or placebo. End points included biomarkers within the colonic mucosal and fecal water as well as immunological and inflammatory response markers. Preliminary results show that subjects in the treatment group had decreased DNA damage and cell proliferation *(72)*. Synergy-1 is currently being studied in a multi-center Phase II study in the United States using ACF as an end point.

7.3. Epidermal Growth Factor Receptor Inhibitors

Inhibitors of epidermal growth factor receptor tyrosine kinase (EGFR-TK) are thought to be chemoprotective through their effects on transforming growth factor-α, which increases as neoplasms progress from adenomas to *in situ* disease to invasive cancer *(73)*. Nearly half of the Min mice treated with a combination of sulindac and EKI-569, a newly developed EGFR inhibitor, showed reduced polyp formation *(73,74)*. When the Min mice were crossed with mice with significantly reduced levels of EGFR (the EGFRWA model), intestinal polyps were reduced by 90% as compared to those with the wild-type allele *(75)*. Both findings support the role of EGFR in intestinal polyp formation and carcinogenesis *(76)*.

7.4. Nitric Oxide-Releasing NSAIDs

Nitric oxide-releasing NSAIDs (NO-NSAIDs) are a class of anti-inflammatory agents that contain an NSAID molecule linked to a nitric oxide-donating group. Preliminary studies in animals and healthy humans suggest that these drugs may have less gastrointestinal toxicity than their traditional counterparts. In vitro studies indicate that NO-ASA, NO-sulindac, and NO-ibuprofen reduce colon cancer cell growth more effectively than their corresponding NSAIDs, making these potential chemopreventive agents worthy of further study *(77)*.

7.5. Future Directions in Colorectal Cancer Prevention Research

The therapeutic dilemmas surrounding the use of COX-2-selective inhibitors highlight several of the challenges in chemoprevention research. Potential agents must have both chemopreventive effects and acceptable risk–benefit profiles for the target population. As scientists continue to dissect both the molecular pathways altered during the colon carcinogenesis sequence and the mechanisms of action of potential chemopreventive agents using animal models, it is anticipated that it will be possible to combine agents at doses that are low enough to limit

toxicity while still preserving or enhancing their antitumor activity. Progress in the establishment of a clinical regimen for the chemoprevention of colorectal cancer continues to be hindered by the lack of validated biomarkers of cancer risk and chemopreventive response. These markers are essential to select a high-risk population who will benefit most from clinical intervention and to determine if use of a test agent confers significant protection from cancer, respectively. A number of strategies are currently being implemented in clinical trials to expedite the discovery of an efficacious regimen for the prevention of colon cancer. These include the use of ACF and other early neoplastic features (i.e., mutations in fecal DNA) as primary end points, "nesting" secondary prevention and toxicity end points within therapeutic trials, and establishing a cooperative network of leading gastroenterologists who are dedicated to the conduct of large, cost-effective clinical chemoprevention trials.

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3 Colorectal Cancer Screening and Surveillance

Arnold J. Markowitz, MD

Summary

Colorectal cancer (CRC) is the second most common cause of cancer death among American men and women. In 2005, it accounted for more than 145,000 new cancer cases, and resulted in more than 56,000 deaths. Currently available screening tests are effective in detecting early-stage CRC and its premalignant precursor lesion, the adenomatous polyp. Detection of early-stage disease at the time of cancer diagnosis is associated with significantly improved survival, with a 5-yr survival rate of 90% in those with localized disease. Removal of adenomatous polyps by colonoscopic polypectomy has been demonstrated to significantly reduce the incidence of developing CRC. Routine screening of asymptomatic average-risk individuals will result in a reduction in CRC incidence and mortality. Screening and surveillance recommendations should be based on the individual's CRC risk assessment. High-risk groups with hereditary cancer syndromes such as familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer should be referred for genetic counseling and offered appropriate genetic testing and specialized screening recommendations.

Key Words: Colorectal cancer screening; colorectal cancer surveillance; colonoscopy; adenomatous polyp; familial adenomatous polyposis; FAP; hereditary nonpolyposis colorectal cancer; HNPCC.

1. INTRODUCTION

In 2005, colorectal cancer accounted for more than 145,000 new cancer cases, and resulted in more than 56,000 deaths in the United States *(1)*. The cumulative lifetime risk of developing colorectal cancer in American men and women is approx 6% *(1)*. The detection of early stage disease at the time of diagnosis is associated with significantly improved survival, with a 5-yr survival rate of 90% in those patients diagnosed with localized disease *(2)*. The colonoscopic removal of adenomatous colorectal polyps, its premalignant precursor lesion, has been demonstrated to reduce the incidence of developing colorectal cancer *(3)*.

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Screening and surveillance recommendations for colorectal cancer are based on the individual's risk for development of the disease. Personal risk factors include age, prior history of colorectal adenomatous polyps or cancer, and a long-standing history of inflammatory bowel disease (i.e., ulcerative colitis or Crohn's colitis). An individual's family history of colorectal cancer or adenomatous polyps also significantly impacts their personal risk and screening recommendations. Finally, some individuals are at very high risk for colorectal cancer owing to an underlying hereditary predisposition syndrome, and require specialized recommendations in these cases.

This chapter will review current colorectal cancer screening modalities, individual risk stratification criteria, and currently recommended colorectal cancer screening and surveillance guidelines.

2. SCREENING TESTS

2.1. Fecal Occult Blood Test

The rationale for use of the fecal occult blood test (FOBT) is that detection of occult blood in stool may indicate an underlying colorectal cancer or adenomatous polyp. The support for FOB testing is primarily based on three large, prospective randomized controlled trials that have demonstrated a reduction in colorectal cancer mortality (Table 1). The US Minnesota trial demonstrated a 33% mortality reduction with annual FOB testing, and a 21% mortality reduction with biennial testing *(4,5)*, whereas two population-based European trials, from the United Kingdom and Denmark, demonstrated mortality reductions of 15 and 18%, respectively, with biennial FOB testing *(6,7)*. Furthermore, the Minnesota trial has also demonstrated a reduction in colorectal cancer incidence with FOB testing *(8)*.

2.2. Sigmoidoscopy

Flexible sigmoidoscopy provides direct visualization of the distal large bowel, and allows for biopsy of polyps and mass lesions. The effectiveness of screening sigmoidoscopy is based on two retrospective, case–control studies that demonstrated a reduction in distal colorectal mortality. In one study from California, screening rigid sigmoidoscopy demonstrated a 59% reduction in rectosigmoid cancer mortality, and this risk reduction benefit continued for 10 yr after a single screening examination *(9)*. In a second study from Wisconsin, screening sigmoidoscopy demonstrated an 80% reduction in rectosigmoid cancer mortality *(10)*.

Several prospective screening sigmoidoscopy trials are currently underway, including the US Prostate, Lung, Colorectal, and Ovarian (PLCO) screening trial *(11)*, the UK Flexible Sigmoidoscopy Screening Trial *(12)*, and the Norwegian Colorectal Cancer Prevention (NORCCAP) trial *(13)*.

Table 1
Prospective Controlled Trials of FOBT

Study	*N*	*Duration of Follow-up*	*Mortality Reduction*
Minnesota, 1993 *(4,5)*	46,551	13 yr	33%[a]
United Kingdom, 1996 *(6)*	152,850	7.8 yr	15%[b]
Denmark, 1996 *(7)*	140,000	10 yr	18%[b]

[a]annual FOBT (21% reduction with biennial FOBT).
[b]biennial FOBT.

2.3. Colonoscopy

Colonoscopy provides a complete direct examination of the entire large bowel, and allows for the removal of colorectal polyps and biopsy of mass lesions. Although there are currently no prospective randomized controlled trials to demonstrate that screening colonoscopy reduces colorectal cancer mortality or incidence in average-risk individuals, there is indirect evidence to support its effectiveness as a primary screening test.

Colonoscopy is felt to have played an essential role in the large FOBT screening trials that demonstrated a reduction in colorectal cancer mortality, in that it was used to evaluate those patients who tested positive for occult blood in their stool *(4–7)*. As colonoscopy is similar in both performance and effectiveness to sigmoidoscopy and evaluates the entire colorectum, it is felt that screening colonoscopy is likely to be even more effective that sigmoidoscopy, which has been demonstrated to reduce colorectal cancer mortality *(9,10)*. The US National Polyp Study (NPS) demonstrated that the removal of adenomatous polyps during colonoscopy, which offers the potential for colonoscopic polypectomy, significantly reduces the incidence of colorectal cancer *(3)*.

Furthermore, several recent studies have provided additional support for the effectiveness of screening colonoscopy in asymptomatic individuals by demonstrating that colonoscopy can detect significant proximal colonic neoplasia that is located beyond the reach of the flexible sigmoidoscope. A large multicenter US Veterans Administration cooperative trial of screening colonoscopy in 3196 asymptomatic individuals (97% men, mean age 62.9 yr), demonstrated that 2.7% of 1765 patients who had no distal colorectal polyps did have an advanced proximal colon neoplastic lesion (i.e., adenoma ≥1 cm, villous histology, severe dysplasia, or invasive cancer), and that 52% of the 128 patients with a proximal advanced neoplasm had no distal adenoma *(14)*. Similarly, another large study of 1994 men and women age 50 yr or older who underwent a screening colonoscopy as part of an employer sponsored screening program demonstrated that 2.5% of 1564 patients with no distal polyp had an advanced proximal neoplasm, and 46%

of the 50 patients with an advanced proximal neoplasm had no distal polyp *(15)*. Furthermore, the Colorectal Neoplasia Screening with Colonoscopy in Average-Risk Women at Regional Naval Medical Centers (CONCeRN) study, which evaluated screening colonoscopy in 1483 women of mean age 53.9 yr at four military medical centers, demonstrated that only 35.2% of women found to have advanced colorectal neoplasia would have had their lesion identified if they had undergone flexible sigmoidoscopy only *(16)*.

Finally, a prospective randomized US national screening colonoscopy trial, designed to investigate whether the performance of a single screening colonoscopy would be effective in decreasing the incidence and mortality of colorectal cancer in the average-risk general population, is currently underway.

2.4. Double Contrast Barium Enema

Double contrast barium enema (DCBE) is a radiological test that provides an evaluation of the entire large bowel. There are no randomized studies to evaluate the effectiveness of DCBE in reducing colorectal cancer mortality or incidence in average-risk individuals. DCBE has several significant drawbacks as a screening test. DCBE is not as sensitive for detecting small or flat colorectal polyp or mass lesions, and may misinterpret retained stool as a false positive result. Also, DCBE is a diagnostic study, and thus does not allow for the removal of polyps or biopsies of mass lesions. Furthermore, DCBE has been demonstrated to be less sensitive than colonoscopy in detecting colonic lesions. A large prospective study of surveillance DCBE as part of the NPS in patients who had undergone prior colonic polypectomy demonstrated that DCBE detected only 48% of adenomatous polyps larger than 1 cm, and only 53% of those 0.6–1.0 cm in size *(17)*. Thus, although DCBE continues to be included as an alternative option for average-risk individuals in standard guidelines, other screening options are preferred.

3. RISK STRATIFICATION

3.1. Average Risk

Average-risk individuals are asymptomatic men and women age 50 yr or older who meet the following criteria: (1) no personal history of colorectal cancer or adenomatous polyps; (2) no personal history of inflammatory bowel disease (ulcerative colitis or Crohn's colitis); and (3) no family history of colorectal cancer or adenomatous polyps.

3.2. Increased Risk

3.2.1. History of Colorectal Adenoma

In the NPS, 68% of polyps removed at the initial colonoscopy examination were adenomas, whereas the remainder included hyperplastic (11%) and other

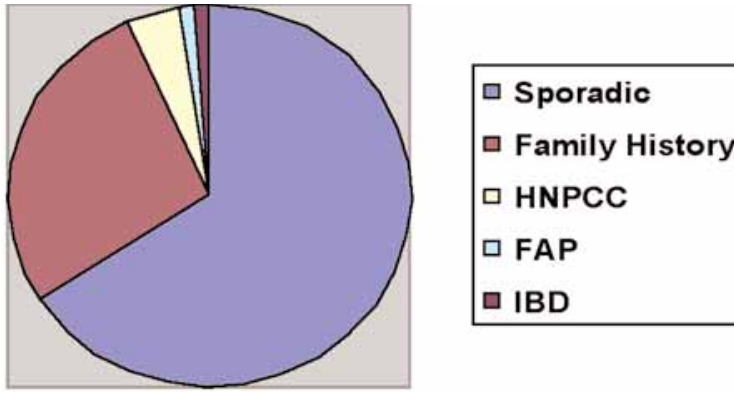

Fig. 1. Factors associated with new cases of colorectal cancer.

non-neoplastic polyps *(18)*. Increased age is associated with an increased risk of multiple synchronous adenomas *(19)*. The precise time course of progression along the adenoma to carcinoma pathway is not certain. However, through indirect evidence it appears to generally be a relatively slow process that, in most cases, occurs over many years. Data from the NPS *(20)* and the St. Mark's Hospital study *(21)*, which described the long-term observation of unresected colorectal adenomas, support an average time course of approx 10 to 15 yr for the progression from a small adenoma to a cancer. In addition, the NPS study has demonstrated that removal of adenomatous colorectal polyps by colonoscopic polypectomy reduces the incidence of developing colorectal cancer *(3)*.

In hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, however, there is suggestion that adenomatous polyps may progress to cancer over a shorter time interval than that commonly seen in sporadic colorectal cancers *(22,23)*. The Netherlands study reported an unexpectedly high incidence of advanced colorectal cancers detected within 3 yr after a negative screening examination (colonoscopy or barium enema) in a large number of patients with HNPCC who participated in a national screening program *(22)*. These findings suggest that HNPCC tumors may demonstrate an accelerated adenoma to carcinoma sequence.

3.2.2. Family History of Colorectal Cancer or Adenomatous Polyp

Approximately 20–30% of cases of colorectal cancer are believed to be associated with familial risk (Fig. 1). A family history of colorectal cancer or adenomatous polyps increases an individual's personal risk for the development of colorectal cancer. If such an individual has a first-degree relative (FDR) who was affected with colorectal cancer, their relative risk of developing colorectal cancer is increased by two- to threefold *(24)*. Furthermore, if an individual has

either two FDRs affected with colorectal cancer or, alternately, a single FDR affected with an early-age colorectal cancer diagnosed anytime before age 50, their risk is increased even further, at three- to fourfold *(24)*. In addition, the NPS has also demonstrated that an individual's risk of colorectal cancer is increased if they have a FDR (sibling or child) who was affected with an adenomatous polyp, particularly if the polyp was diagnosed before age 60 *(25)*. If, however, an individual has more than two FDRs affected with colorectal cancer or, alternately, one affected family member with very early-age onset colorectal cancer diagnosed before age 40, consider the possibility of an underlying hereditary syndrome in this patient's family.

3.2.3. History of Inflammatory Bowel Disease

A long-standing history of ulcerative colitis is associated with an increased risk for colorectal cancer, and its cumulative incidence is increased relative to the duration and anatomic extent of the disease *(26–31)*. The risk of cancer appears to begin after approx 8–10 yr of disease, but thereafter the cancer risk increases at a rate of approx 0.5–1.0% per yr. The risk of cancer is greatest in those patients with pancolitis, which is typically defined as disease involvement extending proximal to the splenic flexure. Primary sclerosing cholangitis is an additional risk factor for the development of colorectal cancer in patients with ulcerative colitis *(32–35)*.

Similarly, the risk of colorectal cancer is also increased in patients with long-standing Crohn's colitis, which, until recently, had remained underappreciated. In fact, the increased risk of cancer is equivalent for both Crohn's and ulcerative colitis of similar duration and anatomic extent *(36–38)*.

There are no randomized controlled studies of surveillance colonoscopy in individuals with long-standing ulcerative or Crohn's colitis. One case–control study of patients with chronic ulcerative colitis undergoing surveillance colonoscopy demonstrated a reduced colorectal cancer-related mortality attributed to the detection of earlier-stage cancer *(39)*.

3.2.4. History of Colorectal Cancer

Individuals with a history of colorectal cancer are at increased risk for both synchronous and metachronous neoplastic lesions. In patients with a colorectal malignancy, the rates for synchronous colorectal cancer and adenoma have been reported to be 2–6% and 25–40%, respectively *(40,41)*. After curative resection, reported rates of subsequent metachronous cancer and adenoma are 3–8% and 25–40%, respectively *(42,43)*. However, prior published rates of metachronous colorectal cancer in these patients are from precolonoscopy era data, whereas now, with colonoscopic clearance of adenomas, it is uncommon to find a metachronous primary colorectal cancer in sporadic patients (i.e., those without an underlying hereditary syndrome).

The primary goal of postsurgical surveillance is to clear the colon of potentially missed synchronous and subsequent new metachronous adenomas. There is no evidence that there is a more rapid progression along the adenoma to carcinoma sequence in patients with a history of sporadic colorectal cancer; therefore, once the colon has been cleared of synchronous neoplastic lesions, the surveillance interval can be every 3 yr. However, no prospective controlled randomized trials have yet been performed to address the issue of appropriate surveillance intervals after curative resection of colorectal cancer.

3.2.5. Hereditary Colorectal Cancer Syndromes

Of the cases of colorectal cancer newly diagnosed each year in the United States, only a small percentage are accounted for by relatively rare inherited colorectal cancer syndromes (Table 2). These syndromes may be classified into adenomatous and hamartomatous polyposis syndromes. The adenomatous polyposis syndromes may be subclassified into hereditary polyposis (such as familial adenomatous polyposis [FAP]) and nonpolyposis (HNPCC) syndromes, and their variants. Patients with FAP and HNPCC are at particularly high risk for the development of colorectal cancer. Hamartomatous syndromes, such as Peutz Jeghers syndrome (PJS) and familial juvenile polyposis (FJP), also carry an increased risk for colorectal cancer development.

3.2.6. Adenomatous Polyposis Syndromes

3.2.6.1. Familial Adenomatous Polyposis. FAP is an autosomal-dominant disorder characterized by the development of hundreds to thousands of colorectal adenomatous polyps. FAP accounts for about 1% of cases of colorectal cancer (Fig. 1). Affected FAP patients have a germline mutation in the adenomatous polyposis coli (*APC*) gene on chromosome 5 *(44–47)*. Adenomatous polyps typically begin to present early in the second decade of life, and if the colon is left intact, cancer will inevitably develop by the fourth to fifth decade of life. The average age of colorectal cancer occurrence in FAP is 39 yr.

Gardner's syndrome, a variant of FAP, is characterized by colorectal adenomatous polyps plus extraintestinal manifestations, including osteomas, particularly of the mandible and skull, soft tissue tumors such as lipomas, fibromas, and epidermoid and sebaceous cysts, supernumerary teeth, desmoid tumors, mesenteric fibromatosis, and congenital hypertrophy of the retinal pigmentation epithelium (CHRPE). Thyroid cancers and adrenal adenomas and cancers have also been associated with this syndrome.

An attenuated form of FAP has also been described that is related to specific mutations at the distal 5′ and 3′ ends of the *APC* gene *(48)*. In contrast to classic FAP, attenuated FAP is associated with a fewer number of adenomatous colorectal polyps (<100), later age of onset of colorectal polyps (44 yr) and cancer (56 yr), and a more proximal distribution of colorectal neoplasia *(49)*.

Table 2
Factors Associated With Increased Risk of Colorectal Cancer

Increased Risk
Age greater than 50 yr
Prior colorectal cancer or adenomatous polyp
Family history of colorectal cancer or adenomatous polyp
Long-standing inflammatory bowel disease (ulcerative colitis or Crohn's colitis)
High-Risk Syndromes
Adenomatous Polyposis
Familial adenomatous polyposis syndrome (FAP)
Gardner's syndrome (GS)
Turcot's syndrome (TS)
MYH-associated adenomatous polyposis (MYH)
Hereditary nonpolyposis colorectal cancer syndrome (HNPCC)
Turcot's syndrome (TS)
Muir-Torre Syndrome (MTS)
Hamartomatous Polyposis
Peutz-Jeghers syndrome (PJS)
Familial Juvenile Polyposis (FJP)

Turcot's syndrome, another variant of FAP, may be associated with mutations in the *APC* gene, and is characterized by colorectal adenomatous polyposis and brain tumors (medulloblastomas).

3.2.6.2. MYH-Associated Adenomatous Polyposis. Recently, some patients with multiple colorectal adenomatous polyps (<100) who tested negative for a mutation in the *APC* gene have been found instead to have a biallelic mutation in the *MYH* gene *(50,51)*. In contrast to FAP, MYH-associated adenomatous polyposis also demonstrates an autosomal-recessive mode of inheritance.

3.2.7. HNPCC Syndrome

HNPCC is an autosomal-dominant disorder in which affected patients develop small numbers of colorectal adenomatous polyps and are at increased risk for colorectal cancer. HNPCC accounts for about 3–5% of cases of colorectal cancer (Fig. 1). HNPCC is associated with germline mutations in several DNA mismatch repair (MMR) genes, including *hMSH2* on chromosome 2p16, *hMLH1* on chromosome 3p21, *hPMS1* on chromosome 2q31–33, *hPMS2* on chromosome 7p22, and *hMSH6* on chromosome 2p16 (52–56). Mutations in these MMR genes result in genomic instability in these patients. Patients with HNPCC are at increased risk for early-onset colorectal cancer, diagnosed at an average age of 40–45 yr. Colorectal cancers in HNPCC are predominantly right-sided, with 60–70% proximal to the splenic flexure. Patients often present

with multiple primary colon cancers, and are also at increased risk for metachronous cancers. HNPCC is also associated with extracolonic cancers of the endometrium, ovary, stomach, small intestine, renal pelvis and ureter (transitional cell cancer), and the pancreaticobiliary system *(57)*. Another subset of Turcot's syndrome may present as a variant of HNPCC, demonstrates a mutation in one of the MMR genes, and is associated with adenomatous colorectal polyps and brain tumors (glioblastomas). Muir-Torre syndrome is another variant of HNPCC and is associated with sebaceous gland adenomas and keratoacanthomas of the skin.

HNPCC syndrome has traditionally been diagnosed based on family history of malignancy. Initial Amsterdam criteria were established that defined an HNPCC family as one in which three or more close relatives, one being an FDR of the other two, from two or more generations were affected with colorectal cancer, and with at least one cancer diagnosed before age 50, in the absence of gastrointestinal (GI) polyposis *(58)*. Subsequently, revised Amsterdam criteria were established to include associated extracolonic malignancies (endometrial cancer, small bowel cancer, and transitional cell carcinoma of the ureter or renal pelvis), as well as colorectal cancer, in the clinical definition of this syndrome *(59)*.

In addition, the Bethesda guidelines are another set of criteria that have been established to identify individuals at risk for HNPCC in whom to recommend testing of their colorectal tumors for evidence of microsatellite instability (MSI) *(60)*. More recently, revised Bethesda criteria have also been established, recommending molecular testing for MSI in colorectal tumors in patients meeting any of the following criteria: (1) patient with colorectal cancer diagnosed before age 50; (2) presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors (i.e., colorectal, endometrial, gastric, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain [typically glioblastoma, as seen in Turcot syndrome], sebaceous gland adenomas and keratoacanthomas [i.e., as seen in Muir-Torre syndrome], and small bowel cancer), regardless of age of diagnosis; (3) colorectal cancer with MSI-associated histology (i.e., presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern), diagnosed in a patient younger than age 60; (4) colorectal cancer diagnosed in one or more FDRs with an HNPCC-associated cancer, with one of the cancers diagnosed before age 50; or (5) colorectal cancer diagnosed in one or more first- or second-degree relatives with HNPCC-associated cancers, regardless of age *(61)*.

Support for the effectiveness of colorectal cancer screening in HNPCC is provided by a long-term Finnish study of colonoscopy screening at 3-yr intervals in 251 at-risk individuals from 22 HNPCC families that demonstrated a reduction in the rate of colorectal cancer by 62%, prevented colorectal cancer-associated deaths, and decreased overall mortality by approx 65% in HNPCC families *(62)*.

3.2.8. Hamartomatous Polyposis Syndromes

3.2.8.1. Peutz-Jeghers Syndrome. Peutz-Jeghers Syndrome (PJS) is an autosomal-dominant inherited disorder characterized by multiple GI hamartomatous polyps and mucocutaneous melanin pigmentation *(63)*. The gene responsible for PJS has been identified on chromosome 19p *(64,65)*. A review of the Johns Hopkins Polyposis Registry showed that the relative risk of a patient with PJS developing a cancer was 18 times greater than expected in the general population *(66)*. A review of the St. Mark's Polyposis Registry found that 22% developed cancer, and that the relative risk of death from GI cancer was 13%, and from all cancers was 9% *(67)*.

3.2.8.2. FJP Syndrome. FJP syndrome is an autosomal-dominant condition that is characterized by multiple juvenile polyps, ranging in number from 25 to 40 or more, located throughout the GI tract *(68–70)*. Extra-intestinal congenital abnormalities may also occur. Patients commonly present during childhood with anemia caused by chronic GI blood loss, crampy abdominal pain, recurrent intussusceptions, or rectal bleeding. Although the juvenile polyps found in FJP are typically benign, affected patients are now recognized to have an increased risk of colorectal cancer of at least 9% *(71)*, and perhaps even much higher *(72)*. The mean age of cancer onset is 40 yr. Unaffected family members are also thought to have an increased risk for colorectal cancer *(71)*.

3.2.9. Emerging Screening Technologies

Computed tomographic colonography (CTC), also known as "virtual colonoscopy," is a new evolving screening technology. CTC is a radiographic test that utilizes CT scan imaging technology, and specialized computer software that can create virtual three-dimensional images of the colorectum to evaluate the entire large bowel. In one early prospective study of 100 increased-risk patients, CTC identified three of three cancers, 91% of polyps ≥1 cm, and 82% of polyps 0.6 to 0.9 cm in size *(73)*. In a recent large prospective screening study of 1233 asymptomatic patients, CTC demonstrated a sensitivity of 93.8% and a specificity of 96% for detecting adenomatous polyps ≥1 cm *(74)*. To date, reports of CTC are somewhat variable in regards to test sensitivity and performance, and this is felt to be related to differences in study protocol methodologies/patient populations, radiologist experience, CTC technique, and use, in some studies, of fecal tagging/electronic bowel cleansing. Although CTC is currently not included as a standard screening option in recommended colorectal cancer screening guidelines, it appears to demonstrate significant potential to possibly develop into an alternative screening option at some point in the future.

DNA-based stool testing is another emerging colorectal cancer screening technology that is undergoing extensive evaluation. The rationale for this type of testing is that neoplastic cells are sloughed from colorectal cancers and adenomatous polyps, and that human DNA can be isolated from the stool and

analyzed for specific DNA mutations. Numerous studies of DNA-based stool testing, using various combinations of multiple target markers (i.e., *APC*, *K-ras*, and *p53* genes, MSI, long DNA, and loss of heterozygosity [LOH]) have demonstrated sensitivity values ranging from 62 to 97% and specificity ranging from 93 to 100% for the detection of colorectal cancer *(75)*. The development of DNA-based stool testing is still ongoing.

4. SCREENING AND SURVEILLANCE GUIDELINES

The approach to colorectal cancer screening relies on a thorough risk assessment of the patient. Asymptomatic average-risk individuals are candidates for routine screening recommendations, whereas those at increased risk owing to a personal or family history of colorectal cancer or adenoma, or inflammatory bowel disease, or at particularly high risk owing to an underlying hereditary colon cancer syndrome, require individualized risk-specific recommendations for screening and surveillance.

4.1. Average-Risk Guidelines

Average-risk asymptomatic men and women should begin routine colorectal cancer screening at age 50. Several screening options are currently recommended for average-risk individuals *(76–78)*.

The standard option has been a FOBT annually combined with a flexible sigmoidoscopy every 5 yr. With this screening approach, if a single positive stool blood test is detected the patient should undergo a complete colorectal evaluation by colonoscopy. Colonoscopy provides the opportunity for direct visualization of the entire large bowel and allows for polypectomy and/or biopsy of suspicious lesions that may be detected. Furthermore, at the time of routine screening sigmoidoscopy if a small ($<$1 cm) benign-appearing polyp is detected, a biopsy is taken and further management is based on the histological assessment of the polyp. If the polyp is an adenoma, then a colonoscopy should be scheduled to perform polypectomy and assess the more proximal colon for potential synchronous neoplastic lesions. In contrast, if the polyp is a benign hyperplastic polyp, no additional tests are necessary. If, however, on screening sigmoidoscopy either a large ($\geq$1 cm) polyp or multiple polyps are detected, then a biopsy is not necessary and the patient should be scheduled directly for colonoscopy and polypectomy.

A second approach to screening the average-risk individual is the choice of a complete colorectal evaluation by colonoscopy, which if negative for neoplasia can be repeated at 10-yr intervals. Although there are currently no prospective randomized trials to support the effectiveness of this option, it is currently believed that the indirect evidence of its benefits and effectiveness support it as an appropriate screening option for this population *(76–78)*. Of note, colonoscopy is the author's preferred screening option.

A third, although less desirable and also unsupported, option included in standard screening guidelines for this population is a DCBE every 5 yr. Any positive DCBE test should be followed up by a colonoscopy. Other screening options are preferred over DCBE.

4.2. Increased-Risk Guidelines

4.2.1. History of Colorectal Adenoma

Colonoscopy is the preferred surveillance examination in patients who have had a colorectal adenoma removed in the past *(77–79)*. The recurrence rate of adenomas in patients after initial polypectomy is high enough to justify periodic follow-up. Ideally, all synchronous adenomas are removed at the time of the initial polypectomy. The patient's colon should be cleared of all adenomas prior to embarking on routine long-term surveillance follow-up.

Current guidelines recommend that after removal of one or two small ($<$1 cm) colorectal tubular adenomatous polyps, a repeat examination can be performed in 5 yr. If, however, either multiple (three or more) adenomatous polyps, or an advanced adenoma (i.e., large size, $\geq$1 cm, villous histology, or high-grade dysplasia) are removed at colonoscopy, then repeat surveillance colonoscopy should be performed in 3 yr. The interval for subsequent follow-up surveillance colonoscopy examinations should be based on findings at time of initial followup examination; for example, if the initial follow-up colonoscopy is negative for neoplasia, or if only —one to two small tubular adenomatous polyps are removed, then the next colonoscopy may be performed in 5 yr.

However, a shorter follow-up surveillance interval may be necessary following any colonoscopy examination in which there was removal of multiple adenomas, excision of an adenoma with invasive cancer, incomplete or piecemeal removal of a large sessile adenoma, or a suboptimal examination owing to a poor colonic preparation. In addition, individual patient considerations such as significant medical comorbidities or pathological predictive factors may also affect decisions regarding continued follow-up.

Following complete colonoscopic removal of an adenoma with invasive cancer ("malignant polyp"), judged by combined gross endoscopic and histological grounds, most endoscopists perform a repeat examination in 3 to 6 mo, and then again at 1 yr, before reverting back to 3-yr follow-up intervals. Surgical resection is indicated if the polyp has cancer invading close to the cautery margin, demonstrates lymphatic or blood vessel invasion, or is poorly differentiated.

4.2.2. Family History of Colorectal Cancer or Adenoma

Individuals who have one or two FDRs who have been affected with colorectal cancer or adenomas are at increased risk. These patients should undergo screening of their entire large bowel beginning at 40 yr of age, or, if earlier, 10 yr younger than the earliest diagnosed cancer in their affected family member(s).

Screening options include the same as those for average-risk individuals, just beginning at an earlier age. However, the high lifetime probability of colorectal cancer in such families has led to the more aggressive option of colonoscopy, particularly in those families where the affected FDR was diagnosed with cancer or adenomatous polyp before age 60. Thus, in patients with a FDR diagnosed with colorectal cancer or adenomatous polyp before age 60, or alternatively, two FDRs diagnosed with colorectal cancer at any age, current recommendation is for colonoscopy beginning at age 40, or 10 yr younger than the age of earliest cancer diagnosis in the family, and if negative for neoplasia may be repeated every 5 yr *(77,78)*.

For patients who have more than two FDRs affected with colon cancer, and no history of a polyposis syndrome, one should consider a diagnosis of HNPCC and recommend screening guidelines as outlined for HNPCC, along with formal genetic counseling and possible gene testing. In addition, if a patient has a FDR affected with colon cancer at an age less than 40 yr, an inherited syndrome, such as one of the polyposes or HNPCC, should be suspected and shorter surveillance intervals and formal genetic counseling should be considered.

4.2.3. History of Inflammatory Bowel Disease

Patients with long-standing inflammatory bowel disease are at increased risk for colorectal cancer and should undergo routine surveillance examinations *(77,78)*. Because the cancer risk in chronic Crohn's colitis appears to be the same as that in ulcerative colitis, these patients should be approached similarly. In patients with pancolitis, typically defined as disease extending proximal to the splenic flexure, surveillance colonoscopy should begin after 8 yr of symptoms, whereas in patients with left-sided colitis, typically defined as disease involvement distal to the splenic flexure, colonoscopy may start after 15 yr of symptoms. The frequency of surveillance colonoscopy examinations should be every 1–2 yr.

At colonoscopy, mucosal biopsies should be routinely taken from grossly normal-appearing mucosa at 10- to 12-cm intervals throughout the colon. In addition, biopsies should also be taken from any areas of mucosal irregularity or plaque-like lesions. Expert pathological consultation should be obtained. If the biopsies are classified as negative or indefinite for dysplasia, surveillance should be continued at 1–2 yr intervals.

Colectomy is indicated for findings of confirmed unequivocal low- or high-grade dysplasia. In addition, colectomy should also be considered in patients with colitis that is difficult to control medically and in those patients who will not comply with surveillance.

4.2.4. Personal History of Colorectal Cancer

In patients who have recently undergone a curative resection for colorectal cancer, the entire colon should be cleared of any potential synchronous cancers

or adenomas by colonoscopy. If this was not performed preoperatively, or if this examination was suboptimal, then the first surveillance colonoscopy is usually performed within 6 mo postresection; otherwise the first screening colonoscopy is typically at approx 1 yr after resection. If this postoperative examination is normal, then subsequent follow-up surveillance colonoscopy is typically performed at 3-yr intervals.

4.2.5. Hereditary Colorectal Cancer Syndromes

4.2.5.1. Familial Adenomatous Polyposis. In FAP, routine colorectal screening for adenomatous polyposis should be performed by annual flexible sigmoidoscopy in all at-risk individuals beginning at approx age 10–12, and may be decreased in frequency to every 3 yr years after age 40 *(77,78)*. Genetic counseling and gene testing should also be offered to members of these families. Surveillance for gastric, duodenal, and periampullary adenomas should begin at the time of diagnosis of colonic polyposis, and continue every 1–3 yr thereafter. At the time of routine upper GI endoscopy, a side-viewing endoscope should also be used to assess the periampullary region of the duodenum and to provide optimal visualization of the major papilla (ampulla of Vater).

4.2.5.2. Hereditary Nonpolyposis Colorectal Cancer. Colorectal screening in patients with HNPCC should be performed by colonoscopy because of the increased incidence of proximal cancers and adenomas. At-risk individuals should have colonoscopy every 1–2 yr beginning by age 20, or 10 yr younger than the earliest age of cancer diagnosis in their affected family members *(77,78)*. Additionally, special screening for extracolonic malignancies is also recommended. HNPCC families should also be referred for genetic counseling and possible gene testing.

4.2.5.3. PJS and FJP Syndrome. In patients at risk for PJS and FJP, colorectal screening should be performed by colonoscopy beginning by the late teenage years, or earlier if symptomatic *(24)*. Follow-up surveillance colonoscopy may be performed every 2 to 3 yr, or as symptoms require. Specialized recommendations should also be offered for screening of the upper GI tract/small bowel in these patients. PJS and FJP families should be referred for genetic counseling. Gene testing is currently available for both PJS and FJP.

5. CONCLUSIONS

Currently available screening and surveillance techniques are effective in detecting early stage colorectal cancer and its premalignant precursor lesion, the adenomatous polyp. Evidence demonstrates that screening tests reduce colorectal cancer mortality. Removal of adenomatous polyps by colonoscopic polypectomy has been demonstrated to significantly reduce the incidence of colorectal cancer.

Appropriate screening and surveillance recommendations should be based on the individual's colorectal cancer risk stratification. Asymptomatic average-risk individuals should begin colorectal cancer screening at age 50. Increased-risk individuals should be identified and offered more aggressive screening recommendations, beginning at an earlier age. High-risk groups, such as FAP and HNPCC, should be offered genetic counseling and specialized screening recommendations for colorectal and associated extracolonic malignancies.

At the present time, patients need to be encouraged to engage in and benefit from currently proven and available screening and surveillance strategies in order to reduce their risk of developing and dying from colorectal cancer.

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4 Cytotoxic Chemotherapy for Metastatic Colorectal Cancer

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Summary

Over the past 10 yr, significant advances have been made with respect to new anticancer agents for metastatic colorectal cancer. These compounds include the topoisomerase I inhibitor irinotecan, the third-generation platinum analog oxaliplatin, and the oral fluoropyrimidine capecitabine, in addition to the mainstay, fluorouracil. The use of these agents is discussed in this chapter.

Key Words: Fluorouracil; irinotecan; oxaliplatin; capecitabine.

1. INTRODUCTION

Chemotherapy has been the mainstay approach for patients with advanced colorectal cancer (CRC) *(1,2)*. For nearly 40 yr, the main drug used for this disease was the fluoropyrimidine, 5-fluorouracil (5-FU). In general, the clinical efficacy of 5-FU as monotherapy has been modest with overall response rates in the range of 10–15% and median overall survival on the order of 6–8 mo. Over the past 10 yr, significant advances have been made with respect to the approval of three new anticancer agents for metastatic CRC. These compounds include the topoisomerase I inhibitor irinotecan, the third-generation platinum analog oxaliplatin, and the oral fluoropyrimidine capecitabine. The use of these agents will be discussed in this chapter. Significant advances have also been made in the development of novel biologic agents, and the anti-epidermal growth factor receptor antibody cetuximab and the anti-vascular endothelial growth factor (VEGF) bevacizumab will be discussed elsewhere in this book.

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2. 5-FLUOROURACIL

For nearly 40 yr, 5-FU was the only active anticancer agent available to treat advanced CRC *(2)*. In general, response rates to 5-FU in patients with advanced disease are low, in the range of 10–15%. To improve the clinical efficacy of 5-FU, the addition of certain biomodulating agents such as the reduced folate leucovorin (LV) and/or a change in the schedule of administration of 5-FU from bolus to continuous infusion have been actively investigated *(3,4)*.

A meta-analysis incorporating 3300 patients from 19 different clinical trials revealed that treatment with 5-FU/LV yielded a significantly improved response rate of 21% compared with an 11% response rate for treatment with 5-FU alone ($p < 0.0001$). Of note, this analysis showed a statistically significant survival benefit for 5-FU/LV compared with 5-FU alone, albeit of only 1 mo (11.7 vs 10.5 mo; $p < 0.004$) *(5)*. However, the survival advantage for LV was limited to only those trials that used essentially the same dose of 5-FU in each arm, ± LV. An analysis of the clinical studies that attempted to explore roughly equitoxic regimens, with a higher 5-FU dose in the non-LV containing arm, did not document a benefit on addition of LV.

The bolus schedules that had been most popular in the United States include the monthly regimen developed by the Mayo Clinic with 5-FU at a dose of 425 mg/m^2 and LV at 20 mg/m^2 repeated every 4 wk, and a weekly regimen developed by the Roswell Park Cancer Institute (RPCI) and the National Surgical Adjuvant Breast and Bowel Project (NSABP) employing 5-FU at 600 mg/m^2 and LV at 500 mg/m^2. The monthly and weekly schedules of LV-modulated 5-FU showed similar clinical activity with respect to response rates (35 vs 31%) and median overall survival (OS) (9.3 vs 10.7 mo). However, their respective toxicity profiles differed substantially, as the Mayo Clinic regimen was associated with increased rates of neutropenia and stomatitis, whereas the RPCI regimen resulted in a higher incidence of diarrhea *(6)*.

The three commonly used infusional schedules include the protracted venous infusion (PVI) schedule of Lokich et al: 5-FU at 300 mg/m^2 for 28 d; and the two high-dose intermittent infusion schedules: the Arbeitsgemein Schaft Internistische Onkologie (AIO) German regimen of 5-FU at 2000 to 2600 mg/m^2 over 24 h with LV at 500 mg/m^2 administered once weekly for 6 wk with a 1-wk rest and the LV5FU2 de Gramont regimen, a combination of bolus and infusional 5-FU/LV administered on days 1 and 2 on a biweekly schedule. Infusional 5-FU, as developed by de Gramont and colleagues, has been shown to be clinically superior to the Mayo Clinic regimen. The infusional regimen was associated with a significantly higher response rate (33 vs 14%) and median progression-free survival (PFS; 28 vs 22 wk), and a trend toward longer median OS (62 vs 57 wk; $p = 0.067$). With respect to safety profile, patients treated with infusional therapy experienced less hematological and gastrointestinal (GI)

toxicity *(7)*. Subsequently, de Gramont et al. developed a simplified LV5FU2 schedule, which administers LV 400 mg/m^2 and 5-FU 400 mg/m^2 day 1, followed by a single 46-h infusion of 5-FU for a total infusion dose of 2400 mg/m^2.

A meta-analysis analyzed the clinical data of six randomized trials (1219 patients) that investigated the clinical efficacy of bolus vs infusional 5-FU regimens *(8,9)*. This analysis included more than 1200 patients and showed that response rates were significantly higher in patients treated with infusional 5-FU when compared with those treated with bolus schedules of 5-FU (22 vs 14%; $p = 0.0002$). Although a statistically significant difference in response rate was observed, median OS was similar (12.1 vs 11.3 mo, $p = 0.04$) between the two 5-FU schedules. With respect to safety profile, hematological toxicity, mainly in the form of neutropenia, was more frequent with 5-FU bolus regimens than with 5-FU CI (31 and 4%, respectively; $p < 0.0001$).

3. CAPECITABINE

Capecitabine is an oral fluoropyrimidine carbamate prodrug of 5-FU. On a pharmacological basis, administration of this oral agent closely simulates infusional administration of 5-FU *(10)*. In contrast to 5-FU, capecitabine is reliably and more completely absorbed from the GI tract. It is inactive in its parent form and requires conversion to 5-FU by three successive enzymatic steps. The third and final step is catalyzed by the enzyme thymidylate phosphorylase. Several studies suggest that the expression of thymidine phosphorylase may be higher in tumor tissue when compared with corresponding normal tissue. This differential expression could lead to enhanced selective activation of capecitabine in tumors.

Two randomized phase III trials were performed comparing capecitabine with bolus 5-FU/LV (Mayo Clinic regimen) *(11,12)*. An integrated analysis of the two studies showed that the overall response rate was significantly greater with capecitabine than with 5-FU/LV (25.7 vs 16.7%; $p < 0.0002$), whereas secondary measures of time to tumor progression and survival were equivalent *(13)*. Moreover, patients treated with capecitabine displayed an improved safety profile and experienced a significantly lower incidence of side effects with respect to diarrhea, stomatitis, nausea, alopecia, and grade 3/4 neutropenia. The only side effect that was observed with higher incidence on the capecitabine arm was hand–foot syndrome. Of note, the improved safety profile associated with capecitabine resulted in a marked reduction of hospitalizations for adverse events when compared to treatment with bolus 5-FU/LV. Based on these trials, capecitabine was approved in the United States as first-line treatment for metastatic CRC when fluoropyrimidine monotherapy was being considered. Although the approved dose is 1250 mg/m^2 twice daily for 14 of every 21 d, clinical experience in the United States has identified doses of 900–1000 mg/m^2

twice daily to be better tolerated, and retrospective analysis does not indicate a reduction in activity in those patients requiring dose reduction.

To date, no randomized clinical trials have been conducted to compare the clinical efficacy and toxicity of single-agent capecitabine with any infusional 5-FU regimen in the first-line treatment of metastatic CRC. Although there are clear limitations to a nonrandomized comparison against historical controls comparison of the clinical efficacy of capecitabine monotherapy with historical control studies of continuous infusion 5-FU, such an analysis suggests that response rates, time to progression, and median survival appear to be similar between infusional 5-FU regimens and oral capecitabine *(14)*.

4. IRINOTECAN

Irinotecan is a semisynthetic derivative of camptothecin, a natural alkaloid first extracted from the *Camptotheca acuminata* tree, and it is a member of the topoisomerase I inhibitor class of anticancer agents *(15)*. Irinotecan is essentially inactive in its parent form and requires conversion to its active metabolite, SN-38, by a carboxylesterase enzyme in the liver. This metabolite forms a stable, covalent complex with DNA and topoisomerase I that then interrupts the breakage–reunion cycle associated with topoisomerase I activity, a process that eventually leads to cell death.

Irinotecan as first-line monotherapy for metastatic CRC has similar response rate as 5-FU/LV. This agent was initially developed in the second-line setting, and it was first approved by the US Food and Drug Administration (FDA) for this indication. Two randomized phase III studies in patients with metastatic CRC provided the initial evidence that first-line combination therapy with irinotecan plus 5-FU/LV (IFL) resulted in improved clinical efficacy in terms of higher response rate and greater PFS and OS when compared to 5-FU/LV monotherapy. In North America, Saltz et al. investigated the role of IFL administered via the bolus, weekly schedule vs 5-FU/LV, and observed that clinical activity was significantly improved with the IFL regimen. In terms of response rates (39 vs 21%; $p < 0.001$), median PFS times (7.0 vs 4.3 mo; $p = 0.004$), and OS (14.8 vs 12.6 mo; $p = 0.04$) *(16)*. In Europe, Douillard et al. investigated the clinical activity of infusional 5-FU with irinotecan, and reported a response rate of 35% in the infusional IFL group and 22% in the 5-FU/LV group for the intent-to-treat analysis ($p = 0.005$); OS was 17.4 and 14.2 mo, respectively ($p = 0.031$) *(17)*. Time-to-treatment failure (TTF) or tumor progression (TTP) was 6.7 mo with infusional IFL and 4.4 mo with bolus IFL ($p < 0.001$). Taken together, these studies demonstrated the clear superiority of the addition of irinotecan to 5-FU/LV, whether it be administered via an infusional or bolus schedule, over 5-FU/LV alone and established this combination as a standard regimen in the first-line treatment of metastatic CRC in the United States.

Kohne et al. reported the results of a randomized phase III study comparing the weekly infusional schedule of 5-FU/LV vs the same weekly infusional schedule of 5-FU/LV combined with weekly irinotecan *(18)*. This regimen was administered weekly for 6 wk and repeated every 7 wk. In terms of safety profile, the infusional IFL regimen was relatively well tolerated, with no significant increase in observed side effects. Patients treated with the infusional all in one IFL regimen experienced significantly improved response rates (54.2 vs 31.5%; $p < 0.0001$) and TTP (8.5 vs 6.4 mo; $p = 0.0001$) when compared with patients treated with 5-FU/LV. Although patients on the irinotecan-containing arm had an improved overall survival (20.1 vs 16.9 mo) when compared with 5-FU/LV, this difference did not reach statistical significance ($p = 0.2279$ log-rank).

Most oncologists in the United States had initially favored the use of the weekly bolus IFL regimen as a results of their familiarity and increased comfort level with the 5-FU bolus schedules. In contrast, European oncologists have preferred infusional schedules of 5-FU in combination with irinotecan (Douillard regimen). However, given the increased incidence of diarrhea, dehydration, and myelosuppression observed with the 6-wk on, 2-wk off IFL schedule *(19)*, US oncologists have now embraced the use of a modified IFL schedule consisting of 2-wk on, 1-wk off as well as the infusional IFL strategies. A randomized trial comparing the modified bolus schedule vs the infusion schedule has completed accrual and data are maturing.

5. IRINOTECAN IN COMBINATION WITH CAPECITABINE

The combination of irinotecan/capecitabine is being actively investigated in an attempt to replace the 5-FU/LV backbone, which is more cumbersome and potentially more toxic to patients, with the oral fluoropyrimidine capecitabine. One approach has been to administer irinotecan in a split-dose fashion on days 1 and 8 every 3 wk or to administer irinotecan on day 1 on an every-3-wk schedule. Patt et al. conducted a phase II study in the United States in which patients under age 65 received capecitabine at 1000 mg/m^2 twice daily (days 1–14) plus irinotecan at 250 mg/m^2 (d 1) in a 21-d cycle, whereas those over age 65 received capecitabine at 750 mg/m^2 twice daily plus irinotecan at 200 mg/m^2 *(20)*. Treatment with CAPIRI yielded a 42% overall response rate and a median time to tumor progression of 7.1 mo. Of interest, no apparent differences in clinical activity were observed between patients older than or younger than 65 yr of age. Disease control (i.e., complete response/partial response plus stable disease) was achieved in 71% of evaluable patients. The CAPIRI regimen was relatively well tolerated, with the most common grade 3/4 toxicities being diarrhea (20%) and neutropenia (18%). However, patients older than 65 experienced a nearly twofold higher incidence of neutropenia and dehydration when compared with those younger than 65 yr. In contrast, no significant differences were observed with respect to GI toxicity.

Bajetta et al. conducted a multicenter phase II trial of two different schedules of irinotecan combined with capecitabine in the first-line treatment of metastatic CRC *(21)*. A total of 140 patients received capecitabine at a dose of 1250 mg/m^2 twice daily on days 2–15 and irinotecan at a dose of either 300 mg/m^2 on day 1 (arm A) or 150 mg/m^2 on days 1 and 8 (arm B), and cycles were given on an every-3-wk schedule. The doses of capecitabine and irinotecan were subsequently reduced during the course of the trial to improve the safety profile of the combination. The dose of capecitabine was reduced to 1000 mg/m^2, whereas the irinotecan dose was reduced to 240 mg/m^2 on arm A and 120 mg/m^2 on arm B. Overall response rates and median PFS were similar in the treatment groups: 47% and 8.3 mo for arm A and 44% and 7.6 mo for arm B. In patients treated on arm A, the incidence of grade 3/4 GI toxicity in the form of diarrhea was nearly 36%; however, upon dose reduction, the incidence of grade 3/4 diarrhea dropped to 25%, which was much lower than the nearly 38% incidence of grade 3/4 diarrhea observed in patients treated on arm B (days 1 and 8 schedule).

Ahn et al. investigated the combination of capecitabine (1000 mg/m^2 orally, twice daily on days 2–15 of each 3-wk cycle) + irinotecan (100 mg/m^2 iv on days 1 and 8) in a phase II trial in the first-line treatment of metastatic CRC *(22)*. With respect to clinical efficacy, the overall response rate was 51.4% (95% confidence interval [CI] 35.3–67.5%), the median TTP was 7.1 mo (95% CI 4.6–9.6 mo), and median OS was 24.8 mo (95% CI 10.5–39.1 mo). This regimen had a manageable safety profile, with the incidence of grade 3/4 neutropenia being 38% with no patients experiencing neutropenic fever. Significant nonhematological toxicities encountered were nausea/vomiting 40%, diarrhea, 27%, hand–foot syndrome 21%, and stomatitis 13%. No treatment-related deaths were reported in this study.

Garcia-Alfonso et al. recently presented the preliminary results of a Spanish study of biweekly schedule of capecitabine and irinotecan (irinotecan 175 mg/m^2 day 1 and capecitabine 1000 mg/m^2 twice daily orally on dayas 2–8). The overall response rate was 50% (95% CI, 33–67%), and tumor growth control (response rate + standard deviation) was seen in 87% of patients *(23)*. Overall, this regimen was well tolerated. The incidence of grade 3/4 neutropenia was 1% in patients above and below 65 years of age, whereas grade 3/4 diarrhea was experienced in 3% of patients older than 65 yr and in 0.5% of patients younger than 65 yr.

6. OXALIPLATIN

Oxaliplatin is a third-generation platinum compound that exerts its cytotoxic effects through the formation of intrastrand and interstrand DNA crosslinks. It is the only platinum analog with demonstrated clinical activity against CRC. In contrast to cisplatin, it does not cause nephrotoxicity, nor does it give rise to the same

degree of myelosuppression and alopecia commonly observed with carboplatin *(24)*. The main dose-limiting toxicity is neurotoxicity, and this specific adverse event presents as both an acute and chronic sensory neuropathy. The acute form is experienced by nearly all patients, and most typically manifested as transient paresthesias that are exacerbated upon exposure to cold. In addition, approx 3 to 4% of patients experience laryngopharyngeal dysethesias. In contrast, the chronic form is a dose-dependent sensory neuropathy that develops in up to 12 to 15% of patients, especially when the cumulative dose is larger than 850 mg/m^2.

In a randomized phase III study, de Gramont et al. compared the FOLFOX4 regimen (oxaliplatin at a dose of 85 mg/m^2 as a 2-h infusion on day 1, every 2 wk, plus LV5FU2) with the LV5FU2 alone in patients with previously untreated advanced CRC *(25)*. This study set the stage for the future clinical development of FOLFOX, as this combination showed significantly longer median PFS times (9 vs 6.2 mo; $p = 0.0003$) and higher response rates (50.7 vs 22.3%; $p = 0.0001$). Although the difference in median OS did not reach statistical significance (16.2 vs 14.7 mo; $p = 0.12$), this trial was not sufficiently powered to detect such a difference. Moreover, both treatment groups were able to receive active salvage therapies, thereby potentially obscuring any potential survival difference. With respect to safety profile, grade 3/4 neutropenia, diarrhea, and neurosensory toxicity were more frequent with FOLFOX4 than with LV5FU2, but were at clearly manageable levels.

Intergroup trial N9741 was a randomized phase III trial in the first-line therapy for metastatic CRC with the bolus, weekly IFL regimen as the control arm *(26)*. The two experimental arms of this trial included FOLFOX4 and a nonfluoropyrimidine-containing arm of irinotecan and oxaliplatin (IROX). This pivotal study showed that FOLFOX4 had significantly greater clinical efficacy than IFL in terms of response rate (45 vs 31%; $p = 0.002$), TTP (8.7 vs 6.9 mo; $p = 0.0001$), and median OS (19.5 vs 14.8 mo; $p = 0.0001$). In addition, when compared with IFL or IROX, FOLFOX4 was associated with a markedly lower incidence of febrile neutropenia and fewer GI side effects in terms of nausea/vomiting, diarrhea, and dehydration. However, peripheral sensory neuropathy and myelosuppression were more common with both FOLFOX4 and IROX when compared with IFL. Based on the results from this large phase III clinical trial, FOLFOX4 was approved for use in the United States as first-line treatment of patients with advanced CRC in January 2004.

de Gramont and his colleagues in France subsequently developed the FOLFOX7 regimen in an effort to maximize the dose intensity of oxaliplatin. This regimen incorporated the same simplified infusion schedule of 5-FU as was delivered with FOLFOX6 without bolus 5-FU, but used a higher dose of oxaliplatin (130 mg/m^2). In a phase II study in previously treated patients, FOLFOX7 resulted in a 42% response rate and a median overall survival of 16.1 mo *(27)*. The FOLFOX7 regimen was subsequently selected for further testing in the first-line

setting in the OPTIMOX trial, where it was compared with the FOLFOX4 regimen *(28)*. This important trial is discussed in further detail in Section 11 of this chapter.

7. FOLFIRI VS FOLFOX

The pivotal N9741 trial demonstrated clear superiority of FOLFOX4 over the bolus, weekly IFL regimen. However, the FOLFOX4 regimen used the infusional schedule of 5-FU/LV, whereas the IFL regimen used a bolus 5-FU/LV schedule. One question that remained unaddressed was whether oxaliplatin was a more active agent than irinotecan when an identical 5-FU-based schedule was employed, and when equal access to active second-line therapy was available to all patient groups. A major limitation of the N9741 study was that oxaliplatin was not commercially available during the conduct of the trial. As a result, patients treated with FOLFOX had ready access to second-line irinotecan monotherapy, whereas patients treated with IFL had limited access to oxaliplatin-based chemotherapy. To address this important issue, Tournigand et al., representing the GERCOR cooperative group in France, conducted a randomized, multicenter, open-label prospective phase III trial *(29)*. This study used a simplified LV5FU2 regimen with a single 46-h infusion rather than two 22-h infusions on days 1 and 2 as had originally been developed by de Gramont and colleagues. In one arm, patients received FOLFIRI (biweekly irinotecan 180 mg/m^2, LV 200 mg/m^2, 5-FU 400 mg/m^2 on day 1 followed by a 46-h continuous infusion of 5-FU at 2.4–3.0 g/m^2) followed at progression by FOLFOX6 (biweekly oxaliplatin 100 mg/m^2 and the same dose and schedule of 5-FU/LV), whereas patients in the second arm received the reverse sequence of FOLFOX6 as first-line therapy followed by FOLFIRI at the time of progression. The primary endpoint of this study was time to progression from initiation of first-line therapy to time of progression after second-line treatment. The response rates for first-line FOLFIRI and FOLFOX6 were virtually identical at 56 and 54%, respectively. Median TTP was 14.4 mo for FOLFIRI followed by FOLFOX6 and 11.5 mo for FOLFOX6 followed FOLFIRI, which was not statistically significant. Median OS for the two arms was also virtually identical being 20.4 and 21.5 mo, respectively. In terms of 2-yr survival, 45% of patients treated with FOLFOX6 as first-line were alive at 2 yr vs 41% of patients who were initially treated with FOLFIRI. Although the numbers were relatively small for patients treated with second-line therapy, FOLFOX6 treatment gave a higher response rate (15 vs 4%), higher incidence of stable disease (67 vs 39%), and a longer time to progression than FOLFIRI (4.3 vs 2.5 mo).

In terms of safety profile, both treatment arms were relatively well tolerated. Patients treated with first-line FOLFIRI experienced a higher incidence of grade 3/4 events in the form of nausea (13 vs 3%), mucositis (10 vs 1%), and grade 2 alopecia (24 vs 9%). The incidence of grade 3/4 diarrhea was comparable at

approx 14% in both arms. Grade 3/4 myelosuppression was observed in a higher number of patients treated initially with FOLFOX6 (44 vs 25%), although the incidence of febrile neutropenia remained low in both arms being less than 10%. Not surprisingly, given the use of oxaliplatin, 34% of those treated with FOLFOX6 experienced grade 3/4 neurotoxicity.

Of note, this study was the first randomized trial to provide a direct comparison between the ability of FOLFIRI and FOLFOX to allow for subsequent surgical resection of liver metastases. In patients treated with FOLFOX6 as first-line therapy, 21 of 111 patients (19%) were able to undergo surgical resection, and complete resections were performed in 13 of these patients. In contrast, only 8 of 109 patients (7.3%) treated with FOLFIRI had an attempt at surgical resection, and histologically complete resections were performed in only 6% of these patients. Although the precise reasons for this difference in surgical resection between the two arms of the study are not entirely clear, these findings suggest that an oxaliplatin-based regimen may be more effective as upfront chemotherapy than an irinotecan-based regimen in terms of downstaging disease and thereby allow for curative resection of metastatic disease. However, further studies are in progress to more precisely address this issue.

The Tournigand study is important as it documented equivalent clinical efficacy between irinotecan and oxaliplatin in the first-line setting using the same 5-FU/LV backbone, in this case an infusional 5-FU/LV regimen developed by de Gramont and colleagues. The second important point to note is that there does not appear to be an optimal sequence of regimens as the overall survival at the end of two treatment arms is virtually identical. In support of the Tournigand trials is the Italian study conducted by Colucci et al., in which the clinical efficacy of FOLFOX4 and FOLFIRI was investigated *(30)*. No significant differences were reported in response rate between FOLFOX (34%) and FOLFIRI (31%). TTP (7.0 vs 7.0 mo), duration of response (9.0 vs 10.0 mo), and OS (14.0 vs 15.0 mo) were virtually identical between patients treated with FOLFIRI and FOLFOX, respectively. In general, both treatment arms were relatively well tolerated with manageable safety profiles.

8. OXALIPLATIN IN COMBINATION WITH CAPECITABINE

A long-standing question has been whether capecitabine can be effectively substituted for infusional 5-FU in combination with oxaliplatin in the treatment of advanced CRC. Scheithauer et al. conducted a study in the first-line setting using a dose-intensified bimonthly schedule for capecitabine (3500 mg/m^2 days 1–7 and 14–21) plus oxaliplatin (85 mg/m^2 days 1 and 14) every 4 wk vs a conventional dose regimen *(31)*. Patients receiving high-dose therapy experienced a higher response rate (54.5 vs 42.2%) and a significantly longer median PFS than those receiving the every-3-wk schedule (10.5 vs 6.0 mo; $p = 0.0013$).

Quite surprisingly, the safety profile was similar to that observed with the lower intensity regimen. Although diarrhea was the most frequent side effect, this regimen, in general, was well tolerated.

A multicenter international phase II study was conducted to investigate the combination of capecitabine and oxaliplatin *(32)*. The dosing regimen of capecitabine 1000 mg/m^2 twice daily (days 1–14) plus oxaliplatin at 130 mg/m^2 iv (days 1) every 3 wk yielded an overall response rate of 55%, similar to that observed with FOLFOX4, and a median OS of 19.5 mo. The most common grade 3/4 adverse events observed were GI toxicity in the form of diarrhea, myelosuppression, and neurotoxicity. The incidence of grade 3/4 myelosuppression was less than 10%, which was significantly lower than the 40 to 45% incidence typically observed with the FOLFOX4 regimen. These findings suggest that the CAPOX combination is an effective and well-tolerated regimen in the first-line treatment of patients with metastatic CRC.

Grothey et al. conducted a randomized phase II in Germany comparing the combination of capecitabine and irinotecan (CAPIRI) with capecitabine and oxaliplatin (CAPOX) in the first-line setting *(33)*. The study protocol included a recommended crossover as second-line therapy. The clinical efficacy of CAPIRI vs CAPOX as first line was as follows: response rate 41% vs 5%, PFS 7.1 vs 6.2 mo. The clinical efficacy of CAPIRI vs CAPOX as second line was as follows: response rate 20.6 vs 12.7%, PFS 5.1 vs 4.3 mo, and OS (after start of second-line) 9.6 vs 10.6 mo. OS for patients with first- and second-line sequential therapy were virtually identical: CAPOX→CAPIRI 17.8 mo, CAPIRI→CAPOX 17.7 mo. In general, the safety profiles of both capecitabine-based regimens were manageable, and the overall incidence of grade 3/4 hematologic and nonhematologic toxicities was relatively low.

9. ORAL VS INFUSIONAL 5-FU IN COMBINATION WITH OXALIPLATIN

The TREE-1 trial was designed to compare the relative safety/efficacy of oxaliplatin in combination with infusional, bolus, and oral fluoropyrimidine regimens in patients with metastatic or recurrent CRC *(34)*. This was a randomized phase II study of 150 patients enrolled onto three treatment arms: modified (m)FOLFOX6 (oxaliplatin 85 mg/m^2 on day 1, LV 400 mg/m^2 on day 1, and 5-FU 400 mg/m^2 bolus followed by 2400 mg/m^2 continuous iv infusion [CIVI] over 46 h beginning on day 1, every 2 wk); bFOL (oxaliplatin 85 mg/m^2 on days 1 and 15, LV 20 mg/m^2 on days 1, 8, and 15, and 5-FU 500 mg/m^2 bolus on days 1, 8, and 15, every 4 wk); or XELOX (oxaliplatin 130 mg/m^2 on day 1 and capecitabine 1000 mg/m^2 po bid on days 1–14 every 3 wk). Patients treated with the CAPOX combination experienced a higher incidence of grade 3/4 nonhematological toxicity (e.g., nausea, vomiting, and diarrhea), and more often

discontinued therapy because of toxicity. The dose of capecitabine was subsequently reduced to 850 mg/m^2 po bid in the TREE-2 trial. The updated toxicity and preliminary clinical efficacy results were reported at the 2005 American Society of Clinical Oncology meeting. In general, the confirmed response rates were highest for the infusional oxaliplatin-based regimens, including mFOLFOX6 (46.9%) and CAPOX (37.5%). In contrast, the bFOL regimen, which was a bolus schedule of 5-FU/LV and oxaliplatin initially developed by Hochster and colleagues at NYU, yielded the lowest response rate (32%) *(35)*.

In a randomized phase III trial conducted by the AIO cooperative group in Germany, Arkenau et al. compared the CAPOX combination with a regimen consisting of weekly infusional 5-FU/LV/oxaliplatin (FUFOX) *(36)*. This particular CAPOX regimen was somewhat different than others previously reported, as oxaliplatin was administered on a weekly basis. In terms of clinical efficacy, overall response rates (47 vs 42%), PFS (7.0 vs 8.0 mo), and median survival (17 vs 18 mo) were nearly equivalent between the CAPOX and FUFOX arms. Moreover, both arms were well tolerated with manageable safety profiles, respectively. A 1600 patient randomized trial of CAPOX vs FOLFOX4, with a 2 $\times$ 2 randomization $\pm$ the anti-VEGF antibody bevacizumab, has completed accrual and data are maturing. As with capecitabine monotherapy, capecitabine-based combination regimens appear to be better tolerated by European than North American patients, perhaps owing to differences in nutritional status and the level of dietary folate supplementation. With this in mind, the toxicity data from European trials should be extrapolated with caution as it relates to the potential safety profile in the North American population.

10. SEQUENTIAL VS COMBINATION THERAPY

To date, only a small number of clinical studies have directly addressed the important issue as to whether combination chemotherapy is superior to sequential use of individual drugs and/or combination regimens in the treatment of advanced CRC. As noted above, Tournigand et al. were one of the first to address the issue of whether there might be an optimal sequence of combination regimens. Based on their study, there does not appear to be an optimal sequence with respect to the use of combination regimens.

An intriguing study was conducted by Maindrault et al. investigating the sequential approach to chemotherapy *(37)*. To evaluate the impact of second- and third-line therapy on patient survival, they used time to disease control (TDC) as their primary clinical endpoint. All patients received the de Gramont biweekly bolus plus infusional 5-FU and LV (LV5FU2) as first-line therapy. At the time of progression, oxaliplatin was added to the de Gramont regimen as second-line therapy, and with subsequent progression, irinotecan was combined with the biweekly 5-FU/LV infusional schedule as third-line

treatment. Ninety-three patients with chemotherapy-naive disease were entered onto this study. Of note, a significant number of patients were able to receive both second- (77%) and third-line (66%) therapy. Response rates of 41, 30, and 5% and median TDC of 9.7, 16.3, and 18.6 mo were observed with first-, second-, and third-line therapies, respectively. This study confirmed that a high number of patients could, in fact, receive three consecutive regimens, and that an impressive median OS of 26 mo could be achieved in this setting.

The Fluorouracil, Oxaliplatin, CPT-11 Usage Study (FOCUS) trial was a large five-arm randomized phase III trial conducted by the Medical Research Council, whose main goal was to determine whether sequential therapy was equivalent to the upfront use of combination regimens *(38)*. Patients were randomized to receive infusional 5-FU/LV followed by irinotecan monotherapy (arm A), infusional 5-FU/LV followed by FOLFIRI (arm B1) or FOLFOX (arm B2), or first-line FOLFIRI (arm C1) or FOLFOX (arm C2). The primary endpoint of this study was OS, and the secondary endpoints included PFS, TTF of the full treatment plan, response rate, toxicity, and quality of life (QL) (QLQ-C30). More than 2000 patients were entered into the trial. OS was 13.7 mo for arm A, 14.8 mo for arm B1 (irinotecan), 15.1 mo for arm B2 (oxaliplatin), 16.2 mo for arm C1 (irinotecan), and 15 mo for arm C2 (oxaliplatin). The use of sequential therapy resulted in inferior survival when compared with the other treatment arms, and initiation with combination chemotherapy yielded higher response rate, PFS, and median OS. However, the survival of 16.2 mo observed with either FOLFIRI or FOLFOX was much lower than the results of previously published trials in the first-line setting. Of note, only 20% of patients had access to all three active anticancer agents, 5-FU, irinotecan, and oxaliplatin, during the course of their disease. This latter point may be especially relevant as Grothey et al. have shown that access to the three main anticancer agents used to treat advanced CRC is a critical predictor of OS in this setting *(39)*. The immediate relevance of the FOCUS trial to our current standards of care for advanced CRC in the United States is not entirely clear, as none of the treatment arms included a biologic agent, such as bevacizumab in the first-line setting, or cetuximab in the second-line or salvage setting.

Sequential monotherapy starting with capecitabine or 5-FU/LV and then proceeding to either oxaliplatin- or irinotecan-based chemotherapy as second-line may be considered in patients who are asymptomatic, in those with relatively slow-growing disease, and/or in those with multiple sites of disease that are deemed to be unresectable. In addition, monotherapy may be more appropriate in elderly patients and in those with significant comorbidities. In contrast, initiation with combination therapy would seem more appropriate in patients with excellent performance status and clinically aggressive disease, in those with significant symptoms, and/or in those who may be considered for salvage via surgical resection.

11. CONTINUOUS VS INTERMITTENT CHEMOTHERAPY

With the rapid development of new advances in the treatment of metastatic CRC, an important issue to address is the optimal duration of chemotherapy. Maughan et al. *(40)* randomized patients responding or having stable disease after 12 wk of therapy with the 5-FU/LV de Gramont regimen, infusional 5-FU therapy as per the Lokich regimen, and single-agent raltitrexed therapy to receive intermittent or continuous chemotherapy until the time of disease progression. A total of 354 patients were enrolled into this study. Patients entered on the intermittent therapy arm showed reduced toxicity in terms of all-grade side effects as well as serious grade 3/4 events when compared to those treated with continuous therapy. Patients in the continuous treatment group remained on therapy for a median of a further 92 d compared to intermittent treatment. However, no differences in overall survival were observed between the treatment arms. This study provided evidence that it is appropriate to stop chemotherapy after a 12-wk treatment period and to then restart the same treatment regimen at the time of disease progression in patients with chemosensitive disease.

The OPTIMOX trial was designed to compare the clinical efficacy and toxicity of continuous vs intermittent oxaliplatin-based chemotherapy in the first-line setting *(28)*. In this trial, the FOLFOX7 regimen was compared directly to the FOLFOX4 regimen in the first-line setting. FOLFOX4 was administered continuously until evidence of disease progression. In contrast, patients enrolled in the FOLFOX7 arm of the OPTIMOX trial received intermittent exposure to oxaliplatin: 6 cycles of FOLFOX7, followed by 12 cycles of 5-FU/LV, and then reintroduction of oxaliplatin for an additional 6 cycles of FOLFOX7. The modified schedule in FOLFOX7 resulted in lower cumulative doses of oxaliplatin, potentially reducing the development of chronic neurotoxicity. Overall, the intermittent FOLFOX7 regimen appeared to be less toxic compared to FOLFOX4. The incidence of grade 3/4 neutropenia was reduced significantly in patients treated with the FOLFOX7 regimen (21.9%) compared to FOLFOX4 (33.2%) ($p = 0.013$). However, there was a higher incidence of grade 3/4 thrombocytopenia with the FOLFOX7 regimen (10.6%) than FOLFOX4 (3.1%) ($p = 0.0006$). With respect to clinical efficacy, the median OS, at the time of the original analysis, was not significantly different between the two treatment arms at 20.7 mo for patients in the FOLFOX4 arm and 21.4 mo for the patients in the FOLFOX7 arm. In addition, both the FOLFOX7 and FOLFOX4 regimens achieved comparable response and surgical resection rates in patients with advanced CRC as well as similar total disease control rates. However, a follow-up analysis has revealed a series of protocol violations relating to the reintroduction of FOLFOX, which may have masked differences in outcome between the two regimens. Only 40% of the patients randomized to the FOLFOX7 arm received the scheduled reintroduction of FOLFOX7.

Moreover, depending on the particular treatment center, as many of 50% of patients who were randomized to FOLFOX4 received additional cycles of oxaliplatin beyond the six courses specified by the protocol in the FOLFOX4 treatments. In the multivariate analyses, patients who were re-exposed to oxaliplatin had significantly improved median overall survival compared to those who received only six courses of treatment. In summary, this trial suggests that intermittent use of oxaliplatin-based chemotherapy is as effective with an improved safety profile when compared with continuous treatment with combination chemotherapy.

12. NOVEL TARGETED AGENTS

The significant advances in molecular oncology have provided an enhanced understanding of the critical signaling pathways involved in tumor growth and proliferation. These insights have served as the rational basis for the development of novel targeted therapies for solid tumors. Such agents are designed to modulate, inhibit, and interfere with the function of specific molecular targets that are essential to the malignancy of tumors. The biological agents that are currently approved for metastatic CRC are the monoclonal antibodies bevacizumab and cetuximab. These agents will be discussed in greater detail in other chapters in this book.

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5 Integration of Antiangiogenic Strategies Into Colorectal Cancer Treatment

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Summary

Angiogenesis is an essential step in the growth and metastases of many solid tumors, including colorectal cancer (CRC). Efforts to inhibit angiogenesis as a potential antineoplastic strategy began more than 30 yr ago, and numerous angiogenic growth factors have subsequently been identified in large bowel tumors. The most successful antiangiogenic strategy to date has focused on inhibiting vascular endothelial growth factor (VEGF).

Bevacizumab (Avastin), a humanized antibody directed against VEGF, has demonstrated significant survival benefits when added to traditional chemotherapy in both the first- and second-line setting in metastatic CRC. The toxicities of bevacizumab appear modest relative to those of most cytotoxic chemotherapy. In addition to bevacizumab, several other promising agents are in late stages of clinical development. Important questions, particularly regarding duration of use, remain to be addressed in well-designed clinical trials.

Key Words: Angiogenesis; vascular endothelial growth factor; VEGF; bevacizumab; colorectal cancer; CRC.

1. INTRODUCTION

Angiogenesis is vital for the growth and metastasis of a variety of solid tumors, including colorectal cancer (CRC). Establishment of a neovascular blood supply derived from existing blood vessels is essential for the growth of tumors beyond 1 to 2 mm in size, when oxygen diffusion alone is no longer sufficient to maintain an adequate tissue oxygen level *(1,2)*. Microvessel count is significantly correlated with tumor size in human colorectal carcinomas and, in CRC patients resected for cure, increased angiogenesis in the primary tumor is

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associated with greater incidence of subsequent hematogenous metastasis, increased relapse of disease, and shorter survival *(3,4)*. Inhibition of angiogenesis prevents the growth of tumor cells at the primary site and can prevent the emergence of metastases *(5)*.

Efforts to inhibit angiogenesis as a means of controlling the growth and spread of cancer cells began more than 30 yr ago *(6)*. CRC is one of the best-studied models of tumor angiogenesis, and numerous angiogenic growth factors, many of which may be targeted with modern drugs, have been identified in large bowel tumors. These include vascular endothelial growth factor (VEGF), platelet-derived endothelial cell growth factor (PD-ECGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), insulin-like growth factors (IGFs), angiogenin, thrombospondin, angiopoeitins, and integrins *(7)*.

The most successful antiangiogenic strategy to date has focused on neutralizing VEGF, a soluble glycoprotein that is an important regulator of physiological and pathological angiogenesis *(8)*. There are two VEGF receptor tyrosine kinases, VEGFR-1 (also known as Flt-1) and VEGFR-2. VEGFR-2 appears to be the major mediator of the angiogenic effects of VEGF, whereas VEGFR-1 plays important roles in hematopoesis *(9)*. This chapter will discuss standard of care antiangiogenic therapy for metastatic CRC and explore promising agents in both the advanced and adjuvant settings.

2. ANTIANGIOGENIC THERAPY IN METASTATIC CRC

2.1. First-Line Therapy

Bevacizumab (Avastin®) is a humanized antibody directed against the VEGFR-1 and -2 ligands. Preclinical studies demonstrated that bevacizumab binds to and neutralizes all human VEGF-A isoforms. In addition to its direct antiangiogenic effects, bevacizumab may also improve the delivery of chemotherapy by reducing interstitial pressure in tumors *(10,11)*. Phase I clinical trials initiated in 1997 showed that the antibody was relatively nontoxic as a single agent, and that adding it to standard chemotherapy did not significantly increase chemotherapy-associated toxicities *(12,13)*. More encouraging efficacy results were seen when bevacizumab was combined with standard chemotherapeutic agents in CRC.

In an early randomized phase II trial, 104 previously untreated patients with metastatic CRC were randomly assigned to 5-fluorouracil (5-FU)/leucovorin (LV) (5-FU 500 mg/m^2 and LV 500 mg/m^2 once weekly for the first 6 wk of an 8-wk cycle) alone or with bevacizumab at one of two different doses (either 5 or 10 mg/kg every 2 wk) *(14)*. Comparing the 5 mg/kg dose with 5-FU alone demonstrated differences in response rate (40 vs 17%), time to tumor progression (9 vs 5.2 mo), and median OS (21.5 vs 13.8 mo), and this dose was

recommended for further use/testing. Higher-dose bevacizumab therapy plus chemotherapy did not offer further potential gains and indeed might have been inferior to lower dose drug. Thrombosis was the most significant adverse event (10.4% grade 3/4) and was fatal in one person, and grade 3/4 hypertension was seen in 16.4% of patients. In addition, 25.3% of patients receiving bevacizumab developed proteinuria or experienced worsening proteinuria. It is not clear why the 5 mg/kg dose of bevacizumab seemed to be more effective than the 10 mg/kg dose, though this was not a phase III trial designed to specifically compare the three arms in terms of efficacy, and the authors pointed out that any imbalance in randomization in this relatively small study could have led to a higher number of poor-prognosis patients in the high-dose arm *(14)*.

The Eastern Cooperative Oncology Group (ECOG) has evaluated the combination of irinotecan, 5-FU/LV (IFL), and bevacizumab in patients with previously untreated advanced CRC in a phase II study (E2200) *(15)*. The first 20 patients received irinotecan (125 mg/m^2), 5-FU (500 mg/m^2), and LV (20 mg/m^2) weekly for 4 of 6 wk and higher-dose bevacizumab (10 mg/kg) every other week. Following a toxicity review of other trials using IFL, subsequent patients were enrolled at reduced starting doses of irinotecan (100 mg/m^2) and 5-FU (400 mg/m^2). Preliminary results are available for the first 87 patients. Although 36% of patients experienced grade 3/4 neutropenia, febrile neutropenia was uncommon (5%). There was one grade 4 epistaxis (requiring tamponade but no transfusion) and two grade 3 melena. There were six grade 3 thrombotic events and three grade 4 events (pulmonary embolism). Proteinuria and hypertension were infrequent. Overall response rate was 49%, and median progression-free survival (PFS) was 10 mo. Median overall survival (OS) has not been reported, although the 1-yr OS rate was 85%.

In a subsequent pivotal phase III trial, 813 patients with previously untreated metastatic CRC were randomized to receive IFL plus either bevacizumab (5 mg/kg every 2 wk) or placebo *(16)*. A third treatment arm consisting of 5-FU/LV plus bevacizumab enrolled 110 patients before a planned interim safety analysis established an acceptable safety profile for the IFL plus bevacizumab treatment group; at that time, 100 patients had also been randomized to the IFL plus placebo treatment arm. The addition of bevacizumab improved median OS (20.3 vs 15.6 mo), PFS (10.6 vs 6.2 mo), and response rate (44.8 vs 34.8%) compared with bolus IFL alone. Grade 3 hypertension was more common during treatment with IFL plus bevacizumab than with IFL plus placebo (11 vs 2.3%), but was easily managed with standard antihypertensives. Unlike in the previously discussed phase II trial, no appreciable increases in thrombosis, bleeding, or proteinuria occurred with the addition of bevacizumab. However, bowel perforation occurred in 1.5% of patients receiving IFL plus bevacizumab, and one patient died as a direct result of this event. Based on the convincing proof of efficacy when added to IFL and 5-FU/LV as first-line

treatment, bevacizumab was approved by the Food and Drug Administration (FDA) in 2004 for use in the first-line setting in combination with intravenous 5-FU-based therapy.

An interesting randomized, placebo-controlled phase II study was conducted concurrently with the pivotal trial in patients deemed nonoptimal candidates for first-line irinotecan-containing regimens *(17)*. Patients were required to have a least one of the following adverse characteristics: age 65 years or older, ECOG performing status (PS) of 1 or 2, serum albumin no more than 3.5 g/dL, or prior radiotherapy to abdomen or pelvis. A total of 209 patients were randomized to weekly 5-FU/LV plus either lower dose bevacizumab or placebo. Despite this higher risk study population, the regimen of 5-FU/LV plus bevacizumab appeared to be well tolerated. Patients receiving bevacizumab had a higher incidence of grade 3 hypertension (16 vs 3%) and arterial thrombotic events (10 vs 5%), but no differences were seen in venous thrombosis, grade 3/4 bleeding, or clinically significant proteinuria. The addition of bevacizumab to 5-FU/LV was associated with numerically superior median PFS (9.2 vs 5.5 mo) and response rates (26 vs 15.2%) compared to 5-FU/LV alone. OS, the primary end point of the study, was longer in the group receiving bevacizumab (16.6 vs 12.9 mo), but this difference did not reach statistical significance. Despite the fact that these patients were deemed unfit for first-line irinotecan, the authors suggested that one possible explanation for the lack of a survival benefit is that more than 40% of patients received potentially effective post-progression therapy with irinotecan and/or oxaliplatin, conceivably diluting any survival advantage from first-line therapy containing bevacizumab.

A combined analysis of raw primary data from three clinical trials further evaluated the addition of bevacizumab to 5-FU/LV *(18)*. These three studies consisted of the two phase II studies by Kabbinavar et al. discussed previously and the third treatment group in the pivotal randomized phase III trial by Hurwitz et al. (5-FU/LV plus bevacizumab) *(16)*. In the combined analysis of the three studies, 249 patients had received 5-FU/LV plus bevacizumab, and 241 patients had received either 5-FU/LV (n = 141 patients) or bolus IFL (n = 100 patients) without the addition of the antiangiogenic agent. Stratified analysis showed that the patients receiving bevacizumab realized a significant increase in median survival (17.9 vs 14.6 mo), PFS (8.8 vs 5.6 mo), and response rate (34.1 vs 24.5%) relative to patients receiving cytotoxic chemotherapy alone.

Oxaliplatin-containing regimens are also highly active against CRC, and investigators have combined FOLFOX with bevacizumab in the front-line setting. The TREE1 study randomized 147 previously untreated patients to one of three oxaliplatin/fluoropyrimidine regimens (mFOLFOX6, bolus 5-FU/oxaliplatin [bFOL], or capecitabine + oxaliplatin [CapOx]) *(19)*. Objective tumor responses were seen in 24–39% of patients in the three arms. The

Table 1
TREE1 and TREE2 Studies

TREE1	*mFOLFOX6*	*bFOL*	*CapOx*
Response rate (%)	39	24	29
TREE2			
	mFOLFOX6 + bevacizumab 5 mg/kg q2 wk	BFOL + bevacizumab 5 mg/kg q2 wk	CapOx + bevacizumab 7.5 mg/kg q3 wk
Response rate (%)	49	34	43

TREE2 study had identical eligibility criteria and then randomized 210 patients to one of the same three regimens (although the capecitabine dose was reduced from 1000 mg/m^2 twice daily [TREE1] to 850 mg/m^2 twice daily [TREE2] after excess toxicity was seen at the higher dose level), plus bevacizumab at a dose of either 5 mg/kg every 2 wk (FOLFOX, bFOL) or 7.5 mg/kg every 3 wk (CapOx) *(20)* (Table 1). The addition of bevacizumab resulted in higher response rates in all three arms of the TREE2 study compared to the TREE1 study, as overall response rates were seen in 34–49% of patients. There appeared to be no significant additive toxicity with bevacizumab. It should be noted that patients were randomized within TREE1 and TREE2, but that patients were not randomized *between* these two studies. Therefore, conclusions regarding the benefit of bevacizumab should be made cautiously.

Numerous additional studies are either ongoing or planned to further examine the role of antiangiogenesis strategies in the upfront treatment of advanced CRC. An industry-sponsored trial (NO 16966) randomized approx 1500 previously untreated patients to either CapOx or FOLFOX4, with or without bevacizumab (dosed at 5 mg/kg every 2 wk with FOLFOX4 or at 7.5 mg/kg every 3 wk with CapOx), with its primary end point being PFS (efficacy and toxicity not data not yet available). This predominantly European study easily completed accrual prior to the commercial availability of bevaciumab in Europe, despite the fact that a similar National Cancer Institute (NCI) Intergroup study opened in the United States and closed prematurely when it became apparent that American patients, who had access to bevacizumab commercially, would not accept potential treatment on a study with nonbiological containing arms. A large, phase III Intergroup trial coordinated by the Southwest Oncology Group (SWOG) and the Cancer and Leukemia Group B (CALGB) activated in September 2005. This trial permits investigators to select upfront chemotherapy for individual patients (either mFOLFOX6 or FOLFIRI), and then randomizes patients among concurrent biological therapy with bevacizumab, cetuximab, or both.

2.2. Salvage Therapy

Bevacizumab has also been studied in previously treated advanced CRC patients. ECOG study E3200 was a randomized phase III trial of FOLFOX4 plus higher-dose bevacizumab (10 mg/kg every 2 wk) compared to FOLFOX4 alone, in patients whose metastatic CRC had progressed despite previous therapy with a fluoropyrimidine and irinotecan. A third arm consisting of bevacizumab alone was closed early for lack of efficacy. A total of 822 patients were randomized *(21)*. The addition of bevacizumab significantly improved median OS (12.5 vs 10.7 mo), PFS (7.2 vs 4.8 mo), and response rate (21.8 vs 9.2%). Importantly, this study confirmed the efficacy of bevacizumab in two novel situations—both in combination with oxaliplatin as well as in the second-line setting. Bevacizumab appeared to be inactive as a single-agent when used in this situation, with a response rate of just 3% in the third arm, which was closed early.

There is also interest in evaluating bevacizumab in conjunction with other biological agents. Cetuximab, a monoclonal antibody that targets the epidermal growth factor receptor, has been approved for use in patients with irinotecan-refractory CRC, as well as those intolerant of irinotecan. A randomized phase II trial (Study EMR 62202-007, or "BOND") demonstrated a 22.9% response rate and median time to progression of 4.1 mo for those given both irinotecan and cetuximab *(22)*. A similar, albeit samller, study added low-dose bevacizumab (5 mg/kg every 2 wk) to treatment arms of cetuximab/irinotecan (arm A) or cetuximab monotherapy (arm B) *(23)*. Seventy-six patients with irinotecan-refractory, advanced CRC have been randomized to cetuximab/irinotecan/bevacizumab (arm A) or cetuximab/bevacizumab (arm B). To date, arm A has demonstrated a superior response rate (35 vs 23%) and median time to progression (5.8 vs 4 mo) relative to arm B. In addition, the response rates and time to progression seen with the addition of bevacizumab to either cetuximab/irinotecan or cetuximab alone are promising relative to those seen in the initial BOND trial; however, it should be noted this is a retrospective comparison of two different studies, and conclusions are again limited.

Bevacizumab alone has also been evaluated in advanced CRC patients who have experienced disease progression after receiving all standard chemotherapy. A phase II Treatment Referral Center trial of 5-FU/LV plus bevacizumab (5 mg/kg every 2 wk) was conducted in patients with tumor progression after (or inability to tolerate) oxaliplatin-based and irinotecan-based chemotherapy *(24)*. Because of tremendous patient and physician interest in gaining access to bevacizumab prior to FDA approval, 337 patients were enrolled in just 4 mo. Preliminary results included a median time to progression of 3.7 mo with an objective response rate of only 1%. Given the apparent lack of efficacy as a single agent of bevacizumab in both this trial as well as the ECOG 3200 trial discussed previously, single-agent bevacizumab is not recommended in this setting. In 2005, the FDA added a prescribing label stating that bevacizumab should not be used as a single agent in CRC.

3. IS THERE A ROLE FOR ANTIANGIOGENIC THERAPY IN THE ADJUVANT SETTING?

With the recognition that bevacizumab prolongs survival and improves tumor response rates when added to chemotherapy in patients with metastatic disease, several large studies are underway to evaluate this agent in the adjuvant setting. However, uncertainty regarding the potential role of antiangiogenic agents in the adjuvant setting arises from several factors. If bevacizumab improves delivery of adjuvant chemotherapy to micrometastatic sites by altering tumor vasculature, then cure rates could be increased with the addition of the biological agent. Alternatively, if microvascular collapse and reduction in chemotherapy delivery is induced in some patients, then cure rates could be *lowered*. Further, the effects of bevacizumab on wound healing are not fully studied. Finally, the high cost of bevacizumab adds urgency to the search for reliable predictive factors that could allow rationale and more selective use of the agent.

National Surgical Adjuvant Breast and Bowel Project (NSABP) C-08 is a phase III randomized study of mFOLFOX6 with bevacizumab (5 mg/kg every 2 wk) vs mFOLFOX6 alone in patients with resected stage II or III colon cancers. Patients randomized to receive antiangiogenic therapy will receive 6 additional mo of single-agent bevacizumab (5 mg/kg every 2 wk) following the completion of cytotoxic chemotherapy. The primary end point is disease-free survival. This study, with a target accrual of 2650 patients, has been entering more than 100 patients per month and will likely close in the near future.

The University of California Los Angeles (UCLA) is coordinating a large, multicenter, industry-sponsored phase III trial (AVANT) of adjuvant chemotherapy in patients with clinical high-risk stage II or stage III colon cancer. Eligible stage II patients must have one of the following: T4 tumor, bowel obstruction or perforation, histological signs of perivascular or perineural invasion, age less than 50 yr, or suboptimal surgery ($<$12 lymph nodes analyzed). A total of 3450 patients will be randomized to FOLFOX4 plus bevacizumab (5 mg/kg every 2 wk), CapOx plus bevacizumab (7.5 mg/kg every 3 wk), or FOLFOX4 alone. Similar to the NSABP trial, patients randomized to therapy with bevacizumab will continue to receive the drug as a single agent for 6 additional mo following completion of traditional cytotoxic chemotherapy. Disease-free survival is the primary end point of the study.

ECOG is also examining the role of bevacizumab in high-risk stage II colon cancer. E5202 will randomize patients with molecular high-risk stage II disease (tumors with both 18q loss of heterozygosity and either microsatellite stability or low-frequency microsatellite instability) to mFOLFOX6 plus bevacizumab (5 mg/kg every 2 wk) or mFOLFOX6 alone, whereas patients with low-risk stage II disease will be assigned to observation alone. Patients randomized to receive bevacizumab will continue to receive the drug as a

single agent following the completion of chemotherapy, for a total of 1 yr of antiangiogenic therapy. The primary end point is 3-yr disease-free survival, and 2600 patients will be enrolled.

4. ANTIANGIOGENIC THERAPY FOR RECTAL CANCER

Antiangiogenic therapy is also being evaluated in the treatment of rectal cancer. A small, single-institution study of preoperative bevacizumab (5 mg/kg every 2 wk) in combination with chemoradiation using continuous infusion 5-FU was completed *(11)*. Preoperative functional CT scans following neoadjuvant therapy identified a significant decrease in tumor blood perfusion, and five out of six patients experienced a tumor response. ECOG is planning a larger, phase II study of bevacizumab in rectal cancer. In E3204, 58 patients will be given neoadjuvant chemoradiotherapy with CapOx and bevacizumab (5 mg/kg every 2 wk). Following surgery, patients will then receive 6 mo of adjuvant systemic chemotherapy with mFOLFOX6 and bevacizumab (5 mg/kg every 2 wk). Pathological complete response will be the primary end point.

The North American Intergroup also is planning to study bevacizumab in the adjuvant treatment of rectal cancer. E5204 will randomize patients with resected stage II or III rectal cancer who received preoperative chemoradiation to adjuvant systemic therapy with mFOLFOX6 plus bevacizumab or mFOLFOX6 alone. OS is the primary end point, and patients who enrolled in NSABP R-04 (preoperative chemoradiotherapy with either capecitabine or continuous infusion 5-FU) will be eligible to participate in E5204. This trial is at the NCI and is expected to open shortly.

5. UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

5.1. Most Effective Dose of Bevacizumab

Bevacizumab is definitely effective in treatment of advanced CRC (at least in combination with chemotherapy); however, the most appropriate dose is not clearly defined. An initial phase II trial evaluated both lower dose (5 mg/kg) and higher dose (10 mg/kg) bevacizumab when added to 5-FU/LV *(14)*. The addition of lower dose bevacizumab led to significant improvements in response rate, time to progression, and OS when compared to 5-FU/LV alone. However, 5-FU/LV was not improved by the addition of higher dose bevacizumab. The authors speculated that perhaps lower doses of bevacizumab reduced intratumor pressure and improved delivery of chemotherapy, whereas higher doses resulted in vascular collapse and limited delivery of chemotherapy. Alternatively, the differences between treatment arms in this relatively small study may have been a result of chance. Lower dose bevacizumab was chosen in the pivotal phase III study evaluating IFL plus bevacizumab vs IFL alone *(16)*. This trial showed a

significant improvement in response rate, time to progression, and OS with the addition of bevacizumab at the 5 mg/kg dose level.

Higher dose bevacizumab (10 mg/kg) has been further evaluated. As discussed previously, E3200 was a randomized phase III trial including arms with FOLFOX4 plus higher dose bevacizumab and FOLFOX4 alone in patients with previously treated advanced CRC. The addition of higher dose bevacizumab improved median OS, progression-free survival, and response rate *(21)*. Questions regarding the optimal bevacizumab dose are not likely to be answered immediately; dosages of 5 to 15 mg/kg delivered every 2 to 3 wk are under investigation in phase III trials, but none of the ongoing studies are designed to directly address the dose issue. At this time, approx 98% of physicians using bevacizumab in both the first- and second-line setting choose the 5 mg/kg dose.

5.2. Continuing Bevacizumab With Second-Line Therapy After Failure in the First-Line

Bevacizumab provides survival benefit when added to cytotoxic chemotherapy in either the front-line or second-line setting in metastatic CRC *(16,21)*. It has been postulated that one of bevacizumab's mechanisms of action may be to improve the delivery of cytotoxic chemotherapy to tumor cells. Thus, patients failing first-line chemotherapy combined with bevacizumab may not actually be resistant to the antiangiogenic agent itself; therefore, it may potentially be beneficial to continue bevacizumab with second-line chemotherapy, even after progression on bevacizumab-containing regimens in the first-line setting. This concept will be tested in clinical trials.

A single-arm, phase II study of cetuximab, irinotecan, and bevacizumab in patients with metastatic CRC who progressed after receiving bevacizumab in the first-line setting has recently been activated. As discussed previously, the original BOND study randomized patients with irinotecan-refractory CRC to either cetuximab plus irinotecan or cetuximab alone *(22)*. The phase II "BOND 2" trial had a similar design and added bevacizumab to both treatment arms, but limited enrollment to patients who had not received prior bevacizumab *(23)*. This new study will allow us, in a preliminary manner, to evaluate the concept of continuing bevacizumab in the second-line setting in patients with "bevacizumab-refractory" disease, although it will not be a randomized study and will therefore be merely exploratory in nature.

SWOG is also evaluating the concept of continuing bevacizumab with second-line chemotherapy. S0600 will enroll 611 patients who failed oxaliplatin and bevacizumab-containing front-line chemotherapy. All patients will receive FOLFIRI and cetuximab, with or without bevacizumab. The primary end point is OS. The concept is at Cancer Therapy Evaluation Program (CTEP) and this study should open for enrollment very soon. Until we have evidence to guide us otherwise,

however, bevacizumab should not be continued with future chemotherapy once progression has occurred on a bevacizumab-containing regimen.

5.3. Duration of Bevacizumab Therapy in the Adjuvant Setting

As discussed previously, bevacizumab is currently being evaluated in the adjuvant setting in several large trials (NSABP C-08, AVANT, E5202). Even if adding antiangiogenic therapy to standard chemotherapy is shown to improve outcomes, the most appropriate duration of bevacizumab therapy will still remain unknown. Prior efforts to improve adjuvant therapy in colon cancer have often focused on *reducing* the dose and/or *shortening* the duration of adjuvant therapy. Standard adjuvant chemotherapy for colon cancer was reduced from 12 to 6 mo when the two durations were shown to have no significant differences in outcome *(25,26)*. However, all of the current adjuvant colorectal trials investigating antiangiogenic therapy involve 1 full yr of bevacizumab, despite a general lack of data to support this long duration. Proponents maintain that the cytostatic nature of antiangiogenic therapy warrants prolonged treatment in the adjuvant setting. A trial comparing 6 vs 12 mo of adjuvant bevacizumab would be a very large and expensive study, and it is unreasonable to believe that such a trial would ever be successfully done. We may therefore never know the most appropriate duration of bevacizumab therapy in the adjuvant setting.

5.4. Surgical Resection of Liver Metastasis After Bevacizumab

Adding irinotecan or oxaliplatin to 5-FU based chemotherapy has led to hepatic metastases resectability rates of up to 22% in patients deemed initially unresectable *(27–31)*. Resected patients are potentially cured; 5-yr survival rates are as high as 35% *(27,28)*. The addition of bevacizumab to cytotoxic chemotherapy improves response rates and could potentially drive resection rates even higher. However, questions have been raised regarding the effect of antiangiogenic agents on wound healing and hepatic regeneration in patients with liver metastases receiving neoadjuvant therapy.

Preclinical studies have demonstrated that inhibition of angiogenesis can inhibit wound healing *(32–34)*. In the pivotal phase III clinical trial comparing IFL with or without bevacizumab in patients with untreated advanced CRC *(16)*, patients who subsequently underwent surgery had a higher complication rate if they had been exposed to bevacizumab (5 of 55 vs 0 of 25); one of these surgical complications occurred 89 d after the last bevacizumab dose *(35)*. The half-life of bevacizumab is relatively long (approx 20 d) and even a relatively low dose of bevacizumab (0.3 mg/kg) can produce undetectable levels of circulating free VEGF *(13)*. The current dose of bevacizumab approved by the FDA in patients with metastatic CRC is 5 mg/kg. Waiting three half-lives (i.e., approx 8 wk) after this standard dose could leave the equivalence of 0.67 mg/kg in the circulation—a dose well above the level of bevacizumab shown to

completely remove free VEGF from the circulation *(13)*. Although it is not known whether circulating VEGF levels are the correct predictor for the biological activity of this molecule, it is clear that close study is warranted and caution is necessary in its use.

Unfortunately, there are no strong preclinical or clinical data to guide recommendations on timing of hepatic resection following neoadjuvant therapy with bevacizumab. Many leaders recommend waiting 8 wk after the last dose of bevacizumab before performing an elective hepatic resection *(36)*, whereas others have advised a shorter period of two half-lives (approx 6 wk). During the waiting period, another course of cytotoxic chemotherapy without bevacizumab can be considered, although the risk of hepatic steatosis from cytoxic chemotherapy and its associated complications must be kept in mind. A SWOG neoadjuvant hepatic resection trial using capecitabine/oxaliplatin and bevacizumab (S0408) will give three cycles of CapOx with bevacizumab but withholds the antiangiogenic agent for a fourth preoperative cycle (thus allowing 6–8 wk between bevacizumab and surgery); after hepatic resection CapOx alone is given for three cycles and bevacizumab is added back during the fourth (at least 7 wk after the procedure).

5.5. Additional Antiangiogenic Agents

Although bevacizumab is the first antiangiogenic drug to be approved for the treatment of CRC, there are several other promising agents.

PTK787 is an oral inhibitor of all known VEGF receptors, platelet-derived growth factor receptor (PDGFR), and cKit. Phase I/II studies demonstrated that chemotherapy and PTK787 could be safely administered as combination therapy. Preliminary data from CONFIRM-1, a large randomized phase III trial comparing FOLFOX4 plus PTK787 to FOLFOX4 alone were presented at the 2005 American Society of Clinical Oncology (ASCO) annual meeting *(37)*. A total of 1168 patients were randomized, and PFS was the primary end point. There was a trend toward improved PFS with the addition of PTK787, but this difference only achieved statistical significance when radiographic response was assessed by investigators ($p = 0.026$) and not by central reviewers ($p = 0.118$). There was no difference in response rates between the two groups. Some have questioned whether once daily dosing was appropriate for a drug with a half-life of just 3–6 h. In addition, a press release detailing a planned interim analysis of CONFIRM-2 (FOLFOX4 plus either PTK787 or placebo in second-line CRC) released in July 2005 indicated low probability of identifying a survival benefit at final analysis. Whether PTK787 will have a role in CRC therapy remains to be seen.

SU11248 is an oral multitargeted tyrosine kinase inhibitor with potential antitumor activity through inhibition of PDGFR, VEGFR, cKIT, and FLT3. A single-institution phase II study of SU11248 in patients with metastatic CRC who had not responded to previous treatment with irinotecan, oxaliplatin, and a

fluoropyrimidine with or without bevacizumab has completed enrollment, but results are not yet available.

Numerous other agents with antiangiogenic properties are in various stages of clinical development. Many of these drugs represent novel approaches to angiogenesis inhibition. VEGF-Trap is a soluble receptor that sequesters free VEGF in the circulation. IMC-11211b is a VEGFR-2 antibody. AD6474, CP-574, and AXP2171 are small molecule inhibitors. ABT-510, angiostatin, and thalidomide are all direct inhibitors of endothelial cell proliferation. Medi-522 and Cilengitude (EMD 12194) are inhibitors of integrin activity, while vascular targeting agents include Combretastatin A4, AVE8062A, ZD6126, and ASI404. Finally, several targeted agents are being evaluated for potential antiangiogenic activity (e.g., cetuximab, erlotinib, bortezomib, CCI-779, and COX2 inhibitors).

6. CONCLUSIONS

Antiangiogenic therapy has made tremendous strides in the 35 yr since the idea was first proposed by Judith Folkman as an antineoplastic strategy. We now have a clinically available monoclonal antibody, bevacizumab, which is targeted against the VEGF. Bevacizumab has demonstrated significant survival benefits when added to traditional chemotherapy in both the first- and second-line setting in metastatic CRC, and it is effective when combined with oxaliplatin, irinotecan, and 5-FU-containing regimens. Bevacizumab is also effective in the frontline treatment of other common malignancies (breast cancer, lung cancer). The toxicities of bevacizumab appear modest relative to those of most cytotoxic chemotherapy. In addition to bevacizumab, several other promising agents are in late stages of clinical development.

However, important questions remain. What is the most effective dose of bevacizumab? Should we continue the biologic agent in the second-line setting after first-line failure? Is resection of liver metastases safe after bevacizumab therapy? Finally, given the relatively high cost of bevacizumab, can we identify predictive markers that will allow us to select patients most likely to benefit from antiangiogenic therapy? These questions and others mandate that we continue to enroll patients in well-designed clinical trials.

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6 The Role of EGFR Inhibition in Colorectal Cancer

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Summary

The epidermal growth factor receptor (EGFR), also known as the ErbB1 or HER1 receptor, triggers intricate and interrelated downstream signaling pathways that, when deregulated, can lead to malignant transformation. The EGFR has now become validated as a target for anticancer therapy, with both small-molecule tyrosine kinase inhibitors and monoclonal antibodies showing clinical activity in some cancers. The biology of EGFR and the clinical data supporting its use as a target in colorectal cancer are explored in this chapter.

Key Words: Epidermal growth factor receptor; EGFR; monoclonal antibody; tyrosine kinase inhibitors.

1. INTRODUCTION

The epidermal growth factor receptor (EGFR), also known as the ErbB1 or HER1 receptor, is a member of the ErbB family of proteins that also consists of ErbB2 (HER2/*neu*), ErbB3 (HER3), and ErbB4 (HER4). These receptors trigger intricate and interrelated downstream signaling pathways that, when deregulated, can lead to malignant transformation *(1)*. EGFR was identified in the 1980s as a cellular oncogene, with homology to the v-erbB viral oncogene *(2)*. It is expressed on normal epithelial cells as well as malignant tumors of epithelial origin, including the majority of human colorectal cancers (CRCs). Expression of EGFR has been correlated with various cellular processes involved in carcinogenesis, such as cell proliferation, inhibition of apoptosis, angiogenesis, cell motility, and metastasis, and its overexpression has been associated with poor prognosis *(3–5)*. EGFR activation also plays a role in resistance to chemotherapy and radiation therapy.

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2. MOLECULAR STRUCTURE AND FUNCTION

The product of a gene on the short arm of chromosome 7, EGFR is a 170-kDa glycoprotein consisting of a single polypeptide chain of 1186 amino acids. The EGFR molecule consists of four regions: an extracellular ligand-binding region consisting of glycosylated domains, a transmembrane domain with a single hydrophobic anchor sequence, an intracellular region containing the catalytic tyrosine kinase domain, and a carboxyl-terminal region containing several tyrosine residues that become phosphorylated upon receptor activation.

EGFR has several known ligands, including the epidermal growth factor (EGF) and the transforming growth factor (TGF)-α. Upon ligand binding to the extracellular domain, EGFR forms homodimers or heterodimers with other members of the ErbB family. Dimerization leads to conformational changes that allow activation of kinases and phosphorylation of key tyrosine residues in the carboxyl-terminal region. The phosphorylated tyrosine residues serve as docking sites for proteins that initiate multiple complex intracellular signal-transduction cascades. Major downstream signaling pathways of EGFR include the mitogen-activated protein kinase (*MAPK*), the phosphatidyl inositol 3 kinase/Akt (*P13K/Akt*), and the Janus kinase/signal transducers and activators of transcription pathways.

3. MECHANISMS OF EGFR ACTIVATION AND DEREGULATION IN TUMOR CELLS

EGFR and its signaling pathways can be activated through several mechanisms, both ligand-dependent and -independent. The presence of heterodimerization, cross talk, and redundancy of pathways leads to a great diversification of the signaling pathways. The major mechanisms of EGFR activation in CRC include coexpression of EGF, TGF-α, EGFR, or a combination of these, suggesting an autocrine loop of ligand production and receptor activation *(6)*; EGFR overexpression leading to ligand-independent receptor dimerization *(7)*; heterodimerization with other HER receptors; and the possible presence of mutant forms of EGFR resulting in ligand-independent constitutive activation *(8)*. Other less-investigated mechanisms have also been proposed *(9,10)*.

4. METHODS OF TARGETING EGFR

EGFR can be targeted through small-molecule tyrosine kinase inhibitors (TKIs), monoclonal antibodies, antisense oligonucleotides, ligand–toxin and immunotoxin conjugates, and inhibitors of downstream effectors of EGFR signaling pathways. TKIs and monoclonal antibodies are the furthest ahead in clinical development and are available and approved for the treatment of various human tumors, including CRC. There are important differences in the

sites of action and in mechanisms of action of monoclonal antibodies and TKIs, leading some to postulate that they could possibly be used in combination *(11)*.

5. EGFR MONOCLONAL ANTIBODIES

Monoclonal antibodies (MAbs) bind to the extracellular domain causing competitive inhibition of ligand binding, thus preventing dimerization and activation of the receptor and the subsequent downstream pathways. EGFR MAbs include cetuximab (IMC-C225), panitumumab (ABX-EGF), matuzumab (EMD 72000), hR3, and ICR62. The first MAb produced against EGFR was the murine MAb 225 (mAb 225) *(12)*; its human:murine chimeric version is cetuximab *(13)*. The mechanisms of action of EGFR MAbs include cell-cycle arrest in the G1 phase *(14)*, increase in the levels and activity of proapoptotic factors and decreased levels of antiapoptotic factors, and inhibition of angiogenesis through either inhibition of tumor-cell production of proangiogenic factors or direct cytotoxic activity on endothelial cells *(15,16)*. A potential contribution by antibody-dependent cellular cytotoxicity has been suggested *(17,18)* but has not been fully defined.

6. CETUXIMAB

Cetuximab is a human:murine chimeric immunoglobulin (Ig)G1 anti-EGFR MAb. It has shown antitumor activity in EGFR-expressing tumors in vivo and in tumor cell lines in vitro. Synergistic activity against human tumor xenografts was demonstrated in combination with several chemotherapy agents *(19)* as well as with radiation therapy *(20,21)*. Cetuximab showed clinical benefit in several solid tumors, including CRC and head and neck squamous cell carcinoma, and its initial development was based on those two malignancies.

The combination of cetuximab and irinotecan demonstrated enhanced antitumor activity against human colorectal tumor xenografts and significantly inhibited growth of established tumors that were poorly responsive to either agent alone *(22)*. Histological examination of those tumors showed extensive tumor necrosis, decreased tumor-cell proliferation and increased apoptosis, and a marked decrease in tumor vasculature. Phase I trials with cetuximab showed that the major toxicity was acneiform rash and that there was little evidence of immunogenicity, with 7% of patients having allergic reactions during the first infusion. Only 2% of the patients had severe anaphylactic reactions. Other side effects of cetuximab included fever, asthenia, and elevation of hepatic transaminases *(23)*. The optimal biological dose, as determined by saturation of antibody clearance, was in the range of 200–400 mg/m^2 per week *(23)*. The recommended phase II and III dose was 400 mg/m^2 loading dose followed by 250 mg/m^2 per week maintenance dose.

Table 1
Anti-EGFR Monoclonal Antibodies

Antibody	*Type*	*Development stage*
Cetuximab	Chimeric	Available
ABX-EGF	Human	Phase III
EMD72000	Humanized	Phase II
hR3	Humanized	Phase II

In the first phase II trial of cetuximab in colon cancer, the drug was administered in combination with irinotecan *(24)*. The study included 121 patients with EGFR-positive CRC previously shown to be refractory to irinotecan. The patients were treated with the standard dose of cetuximab and the same dose and schedule of irinotecan they had previously received. Side effects included allergic reactions (3% grade 3 and 4 allergic reactions) and acneiform rash (61%). Side effects similar to those experienced after treatment with irinotecan alone were also noted. Partial response was achieved in 17% of patients, and 31% had stable disease or a minor response *(24)*. Fifty-seven patients whose tumors had progressed after treatment with irinotecan were enrolled in a phase II trial of single-agent cetuximab. Partial response was achieved in 9% of the patients, with a median survival of 6.4 mo *(25)*. The survival rate of patients with skin rash of any grade was superior compared to patients with no skin rash *(25)*. In the pivotal registration trial, 329 patients with CRC were randomly assigned in a two-to-one fashion to receive either cetuximab plus irinotecan or cetuximab monotherapy with crossover allowed after progression. The response rate was significantly higher in the combination arm (22.9 vs 10%, respectively), as was the time to progression (4.1 vs 1.5 mo, respectively). The median survival duration was 8.6 vs 6.9 mo. The number of previous regimens and the previous use of oxaliplatin did not affect the efficacy of the combination regimen. These study results confirmed the correlation between the severity of rash and the rates of response and survival *(26)*.

On the basis of results of these phase II trials, the US Food and Drug Administration (FDA) approved cetuximab for use in combination with irinotecan in the treatment of EGFR-expressing, metastatic colorectal carcinoma in patients whose tumors are refractory to irinotecan-based chemotherapy. Cetuximab was also approved for use as a single agent for patients who are intolerant to irinotecan-based chemotherapy.

Phase II and III clinical trials of cetuximab as a first-line treatment for metastatic CRC are presently being conducted. A small phase II trial of cetuximab in combination with FOLFOX4 chemotherapy (oxaliplatin, 5-fluorouracil [5-FU], and folinic acid) yielded a partial response rate of 81% *(27)*. The combination of cetuximab with weekly administration of 5-FU, folinic acid, and irinotecan

resulted in a partial response rate of 44%. The main side effects were diarrhea and neutropenia *(28)*. The combination of cetuximab with FOLFIRI (irinotecan, folinic acid, and 5-FU) as first-line treatment in CRC yielded a response rate of 46%, with 41% of patients having stable disease *(29)*. Phase III trials of cetuximab plus different chemotherapy regimens as treatment for colon cancer are ongoing, as are studies of cetuximab in the adjuvant and neoadjuvant settings.

7. EGFR TESTING AND CETUXIMAB USE

Although a common requirement in most clinical trials, the need for EGFR testing has recently been questioned. No correlation was observed between EGFR expression and clinical outcome in the phase II trials of cetuximab, with comparable response rates among patients with expression levels of 1+, 2+, or 3+ *(24,26)*. An expanded-access trial of cetuximab, which included 350 patients with CRC refractory to both irinotecan and oxaliplatin, resulted in a partial response rate of 12%. Whereas in most patients the tumors were EGFR positive, 9 patients had EGFR-negative tumors, and 2 of them had a partial response *(30)*. Supporting this finding, in a recent single-institution retrospective review, there were 4 responses among 16 patients with EGFR-negative, irinotecan-resistant CRC treated with cetuximab *(31)*. The level of tumor EGFR expression required to achieve clinical benefit is unknown, and tumors shown to be negative on immunohistochemical analysis may have the threshold level of EGFR expression required for response but not enough to stain with current methods. In addition, activation of critical downstream signals via crosstalk mechanisms, regardless of EGFR expression, may lead to EGFR independence. The limited data available on patients with EGFR-negative disease suggest a degree of clinical activity not much different from that seen in patients with EGFR-positive tumors, and the use of immunohistochemical negativity to exclude patients with CRC from clinical trials of anti-EGFR agent and from receiving therapy with those agents should be reconsidered. Prospective studies with alternative predictive methods are clearly indicated.

8. MONOCLONAL ANTIBODIES

8.1. Panitumumab (ABX-EGF)

Panitumumab is a fully human IgG2 anti-EGFR MAb with a relative long half-life, which allows for dosing every 2 wk. In a phase I study of panitumumab monotherapy in patients with advanced solid malignancies, the drug was well tolerated and showed antitumor activity with a partial response rate of 12.8%, most notably in advanced CRC *(32)*. Phase II studies of panitumumab monotherapy were conducted in patients with EGFR-expressing CRC in whom 5-FU plus irinotecan, oxaliplatin, or both failed to produce response.

The response rates in two studies were 10 and 13% *(33,34)*. Side effects included rash, fatigue, nausea, vomiting, pruritus, and infusion-related adverse effects, but no development of human antihuman antibodies was observed *(34)*. In a pharmacokinetic analysis of a phase II study of panitumumab in combination with irinotecan, 5-FU, and folinic acid (IFL) chemotherapy as first-line treatment of metastatic CRC, no significant pharmacokinetic interaction between panitumumab and irinotecan was observed *(35)*. Clinical development of panitumumab continues with the phase III Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) trial, in which bevacizumab plus chemotherapy is being compared with this combination plus panitumumab.

8.2. Matuzumab (EMD72000)

Matuzumab is a humanized IgG1 CRC against EGFR. In preclinical studies in xenografts, matuzumab achieved antitumor activity as a single agent and in combination with chemotherapy. Phase I studies of matuzumab in advanced solid tumors showed it to be well tolerated, with the main side effects being acneiform skin rash, headache, and fever. There were no allergic reactions or diarrhea. Matuzumab showed evidence of activity with an objective response rate of 23% *(36)*. In another phase I study of matuzumab given in different schedules, 15 of the 22 patients had colon cancer. In total, a partial response, a minor response, or stable disease was achieved in 5 patients, 4 of whom had colon cancer. These data support a 3-wk dosing schedule *(37)*. Currently, no results from phase II or III trials of matuzumab in colon cancer are available.

8.3. Pertuzumab (2C4)

Pertuzumab is the first of a new class of agents called HER dimerization inhibitors. It is a recombinant IgG1 humanized MAb that binds to the extracellular domain of HER2 and blocks its dimerization with other HER receptors. Dimerization is essential for HER receptor activity, and HER2-containing heterodimers elicit greater mitogenic responses than homodimers. Pertuzumab was shown in preclinical studies to inhibit downstream signaling pathways, and its development continues. However, we have no significant clinical data regarding its use in CRC *(38)*.

8.4. Other Monoclonal Antibodies

In addition to the above antibodies, bispecific MAbs are being studied. They have two different antigen-binding arms and therefore have dual specificity. One arm is specific for EGFR and the other for an immunological effector cell, leading to enhanced host antitumor cellular immune response *(1)*. Examples include M26.1 F(ab′)2, which targets EGFR and CD3; and MDX-447 and H22-EGF, which target EGFR and CD64 (the IgG receptor). Data from a phase I study of

MDX-447 showed the antibody to have good tolerability and good biological response in patients whose tumors were refactory to previous treatment *(39)*.

9. TYROSINE KINASE INHIBITORS

Small-molecule TKIs are orally administered agents that inhibit EGFR phosphorylation by binding to the adenosine triphosphate binding site and thereby inhibiting phosphorylation, receptor activation, and signal transduction. Several EGFR TKIs are available, and many have been tested for their activity against CRC. So far, TKIs have been less effective than the MAbs; however, the search for an effective EGFR TKI inhibitor with activity against CRC continues. The number of TKIs under investigation is vast, and it would be impossible to cover all of them in this chapter. Here we review a select few.

9.1. Gefitinib (ZD1839; Iressa®)

Gefitinib is an oral, reversible, EGFR-specific TKI originally approved as a treatment for refractory non-small cell lung cancers (NSCLCs). In preclinical studies, gefitinib demonstrated growth inhibition in colon cancer cell lines and xenografts *(40)*, and its antitumor activity was potentiated by its combination with different cytotoxic agents, including irinotecan *(40,41)*. In a phase I study of single-agent gefitinib therapy in patients with pretreated metastatic CRC, no objective responses were observed. However, some patients had stable disease *(42)*. The most common side effects were acneiform rash, diarrhea, nausea, vomiting, fatigue, and dry skin.

The combination of gefitinib with the FOLFOX regimen resulted in a response rate of 74–78% in the first-line setting *(43,44)* and 24–36% in previously treated patients *(43,45)*. The main side effects included neutropenia, diarrhea, nausea, and fatigue. Because of the treatment-related toxic effects, the combination of gefitinib and irinotecan in metastatic colon cancer was not feasible in either the first-line setting or in previously treated patients *(46–48)*.

9.2. Erlotinib (OSI-774, Tarceva®)

Erlotinib is another oral, reversible, EGFR-specific TKI also originally developed as an optional treatment for refractory non-small cell carcinomas. Erlotinib is FDA-approved for use against both lung and pancreatic cancers. However, in a study of single-agent erlotinib in previously treated metastatic colon cancer, there were no objective responses *(49)*. Combination studies have addressed the use of erlotinib with oxaliplatin as well as irinotecan-based regimens. The use of erlotinib in combination with oxaliplatin and capecitabine in previously treated metastatic colon cancer resulted in a response rate of 20% *(50)*, whereas a phase I trial of erlotinib with FOLFIRI had to be stopped early

Table 2
Anti-EGFR Tyrosine Kinase Inhibitors

Agent	*Target*	*Reversibility*	*Stage*
Gefitinib	HER1	Reversible	Available
Erlotinib	HER1	Reversible	Available
EKB-569	HER1	Irreversible	Phase II
Lapatinib	HER1/2	Reversible	Phase III
ZD6474	HER1/VEGFR-2	Reversible	Phase II

because of unacceptable toxicity. Erlotinib remains of interest in CRC, and several combination trials are currently ongoing.

9.3. EKB-569

EKB-569 is an EGFR-specific, irreversible TKI. In preclinical studies, EKB-569 in combination with chemotherapy agents demonstrated, at minimum, an additive effect on the inhibition of the growth of colorectal tumors. In a phase I study of EKB-569 in advanced solid tumors, the most common side effects were diarrhea and rash *(51)*. Two studies explored the use of first-line EKB-569 in combination with FOLFIRI or FOLFOX as treatment for advanced CRC. The combinations resulted in response rates of 38 and 36%, respectively *(52,53)*.

9.4. Lapatinib (GW572016)

Lapatinib is an oral, reversible, dual TKI of EGFR and HER2. In a phase I study in heavily pretreated patients with EGFR-expressing or HER2-overexpressing tumors or both, responses were seen in trastuzumab-refractory breast cancer, with the main side effects being diarrhea and rash *(54)*. In a phase II study of lapatinib in patients with metastatic CRC refractory to first-line therapy with 5-FU in combination with irinotecan, oxaliplatin, or both, lapatinib had very limited activity, with a partial response rate of 1% *(55)*. In a phase I study of lapatinib plus FOLFIRI in solid tumors, the combination was tolerable and active, but dose reductions of both FOLFIRI and lapatinib were required *(56)*.

9.5. ZD6474

The development of dual TKIs targeting EGFR and vascular EGFR (VEGFR) is generating great interest. ZD6474 is an oral, small-molecule TKI that inhibits VEGFR-2 and EGFR. ZD6474 inhibits endothelial-cell proliferation by blocking VEGFR, and inhibits cancer-cell growth by blocking EGFR signaling. ZD6474 inhibited growth of colon cancer xenografts that were rendered resistant to cetuximab or gefitinib *(57)*. In phase I studies, ZD6474 was

well tolerated when given once daily, with the most common side effects being diarrhea, hypertension, and rash *(58)*. Clinical development of ZD6474 in several tumors, including CRC, continues.

10. SURROGATE AND PREDICTIVE MARKERS OF CLINICAL RESPONSE TO ANTI-EGFR THERAPY

A predictive marker of response is a marker that is measurable before the start of treatment and provides information about the possible response of a tumor to the therapeutic agent *(59)*. A surrogate marker, on the other hand, is measured after the start of treatment and correlates with a clinically relevant endpoint of therapy.

The availability of a reliable predictive marker would allow the tailoring of therapy to each individual patient and a more rational use of resources. Unfortunately, EGFR immunohistochemistry and gene amplification, which were the most obvious predictive markers of EGFR therapy, were found to be poor markers in CRC. In contrast to CRC, research in lung cancer has been more successful. The presence of activating tyrosine kinase domain mutations in non-small cell carcinomas are thought to lead to increased sensitivity to TKI therapy. Unfortunately, EGFR kinase domain mutations are rare and not thought to play a significant role in response to therapy in CRC. Several other predictive markers have been and continue to be explored. So far, however, none has been confirmed, and the use of anti-EGFR therapy against CRC remains largely empirical.

A specific class effect of anti-EGFR agents is a papulopustular rash that is dose-dependent and that affects about two-thirds of patients. It occurs mostly on the face, neck, and upper torso, usually starting during the first 2 wk of therapy. The etiology of the rash is not well understood. It may be caused by EGFR inhibition in the skin, but it has also been postulated that the rash could be caused by an inflammatory reaction developing in response to therapy *(60)*. The presence and severity of EGFR-inhibitor-induced rash has been shown to be predictive of response and survival rates in patients with colon cancer *(61)*. Analysis of four phase II studies, including two studies in CRC, one of cetuximab monotherapy, and one of cetuximab in combination with irinotecan *(24,62)*, showed that patients who developed the rash survived longer than those who did not, and those with more intense rash survived longer. These findings strongly suggest that the rash may be an important clinical surrogate of efficacy and raised the question of the importance of investigating the value of tailoring patient dosing to stimulate or enhance skin toxicity as a way of maximizing the activity of cetuximab. This concept is being explored in the EVERST study, but no preliminary results have been released to date. Studies have demonstrated the predicted effects of anti-EGFR agents on the EGFR signaling pathway, such

as phosphorylated EGFR and phosphorylated AKT, Ki67, and p27 in the skin; however, a clear association between signal inhibition in skin and antitumor response has not been found *(36)*.

11. EGFR AND ANGIOGENESIS

Preclinical data support an antiangiogenic mechanism as a component of the antitumor effects of anti-EGFR MAbs. Expression of EGF and TGF-α was shown to correlate with microvessel density in breast cancer *(63)*, and one important mechanism by which EGFR may affect tumor progression and metastasis is promotion of angiogenesis *(64)* through upregulation of VEGF and possibly other mediators of angiogenesis. Cetuximab leads to downregulation of angiogenic factors in vitro and in vivo, thus inhibiting tumor growth and metastasis *(40)*.

Upregulation of angiogenesis is thought to play a role in developing resistance to anti-EGFR agents. In a study of human squamous cell carcinoma xenografts, resistance to anti-EGFR MAbs was noted *(65)*, and the resistant cell variants demonstrated increased levels of VEGF expression. The mechanism of resistance is thought to involve selection of tumor-cell subpopulations capable of constitutive VEGF upregulation *(65)*. Those genetic changes could be present in a small subpopulation of resistant cancer cells at the start of therapy or could be acquired during treatment.

In a mouse model of colon cancer carcinomatosis, the combination of cetuximab and DC101 (a murine anti-VEGFR-2 antibody) led to greater reduction in angiogenesis and ascites than DC101 alone *(66)*. Treatment of colon cancer xenografts with cetuximab in combination with anti-VEGF therapy led to prolonged inhibition of growth and increased survival duration compared to treatment with the single agents *(40)*.

A phase II randomization trial of CBI (cetuximab, bevacizumab [a MAb that targets VEGF], and irinotecan) vs CB (cetuximab and bevacizumab) was recently conducted in patients with irinotecan-refractory CRC. All patients were naive to both MAbs, and EGFR expression was not required for study entry. Bevacizumab was given at 5 mg/kg every 2 wk, and cetuximab was given at the standard dose. The study was closed early after both agents were commercially approved, with 76 patients randomly assigned to one of the treatment groups. There were no unexpected toxicities, and the partial response rates were 37% in the CBI group and 23% in the CB group. The progression-free survival duration of patients in the CBI arm was an impressive 7.6 mo *(67)*. The results of this small phase II trial led to the development of a large intergroup phase III trial, in which untreated patients receive either FOLFIRI or FOLFOX at their physician's discretion, and are randomly assigned to receive concomitant bevacizumab, cetuximab, or both.

12. CROSSTALK BETWEEN EGFR AND OTHER RECEPTORS AND SIGNALING PATHWAYS

Crosstalk between different signaling systems allows for the integration of a diversity of stimuli received by cells under various physiological situations *(9)*. Activation of EGFR can occur by indirect mechanisms not involving direct binding of EGFR ligands. These mechanisms include unphysiological stimuli, such as exposure to radiation or oxidative stress, and interactions with G protein-coupled receptors (GPCRs), chemokines, and cell-adhesion molecules.

12.1. EGFR Transactivation

EGFR signal transactivation is a crosstalk mechanism whereby a receptor is activated by components of the signaling cascade stimulated by ligands binding to a different class of receptors *(68)*, GPCRs. The GPCRs are a large group of cell-surface receptors with a variety of biological functions. GPCRs are able to utilize EGFR as a downstream signaling partner in generating mitogenic signals. EGFR ligands are synthesized as membrane-spanning precursor molecules that are proteolytically processed to become fully active *(9,69)*. Examples of receptors and pathways involved in GPCR-induced EGFR transactivation that might be relevant to colon cancer include the protease-activated receptors (PARs) *(70)*, prostaglandin E_2 (PGE_2) *(71)*, and cholinergic agonists *(72)*. Other examples of EGFR transactivation include interaction with insulin-like growth factor receptor 1 (IGF-1R) *(73,74)*, platelet-derived growth factor receptor (PDGFR) *(75)*, and type I protein kinase A *(76)*.

12.2. Interaction With Other Members of the ErbB Family

The ErbB family is a diverse and flexible system of receptor attributable to heterodimerization and the presence of a variety of stimulating ligands. Heterodimers lead to more potent mitogenic stimuli than homodimers. Overexpression of both EGFR and HER2 leads to worse prognosis. Pertuzumab is an MAb against the extracellular domain of HER2 that prevents heterodimerization. This blockade has divergent downstream signaling and growth effects *(77)*. A study of colon cancer cell lines that express EGFR, HER2, and HER3 demonstrated that pertuzumab blocked heregulin-mediated activation of MAPK and PI3K/Akt and tumor proliferation and growth. On the other hand, pertuzumab blocked EGF-mediated phosphorylation of HER2, activation of PI3K/Akt, and growth, but did not affect MAPK *(77)*. It was concluded that HER2 is a critical component of EGF signaling to the Gab1/Gab2-PI3K-Akt pathway, but EGF stimulation of MAPK can occur independent of HER2.

12.3. Cross-Talk Between EGFR and Src

Src is a nonreceptor tyrosine kinase localized to the intracellular membrane, which is intimately connected to the EGFR pathway *(78,79)*. Upon ligand binding, Src associates with members of the HER family and phosphorylates downstream targets. In addition, Src can phosphorylate itself following ligand binding and through GPCR-mediated transactivation of EGFR. Src activity has been associated with a poor prognosis in colon cancer *(80)*.

12.4. Cross-Talk With Cell Adhesion Molecules

E-cadherin is a mediator of cell–cell adhesion with more recently recognized signaling functions. It interacts with the extracellular domain of EGFR, thereby inhibiting it. Activation of EGFR is implicated in the loss of cell adhesion and increased cell migration and invasion. Downregulation of E-cadherin may contribute to the frequently observed activation of receptor tyrosine kinases in tumors *(81)*.

13. RESISTANCE TO ANTI-EGFR AGENTS

Refractory disease will eventually develop in nearly all patients who are treated with anti-EGFR agents, including patients who are initially unresponsive to those agents and patients who progress after an initial period of response or stable disease. Chronic exposure to EGFR inhibitors may select clones of preexisting cells that rely on redundant signaling pathways or that can overactivate survival pathways in response to therapy. Research into the mechanisms of resistance to anti-EGFR agents and the strategies to overcome this resistance has recently been intensified.

13.1. Mechanisms of Resistance to Anti-EGFR-Targeted Therapies

Several genetic and epigenetic changes in cancer cells have been postulated as mechanisms for resistance *(82–84)*. EGFR can be activated through interaction with integrins *(85)*. This mechanism of ligand-independent activation could bypass the inhibitory effects of anti-EGFR antibodies, leading to resistance, and thus may represent a therapeutic target *(86)*.

Certain EGFR mutations determine the sensitivity of tumor cells to anti-EGFR by altering the conformation and activity of the receptor. EGFR mutations can occur in the tyrosine kinase domain or in the extracellular domain. Mutations in the tyrosine kinase domain can be either activating mutations or mutations that lead to resistance to TKIs. Activating tyrosine kinase domain mutations have been identified in NSCLC and seem to result in greater sensitivity to TKIs. Unfortunately, it does not seem to be very important in CRC *(87,88)*. On the other hand, the T790M missense mutation in exon 20 of the *EGFR* gene is associated with resistance to TKIs *(89,90)*. EGFR kinase domain

mutations are not thought to play a role in response to therapy in CRC. The best-characterized extracellular domain mutation is EGFR variant III (EGFRvIII), which is identified in 40% of glioblastomas and in some breast and ovarian cancers. This mutation is caused by an in-frame deletion in the extracellular domain of EGFR, resulting in a truncated receptor that is constitutionally active *(91)*. However, it is not frequently seen in CRC.

The downstream signaling pathways of EGFR can also be constitutively activated by genetic or epigenetic mechanisms, bypassing the need for EGFR activation and reducing the efficacy of EGFR inhibitors. Loss of PTEN *(92,93)* and activation of the Akt pathway owing to gene amplification of PI3K or Akt *(94,95)* seem to be relatively common in CRC, as is increased Src activation *(80)*.

It is well known that the activation of other tyrosine kinase growth factor receptors can also bypass EGFR by activating similar and overlapping signal transduction pathways. Examples include the IGF-1R *(96)*, PDGFR, and c-MET (hepatocyte growth factor receptor). This redundancy is important because these receptors regulate cell growth and survival, and understanding it could lead to more effective therapies.

Other mechanisms, such as loss of or low levels of $p27^{Kip1}$, are still being investigated *(97)*, and it will be some time before a complete picture of the way the different mechanisms interact emerges. Nevertheless, this rapid accumulation of knowledge will hopefully lead to more effective therapies for CRC.

14. EGFR-RELATED PEPTIDE

EGFR-related peptide (ERRP) is a negative regulator of EGFR *(98)* and is present in most normal human epithelial cells. It has about 90% homology to the extracellular ligand-binding domain of EGFR and significant homology to HER2, HER3, and HER4. The inhibition of basal and ligand-induced phosphorylation of EGFR is at least partly modulated by sequestration of EGFR ligands by ERRP, leading to inactive heterodimers between EGFR and ERRP. The expression of ERRP is high in benign epithelia but low in adenocarcinomas.

Transfection of ERRP c-DNA into a colon cancer cell line resulted in marked inhibition of proliferation and colony formation. In another colon cancer cell line, induction of ERRP expression led to a marked decrease in EGFR activation and proliferation *(98)*. Intratumoral or subcutaneous injection of ERRP has also been shown to cause regression or arrested growth of colon cancer xenograft tumors in mice *(99)*. Recombinant ERRP was shown to inhibit growth of colon and breast cancer cells expressing varying levels of EGFR, HER-2, and HER-4 *(100)*. It significantly induced apoptosis, inhibited ligand-induced EGFR activation, and induced heregulin-α activation of colon and

breast cancer cells with high levels of HER-2 *(100)*. ERRP is an effective pan-ErbB inhibitor that is capable of inhibiting multiple members of the EGFR family, and may be a potential therapeutic agent for a wide variety of epithelial cancers *(100)*.

15. CHALLENGES AND FUTURE DIRECTIONS IN THE USE OF EGFR-DIRECTED THERAPY

Many challenges face the current use of EGFR-directed therapy *(101)*. Improving patient selection is an important step toward the optimal use of those agents. Although it is important to enroll patients with EGFR-dependent tumors in clinical trials of anti-EGFR agents to avoid a dilution of the clinical benefits *(102)*, determining who these EGFR-dependent patients are is currently not possible. The solution for this conundrum involves validation of more accurate methods of measuring EGFR, because immunohistochemistry techniques have been proven faulty. Measuring the activated form of EGFR in addition to its downstream effectors may eventually become a better way of making this determination, and collecting tumor samples before and after treatment will allow us to make biological measurements that may identify predictive markers and serve as surrogate markers of therapy. Such a task, however, will involve repeat biopsies of primary tumor or metastatic sites, which presents medical and ethical concerns. It is uncertain whether surrogate tissues that are easy to obtain, such as skin, are adequate alternatives for use in correlative studies. Hopefully, with current advances in the field of genomics and proteomics, colon cancer "signatures" may evolve as important predictors of response to therapy.

Another consideration in clinical trials of EGFR-directed agents is the selection of the appropriate endpoints. The selection of an optimal biological dose is more important than the determination of a maximum-tolerated dose, and time to progression may be more important than objective tumor responses *(103)*.

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7 Second-Line Strategies in the Treatment of Patients With Metastatic Colorectal Cancer

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Summary

Given the number of active drugs and combinations available, the choice of second-line therapy in metastatic colorectal cancer can be complicated. It is influenced by many factors, such as the nature of the first-line therapy, the potential toxicity of one regimen vs another, and the overall goal of treatment for the patient. In this chapter, we focus our discussion on the therapeutic strategies that can be used in the treatment of colorectal cancer after the failure of first-line therapy.

Key Words: Chemotherapy; oxaliplatin; irinotecan; cetuximab.

1. INTRODUCTION

The multitude of effective cytotoxic and targeted agents given in different combinations has brought new hope to patients and significant challenges to the treating oncologist *(1–8)*. The sequencing of the different combinations and the incorporation of the targeted agents have to be done in a rational manner that provides the highest efficacy and least toxicity. For this purpose, treating oncologists find themselves faced with the task of assimilating a large body of data and trying to formulate a treatment approach that best suits the patient and allows him or her the benefit of exposure to all or most of the active compounds.

In this chapter, we will focus our discussion on the therapeutic strategies that can be used in the treatment of metastatic colorectal cancer (CRC) after the failure of first-line therapy. Given the number of active drugs and combinations available, the choice of second-line therapy can be complicated, because it is

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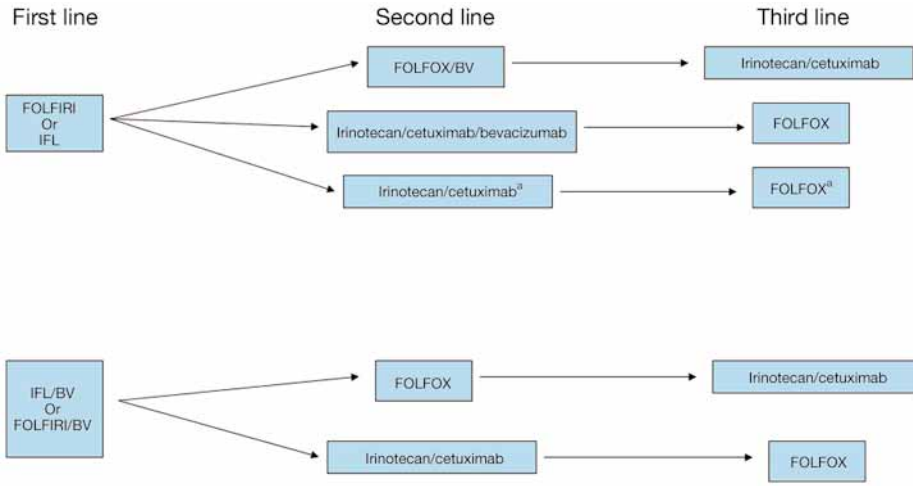

Fig. 1. Potential second- and third-line therapies after failure of an innotecan-based regimen. ([a]This sequence, which excludes bevacizumab, may be most appropriate for a patient with contraindications to BV such as a recent myocardial infarction or cerebrovascular accident.)

influenced by many factors such as the nature of the first-line therapy, the potential toxicity of one regimen vs another, and the overall goal of treatment for the patient.

2. OVERVIEW OF SECOND-LINE THERAPEUTIC OPTIONS AFTER FAILURE OF A FRONT-LINE IRINOTECAN-BASED COMBINATION

The most common irinotecan-based combinations used in the first-line treatment of patients with metastatic CRC include infusional 5-FU and leucovorin (LV) with irinotecan (FOLFIRI) or FOLFIRI with bevacizumab (BV). Several second-line treatment options exist in case of failure of FOLFIRI or FOLFIRI/BV. As we review the data related to these options, we will highlight the factors that may favor one over another (Fig. 1).

2.1. FOLFOX

Prior to the incorporation of BV in the first-line treatment of metastatic CRC, Tournigand had shown that the sequence of FOLFIRI followed by 5-FU/LV/oxaliplatin (FOLFOX) was equivalent to FOLFOX followed by FOLFIRI. Both sequences resulted in an overall survival of 21 mo. Specifically, after progression of disease on FOLFIRI, FOLFOX had a response rate of 15% and a progression-free survival (PFS) of 4.2 mo *(9)*. The sequence of FOLFIRI followed by FOLFOX or vice versa is not commonly used anymore given the

incorporation of BV into first- or second-line chemotherapy. It is now uncommon for a patient to undergo two lines of therapy without being exposed to BV as part of the first- or second-line combination regimen. One of the earlier indications of the impact of receiving all four drugs—5-FU, irinotecan, BV, and oxaliplatin—as part of the first- and second-line treatments of metastatic CRC can be found in a retrospective subgroup analysis performed by Hedrick et al. *(10)*. In this analysis, patients who received oxaliplatin-based therapy after failure of bolus 5-FU/irinotecan and BV had an impressive median overall survival (OS) of 25.1 mo. Consequently, FOLFOX is an effective second-line regimen after failure of a 5-FU/irinotecan and BV combination. For patients who did not receive BV in first line with an irinotecan-based combination, the combination of FOLFOX and BV as a second-line therapy was recently evaluated in the study E3200, which is discussed next.

2.2. FOLFOX/BV

BV, a humanized antibody against vascular endothelial growth factor (VEGF), has been shown to inhibit VEGF-induced angiogenesis and tumor cell growth *(11)*. BV is approved for the first-line treatment of metastatic CRC in combination with iv 5-FU-based chemotherapy. BV in second-line therapy has been evaluated in combination with FOLFOX4 in Eastern Cooperation Oncology Group (ECOG) 3200 *(12)*. Eight-hundred and thirty patients with metastatic CRC who had been treated with 5-FU and irinotecan in sequence or combination were randomized to FOLFOX4 (arm A) vs FOLFOX4 and BV (arm B) vs BV alone (arm C). The BV-alone arm was closed because of concern for lack of efficacy. Patients on arm B had a significant OS benefit of 12.9 mo vs 10.8 mo for patients on arm A ($p = 0.0018$). The response rate for FOLFOX4 + BV was 21.8 vs 9.2% for FOLFOX4 alone. Based on these data, FOLFOX + BV is a superior second-line treatment to FOLFOX alone for patients who have failed first-line treatment with 5-FU and irinotecan. Despite a higher frequency of hypertension, bleeding, neuropathy, and vomiting, FOLFOX4 + BV was overall well tolerated with an all-cause mortality of 6% at 60 d compared with 4% for FOLFOX4 alone (Table 1). It is important to note that this regimen has been evaluated only in patients who had not received any prior BV. To date, there are no data to support the continued usage of BV as part of a second-line regimen after progression of disease on a regimen containing BV. In other words, if a patient had received FOLFIRI and BV in first line, BV should not be continued in the second-line treatment in combination with FOLFOX.

2.3. Irinotecan/Cetuximab

Two combination regimens that have been studied in the third-line setting deserve mention and consideration in the second-line setting after irinotecan failure. These include the combination of irinotecan/cetuximab or the combination

Table 1
Grade 3 and 4 Toxicities Associated With FOLFOX and FOLFOX/BV in Second-Line Therapy Based on ECOG 3200 *(12)*

	FOLFOX/BV		*FOLFOX*		
	Grade 3	*Grade 4*	*Grade 3*	*Grade 4*	*p-Value*
Hypertension	5%	1%	2%	<1%	0.018
Bleeding	3%	<1%	<1%	0	0.011
Neuropathy	16%	<1%	9%	<1%	0.016
Vomiting	9%	1%	3%	<1%	0.010
Bleeding	3.1%	<1%	<1%	0	

of CBI. Cetuximab is a chimeric monoclonal antibody that binds to epidermal growth factor receptor, inhibits its phosphorylation, and prevents the initiation of several intracellular events related to angiogenesis, proliferation, and invasion *(13)*. The combination of irinotecan and cetuximab was compared with cetuximab alone in a randomized trial involving 329 patients who had disease progression after irinotecan therapy *(8)*. Sixty percent of these patients had received two previous lines of therapy containing both irinotecan and oxaliplatin. The response rate was 22.9% for the combination arm vs 10.8% for cetuximab monotherapy ($p = 0.007$). There was an impressive rate of disease control (complete response + partial response + stable disease) that reached 55% with the combination arm vs 36% with cetuximab alone ($p < 0.001$). PFS was significantly better in the combination arm (4.1 vs 1.5 mo). Median OS reached 8.6 mo for irinotecan/cetuximab but was not significantly different than the median OS of 6.9 mo with cetuximab alone. This may be partially attributed to the fact that 50% of patients in the monotherapy group received combination therapy with irinotecan after progression. Irinotecan/cetuximab could be used as a second-line regimen after the failure of an irinotecan-based therapy, including one that contained BV. For example, irinotecan/cetuximab is an appropriate second-line regimen after failure of first-line FOLFIRI and BV. There is no available direct comparison of the combination of irinotecan/cetuximab with FOLFOX or FOLFOX/BV in the second-line setting. However, based on nonrandomized cross-study data, irinotecan/cetuximab had a response rate of 23% after 5-FU and irinotecan failure, whereas FOLFOX had a response rate of 10–15% *(9,14)*.

2.4. CBI or CB

The combinations of CBI and cetuximab/BV (CB) were evaluated in BV-naive patients who had failed prior therapy with irinotecan or oxaliplatin and 5-FU combinations. Eighty-seven percent of patients had received two prior

lines of therapy. CBI manifested an impressive response rate of 37% and a median time to progression of 7.9 mo. Interestingly, the combination of the two targeted agents, cetuximab and BV, without any cytotoxic drug, was shown to have a partial response rate of 23% and a stable disease rate of 54% *(15)*. Both CBI and CB could be used as second-line therapies after the failure of an irinotecan-based regimen in a BV-naive patient. For example, CBI is a reasonable second-line therapy after failure of FOLFIRI. Choosing CBI or CB rather than FOLFOX/BV as second-line treatment after failure of FOLFIRI would be arbitrary given the absence of a direct prospective comparison. However, as discussed in section 4.1. and 4.2., the toxicity profile of one regimen over another and the overall goal of therapy may influence the selection.

3. SECOND-LINE THERAPY AFTER FAILURE OF A FRONT-LINE OXALIPLATIN-CONTAINING REGIMEN

The combination of infusional FOLFOX with or without BV has become a commonly used first-line therapy for metastatic CRC. The choice of second-line therapy after failure of an oxaliplatin-containing regimen depends on whether BV was included in the oxaliplatin-based combination as well as on the differential toxicities and efficacy of the potential second-line therapeutic options (Fig. 2).

3.1. FOLFIRI

As noted earlier, Tournigand et al. *(9)* had demonstrated the equivalence of the FOLFOX followed by FOLFIRI to FOLFIRI followed by FOLFOX. Specifically, FOLFIRI after FOLFOX failure had a response rate of 4% and a PFS of 2.5 mo. Even though not evaluated directly, FOLFIRI may be used as a second-line regimen after progression on FOLFOX + BV.

3.2. Irinotecan Monotherapy

The data related to the usage of irinotecan monotherapy in second line is mostly derived from patients who had failed 5-FU and LV *(16,17)*, rather than FOLFOX. It is unclear whether irinotecan monotherapy is equivalent to FOLFIRI after FOLFOX failure. There has not been a study specifically designed to answer this question. However, the FOCUS trial sheds light on a potential difference between irinotecan alone vs irinotecan with 5-FU as a second-line therapy *(18)*. FOCUS is a large trial involving 2100 patients with five arms; arm 1 assigned patients to 5-FU monotherapy with subsequent treatment with irinotecan alone upon progression; arms 2 and 3 assigned patients to 5-FU monotherapy followed by the addition of irinotecan (arm 2) or oxaliplatin (arm 3) while 5-FU was continued; the last two arms gave 5-FU in combination with irinotecan or oxaliplatin at the time of initiation of therapy. Although the trial did not reveal any difference in OS among the five arms, irinotecan appeared to be more effective in second line if 5-FU was continued rather than

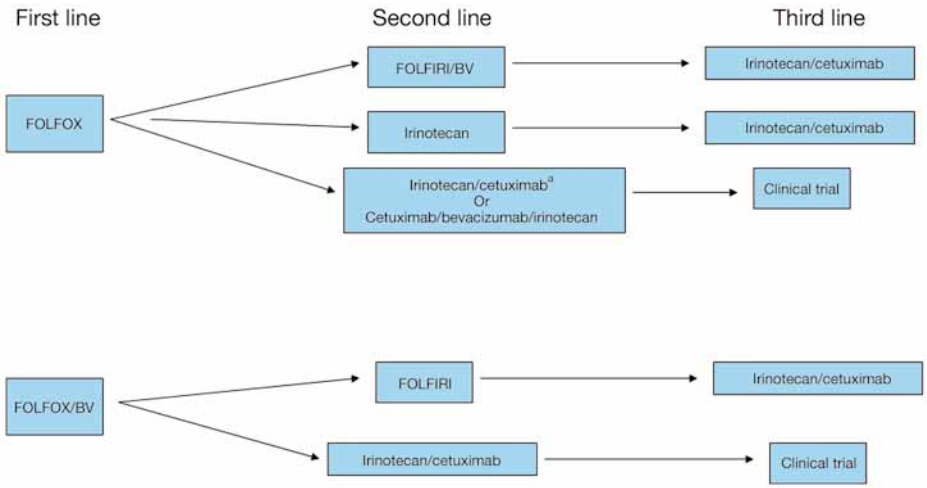

Fig. 2. Potential second- and third-line therapies after failure of an oxaliplatin-based regimen. ([a]This sequence, which excludes bevacizumab, may be most appropriate for a patient with contraindications to BV such as a recent myocardial infarction or cerebrovascular accident.)

stopped (response rate of 21 vs 11%). OS was 14.8 mo if 5-FU was continued after the addition of irinotecan vs 13.9 mo if 5-FU was stopped upon progression and irinotecan used alone in second line, but the difference was not statistically significant. The question remains as to whether it makes sense to continue 5-FU after a patient failed a 5-FU-containing regimen such as FOLFOX. The answer is not readily available at this point. On one hand, the FOCUS trial suggests that continuing 5-FU with irinotecan in second line is superior to using irinotecan alone. On the other hand, this was a five-arm trial powered to look for a difference in OS between different sequencing modalities and not specifically powered to address the question of using irinotecan monotherapy vs 5-FU and irinotecan in second line. In conclusion, it remains uncertain whether irinotecan monotherapy is equivalent to 5-FU and irinotecan in combination after the failure of a 5-FU-containing regimen such as FOLFOX.

3.3. CBI and CB

As noted in the case of irinotecan failure, irinotecan/cetuximab, CBI, and CB are three regimens that were evaluated in the third-line setting but could have an important role in second-line therapy after oxaliplatin failure. The study by Cunningham et al. *(8)* had demonstrated the efficacy of cetuximab/irinotecan after the failure of irinotecan-based therapy. Based on these data, cetuximab has been traditionally added to irinotecan in third line after patients failed an oxaliplatin- and an irinotecan-based combination. However, the question arises

whether there is benefit to the usage of cetuximab in combination with irinotecan in second-line therapy after progression with oxaliplatin rather than waiting for progression of disease with second-line irinotecan. An ongoing trial that randomizes patients to irinotecan alone vs irinotecan and cetuximab after oxaliplatin failure is intended to answer this question. One argument against the usage of irinotecan/cetuximab in second line before irinotecan monotherapy failure is that it consumes two potentially active regimens that have activity independently, thereby limiting the number of long-term options. In other words, a patient could receive irinotecan monotherapy or FOLFIRI after FOLFOX or FOLFOX/BV and subsequently receive irinotecan/cetuximab in third line. However, the sequential usage of these regimens may deprive some patients from the earlier introduction of therapy that could potentially improve their chance of response or palliation. The sequential usage may also result in missing a window of opportunity after which the performance status of the patient worsens, making them less likely to tolerate therapy.

CBI and CB have both resulted in significant clinical activity, as discussed previously *(15)*, which makes them potential choices for second-line treatment after first-line oxaliplatin-based treatment that does not include BV.

4. STRATEGIES AND CONCERNS TO BE CONSIDERED IN THE CHOICE OF SECOND-LINE THERAPY

4.1. Is Cure Still Possible After Second-Line Therapy?

It is logical to conclude from our discussion so far that the choice of second-line therapy is influenced, to a large extent, by the type of first-line therapy. Another factor to be considered is the overall goal of therapy for each individual patient. In patients with limited disease to the liver or lung, surgical resection of metastases has been shown to offer long-term cancer control and potential cure *(19–22)*. Even though the resection of single organ metastases is traditionally done on diagnosis or after effective first-line therapy, this may not be successful if the patient has progression of disease on first-line treatment. If the disease is still limited to a single organ and still potentially resectable after failure of first-line therapy, it is not unreasonable to adopt a second-line regimen that has the highest chance of tumor response with the hope of proceeding to surgical resection of metastases. For example, if a patient with metastatic disease limited to the liver receives FOLFOX as first-line therapy and experiences an increase in the size of two existing liver lesions at 6 wk, it may be appropriate to choose second-line treatment with CBI because this is the combination with the highest response rate and, therefore, the highest chance of allowing the patient to have surgical resection of the liver metastases. It is important to note that such an approach is not based on any direct randomized comparisons of second-line therapies and their effectiveness in allowing for tumor downstaging.

Table 2
Common Toxicities Associated With the Combination of CBI vs CB *(15)*

	CBI	*CB*
Grade 3/4 neutropenia	22%	0%
Grade 3/4 diarrhea	24%	0%
Grade 2 diarrhea	29%	5%
Grade 3 fatigue	10%	0%
Grade 2 fatigue	32%	5%
Grade 3 nausea	2%	0%
Grade 3 skin rash	17%	20%
Grade 2 skin rash	60%	65%

Furthermore, there is no available published data on resection of metastases after second-line therapy. Despite these facts, the effectiveness of the second-line therapy may influence the treating oncologist's choice if the aim of treatment is to perform surgical resection of metastases.

4.2. Toxicity Concerns

As the number of options for second-line therapy expands, it becomes increasingly possible to tailor the choice of therapy to the patient's lifestyle and comorbidities. Tables 1 and 2 present an overview of the potential toxicities with some of the second-line regimens discussed. While understanding the limitations of cross-study comparisons, one is still able to identify some of the more concerning toxicities related to a specific regimen, which could potentially influence the use of one over another. For instance, a patient with peripheral sensory neuropathy from diabetes may not be the best candidate for FOLFOX in second line after failure of FOLFIRI. The risk of neuropathy is even greater if FOLFOX is combined with BV in second line *(12)* (Table 1). The same argument could apply to other patients for whom neuropathy is a concern such a musicians, surgeons, anyone who uses a computer keyboard, and laborers in outdoor cold climates *(23)*. Irinotecan/Cetuximab may offer such a patient an effective alternative regimen that does not exacerbate existing symptoms *(8)*. Irinotecan has been associated with diarrhea and neutropenia *(24)*. Patients with predisposition to diarrhea and a poor performance status may benefit from therapy with CB without irinotecan, given the significantly lower incidence of diarrhea, neutropenia, and fatigue with CB *(15)* (Table 2).

Although this discussion is not intended to be a comprehensive review of toxicities, it is meant to illustrate the fact that they could influence the selection of one second-line treatment over another based on the known toxicity profiles of the different regimens.

4.3. The Promise of Pharmacogenomics and Tailored Therapy

The ability to predict response and toxicity to a particular therapy based on the expression level or polymorphisms of drug target genes or genes involved in drug metabolism, transport, DNA repair, and cell cycle control has been the focus of intense research *(25,26)*. Molecular predictors of response may play a role in determining the first line of therapy that a patient should receive with the hope of improving efficacy and maximizing the possibility of long-term control. Such an approach may also lead to minimizing toxicity through the avoidance of regimens with a low likelihood of response and through the ability to predict the type and severity of side effects. An extensive discussion of the concept of tailored therapy based on pharmacogenomics is beyond the scope of this chapter. However, we will utilize a couple of examples to highlight the potential future impact of this field on the choice of second-line therapy. For instance, excision repair complementation group 1 (*ERCC1*) gene family is thought to prevent DNA injury and mutations via the nucleotide excision repair pathway. Given the mechanism of action of oxaliplatin, which forms bulky DNA adducts, *ERCC1* has been evaluated for its role in predicting clinical outcome with oxaliplatin-based therapy *(27,28)*. Thymidilate synthase (TS) is the target of fluoropyrimidines like 5-FU. TS inhibition prevents the cell from its sole *de novo* source of thymidine, which is essential for DNA replication and repair. Shirota et al. *(28)* evaluated tumors of patients treated with 5-FU/oxaliplatin for the messenger RNA (mRNA) expression levels of TS and *ERCC1*. Both TS and *ERCC1* mRNA expression levels had a statistically significant association with survival in these patients. *ERCC1* and *ERCC2* single-nucleotide polymorphisms have also been found to be associated with survival or response to oxaliplatin-based therapy *(29)*. These data are derived from retrospective studies with small numbers of patients. However, if they are validated prospectively, they may allow us to predict the likelihood of response to oxaliplatin. In such case, a patient with elevated TS and *ERCC1* gene expression levels or with an *ERCC1* polymorphism associated with resistance to oxaliplatin may receive irinotecan-based therapy in first line (FOLFIRI or FOLFIRI and BV) followed by irinotecan and cetuximab rather than FOLFOX.

Tailored therapy can also be aimed at minimizing toxicity based on known genetic predispositions to increased complications with one drug over another. For instance, *glutathione S-transferase P1 I105V* polymorphism was found to be associated with early onset of oxaliplatin-induced neurotoxicity *(30)*. Variations in drug metabolism and transport genes have been associated with the risk irinotecan toxicity. UDP-glucoronosyltransferase, UGT1A1, is known to glucoronidate SN-38, the active metabolite of irinotecan, to an inactive product. The UGT1A1 7/7 variant has been shown to be associated with the risk of neutropenia *(31)*. Because the drug metabolism pathway of irinotecan is polygenic, it is unlikely that UGT1A1 polymorphisms alone will allow for adequate toxicity

risk stratification. Current and future efforts need to identify other relevant genes that may be assessed and analyzed together in order to delineate different risk groups. An example of this approach can be found in a study by Innocenti et al. *(32)*, in which patients were assigned to low-, intermediate-, and high-risk groups for neutropenia based on polymorphisms in UGT1A1, *SLCO1b1* (an organic anion transporter gene expressed in liver) and gender. These data could complement the observed toxicity profiles in clinical studies and help guide the choice of first- and second-line treatments for metastatic CRC.

5. CONCLUSION

The second-line treatment of metastatic CRC has been complicated by the advent of multiple effective combinations of cytotoxic and targeted agents. We believe that the treating oncologist should have a "road map" designed for each patient based on the patient's performance status, the goal of therapy, and the known toxicities. Such an approach will result in the sequencing of the different therapeutic agents and combinations based on the currently available data and in a manner that best fits the individual patient. Knowledge of molecular predictors of response and toxicity should complement and refine the clinical decision-making process as we move into the future. Ideally, a better understanding of the mechanisms of resistance to specific drugs and the molecular basis of synergy of certain combinations is needed to maximize the benefit of each drug. This will help determine the best place for a drug in the sequence of therapeutic options and its optimal use as a single agent or in combination.

Meanwhile, we continue to rely on the available data to choose the appropriate second-line therapy based on the type of first-line therapy and the nondirect comparisons of efficacy and toxicity of the second-line regimens.

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8 Adjuvant Chemotherapy for Colon Cancer

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Summary

Colon cancer is curable by surgery, but frequently recurs despite apparently complete surgical resection. The risk of such recurrence is closely linked to pathological stage. The concept of adjuvant chemotherapy was conceived many years ago as a way of eradicating microscopic residual disease, and proven by randomized clinical trials in colon cancer in the 1980s. Since that time, many important trials have been completed, and both our understanding of and armamentarium against locoregionally confined colon cancer have improved as a result. Despite this, certain aspects of adjuvant therapy remain controversial, particularly the use of adjuvant therapy in patients with node-negative colon cancer. In this chapter we review the studies that proved adjuvant 5-FU-based therapy effective, discuss controversies in adjuvant therapy, review recent trials with additional agents, and finally discuss the potential for molecular markers to augment stage in determining risk and response to therapy.

Key Words: Adjuvant therapy; stage II; stage III; drug therapy; 5-fluorouracil; 5-FU; oxaliplatin; microsatellite instability/prognosis; 18q loss of heterozygosity/prognosis.

1. INTRODUCTION

A considerable proportion of patients with apparently localized colon cancer experience recurrence of disease despite complete surgical resection. Adjuvant therapy was conceived more than 40 yr ago as a way to decrease recurrence by eliminating small volume or microscopic residual disease. In this chapter we will highlight results from 25 yr of clinical trials in colon cancer that led to acceptance of the long-time standard of 5-FU modulated by leucovorin (LV) for patients with node-positive colon cancer. We will then focus on recent advances

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that support the incorporation of oxaliplatin with 5-FU as a new standard of care. We will also attempt to clarify the still-controversial issue of the effectiveness of chemotherapy for patients with node-negative colon cancer, providing an evidence base for discussion of this complex issue with patients. We will discuss the present and potential future roles of molecular and genetic markers in the selection or omission of drugs for adjuvant therapy of colon cancer. Finally, we will examine the potential of new targeted agents to impact on adjuvant therapy.

2. ADJUVANT CHEMOTHERAPY FROM MOF TO FOLFOX

2.1. Fluorouracil and LV

The combination of methyl-CCNU, vincristine, and 5-FU (MOF) was the first chemotherapy regimen to convincingly demonstrate clinically and statistically significant disease-free (DFS) and overall survival (OS) improvement over surgery alone in an adequately powered, randomized study of patients with locoregional colon cancer *(1)*. At the same time as this first National Surgical Adjuvant Breast and Bowel Project (NSABP) trial, an era of schedule modification and study of a variety of 5-FU modulating agents began, spurred by advances in studying patients with metastatic colon cancer *(2–7)*. The first agent felt to have 5-FU modulation as its principal activity was levamisole, which significantly decreased the risk of disease recurrence when given with 5-FU in an early North Central Cancer Treatment Group (NCCTG) adjuvant trial *(2)*. The mechanism of action of levamisole, principally utilized as an antihelminthic for sheep, remains unknown to this day. In retrospect, this agent may not have had activity at all, but rather may have been part of the first adequately powered study with adequate doses and duration of 5-FU to allow the modest activity of 5-FU to show a survival benefit.

LV, which works to stabilize the complex between 5-FU, thymidylate synthase (TS), and reduced folates *(8,9)*, emerged as an alternative, mechanism-based 5-FU modulator. When given with 5-FU in patients with stages II and III disease, LV modestly improved outcomes over 5-FU alone, and added relatively little toxicity *(10–17)*.

First presented in 1998, the results of the Intergroup 0089 adjuvant colon cancer trial settled several important questions and led to widespread adoption of 6 mo of 5-FU with LV modulation as the standard of care *(17)*. The Intergroup 0089 study randomized 3759 high-risk stage II and stage III patients to one of four treatment arms: 5-FU plus levamisole for 12 mo, 5-FU plus high-dose LV (Roswell Park Memorial Institute [RPMI] Regimen, weekly for 6 of 8 wk for 4 cycles), 5-FU plus low-dose LV (Mayo regimen, daily for 5 d every 4–5 wk for 6 cycles), and 5-FU plus low-dose LV plus levamisole (Table 1). In final analysis (which differs slightly from the originally presented data), there were no significant differences in outcomes between the four groups *(17a)*. Overall,

Table 1
Results of Intergroup Adjuvant Colon Study 0089

		DFS (%)		*OS*	
Treatment Arm:	***N***	**5-yr**	**10-yr**	**5-yr**	**10-yr**
5-FU/LV Mayo Sched. (6 mo)	953	60	49	66	52
5-FU/LV Roswell Park (6 mo)	946	58	47	66	52
5-FU/levamisole (12 mo)	835	55	45	64	50
5-FU/LV/levamisole (6 mo)	827	49	68	54	59

the study results suggested that either of the 6-mo 5-FU/LV regimens could be considered appropriate therapy for stage III colon cancer, and that levamisole is not a necessary component of therapy. Importantly, this study also demonstrated that 6 months of systemic 5-FU and LV is as effective as 12 mo of therapy. Because the Mayo regimen gave more stomatitis and neutropenia, the weekly RPMI regimen, which consequently produces a higher rate of diarrhea, became the regimen that many oncologists preferred.

The benefits of adjuvant 5-FU-based chemotherapy, both overall and for various risk groups, have recently been estimated based on a pooled analysis of seven pivotal adjuvant trials that included a surgery-only control arm *(18)*. Adjuvant modulated 5-FU is estimated to decrease the risk of death at 5 yr by 26% (for the pooled populations of patients with stages II and III disease), corresponding to an absolute improvement in 5-yr survival from 64% without treatment to 71% with therapy. For stage III patients, the hazard ratio (HR) for death associated with 5-FU-based chemotherapy is 0.60 (or 40% reduction in risk of death, for both N1 and N2 disease) and OS improves from approx 58 to 71% for N1 disease and from 29 to 44% for N2 disease. These data unequivocally demonstrate the value of adjuvant chemotherapy for stage III patients who are medically appropriate for chemotherapy. The benefit of chemotherapy for the stage II population is proportionately lower (HR for death 0.83, or a 17% decrease in risk of death), and is discussed at length in Section 2.8.

2.2. Continuous Infusion and Mixed Continuous-Bolus Schedules

The concept of administering 5-FU over several days or continuously, a practice pioneered in the United States but later more closely embraced by European medical oncologists, is at least as effective as bolus administration,

and causes fewer high-grade toxicities *(19)*. A small trial conducted by Seifert et al. in the 1970s suggested benefit of infusion over bolus 5-FU administration in the metastatic disease setting *(20)*. Most studies of continuous 5-FU subsequent to the Seifert trial were also conducted in patients with metastatic colorectal cancer (CRC), and a meta-analysis of these trials suggested similar outcomes between bolus and infusion 5-FU strategies *(21)*. Intergroup 0153, the results of which were published in 2005, was the largest trial to explore the infusion vs bolus question in the adjuvant setting *(22)*. In this trial of 1135 patients (which included administration of levamisole in both arms), continuous 5-FU 250 mg/($m^2 \cdot d$) for 27 wk was compared with the Mayo 5-FU/LV schedule for six cycles (approx 32 wk). No improvement in DFS or OS was noted in the study, but continuous intravenous infusion (CIV) 5-FU was noted to have a significantly improved toxicity profile.

More recently, a relatively small study of continuous vs bolus 5-FU performed in the United Kingdom has been published *(23)*. It compared a short duration of continuous 5-FU (12 wk) with a standard 6-mo course of bolus 5 FU/LV (Mayo Clinic regimen). The trial found that 12 wk of CIV 5-FU was at least equivalent to 6 mo of bolus 5-FU/LV, and in fact demonstrated a trend toward superiority of the continuous regimen (HR for OS 0.79; 95% confidence interval [CI] 0.61–1.03, $p = 0.083$). Unfortunately, at 400 patients per arm, the trial was underpowered to demonstrate superiority statistically, and was not a true equivalence trial by design. In spite of that, the data regarding the potential benefit of therapy limited to 3 mo is compelling.

Germane to the upcoming discussion about oxaliplatin-5-FU combination therapy is the history of the bolus-infusion regimen of 5-FU and LV developed by Aimery de Gramont of the Hopital Saint-Antoine in Paris. The LV5FU2 regimen forms the backbone for what is currently known as the FOLFOX4 regimen. The design of this regimen was based on several factors: the cell cycle specificity and pharmacokinetics of 5-FU, and the observation over time that resistance to bolus-administered 5-FU can occasionally be overcome by administration of the same drug using a continuous infusion schedule *(24)*. It was therefore surmised that combining bolus and infusion administration might prove superior to either alone. A randomized study of the LV5FU2 regimen (d 1 and 2 of 14: LV 200 mg/m^2, 5-FU 400 mg/m^2 bolus / 600 mg/m^2 continuous $\times$ 22 h) vs the Mayo Clinic regimen was conducted in Europe, with the LV5FU2 given for 24 wk in comparison with 36 wk for the Mayo Clinic regimen *(25)*. The study enrolled 905 patients, and demonstrated no clinically or statistically significant differences between the arms. It should be noted that the design was not a true equivalence design, and is therefore underpowered to prove noninferiority. Nevertheless, on the basis of this trial, LV5FU2 is widely considered to be a reasonable control arm for the MOSAIC trial, which is discussed in detail in Section 2.4.

2.3. Oral Fluoropyrimidines

The ease of administration of oral fluoropyrimidines makes them attractive alternatives to both bolus and infusional 5-FU. Recent clinical trials have proven the oral route to be as effective as bolus 5-FU/LV in adjuvant therapy. A number of oral formulations are available worldwide. Tegafur/uracil (UFT) is a combination drug that contains both an active drug (tegafur, a 5-FU prodrug) and an inhibitor of dihydropyrimidine dehydrogenase (DPD) to prevent degradation of 5-FU in the gastrointestinal (GI) tract. S-1 incorporates a more potent DPD inhibitor (5-chloro-2,4 dihydroxypyridine) with tegafur and has been used exclusively in Japan *(26,27)*. The third, capecitabine, is an oral prodrug of 5-FU that is preferentially metabolized within tumor cells to 5-FU *(28)*.

In 2004, the results of the NSABP C-06 were presented. C-06 compared UFT to bolus 5-FU/LV (RPMI) in resected stage II and III colon cancer; there was no difference in outcomes or in toxicity in either arm, though complete toxicity data have not been published *(28a)*. UFT has never been approved in the US, and is not likely to become available. Presented at the same meeting as NSABP C-06, the X-ACT trial also explored the potential equivalence of an oral agent to intravenous 5-FU/LV. In X-ACT, capecitabine at a dose of 1250 mg/m^2 BID was as effective as the Mayo Clinic regimen in resected stage III colon cancer (*see* Table 1) *(29)*. This study was powered for a primary endpoint of equivalence in DFS to 5-FU/LV, though the hazard ratio for DFS of 0.87 (95% CI 0.75,1.00) suggested capecitabine may be superior to the Mayo Clinic regimen. In subgroup analyses, all cohorts of patients, including those older than 70 yr, appear to benefit equally from capecitabine. Toxicities of these drugs were different, with more neutropenia and stomatitis in patients on the 5-FU/LV arm, but more hand–foot syndrome and severe (albeit reversible) hyperbilirubinemia in patients receiving capecitabine.

Though the results of the X-ACT trial and C-06 trial support the use of oral fluoropyrimidines as single agents in adjuvant colon cancer, claims of superior efficacy and decreased toxicity of capecitabine may be overstated. It is difficult to directly translate the results of this study into clinical practice in North America, as clinicians (particularly those in the United States) often prescribe a lower starting dose of capecitabine (typically 1000 mg/m^2 twice a day), which could potentially be less effective than the dose prescribed in the X-ACT trial (In that study, ~40% of patients required dose reduction from their starting dose of 1250 mg/m^2 twice daily). Furthermore, few medical oncologists presently choose the Mayo Clinic schedule of 5-FU/LV in practice because of toxicity and convenience issues. Despite these minor reservations, capecitabine can be seen as a reasonable alternative to bolus 5-FU/LV in patients who are either poor candidates for combination chemotherapy, or who prefer to avoid intravenous therapy. It should be noted that strong patient compliance is necessary

for successful oral treatment and only highly motivated and reliable patients should be considered for oral adjuvant therapy.

2.4. Oxaliplatin, 5-FU, and LV

Combinations of bolus plus infusional 5-FU/LV with oxaliplatin (FOLFOX) and bolus and/or infusional 5-FU/LV with irinotecan (IFL and FOLFIRI, respectively) have led to significant improvements in median survival of patients with metastatic CRC *(29a–32)*. The success of combination chemotherapy regimens in metastatic disease has led to clinical trials of these combinations as adjuvant therapy in patients with resected locoregional disease. These adjuvant trials began to mature in 2004 and 2005.

In 2004, the European MOSAIC trial was the first trial ever to demonstrate the superiority of a combination chemotherapy regimen over 5-FU/LV alone. The MOSAIC study randomized 2246 patients with stages II and III colon cancer between FOLFOX4 and LV5FU2. At time of analysis for the first report, with 38 mo median follow-up, patients in the FOLFOX group had a 23% decrease in the relative risk of recurrence compared with those treated with LV5FU2, corresponding to a 3-yr DFS survival of 78% in the FOLFOX group and 73% in the LV5FU2 group *(33)*. A subsequent update of the study with 56 mo of follow-up confirmed that the addition of oxaliplatin confers a 6.6% absolute reduction in the risk of relapse, with 4-yr DFS of 76% in the FOLFOX arm and 69% in the LV5FU2 arm (HR 0.77, 95% CI 0.65–0.90) *(34)*. To date, OS in the two arms is statistically equivalent, but the study is not mature for 5-yr OS analysis. There is an (as yet) nonsignificant 3.2% difference in OS for patients with stage III disease. The number of patients with stage II disease is likely too small to detect a survival difference for that subgroup, a fact that will be discussed in more detail below.

The addition of oxaliplatin to the 5-FU/LV backbone did result in additional toxicity, but overall this toxicity is considered acceptable. Oxaliplatin caused significantly more grade 3 and 4 neutropenia, febrile neutropenia, thrombocytopenia, nausea and vomiting, diarrhea, and allergic reactions (*see* Table 2). Ninety-two percent of patients suffered from parasthesias (peripheral neuropathy) of any grade, although half of these were grade 1—not interfering with function. For the most part, patients who developed severe neuropathy had fairly rapid improvement in symptoms after completing or withdrawing from therapy. Only 1% of patients had residual grade 3 neuropathy (defined as having severity such as to interfere with activities of daily living [ADL]) 12 mo after completion of therapy. A number of patients did continue to suffer from persistent grade 1 or 2 neuropathy after the use of oxaliplatin, and physicians recommending the oxaliplatin-containing regimen should be aware that grade 2 neuropathy, that which interferes with function but not ADLs, can be a persistent and bothersome issue for many patients well after completion of adjuvant therapy.

Table 2
Grade 3 and 4 Toxicities of Acceptable Regimens From Recent Trials of Adjuvant Chemotherapy

	X-ACT[a] *(n = 1987)*		*MOSAIC (n = 2246)*		*NSABP C07*[b] *(n = 2492)*	
	Mayo	**Capecitabine**	**LV5FU2**	**FOLFOX4**	**RPMI**	**FLOX**
3-yr DFS	61%	64%	73%	78%	72%	77%
Neutropenia	26%	2%	5%	41%	—	4%
Febrile Neutropenia	—	—	<1%	2%	—	—
Diarrhea	13%	11%	7%	11%	—	38%
Vomiting	3%	3%	1%	6%	—	—
Stomatitis	14%	2%	2%	3%	—	—
Parasthesias	—	—	<1%	12%	1%	8%
Hand-Foot Syndrome	<1%	17%	—	—	—	—
Hyperbilirubinemia	6%	20%	—	—	—	—

—, denotes unreported; [a], X-ACT included stage III patients only; [b], C07 has been presented in abstract form only, full toxicity data not available;

The benefits of combining oxaliplatin with 5-FU/LV have been confirmed by a second large study, NSABP C-07. C-07 was reported in abstract form at the annual meeting of the American Society of Clinical Oncology (ASCO) in 2005. C-07 randomized 2492 patients with stages II and III colon cancer to receive the RPMI regimen of weekly bolus 5-FU/LV with or without oxaliplatin (FLOX) (Fig. 1) *(35)*. The trial confirmed that the addition of oxaliplatin improves 3-yr DFS after resection of stage II and III colon cancer. Three-year DFS was prolonged from 72 to 77% (HR for DFS 0.79). As in the MOSAIC trial, oxaliplatin treatment led to modestly increased toxicity from adjuvant chemotherapy, causing any grade 3 or greater side effect in 51% of patients getting 5-FU/LV compared with 61% getting FLOX. As in the MOSAIC study, there appeared to be a proportionally greater relapse-free survival benefit for stage III patients compared with stage II patients.

Toxicities of combination chemotherapy clearly differ depending on the mode of fluoropyrimidine administration. When comparing MOSAIC and C-07, diarrhea appears to be more prominent in patients treated with bolus 5-FU. The incidence of grade 3 or 4 diarrhea in the FLOX arm of C-07 was 38%, but only 11% on the MOSAIC trial. Furthermore, on C-07 56 (4.5%) patients treated with FLOX and 34 (2.7%) patients treated with 5-FU/LV were hospitalized for a GI toxicity syndrome characterized by either diarrhea, dehydration, and bowel wall thickening or grade 3 or 4 diarrhea in conjunction with neutropenia and sepsis *(35,36)*. The description of this enteropathy is very similar

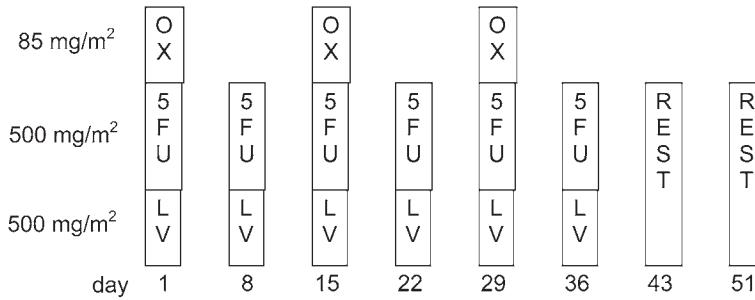

Fig. 1. The bolus FLOX regimen of NSABP C-07 (one cycle of three).

to the life-threatening GI toxicity associated with the use of irinotecan and bolus 5-FU/LV (IFL) *(37)*. The incidence of neutropenia caused by FLOX was less than resulted from FOLFOX on the MOSAIC study: 4 vs 41% (although the incidence of febrile neutropenia with FOLFOX was only 2%). As might be expected given the lower cumulative dose of oxaliplatin given in C-07 than in the MOSAIC trial (765 mg/m^2 vs 1020 mg/m^2, respectively), the incidence of peripheral neuropathy was lower with FLOX: 85 and 92% had any grade of neuropathy, 8 and 12% had grade 3 neuropathy on FLOX and FOLFOX, respectively *(33,35)*.

Based on the combined results of MOSAIC and C-07, the addition of oxaliplatin to adjuvant 5-FU/LV has convincingly been shown to be more effective than adjuvant 5-FU/LV, with a comparable improvement in the relative risk of relapse of 24% in MOSAIC and 21% in C-07. However, whether infusional and bolus 5-FU administration are equally safe and effective is, at present time, uncertain. The effectiveness of different methods of fluoropyrimidine administration in combination with oxaliplatin is currently being examined in randomized studies in the metastatic setting. Preliminary reports of one randomized phase II trial suggest that the bolus oxaliplatin/5-FU/LV regimens may result in a lower response rates than do infusion-5-FU or capecitabine-based regimens *(38)*. Whether this increased response rate will translate into improved survival in the metastatic setting, or to improved DFS and OS in the adjuvant setting remains to be seen. At the time of this writing, FOLFOX is the appropriate standard for adjuvant oxaliplatin-based treatments. FLOX should be regarded at this time as an alternative for those few patients in whom there are substantial impediments to infusional drug administration.

Given the convenience of administration of capecitabine, and the (at least) equivalence of capecitabine to bolus 5-FU/LV in the X-ACT study described previously, it is tempting for many physicians to recommend a combination of oxaliplatin and capecitabine to adjuvant colon cancer patients. Such a recommendation would be premature in the absence of direct data. To partially

address this question, a phase III trial comparing capecitabine and oxaliplatin (cape/ox or XELOX) to bolus 5-FU/LV has completed accrual, and preliminary safety data have shown no unexpected adverse effects *(39)*. However, whether cape/ox is incrementally better than bolus 5-FU/LV is no longer the pertinent question, but rather its comparative efficacy to FOLFOX will need to be addressed in future trials. Such an equivalence trial would by necessity be very large, and may in fact never occur. For now, there is no phase III data to support the equivalency of capecitabine substitution for 5-FU in combination regimens in the adjuvant setting, and the authors would recommend caution in doing so outside of clinical trials in patients in whom the goal of treatment is the cure of their disease.

2.5. IFL

In contrast to the success of the oxaliplatin/5-FU combination chemotherapy in adjuvant trials, the experience of adding irinotecan to 5-FU/LV in the adjuvant setting has been somewhat disappointing. Three studies have been reported, the first being Cancer and Leukemia Group B (CALGB) 89803, which compared the bolus IFL regimen to bolus 5-FU/LV *(40)*. Data from this trial were released after a planned interim analysis crossed the prespecified futility boundaries such that even with continued follow-up to the trial, IFL could not be proven superior to 5-FU/LV *(37,40)*. A second trial, the Pan European Trial in Adjuvant Colon Cancer (PETACC)-3, failed to meet its primary endpoint of improvement in 3-yr DFS with the addition of irinotecan to infusional 5-FU/LV, with a HR for DFS of 0.89 ($p = 0.091$) *(41)*. The authors preformed a post hoc analysis, adjusting for imbalances in T stage and number of positive lymph nodes between arms (stage factors that were not stratification variables at randomization). This analysis resulted in an adjusted hazard for DFS favoring the irinotecan-containing arm (HR 0.86, $p = 0.026$). Although this analysis suggested possible additional benefit to irinotecan, it should not be considered as definitive evidence of the superiority of FOLFIRI over 5-FU/LV alone. Furthermore, the magnitude of benefit from irinotecan is significantly smaller even in the adjusted analysis than was the benefit from oxaliplatin. The final study examining the potential benefits of adjuvant irinotecan was the French ACCORD2 trial *(42)*. Though smaller than the other trials, the study was designed to be enriched for high-risk patients, enrolling 400 patients with N2 disease or high-risk presentations such as perforation or obstruction. The study showed no benefit to the irinotecan-containing combination even after adjustment for potential prognostic factor imbalances on the treatment arms. In contrast, this group of high-risk patients is the group in whom FOLFOX has shown to have the greatest impact on DFS *(34)*.

When the results of the PETACC-3 trial is taken in the context of the results from both CALBG 89803 and ACCORD2, the data simply do not

support the use of adjuvant irinotecan. Furthermore, any small benefit may be outweighed by the toxicity that comes from the inclusion of irinotecan to adjuvant therapy. The most disconcerting toxicities among these trials occurred with the IFL regimen, which led to a marked increase in the incidence of neutropenia, febrile neutropenia, and death on treatment (2.8 vs 1%). Deaths related to therapy with IFL have been attributed to an enteropathy characterized by diarrhea, dehydration, and sepsis *(37)*. The incremental toxicity from the addition of irinotecan to infusional 5-FU regimens is smaller, and similar in magnitude to that of FOLFOX; however, the benefit of adjuvant irinotecan does not appear to be great enough to merit even this smaller incremental risk. Based on the results of these trials, the authors cannot recommend the addition of irinotecan to 5-FU/LV in the adjuvant setting regardless of which schedule is chosen. The irinotecan story is a puzzling one, as in the metastatic colorectal setting there appears to be essentially no difference in outcome between irinotecan- and oxaliplatin-based combinations *(30)*. Hopefully, evaluation of tumor markers being carried out on tissues from patients enrolled in CALGB 89803 and PETACC-3 will provide some clues as to why this difference between adjuvant and metastatic therapy has been observed.

2.6. Duration of Adjuvant Therapy

The current standards of care in terms of duration of therapy are informed by three trials—the MOSAIC trial investigators specified 24 wk of therapy *(33)*, as this has generally been considered the current standard of care for most patients with stage III colon cancer. For patients receiving 5-FU/LV alone, studied durations are six cycles of Mayo schedule 5-FU/LV based on Intergroup-0089 (which amounts to between 24 and 30 wk of therapy) *(17a)*, or four cycles of 5-FU/LV on the RPMI schedule, amounting to approx 30 wk. For patients treated with capecitabine, the X-ACT study specified eight cycles of capecitabine (also 24 wk) *(29)*. Thus, the largest body of current literature has been based on a 24-wk adjuvant treatment period. It is certainly possible, particularly in regards to combination chemotherapy, that this duration of therapy is longer than necessary to produce the results that have been realized in these trials. In support of that possibility are the previously mentioned results of the UK trial that compared 6 mo of bolus 5-FU/LV to 3 mo of protracted-infusion 5-FU, and actually produced a trend favoring the shorter-duration therapy *(23)*. However, the small size of this study suggests that the issue of short-duration continuous 5-FU remains an open question. Current cooperative group trials maintain the 24-wk duration of chemotherapy, and explore the potential benefits of so-called targeted therapies that are discussed in Section 4, rather than focusing on questions of optimal duration of therapy.

2.7. Adjuvant Chemotherapy and Elderly Patients

Three meta-analyses have been published on the subject of the relative benefits of 5-FU/LV for elderly patients, generally defined as those patients older than 70 yr of age, two of which were focused on adjuvant therapy. This subgroup represents a substantial fraction of all patients diagnosed with CRC. All three meta-analyses have come to the same conclusion, which is that the benefits of 5-FU-based chemotherapy extend to the elderly, who glean equal survival benefit as do younger patients with little, if any, evidence of increase in toxicity (with the possible except of a slight increase in the incidence of leucopenia) *(43–45)*. No such analysis to date has been performed in patients treated with oxaliplatin/5-FU combinations, but based on limited information on relative toxicities of FOLFOX in elderly patients with metastatic disease *(46)*, it would seem unnecessary to exclude patients for consideration of treatment with FOLFOX on the basis of age alone.

2.8. Benefit of Adjuvant Therapy in Stage II

The prognosis of stage II colon cancer is generally good, with up to 83% of patients experiencing long-term survival in more recent literature *(47)*. Whether survival is improved by adjuvant chemotherapy is a matter of considerable debate. Many adjuvant colon cancer trials have included stage II patients, although the stage II subgroup has generally comprised only a quarter to a third of the study population. It has been estimated that 4000 stage II patients would be required to definitively demonstrate benefit of a particular treatment strategy, assuming a reasonable 2% improvement in OS *(48)*. As trials of that size are uncommonly completed, the decision to use chemotherapy in stage II patients must be based on the best available evidence.

Three pooled analyses of stage II patients enrolled on adjuvant trials of 5-FU-based chemotherapy have been reported. The largest of these pooled patient data from seven trials that compared adjuvant 5-FU (two trials with levamisole, five with LV) to best supportive care; 1440 of these were node-negative patients *(18)*. Though in this investigation patients appeared to derive more benefit from chemotherapy as the number of positive lymph nodes increased, the node-negative patients still had a statistically significant 17% reduction in the risk of relapse. However, the improvement in OS was estimated at only 1% and was not statistically significant. The IMPACT B2 meta-analysis, an earlier analysis that included some of the same trials, demonstrated a comparable decrease in the risk of recurrence and a 2% absolute improvement in OS, neither of which were statistically different from the observation group *(49)*. A third combined analysis of four trials carried out by the NSABP (C01, C02, C03, C04) assessed the magnitude of benefit in Dukes B patients receiving the superior treatment on each of these trials *(50)*. This analysis pooled

patients from a heterogeneous group of treatments into inferior and superior arms, a design that has been criticized. That said, the NSABP meta-analysis demonstrated a decrease in the risk of relapse of 30% in the superior group, and an approx 5% improvement in OS that reached statistical significance.

One large randomized trial that was powered to detect small differences in outcome for stage II patients has been performed in the UK by the Quick and Simple and Reliable (QUASAR) study group. The QUASAR investigators enrolled 3238 patients, 91% of whom were Dukes B and 71% of whom had colon cancer (the remaining patients had rectal cancer) *(51)* to one of three 5-FU treatment arms or to observation. The study demonstrated significant improvement in DFS, with an 18% reduction in the risk of recurrence associated with 5-FU-based therapy. The authors estimated that the true survival benefit is between 1–5%. One difficulty in interpreting this result is the inclusion of a significant number of stage II rectal cancer patients, a group of patients known to experience a higher rate of distant failure compared with like-staged colon cancer patients. Inclusion of these higher-risk patients in an observation arm could have resulted in an overestimation of adjuvant benefit.

Although confidence intervals around the critical outcomes of relapse-free and OS are wide in the pooled analyses of trials that attempt to assess the benefit of chemotherapy in stage II patients, point estimates have been fairly consistent. As such, patients can be counseled that adjuvant chemotherapy for pooled node-negative colon cancer likely decreases the absolute risk of death by 2–4%. ASCO has recently convened an expert panel that provided recommendations for adjuvant therapy of stage II colon cancer. They concluded that its routine use was not indicated, but that it might be considered for patients with higher than average risk, including those with inadequately sampled nodes ($<$13), T4 primary lesions, perforation, obstruction, lymphovascular invasion, or poorly differentiated tumors *(48)*. None of these higher-risk groups has sufficient numbers in a single trial or meta-analysis to definitively assess the impact of chemotherapy, but given the general trend in most studies of proportionately larger benefit for proportionately higher risk, this recommendation seems prudent.

Subgroup analysis of stage II patients from the MOSAIC trial suggests that the use of oxaliplatin-containing chemotherapy may reduce the risk of recurrence and death even further than does 5-FU/LV. However, the relative reduction in the risk of recurrence gleaned from the use of FOLFOX over LV5FU2 in stage II patients was smaller than that achieved for higher-risk (stage III) patients in the study (18% compared with 23% for stage III), and was not statistically significant in the stage II subpopulation *(52)*. If one adds the potential benefit of 5-FU/LV over observation and the potential benefit of oxaliplatin on top of that, it is possible that the difference in survival would be meaningful. Unfortunately, the difference between FOLFOX therapy and no therapy can

only be estimated in rough fashion based on currently available data. To conclude, although no definitive statement can be made regarding the treatment of stage II colon cancer, it is reasonable based on the available data to at least discuss chemotherapy, including combination chemotherapy, with all stage II patients. No clear standard of care can be stated, however, and care must be individualized in these instances.

3. MOLECULAR MARKERS OF PROGNOSIS AND MARKERS FOR PREDICTION OF RESPONSE TO THERAPY

In this section we discuss prognostic and predictive markers, and their potential use in determining whether: (A) a patient is at higher or lower than average (for his or her stage) risk of recurrence, (B) if this stage-independent prognostic information can help determine need for adjuvant therapy, and (C) given a choice of agents, can we predict what is the best (or least toxic and still effective) therapy for an individual or against an individual tumor. Questions A and B are determined by prognostic markers, that is, markers that can separate apparently equivalent groups of patients (in terms of pathological features) into higher- and lower-risk subgroups. The answer to question C is determined using predictive markers, that is, those that can predict either response or toxicity to a given therapy, whether or not the marker has independent prognostic value.

The therapeutic regimens utilized for leukemias, lymphomas, and breast cancers are routinely chosen based on both molecular markers predictive of response to specific agents or classes of agents and on prognostic markers. The obvious and most dramatic example of this is in breast cancer, where decisions between chemotherapy and hormone-based therapies (or combinations thereof) are strongly influenced by the presence or absence of estrogen and progesterone receptors in addition to stage (therefore estrogen receptor [ER] here is considered a predictive marker). The intensity of therapy for acute myelogenous leukemia is determined largely by a given leukemia's cytogenetics rather than its morphology. For example, patients with 8:21 translocations may be treated with chemotherapy alone whereas patients with trisomy 8 might be treated initially with bone marrow transplantation (in this case, cytogenetics serves primarily as a prognostic marker). As discussed at length in Section 2.8, one area where prognostic markers could be of great utility is in stage II colon cancer. Medical oncologists currently treat 25–45% of patients with therapy that benefits only approx 3% *(53,54)*. Additionally, now that oxaliplatin has been shown to add benefit when combined with 5-FU but carries risks of long-lasting neuropathy, a means of determining which patients benefit from this addition (a predictive marker) is highly desirable.

We will focus here on three markers that have been studied extensively in colon cancer, and their roles as predictive and/or prognostic markers in the

adjuvant setting. These include: (1) presence of microsatellite instability (MSI), (2) loss of chromosome 18q, and (3) levels of tumor TS. Each of these molecular markers is also discussed in more detail in other chapters of this text.

3.1. MSI—A Prognostic Marker With Unclear Predictive Capability

MSI is the defining molecular feature of the hereditary nonpolyposis CRC familial syndrome, and also occurs in sporadic (nonfamilial) CRC at a frequency of 10–15% (reviewed in ref. *55*). The presence of high-frequency MSI (also termed MSI-H) is a positive prognostic factor, with a stage-independent improvement in survival and lower risk of metastasis than is associated with tumors without MSI *(56)*. Although this fact is relatively consistent between studies, there is more controversy surrounding the potential of MSI to serve as a predictive factor for response to chemotherapy. A number of studies have been published that suggested tumors with high frequency of MSI may benefit more from 5-FU-based chemotherapy than microsatellite stable tumors *(55)*. However, most of these studies were retrospective in design, and suffered from the possibility that selection bias (for example, with regard to which patients were chosen to have chemotherapy vs not) resulted in the reported association between MSI and better outcome with chemotherapy. A systematic review of all available studies suggested no benefit of 5-FU for patients with MSI-H tumor phenotypes *(55)*. A study with a design less subject to bias was reported in 2003 *(57)*. In that study, tumor samples from patients enrolled in multiple phase III trials comparing 5-FU-based therapies to observation after surgery were studied for presence or absence of MSI. Results from this relatively large patient group, which had been prospectively randomized to treatment or observation, revealed, somewhat surprisingly, that the small population of patients treated with adjuvant chemotherapy who had MSI-H tumors actually fared significantly worse than those patients with MSI-H tumors who were observed after surgery. The important limitation of this study was the number of patients studied, which was constrained by the number of tumors available for analysis. Though these data are intriguing, the authors and editorialist suggested that MSI testing should not yet be used on a routine basis for adjuvant therapy decision making.

3.2. Loss of Chromosome 18q (or the DCC *Gene): A Strong Prognostic Marker*

Chromosomal gains and losses are a frequent hallmark of cancer, and occur in the majority of patients with CRC. Study of such changes has brought about very important advances. It has long been noted that losses of chromosomal arms of chromosome 5, 17, and 18 are particularly common in CRC *(58,59)*. In 1994, a landmark study was published by Jen et al. in which tumors from 136

Table 3
5-Yr DFS for Patients With or Without 18q and *DCC* Loss

	18q present	*18q absent*	*DCC present*	*DCC absent*
Stage II	93%	54%	94%	62%
Stage III	52%	38%	59%	33%

patients (none of whom received chemotherapy) with stage II and III colon cancer were assayed for loss of the long arm of chromosome 18 (18q) using polymerase chain reaction methodology *(60)*. In this study, 67% of the tumors had complete or partial loss of 18q. There were large and statistically significant differences in OS for each stage, favoring patients with an intact chromosome 18 (Table 3). Subsequent to this, a gene called deleted in colon cancer (*DCC*) was identified as a candidate tumor suppressor gene that resides on chromosome 18q61. A retrospective study of 132 archived stage II and III CRC samples again revealed an absence of *DCC* in 50% of the patients' tumors, using immunohistochemistry to detect *DCC* (probably a less accurate method than that used to detect 18q loss in the earlier studies) *(62)*. Again, survival was dramatically better for those patients with stage II disease and retained *DCC* than for those patients with the same tumor stage whose tumors had lost *DCC* expression. To this day, it has not been clarified whether *DCC* is the true tumor suppressor gene on chromosome 18q. Interestingly, in a study of the effect of *DCC* expression on survival in patients with metastatic colorectal cancer treated with 5-FU, patients with *DCC* loss had worse survival, but there was no difference in the rate of response to chemotherapy *(63)*. This unfortunately suggests that 5-FU-based chemotherapy might not be able to overcome the negative biological effects of *DCC*/18q loss; whether other drugs such as oxaliplatin or irinotecan might overcome this risk will need to be determined as well.

In 2005, we are left without a prospective database on which to base a clear sense of what to do with the information that 18q or *DCC* are missing or that a tumor exhibits MSI, but an important study that will attempt to bring us closer to utilizing these markers is now underway. ECOG 5202 (Fig. 2) has been designed to randomize stage II patients with molecular high-risk features to receive FOLFOX chemotherapy or FOLFOX with the vascular endothelial growth factor (VEGF) inhibitor bevacizumab (Avastin®, Genentech, San Francisco, CA). In this trial, patients considered high-risk are those whose tumors exhibit loss of chromosome 18q, with the exception of patients with high-level MSI, all of whom are in the low-risk group. Low-risk patients are not randomized, but simply observed to try and confirm the good prognosis of the group. Tumors from all patients will be collected for extensive prospective investigation of multiple predictive and prognostic factors.

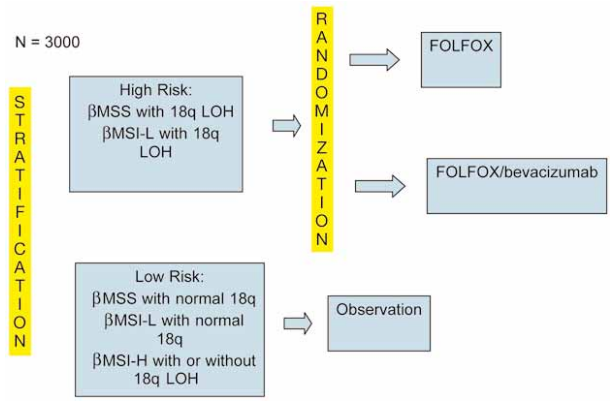

Fig. 2. Schema of ECOG 5202.

3.3. TS as a Predictive Marker for 5-FU Efficacy

As discussed previously in this chapter and in others, TS (along with DNA and RNA) is considered one of the main molecular targets of 5-FU. Accumulation of TS is a known mechanism of acquired resistance to 5-FU in human colorectal cancer xenograft models *(64)*, and is presumed to be at least one mechanism of resistance in vivo. A number of studies have now described varying baseline levels of TS, both at the protein and message (RNA) level, in human colon cancers and have correlated higher TS levels with decreased chance of radiographical response to 5-FU in the metastatic disease setting (reviewed in ref. *65*). Such studies have piqued interest in TS as a predictive marker for 5-FU efficacy in the treatment of metastatic disease, but individually the trials have been small and the results subject to similar biases as those mentioned previously for investigations of MSI. In order to more definitively study the question of the utility of TS as a predictive marker, an analysis of 706 patients treated in NSABP protocols C-01 to C-04 was conducted, in which TS was one marker examined (immunohistochemically) *(66)*. In this study, TS staining intensity had negative prognostic implications (response rate 1.46, $p = 0.01$), but no interaction between marker status and treatment outcome was found. These data would argue against present use of TS staining as a decision-making tool for patients with stage II and III colon cancer.

Markers with potential to predict response and toxicity for irinotecan and oxaliplatin exist as well, but still require validation in metastatic settings before there will be attempts to apply them to the adjuvant circumstance. Retrospective analysis of such markers has been built into the recent and upcoming adjuvant trials in hopes that the future of colon cancer therapy will be one in which treatments are optimally tailored to individual patients based on optimizing both their outcome and their toxicity profile. Also on the near horizon there are a

number of pharmacogenetic markers, or inherited differences in genes related to drug response or metabolism, that result in individual differences in response (toxic or therapeutic) to drugs and are independent of the genetic abnormalities of the tumor itself. The best examples of these to date are deficiency of the 5-FU metabolizing enzyme DPD (which occurs through a variety of genetic mechanisms), and polymorphism of the irinotecan-metabolizing gene *UGT-1A1*, a gene responsible for inactivation of SN-38 by glucuronidation, which results in severe toxicity after exposure to irinotecan. The relevance of these pharmacogenetic variations is further discussed in other chapters.

4. THE FUTURE: INCORPORATION OF TARGETED THERAPIES INTO ADJUVANT TREATMENT STRATEGIES

4.1. Angiogenesis Inhibition

As will be discussed extensively in the chapters on treatment of metastatic colorectal cancer, the concept that angiogenesis inhibition can be an effective therapy for cancer has been proven in principal by the clinical success of the VEGF-inhibiting antibody, bevacizumab. The concept of angiogenesis inhibition as an adjuvant therapy strategy, however, poses some very interesting mechanistic problems. By its very nature, inhibition of angiogenesis is tumoristatic (although uncommonly in colon cancer, some degree of tumor regression can be seen with VEGF inhibition alone). Oncologists have generally considered the success of adjuvant chemotherapy to be caused by the tumoricidal effects of drugs, leading to the eradication of micrometastatic tumor deposits. In metastatic tumor models, VEGF inhibition by itself can limit the bulk of metastatic tumors, but not eradicate them *(67)*. One of the theoretical benefits of angiogenesis in advanced tumors is improvement of drug delivery to tumors with disordered vasculature and high interstitial pressure *(68)*, factors that are very unlikely to be issues in submillimeter metastatic deposits. The quandary in this case, then, is that if we are to assume that angiogenic agents can inhibit growth of tumors that are present for some period of time (until the tumor has obtained a resistance mechanism to the anti-angiogenic strategy, something that is likely inevitable), then we must consider significantly important questions of duration of therapy that would result in clinically meaningful benefit. For example, do we choose 6 mo? 1 yr? 2 yr? 10 yr? What improvement in freedom from relapse (perhaps without an increase in the number of patients actually cured of disease) would be needed in order to consider this strategy acceptably efficacious for widespread use in the adjuvant setting? Such questions can only be answered by carefully constructed clinical trials, the first generation of which have already begun.

The NSABP has incorporated bevacizumab into clinical trial C-08, which randomizes both stage II and stage III patients to modified FOLFOX6 alone or

the same chemotherapy with bevacizumab. In this case, the chemotherapy is to be administered for the now-customary 6 mo, and the bevacizumab for an additional 6 mo as a single agent. Results of this first study will likely not be available before 2009. Similarly, the AVANT study is randomizing patients with stage III and high-risk stage II colon cancer to one of three treatment arms: FOLFOX4, FOLFOX4 plus bevacizumab, or CapeOx plus bevacizumab. Again, a duration of 6 months of chemotherapy and a further 6 mo of bevacizumab has been chosen by the investigators. For now, it is not possible to recommend bevacizumab in completely resected, stage II or stage III (or even resected stage IV) colorectal cancer outside the setting of a clinical trial.

4.2. Epidermal Growth Factor Receptor (EGFR) Inhibition

Antibody-based inhibition of the EGFR, part of a paracrine and autocrine signaling pathway that is dysregulated in many cancers *(69)*, has been demonstrated to have efficacy in the treatment of advanced colorectal cancer *(70)*. Importantly, the EGFR-antagonizing antibody cetuximab has been shown to improve sensitivity of colorectal cancer cells (in model systems) and colon cancers in humans to the effects of chemotherapy. The apparent synergism between cetuximab and certain chemotherapy agents (particularly irinotecan, but probably platinum agents as well) makes cetuximab a very appealing consideration for adjuvant therapy. In contrast to an anti-angiogenic agent, there would be no scientifically based rationale for consideration of continuing EGFR-targeted therapy beyond the time of chemotherapy. The possible benefits of EGFR antagonism with the therapeutic antibody cetuximab are now being studied in an Intergroup trial initiated by the NCCTG. This trial underwent several revisions in design during the years 2004 and 2005, but in final form is a comparison between modified FOLFOX6 or modified FOLFOX6 plus cetuximab in patients with completely resected stage III colon cancer.

4.3. Current Evidence-Based Management Recommendations

4.3.1. Stage III Colon Cancer

There is clear evidence of a survival benefit for 5-FU-based chemotherapy when compared with no therapy amounting to a relative reduction in risk of death at 5 yr of 40% in stage III patients according to a large pooled analysis of major trials comparing 5-FU/LV to surgery alone *(18)*. The absolute benefit for an individual patient can be calculated either using a web-based model (*see* next section), or estimated by multiplying the approximate risk of death given the stage by 0.40, with the resultant percentage subtracted from the original risk of death. As an example, a patient with a low-grade T3N1 cancer starts with recurrence risk of 51%, and a risk of death at 5 yr of 34%. To estimate the absolute survival benefit from adjuvant 5-FU/LV, the risk of death (34%) is multiplied by the proportional risk reduction (0.40), resulting in a 13.6% absolute

reduction in the risk of death for this patient (34% × 0.40% = 13.6%), or a risk of death at 5 yr of 34% – 13.6% = 20.4% as a result of chemotherapy.

There is very strong evidence of a benefit in DFS at 4 yr favoring the addition of oxaliplatin to 5-FU/LV either administered by a bolus-infusion (i.e., LV5FU2) or bolus method, with side effect profile favoring the infusion-based 5-FU administration. This DFS benefit for FOLFOX compared with LV5FU2 is approx 24%. At this point there is not a statistically significant OS benefit, but analysis of large trials has demonstrated that DFS nearly always predicts an OS benefit *(71)*. The authors therefore recommend FOLFOX as adjuvant therapy for any stage III colon cancer patients who do not have a strong contraindication for receiving oxaliplatin.

Based on currently available data, there is no role for the addition of irinotecan to 5-FU/LV for adjuvant therapy of stage III colon cancer patients.

At this point in time, no biological or genetic markers should be utilized for treatment-related decisions for patients with stage III colorectal cancer.

4.3.2. Stage II Colon Cancer

Adjuvant therapy of stage II colon cancer remains controversial. To date, only one individual study has demonstrated a benefit to 5-FU-based chemotherapy over observation after surgical resection *(51)*. That study (the large, UK-based QUASAR study) showed an absolute 5-yr survival benefit of 3%, but may have overestimated the benefit on the basis of inclusion of patients with T3N0 rectal cancer in that trial. This number is similar in magnitude to that obtained in two meta-analyses, one of which did and one of which did not reach statistical significance. The expected survival benefit that should be used when discussing adjuvant chemotherapy with patients is 2–4%. An ASCO expert panel has recommended strong consideration of adjuvant chemotherapy for stage II patients with high-risk clinical features including: presentation with bowel obstruction or perforation, T4 primary, lymphovascular invasion, poorly differentiated histology, and inadequate lymph node sampling (<12 nodes) *(48)*. All of these features have been shown to worsen prognosis, but no direct evidence exists that chemotherapy actually improves survival in any of these high-risk subgroups.

Regarding use of oxaliplatin in stage II colon cancer, both the MOSAIC and NSABP C-07 studies included stage II patients, but neither had power to detect a difference in DFS or OS in the stage II subgroup. Therefore, in spite of a trend toward DFS benefit, the authors do not recommend routine addition of oxaliplatin to 5-FU for patients with stage II colorectal cancer. The authors would, however, recommend consideration of the addition of oxaliplatin to 5-FU for the high-risk stage II patients as defined previously. It should be recognized by clinicians that patients with T4N0 colon cancer have a risk of recurrence that is actually slightly higher than patients with T2N1 (less than four positive nodes) *(18,47)*.

Presently, there is insufficient data regarding the use of molecular markers on which to base clinical decisions about treatment of patients with stage II colon cancer. However, in the specific case of a patient with stage II disease without high-risk features, and with known MSI-H in the tumor, the patient might be counseled that the expected risk of recurrence is less than or equal to 10% (assuming T3N0) and that the expected benefit of chemotherapy, if any exists, would be exceedingly low.

Several on-line adjuvant risk calculators now exist. The Mayo Clinic adjuvant colorectal calculator is based on the combined analysis trial published by Gill et al. *(18)*, and can be found at http://www.mayoclinic.com/calcs. A second evidence-based risk calculator can be found at http://www.adjuvantonline.com. These calculators can provide visual guidelines for physicians to present to patients during discussions of adjuvant therapy, and can be particularly useful in discussing therapy in patients with stage II disease.

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9 Management of Locally Advanced Rectal Cancer

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Summary

The management of locally advanced rectal cancer remains a challenge because of relatively high local recurrence rates, even with optimal surgery. This chapter reviews the evidence from clinical trials for the use of various perioperative strategies based on radiotherapy, chemotherapy, or both in these patients, as well as discusses how these results may inform clinical practice. In particular, two treatment strategies that have been shown to be beneficial and that are standard treatment options for patients with locally advanced disease are short-course preoperative radiotherapy and long-course preoperative chemoradiotherapy. In clinical trials in which these treatments were used in addition to surgery, improvements in local recurrence rates have been observed, although overall survival has less frequently been prolonged. These patients should also receive postoperative adjuvant chemotherapy.

Other issues that are discussed include the selection of patients with locally advanced disease for treatment, the use of total mesorectal excision as the optimal surgery for these patients, the impact of various preoperative treatments on sphincter preservation rates, and ongoing areas of research in this disease. Although the management of these patients continues to be controversial, it is clear that surgery alone is inadequate for patients with locally advanced rectal cancer and that they are best managed in a multidisciplinary setting.

Key Words: Rectal cancer; locally advanced; radiotherapy; chemoradiotherapy; preoperative treatment.

1. INTRODUCTION

The management of locally advanced rectal cancer is controversial, with opinions and practices varying between and within countries, and particularly between the United States and Europe. Although many clinical trials have explored various strategies for using radiotherapy (RT), chemotherapy, or both

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for improving outcomes from surgery, the main therapeutic intervention for these patients, relatively few of these trials have been randomized studies. The interpretation of results and cross-study comparison for the purposes of informing a universally acceptable standard treatment plan have been complicated by issues such as variability in patient selection criteria and technique (e.g., clinical assessment, endorectal ultrasonography [EUS], or magnetic resonance imaging [MRI]), RT techniques (e.g., field techniques and small bowel protection), surgery, and reporting of results. Some of these factors have become less prominent with more consistent use of imaging techniques to aid patient selection, better understanding of the importance of various pretreatment prognostic factors, and improved standardization of RT and surgical techniques.

1.1. Total Mesorectal Excision Surgery

Local recurrence rates of 25–40% have been reported in large series of patients undergoing conventional resections *(1,2)*. An early advance in the treatment of rectal cancer has been that of total mesorectal excision (TME) surgery, which was developed in the United Kingdom in the late 1970s as a response to the poor results with the conventional anterior resections (ARs) and abdomino-perineal resections (APRs) of the time *(3)*. The surgical technique involves the sharp dissection under direct vision of the avascular plane between the mesorectum and the surrounding parietal tissues down to the distal extremities of the pelvis. The resected specimen should ideally have a smooth unbroken surface, a factor in assessing the quality of the procedure performed. In a case series by Heald et al., which included 405 curatively resected patients, the local recurrence rate at 5 and 10 yr was reported to be 3 and 4%, respectively. TME surgery has been widely adopted in Europe as standard treatment for patients with operable rectal cancer. In the United States, TME is regarded by the academic surgical community as appropriate standard treatment; however, actual surgical management of patients in the United States is less standardized, and unfortunately some patients are still receiving substandard surgical procedures.

In two nonrandomized comparisons of the outcomes of patients operated by TME or conventional surgery, it was found that TME was associated with an improved overall survival (OS) and local recurrence rate *(1,2)*. When the results of a Dutch randomized trial that specified that all patients should have TME surgery *(4)* were compared with those of historical controls from an earlier study of conventional surgery *(5)*, it was found that TME surgery decreased local recurrence rates from 16 to 9% and that the type of surgery (TME vs non-TME) was an independent predictor for local recurrence ($p = 0.002$) *(6)*. Although survival was also higher in the TME cohort (2-yr survival of 86 vs 77% for the non-TME cohort, $p = 0.002$), the risk of distal recurrence was not affected by the type of surgery and was predicted only by the tumor-node

metastases (TNM) stage. In order to ensure the two cohorts were comparable, patients who had received preoperative treatment were excluded from analysis.

A more recent study has evaluated the effect of TME surgical principles when applied to ARs and APRs *(7)*. In this case series, it was found that even with TME surgery the incidence of circumferential resection margin (CRM) involvement was still higher in patients who underwent APR and that the advent of TME surgery had not diminished the frequency of CRM involvement in this group. Patients undergoing APR had a higher local recurrence rate (22.% vs 13.5%, $p = 0.002$) and lower survival than patients undergoing AR. Therefore, although TME surgery has improved the outcomes for patients with rectal cancer, there are still subgroups of patients at significant risk of local and/or distal failure who may benefit from more aggressive treatment approaches. Therefore, despite improved results with TME, adjuvant therapy remains important in rectal cancer *(8)*.

1.2. Defining Locally Advanced Rectal Cancer

A problem that arises when attempting to interpret the results of reported clinical trials and case series of patients with locally advanced rectal cancer is that of the range of possible definitions of "locally advanced" disease. The eligibility of patients may also be determined by pretreatment imaging, clinical assessment, or assessment of the surgical resection specimen. The aim of defining locally advanced disease should be to identify the group of patients who are more likely to suffer local recurrence with surgery alone, and who therefore might be most likely to benefit from adjuvant treatment.

The most commonly inclusion criteria is based on TNM staging, requiring patients to have T3 or T4 disease and/or local lymph node metastases (N+) *(9–16)*. In fact, T3-stage patients predominate in many studies. In a pooled analysis of five randomized studies that included patients with rectal cancer treated with surgery only, or surgery followed by postoperative RT and/or chemotherapy, T and N stage were found to have independent prognostic significance for overall and disease-free survival (DFS). Patients could be grouped into intermediate, moderately high, or high risk. In terms of outcome, intermediate-risk (T1-2/N1, T3/N0) patients seemed to do as well with surgery and chemotherapy, so that adding RT to treatment may be excessive *(17,18)*.

Other factors that potentially impact the risk of local recurrence are the resectability of the tumor and the involvement of the CRM. A tumor can be regarded as resectable if it can be excised without involvement of the surgical margins with tumor. Therefore, a tumor that infiltrates an adjacent structure that can still be resected, at least in part, with margins clear of tumor, such as uterus, bladder, prostate, or sacrum (T4 disease), is still resectable. Some studies have specified either tethering or fixation of tumor as either the main or a subsidiary inclusion criteria in order to evaluate the risk of irresectability *(19,20)*. Tethering or fixation can be assessed either clinically or by imaging, and is

perhaps less objective than CRM involvement (discussed later), which has been found to predict for incomplete resection and/or high risk of local recurrence.

Several notable studies have not restricted study entry to a particular stage of rectal tumors *(4,21–23)*. These are mostly studies of preoperative treatment. Although several studies report on the rate of sphincter-preserving surgery as an important study endpoint, Fewer studies restrict study entry or reporting of rates of sphincter preservation to patients with low-rectal tumors (lower border within 6 cm of anal verge) or to patients who have been determined at study entry to require APR *(24,25)*. In addition to the prognostic significance of tumor stage, serosal invasion, and venous invasion, one Japanese study of patients who had surgery without adjuvant chemotherapy as treatment for rectal cancer found that lower rectal tumors were associated with a higher risk of recurrence *(26)*.

In summary, factors that potentially predict for the risk of local recurrence and may therefore contribute to the definition of locally advanced disease include: T3c (invasion into perirectal fat >5 mm) or T4 disease, N+ disease, inferior margin of tumor no more than 6 cm from the anal verge, CRM involved or threatened, and extramural venous invasion.

1.3. CRM Involvement

In rectal cancer surgery, CRM involvement is defined as tumor observed no more than 1–2 mm from the resection margin and has been associated with higher rates of local recurrence and poorer survival *(27–32)* even with TME surgery *(27,28)*. In a study of patients who had TME surgery without preoperative RT, a margin of no more than 2 mm was associated with a local recurrence risk of 16%, compared to only 5.8% in patients with more tissue surrounding tumor ($p < 0.0001$) *(27)*. Similarly, other studies have shown differences in local recurrence rates for CRM-positive and -negative patients of 38.2 vs 10% *(29)*, and 22 vs 5% *(28)*. The latter study also observed that 40% of patients with CRM involvement developed distal metastases compared with 12% of patients without CRM involvement. More recently, an analysis of 122 patients with locally advanced rectal cancer who received preoperative chemoradiotherapy with 5-fluorouracil (5-FU) and leucovorin (LV) before surgery found a significant difference in 5-yr DFS and OS between patients who had undergone complete (R0) or incomplete (R1/R2) resections (68 vs 25%, $p = 0.0032$ and 72 vs 30%, $p = 0.02$, respectively) *(32)*.

1.4. Pretreatment Assessment of Patients With Locally Advanced Rectal Cancer

Pretreatment assessment of patients is particularly important when considering treatment with a preoperative or neoadjuvant strategy, in order to select patients most likely to benefit from treatment and to not overtreat patients with early-stage disease, estimated to be 18% in one randomized trial *(15)*. Whereas in the past,

clinical assessments, including digital rectal examination (DRE) and computed tomography (CT), were important means of assessing these patients, this role has been assumed by techniques such as thin slice MRI and EUS. Indeed, MRI has been found to be superior to CT for assessing pelvic disease *(33)*.

Various studies have been performed in which pretreatment MRIs were correlated with the histopathology of surgical resection specimens. In one study, MRI findings agreed with histopathology for T-stage in 94% of cases and nodal status in 85% of cases *(34)*. Another study, which correlated the lymph node appearance on MRI with that of the TME specimens, found that the sensitivity for detecting lymph nodes involved with local metastases was improved if the border contour and signal intensity characteristics of lymph nodes were used, rather than size criteria, to identify involved nodes *(35)*. When the different modalities were compared, MRI staging correlated favorably with histopathology in 94% of cases, compared to only 65% for DRE and 69% for EUS *(36)*. MRI also accurately predicted CRM involvement (92% of cases in one study) *(34,37)*.

EUS is most accurate for assessing superficial rectal cancers and is less suitable when tumors are more advanced *(33)*. It has a role in determining depth of tumor invasion, but is less accurate for assessing local lymph node involvement *(38,39)*. Although it has the advantage of being portable and office based, the quality of the results is dependent on operator skill, as is the case with other ultrasound-based imaging techniques.

The authors are of the opinion that thin slice MRI is the preferred method of assessing patients with rectal cancer. The advantages in terms of accuracy in assessing patients before treatment have been demonstrated and MRI images can be reproduced for the purposes of multidisciplinary team reviews or centralized reporting in clinical trials.

2. POSTOPERATIVE ADJUVANT TREATMENT

Based on the results of randomized trials by the Gastrointestinal Study Group (GITSG) *(9)* and the North Central Cancer Treatment Group (NCCTG) *(11)*, the US National Cancer Institute recommended in 1991 that postoperative adjuvant chemoradiotherapy was the standard of care in patients with rectal cancer *(40)*. Postoperative treatment has the advantage of selecting patients for treatment on the basis of well-validated histopathological staging of the surgical specimen. Surgical resection margins are also more easily assessed in patients who have not received preoperative therapy. Postoperative RT generally consists of hyperfractionated RT with approx 45 Gy delivered to the pelvis followed by a boost dose to the tumor bed over 5–6 wk. Table 1 summarizes the randomized trials of postoperative adjuvant treatment.

GITSG randomized 202 patients with curatively resected rectal cancer to four treatment options: observation only; chemotherapy with 5-FU and

Table 1
Summary of Randomized Trials of Postoperative Adjuvant Treatment

Study, N =	*Inclusion criteria*	*Treatment*	*Recurrence*	*DFS*	*Overall survival*
GITSG *(9,41)*, *N* = 202	T3/T4 and/ or N+	Observation, OBS Chemotherapy, CT: 5-FU/MeCCNU Radiotherapy, RT: 40/48 Gy Combination, COM: chemoradiotherapy followed by chemotherapy	LR: OBS 55% CT 46% RT 48% COM 33%	—	Significant difference seen after longer follow up only in comparison between COM and OBS ($p = 0.005$)
NSABP R-01 *(10)*, *N* = 555		Observation, OBS Chemotherapy, CT: MeCCNU/5-FU/ vincristine (MOF) Radiotherapy, RT: 46–47 Gy	LR: RT 16% OBS 25%, $p = 0.06$	5-yr DFS: (RT vs OBS, $p = 0.4$) CT 42% OBS 30%, ($p = 0.006$)	5-yr OS: (RT vs OBS, $p = 0.7$) CT 53% OBS 43%, ($p = 0.05$)
NCCTG 79-47-51 *(11)*, *N* = 204		Radiotherapy, RT: 50.4 Gy Chemoradiotherapy, CRT: 5-FU/MeCCNU followed by RT with 5-FU followed by 5-FU/MeCCNU	5-yr recurrence: RT 62.7% CRT 41.5%	—	29% reduction in death rate with CRT (95% CI 7–45%)
NSABP R-02 *(45)*, *N* = 694	Dukes B and C	Chemotherapy, CT: 5-FU/LV or MeCCNU/5-FU/vincristine (MOF) Chemoradiotherapy, CRT: CT with 50.4 Gy	LR: relative risk of 0.57 (CRT vs CT, $p = 0.02$)	Minimum of 3-yr follow-up: DFS HR 0.99 (95% CI 0.80–1.22, $p = 0.90$)	Minimum of 3-yr follow-up: OS HR 0.98 (95% CI 0.78–1.24, $p = 0.89$)

All radiotherapy included a boost dose. These results show that adjuvant treatment improves local recurrence rates compared to surgery alone, and support the use of combined-modality treatment of RT with concurrent chemotherapy.

LR, locoregional recurrence; DFS, disease-free survival; OS, overall survival; GITSG, Gastrointestinal Study Group; NSABP, National Surgical Adjuvant Breast and Bowel Project; NCCTG, North Central Cancer Treatment Group.

methyl-CCNU (MeCCNU or semustine); RT; or chemoradiotherapy followed by chemotherapy (combination treatment) *(9)*. With a minimum of 5-yr follow-up of all living patients at the time of analysis, the recurrence rates for each of the arms were 55, 46, 48, and 33%, respectively. Locoregional recurrence was less frequent in the arms that received any RT compared to those that did not (15/96 vs 27/106). At the time of analysis, the percentage of patients who had died was 64, 54, 54, and 44%, respectively. When the OS of the four groups was compared, none was found to be superior ($p = 0.20$). The largest difference was between combination treatment and surgery only, but this was not initially statistically significant ($p = 0.07$). However, after a median postoperative follow-up of 94 mo was reached for surviving patients, the differences in survival between the groups was maintained ($p = 0.1$) and the pairwise comparison between combination treatment and surgery only, adjusted for prognostic variables, had become statistically significant ($p = 0.005$) with a survival advantage of 24% in favor of combination treatment *(41)*.

Another study (NCCTG 79-47-51) subsequently reported the results of 204 patients with curatively resected rectal cancer who were randomized to either postoperative RT, or the same with concurrent 5-FU, and preceded and followed by treatment with 5-FU and semustine *(11)*. With a median follow-up in excess of 7 yr, the 5-yr recurrence rate was 62.7% for RT compared to 41.5% for combination treatment. Fewer patients who received combination treatment failed initially with local recurrence (13.5 vs 25.0%, $p = 0.036$) and also had less distal metastases (28.8 vs 46%, $p = 0.011$). There was a significant difference in OS in favor of combination treatment with a 29% reduction in death rate (95% confidence interval [CI], 7 to 45%). Acute toxicity was higher in the combination treatment arm, in particular gastrointestinal and haematological toxicity. However, the incidence of severe delayed toxicity was similar between arms. A further study by the same group (NCCTG 86-47-51) found that MeCCNU did not improve local control or OS, whereas the use of continuous infusion 5-FU was found to decrease tumor relapse rates (37 vs 47%, p=0.01), decrease the occurrence of distal metastases (31 vs 40%, $p = 0.03$) and improve 4-yr survival (70 vs 60%, $p = 0.005$) compared to bolus 5-FU *(42)*. The issue of whether RT should be administered early or late in combination therapy with 5-FU/LV has also been assessed in a randomized study, in which a significantly superior 4-yr DFS (81 vs 70%, $p = 0.043$) was found with early administration *(43)*. However, there was no significant difference in 4-yr OS between arms (84 vs 82%, $p = 0.387$).

On the other hand, a study that included a larger number of patients ($N = 555$) conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP) R-01, which randomized a similar group of patients to observation, postoperative chemotherapy (with 5-FU, MeCCNU and vincristine; MOF), or postoperative RT, failed to show a significant improvement in 5-yr DFS ($p = 0.4$) and OS

($p = 0.7$) for RT compared to surgery alone, with a lower locoregional failure that was of borderline significance (16 vs 25%, $p = 0.06$) *(10)*. However, when chemotherapy was compared to surgery alone, there was a significant improvement in 5-yr DFS (42 vs 30%, $p = 0.006$) and OS (53 vs 43%, $p = 0.05$). The cumulative odds at 5-yr comparing the disease-free interval in the chemotherapy group to the disease-free interval in the control group were 1.50 (95% CI 1.13–1.99). Patients from the TME-only arm of the Dutch Colorectal Cancer Group randomized trial of preoperative radiotherapy *(4)* who were found to have involved CRM (defined as no larger than 1 mm) could also receive 50.4 Gy of postoperative irradiation *(44)*. No difference in local recurrence rate was observed between irradiated and nonirradiated patients (17.3 vs 15.7%, $p = 0.98$).

The NSABP have also conducted a randomized trial to evaluate the addition of RT to postoperative chemotherapy with either 5-FU/LV or MOF in patients with curatively resected Dukes B and C rectal cancers *(45)*. Although RT improved locoregional control (relative risk 0.57 [95% CI 0.36–0.92, $p = 0.02$]), there was no significant effect on relapse-free survival ($p = 0.38$), DFS ($p = 0.90$), or OS ($p = 0.89$). The study also compared the efficacy of the two chemotherapy regimens used and found that the use of 5-FU/LV was associated with a significantly superior relapse-free survival ($p = 0.046$) and DFS ($p = 0.009$), but with no significant difference in 5-yr OS ($p = 0.17$). The US Intergroup study 0114 evaluated the addition of difference modulators to adjuvant bolus 5-FU (low-dose LV, levamisole, or LV/levamisole) compared to bolus 5-FU alone *(12)*. No significant difference in local control or survival was observed across the four arms.

The benefits of postoperative adjuvant chemotherapy are also supported by the results from adjuvant studies in colon cancer, and from trials that included rectal, as well as colon, patients such as the UK QUASAR study *(46)*. In this study, adjuvant chemotherapy was shown to significantly improve 5-yr survival (80.3 vs 77.4% for observation [relative risk 0.83, 95% CI 0.71–0.97; $p = 0.02$]) and recurrence rates (22.2 vs 26.2% [relative risk 0.78, 95% CI 0.67–0.91; $p = 0.001$]) in patients with colorectal cancer. In the subgroup of patients with rectal cancer (approx 30% of the study cohort), adjuvant treatment improved recurrence (19.6 vs 26.8%, $p = 0.005$) and survival ($p = 0.06$). Therefore, patients with curatively resected rectal cancer should receive adjuvant chemotherapy after completion of postoperative chemoradiotherapy, as was the case in some of the studies described previously.

The benefits of postoperative adjuvant treatment are supported by the results of a meta-analysis by the UK Colorectal Cancer Collaborative Group (CCCG), which included individual patient data for 2157 patients from 8 randomized controlled trials of postoperative RT vs surgery alone, and which commenced before 1987 *(47)*. The study also included an analysis of preoperative RT, which will be discussed later, as will the comparison between pre- and postoperative

RT. Survival was overall marginally better in patients allocated to any RT (pre- or postoperative) compared to surgery alone (45.0 vs 42.1% at 5 yr, and 26.9 vs 25.3% at 10 yr, $p = 0.06$) with similar reductions in yearly death rate observed for pre- and post operative treatment. Postoperative RT reduced the risk of isolated local recurrence at 5 yr (15.3 vs 22.9%, $p = 0.0002$), but not the risk of any recurrence (50.3 vs 53.8%, $p = 0.10$). A US survey of the practice of 73 institutions in the 1988–1989 Patterns of Care Study also found that the outcomes of patients treated with adjuvant chemoradiotherapy were better than those treated with RT alone *(48)*.

The Radiation Therapy Oncology Group has conducted a trial (RTOG 76-16) in which 147 patients with residual, inoperable, or locally recurrent rectal cancer were randomized to postoperative RT with 45–51 Gy (with boost to a maximum of 70 Gy) or the same with concurrent 5-FU followed by maintenance 5-FU and MeCCNU *(49)*. The addition of chemotherapy did not improve the outcome of these patients. Several phase I studies have also explored the use of raltitrexed as a radiosensitizer for postoperative adjuvant chemoradiotherapy *(50,51)*.

In summary, postoperative treatment for curatively resected locally advanced rectal cancer should consist of combination therapy with RT and concurrent chemotherapy, followed by further adjuvant chemotherapy. The role of single modality RT in this setting is limited. The rest of this chapter will discuss the use of preoperative treatment strategies and the comparison of pre- and postoperative treatment.

3. PREOPERATIVE RADIOTHERAPY

3.1. Background and Rationale

Preoperative treatment strategies for locally advanced rectal cancer are favored in Europe, where several of the large randomized trials of preoperative treatment have been conducted. There is also increasing interest in this type of treatment in the United States, which is reflected in trials such as the NSABP R-03 52 and R-04 studies. The potential benefits of using preoperative treatment include the following *(53–56)*:

1. Tumor downstaging and reduction in tumor size, increasing the likelihood of a complete resection and sphincter-preserving procedure *(57)*; unresectable tumors may also become resectable with a response to preoperative treatment.
2. The effects of radiotherapy may be enhanced as a result of better tissue oxygenation preoperatively.
3. Preoperative treatment may be better tolerated with less acute toxicity. There may be less risk of small bowel toxicity from radiation, as it is more mobile preoperatively and more easily displaced from RT field. Therefore, there may be a greater likelihood of completing planned treatment.
4. Improvement of tumor-related symptoms.

The disadvantage of preoperative treatment is that it is commenced without pathological staging and depends on the quality of pretreatment imaging and clinical assessment in order to ensure appropriate patient selection. There is therefore a risk of overtreating early-stage patients or patients with occult metastases. The surgical specimen can also be difficult to assess after preoperative RT. For example, assessment of adequate lymph node harvesting is made difficult by radiation-induced regression, and the completeness of surgery can be difficult to assess if the resection margin transects an area of fibrosis where previously there was tumor involvement.

3.2. Significance of Response and Downstaging

Several studies have assessed whether factors such as radiological response, pathological complete response (pCR), and tumor downstaging have predictive significance in patients with locally advanced rectal cancer treated with preoperative treatment. Pathological stage has been found to predict survival after preoperative treatment (both RT and chemoradiotherapy). In addition, patients who were downstaged by treatment survived longer than those who were not downstaged *(58–61)*. Downstaging occurred in 26–62% of patients and was assessed by comparing the stage on pretreatment imaging with the histopathological staging of the surgical specimen in these series. A recent analysis reported a significant difference in 3-yr DFS and OS between patients who were and were not pathologically downstaged (52 vs 9%, $p < 0.001$, and 64 vs 25%, $p = 0.0001$, respectively) *(62)*. An earlier study of patients treated with preoperative chemoradiotherapy and postoperative adjuvant 5-FU/LV found that the 3-yr DFS of patients was significantly better if they had any response to ($p = 0.01$) or were downstaged ($p = 0.03$) by treatment, even though this did not result in a difference in OS or local control *(60)*.

Good outcomes have been reported in patients who have achieved pCR in two case series, one of which reported 5- and 10-yr survival rates of 91 and 86%, respectively, in a patient cohort that included some patients with initially fixed or tethered tumors *(63)*. The pCR rate was 16%. A second series of patients with unresectable T3/T4 disease has not observed any pelvic recurrences in the 23 out of 143 (18%) patients who achieved pCR, with a median follow-up of 24 mo *(64)*. Various qualitatively based systems for grading downstaging or response to preoperative treatment have also been correlated with outcome *(65,66)*. For example, the response grading proposed by Dworak et al. *(67)* has been shown to predict 2-yr DFS in patients treated with preoperative fluoropyrimidine-based chemoradiotherapy.

3.3. Clinical Trials of Preoperative RT

Preoperative single modality RT consists predominantly of hypofractionated RT with a dose of 25 Gy delivered in five fractions, followed shortly by surgery.

This has been adopted as a standard treatment in many centers in Europe following the results of the Swedish Rectal Cancer Trial, which reported an improvement in local control and survival in completely resected patients *(22)*. This schedule can be administered with minimal acute toxicity, but because of the short interval between RT and surgery, does not usually result in significant tumor downstaging, even though downsizing may occur *(68)*. It is possible that tumor downstaging may be observed if a longer interval was allowed. The main randomized trials of preoperative RT are summarized in Table 2.

The Swedish Rectal Cancer Trial randomized 1168 patients to preoperative RT or surgery alone *(22)*. A similar proportion of patients in both arms had curative resections. There was no difference in in-hospital mortality between both groups (4 and 3%). With a minimum follow-up of 5-yr, local recurrence rates were significantly lower in the group who received RT (11 vs 27%, $p < 0.001$ overall and 9 vs 23%, $p < 0.001$ in curatively resected patients). Only the survival of patients who were curatively resected was reported. The 5-yr survival of the two subgroups was 58 vs 48% ($p = 0.004$). There was some overlap with the patients included in the 557-patient Stockholm II study, which had a similar study design *(23)*. With a median follow-up 8.8 yr, the local recurrence rate was reduced in the RT arm compared to surgery only (12 vs 25%, $p < 0.001$), but the distal recurrence rate was not significantly different ($p = 0.8$). There was no significant difference in OS between both groups (39 vs 36%, $p = 0.2$), although a significant survival difference was again observed if only the patients who underwent curative resection were included in the analysis (46 vs 39%, $p = 0.03$). In a subgroup analysis of patients who were curatively resected evaluating the rate of local recurrence by tumor location, it was found that the local recurrence rate was reduced from 30 to 20% in low-rectal tumors (0–5 cm from the anal verge), 25 to 11% in mid-rectal tumors (6–10 cm from the anal verge), and 21 to 5% in upper-rectal tumors (>10 cm from the anal verge) *(69)*. Both studies excluded patients who were older than 80 yr in age as it was thought that an excess in postoperative mortality observed in the earlier Stockholm I trial may have reduced the survival benefit from preoperative RT *(21)*.

In the earlier 849-patient Stockholm I study, a similar proportion of surgery was considered "curative" in both groups (78 vs 82%) *(21)*. More patients who received preoperative RT had postoperative complications (28 vs 20%, $p < 0.01$) mainly because of an increased incidence of wound sepsis among irradiated patients. Postoperative mortality was also increased in these patients (8 vs 2%, $p < 0.01$), mainly because of increased cardiovascular deaths, particularly in patients older than 75 yr. Preoperative RT significantly improved the incidence of local recurrence (hazard ratio [HR] 0.51, 95% CI 0.37–0.69; $p < 0.01$) leading to a longer disease-free interval in this group (HR 0.76, $p < 0.01$). However, the incidence of distal metastases was not significantly different between the groups, and there was no significant difference in OS for all

Table 2
Summary of Randomized Trials of Preoperative Single Modality RT

Study, N =	*Inclusion criteria*	*Treatment*	*Recurrence*	*DFS*	*Overall survival*
Stockholm I *(21)*, $N = 849$	Resectable rectal cancer	Radiotherapy, RT: 25 Gy in five fractions Surgery, S	LR: RT 14% S 28% (HR 0.52, 95% CI 0.37–0.69, $p < 0.01$)	HR 0.51, $p < 0.01$	No difference
Swedish Rectal Cancer Trial *(22)*, $N = 1168$	Resectable rectal cancer, age > 80 excluded	Radiotherapy, RT: 25 Gy in five fractions Surgery, S	LR: RT 11% S 27% ($p < 0.001$)	—	5-yr OS: RT 58% S 48% ($p = 0.004$, curatively resected patients only)
Stockholm II *(23)*, $N = 557$	Resectable rectal cancer, age > 80 excluded	Radiotherapy, RT: 25 Gy in five fractions Surgery, S	LR: RT 12% S 25% ($p < 0.001$)	—	OS: RT 46% S 39% ($p = 0.03$, curatively resected patients only) RT 39% S 36% ($p = 0.2$, all patients)

Dutch TME *(4,70)*, $N = 1861$	Rectal cancer, fixed tumors excluded	Radiotherapy, RT: 25 Gy in five fractions TME surgery, S(TME)	LR: RT 2.4% S(TME) 8.2% ($p < 0.001$, 2-yr follow-up) RT 5.8% S(TME) 14.9% (5-yr follow-up)	—	OS: RT 82.0% S(TME) 81.8% ($p = 0.84$, 2 yr) RT 64.3% S(TME) 63.5%

All used short course radiotherapy (25 Gy in five fractions), followed by surgery. This treatment results in a significant reduction in local recurrence compared to surgery (TME or non-TME). An overall survival benefit was only shown in the subgroup of curatively resected patients.

LR, locoregional recurrence; DFS, disease-free survival; OS, overall survival; TME, total mesorectal excision.

patients and for the subgroup of patients who had curative resections between arms. In Stockholm II, the incidence of postoperative complications was again increased in the preoperative RT group, also mainly because of wound infections (41 vs 28%, $p < 0.01$), but the postoperative mortality rate was similar between arms (2 and 1%) *(23)*.

Whereas the Swedish studies did not require patients to have TME surgery, the Dutch Colorectal Cancer Group conducted a 1861-patient randomized trial in which patients were treated with short-course preoperative RT followed by TME surgery or TME alone *(4)*. Histopathology assessment was also standardized. Patients with fixed tumors were excluded and most patients were TNM stage III. APR surgery occurred in a similar proportion of patients in both arms. After a median follow-up of 2 yr, the local recurrence rate was significantly improved by preoperative RT (2.4 vs 8.2%, $p < 0.001$). The overall benefit to local recurrence was also maintained after a median follow-up of 5 yr (5.8 vs 11.4%) *(70)*. Treatment group assignment was an independent prognostic factor ($p < 0.001$) in a multivariate Cox regression analysis. In a more recent univariate analysis of the different subgroups by distance from the anal verge, only patients with tumors between 5 and 10 cm from the anal verge maintained a statistically significant reduction in local recurrence risk (3.9 vs 14.9%) *(70)*. The rate of distal recurrence was not different between the groups at 2 yr (14.8 vs 16.8%, $p = 0.87$) but the overall recurrence rate trended towards being lower at 2 yr in the RT group (16.1 vs 20.9%, $p = 0.09$), although this was not statistically significant. Although local recurrence was reduced in patients with tumors within 1.1–2 mm of the CRM (0 vs 14.9%, $p = 0.02$) and more than 2 mm of the CRM (0.9 vs 5.8%, $p < 0.0001$), local recurrence was not significantly reduced in those with positive margins, defined as tumor no greater than 1 mm of the CRM (9.3 vs 16.4%, $p = 0.08$) *(44)*. There was no significant difference in OS at 2 yr (82.0 vs 81.8%, $p = 0.84$) 4 or 5 yr (64.3 vs 63.5%) *(70)*.

Patients who had more than a 10-d delay between the start of RT and surgery were excluded from an analysis of the distribution of TNM staging of pathology specimens, which found that both arms were similar, suggesting that hypofractionated preoperative RT does not lead to tumor downstaging in patients when the interval between RT and surgery is short *(68)*. However, the mean diameter of tumors in the irradiated group was smaller (4.0 vs 4.5 cm, $p < 0.001$) and the total number of lymph nodes examined was less (7.7 vs 9.7, $p < 0.001$). More blood loss during surgery occurred in those who had been irradiated (1000 mL vs 900 mL, $p < 0.001$) and in patients who had APRs; more perineal complications also occurred in this group (29 vs 18%, $p = 0.008$) *(71)*. No difference was observed in postoperative mortality (4.0 vs 3.3%) or in the number of reoperations. No other excess toxicity was observed in the RT arm. However, more patients who had received RT suffered long-term problems of fecal incontinence (62 vs 38%, $p < 0.001$) and other problems of bowel function, and

fewer patients remained sexually active ($p = 0.05$ in males and $p = 0.02$ in females) *(70,72)*.

Several nonrandomized studies or case series have also reported results on the use of hyperfractionated preoperative RT of between 45–55 Gy given over 5–6 wk, including a boost *(19,73,74)*. These have often included patients with poor prognostic features, such as low-rectal tumors, or tethered or fixed tumors. Others have specifically included patients with initially unresectable disease *(75–77)*.

Camma et al. performed a meta-analysis of the results from 6426 patients from 14 randomized trials of preoperative RT compared to surgery alone *(78)*. The proportion of patients who underwent curative resection was similar among the studies, but the proportion of patients undergoing APR was highly variable (between 0 and 94%). A variety of irradiation schedules was used. Although a survival benefit from RT was observed in 11 out of the 14 trials, only 3 had statistical significance. However, the pooled estimate of the treatment effect on survival was significant (odds ratio [OR] 0.84 [95% CI 0.72–0.98, $p = 0.03$]). Significance was lost when the largest trial (the Swedish Rectal Cancer Trial) was excluded from the analysis. A subgroup analysis by stage showed that Dukes B and C patients had significant survival benefit from treatment (OR 0.67 [95% CI 0.52–0.88, $p = 0.004$] and OR 0.76 [95% CI 0.59–0.97, $p = 0.03$]) but not Dukes A patients (OR 0.84 [95% CI 0.58–1.21, $p = 0.34$]). Eleven trials were evaluable for 5-yr local recurrence rates; preoperative RT was superior to surgery only in all but one study and reaching statistical significance in six studies. The pooled OR was 0.49 (95% CI 0.38–0.62, $p < 0.001$). The combined data from nine trials confirmed that there was no statistically significant difference in the occurrence of distal metastases (OR 0.93 [95% CI 0.73–1.18, $p = 0.54$]). There was, however, a significant increase in postoperative adverse events in the preoperative RT group (57.4 vs 42.3%, $p < 0.001$). Although postoperative mortality risk was also higher for irradiated patients in some studies, it did not reach statistical significance in the pooled analysis ($p = 0.22$).

The CCCG analyzed individual patient data from 6350 patients in 14 randomized trials where preoperative RT was compared to surgery only *(47)*. Most studies did not included TME surgery. As previously mentioned, overall survival was marginally better in patients allocated to any RT (pre- or postoperative) compared to surgery alone (45.0 vs 42.1% at 5 yr and 26.9 vs 25.3% at 10 yr, $p = 0.06$) with a similar reduction in yearly death rate observed for pre- and postoperative treatment. The absolute risk of any recurrence and isolated local recurrence at 5 yr and 10 yr was significantly lower in patients who had preoperative RT compared to those who did not (5-yr: any recurrence 45.9 vs 52.9%, $p < 0.00001$ and isolated local recurrence 12.5 vs 22.2%, $p < 0.00001$; 10 yr: 55.1 vs 66.8%, $p < 0.00001$ and 16.7 vs 25.8%, $p < 0.00001$, respectively). There was also a highly significant trend towards greater efficacy of preoperative

RT with higher biologically effective doses (>30 Gy) ($p < 0.0001$). Studies in which high (>30 Gy) biologically effective doses were used showed a halving of risk of local recurrence (57%, $p < 0.0001$), which was significantly larger than the 37% reduction observed studies of postoperative RT ($p = 0.01$). Of patients who had preoperative RT, 46% died of rectal cancer compared to 50% of patients who had surgery only ($p = 0.0003$), with the largest effect seen in those who received more than 30 Gy of preoperative treatment (overall reduction in deaths was 22%, $p = 0.00002$). However, most of the patients who died from nonrectal cancer causes were in trials of preoperative RT, the 15% increased risk in the combined trials of preoperative RT being significant ($p = 0.02$). In the patients who received more than 30 Gy of RT, the excess deaths were all seen in the first year after randomization, mainly as a result of vascular and infective causes.

A 471-patient randomized trial has compared pre- and postoperative RT in patients with operable rectal cancer *(79)*. All patients in the preoperative treatment group received short-course RT at a dose of 25.5 Gy over five fractions, followed by surgery within a week. In the postoperative treatment group, only patients with stage B2 (T4N0) or worse (TxN+) disease were irradiated, starting 4–6 wk after surgery, with 60 Gy in a split dose over 8 wk, including a boost to the tumor bed for the last 10 Gy. Out of the 235 patients randomized to this arm, 137 were suitable for RT after surgery, but only 115 (84% of 137) actually commenced treatment. Postoperative treatment was not as well tolerated as preoperative treatment, with nine patients having treatment interrupted prematurely because of adverse events and only nine patients completing treatment without any adverse effects. However, more patients in the preoperative group suffered perineal wound sepsis after APR (33 vs 18%, $p < 0.01$), although postoperative complications were otherwise similar between arms. The types of resections performed and the pathological staging of both groups were well matched. More resected patients in the postoperative group developed local recurrence (21 vs 12%, $p = 0.02$) and this difference was more marked in patients with stage B2 or worse disease. The probability of developing local recurrence at 5 yr was 14.3% (preoperative) and 26.8% (postoperative). There was no difference in the occurrence of distal metastases ($p = 0.3$), and OS ($p = 0.4$) and cancer-specific survival ($p = 0.2$) were similar, with a minimum follow-up of 3 yr. A report on the long-term side effects from this study, which included an additional 58 patients from an earlier pilot study treated with the same regimen *(80)*, found no increase in long-term side effect with preoperative RT. Patients who received postoperative treatment were also at greater risk of developing bowel obstruction (11% in patients who received postoperative RT vs 5% for preoperative patients vs 6% for patients who had surgery alone).

4. PREOPERATIVE CHEMORADIOTHERAPY

4.1. Background and Rationale

Preoperative chemoradiotherapy is most frequently based on 45–54 Gy of pelvic RT, including a boost to the tumor volume, delivered over 5–6 wk. The majority of clinical trials have used fluoropyrimidine-based chemotherapy as a radiosensitizer, which is also the preferred combination in clinical practice. However, with the efficacy of other cytotoxics and the targeted agents demonstrated in advanced disease, more recent studies are exploring using more than one agent concurrently with RT. The interval between completion of RT and surgery is also typically approx 6 wk to allow for a more optimal effect of treatment. A study comparing intervals between chemoradiotherapy and surgery of 4–8 wk and 10–14 wk found that there was no benefit from the longer interval *(81)*. The rationale for chemoradiotherapy is that the use of a radiosensitizer may improve the response rates to preoperative RT with only a modest increase in RT-related toxicity. Therefore, more studies of chemoradiotherapy have included patients with unresectable or borderline resectable tumors who may benefit from downstaging. Chemotherapy may also have a systemic effect, potentially improving control of distal metastases, although this is only likely to be significant in schedules in which the dose intensity of concurrent chemotherapy is close to that which is efficacious without RT, or which includes a component of full-dose chemotherapy alone.

4.2. Clinical Trials of Preoperative Chemoradiotherapy in Resectable Rectal Cancer

Four randomized trials, NSABP R-03, the German CAO/ARO/AIO-94, the European Organization for Research and Treatment of Cancer (EORTC) 22921, and the Fédération Francophone de Cancérologie Digestive (FFCD) 9203 have evaluated preoperative chemoradiotherapy (Table 3). NSABP R-03 was a 267-patient trial that closed prematurely because of poor accrual, comparing preoperative chemoradiotherapy (with 5-FU/LV) followed by surgery, with surgery followed by the same chemoradiotherapy *(52,56,82)*. In both arms, study treatment was completed by the administration of four cycles of postoperative adjuvant chemotherapy with 5-FU/LV. The chemotherapy schedule for concurrent use with RT was determined in an earlier phase I study that found that the use of high-dose LV during chemoradiotherapy was not optimal *(83,84)*. With a median follow-up of 78 mo, there was no significant difference in 5-yr DFS (64 vs 53%, $p = 0.08$) and OS (74 vs 66%, $p = 0.14$) between treatment groups *(52)*. Although clinical response to preoperative treatment (complete response [CR] vs partial response [PR] vs stable disease [SD]) correlated significantly with DFS (95, 72, and 66%, respectively, $p < 0.03$), there was no significant

Table 3
Summary of Randomized Trials of Preoperative Chemoradiotherapy

Study, N =	*Inclusion criteria*	*Treatment*	*Recurrence*	*DFS*	*Overall survival*
NSABP R-03 *(52)*, $N = 267$	Operable rectal cancer	Preoperative: 5-FU/LV (one cycle) + radiotherapy 50.4 Gy with 5-FU/LV + surgery + 5-FU/LV (four cycles) Postoperative: Surgery + radiotherapy 50.4 Gy with 5-FU/LV +5-FU/LV (four cycles)	9 vs 5% ($p < 0.5$)	5-yr DFS: 64 vs 53% ($p = 0.08$)	5-yr OS: 74 vs 68% ($p = 0.14$)
CAO/ARO/ AIO-94 *(15)*, $N = 823$	Resectable T3/T4 and/ or N+ rectal cancer	Preoperative: 50.4 Gy with 5-FU Postoperative: 50.4 Gy with 5-FU + 5.4-Gy boost (all patients had TME surgery and received postoperative adjuvant bolus 5-FU for four cycles)	5-yr cumulative LR: 6 vs 13% ($p = 0.006$)	5-yr DFS: 68 vs 65% ($p = 0.32$)	5-yr OS: 76 vs 74% ($p = 0.80$)
EORTC 22921 *(87)*, $N = 1011$	T3, resectable T4M0 rectal cancer	2×2 randomization to: Preoperative chemoradiotherapy, CRT: 45 Gy with 5-FU/LV Preoperative radiotherapy, RT: 45 Gy Adjuvant chemotherapy,	5-yr LR: RT 17.1% CRT 8.7% RT + adj-CT 9.6% CRT + adj-CT 7.6%	—	5-yr OS: CRT vs RT, 65% both arms Adj-CT vs S, ($p = 0.11$)

		adj-CT: 5-FU/LV (four cycles) Surgery only, S	($p = 0.0016$ for comparison of RT against any arm with chemotherapy)		
FFCD 9203 *(88)*, $N = 762$	Resectable T3/T4 rectal cancer accessible by DRE	Preoperative chemoradiotherapy, CRT: 45 Gy with 5-FU/LV Preoperative radiotherapy, RT: 45 Gy (All patients received four cycles after surgery 5-FU/LV)	5-yr LR: 8 vs 16.5%	5-yr DFS: 59 vs 56%	5-yr OS: 67 vs 66%

Preoperative chemoradiotherapy improves local control compared to postoperative chemoradiotherapy and to preoperative RT, but not overall survival.
LR, locoregional recurrence; DFS, disease-free survival; OS, overall survival.

correlation between pathological response and outcome ($p < 0.09$ for DFS and $p < 0.28$ for OS). Local recurrence was similar between arms (9 vs 5%, $p < 0.5$). Prior to treatment, 35 and 39% of patients from each group were expected to be suitable for sphincter-preserving surgery prior to treatment. There was no significant improvement in the rate of sphincter-preserving surgery with preoperative treatment (48 vs 39%, $p < 0.17$). The incidence of treatment-related deaths was similar in both arms (2.6% overall) and grade 3 or worse diarrhea was the predominant toxicity (34 and 26% in each arm).

In the German CAO/ARO/AIO-94 study, 823 patients with T3–4 and/or N+ rectal cancer within 16 cm of the anal verge assessed on EUS or CT were randomized to preoperative chemoradiotherapy (50.4 Gy in 28 fractions with 5-FU administered concurrently on wk 1 and 5), followed by surgery after 6 wk, or the same chemoradiotherapy postoperatively with an additional 5.4-Gy boost to the tumor bed *(15)*. In addition, all patients received four cycles of bolus 5-FU-based adjuvant chemotherapy after surgery or chemoradiotherapy, respectively. All patients had TME surgery with randomization stratified by surgeon. The arms were well balanced except for significantly more patients in the preoperative treatment group with tumors less than 5 cm from the anal verge (39% of 405 evaluable patients and 30% of 394 evaluable patients, respectively, $p = 0.008$). An intention-to-treat analysis showed that there was no significant difference in OS (HR 0.96, $p = 0.080$) or DFS (HR 0.87, $p = 0.32$) at 5 yr. However, preoperative treatment improved the cumulative incidence of local recurrence at 5 yr (6 vs 13%, relative risk 0.46 [95% CI 0.26–0.82, $p = 0.006$]). The cumulative incidence of distal recurrence was similar in both arms (36 and 38%, $p = 0.84$). A pCR rate of 8% was observed in the preoperative treatment arm (compared to 0%, $p < 0.001$), with downstaging suggested by the greater likelihood of patients in this arm having disease of earlier histological stage ($p < 0.001$). These effects did not seem to influence the type or completeness of resections performed, which was similar in both arms. However, in the subgroup of 194 patients (116 and 78 in the pre- and postoperative treatment groups, respectively) determined at study entry by a surgeon to require APR, the incidence of sphincter-preserving surgery was higher in the preoperative treatment arm (39 vs 19%, $p = 0.004$). Perioperative morbidity and mortality was similar between groups, whereas there was a greater incidence of acute grade 3/4 (27% for preoperative vs 40% postoperative, $p = 0.001$) and long-term (14 vs 24%, $p = 0.01$) toxicity in the postoperative treatment group. These patterns of toxicity are similar to those observed in an earlier study comparing pre- and postoperative treatment, in which significantly fewer patients in the former group experienced grade 3/4 toxicities (13 vs 48%, $p = 0.045$) despite a higher dose level of 5-FU administered to the preoperative treatment patients *(53)*.

EORTC 22921 included only patients with T3 or resectable T4M0 rectal cancer *(85–87)*. In this study, 1011 patients ($N = 1011$) were treated within a 2×2

randomization of preoperative chemoradiotherapy vs RT alone, and postoperative chemotherapy vs surgery alone. Preoperative RT consisted of 45 Gy over 5 wk, with the RT volume limited to the main field of tumor spread. Patients who received chemoradiotherapy were also given concurrent 5-FU/LV on wk 1 and 5 of preoperative treatment. Postoperative chemotherapy consisted of four cycles of 5-FU/LV chemotherapy. TME surgery was recommended for all patients. More than 90% of patients had low- to mid-rectal tumors, with a similar proportion being T3 tumors. The addition of concurrent chemotherapy increased the pCR rate from 5.3 to 13.7% ($p < 0.001$). There were more tumors of lower T stage ($p < 0.0001$) and less nodal involvement ($p < 0.0001$) in patients who received chemoradiotherapy. The tumors in this group were also smaller (median 30.0 mm vs 25.0 mm, $p < 0.0001$) with less lymphatic and venous invasion ($p = 0.008$). Slightly more patients who had chemoradiotherapy had ARs (55.3 vs 52.8%, $p = 0.05$). In the comparison between preoperative chemoradiotherapy and RT only, there was no significant difference in 5-yr OS (65% for both arms, $p = 0.84$) and progression-free survival. Similar, when postoperative chemotherapy was compared with surgery alone, there was no significant difference in 5-yr OS ($p = 0.11$) and progression-free survival ($p = 0.13$) *(87)*. The rate of local relapse at 5-yr was as follows for each arm: 17.1% (preoperative RT only), 8.7% (preoperative chemoradiotherapy only), 9.6% (preoperative RT with adjuvant chemotherapy), and 7.6% (preoperative chemoradiotherapy with adjuvant chemotherapy). These results were significant for the comparison of the preoperative RT-only arm with any of the other arms that contained chemotherapy ($p = 0.0016$). Distal recurrence rates were similar for all the arms. The addition of chemotherapy to preoperative treatment increased the incidence of grade 2 or worse toxicity (54.3 vs 37.7%, $p < 0.005$), mainly owing to diarrhea (34.3 vs 17.3%) and perineal dermatitis (26.0 vs 20.1%) *(85)*.

A French study (FFCD 9203) with a similar design of preoperative chemoradiotherapy compared to preoperative RT, but with all patients receiving four cycles of postoperative adjuvant chemotherapy after surgery, enrolled a total of 762 patients *(88)*. Treatment arms were well balanced, with 51 and 52% of patients in the chemoradiotherapy and RT arms with tumors less than 5 cm from the anal verge. The pCR rate was improved with the addition of chemotherapy, from 3.7 to 11.7% ($p < 0.05$), but the rate of sphincter preservation was similar between arms (53 and 52%). With a median follow up of 69 mo, the chemoradiotherapy group had an improved 5-yr local failure rate (8 vs 16.5%), which will need to be confirmed in the final study analysis. However, there was no improvement in DFS or OS .

One case series of 297 patients treated with preoperative chemoradiotherapy and TME surgery has reported a 10-yr OS for the cohort of 58%, with a relapse-free survival of 62%. These results were significantly better in patients who

achieved a greater than 95% pathological response ($p = 0.003$ for OS and $p = 0.002$ for relapse-free survival) *(16)*. Another small case series reported an overall 3-yr survival of 88% with no difference in survival between patients who had APRs or sphincter-preserving surgery *(89)*.

4.3. Preoperative Chemoradiotherapy in Initially Unresectable Rectal Cancer

Preoperative chemoradiotherapy has also been used to downstage patients with initially unresectable disease prior to surgery with curative intent. Results from randomized trials specifically for this indication are lacking. Phase I/II studies and retrospective series have reported pCR rates of up to 28% with 5-FU-based chemoradiotherapy and complete (R0) resection rates in excess of 90% *(13,90–96)*.

One phase II study reported a 5-yr OS of 60% and local recurrence-free rate of 96% for patients who had R0 resection *(13)*. The local recurrence-free rate for the whole cohort was 92%. A study that evaluated adding bolus 5-FU with sequential methotrexate as a modulator to preoperative RT found that chemoradiotherapy reduced local recurrence (17 vs 44%, $p = 0.05$ in patients who had any resection and 4 vs 35%, $p = 0.02$ in patients who had locally complete resection) *(94)*. This only resulted in a nonsignificant difference in 5-yr OS (29 vs 18%, $p = 0.3$). Of note, 18% of patients did not complete chemoradiotherapy because of toxicity, with 82% of patients in this arm having grade 2 or higher toxicity, compared to 67% in the RT-only group having a grade 1 or less toxicity. Another randomized phase II study found that chemoradiotherapy resulted in an increased pCR and induced greater downstaging compared to RT alone in this setting *(97)*.

Although these results are promising, they need to be evaluated further in well-designed phase III trials. Previous studies have tended to also include patients with locally recurrent rectal cancer, resulting in a biologically heterogenous cohort. A more potentially significant issue is that of how patients are selected for study entry and on what basis irresectability is defined. These factors account for the variable results seen in noncomparative studies.

Another treatment modality that has been used particularly in patients with unresectable or locally recurrent rectal cancer is intraoperative RT (IORT), often delivered in addition to external beam RT *(98–102)*. IORT should be regarded as experimental and will not be discussed further.

4.4. Other Radiosensitizers for Preoperative Chemoradiotherapy

Several studies are evaluating the use of different agents as radiosensitizers for chemoradiotherapy, either as single agents or in combination with a fluoropyrimidine. This is the obvious developmental pathway, given the superior efficacy of combination chemotherapy observed in advanced colorectal cancer, in particular with treatment containing either oxaliplatin or irinotecan. 5-FU has

been replaced in a few studies by oral agents such as capecitabine *(103,104)* or tegafur-uracil *(105)*, or with raltitrexed *(106,107)*. Based on results from studies in advanced disease, these agents are expected to have similar efficacy to 5-FU, but with easier administration or improved toxicity. Single-agent capecitabine at a dose of 825mg/m^2 twice daily administered concurrently for the duration of RT is tolerable with RT. Raltitrexed as a single agent with RT should be used at a dose of 2.6mg/m^2 every 3 wk. However, these agents should be evaluated in randomized trials before their role in chemoradiotherapy can be confirmed. The ongoing NSABP R-04 study is comparing preoperative chemoradiotherapy with concurrent capecitabine with concurrent continuous infusion 5-FU in patients with resectable rectal cancer.

Oxaliplatin is usually combined with a fluoropyrimidine and appears to be tolerated when used for chemoradiotherapy, for example, at a dose of 130mg/m^2 given on wk 1 and 5, most commonly with 5-FU *(107–114)*. Treatment is generally well tolerated, with minimal dose-limiting toxicity seen at the prespecified target dose, the most common additional toxicity appearing to be neurotoxicity *(109)*. With a significant benefit to 3-yr DFS demonstrated in colon cancer with oxaliplatin in combination with 5-FU *(115,116)*, it is also likely that future trials may evaluate the use of similar combinations for postoperative adjuvant treatment in this disease. Irinotecan has been used as a single agent and in combination with either 5-FU or capecitabine in chemoradiotherapy trials in a wide variety of schedules *(117–123)*. The main toxicities increased in these combinations are diarrhea and myelosuppression. Both oxaliplatin and irinotecan have shown adequate efficacy in the phase II setting to warrant further evaluation in randomized trials. Other agents that have been explored with chemoradiotherapy for rectal cancer include cisplatin124, 125, and mitomycin-C 20.

It is also likely that future clinical trials will evaluate the use of the novel targeted agents with RT or chemoradiotherapy. At present, the two most likely agents for evaluation are the antiepidermal growth factor receptor monoclonal antibody, cetuximab, and the antivascular endothelial growth factor monoclonal antibody, bevacizumab. Both have been shown in to improve survival and response rates in advanced colorectal cancer *(126–131)*. Cetuximab has shown to prolong survival in patients with locally advanced head and neck squamous cell carcinoma when added to RT compared to RT alone *(132)*. Synergy between bevacizumab and RT has been demonstrated in preclinical models, and is currently being assessed in a phase I trial *(133)*.

5. SPHINCTER PRESERVATION WITH PREOPERATIVE TREATMENT

Surgery for rectal cancer can generally be classified as APRs or ARs. The type of surgery performed is usually determined by the surgeon, based on the distance of the inferior border of the tumor from the anal verge, the aim being

to perform a surgical procedure with an adequate margin of clearance distally. In general, APR is usually considered when the inferior border of the primary tumor is 6 cm or less from the anal verge. If tumor regression can be induced with preoperative treatment in low-rectal tumors, then a sphincter-preserving procedure such as an low or ultra-low anterior resection or coloanal anastamosis may be performed instead. Although sphincter preservation is often reported in studies of preoperative treatment, patient recruitment has not necessarily been limited to patients with low tumor, nor has the rate of sphincter preservation in the subgroup of patients with low tumors been reported separately. An important secondary issue when considering sphincter-preserving surgery is whether patients will actually benefit from the point of view of preserved sphincter function and quality of life. For example, one study that assessed patients who were undergoing low anterior resections or coloanal anastamosis after combined modality treatment using a function assessment and quality of life questionnaire suggested that such patients might be better served by permanent diversion *(134)*.

In single arm studies or case series, which reported the sphincter preservation rates of the subgroup of patients with tumors no more than 6 cm from the anal verge or of patients who were predetermined at study entry to require APR, sphincter preservation rates between 42–89% were achieved with preoperative chemoradiotherapy with 45–50.4 Gy and concurrent 5-FU *(14,25,135,136)*. Tumor response to preoperative treatment was a predictive factor for sphincter preservation in tumors less than 6 cm from the anal verge, but not for higher tumors *(135)*. The highest rate of sphincter preservation (89%) was reported by a retrospective analysis, which also found a 3-yr local failure rate of 2%, and 5-yr DFS and OS of 72 and 88% for these patients, respectively *(136)*. In another analysis with low-rectal T3 or T4 tumors, sphincter preservation occurred in 49% *(25)*. Factors that independently predicted the likelihood of sphincter preservation were increased distance of tumor from the anal verge, more recent year of treatment, and complete clinical response to preoperative treatment. The duration of the interval between completion of preoperative treatment and surgery (4–8 wk) did not significantly predict sphincter preservation. The overall 5-yr pelvic recurrence rate was similar in patients who underwent sphincter preservation compared to those who did not (13 and 14%, p = not significant), suggesting the efficacy of surgery was not compromised by a conservative procedure.

As previously discussed, the German study comparing pre- and postoperative chemoradiotherapy was able to demonstrate a statistically significant improvement in sphincter preservation in the subgroup of patients who were determined at randomization to require APR (39% for preoperative treatment vs 19%, $p = 0.004$) *(15)*. The significance of the difference was not maintained when sphincter preservation rates for the whole cohort were compared, but this may have been because the preoperative treatment arm had significantly more

patients with low rectal tumors (39 vs 30%, $p = 0.008$). There was no significant difference in sphincter preservation between arms in the NSABP R-03 in the similar subgroup of patients *(52)*.

The two randomized trials of preoperative chemoradiotherapy vs preoperative RT (EORTC 22921 and FFCD 9203) have not thus far reported separate results for the subgroup of patients with low tumors or who were predetermined to require APR. In EORTC 22921, slightly more patients who had chemoradiotherapy had ARs (55.3 vs 52.8%, $p = 0.05$) *(87)*. However, this difference was of borderline significance. The rate of sphincter-preserving surgery in FFCD 9203 was similar in both arms *(88)*.

6. NEOADJUVANT CHEMOTHERAPY

Neoadjuvant chemotherapy is the use of a course of full-dose systemic chemotherapy before preoperative chemoradiotherapy and surgery. In many of the previously discussed studies of preoperative treatment, improvements in local disease control and local disease-free recurrence have not generally resulted in prevention of the development of distal metastases and improvements in survival. This is likely to be caused by micrometastases outside the RT field, which are also not well treated by the reduced doses of chemotherapy required to make concurrent administration feasible. In theory, early systemic treatment may lead to improved disease control by delaying the development of or eliminating these micrometastases. Treatment can often be commenced immediately, avoiding potential delays while waiting for definitive chemoradiotherapy, resulting in a reduction in tumour bulk and an early improvement in tumor-related symptoms.

Neoadjuvant chemotherapy is a treatment strategy of interest to the authors. Initially, we reported the use of 3 mo of neoadjuvant capecitabine and mitomycin-C prior to 54-Gy RT (including a boost dose) with concurrent protracted venous infusion 5-FU followed by surgery *(137)*. More recently, the preliminary results of another phase II study (EXPERT) with similar design, in which capecitabine and oxaliplatin was used for neoadjuvant chemotherapy and capecitabine was administered concurrently with preoperative chemoradiotherapy, were reported *(138)*. Both studies included patients with a high risk of local recurrence if treated with surgery alone, defined by characteristics such as: T3c (tumour invading ≥5mm into perirectal fat) or T4 disease, T3 tumors at or below the levator plane, involvement of more than four lymph nodes (N2 disease), or tumors extending within no more than 1 mm of the mesorectal fascia. Most patients in the earlier were assessed with MRI and had TME surgery, whereas both MRI assessment and TME surgery were compulsory in EXPERT. In both studies, patients received postoperative adjuvant chemotherapy with 5-FU/mitomycin-C or capecitabine, respectively. In the

preliminary results of EXPERT, response rates of 88.2 and 97.1% were documented on MRI in 68 evaluable patients after neoadjuvant chemotherapy and RT, respectively *(138)*. Of the 62 patients who proceeded to TME at that time, 61 had R0 resections, with pCR rate of 24.2% and microscopic tumor foci-only found in a further 42%. The toxicity of this treatment was generally acceptable. Recurrence and survival outcomes will be reported in the future when adequate follow-up has been achieved. We have recently opened to recruitment a multicenter randomized phase II study, in which cetuximab is added to the treatment in EXPERT.

Also recently reported were the results of another phase II study in which patients with T3 or worse disease, selected by EUS and CT, were treated with neoadjuvant FOLFOX4 followed by 45–50.4 Gy of RT with concurrent tegafur. Some of these patients also were treated with IORT *(139)*. The 2-yr local control rate, DFS, and OS reported by this study were 95, 88, and 93%, respectively.

It should be emphasized that although neoadjuvant chemotherapy appears to be promising, it remains an experimental treatment strategy. Therefore, patients should not be treated with neoadjuvant chemotherapy outside a clinical trial.

7. CONCLUSIONS

Even with optimal surgery (TME), local recurrence rates from locally advanced rectal cancers remain significant. In general, strategies for improving the outcomes of these patients have been based on RT (whether pre- or postoperative), to which concurrent and/or sequential chemotherapy may be added. These treatments have often resulted in improvements in local recurrence rates with a less frequent impact on survival.

Short-course preoperative RT improves local recurrence compared to surgery alone (TME or non-TME). An improvement in OS has only been observed in the curatively resected subgroup of patients *(22,23)* and in meta-analysis. Although acutely well tolerated, preoperative RT is associated with an increase in perioperative morbidity, particularly wound sepsis. At least one large study has also documented long-term bowel function-related morbidity *(72)*. Noncancer deaths are also increased, particularly as a result of cardiovascular causes, in the first year after treatment.

The addition of concurrent chemotherapy with 5-FU/LV to preoperative long-course RT (45–54 Gy RT delivered over 5–6 wk) appears to improve local recurrence rates compared to RT alone *(87,88)*. Similarly, at least in one randomized trial, preoperative chemoradiotherapy resulted in a reduction in local recurrence compared to postoperative chemoradiotherapy (15), which was not reproduced in another randomized trial (52). In neither comparison did

preoperative chemoradiotherapy improve distal recurrence rates or survival. The addition of chemotherapy to preoperative RT results in a modest increase in acute treatment-related morbidity, particularly diarrhea and skin complications, but no increase in perioperative complications. Compared to postoperative chemoradiotherapy, preoperative combined-modality treatment is generally better tolerated.

In patients with low-rectal tumours (≤6 cm from the anal verge), preoperative treatment may lead to tumor regression, in order to make sphincter-preserving surgery feasible. Indeed, patients who underwent sphincter-preserving surgery after preoperative treatment appeared to have similar outcomes to those who had APRs. Patients initially determined to require APR who are treated with preoperative chemoradiotherapy appear to be more likely to have a sphincter-preserving procedure than if they had proceeded to directly to surgery *(15)*. However, the additional benefit of using chemotherapy with preoperative long-course RT appears to be small, with only one study showing a small improvement in sphincter-preserving surgery of borderline significance *(87)*.

No studies have directly compared preoperative long-course treatment with short-course RT. However, in view of the lack of downstaging with short-course treatment (at least with 25 Gy RT over 5 d with an interval of less than 10 d between the start of RT and surgery), long-course treatment is more likely to be useful when preoperative tumor downstaging is a significant goal of treatment. On the other hand, short-course RT is associated with minimal acute morbidity and may be suitable for patients without poor prognostic features or patients who may not tolerate long-course chemoradiotherapy. It is also a treatment option for patients who may not want to undergo 5–6 wk of treatment before surgery.

Postoperative adjuvant chemotherapy is in widespread clinical use in patients with curatively resected rectal cancer and supported by results such as those of the UK QUASAR study, in which a reduction in recurrence and improvement in survival was observed in these patients *(46)*. Therefore, patients with curatively resected rectal cancer should receive postoperative adjuvant chemotherapy, either after postoperative chemoradiotherapy or after surgery, if patients have received preoperative treatment.

Future trials will evaluate the use of more potent radiosensitizers with preoperative long-course RT, including the novel targeted agents, as well as the use of neoadjuvant chemotherapy prior to preoperative chemoradiotherapy. Other factors that may improve the outcomes of patients with locally advanced rectal cancer include management of patients by specialist multidisciplinary teams, standardization of surgery (preferably TME) within an appropriate training scheme, rectal cancer surgery occurring only in specialized high-throughput units, standardization of histopathology assessment, and access to imaging modalities such as thin slice MRI.

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10 Rationale for Adjuvant and Neoadjuvant Chemotherapy in the Resection of Liver Metastases

Axel Grothey, MD and Steven A. Alberts, MD

Summary

Since chemotherapy of metastatic colorectal cancer (CRC) is palliative by definition, hepatic resection is the only potentially curative option for liver metastases. The demonstration of a curative potential of liver surgery in liver-limited stage IV CRC abrogated the need for randomized trials to address the value of surgery in the setting of resectable liver metastasis; in fact, such trials would be unethical. Methods to improve resectability of metastases and long-term outcome utilizing chemotherapy are explored in this chapter.

Key Words: Liver resection; neoadjuvant; adjuvant; chemotherapy.

1. INTRODUCTION

Of the approx 145,000 patients diagnosed with colorectal cancer (CRC) every year in the United States and the 1 million patients with CRC worldwide, 20% will have distant metastasis at diagnosis. In addition, 25–30% of patients with stage II/III disease will eventually develop metastasis. The liver is the most common site of metastasis of advanced CRC, at least in part owing to hematogenous spread via the portal vein system. In about one-third of patients with metastatic disease, the liver will be the only site of metastasis. Since the initial reports of long-term overall survival after complete resection of liver metastases of CRC in the 1980s *(1)*, multiple series have shown the feasibility, safety, and clinical value of metastasectomy for the overall prognosis of patients with metastatic CRC (Table 1, adapted from *[2]*). Overall, approx 30–35% of patients who underwent liver surgery for initially resectable disease in the 1980s and 1990s survived for 5 yr. Because chemotherapy of metastatic CRC is palliative by definition, hepatic resection is the only potentially curative option for liver metastases, with contemporary

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Table 1
Outcome of Resection of Liver Metastasis of Colorectal Cancer

Study	*Year*	*No. of Patients Survival (%)*	*5-Yr Overall*	*Synch (%)*	*Multiple Mets (%)*	*Bilateral (%)*	*>5-cm Lesion (%)*	*Node+ Primary (%)*
Adson	1984	141	24	26	25			
Ekberg	1986	72	16	58	51	24		69
Stehlin	1987	43	22		63	42		71
Registry	1988	859	33	58	35	32		58
Schlag	1990	122	30	39	48			
Gazzaniga	1991	66	25	52				
Fegiz	1991	47	12	59	23	27		76
Doci	1991	100	30	32	42	14		63
Yamaguchi	1993	40	41	55	58	30	30	
Gaywoski	1994	204	32	44	55	39	64	63
Scheele	1995	434	33	44	42	16	33	65
Nordlinger	1996	1568	28	40		22	45	54
Jenkins	1996	131	25	20	39			
Rees	1997	89	37		54	27		
Jamison	1997	280	27	39	33			52
Fong	1999	1001	37	49	51	40	44	60
Choti	2002	226	40	30	38	24	41	63
Liu	2002	72	32	35				
Bramhall	2003	212	28	24	35		57	72
Kato	2003	585	39	45	50			70

Synch, synchronous liver metastases and primary tumor; node + primary, primary tumor stage with lymph node involvement.

operative mortality rates as low as 1 to 2%. The demonstration of a curative potential of liver surgery in liver-limited stage IV CRC abrogated the need for randomized trials to address the value of surgery in the setting of resectable liver metastasis; in fact, such trials would have been unethical.

2. METHODS TO IMPROVE RESECTABILITY OF METASTASES AND LONG-TERM OUTCOME

Until recently, resection of liver metastasis was deemed feasible in only a small subset of patients with advanced disease and only approx 10–15% of patients with liver-only disease were amendable to upfront resection (Fig. 1A). Major advances in the medical treatment of CRC with significant increase in the quantity and quality of responses, as well as better patient selection, advances in surgical methods, and the emergence of nonsurgical tumor ablation techniques have significantly

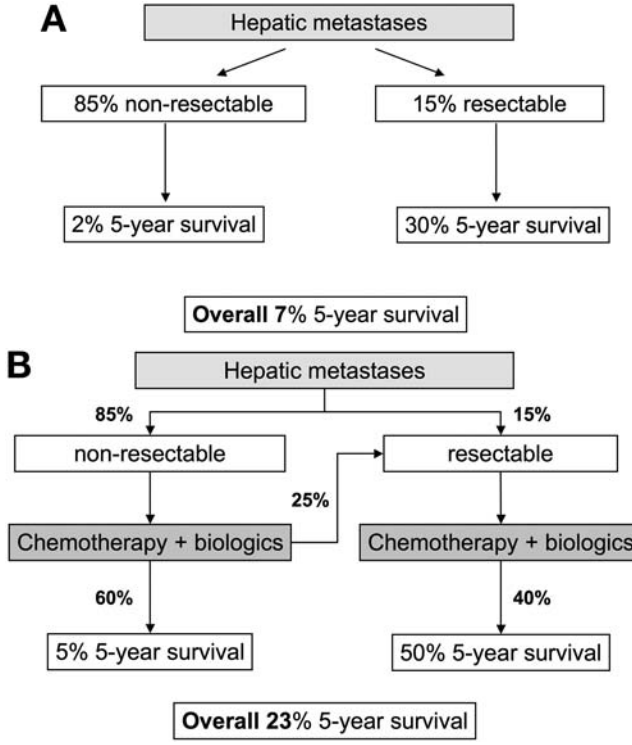

Fig. 1. (**A**) Treatment of CRC liver metastases: the past and (**B**) treatment of liver metastases: the future.

changed the modern approach toward liver-limited metastatic CRC. Using a multimodality strategy involving medical oncologists, liver surgeons, and interventional radiologists, the number of patients rendered free of metastatic disease as a prerequisite for a curative chance will substantially increase in the future (Fig. 1B).

3. PATIENT SELECTION FOR LIVER RESECTION

The definition of surgical resectability has undergone dramatic changes in recent years and continued to evolve. Several scoring systems have been developed over the years to predict the outcome of patients after liver resection *(3,4)*. However, these scoring systems can only provide general information on the prognosis of patients with certain characteristics. In this context, one has to distinguish between tumor characteristics that influence the technical resectability of metastases, the presence of prognostic factors associated with a poor outcome even after successful liver resection (oncological contraindications), and patient-associated factors such as preexisting comorbidities (Table 2).

Although the presence of unresectable extrahepatic metastases is an absolute contraindication for liver resection, a two-stage approach with liver resection

Table 2
Factors Influencing Decision on Resectability of Liver Metastases

Technical resectability
Location of metastases
Intrahepatic distribution of metastases
Involvement of essential anatomical structures by metastases
Number of metastases
Size of metastases
Functional hepatic reserve after resection
Adverse prognostic factors
Presence of extrahepatic disease (e.g., lung, lymph nodes, peritoneum)
Indicators of aggressive biology (e.g., high preoperative CEA)
Synchronous presentation of liver metastasis with primary tumor
Lymph-node positive primary stage
Short disease-free interval since resection of primary tumor
Progression on neoadjuvant chemotherapy
Severe, preexisting comorbidities of affected patient

CEA, carcinoembryonic antigen.

followed by resection of extrahepatic disease (e.g., by pulmonary metastasectomy) can result in long-term disease-free survival *(5)*. As further detailed here, resectability of metastases in this aggressive setting can conceivably be increased by systemic neoadjuvant treatment.

It is important to note that the factors determining the technical resectability can potentially be overcome by advances in surgical techniques and by the use of neoadjuvant chemotherapy to downsize liver metastases.

4. IMPROVED SURGICAL TECHNIQUES

A major milestone in the development of liver surgery was the identification of functionally and anatomically independent liver segments, which allowed a more sophisticated surgical approach toward multifocal, bilobar disease (Fig. 2). In combination with new nonanatomical resection techniques, the limitation for liver resection is now mainly defined by the functional liver volume that remains after surgery. Computed tomography (CT)-assisted volumetry is able to predict the future liver remnant volume as indicator of the functional hepatic reserve (FHR). Current concepts indicate that the FHR should not be less than 20–25% to limit postoperative morbidity and mortality.

If the calculated FHR is less than 20%, selective portal vein embolization might improve the preconditions for liver resection via induction of compensatory

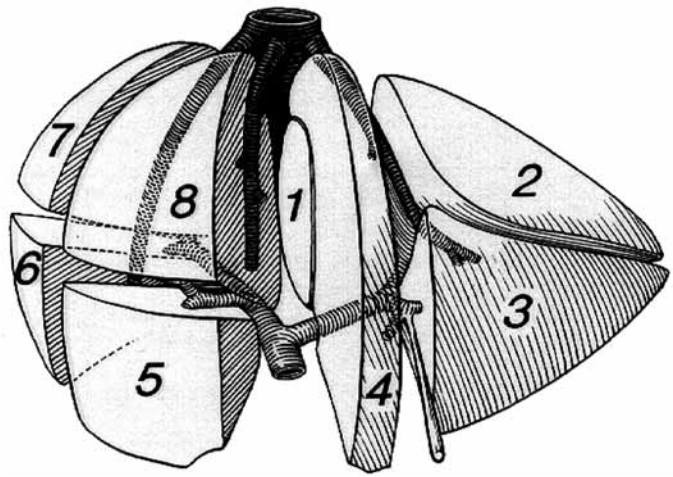

Fig. 2. Functionally and anatomically independent liver section.

hypertrophy of the remnant liver, which is commonly achieved within 6 wk after embolization *(6)*. This technique can also be of value in patients with liver dysfunction, for example, after neoadjuvant chemotherapy (*see* Section 6).

An alternative way to address the problem of insufficient postoperative liver volume is a planned two-stage resection approach, in which the part of the liver bearing the dominating tumor mass is resected as initial step followed by subsequent resection of the less tumor-affected residual liver after hypertrophy of the liver parenchyma is achieved. The time to second resection can be bridged by chemotherapy.

Further advances in the surgical management of liver metastases include the routine use of intraoperative ultrasound (IOUS) to visualize liver metastases not detected by preoperative imaging studies. The results of IOUS influence the surgical approach in 10 to 40% of cases and is thus an important tool in the surgical approach toward complete metastasectomy *(7)*.

Ablative treatment such as cryoablation or radiofrequency ablation (RFA) has expanded the approach of hepatic surgery in patients not considered optimal candidates for surgical resection. The efficacy of ablative techniques is, however, limited by the size of the metastasis, which should not exceed 3–4 cm, and the contact with major biliary or vascular structures. The higher rate of local and intrahepatic recurrence observed with RFA compared to conventional resection clearly defines RFA as an adjunct and not as substitute for surgical resection of liver metastases *(8)*. Locally destructive methods are useful in the context of extended hepatectomy with residual, surgically inaccessible intrahepatic disease.

5. ADJUVANT CHEMOTHERAPY AFTER LIVER RESECTION

Patients who have undergone complete resection of colorectal metastases to the liver may be candidates for adjuvant therapy because they are at high risk for recurrence. Following surgery, the 5-yr survival rate is 25–40%. Despite this observation, no prospective randomized trials of adjuvant chemotherapy in resected stage IV patients have yet been reported. In fact, such a trial, though scientifically interesting, will almost certainly never be done, since the rationale for an adjuvant approach is compelling enough to dissuade most investigators and patients from participation in a surgery-only control arm.

The rationale for administering adjuvant chemotherapy in patients after resection of liver metastases is based on the observation that, in localized colon cancer, patients with higher risk of recurrence show the greatest relative and absolute benefit from adjuvant treatment. Patients with stage III disease benefit more than patients with stage II disease, and patients with high-risk stage III (e.g., N2 disease) benefit more than patients with low-risk stage III tumors *(9)*. Translated into the setting of resected stage IV patients with no evidence of disease after surgery, this observation forms a strong rationale for the use of adjuvant chemotherapy after liver resection. Some, admittedly retrospective, analyses of clinical trials support this notion.

In the context of adjuvant therapy after liver resection, two different modalities and three different scenarios can be identified. The fact that 40% of recurrences after liver surgery exclusively involve the liver forms the rationale for adjuvant locoregional approaches (hepatic arterial infusion [HAI]). In turn, the fact that 60% of patients will present with extrahepatic (± intrahepatic) relapse makes the case for the use of optimized systemic chemotherapy after liver surgery.

Different clinical scenarios include the following:

1. Adjuvant therapy after primary resection of resectable metastases.
2. Adjuvant therapy after neoadjuvant therapy for resectable metastases followed by resection.
3. Adjuvant therapy after neoadjuvant therapy and resection of initially unresectable metastases.

Scenario 1 was mainly addressed in trials using HAI with or without systemic chemotherapy as adjuvant therapy. Although a definitive effect on the risk of hepatic recurrence could be observed in most studies on adjuvant HAI, the use of HAI did not, in studies thus far, translate into significantly improved overall survival. In a study from Memorial Sloan-Kettering Cancer Center, patients were randomized to systemic chemotherapy alone (82 patients) vs systemic chemotherapy combined with HAI with 5-fluorouridinedeoxyribose (FUDR) (74 patients) *(10)*. In the most recent update of this trial, the median

survival in the group receiving combined therapy was 68.4 mo compared to 58.8 mo for those receiving systemic therapy alone, a difference that was not statistically significant *(11)*. At 2 yr, however, the rate of survival free of hepatic recurrence was 90% in the combined therapy group compared to 60% in the systemic therapy-only group ($p < 0.001$). As expected, recurrence outside the liver appeared similar in both groups.

The technical challenges associated with pump and catheter placement, the challenges of administration of HAI chemotherapy, and the increased efficacy of modern systemic combination therapy have made most oncologists abandon HAI as adjuvant therapy. However, investigations continue, and HAI might still play a role in the adjuvant therapy of patients with resected liver metastases when combined with modern systemic combination therapy. In a phase II trial that recently completed accrual (North Central Cancer Treatment Group [NCCTG] N9945) the potential benefit of capecitabine and oxaliplatin alternating with HAI of FUDR was assessed. Building on this study, a phase III trial (National Surgical Adjuvant Breast and Bowel Project [NSABP] C-09) will definitively evaluate the necessity of adding HAI of FUDR to systemic therapy with capecitabine and oxaliplatin by randomizing patients to systemic therapy alone or to the combination with HAI.

Scenario 2 is addressed by the experimental arm of the recently fully accrued European Organisation for Research and Treatment of Cancer (EORTC) Intergroup trial 40983, which awaits final analysis in 2006.

The pertinent question for the third scenario is how long overall chemotherapy should be conducted. In analogy to the experience in stage III colon cancer, current consensus would suggest to limit the total duration of neoadjuvant and adjuvant therapy combined to 6 mo. If an oxaliplatin-based regimen is used as neoadjuvant therapy, the cumulative neurotoxicity associated with this drug would hardly allow longer administration than 6 mo of FOLFOX anyway *(12)*. It is unclear, though, if patients who underwent neoadjuvant oxaliplatin-based therapy should rather receive postoperative adjuvant treatment with irinotecan to further the eradication of residual, at that time presumably oxaliplatin-resistant, micrometastases. Although the data on irinotecan as adjuvant therapy in stage III colon cancer are rather disappointing, resected stage IV disease after oxaliplatin-based neoadjuvant therapy might represent a different situation, in which irinotecan could be considered. On the other hand, the in vivo test of sensitivity to a certain neoadjuvant chemotherapy might serve as argument to continue an effective treatment regimen in the postoperative setting. Another open question is the role of the new biological agents in the adjuvant setting, and in particular, the duration of their use. As before with conventional chemotherapy, the data of ongoing phase III trials in the adjuvant setting in stage II and III colon cancer will conceivably influence the future practice pattern in resected stage IV disease.

6. NEOADJUVANT CHEMOTHERAPY TO IMPROVE RESECTABILITY

The emergence of highly active chemotherapy regimens for the treatment of metastatic CRC has substantially changed the approach to patients with initially unresectable, liver-limited metastases. Because complete surgical resection with free margins is the only modality that offers patients a chance of long-term, tumor-free survival, strategies to downsize technically unresectable liver metastases by primary chemotherapy to allow for subsequent curative resection are of high clinical relevance.

The main predictor of whether or not a given chemotherapy regimen is able to lead to increased respectability rates in liver-limited disease is the direct antitumor activity of the regimen measured by its response rate *(13)*. It is thus easy to understand that these neoadjuvant approaches only emerged with the advent of chemotherapies that reliably induced response rates at or above 50%, much higher than the 15–20% response rate associated with systemic biomodulated 5-fluorouracil (5-FU) alone. In the era of 5-FU, locoregional approaches using HAI with 5-FUDR combined with systemic 5-FU/leucovorin (LV) were applied to maximize response. In fact, several phase II trials documented response rates of 22–62% *(14)*, but toxicities related to 5-FUDR (biliary sclerosis) and catheter-related complications, which can conceivably interfere with a subsequent surgical approach, have so far precluded HAI from becoming standard-of-care as neoadjuvant treatment of unresectable liver metastases. In addition, modern systemic chemotherapy regimens achieve response rates in the same range as or exceeding HAI.

Oxaliplatin-based regimens have so far been most widely studied as neoadjuvant therapy in patients with initially unresectable liver metastases. Studies presented in the mid-1990s by French investigators established the concept of downsizing unresectable liver metastases with systemic 5-FU/oxaliplatin to obtain surgical resectability when they could demonstrate that the long-term prognosis of these patients did not differ from historical controls with initially resectable metastases *(15,16)*. In fact, to date several countries have only approved oxaliplatin as part of a neoadjuvant strategy as first-line therapy for advanced CRC. It is conceivable that the capability of a certain chemotherapy regimen to downsize metastasis in a neoadjuvant setting is most closely related to the reported overall response rate obtained with the given regimen. Because response rates of oxaliplatin- and irinotecan-based combination protocols are equivalent in advanced CRC, FOLFIRI (infusional 5-FU/LV plus irinotecan) should be regarded as an alternative to FOLFOX as neoadjuvant therapy, even if FOLFIRI has not been as widely studied in this setting.

Prospective trials focusing on a neoadjuvant therapeutic approach in liver-limited, initially nonresectable metastatic disease demonstrate that FOLFOX is

able to render approx 20–35% of cases resectable. A recent phase II trial in 42 patients with initially unresectable, liver-limited disease demonstrated that 17 of 25 patients with partial response to FOLFOX subsequently underwent surgery with planned hepatic resection *(17)*. In 14 of these patients, complete surgical resection of metastases was achieved, 1 patient had a partial resection, and 2 patients were considered unresectable after laparotomy. The median overall survival of the patients undergoing complete or partial metastasectomy was 26 mo. Even though 11 of 15 patients who underwent liver resection relapsed, the aggressive surgical approach appeared to be associated with a survival benefit compared with historical controls on palliative chemotherapy.

7. NOVEL BIOLOGICAL AGENTS IN THE NEOADJUVANT SETTING

The standard-of-care of systemic treatment in advanced CRC has recently moved to a combination of conventional chemotherapy plus biological, targeted agents such as the vascular endothelial growth factor-inhibitor bevacizumab and the endothelial growth factor receptor (EGFR)-antibody cetuximab. Based on the results of pivotal trials in bevacizumab-naive patients *(18)*, bevacizumab has emerged as a standard component of systemic therapy in first- and second-line. The addition of bevacizumab to conventional chemotherapy has consistently shown to increase response rates by approx 10–15% with a more dramatic effect on time-to-tumor progression of approx 4.5 mo in the first- and approx 2 mo in the second-line setting. Although the increased response rate observed with bevacizumab-containing regimens in principle favors its use in a neoadjuvant setting, bevacizumab itself has been shown to cause delayed wound healing, a factor that has to be considered when bevacizumab is part of a neoadjuvant treatment strategy. The long half-life of bevacizumab of approx 3 wk, and its protracted biological effects have led to recommendations to discontinue bevacizumab approx 6–8 wk before a planned liver resection *(19)*. In clinical practice, an appropriate neoadjuvant strategy for liver-limited metastases could consist of administering 4–6 cycles (i.e., 2–3 mo) of FOLFOX (or FOLFIRI) plus bevacizumab, restaging patients by CT scan and then deciding—in conjunction with an experienced liver surgeon—if patients are candidates for liver resection with curative intent. If a surgical approach is planned, bevacizumab should be omitted from the next two cycles of therapy so that surgery can be performed 6–8 wk after discontinuation of bevacizumab. In the postoperative phase, bevacizumab-containing therapy can be resumed 4 wk after surgery.

Although the major strength of bevacizumab appears to be delaying tumor progression rather than inducing tumor shrinkage, and though it has very limited single-agent activity, cetuximab, with its direct attack on tumor cells, is able to induce tumor regression as single agent as well as to enhance activity

of chemotherapy *(20)*. Cetuximab, as well as another anti-EGFR monoclonal antibody, panitumumab (*see* Chapter 6), are currently undergoing phase III testing in the first-line setting in advanced CRC. Phase II data on cetuximab in combination with modern oxaliplatin- and irinotecan-based combination chemotherapy show encouraging results, with response rates of up to 80% in chemotherapy-naive patients *(21)*. In conjunction with the fact that cetuximab does not appear to impair wound healing, combination regimens with cetuximab could emerge as optimal neoadjuvant treatment approach in patients with unresectable liver metastases. An ongoing NCCTG phase II trial is currently testing this hypothesis by investigating FOLFOX plus cetuximab as neoadjuvant therapy in patients with unresectable, liver-limited metastases.

It is conceivable that in the future, regimens consisting of conventional combination chemotherapy plus dual antibodies (e.g., bevacizumab and cetuximab) will emerge as the most effective tools to downsize liver metastases for a subsequent curative surgical approach. The high costs associated with these regimens would clearly be offset by the curative goal of the strategy and the limited duration of therapy until the definite decision on resectability is made.

8. DURATION OF NEOADJUVANT TREATMENT

It is well established that tumor response on chemotherapy is most dramatic in the initial phase of treatment. A recent analysis of the FOLFOX arm in Intergroup trial N9741 indicated that median time to response on FOLFOX was 2.2 mo and no patient converted from nonresponder to responder after 6 mo of treatment. In addition, the use of chemotherapy, in particular, oxaliplatin- and irinotecan-based chemotherapy, has been associated with a form of liver toxicity termed chemotherapy-associated steatohepatitis (CASH). Another hepatic toxicity associated with oxaliplatin is the development of sinusoidal obstruction, which can be accompanied by splenomegaly. Although the development of CASH and sinusoidal obstruction *per se* does not necessarily preclude liver resection and is apparently not associated with significant increases in postoperative morbidity or mortality, most liver surgeons prefer to limit the duration of chemotherapy before liver surgery to less than 4–6 mo to avoid CASH and thus facilitate liver resection. Chemotherapy-induced changes in liver architecture are not exclusive to oxaliplatin. Other commonly used agents in the treatment of CRC have also been associated with histological features of liver toxicity (Table 3).

9. ROLE OF IMAGING STUDIES IN THE NEOADJUVANT SETTING

Another parameter potentially affecting the duration of neoadjuvant therapy is the notion that treatment should be discontinued and a surgical approach

Table 3
Chemotherapy-Induced Changes in Liver Architecture

Histopathology	*Agent(s)*
Steatosis	Multiple agents
Chemotherapy-associated steatohepatitis (CASH)	Oxaliplatin, irinotecan
Sinusoidal obstruction syndrome	Oxaliplatin
Biliary structures, biliary sclerosis	FUDR

initiated before a complete response by imaging studies is achieved. This at first glance counterintuitive strategy facilitates the identification and resection of the tumor-bearing part of the liver during surgery. If CT and magnetic resonance imagine (MRI) scans are not able to detect tumor manifestations anymore, IOUS might still be able to detect tumor residues. Even if no tumor is detected by IOUS, the previously tumor-bearing part of the liver should be resected because in a significant number of patients viable tumor cells will be found microscopically. It is important to note in this context that positron emission tomography (PET)-negativity of liver metastases after chemotherapy is not equivalent to a pathological complete response. Tumors can become metabolically inactive (and thus PET-negative) on chemotherapy, but still remain viable. Thus, PET-negative liver metastases should be resected if feasible. Whether an aggressive surgical approach on liver metastases is warranted once extrahepatic metastases have become PET-negative on chemotherapy is an open question, but, again, the usability of PET to reliably assess viability on chemotherapy is limited.

10. NEOADJUVANT THERAPY OF RESECTABLE METASTASES

Modern chemotherapy combinations, including biological agents, show an impressive antitumor activity and only a small minority of patients will have primary progressive disease on therapy. Recent phase II trials have reported tumor control rates (tumor response plus stable disease) of up to 98% *(21)*. Thus, upfront chemotherapy in patients with initially resectable liver metastases would not harm the overall outcome. In addition, in view of the high rate of relapse even after potentially curative metastasectomy, upfront initiation of systemically active chemotherapy constitutes early treatment of intra- or extrahepatic micrometastases, which could be responsible for relapse after liver resection. Furthermore, neoadjuvant chemotherapy can serve as in vivo test of the chemosensitivity of the tumor, which could guide postresection adjuvant therapy. Response to neoadjuvant therapy can also provide information on the biology of the tumor and the prognosis after liver resection. A recent retrospective analysis in patients with

neoadjuvant therapy in resectable liver metastases indicated that patients who showed progressive disease on chemotherapy had a much lower chance to remain disease-free and alive after complete metastasectomy compared with patients who showed tumor shrinkage or stable disease on chemotherapy *(22)*. It is unclear, though, if these data would justify withholding liver resection in patients with principally resectable disease who show progressive disease on neoadjuvant chemotherapy.

The role of neoadjuvant and adjuvant chemotherapy will be further clarified by the results of a recently completed EORTC Intergroup trial 40983, in which 364 patients with principally resectable liver metastases were randomized to surgery only, or six cycles of neoadjuvant FOLFOX4 followed by liver resection and subsequent six adjuvant cycles of FOLFOX4. Unfortunately, the trial design will not allow distinguishing between the relative contributions of the neoadjuvant and adjuvant component of the chemotherapy arm for the overall outcome of patients.

11. SUMMARY AND CONCLUSION

Patients with liver-limited metastases from CRC have a curative chance if their metastases can be surgically removed with free margins. The high efficacy of modern systemic chemotherapy has allowed for the development of neoadjuvant treatment approaches in liver metastases not amendable for surgical resection upfront. The addition of biological agents such as bevacizumab and anti-EGFR antibodies to conventional chemotherapy will conceivably further enhance the activity of neoadjuvant therapies, thereby making more patients candidates for secondary surgery with curative intent (Fig. 1B). Even in the absence of data of prospective clinical trials in this setting, postoperative, adjuvant systemic chemotherapy is routinely used in view of its convincing efficacy in high-risk stage III colon cancer. A multimodality/multidisciplinary approach integrating highly active chemotherapy and surgery will allow patients to experience the greatest chance of long-term disease-free survival.

The greatest clinical challenge right now is creating an increased awareness of the curative potential of primary and secondary surgery of metastases in advanced CRC among surgeons, medical oncologists, and patients.

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11 Percutaneous Radiofrequency Ablation in the Management of Patients With Colorectal Cancer

Karen Brown, MD, FSIR

Summary

This chapter provides a brief overview of the methods of percutaneous thermal ablation including high-intensity focused ultrasound, interstitial laser, microwave, cryo and radiofrequency currently in use, followed by a discussion of the potential use of these modalities in patients with colorectal cancer. The most common indication for thermal ablation in this group of patients is the treatment of metastatic disease to the liver. Ideal candidates for this therapy will have disease limited to the liver, with no more than three lesions, all less than 3 cm in diameter. Indications may be expanded to treat patients with a single larger lesion, or in other instances where the objective is palliation rather than "cure." Technical issues that make some lesions treatable and other similar lesions not are outlined. The use of ablative therapies in other areas of the body such as lung and bone are also discussed.

Key Words: Percutaneous ablation; radiofrequency ablation; cryoablation; hepatic radiofrequency ablation; results; radiofrequency ablation; complications.

1. INTRODUCTION

The object of percutaneous thermal ablation is to destroy an entire tumor, killing the malignant cells in a minimally invasive manner, without damaging adjacent vital structures. Treatment is usually planned to encompass the entire mass, plus a 5–10 mm surgical margin. Although this chapter deals predominantly with radiofrequency ablation (RFA), as the largest body of clinical experience has been gained with this method, it is important to recognize that other methods of thermal ablation are coming to market, including three methods of heat deposition (high-intensity focused ultrasound [HIFU], interstitial laser photocoagulation [ILT], and microwave) as well as one method of freezing, cryoablation *(1)*. Any of these may

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gain widespread acceptance in the next few years if clinical studies demonstrate either clinical or technical advantages over RFA.

1.1. High-Intensity Focused Ultrasound

HIFU has the advantage of being the least invasive technique, as nothing penetrates the patient's skin. A parabolic transducer is used to focus ultrasound energy at a distance. This focused energy is transmitted transcutaneously into the targeted tissue. The absorbed energy is converted to heat, which ablates tissue with coagulative necrosis. Tissue destruction can be induced at depths of 10 cm, destroying a selected target without causing damage to intervening structures. This technique requires an acoustic window, and only small volumes of tissue are ablated with each application. Image tracking with sequential overlap would be required in order to ensure complete ablation, and the technique requires approx 1 h to ablate a 2-cc^3 volume.

1.2. Interstitial Laser Therapy

ILT is easily performed with small applicators, and 1–2 s of high-power laser output results in immediate vaporization of tissues in contact with the laser fiber. With lower power and times of 10 min and longer, deeper penetration of light and heat are possible. However, even under the best of circumstances, coagulation of greater than 1.6 cm cannot be achieved with a single fiber.

1.3. Microwave

Microwave is the third form of heat energy that can be used to ablate tissue, and is largely used in the Far East. The inserted microwave probes (often 14 gauge) function as antennae for externally applied energy. When microwave energy is applied, heat is created at the probe tip. It seems that microwave energy results in greater tissue penetration, and thus a larger volume of coagulation necrosis than either RFA or laser energy. Currently, the application of microwave energy by means of a single probe results in necrosis measuring no more than 2.5 cm, so size of the coagulum remains a limitation.

1.4. Cryoablation

Cryoablation works by using a cryoprobe to cool tissues to between –20 and –40°C, resulting in instantaneous cell death. Although cryotherapy has had many medicinal uses and has been used in the operating room to ablate tumors for years, it is only recently that cryoprobes have been made small enough to be used percutaneously. There are three advantages to cryotherapy: it is faster in many cases, and may be less painful than RFA, theoretically allowing more cases to be performed with conscious sedation. The "ice ball" created during treatment is readily seen with computed tomography (CT), making it easier to know that the target lesion has been completely treated. In addition, up to eight

probes can be inserted and cooled simultaneously. The ability to freeze eight probes at once allows for the treatment of a larger volume of tissue. This may enable successful treatment of a larger single lesion, or treatment of more than one lesion at the same time.

2. RADIOFREQUENCY ABLATION

Percutaneous RFA refers to the technique of devitalizing tissue with alternating electrical current. RFA has come into widespread use, and has many applications *(2)*. Patients essentially become electrical circuits when RF electrodes are inserted into their bodies, and grounding electrodes are applied to their thighs. A generator then produces alternating current in the RF range, 300 to 500 kHz. This current causes ionic agitation, which result in the production of frictional heat at the electrode tip. This heat is deposited in the surrounding tissues, and as temperatures rise cell death occurs. Heat causes protein denaturation, desiccation, and coagulative necrosis that occurs after 45 min at 48°C, but occurs within 4 to 6 min at 50–52°C. When temperatures are at or above 60°C, cell death is instantaneous.

Although this seems very straightforward, there are some problems with creating an area of necrosis that is clinically useful. First of all, as the temperature rises at the electrode tissue interface, the tissues begin to desiccate. This causes the impedance of the neighboring tissue to begin to rise. When the impedance becomes too high, current no longer flows and energy can no longer be deposited in the tissues. With just a bare electrode, ablation zones of less than 1 cm are common. Adequate treatment of a 1 cm lesion with a 1cm surgical margin requires ablation of a 3-cm spherical volume of tissue. The goal becomes to continue energy deposition while the heat is conducted into the surrounding tissues in order to create a "kill zone" of sufficient size to be clinically useful. Various methods are used in an effort to enlarge the zone of ablation. One is to cool the needle electrode with circulating water so as to keep the temperature from rising high enough to desiccate the tissues and short circuit the system. Another approach is to use more than one parallel electrode (Fig. 1), or to use an electrode array. Electrode arrays are contained within 14–17 gauge needles, and when deployed resemble the skeleton of an umbrella (Fig. 2). These array-type electrodes may also be combined with hypertonic saline infusion to further enlarge the amount of tissue ablated *(3)*.

Efforts to increase heat deposition may also be made from the tissue side. Some authors have demonstrated that larger treatment areas can be obtained when there is concomitant hepatic artery and/or portal vein occlusion. Intraoperatively, this would be accomplished by the "Pringle maneuver", whereby the afferent hepatic blood supply is occluded by compressing the hepatic artery and portal vein at the porta hepatis. There are percutaneous methods of temporarily occluding the blood flow within these vessels; however, the added complexity does not seem clinically

Fig. 1. Radionics Electrode Cluster consists of three parallel electrodes attached at the base and inserted as a single unit.

Fig. 2. Boston Scientific RF 3000 electrode array contained within introducer, and then in deployed state.

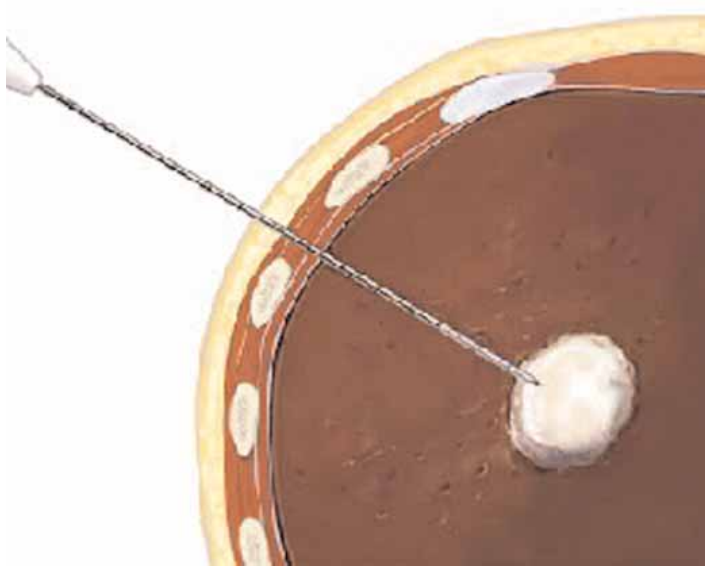

Fig. 3. Array electrode contained within introducer being inserted into liver lesion.

justified. Pharmacological methods of diminishing blood flow have been studied as well *(4)*, but have not gained widespread acceptance. When hypervascular tumors are being treated, the combination of hepatic artery occlusion or embolization with RFA has been shown to result in larger areas of coagulative necrosis *(5)*. Unrelated to blood flow, the adjuvant administration of liposomal doxorubicin prior to RFA has been shown to increase local tumor destruction in animal tumor models *(6)* as well as in humans *(7)*.

The second problem is the geometry of the burn. Electrodes can be made in a variety of shapes, but must be capable of being inserted into a tumor. That implies a needle-like configuration (Fig. 3). When a straight electrode with a bare tip is inserted into tissue, the devitalized area that is created is typically an ellipse (Fig. 4), whereas metastases are generally spherical. This geometry problem can be solved in a variety of ways. Several overlapping burns or applications of RF may be performed in order to construct the proper geometry. Multiple electrodes may be inserted simultaneously, or an appropriately configured electrode array (Fig. 5) may be used.

Finally, one must be aware of the structures in the neighborhood of the RFA target. There are two potential problems associated with adjacent structures. One concerns the safety of RFA, and one the efficacy. In attempting to achieve a 1-cm margin around the target lesion, adjacent organs can be damaged by heat. When treating colorectal metastases in the liver, structures that might be affected include the heart, the bowel, the bile ducts, the pancreas, the kidney,

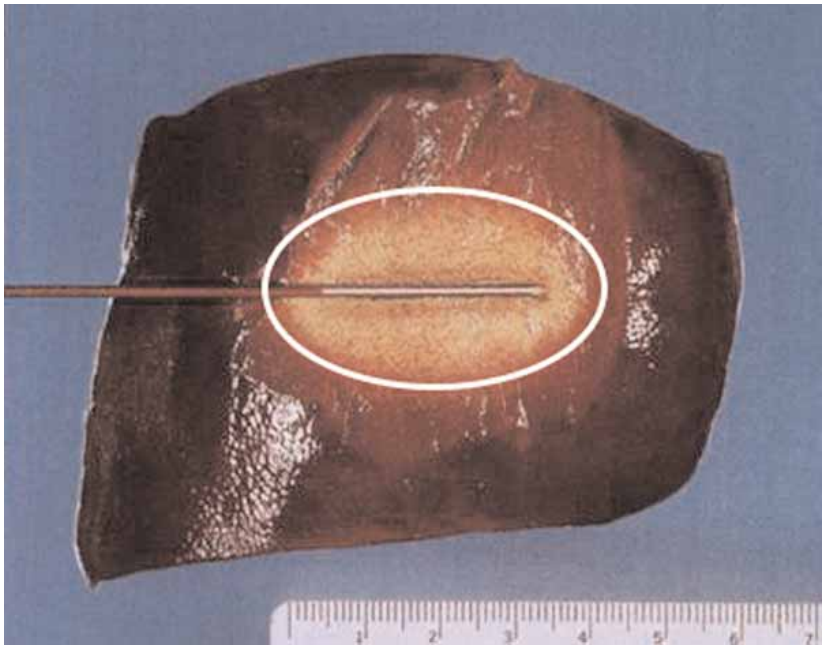

Fig. 4. Pale area around single electrode represents region of burn. Note its elliptical shape (white line).

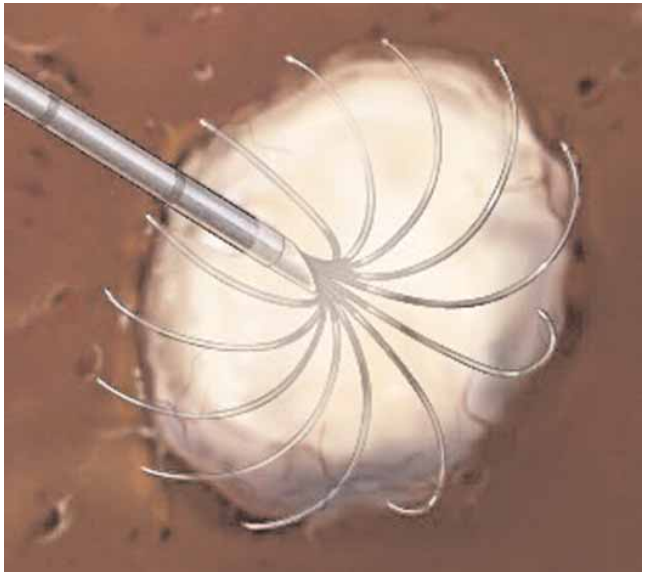

Fig. 5. Schematic of electrode array deployed so as to encompass liver lesion.

and the diaphragm. Concerns about ablating lesions adjacent to the gallbladder have been dispelled by the growing body of experience with RFA *(8)*. When lesions adjacent to the diaphragm are treated, large reactive pleural effusions may develop and, if symptomatic, require drainage. If ablation is being considered outside the liver (e.g., within the pelvis for recurrent disease after a low anterior resection), one must also be concerned about the ureter and adjacent nerves. In some cases where the soft tissue mass is thought to be contiguous to a major nerve, it is prudent to begin the procedure with only light sedation so that the patient can indicate if there is radicular pain, such as what might be anticipated with the application of electrical current or heat to a nerve.

With regard to the efficacy of RFA, adjacent structures such as large blood vessels create "heat sinks." These "heat sinks" are places where one can deposit a lot of energy without being able to heat the tissue above 60°C because the flowing blood is continually carrying the heat away from the tumor blood vessel interface. Temperatures resulting in cell death can be difficult, if not impossible, to achieve at the tumor–vessel interface *(9)*; however, with more powerful generators some of these heat sinks may be overcome (Fig. 5A,B). Within the liver the primary culprits are primary and secondary hepatic vein and portal vein branches, as well as the inferior vena cava.

3. INDICATIONS

3.1. Curative

Ablation should be considered with curative intent when it is used in a realistic effort to eradicate all visible disease. Patients with lesions 2.5 cm or smaller are ideal candidates for thermal ablation, as we can reliably produce coagulative necrosis volumes 3 cm in diameter. In general, patients should have three or fewer lesions, all less than or equal to 3 cm in diameter. In 1997 Livraghi et al. reported 75% complete necrosis in hepatic colorectal metastases 3 cm or less (88% for tumors ≤2 cm *[3]* with a single application of RF). Techniques and tools have improved since then, more powerful generators capable of producing up to 300 W of power are now available, and we should be able to produce better results. In addition, a better understanding of local recurrence has lead to recognition of the importance of timely follow-up imaging. An incompletely ablated margin can be retreated as soon as it is identified. Patients with lesions up to 5 cm can be treated with ablation; however, this is less likely to result in complete necrosis, and more likely to require more than one RF application. As the number and size of lesions increase, the duration and complexity of the ablation procedure and risk of complication increases, eventually becoming prohibitive. It is likely that the impact on patient survival is inversely proportional to the number and size of lesions ablated. When used with curative intent, this is a technique best suited to one or two small lesions.

3.2. Adjunctive

Ablative techniques may be used in situations that are not considered primarily curative, but rather adjunctive or palliative. A patient may demonstrate a response to chemotherapy with decrease in size or disappearance of all lesions except one. The lesion that is resistant to chemotherapy could potentially be ablated. Patients with multiple lesions who would be candidates for anatomic resection except for a single lesion that would not be encompassed by the planned resection could have that single lesion treated with ablation either percutaneously or intraoperatively. Patients who present with one to three metastases but who are suspected of harboring undetectable disease based on the pathological stage of their resected primary, or some other factor, may be treated with percutaneous ablation while they concomitantly receive chemotherapy and are observed for development of additional metastases. Patients with metastatic colorectal cancer might develop lesions at extrahepatic sites, such as the lung, or local recurrence in the abdomen or pelvis. As long as the lesion is small, and thought to be the only site of disease, ablation might be indicated.

3.3. Palliative

Patients who have otherwise untreatable metastatic disease might have a single hepatic lesion that is thought most likely to impact their overall survival or quality of life. An example of this would be a centrally located metastasis likely to cause bile duct obstruction. Bone metastases are not common from colorectal cancer but, when they occur, may cause pain. Bone metastases respond well to RFA.

4. CONTRAINDICATIONS

Because the procedures may be lengthy, and in some cases painful, most RFA is performed with either deep sedation or general anesthesia, so patients must be acceptable anesthesia risks, and cardiac clearance may be warranted. The usual contraindications to any percutaneous procedure, such as an uncorrectable coagulopathy, apply to RFA as well. In addition, there are specific issues that come into play relative to the use of electrical current and the production of heat. Patients with implanted defibrillators or who require constant external pacing cannot undergo RFA, but cryoablation might be considered. Demand pacemakers need to be disabled during the RFA procedure.

Structures adjacent to the target lesion that may be damaged by thermal application, such as bowel or pancreas, must either be protected by instilling fluid or air to move them away from the target, or in some cases require laparoscopic ablation in order to be physically moved. There are two issues to consider when a lesion is close to the heart. If a liver lesion is directly adjacent to the heart, accurate probe placement may be difficult, and if a multitine array is

to be used, care must be taken to ensure that the tines remain contained within the liver. In addition, RFA-induced arrhythmias can be seen, and may preclude complete treatment.

5. RESULTS

In 1997, Solbiati et al. *(10)* reported on 16 patients having 31 liver metastases treated during 75 treatment sessions. Complete response, by imaging, was achieved in 18 of 31 lesions (58%). All 18 were less than 3 cm in diameter, and 13 were less than 2 cm. Residual viable tumor was seen in the other 13 lesions, typically at the margin and in some cases in proximity to a blood vessel. This group published their long-term results in 2001 *(11)*, treating patients with four or fewer lesions. Technical success, defined as no evidence of tumor by contrast-enhanced CT performed 7–14 d after treatment, was achieved in 176 of 179 (98%) tumors in 117 patients. Median survival for all patients was 36 mo, the 1, 2, and 3 yr survival was 93, 69, and 46%, but 67 (57%) patients developed new metastases. Of all treated lesions, 39% recurred during the observation period; no lesion recurred after 18 mo. Gillams and Lees *(12)* reported on 167 patients in 2004. Their overall mean survival for patients with no more than 5 lesions, no more than 5 cm in diameter and with no evidence of extrahepatic disease was 38 mo, with 1-, 3-, and 5-yr survival from the time of diagnosis of 99, 58, and 30%. Not surprisingly, patients with larger tumors, more than five to six tumors, or extrahepatic disease had less encouraging results.

5.1. Follow-Up

One of the most important ways to maximize the effectiveness of RFA, or any ablative method, is with careful follow-up. It is important to understand that this is a minimally invasive treatment that does not always yield the correct ablation geometry on the first attempt, despite our best efforts, but that can be easily repeated to treat an area that was missed. CT scans prior to and after the administration of contrast, or contrast-enhanced MRI, should be used to follow up patients following ablation. The first scan should be obtained within 4–6 wk, with quarterly scans thereafter. After treatment there is typically a low attenuation, non-enhancing region that is bigger than the lesion that was ablated (Fig. 6A,B). Because treatment is most effective when a 5- to 10-mm margin of normal tissue is ablated along with the lesion, the tendency is to over ablate. The fact that the lesion is bigger does not mean the treatment has not been successful; on the contrary, as long as there is no enhancement it is likely the treatment has been quite successful. If there is a focus of contrast enhancement, typically at a margin (Fig. 7A,B), occasionally at an interface with a blood vessel, it is likely recurrent tumor and, if feasible, this region should be retreated promptly.

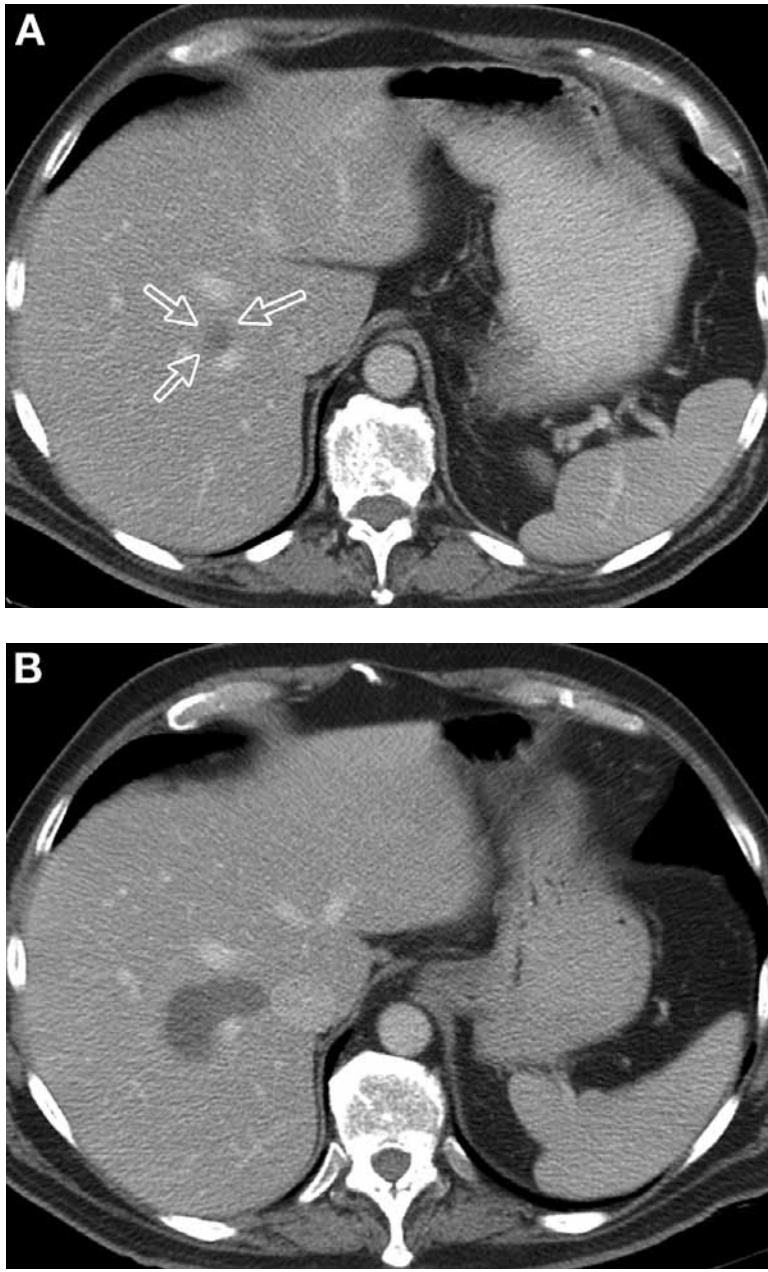

Fig. 6. (A) Pre-RFA CT demonstrates a small colorectal metastasis in between the right and middle hepatic veins. The lesion is directly contiguous with right hepatic vein. **(B)** Post-RFA CT image at same level 6 wk following RFA. The low-density area is larger than the lesion seen on pretreatment scan, and represents the region of ablation.

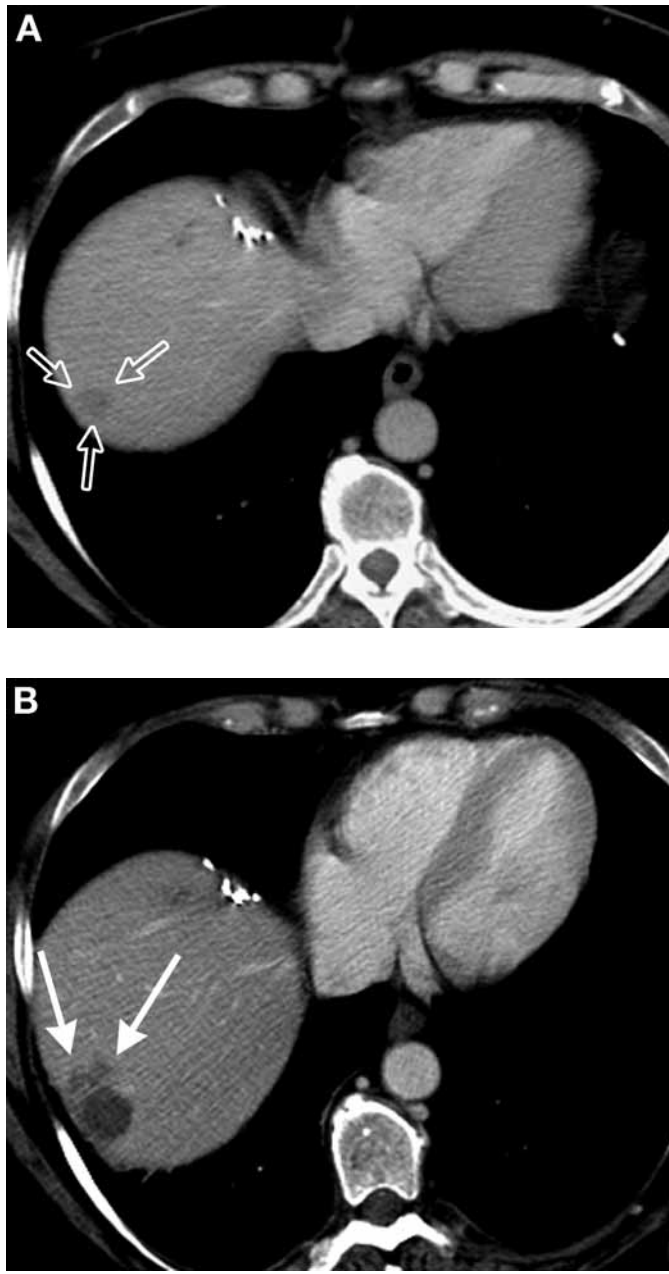

Fig. 7. (A) Small low-density colorectal metastasis in dome of right liver, pre-RFA. **(B)** Uniformly low-density spherical area in region of previous metastasis is non-enhancing ablated tissue; however, there is evidence of irregular enhancement at the margin representing recurrence (two arrows).

On occasion it is difficult to determine if there is recurrent tumor based on CT or MR findings. In these cases PET CT can be particularly helpful. In the immediate posttreatment period, the PET scan may demonstrate increased uptake secondary to metabolic activity caused by the RFA. The increased uptake in this instance is typically rim-like and should resolve within a few weeks, at which point the scan should appear normal (Fig. 8A,B).

5.2. Complications

In a series of 312 patients who underwent 350 sessions of hepatic RFA, de Baere reported 37 adverse events (10.6%) and 5 deaths (1.4%) *(13)*. Of the adverse events, 6.3% were considered minor complications, and 5.7% were major. These included five pleural effusions, five skin burns, four episodes of hypoxemia, three pneumothoraces, two small subcapsular hematomas, one hemoperitoneum, one acute renal insufficiency, and one needle tract seeding. Liver abscess was the most common complication, occurring in seven patients (7/350, 2%). Abscess formation was significantly more frequent in patients with a bilioenteric anastomosis. All three patients with bilioenteric anastomosis developed a liver abscess, as well as one patient with a biliary drainage catheter. The five deaths resulted from liver failure, colon perforation and, in three instances, portal vein thrombosis. Portal vein thrombosis was significantly more frequent in cirrhotic livers, but has been seen in noncirrhotic livers as well. Ultrasound was used for image guidance in 92% of these cases.

Gillams et al. *(12)* reported similar complication rates in their 2002 study that evaluated 354 treatments. There were major complications in 4% of patients, including hollow viscus perforation, abscess, and skin seeding, and minor complications, including hemorrhage requiring transfusion, grounding pad burns, and needle tract seeding in 6%. There were also 15 (4%) systemic complications including postprocedure urinary retention, pneumonia, and myocardial ischemia. In this series ultrasound was the preferred method of electrode placement.

With proper patient selection, careful planning, and appropriate image guidance, many complications can be avoided. In our experience two patients with hepatic metastases from colorectal cancer developed minor complications after 58 sessions of RFA (3.4%). We perform virtually all of our procedures with CT guidance, and this may explain the lower complication rate.

6. SUMMARY

RFA is a useful tool in the armamentarium of those who treat patients with metastatic colorectal cancer. Although ablation of hepatic metastases is most common in this group, lesions in the lung, bone, kidney, and even some soft tissue recurrences, can be treated. Patient selection is very important, and

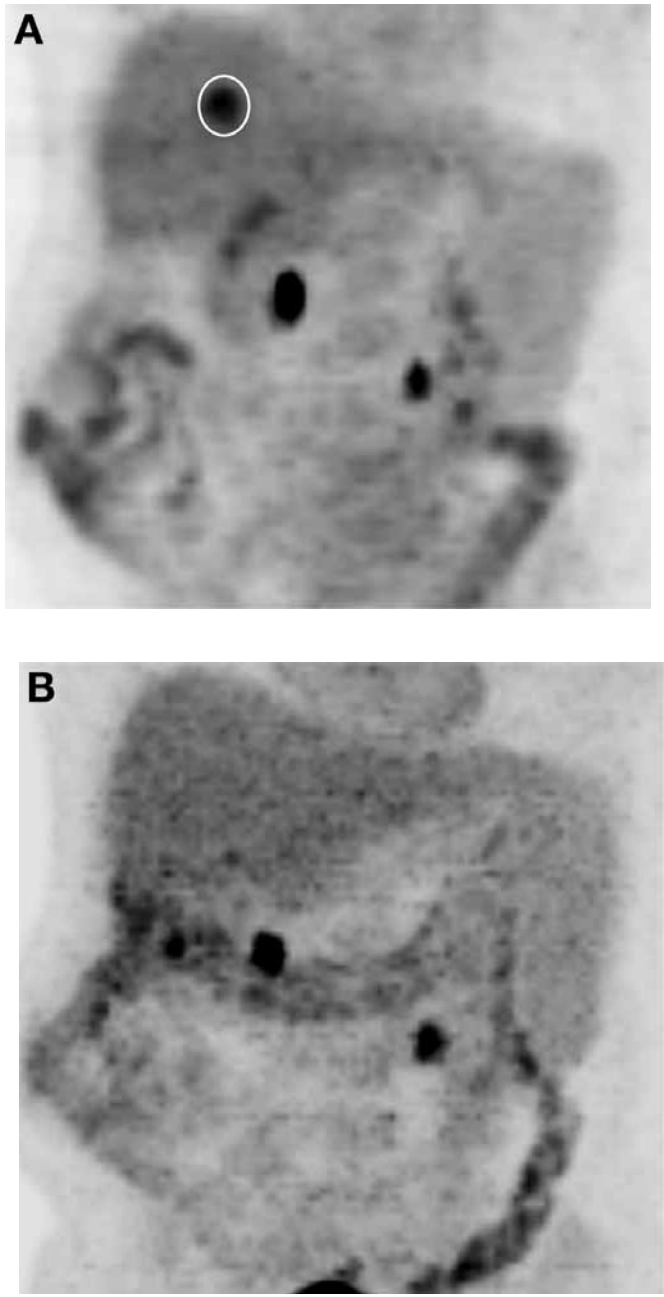

Fig. 8. (A) PET scan 1 d before RFA of colorectal metastasis demonstrates single hypermetabolic focus in liver (inside white oval). **(B)** Follow-up PET scan in same patient 1 mo following RFA is normal.

excellent results can be expected with lesions 3 cm or less in diameter, although lesions up to 5 cm can reasonably be treated. At greater than 5 cm, it is currently more difficult to achieve a geometric treatment region that allows for complete ablation of the tumor, plus a 5–10 mm margin. Treatment of four or more lesions has been reported *(11,12)*, but in these series more than 50% of patients developed new metastases and almost 50% developed new sites of disease or progressive extrahepatic disease. Improved patient selection might be expected to translate the excellent technical results observed into improved survival. Although thermal injury to adjacent structures and specific technical considerations may preclude RFA, there are other ablative methods that might be applicable. Complications do occur, and patient selection should include assessment of anesthetic risk. Use of CT guidance might decrease the complication rate.

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12 Colorectal Cancer Imaging

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and Laurence H. Schwartz, MD

Summary

Imaging of the colorectal cancer patient is employed in three basic settings: evaluation of extent of disease in the preoperative patient, evaluation of the presence or absence of recurrence in the surgically cured patient, and evaluation of the response or progression of disease in the patient with known metastatic cancer. This chapter explores the role of different imaging modalities in each of these scenarios.

Key Words: CT scan; MRI; PET scan; imaging.

1. INTRODUCTION

Imaging of colorectal cancer (CRC) has evolved dramatically over the last two decades, and can be divided into three basic areas: screening, local–regional evaluation of the primary tumor, and evaluation of metastatic disease. It is this latter area on which this chapter will focus. Double-contrast barium enema (DCBE) and computed tomography (CT) colonography (Fig. 1) are used for lesion detection (screening) in healthy individuals. DCBE is useful for exclusion of synchronous primaries preoperatively. Tailored imaging strategies have been developed in rectal cancer, for example, with transrectal ultrasound (TRUS) and magnetic resonance imaging (MRI) having specific roles in the evaluation and management of the primary tumor. CT is used widely for detection and assessment of adjacent organ involvement and distant metastases both preoperatively and during surveillance. ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography combined with CT (PET-CT) is helpful in selected cases where resection of metastatic disease is under consideration.

Imaging of the patient with CRC is employed in three basic settings: evaluation of extent of disease in the preoperative patient, evaluation of the presence or absence of recurrence in the surgically cured patient, and evaluation of the

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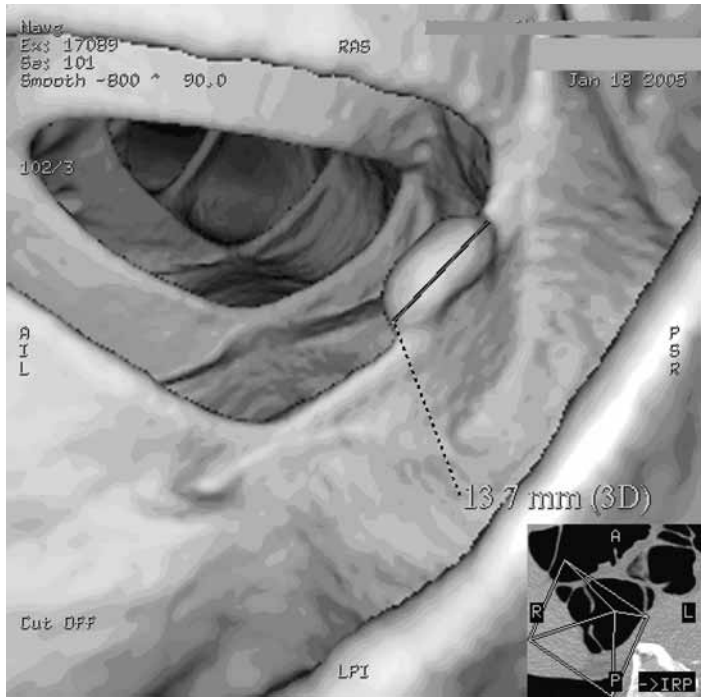

Fig. 1. Computed tomography colonographic multiplanar reformatted three-dimensional endoluminal image (generated on a GE Advantage 4.2 workstation) showing a 1.4 cm polyp in the hepatic flexure.

response to progression of disease in the patient with known metastatic cancer. Detection of local spread and the existence and extent of distant metastases is the major goal of preoperative imaging. Although there is no clearly defined imaging algorithm for postoperative surveillance of patients after curative intent primary treatment, imaging can detect recurrence earlier than laboratory tests alone.

2. PREOPERATIVE STAGING

2.1. Computed Tomography

2.1.1. Technique

The standard CT examination includes the abdomen and pelvis from the domes of the diaphragm (included the lung bases) to the pubic symphysis. Either gastrografin or barium-based oral contrast is given to the patient to drink for 1 h prior to the scan. Patients tolerate this reasonably well unless obstruction is present. In cases where the patient is nauseated, administration of the oral contrast by nasogastric tube is helpful. It is important that there is good opacification in order to differentiate bowel from a possible abscess, as

unopacified bowel can appear exactly like an abscess. Prompt scanning once the drink is finished is appropriate. If a pelvic process specifically is suspected, then it is useful to give a greater amount of contrast (1200 vs 900 cc) and wait a little longer (30 min) to ensure adequate opacification of all pelvic bowel loops. Intravenous contrast is also routinely given (150 cc nonionic at 2 cc/s). If the patient has an allergy to intravenous contrast, then steroids are given 12 h and 2 h before scanning either orally or intravenously as premedication along with diphenhydramine or similar antihistamine. Rectal contrast may also be given in selected cases. Imaging is performed at 5-mm slice intervals and scanning begins at 50 s postcontrast injection (i.e., portal venous phase only). Noncontrast imaging is not routinely performed unless specifically requested, to detect possible steatosis, for example, or as part of a preoperative triphasic CT of the liver. Patients allergic to nonionic contrast may be premedicated with steroids. If there is a history of a severe contrast reaction in the past, it is better to perform a noncontrast CT study or MRI instead.

At most institutions CT of the abdomen and pelvis is the cornerstone of staging, as it is readily available and fast. Chest X-ray is sufficient for evaluating the lungs for metastases in most instances. Neither PET nor PET-CT has a proven role in staging of colon cancer, and neither is recommended for these indications outside of a clinical trial at this time.

2.1.2. CT for Identification of Complications

CT has a very high sensitivity for detection of either small- or large-bowel obstruction and the cause may be identified in 70% of cases *(1)*. A transition point or zone of transition where the bowel narrows from a dilated state indicates mechanical obstruction, distinguishing it from ileus. Assessment of the scout images prior to the full scan can help determine if small-bowel obstruction is likely and so a more detailed three-dimensional reconstruction algorithm may be performed. Coronal reformats may be helpful in identifying a zone of transition if it lies in the axial plane and is therefore difficult to visualize on axial sections (Fig. 2). Abscess formation is relatively easily identified on CT; usually manifesting as a hypoattenuating collection with rim enhancement and presence of gas bubbles. One caveat is early abscesses may not yet have developed an enhancing wall and so correlation with clinical history is important. Fistula formation or perforation is readily seen on CT; the tiniest pockets of free air can be readily identified by viewing the abdominal sections on lung windows. A sealed perforation may be evident or extravasation of oral contrast into the peritoneum is occasionally seen, indicating a perforation.

2.1.3. Staging

Although CT cannot distinguish between T1 and T2 disease, lymph node and liver metastases are easily detected. Limitations of CT include inability to

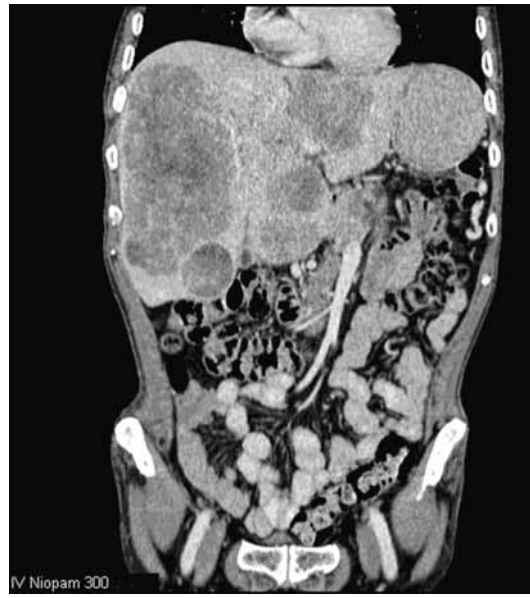

Fig. 2. Coronal reformatted computed tomography of the abdomen and pelvis showing liver metastases and exquisite anatomical detail of the bowel.

determine lymph node activity, which ranges from 38 to 56% *(2)*. On CT, pericolonic fat stranding is nonspecific and may represent T3 disease or merely inflammation. Similarly, loss of fat planes between the colon and adjacent organs suggests extracolic spread. The sensitivity of CT for detection of local spread is approx 50% *(3)*. If extensive T4 disease is seen, however, this may alter the surgical approach. Primary tumors may show central necrosis if large and even contain some air, mimicking an abscess.

Transcoelomic metastases can also be shown at typical locations such as the superior and inferior ileocolic recesses, the intersigmoid recess, and the pouch of Douglas *(4)*. A recent prospective study of 53 patients showed FDG-PET to be equivalent to CT for hepatic assessment, but superior for extraabdominal sites *(5)*.

2.2. Metastatic Disease

2.2.1. Liver Staging

Several modalities may be used to detect liver lesions. CT is the mainstay of liver assessment for metastases with accuracy rates up to 85% reported. CT arterioportography is no more accurate and is invasive (involving insertion of an angiographic catheter into the hepatic artery), has a high false-positive rate *(6)*, and the radiation dose is high; thus it is rarely indicated. Although CT now picks up many more lesions, many of these are "TSTCs" or too small to characterize lesions, 89% of which were shown to be benign in one study in a

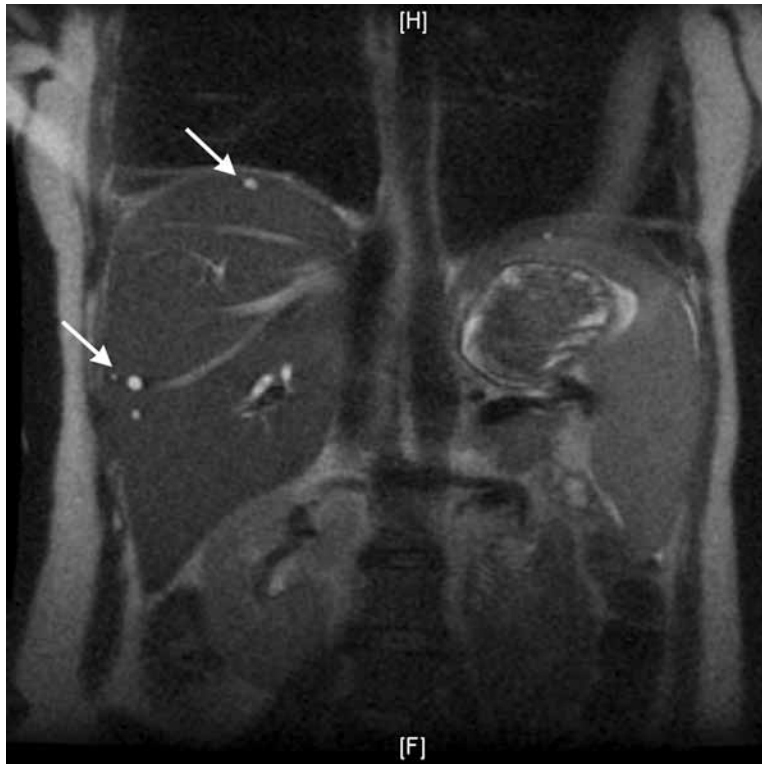

Fig. 3. T2-weighted coronal magnetic resonance image of the liver shows several hyperintense cysts, found to be indeterminate on computed tomography.

cancer population *(7)*. CT characteristics of lesions are not pathognomonic and so in equivocal cases, some are managed with close interval imaging follow-up. There is no advantage to be gained in reducing the slice thickness below 5 mm. It is worth remembering that the number and size of subcentimeter hepatic lesions may vary between examinations if the previous CT was performed using thicker slices. In other words, the lesions may only vary because of technical reasons and not actually show a real change in size. Mucinous adenocarcinoma may occasionally produce cystic or calcified hepatic metastases.

MRI may help to distinguish cysts from other lesions (Fig. 3). Occasionally, multifocal fatty infiltration can mimic metastases, as this shows multiple hypodensities on CT. MRI can show these lesions to be fatty on either in and out of phase or fat-saturated T2 weighted images *(8)*.

Transabdominal ultrasound is mainly used as a problem solver to characterize indeterminate lesions seen on CT. Contrast-enhanced ultrasound is showing promise in small studies but is a routine part of preoperative assessment of

patients. With more effective chemotherapeutic agents now available, along with multiple options for intervention involving surgical or interventional radiology or both, accurate imaging has become essential.

In some instances, where it is unclear from CT whether there is involvement of vessels, targeted ultrasound examination of liver lesions to assess for involvement of portal or hepatic veins is helpful for the surgeon to plan the operation. More specifically, multidetector CT angiography prior to intra-arterial chemotherapy pump placement is extremely accurate in defining the exact arterial anatomy *(9)*.

Ultrasound has a role in guiding biopsy and fine needle aspiration of specific targeted lesions when there is doubt about the diagnosis. In addition, ultrasound helps surgeons decide on resectability when lesions are close to the hepatic and portal veins.

2.3. MRI for Staging

2.3.1. Technique

Axial, sagittal, and coronal sections, in addition to axial oblique sections perpendicular to the rectum along its length, help to clearly define the mesorectum. Whether or nor adding intravenous contrast serves to increase detection is controversial *(10)*. The use of rectal contrast agents such as water for distension of the lumen does not add to the ability to assess extramural spread. Routine use of an endorectal coil would be problematic because of pain and stenosis among other technical difficulties similar to endorectal ultrasound imaging limitations. In addition, there is no accuracy advantage to using an endorectal coil over a standard body coil *(11)*.

High spatial-resolution MRI has recently been shown to be useful for staging of primary rectal tumors *(12)*, with a high positive predictive value for prediction of histological status of the circumferential resection margin (CRM), which allows stratification of therapy. When a clear margin of at least 5 mm is detected on MRI, this results in a high degree of accuracy when correlated with histological specimens *(13)*. The mesorectal fascia and perirectal lymph nodes are well seen on MRI. T2-weighted images are the most useful in detection of local spread. Assessment of nodal morphology and MRI signal characteristics were found to help increase the specificity of MRI for prediction of malignancy as well as size *(12)*. One study also showed MRI may be useful in the identification of other important risk factors preoperatively such as presence of extramural venous extension and peritoneal perforation. If morphological characteristics such as irregularity of node contour and mixed signal intensity are combined with size as criteria for malignancy, then the sensitivity of MRI has been shown to be as high as 85%. Endoscopic ultrasound (EUS) can be used to accurately demonstrate early T1 rectal tumors; however, it has several limitations such as intolerance by the patient, a limited field of view (cannot assess CRM), and

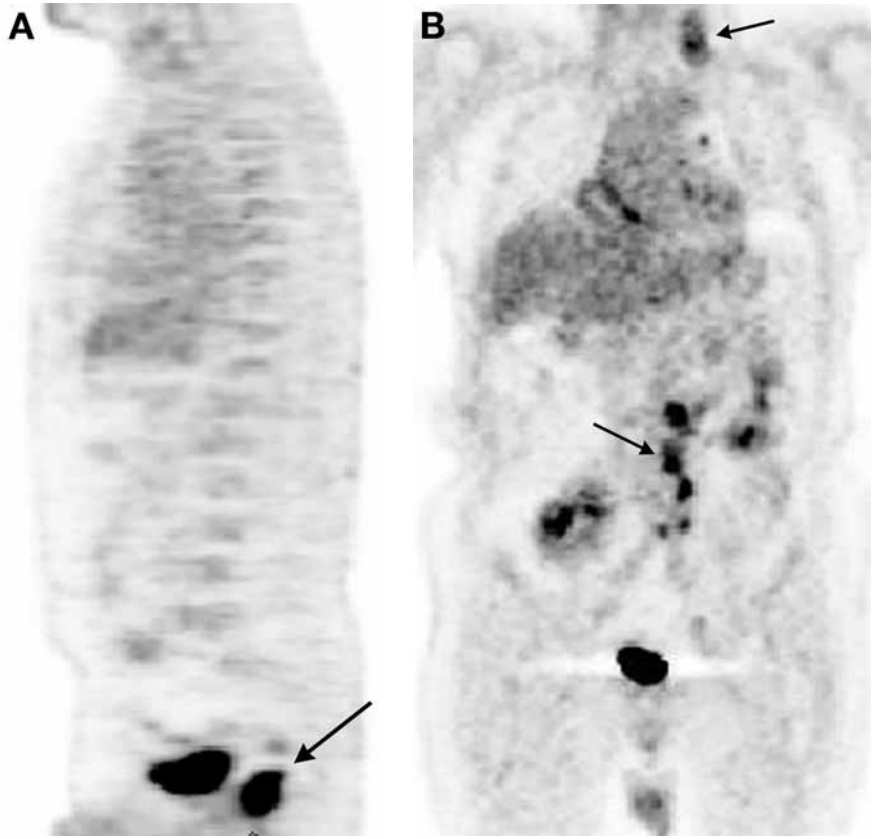

Fig. 4. Sagittal positron emission tomography (PET) image (**A**) showing focally increased FDG uptake in the rectum (arrow) representing the primary tumor without evidence of uptake elsewhere. Another patient (**B**) with diffuse nodal metastases seen on a coronal PET image.

operator dependance. EUS cannot distinguish benign from malignant nodes and 5 mm is the smallest node it can detect.

Liver MRI with addition of liver specific contrast agents such as mangafodipir trisodium (Mn-DPDP) detected 90% of lesions vs 71% for CT in a study of 44 patients with intraoperative ultrasound used as the gold standard *(13a)*.

2.4. Distant Spread

The main goal of CT preoperatively is to detect distant metastases, which would influence whether surgery is undertaken or not. Another subgroup of patients who would likely not be operated on is those with extensive T4 disease. PET may show previously unsuspected extrahepatic spread and influence decision making in up to 29% *(14)*.

Many patients have multiple subcentimeter pulmonary nodules reported on chest CT with the improved quality of scans. The majority of these are of questionable significance. PET-CT has no proven role in the evaluation of these tiny nodules and, given that the resolution of current PET-CT scanners is 5 mm, it is unlikely to be helpful. Pulmonary lesions between 5 and 10 mm considered at least intermediate risk may be sampled if positive on PET; however, if negative will provide no useful information (Fig. 4).

2.5. Recurrent Disease

2.5.1. Surveillance

With the increasing effectiveness of treatments for CRC there is an ever-increasing cohort of patients requiring follow-up. Recurrence at the anastomosis can be found with surveillance endoscopy for metachronous lesions, but CT can show recurrence elsewhere in the mesocolon, mesentery locoregional lymph nodes, or more distant spread.

A major surgical advance in recent years has been total mesorectal excision *(15–17)*, which has helped reduce local recurrence rates to approx 10% *(18)*. One small trial suggested that CT might be more useful in detecting hepatic metastases than carotid endarterectomy (CEA) measurement *(19)*. There are no data to show whether a high vs a low frequency of scanning benefits the patient or leads to earlier detection *(20,21)*, or whether CT of the thorax or pelvis should be included. If scanning occurs at short intervals, an increased radiation hazard must be considered, as well as the increased cost. Cost, radiation dose, and psychological effects from indeterminate findings must all be considered and weighed up in the decision to image with CT more intensively. There is an urgent need for a large well-designed study to decide how often surveillance should be performed, particularly in view of the increasingly effective chemotherapeutic regimens.

In cases where the CEA is rising and CT is negative, PET or PET-CT may help confirm relapse *(20,22–24)*. PET is only useful 6 mo after completion of radiotherapy, as inflammation gives false positives.

2.5.2. Local Recurrence

CT may be performed initially but if unclear, MRI may be required as a problem solver. If both of these tests are negative or equivocal, and there is a high index of suspicion of recurrent disease *(24)*, PET or PET-CT may be indicated when there are rising markers.

3. RESPONSE ASSESSMENT: THE ROLE OF IMAGING

Radiological assessment of rectosigmoid cancer after neoadjuvant chemo- and radiotherapy may be done using CT, MRI, or FDG-PET. CT and MRI are used for morphological evaluation; however, the differentiation between tumor and

Table 1
Guidelines for Evaluation of Tumor Response

Best response	*WHO (change in sum of products)*	*RECIST (change in sum of longest diameters)*
CR	Disappearance[a]	Disappearance[a]
PR	50% decrease[a]	30% decrease[a]
SD	Neither PR nor PD	Neither PR nor PD
PD	25% increase	20% Increase

[a] Must be confirmed at 4 wk.
PR, partial response; PD, progressive disease; SD, stable disease; CR, complete response.

scar tissue after radiation is difficult. Conventional imaging modalities do not provide information on the viability of tumors. Current guidelines for geometric measurement of tumors include only the one-dimensional Response Evaluation Criteria in Solid Tumors (RECIST) or the two-dimensional World Health Organization (WHO) criteria. It is vital to have serial scans of similar quality and to measure lesions that are reproducible. Coalescing lesions in the liver for example may separate on subsequent scans, causing difficulty in measurement. It is recommended that the enhancing rim seen around many liver metastases is included in the measurement. The overall goal is to assess both target (measurable) and nontarget (nonmeasurable) lesions that are representative of the total tumor burden, including up to 5 lesions per organ and a total of 10 lesions using RECIST guidelines. The overall response takes into account target, nontarget, and new lesions. Assessment of best response is summarized in Table 1.

The majority of radiologists; however, do not issue reports in terms of RECIST or other guidelines. It is worth noting that occasionally a patient may be responding well both clinically and biochemically despite worse imaging appearances and so, in these situations, the clinician may overrule RECIST to upgrade the patient's response (for example, in clinical trials). This is an important point, as imaging is increasingly being used as a surrogate endpoint or biomarker during clinical trials for new agents. There are no guidelines for volumetric measurement of tumors, but recent studies have shown a great variation in results for volumetric analysis vs two-dimensional techniques *(25)*. Perfusion studies involving both CT and MRI to better define pharmacological response is currently the focus of intense clinical research *(26,27)*.

4. CONCLUSION

Imaging CRC has evolved such that several modalities play a role in each patient's care, with the choice tailored for patient with close discussion with

the radiologist. CT can assess most patients adequately, with the more time-consuming and expensive modalities such as MRI and PET used in selected cases to problem solve. A combination of conventional and functional imaging will likely be used in the future for better pharmacological response assessment.

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13 Nursing Issues in Colorectal Cancer Chemotherapy

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Summary

Key roles of the oncology nurse in colorectal cancer chemotherapy management are to assess the patient for adverse events, and to provide support by addressing the patient's concerns. In a collaborative practice, the oncology nurse is also directly responsible for the education of the patient in symptom management, especially during chemotherapy. It is imperative for the oncology nurse to be knowledgeable regarding the current regimens, their associated toxicity profiles, and the tools used in symptom management, in order to maximally enhance the patient's safety while receiving chemotherapy, and improve the patient's overall quality of life.

Key Words: Chemotherapy; toxicity; side effects; education.

1. INTRODUCTION: THE ROLE OF THE ONCOLOGY NURSE IN COLORECTAL CANCER TREATMENT

The recent advances in development of new treatment regimens for colorectal cancer (CRC) have made this an exciting and challenging time to be an oncology nurse caring for these patients. Key roles of the oncology nurse are to assess the patient for adverse events and to provide support by addressing the patient's concerns *(1)*. In a collaborative practice, the oncology nurse is also directly responsible for the education of the patient in symptom management, especially while undergoing chemotherapy. It is imperative for the oncology nurse to be knowledgeable regarding the current regimens, their associated toxicity profiles, and the tools used in symptom management. This knowledge will allow the nurse to ensure patient understanding of the regimens, side effects, and self-care measures. Ultimately, this will enhance patient safety while receiving chemotherapy, and will improve the patient's overall quality of life.

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2. NURSING MANAGEMENT OF SPECIFIC ISSUES

2.1. Diarrhea

Diarrhea is a multifaceted and problematic symptom experienced by many patients with CRC. Both irinotecan and fluorouracil-based therapies can cause diarrhea, and patients with CRC may have diarrhea as their baseline pattern secondary to previous colon resections or preexisting conditions (e.g., ulcerative colitis, Crohn's disease). For the oncology nurse caring for these patients, the management of this potentially serious symptom depends on the accurate evaluation of the degree of diarrhea that the patient is experiencing over their baseline pattern of elimination. The National Cancer Institute (NCI) Common Toxicity Criteria v3.0 for reporting adverse events is the most widely used tool *(2)*. This tool grades diarrhea according to the number of increased stools experienced by a patient over their normal daily baseline. It is crucial that the pretreatment number of stools a patient has per day becomes the benchmark for further assessments made during their course of chemotherapy. All too often, a patient will self-report that he or she is having seven loose stools per day and upon further investigation it is determined that the pretreatment baseline pattern is actually five soft stools per day. At first glance, this may easily be reported as a grade 2 diarrhea, which is an increase of four to six stools per day; however, it is actually only a grade 1 toxicity. In addition, when the number of stools is reported, it must be clarified whether these are in fact diarrheal stools or not. Patients may experience four to eight formed or semiformed bowel movements per day postresection; these events do not constitute diarrhea.

On planned treatment days it is important to interview a patient about his or her recent bowel history; it is not enough just to know whether a patient is experiencing diarrhea or not. Also, it is relevant to know if the patient has had any diarrhea in the past 24 h prior to his planned chemotherapy treatment, or if he/she is taking antidiarrheals at the time, because the physician may decide to delay therapy in such instances *(3)*.

Irinotecan is associated with two distinct diarrheal syndromes: early onset and late onset. Acute early onset diarrhea occurring during irinotecan administration is cholinergic in nature and specific to irinotecan. Other symptoms such as increased salivation, watery eyes, diaphoresis, flushing, nasal congestion, and abdominal cramping may also occur during the administration of irinotecan even in the absence of diarrhea. All of these symptoms can be effectively treated with atropine 0.25–1.0 mg iv, assuming of course that no contraindications to atropine are present. This medication may then be given as a premedication with each subsequent irinotecan treatment *(4)*.

Delayed onset diarrhea occurs anytime after 24 h postirinotecan administration and can be more complicated to treat. Patients should have a supply of over-the-counter loperamide (Imodium-AD®) at home prior to starting therapy.

At the first sign of diarrhea, loperamide 4 mg should be taken, followed by loperamide 2 mg every 2 h until the patient is diarrhea-free for a 12-h period. Patients need to be told that this is more loperamide than the package insert allows in a 24-h period. It is also important to stress that patients taking significant amounts of loperamide should follow up with their health care provider over the telephone just to provide an update. Diarrhea that lasts longer than 24 h even with the aggressive loperamide regimen dictates a more in-depth patient evaluation and even possibly an emergency room visit.

Diphenoxylate/atropine sulfate (Lomotil®) may also be used instead of loperamide for mild to moderate diarrhea; however, it is prescribed differently than loperamide. This medication is dosed every 6 h, one to two tablets, for a maximum of eight tablets per day.

2.2. Dietary and Oral Fluid Management of Diarrhea

In the setting of diarrhea, patients need to be instructed to increase their fluid intake over their normal 2 L/d to toleration. This should be a mixture of fluids including water, sports drinks, flat ginger ale, and chicken or beef broth to replace some of the electrolytes that are being depleted. If the patient is on a diuretic, this should be discontinued until the diarrhea resolves. Any complaints of feeling dizzy or lightheaded, or any noted decrease from normal amount of urine output most likely indicates that the patient is not getting enough fluids and intravenous hydration may be needed.

Dietary modifications can be made once diarrhea occurs, but there is no need to implement these in an asymptomatic person as a preventative measure. Patients should eat small frequent meals if they have diarrhea, and should avoid greasy, fried, or spicy foods. The Bananas, Rice, Applesauce, and Toast (BRAT) diet is advisable initially.

However, it is important to note that the BRAT diet is not a diet that a patient stays on for a prolonged period of time, because it does not provide enough calories to sustain an individual. Other common trigger foods that should be avoided once diarrhea occurs include high-fiber foods (e.g., whole grain breads, bran cereal, raw vegetables, cooked vegetables that cause gas, juice with pulp), high-fat foods (e.g., fried or spicy foods, cream sauces, gravies), and lactose-containing products (e.g., milk, ice cream, and processed cheese). Alcohol and caffeinated beverages also should be avoided. As diarrhea starts to resolve, a patient can reintroduce foods such as eggs, chicken or turkey without the skin, baked potato without the skin, pasta without sauce, cooked or canned fruits, white rice, plain yogurt, and sorbet.

Finally, in the setting of diarrhea, the oncology nurse needs to identify any other overlapping toxicity such as abdominal cramping or a fever that the patient may be experiencing that could indicate a more serious problem *(5)*. The combination of diarrhea and fever is of particular concern in the cancer patient,

and patients should be instructed to seek urgent medical attention if this symptom complex develops.

2.3. Neutropenia

Myelosuppression can be experienced with any of the cytotoxic chemotherapies used to treat CRC. Heavily pretreated patients as well as patients with a prior history of pelvic radiation may be more susceptible to neutropenia, but all patients need to be educated about the danger of neutropenic fever *(6)*. Patients often worry about neutropenia, not fully understanding its implications. However, they usually don't "feel" neutropenic and this is where the danger lies, in the form of unreported symptoms such as a fever or signs and symptoms of an infection (e.g., urinary burning). An unreported fever in the setting of neutropenia can easily lead to a life-threatening septic event.

The simple practice of patients monitoring their temperature twice a day while on chemotherapy with instructions to report a fever of 100.5°F (38.0°C) or greater can help patients feel more secure during their course of therapy. Patients should be educated to take their temperature in the early morning as well as in the evening before dinner, with nothing taken by mouth for at least 15 min before the temperature is taken to assure an accurate reading. They need to be reminded to wait at least 4 h after taking acetaminophen (Tylenol®) or any other medication containing it (e.g., Percocet®) before taking their temperature. Also, if a patient experiences a shaking chill, or rigor, he or she should take his or her temperature and, even in the absence of a fever, a telephone call should be made to their health care provider for further instructions.

A majority of patients being treated for CRC will have an indwelling vascular access device (e.g., MediPort®). Others may have an implantable hepatic arterial pump, a biliary catheter, or a urinary stent. These foreign bodies will all need to be evaluated when a patient reports a chill or fever. As noted previously, oncology nurses also need to pay strict attention to overlapping toxicities as can be common in many CRC regimens. Uncontrolled diarrhea in a neutropenic patient may be an indication of an infectious process warranting prompt evaluation *(5)*.

2.4. Chemotherapy-Induced Nausea and Vomiting (CINV)

Most patients with CRC will receive oxaliplatin and/or irinotecan at some point during their course of treatment. Both of these agents are considered moderately emetogenic and require premedication before infusion with dexamethasone and a 5-HT3 blocker such as ondansetron (Zofran®), granisetron (Kytril®), dolasetron (Anzemet®), or palonosetron (Aloxi®). Palonosetron is a highly selective, second-generation, 5-HT3 receptor antagonist with a prolonged plasma elimination half-life of 40 h. Palonosetron, given as a single intravenous dose of 250 mcg (0.25 mg), was shown in a phase III trial to be more effective than single-dose dolasetron in preventing acute and delayed CINV *(7)*.

Some patients, however, may have refractory nausea and vomiting even with dexamethasone plus 5-HT3 blockers, and these patients require further intervention. Aprepitant (Emend®) is a recent antiemetic agent that is a substance P/neurokinin 1 (NK1) receptor antagonist and can be given on days 1–3 of therapy. A single dose of 125 mg capsule of aprepitant given prior to chemotherapy with subsequent doses of 80 mg capsule given once daily in the morning on days 2 and 3 postchemotherapy is approved for the prevention of acute and delayed nausea and vomiting associated with highly and moderately emetogenic chemotherapy agents. Oral dexamethasone doses should be reduced by approx 50% when co-administered with aprepitant to achieve exposures of dexamethasone similar to those obtained when it is given without aprepitant *(8)*. Other antiemetic agents can be used as needed such as prochlorperazine (Compazine®), metoclopramide (Reglan®), or lorazepam (Ativan®).

Oncology nurses should evaluate the effectiveness of an antiemetic regimen and, if the patient is still having breakthrough nausea and vomiting, initiate discussions of appropriate adjustments. Patients should be reminded to have a light meal prior to chemotherapy. All too often patients will withhold food, thinking that this will reduce their chance of having nausea, when in fact may they feel worse with an empty stomach.

2.5. Mucositis

Oral mucositis is a potential problem with any chemotherapeutic agent, but it is more frequently seen with infusional regimens of 5-fluorouracil (5-FU). There is no single "gold standard" regimen for oral care; however, it is important for oncology nurses to reinforce with patients the need to practice good oral hygiene. This can consist of tooth brushing, flossing, and appropriate denture care, as well as adequate routine dentist visits. If significant mucositis does develop while a patient is on therapy, utmost attention must be given to assessing a patient's ability to take in adequate amounts of fluids. Although infrequent, a patient may at times require intravenous hydration. The impact of mucositis on a patient's ability to drink should never be underestimated.

Oral anesthetic gels such as polyvinyl pyrrolidone and sodium hyaluronate (Gelclair®) can be prescribed for the management of the pain associated with mucositis. Such gels are able to adhere to the oral mucosal and form a protective covering, which lessens the severity of the discomfort and allows a patient to drink more easily *(9)*. If an underlying oral candidiasis exists, then nystatin (Mycostatin®), clotrimazole (Mycelex® troche) or fluconazole (Diflucan®) can be prescribed. It should be emphasized, however, that the majority of chemotherapy-associated mucositis is not fungal in nature, and antifungal treatments will not be expected to be useful unless candidiasis is observed. Thus, the majority of oral mucositis in CRC should not be treated with antifungal therapy.

2.6. Skin Rash of Fluorouracil-Based Therapy

A toxicity associated with capecitabine, and to a lesser extent 5-FU is hand–foot syndrome (HFS) or palmar-plantar erythrodysesthesia (PPE). This syndrome occurs in as many as 56% of patients receiving capecitabine, 34% receiving protracted continuous infusions of 5-FU, and 13% receiving intravenous bolus 5-FU with leucovorin (LV). HFS may be at least partially caused by a crushing of the capillaries in the hands and feet. The inflammatory response involved may be at least partially mediated by cyclooxygenase (COX)-2 *(10)*. HFS may begin with a sensation in the hands and feet. This can progress to painful erythema, swelling, blisters, peeling of the skin, and an interruption in the patient's activities of daily living (ADL).

Capecitabine is a therapy that is self-administered at home by the patient. Self-administered oral chemotherapy greatly shifts the responsibility for dose monitoring and adjustments from the provider to the patient. The oncology nurse's role includes patient education, symptom management, and proactive follow-up. Patient education for HFS includes keeping hands and feet clean and moist, using emollients and lubricating lotions and creams such as petroleum-based lotions (e.g., Lubriderm®, Aveeno®, Bag Balm®, or Udder Cream®), avoiding constricting clothing, and avoiding extreme pressure or temperature on hands and feet. The oncology nurse should educate the patient on the recognition of side effects and prompt reporting. Patient education can be effectively reinforced through telephone triage.

Capecitabine therapy may need to be interrupted until the HFS resolves. A dose reduction may be necessary based on the severity of the toxicity and the resolution to baseline. Any necessary dose reduction of dose to that patient will remain in effect with subsequent cycles.

2.7. Acne-Like Rash of Cetuximab and Other EGFR Inhibitors

The most common toxicity observed with cetuximab is an acne-like rash *(11)*. Although the superficial appearance is like that of acne, this rash is not acne. It is a dry rash, not an oily rash, and topical acne medications are, in general, counterproductive. The development of this side effect is attributed to the expression of endothelial growth factor receptor (EGFR) in many normal epithelial tissues (e.g., skin and hair follicle). The rash is characterized as a sterile folliculitis that generally appears on the face, neck, and upper trunk, but can extend to extremities. Patients may also have a drying of the skin, pruritus/itching, inflammation of hair follicles, sometimes by eyelashes, and paronychial inflammation associated with nail folds of hands and toes. The rash tends to appear within the first 2 wk of therapy, reach maximum severity after week 3 or 4, and then decrease in severity as therapy continues. However, the rash can also wax and wane over time during therapy. The rash generally resolves completely 4 to 8 wk after discontinuation of therapy.

In the treatment of the acneiform rash, no specific intervention has been proven to provide significant benefit. Application of emollients and moisturizers such as Vaseline Intensive Care® and similar products may reduce skin peeling, dryness, and soothe the skin. Makeup is safe, does not appear to exacerbate the rash, and can camouflage the rash. Sunscreen, hats, and limited sun exposure are recommended, as sunlight may exacerbate skin reactions *(1)*. Evidence would suggest that topical antibiotics and topical steroids are not effective. Rashes that appear to be superinfected may improve with a course of oral antibiotics with staph/strep coverage, such as first generation cephalosporins, dicloxacillin, or amoxicillin/clavulanic acid (Augmentin®).

An assessment of any skin breakdown around the patient's nail bed is important, because paronychial cracking may occur with cetuximab. Paronychial cracks or inflammation with overt superinfection may require treatment with oral antibiotics. Patients are advised not to use drying agents on the rash such as acne medications (e.g., benzoyl peroxide), as these treatments do not improve the rash and can worsen the rash and make it more painful.

2.8. Sensory Neuropathy Associated With Oxaliplatin

Sensory neuropathy is a dose-limiting toxicity associated with oxaliplatin and manifests itself as acute or persistent. The acute neuropathy, which is precipitated by exposure to cold temperature or cold objects, is reversible, may occur within minutes or 1 to 2 d of administration and resolves within 14 d. The patient experiences transient paresthesia or dysesthesia in the hands, feet, perioral area, or the throat. An acute syndrome of pharyngolaryngeal dysesthesia is seen to a lesser extent upon exposure to cold and is characterized by subjective sensations of dysphagia or dyspnea, without any laryngospasm or bronchospasm *(12)*. Nursing interventions focused on the acute neuropathy involve self-care measures for patients to minimize their exposure to cold such as drinking beverages at room temperature, avoiding cold foods, wearing gloves and a scarf in cold weather, and even avoiding air conditioning. It is also extremely important that patients are educated regarding the possibility of experiencing pharyngolaryngeal dysesthesia because this can be very frightening. Patients need to understand that their airway is not compromised and that the subjective sensation of their throat tightening will resolve in a few minutes *(10)*.

The persistent and cumulative sensory neuropathy is also characterized by paresthesias, dysesthesias, and hypoesthesias, but lasts longer than 14 d. Deficits in proprioception may also occur, which can interfere with ADL (e.g., writing, buttoning, and difficulty walking) *(12)*. Nursing interventions focused on this cumulative neuropathy primarily involve early detection of worsening paresthesias and dysesthesias that persists longer than a few days after oxaliplatin administration. A grade 2 peripheral sensory neuropathy is characterized by sensory

alteration or paresthesia (including tingling) interfering with function but not interfering with ADL *(2)*.

Once a functional impairment exists related to neuropathy, the focus is on patient safety. Patients with decreased temperature sensation need to be cautioned about heat extremes in their environment (e.g., temperature of running water) *(13)*. Nursing intervention for patients with significant neuropathy affecting proprioception interfering with ambulation and also driving involves fall and accident prevention.

2.9. Bevacizumab-Related Toxicity

Bevacizumab is a recombinant humanized monoclonal antibody directed to neutralize vascular endothelial growth factor. Of the more common side effects that must be monitored for, bevacizumab may cause hypertension. Recurrent or persistent hypertension greater than 150/100, classified as grade 2 when previously normal, may require treatment *(2)*. The hypertension seems to be manageable with virtually all oral antihypertensive agents, most often with a single agent, except in patients with a preexisting history of hypertension. Some agents used are the angiotensin II receptor blockers, converting enzyme inhibitors, diuretics, β blockers, or calcium channel blocking agents. Oncology nurses should routinely monitor patient's blood pressure and report any elevated findings. In addition, the nurse needs to educate the patient on taking their antihypertensive medications correctly.

Patients receiving bevacizumab should also be educated to report any signs of bleeding, but also should be advised in advance that mild epistaxis or hemorrhoidal bleeding is common. This generally resolves on its own without intervention. Gastrointestinal perforation is rare but may occur. Patients who report sudden severe abdominal pain should be evaluated in a timely manner. Patients may also be at risk for arterial thrombotic events and need to be educated to report any new central nervous system symptoms, chest pain or dyspnea. These events, fortunately, are rare with bevacizumab.

2.10. Nursing Implications of Patients Receiving Oral Chemotherapy

Increasing numbers of patients are receiving oral chemotherapy at home; specifically capecitabine (Xeloda®) for CRC, and with this move to oral self-administration, there has been a critical shift in responsibility of management from provider to patient. Oral regimens pose new challenges in patient selection and education. Recognition of factors that affect patient compliance will be particularly important with oral chemotherapy. Strategy tools for the patient and provider will need to be developed to ensure optimal compliance and safety *(14)*. Appropriate patient selection is central to the successful and safe administration of any chemotherapy agent. Oncology nurses can play an active role in the identification of appropriately motivated patients for these self-administered therapies.

Oncology nurses who are involved with patients taking oral chemotherapy must understand what factors affect compliance and how identification of these factors can aid in the development of education strategies that will help assure patient compliance.

The quality of the patient-provider relationship can profoundly affect compliance. The patient must feel comfortable with his or her physician or nurse in asking questions and reporting side effects. A patient's support system, including family, friends, or home care nurses also greatly influences the likelihood of compliance with therapeutic regimens. Is there someone to remind the patient to take his or her medication? Are caregivers in the home aware that they need to notice and inquire about side effects of clinical changes?

Compliance will also be influenced by each patient's preexisting beliefs and attitudes about health, disease, and medical treatments. These can affect not only how the patient follows his or her drug schedule, but also what he or she is willing to report or discuss regarding complications and side effects.

Compliance may be variable over time, with motivation and actual compliance potentially diminishing with the increasing duration or a patient's illness. The complexity of the regimen will also affect the patient's ability to comply.

Other factors in patient selection must include a patient's physical limitations, especially in elderly patients. These limitations might include limited sight and limited manual dexterity in handling pills. Also, older patients are more likely to be taking multiple oral medications, and the addition of oral chemotherapy to this patient's regimen may not be feasible, because of either the increased complexity or potential drug interactions. The primary drug interactions with capecitabine are the oral anticoagulant, warfarin (Coumadin®) and the seizure medication, phenytoin (Dilantin®). Patients on warfarin are at an increased risk for bleeding because there can be a significant increase in their prothrombin time and international normalized ratio. Close monitoring of these patients on a weekly basis is usually necessary. Increased phenytoin levels can be seen in patients taking capecitabine and adjustments may need to be made in their maintenance dosage *(15)*. A last consideration in patient selection is simply the patient's ability to tolerate an oral medication. One must evaluate whether or not a patient is able to swallow pills and whether there is adequate gut function and absorption.

The oncology nurse's responsibilities include patient education, symptom management, and proactive follow-up. First and foremost, patients need to know the correct dose and administration schedule. Next, the toxicity profile of the oral agent needs to be discussed. Finally, the importance of early recognition of side effects and prompt reporting needs to be stressed. Patients are often reluctant to notify the nurse of side effects because they fear that their therapy may be interrupted or their dose will be lowered. Patients will be helped if they

can understand the importance of early reporting. This can be remedied by simply explaining to patients that most side effects resolve with a brief interruption of therapy, any necessary dose reduction is simply a customization of dose to that individual's needs, and a dose reduction does not necessarily lessen the chance of antitumor effects.

As educators, it is a challenge for oncology nurses to provide the knowledge and support to ensure the safety of patients taking on the responsibility of taking oral chemotherapy at home.

2.11. Nursing Issues in Pain Assessment and Management

Many patients with CRC have pain related to their tumor burden, bony metastases, or treatment-related side effects such as neuropathy from oxaliplatin (*see* Chapter 14). Oncology nurses interact closely with patients and have become very astute at assessing pain and evaluating the effectiveness of these medications. It is important to understand that a response from chemotherapy may translate into an improvement in tumor-related symptoms such as pain, and adjustments will need to be made accordingly. Likewise, patients with progressive disease may need an increase in their pain medication and a pain consult may be necessary for patients with refractory pain. Also, patients on opioids are at a risk for constipation and, as will be discussed in the next section, a bowel regimen needs to be established from the onset of beginning a course of analgesics.

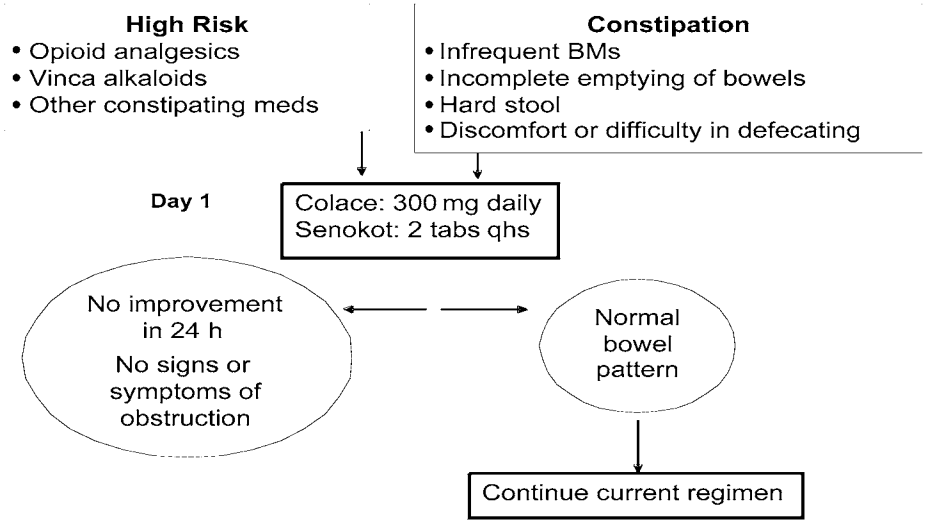

Fig. 1. (*Continued*)

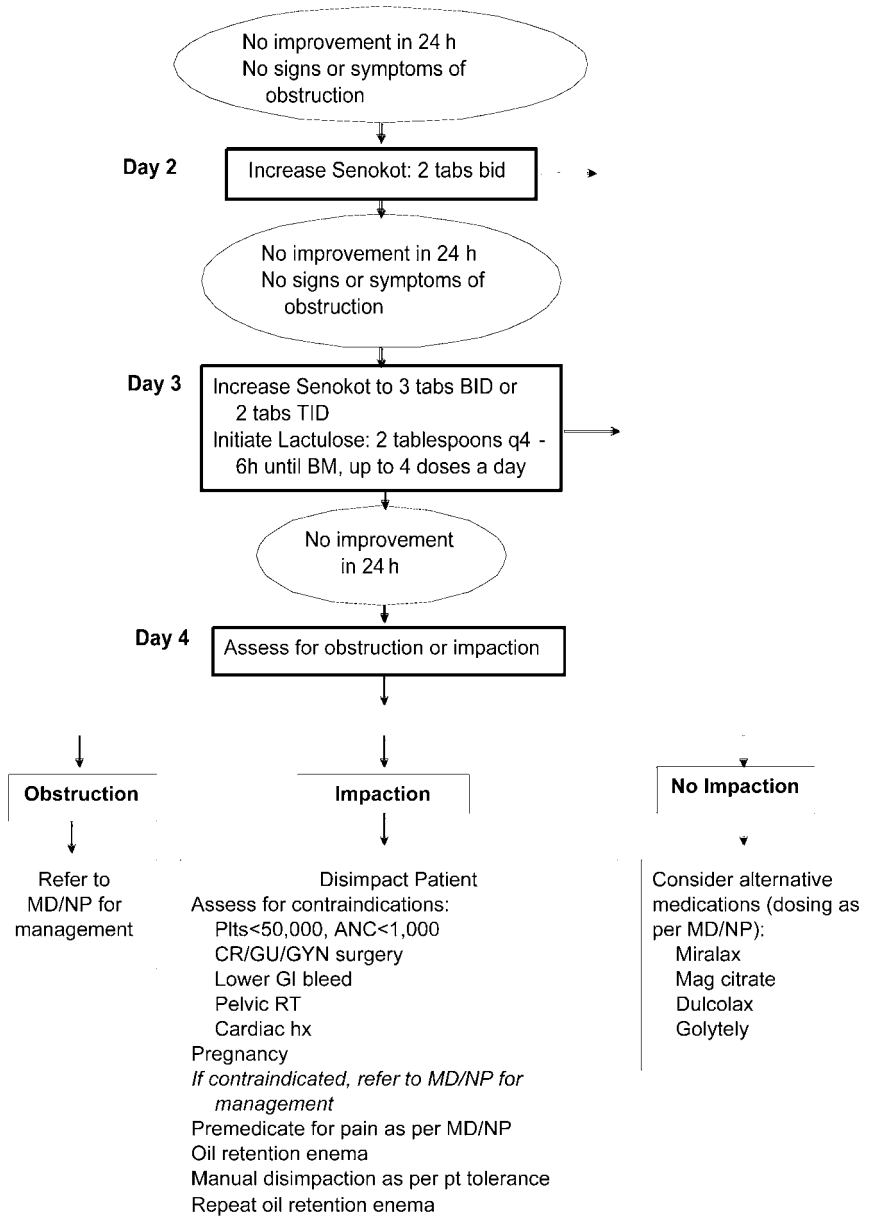

Fig. 1. Constipation Prevention/Treatment Algorithm.

2.12. Constipation

Constipation is one of the most distressing symptoms experienced by cancer patients, and its management can be quite challenging to oncology nurses. Although constipation is not a major side effect of the chemotherapy regimens used to effectively treat CRC, it is a symptom that patients often experience while on therapy.

The causes of primary or simple constipation range from decreased physical activity, inadequate dietary fiber, inadequate fluids, to inadequate time or privacy for elimination. Secondary causes of constipation include disease or treatment-related factors. Disease-related causes of constipation in patients with CRC are intestinal obstruction, peritoneal carcinomatosis, or a more serious complication such as a spinal cord compression. The most common treatment-related causes of constipation are the usage of opioid medications for pain control, with nearly 95% of patients reporting this symptom *(16)*. Other constipating medications include the usage of antiemetics for CINV.

Specifically the 5-HT3 blockers, which include ondansetron (Zofran), granisetron (Kytril), dolasetron (Anzimet), and palonosetron (Aloxi) can cause constipation or aggravate a preexisting condition. Other medications that contribute to constipation are cough suppressants containing codeine, psychotropics (e.g., Elavil®, Prozac®, Zyban®, Xanax®), nonsteroidal anti-inflammatory drugs, antihypertensives, specifically the calcium channel blockers (e.g., Cardizem®, Norvasc®, Procardia®), calcium- and aluminium-based antacids (e.g., TUMS®, Amphogel®), antihistamines (e.g., Benadryl®) and dietary supplements (e.g., iron, calcium). Electrolyte imbalances such as hypercalcemia or hypokalemia can also lead to constipation. Finally, preexisting conditions including depression, diabetes, hypothyroidism, Parkinson's disease, and other neurological conditions affecting the innervation of the intestinal tract can contribute to constipation *(17)*.

All of these factors need to be taken into account when assessing a patient for constipation and formulating an intervention. Consultation with a MD/NP is necessary in patients with a suspected bowel obstruction, an ostomy, or recent gastrointestinal or gynecologic surgery.

Pharmacological intervention is needed in most patients suffering from constipation. The regimen may include stool softeners (Colace®), stimulant laxatives (e.g., Senokot®, Dulcolax®), saline laxatives (e.g., MOM®, Magnesium Citrate®), bulk-forming laxatives (e.g., Metamucil®), synthetic disaccharides (e.g., lactulose), and polyethylene glycol solutions (Miralax®).

A baseline regimen should be established for all patients at risk for constipation. The following algorithm can be used as a guide in most practice settings (Fig. 1) *(18)*.

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14 Pain Management in the Colorectal Cancer Patient

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Summary

Pain management is a critical component of the treatment of advanced colorectal cancer. In this chapter, a set of core principles is outlined to assist in the diagnosis and treatment of most causes of pain in the colorectal cancer patient. An algorithmic approach is described to facilitate expeditious and efficacious treatment.

Key Words: Pain; nociceptive; neuropathic; narcotic.

1. INTRODUCTION

Although pain for patients with colorectal cancer (CRC) may be a "pain in the butt," treating their pain need not be. In this section, a set of core principles will be outlined to assist in the diagnosis and treatment of most causes of pain in the patient with CRC. An algorithmic approach is described to facilitate expeditious and efficacious treatment.

2. TYPES OF PAIN

Pain is often defined as: "*An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*" *(1)*. Thus, pain is not pleasant, can be strictly an emotional experience, and is associated with tissue damage. Although this helps in defining what is and is not pain, it does little to help define pain in a manner that aids treatment. Broadly speaking, pain that meets the above definition may be one of three types: nociceptive, neuropathic (or non-nociceptive), or psychogenic (Fig. 1) *(2–4)*.

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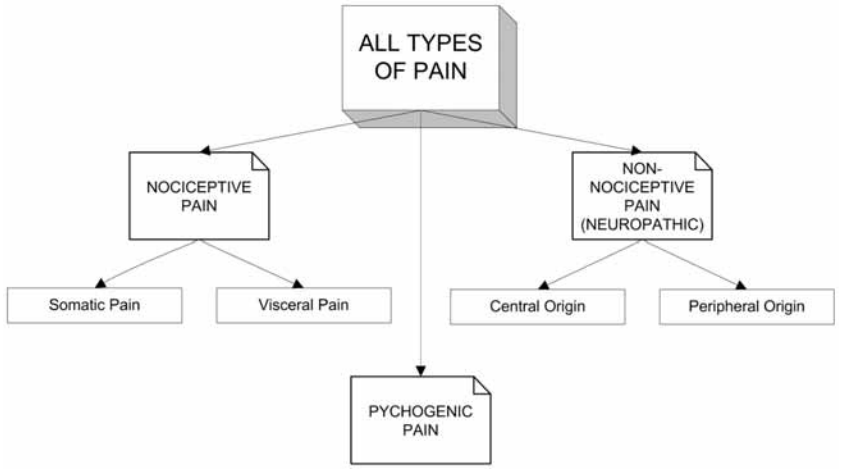

Fig. 1. Classification of pain types.

2.1. Nociceptive Pain

Nociceptive pain is caused by the stimulation of "nociceptors," or pain receptors at nerve endings. It is a "normal" pain that all have experienced and is easily described to others. This type of pain is most easily referrable to a recognizable injury or mass. It may be divided into somatic nociceptive pain, which is a well-localized, constant pain usually involving the body wall. A typical example is the pain associated with bone metastases *(5)*. In contrast is visceral nociceptive pain, as might occur with pancreatic cancer. This pain involves a visceral organ, is typically paroxysmal, and poorly localized. Regardless of the type of nociceptive pain, all involve nociceptors and are responsive to nociceptive blockers such as opioid agents *(6)*.

2.2. Neuropathic (Non-Nociceptive) Pain

Pain of this type is very difficult to describe to others as it is characterized by sensations not typical of pain. Many will complain of pins and needle sensation, a burning feeling, or shooting pain *(7)*. A classic example of this type of pain is peripheral neuropathy resulting from chemotherapy. Neuropathic pain is characterized by dysfunction somewhere in the nervous system *(8)*. This dysfunction may be either in the peripheral or central nervous system (CNS; as may occur from a spinal cord lesion) *(9)*. It need not be associated with a discrete lesion and may persist even after apparent healing. It best thought of as a "short-circuit" in the nervous system that processes pain signals. Opioids are not the treatment of choice. Agents such as antidepressants and anticonvulsants, which aim to minimize the short circuiting, are typical first-line choices *(10)*.

2.3. Psychogenic Pain

This is pain that is "in one's head." It is true pain in that there is an "unpleasant emotional experience"; however, there is no clear tissue damage. It is pain that is neither nociceptive nor neuropathic and thus diagnosing psychogenic pain is a process of excluding other types of pain. It is an uncommon cause of pain in patients with cancer. Therapy is more psychiatric than analgesic.

Although the previous categorization allows for delineation of pain subtypes, it is important to realize that they are not mutually exclusive as mixed nociceptive-neuropathic pain is possible. For example, a lesion involving a vertebral body encroaching on the spine may result in somatic nociceptive pain from its presence in the bone of the vertebral body while also causing neuropathic pain through its impingement on the spine.

3. TREATMENT OF NOCICEPTIVE PAIN

Standard treatment for nociceptive pain involves use of opioids and nonsteroidal anti-inflammatory drugs (NSAIDs); but, where does one start? A very useful starting point is suggested by the World Health Organization's (WHO) analgesic ladder *(11)*. This was developed to assist in the effective management of cancer pain in developing countries. The ladder is depicted in Fig. 2. It relies on using the drugs in a stepwise fashion to maximize analgesia while minimizing side effects.

Initial attempts for pain relief employ the use of NSAIDs. When these are no longer sufficient, step 2 of the ladder advocates the use of weak opioids. If there is still inadequate analgesia, the use of strong opioids is necessary *(6,11,12)*.

The WHO ladder is appropriate in nociceptive pain, both somatic and visceral. It offers little guidance in the management of neuropathic pain.

3.1. NSAIDs

NSAIDs reduce pain by inhibiting the production of prostaglandins. Prostaglandins sensitize nociceptors, which facilitate pain. Thus, if prostaglandins result in pain, prostaglandin inhibition must results in analgesia. NSAIDs form the first step of the WHO ladder. Prostaglandin inhibition is sufficient for some people, but may not be sufficient for all. NSAIDs are often chosen as an initial therapeutic measure because they are not opioids and are therefore without opioid-related side effects. Traditional NSAIDs do result in their own set of side effects such as ulcers, platelet dysfunction, and renal failure.

In recent years, it was discovered that all prostaglandins were not created equal. Whereas "bad" prostaglandins incited pain, "good" prostaglandins maintained the body by lining the gastric mucosa, ensuring platelet function, and perfusing the kidneys. Traditional NSAIDs block production of bad prostaglandins through their inhibition of the enzyme cyclooxygenase (COX)-2 *(13–17)*.

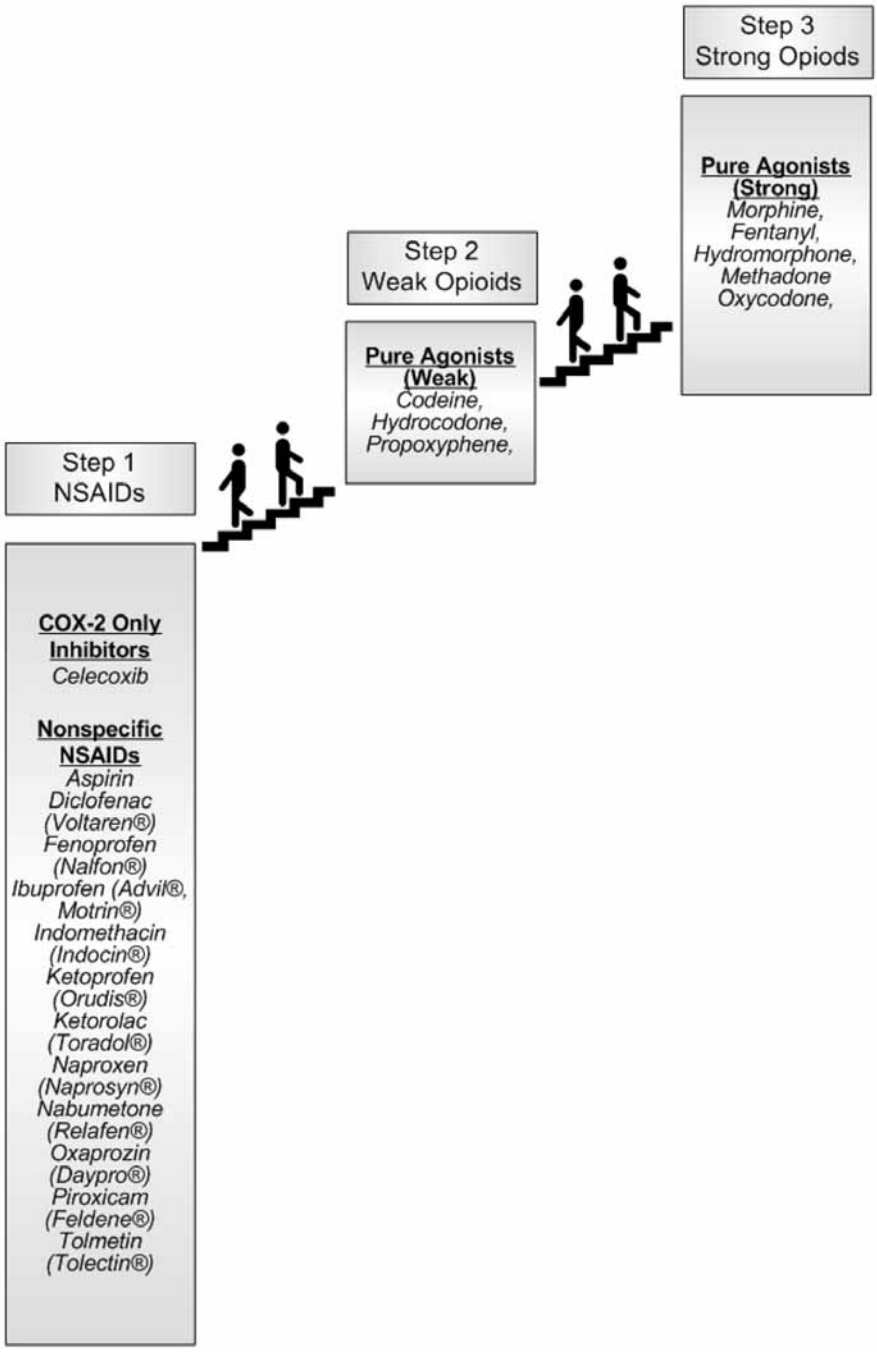

Fig. 2. WHO ladder.

COX-1 catalyzes the production of good prostaglandins but is also inhibited by traditional NSAIDs and results in their unwanted side effects on the stomach and platelets. With the introduction of COX-2-specific inhibitors such as celecoxib, the possibility of obtaining analgesia with a minimum of NSAID-related side effects was realizable *(13,18)*.

Concerns regarding the increased incidence of thrombotic events in patients taking COX-2-specific inhibitors led to the market withdrawal of rofecoxib and valdecoxib. This increase in thrombosis is partly the result of the selective inhibition of prostaglandins by COX-2 agents resulting in a net prothrombotic state *(19–23)*. Nonselective NSAIDs are "equally inhibitory" and do not create such a thrombotic state. Although agents such as celecoxib are useful and provide many benefits, their use must be weighed against the risks they impart to patients with preexisting cardiac or cerebrovascular disease.

Metastatic bone pain treatment is guided by use of the WHO ladder, as it represents a nociceptive type of pain. Bone metastases are a common cause of pain in oncology patients *(2)*. Tumors that typically metastasize to the bone include tumors of the lung, breast, and prostate. Although spread to bone is uncommon for CRC, when it does occur, it may be widespread.

Prostaglandins are a key etiological cause of bony pain. Thus, effective analgesia is sometimes achieved using prostaglandin inhibitors such as NSAIDs in combination with opioids. At times, the bony lesion can abut a nerve and subsequently result in neuropathic pain.

Bisphosphonates are used to reduce bone pain and slow bone damage caused by metastases *(24,25)*. They act by binding to the bone matrix and reducing the solubility of bone, making it resistant to erosion by tumor and resorption by osteoclasts. Pamidronate is the most often used bisphosphonates in cancer and is given monthly (or every 2 wk) at a dose of 60 or 90 mg. Zoledranate is newer alternate bisphosphanate given at a dose of 4 mg. It has the advantage of a significantly faster infusion time. Neither agent should be used in patients with renal insufficiency.

Treatment of pain from bony metastases also involves the use of surgery and radiation as initial measures *(26)*. Prophylactic surgery may be required to prevent pathological fractures in weight-bearing regions. External beam radiation to tumor sites will hopefully result in shrinkage of the tumor and consequent analgesia.

3.2. Weak Opioids

Opioids are the mainstay in the treatment of nociceptive pain. Weak opioids are the initial opioids of choice and include agents such as propoxyphene (Darvocet®), hydrocodone (Vicodin®), or codeine (Tylenol no. 3®). Many of these compounds are combined with acetaminophen to facilitate their effect. Weak opioids are typically prescribed on an "as-needed" basis (one to two tablets every 4 h as needed) as opposed to around the clock. Patients should be advised

to limit intake so that total daily *(27)* usage does not exceed 4000 mg. All weak opioids are fast acting, meaning their onset of action is within 30 min *(6)*.

Weak opioids are typically initiated on an "as-needed" (prn) basis. When pain is persistent despite prn dosing, they are often administered at regular intervals to maintain efficacy. Although they may be given every 4 to 6 h, it is important to limit the total daily acetaminophen dosage to less than 4000 mg per day to avoid liver toxicity.

3.3. Strong Opioids

Analgesia poorly controlled by weak opioids requires step 3 of the WHO ladder, use of strong opioids *(28)*. Commonly used agents include morphine, oxycodone, and hydromorphone. Initial dosing is begun on an as-needed basis with fast-acting (i.e., not sustained release) drugs. Which specific drug to choose initially is often arbitrary and more often based on a patient's previous favorable or unfavorable response to a given medication.

3.4. Sustained-Release Opioids

Strong opioids are initiated on a prn basis and are then advanced to around-the-clock dosing should pain be inadequate. Unlike weak opioids, strong opioids have corresponding sustained-release forms *(29,30)*. It is a common error to initiate a sustained-release opioid (such as a fentanyl transdermal patch) when a prn opioid does not provide adequate analgesia. Although pain is a necessary requirement, it is insufficient by itself to begin a sustained-release opioid. Two criteria must be met: insufficient analgesia and maximal frequency (i.e., every-4-h dosing) of a strong prn opioid. The intent of the sustained-release opioid is to provide higher blood levels of opioid (better analgesia) with a convenient dosing schedule.

In the absence of a sustained-release opioid, an individual could theoretically achieve higher opioid levels by either ingesting a stronger dose or increasing the frequency of an immediate-onset opioid. For a medication conjugated with acetaminophen, the maximal daily dose is 4000 mg of acetaminophen. For pure opioids (without acetaminophen), in the absence of side effects, there is no upper limit to their dose. More medicine does indeed work better; however, this is not often practical. The goal with an as-needed medication is to take between two to five doses per day. A dose does not refer to the number of total pills, but instances of taking the medication; thus, two pills taken at once is a single dose. When a patient must take more than five doses per day to achieve satisfactory analgesia, the patient is working excessively. Rather than imposing this effort on the patient, sustained-release opioids (such as the fentanyl patch, extended-release oxycodone, etc.) are used. By releasing over an extended period of time, a higher dose of medication is maintained at a stable level for longer periods, diminishing the need for frequent dosing. Sustained-release opioids are not fast acting. Fast-acting strong opioids are concurrently prescribed with sustained-

release agents so that booster (or rescue) doses are available should an exacerbation of pain occur at any time. If more than five rescue doses are needed while on a sustained-release opioid, an increase in the sustained-release opioid should be considered. Likewise, if fewer than two rescue doses are needed, a decrease in the sustained-release drug may be warranted.

3.5. Combining Drugs

Some practitioners may combine two sustained-release opioids in the hope of achieving better analgesia. Although this may provide improved analgesia by delivering more total opioid, it may result in more side effects. Two sustained-release opioids allow for two sets of side effects. It is better to continue with a single sustained-release opioid, but at a higher dose. This will achieve improved analgesia with a potential of side effects from only one agent.

It is best to regard nociceptive medications as belonging to one of three groups: NSAIDs, rescue opioids (weak and strong, taken as needed), and sustained-release opioids (taken around the clock). It is acceptable to combine a single medicine from each group, but not acceptable to have multiple medications from a single group. Thus, use of ibuprofen with oxycodone prn and a fentanyl patch is okay; however, use of a fentanyl patch with rescues of oxycodone and morphine would be inappropriate. There should be at most one sustained-release opioid, one rescue opioid, and one NSAID.

3.6. Opioid Rotation

Assessment of a pain patient requires the answer to two questions: how is the pain and are there any side effects (such as nausea, vomiting, sedation)?

Looking at Table 1, four permutations exist of pain and side effects. The ideal situation arises if there is no pain and no side effects. If the patient has continued pain and no side effects, medications need to be increased. Similarly, if there is no pain but side effects are present, medications need to be decreased. An algorithm for doing this is discussed in Table 3.

When pain persists and side effects persist, adjustment of the dose lower or higher will either make the side effects worse or compromise the analgesia. A potential solution is to rotate to another opioid that may "agree" with the patient better, i.e., provide analgesia with no side effects *(31)*.

Such a rotation requires conversion among opioids *(31–34)*. It is not enough to simply stop one opioid and start another; the doses must be of equal potency, an equi-analgesic conversion must occur *(35)*. Fortunately, opioids can be changed among each other in a fashion similar to currency. A conversion table listing the equivalent strengths of each is required and is depicted in Table 2. In this table every dose shown is of equivalent analgesic potency. Note that oxycodone, immediate-release, and OxyContin® are the same drug, but with different pharmacokinetics. The same is true for morphine and MSContin®.

Table 1

	No side effects	*Side effects*
Good pain relief	Do nothing	Treat side effects, decrease dosage
Poor pain relief	Increase dosage	Treat side effects and/or change drug

Table 2

Drug name (PO)	*Conversion factor*	*Frequency*
WEAK PRN OPIOIDS		
Codeine	130 mg	q4hr prn
Hydrocodone	30 mg	q4hr prn
Propoxyphene	200 mg	q4hr prn
STRONG PRN OPIOIDS		
Morphine	30 mg	q4hr prn
Oxycodone	20 mg	q4hr prn
Hydromorphone	8 mg	q4hr prn
Meperidine	300 mg	q4hr prn
SUSTAINED-RELEASE OPIOIDS		
MScontin®	30 mg	q8hr
OxyContin®	20 mg	q12hr
Fentanyl	0.2 mg (200 mcg)	q1hr
Methadone	2 mg	q8hr

A simple worksheet to perform such a conversion is as follows:

1. Write down dose of current opioid (1)
2. Write down conversion factor of current opioid. (2)
3. Write down conversion factor of desired opioid (3)
4. New dose of desired opioid = (1) × (3) / (2)

Thus, to convert 45 mg of morphine to oxycodone, one would perform the following calculations:

1. Write down dose of current opioid (1): 45 mg
2. Write down conversion factor of current opioid. (2): 30
3. Write down conversion factor of desired opioid (3): 20
4. New dose of desired opioid = (1) × (3) / (2) = 45 × 20 /30 = 30 mg of oxycodone

Note that this is usually combined with the adjustment of the dose of a sustained-release opioid with its rescue dose and is more fully described in the next section.

Table 3

1. Determine total current long-acting medication	
1a. Determine the total daily usage of all CURRENT long-acting medications	mg Each dose × frequency per day
1b. Write down conversion factor for CURRENT long-acting medicine	
1c. Write down conversion factor for NEW long-acting medicine	
1d. Convert current long-acting medication to NEW desired long-acting medication (per day)	(Value in 1a.) × (Value in 1c.) / (Value in 1b.)
2. Determine total current short-acting medication	
2a. Determine the total daily usage of all CURRENT short-acting medications	mg Each dose × no. doses actually taken per day
2b. Write down conversion factor for CURRENT short-acting medicine	
2c. Write down conversion factor for NEW long-acting medicine	
2d. Convert current long-acting medication to NEW desired long-acting medication	(Value in 2a.) × (Value in 2c.) / (Value in 2b.)
3. Determine total daily opioid usage in terms of NEW long-acting	
3a. New total daily opioid dose	add (value in 1d.) + (value in 2d.)
4. Determine NEW long-acting dose	
4a. Determine new long-acting portion of total daily dose (75% of daily dose)	Multiply (value in 3) × 0.75
4b. Write down frequency of NEW long-acting agent per day.	(2: bid, 3: tid, 24: qhr, etc.)
4c. Determine new long-acting dosing	Divide (value in 4a.) / (value in 4b.)
5. Determine NEW short-acting dose	
5a. Determine short-acting portion of total daily dose (25% of daily dose)	Multiply (value in 3) × 0.25
5b. Determine new short-acting dose (assuming 5 rescues per day)	Divide (Value in 5a.) / 5
6. Convert short-acting dose	
6a. Write down conversion factor for NEW desired short-acting medicine	
6b. Convert this short-acting medication to NEW desired short-acting medicine	(Value in 5b.) × (value in 6a.) / (value in 2c.)

(*Continued*)

Table 3 (*Continued*)

7. New long-acting dose	
Value in 5b.	Given at desired frequency (Value in 4b.)
8. New short-acting dose	
Value in 6b.	Given q4hr prn

Table 4

1. Determine total current long-acting medication	
1a. Determine the total daily usage of all CURRENT long-acting medications	mg Each dose × frequency per day (200mg) × 3/d = 600 mg/d
1b. Write down conversion factor for CURRENT long-acting medicine	30
1c. Write down conversion factor for NEW long-acting medicine	0.2
1d. Convert current long-acting medication to NEW desired long-acting medication (per day)	(Value in 1a.) × (Value in 1c.) / (Value in 1d.) (600) × (0.2) / (30) = 4 mg/d
2. Determine total current short-acting medication	
2a. Determine the total daily usage of all CURRENT short-acting medications	mg Each dose × no. doses actually taken per day (8 mg) × (10) = 80 mg/d
2b. Write down conversion factor for CURRENT short-acting medicine	8
2c. Write down conversion factor for NEW lon- acting medicine	0.2
2d. Convert current long-acting medication to NEW desired long-acting medication	(Value in 2a.) × (Value in 2c.) / (Value in 2b.) (80) × (0.2) / 8 = 2 mg/d
3. Determine total daily opioid usage in terms of NEW long-acting	
3a. New total daily opioid dose	Add (value in 1d.) + (value in 2d.) (4) + (2) = 6 mg/d
4. Determine NEW long-acting dose	
4a. Determine new long-acting portion of total daily dose (75% of daily dose)	Multiply (value in 3) × 0.75 (6) × (0.75) = 4.5 mg/d
4b. Write down frequency of NEW long-acting agent per day	(2: bid, 3: tid, 24: qhr, etc.) (24)

Table 4 (*Continued*)

4c. Determine new long-acting dosing		Divide (value in 4a.) / (value in 4b.) (4.5) / (24) = 0.1875 mg/h = 187.50 mcg/h fentanyl
5. Determine NEW short-acting dose		
5a. Determine short-acting portion of total daily dose (25% of daily dose)		Multiply (value in 3) × 0.25 (6 mg) × (0.25) = 1.5 mg
5b. Determine new short-acting dose (assuming 5 rescues per day)		Divide (value in 5a.) / 5 (1.5 mg) / 5= 0.3 mg
6. Convert short-acting dose		
6a. Write down conversion factor for NEW desired short-acting medicine		30
6b. Convert this short-acting medication to NEW desired short-acting medicine		(Value in 5b.) × (Value in 6a.) / (Value in 2c.) (0.3) × (30) / (0.2) = 45 mg
7. New long-acting dose		
Fentanyl patch	187.50 mcg/h	Given at desired frequency (value in 4b.)
8. New short-acting dose		
Morphine PO	45 mg	q4hr prn

3.7. Adjusting Opioid Doses

More than five rescue doses per day or less than two requires adjustment of the sustained-release opioid. But how is one to do this? Fortunately, this can be done scientifically. The key is to total all of the opioid usage per day and adjust the dosage so that 75% of the total daily dose is provided by the sustained-release opioid. The remaining 25% is provided by the rescue dose. If we assume that a patient requires approx three rescues per day, each rescue dose amounts to approx 8% of the total daily dose.

By only providing 75% of the total daily dose in the sustained-release opioid, rescue doses become essential. Why not put 100% of the total daily dose into the sustained-release opioid and not require rescues? Rescue doses provide a marker to ensure that the sustained-release dose is not excessive. If no rescue doses are required, it is impossible to know if the sustained opioid dose provides 100 or 300% of the daily dose. If a rescue dose is required, it can be certain that the sustained-release opioid is not complete; however, too many rescues means that the sustained-release opioid is insufficient. Table 3 provides a worksheet to assist in the adjustment of opioid doses.

As an example, imagine that a patient takes MS Contin 200 mg po bid and Dilaudid rescues 8 mg each time, 10 times per day. How much fentanyl patch with morphine rescues is required? (Table 4)

Should the calculations lead to a number more than double the current sustained release opioid, a safe practice is to limit the increase to only double the current dose. It is more prudent to check on the patient in 1 or 2 d and readjust as needed based on rescue doses.

4. TREATMENT OF NEUROPATHIC PAIN

When dysfunction occurs along the course of a pain nerve or in the spinal cord, a pain distinct from somatic pain, termed neuropathic pain occurs. A typical form of this pain is the pain of peripheral neuropathy. It can also arise from tumors abutting neural fibers and is best conceived of as a change in the transmittance of the nerve signal. Common complaints include sensations of "pins and needles," "burning," or "shooting pains" along the course of the affected nerve or in the distal extremities *(7,8,36,37)*. Treatment of this altered nerve function requires medications that minimize the aberrant conduction. Traditional agents used for this are anticonvulsants and antidepressants. Described here are initial measures for the treatment of this type of pain. Further therapy is best directed to a pan specialist.

Medications that have greater efficacy but also greater side effects include antidepressants such as amitriptyline and nortriptyline *(38)*. These agents act on the neurotransmitters norepinephrine and serotonin to alter pain conductivity and thus ameliorate neuropathic pain. It is this same action (as well as their anticholinergic activity) that is responsible for their side effects such as sedation, arrhythmia, dry mouth, urinary retention, and orthostatic hypotension. Unfortunately, other antidepressants, such serotonin selective reuptake inhibitors, though having fewer side effects are disappointing in their efficacy for pain *(39)*. In those who are without significant comorbidities, antidepressants should be considered as first-line agents *(40–42)*. Nortriptyline is the kinder and gentler agent and is typically begun at a dose of 10 or 25 mg nightly. Its side effect of somnolence is sometimes welcome by patients *(10,40,43)*. The dose is doubled at weekly intervals until analgesia is obtained or side effects ensue. Some recommend evaluation of an electrocardiogram prior to initiation. The earliest a response may be seen to these medications is approx 1 wk.

Although many anticonvulsants are used to treat neuropathic pain, one of the most common is gabapentin *(42)*. It is typically initiated at 100 mg po tid and increased weekly by 100 mg tid until analgesia begins or side effects ensue. Although its mechanism of action is unclear, its benefit is clear. A positive response may not be noticed until 1 wk. It is essential that the medicine be administered on a schedule, as it does not act as a prn medication. Typical side effects are difficulty with mentation, fatigue, somnolence, dizziness, and peripheral edema.

Opioids alone have also been found to be beneficial in the treatment of neuropathic pain; however, the dose achieving analgesia is typically much higher than that used for somatic pain *(44,45)*. Although some patients may tolerate opioids with a dearth of side effects, many others may not. In those without side effects, the opioid may be titrated upward to treat the neuropathic pain; but, once side effects begin, it may be necessary to consider using an anticonvulsant or antidepressant.

Multiple neuropathic mediations may be given together, provided they are from different classes. For example, it is common to combine gabapentin with nortriptyline, but it is atypical to combine amitriptyline with nortriptyline.

5. MIXED SOMATIC AND NEUROPATHIC PAIN

In cancer, it is more common for pain to be both somatic and neuropathic in nature. A typical situation is metastases to a rib causing somatic pain by invasion of the bone and neuropathic pain by affecting the underlying intercostals nerve. In such situations, opioids may be combined with neuropathic medications to treat both types of pain. There are single medications capable of treating both types of pain. Tramadol and methadone are both racemic mixtures, with one component effective for somatic pain whereas the other component treats neuropathic pain.

Tramadol is an unusual drug in that its somatic component acts on opioid receptors, yet in the United States it is not classified as an opioid. When used for the treatment of somatic pain only, it may be initiated as a prn medication at 50–100 mg po q 6 h prn. For neuropathic pain, it acts on serotonin and norepinephrine pathways, and requires around the clock dosing to be effective. Typical doses are 50–100 mg po q 6 h. As it lowers the seizure threshold, it should be avoided in those at risk. In addition, when combined with medications affecting serotonin reuptake (such as antidepressants), it may precipitate serotonin syndrome. Tramadol is at best a weak opioid and should be used in that context *(46,47)*.

Methadone has a rich use in cancer pain; however, it is also a drug that generates much respect among pain physicians *(48–50)*. This is, in part, to its potential to be very potent if not used cautiously. A single dose of methadone can be 10 to 15 times more potent than the equivalent does of morphine. It is recommended that this medication be considered when all else fails and only in conjunction with a consultation to a pain practitioner. In a patient taking the equivalent of oral morphine 100 mg or more per day, it is reasonable to initiate a dose of methadone 2.5 mg po q 8 h in addition to the current opioid. It will act synergistically with the current opioid. It is imperative that a concurrent pain consultation be sought so that further adjustments are made by one familiar with all the risks and benefits.

Table 5

	No side effects	*Side effects*
Good pain relief	Do nothing	Treat side effects and decrease dosage
Poor pain relief	Increase dosage	Treat side effects and/or change drug, or perform intervention and decrease dosage

6. CONSIDERATION OF INTERVENTIONS

When should one abandon all medication and pursue a procedure? When side effects persist and analgesia has not been achieved, one may attempt one or two opioid rotations. Should these fail, further opioid rotations may result in a repetition of past failures. At this stage, some means of reducing the pain in the absence of systemic medication must be considered. Typically, this accomplished by a procedure such as nerve block or spinal (or epidural) pump *(51,52)*. The goal is to remove some percentage of the pain with the procedure. Consequently, the systemic medications can be decreased so that side effects will be lessened. Thus, when side effects and analgesia coexist and have not responded to two opioid rotations, referral to a pain specialist is warranted for possible intervention or more specialized management. Thus Table 1 may be revised and appears as shown in Table 5.

7. SIDE EFFECTS AND THEIR TREATMENT

The most significant side effects associated with pain medications, particularly opioids, include constipation *(53)*. So long as a patient is taking an opioid all patients should be prescribed a bowel regimen. Typical regimens include the use of docusate (Colace®) at 100 mg po tid along with senna two tablets po every hour. Should these fail, additional agents that can be added to induce a bowel movement are polyethylene glycol, lactulose, and magnesium citrate.

Treatment for nausea and vomiting induced by opioids is similar to that for chemotherapy-induced nausea and vomiting *(53)*.

The side effect that poses the greatest challenge in pain management is sedation *(53)*. Although it is acceptable to perform an opioid rotation, sometimes it is simpler to treat the sedation. This is most notable when a patient has stable pain relief or has had multiple opioid rotations already. Opioid-induced sedation is countered using CNS stimulants such as methylphenidate and modafinil. Methylphenidate is typically initiated at a dose of 5 mg in the morning. Should this be insufficient or only partially successful, the dose is increased to 10 mg every morning, then 10 mg every morning and 5 mg at noon. The maximal dose

typically given for opioid-induced sedation is 10 mg in the morning and 10 mg at noon. Alternatively one may use modafinil at a dose of 100 mg po in the morning advancing incrementally in a manner similar to methylphenidate to maximal dosage of 200 mg po in the morning and 200 mg at noon.

8. SUMMARY

Successful management of pain in the patient with CRC can be straightforward if approached in a systematic manner. Such an algorithmic approach involves the following stepwise approach:

1. Do a thorough history and physical and identify whether the type of pain is nociceptive or neuropathic. Key descriptors assisting in this determination are descriptions such as "pins and needles," "burning," or "shooting sensations." These suggest pain that is neuropathic, as do radicular symptoms by exam. Deep aching pain is more suggestive of nociceptive pain.
2. For neuropathic pain, it is best to begin with neuropathic medications such as antidepressants and anticonvulsants. Drugs within a class are titrated to maximal tolerability. If pain is till insufficient, drugs from another class are attempted.
3. For pain that is not neuropathic, nociceptive treatment algorithms are employed. This involves initially using NSAIDs only, followed by weak opioids, and then strong opioids. A transition between steps occurs if analgesia is poor.
4. Should side effects occur with poor analgesia, an equi-analgesic opioid rotation is warranted with treatment of the side effects with adjuvant medication.
5. If a patient is taking more than five or six rescue doses per day with continued pain, a sustained-release opioid should be added. In those already on sustained-release opioids, upward adjustments will be necessary.
6. For a patient already on sustained-release opioids who is taking fewer than two rescue doses per day, a downward titration in the sustained-release opioid will be required.
7. For patients with mixed neuropathic and nociceptive pain, first-line agents may include the use of tramadol, or combinations of nociceptive and neuropathic medications.
8. Methadone is adjuvant medication that can be used to augment existing regimens or treat combined neuropathic and nociceptive pain syndromes. Its use should be coordinated with a pain specialist.
9. Procedures such as nerve blocks and implantable pumps should be considered when side effects and poor analgesia persist. By relieving a portion of the pain with a procedure, analgesia is improved and subsequent decreases in systemic medications may improve side effects.

Like so much else in medicine, pain management is as much art as it is science. Although the previous approach provides some measure of success, it is by no means complete. Maintaining close ties with a pain management specialist is essential for continued success.

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15 Novel Agents and New Paradigms for Colorectal Cancer

Beyond EGFR and VEGF

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Summary

The advent of molecularly targeted therapies has fundamentally changed anticancer drug development. Advances in molecular oncology have altered scientific paradigms in drug discovery, and preclinical and early clinical drug development. In the current era, selective therapies are now rationally designed to inhibit specific novel targets that are well characterized at the molecular level. Inherent in this approach are new strategies that include testing agents in pharmacogenetically defined populations, analyzing the molecular profile of tumors prior to treatment, and individualizing anticancer therapy for each unique patient. Examples of promising molecular targets for future therapies for treating patients with colorectal cancer include the PI3K/Akt/mTOR signaling pathway and antiangiogenic inhibition, to name just two. This brief review discusses the new challenges and changing paradigms related to developing novel colorectal cancer therapeutics in the age of molecularly targeted therapies.

Key Words: Targeted therapies; drug development; molecular therapeutics.

1. INTRODUCTION

The recent approval of the monoclonal antibodies bevacizumab and cetuximab for the treatment of colorectal cancer (CRC) has ushered in the new era of targeted therapies for common solid tumor malignancies. As such, it represents the dawn of a new age in oncology therapeutics and it highlights the need to adopt new paradigms for cancer drug development. One needs only to peruse the latest oncology journals or visit any current scientific meeting on targeted therapies to see how these changes are fundamentally altering the landscape of

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applied cancer research. The emphasis is now on molecularly targeted therapies, in contrast to traditional and now presumably outdated nonspecific chemotherapeutic agents. Underlying this change is the rapid growth in our understanding of molecular alterations responsible for the development of a cancer cell *(1)*. Further acceleration of this process is driven by monumental advances in basic human biology, such as the successful sequencing of the entire human genome. Thus, cancer therapeutic development is currently a science-driven process to a greater extent than ever before in its history.

1.1. What Are Targeted Therapies?

Because of this emphasis on new approaches to cancer therapeutics, it is worthwhile to ask several naive-sounding questions. First, what are targeted cancer therapies? Second, how do they differ from conventional cancer chemotherapies that were developed using traditional clinical research strategies? The National Cancer Institute's (NCI) dictionary of cancer terms (http://www.cancer.gov/dictionary/) defines targeted therapy as:

> *a type of treatment that uses drugs or other substances to identify and attack specific cancer cells without harming normal cells. A monoclonal antibody is a type of targeted therapy.*

Although an admirable effort, this definition clearly falls short of the mark. One need only to experience firsthand an acute gastrointestinal perforation associated with bevacizumab therapy or to examine a severe grade 3 skin rash in a patient on cetuximab to appreciate fully the deficiencies of this brief definition. The NCI has provided a better follow-up definition in their internet fact sheet (http://www.cancer.gov/cancertopics/factsheet/Therapy/targeted) stating:

> *Targeted cancer therapies use drugs that block the growth and spread of cancer. They interfere with specific molecules involved in carcinogenesis … and tumor growth. Because scientists call these molecules "molecular targets," these therapies are sometimes called "molecular-targeted drugs," "molecularly-targeted therapies," or other similar names. By focusing on molecular and cellular changes that are specific to cancer, targeted cancer therapies may be more effective than current treatments and less harmful to normal cells.*

Overall, this superior definition emphasizes that the differences that distinguish targeted therapies from conventional anticancer drugs is one of conceptualization and process rather than substance.

Thus, a targeted therapy is less defined by its physical properties than it is by the method by which it is conceived, identified, tested, and clinically developed. It is the unique birthing process, both practical and theoretical, by which a new agent is developed that ultimately defines a targeted therapy. This process is characterized as being inherently rational and science-driven, in contrast to the

sporadic chance discoveries that highlight the development of many historical chemotherapeutic agents. Well-known historical examples of serendipitous advances include the famous non-oncological discovery of penicillin by Sir Alexander Fleming, who noticed that the mold contaminant growing in his bacterial cultures inhibited cell growth *(2)*. In the field of cancer chemotherapy, this seminal discovery was echoed by Barnett Rosenberg's observation that bacterial growth was inhibited near the platinum electrode in his study of electricity effects on cells *(3)*. This ultimately led to the discovery of the anticancer agent, cisplatin.

Thus, the rational design of a targeted therapy requires a thorough understanding of the molecular pathways and events that drive the conversion of a normal cell into a lethal malignant invasive tumor. Targeted therapies attempt to exploit the fundamental differences that exist between these normal and malignant cells. For example, the recognition that an abnormal constitutively activated tyrosine kinase, the Bcr-Abl fusion protein, is responsible for driving the malignant phenotype in chronic myelogenous leukemia (CML) led to a concerted effort to discover agents that inhibit this protein target *(4)*. Such strategies ultimately led to the discovery and rapid development of the prototypic example of a targeted therapy, imatinib (Gleevec®). This oral and generally well-tolerated agent has tremendous antitumor activity in CML and gastrointestinal stromal cell tumors (GIST). Thus, the characterization of promising tumor cell targets allows for the subsequent screening of large libraries of molecules to discover lead compounds ripe for further testing. This paradigm is now driving huge efforts in drug discovery laboratories across the globe.

However, even this practical definition of a targeted therapy has limitations. For example, in the 1950s, Charles Heidelberger noted that tumor cells took up uracil at higher rates than normal tissues. After studying the metabolic pathways involved in uracil and thymidine metabolism, he rationally designed the classic antimetabolic chemotherapy drug, 5-fluorouracil (5-FU), as a targeted therapy *(5)*. Similarly, it is impossible to think of a more targeted therapeutic than the antiestrogen hormonal agent, tamoxifen *(6)*. Nonetheless, current drug discovery and development programs are heavily influenced by the recently developed targeted therapies, such as cetuximab and bevacizumab in CRC and imatinib in CML and GIST *(7)*.

Given this understanding, we can now ask from where the next generation of targeted therapies will arise. Clearly, vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) signaling are validated target pathways for new treatments for CRC. Currently, much of the pharmaceutical industry's resources are geared towards developing drugs that mimic the effects of cetuximab or bevacizumab in hitting these same targets to try to develop better and more effective therapies. Although advances and

refinements in our current treatments will undoubtedly arise from these efforts, a larger question is what new molecular targets will be important for the development of future targeted therapies. For the purposes of this discussion, we wish to speculate as to what types of agents and targets will yield the next generation of therapies for CRC. There is no absolute way to be precise in these predictions, any more than a venture capitalist can reliably pick a winner in the stock market. However, it is possible to make some highly educated guesses. The pathways and the inherent targets discussed here reflect our personal biases and are influenced by our own direct experience in cancer drug development. Thus, it is likely that when this chapter is reviewed in 5 or 10 yr, its likelihood of being highly accurate is small. Nonetheless, it is both exciting and exhilarating to consider select areas of applied scientific research with the highest potential for yielding the next generation of clinical advances in cancer therapeutics. We have great confidence that the current ongoing basic research in cancer biology ultimately will lead to tomorrow's great therapeutic advances.

2. NEW TARGETS FOR TREATING CRC

Cancer is a molecular disease. Hence, the next generation of molecularly targeted therapies will undoubtedly arise from ongoing basic research on the molecular basis of carcinogenesis of solid tumor malignancies *(1)*. Based on the experience with cetuximab and bevacizumab in CRC, two promising areas of targeted therapy development in oncology are signal transduction inhibitors and antiangiogenic agents. In this selective overview, an extremely interesting signaling pathway and a family of angiogenic proteins will be discussed as potential targets for new cancer therapeutics.

2.1. Phosphoinositide 3-Kinase (PI3K)/Akt/mTOR Targeting Agents

The development of the antibody cetuximab and the small molecule tyrosine kinase inhibitors gefitinib and erlotinib, which target EGFR signaling, has highlighted the importance of signal transduction inhibitors as cancer therapeutics. However, many additional pathways beyond the ErbB family of receptors have been implicated in the transformation and growth of malignant tumor cells. One of the most promising targets for future cancer therapies is the PI3K/Akt/mTOR pathway (Fig. 1) *(8)*. The PI3K are a complex family of heterodimers with separate regulatory (p85) and catalytic (p110) protein subunits. There are three general classes, but at present, much of the research in this area has focused on the Class Ia PI3Ks, which are activated by growth factor receptor tyrosine kinases. Class Ia PI3Ks phosphorylate inositol-containing lipids at the 3-OH position and are responsible for the conversion of phosphatidylinositol-4,5-triphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3), a key second messenger lipid signaling molecule involved in the activation of various downstream

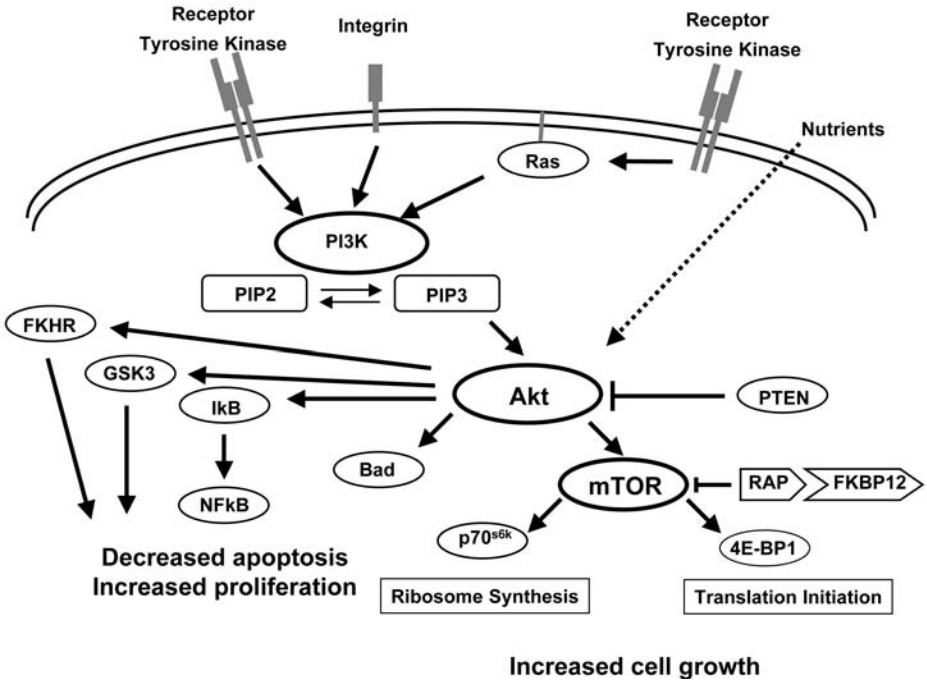

Fig. 1. The PI3K/Akt/mTOR Signaling Pathway. Abbreviations: PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-4,5-triphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; FKHR, forkhead-related transcription factor; GSK3, glycogen synthase kinase 3; IκB, I-kappa-B; NF-κB, nuclear transcription factor-kappa-B; mTOR, mammalian target of rapamycin; p70 s6K, 70-kDa ribosomal S6 kinase; 4E-BP1, elongation-initiation factor 4E-binding protein-1; RAP, rapamycin; FKBP12, FK506-binding protein.

serine/threonine protein kinases including 3-phosphoinositide-dependent protein kinase-1 (PDK1) and Akt *(9)*. Signaling along this pathway is responsible for the regulation of a large number of cellular functions including proliferation, nutrient homeostasis, apoptosis, growth, and motility. Many of these functional effects are important for the propagation growth, invasion, and survival of tumor cells.

In a variety of different cells, enhanced levels of PIP3 activate a 57-kDa cellular homolog of the retroviral oncogene *v-Akt* called Akt *(10)*. Akt is a serine-threonine kinase, also known as protein kinase B. Three members of the Akt family (Akt1, Akt2, and Akt3) have been well characterized; however, specific functional differences between the different Akt isoforms have not been well characterized *(10)*. Akt1 is associated with cell growth and decreased apoptosis and it is activated by a number of pathways, including the platelet-derived growth factor receptor. It has been implicated in cancer cell growth and neuronal

cell development. Akt2 is involved with glucose metabolism, but is also over expressed in some ovarian cancer tumors. The third member of this family, Akt3, has also been implicated in oncogenic transformation *(11)*. Intracellular levels of PIP3 recruit Akt and other proteins to the inner surface of the plasma membrane by interacting with their pleckstrin homology (PH) domains. Once localized to the plasma membrane, Akt is activated via phosphorylation by PDK1, which is also recruited to the membrane by PIP3. Once activated, Akt results in the enhanced transcriptional expression of a very large number of downstream target proteins, such as I-κ-B, Bad, forkhead-related transcription factor (FKHR-L1), glycogen synthase kinase 3, and others. These downstream target proteins are involved in blocking apoptosis, promoting replication events in the cell cycle, and enhancing protein synthesis and cell growth *(8)*.

The intracellular concentrations of the second messenger, PIP3, are strictly controlled by phosphatases such as PTEN, so named because it is the phosphatase and tensin homolog deleted from chromosome 10 *(12)*. This 403-amino acid 3′-phosphatase dephosphorylates PIP3 back to PIP2. It is a dual-function phosphatase active against lipid and protein substrates. As a consequence, Akt activation is negatively regulated by PTEN activity and loss of PTEN function can enhance malignant cell behaviors. Germ-line *PTEN* mutations are associated with Cowden's disease, an inherited disorder associated with a predisposition towards developing breast cancer. Thus, *PTEN* has the characteristics of a classic tumor suppressor gene. Somatic *PTEN* mutations are common in breast, glioblastoma, melanoma, ovary, and prostate cancers. These mutations are associated with hyperactivation of the PI3K/Akt pathway and are associated with enhanced cellular proliferation, growth, and survival.

An important downstream target from Akt is mTOR, also known as the mammalian target of rapamycin *(13,14)*. This 289-kDa serine-threonine kinase is a member of the PI3K-related kinase (PIKK) family, which is highly conserved from yeast to mammalian cells. It modulates cellular transitions between energy-rich and energy-depleted states and appears to be an important regulator of cell growth, in contrast to cell proliferation. Activation of mTOR is also controlled by cellular nutrient levels and by growth factors. It is a major downstream effector of the PI3K/Akt signaling pathway. Activation by Akt increases protein translation by stimulating the 70-kDa ribosomal S6 kinase (S6K1) and by affecting the elongation-initiation factor 4E binding protein-1 (4E-BP1). Inhibition of mTOR causes decreased cell proliferation and G1 cell cycle arrest. Emerging evidence suggestions that tumor cells with hyperactive PI3K/Akt activation caused by *PTEN* mutation are markedly growth inhibited by exposures to mTOR inhibitors.

Thus, the role of PI3K/Akt/mTOR pathway is an attractive target for cancer therapeutic development. Direct activation of this pathway can drive cell growth and proliferation in normal cells and it may block apoptotic signals in

tumors, thereby promoting malignant cell survival *(15)*. Activating mutations in *PI3K*, gene amplification of *PI3K* and/or Akt, and loss of PTEN function have all been characterized in a variety of cancer cells *(16)*. These mutations and gene amplification events suggest that the spectrum of clinical utility of agents that block PI3K/Akt signaling may be quite large *(8)*. Disadvantages include the internal redundancy of this pathway and its complex interaction with other signaling pathways, suggesting that simple inhibitors of single proteins in this cascade may have little overall biological effect. Preliminary studies showing reversal of drug resistance by inhibition of the PI3K/Akt pathway also suggests that chemotherapy drug combinations with molecularly targeted inhibitors may provide a fruitful clinical therapeutic strategy.

Recently, Vogelstein and others reported that *PI3K* mutations were very common in colon cancers. In an analysis of 74 colon tumors, 32% harbored activating *PI3K* mutations in the p110a subunit *(17)*. None of these mutations were associated with *PI3K* gene amplification. Extension of these observations to other tissues and tumor types revealed *PI3K* mutations in only 3% of nonmalignant colon adenomas, 27% of glioblastomas, 8% of breast cancers, 4% of lung cancer, and none in 11 pancreatic tumors and 12 medulloblastomas. Follow-up studies demonstrated that nearly 40% of colorectal tumors had associated alterations at some point in the *PI3K* pathway genes *(18)*. These cumulative findings emphasize the importance of the PI3K/Akt pathway in the development of invasive CRC and it strongly supports ongoing efforts to develop PI3K/Akt/mTOR inhibitors as therapeutic agents for treating this disease.

Inhibitors of PI3K have been difficult to develop clinically; however, laboratory reagents, such as wortmannin and LY294002, can readily inhibit the p110 catalytic subunit. In nonclinical studies, these agents block in vivo tumor growth in breast and pancreatic xenograft models and they are markedly synergistic in combination with chemotherapeutic agents *(19)*. However, their poor selectivity and pharmacological properties have hampered their direct clinical use *(20)*. Several semisynthetic wortmannin-like viridin analogs are in clinical development, including PX-866 (ProLX, Inc), which is due to enter phase I studies in late 2005 *(21)*. Another agent, perifosine (KRX-0401), is an orally active alkylphosphocholine derivative developed by Kerix Pharmaceuticals, and is now in phase II studies. It was initially developed as a putative Akt inhibitor *(22)*, but its precise mechanism of action is unclear. In phase I trials, it was well tolerated on an oral daily dosing schedule with dose-limiting toxicity consisting of nausea, diarrhea, dehydration, and fatigue. It was active inducing a partial response in a patient with leiomyosarcoma *(23)*. Prolonged stable disease was also seen in two patients with renal cell cancer. Further evaluation as a single agent in prostate, pancreatic, and melanoma were disappointing because of lack of efficacy. Further perifosine studies are ongoing in combination with gemcitabine, docetaxel, and radiation therapy.

Another active area of clinical research is the development of mTOR inhibitors. All clinically tested mTOR inhibitors analyzed to date are structurally related to rapamycin (sirolimus), an insoluble lipophilic macrolide natural product isolated over 20 yr ago from the bacterium, *Streptomyces hygroscopicus (13,14,24)*. This organism was found in soil samples taken from Easter Island, also know as Rapa Nui. Rapamycin is a potent fungicide and immunosuppressant that can also inhibit malignant cell growth. Clinical development of sirolimus as an immunosuppressant led to its approval in the United States for antiallograft rejection. Rapamycin-coating of cardiac arterial stents diminishes stent occlusion and these devices are approved for use in patients with coronary artery disease. However, rapamycin's poor solubility and stability problems have limited its use as an anticancer agent.

Rapamycin and it analogs bind to the immunophilin FK506-binding protein 12 (FKBP12). This forms an inhibitor rapamycin/FKB12 complex that blocks mTOR activation. This prevents the activation of the two major effector proteins downstream from mTOR: p70s6K and 4E-BP1. Rapamycin can generate profound antiproliferative and immunosuppressive effects and it is a potent growth inhibitor in human tumor xenografts models *(25)*.

The first rapamycin analog to enter clinical testing, CCI-779 (temsirolimas), is a water-soluble rapamycin ester that has a profile of antitumor activity similar to rapamycin but with better pharmacological properties *(24,25)*. In phase I studies its major toxicities were skin rash, myelosuppression (mainly thrombocytopenia), transient liver function test elevation, and asymptomatic hypocalcemia. Objective responses were seen in patients with soft tissue sarcoma, breast, renal cell, and nonsmall cell lung cancers. Minor responses occurred in patients with endometrial papillary cancer, squamous cell skin cancer and non-Hodgkin's lymphomas. In phase II trials in renal cell cancer, objective responses were seen in 7% of patients, with median survivals reported as 15 mo. In breast cancer, preliminary response rates were seen in 8.5% of patients and excellent activity in patients with mantle cell lymphoma has been reported, with preliminary response rates of 50% seen in relatively small numbers of patients. No objective response activity was seen in phase II trial in glioblastoma multiforme, but radiological improvements were noted in some patients. In phase II studies in melanoma, response rates were 3% or less. Currently, CCI-779 is undergoing development as an intravenously agent administered weekly, but oral formulations are also in development.

RAD001 (everolimus) is an orally bioavailable hydroxyl ethyl ether of rapamycin that is being clinically developed by Novartis *(24,25)*. It has excellent antitumor activity in xenograft models of a wide range of human cancers. In phase I studies administering RAD001 on weekly or daily oral schedules, the major dose-limiting toxicities were stomatitis, neutropenia, and hyperglycemia. Less substantial minor toxicities included anorexia, fatigue, rashes, mucositis,

headache, hyperlipidemia, and dyspepsia. A partial response in these phase I studies was seen in a patients with CRC, and a substantial change in positron emission tomography (PET) scan activity was reported in a patients with nonsmall cell lung cancer. Disease stabilization was also seen in patients with renal cell and breast cancers. Further clinical testing is ongoing in phase II studies in nonsmall cell lung cancers, prostate, and other solid tumors. RAD001 combination studies with gemcitabine, imatinib, and other conventional and targeted therapies are in progress.

Finally, AP23573 is a very promising intravenous and oral rapamycin analog that is also in clinical development. It is not a rapamycin prodrug. Minor toxicities associated with the intravenous formulation include chills, diarrhea, fatigue, rash, anorexia, liver function test abnormalities, and at higher doses, mucositis appears to be dose limiting *(26,27)*. Preliminary antitumor activity was evidenced by partial responses seen in phase I studies in patients with sarcoma, non-Hodgkin's lymphoma, nonsmall cell lung cancer, and minor responses occurred in patients with sarcoma, nonsmall cell lung cancer, renal, mesothelioma, and thyroid cancer *(26,27)*. Weekly dosing induced a minor response in a patient with mesothelioma. In an ongoing phase II, 27% of 188 evaluable sarcoma patients treated with AP23573 demonstrated either sustained tumor regression or disease stabilization *(28)*. These results are highly promising.

Thus, therapies that target the PI3K/Akt/mTOR pathway are in clinical development and preliminary results suggest agents such as the mTOR inhibitors may have promising activity in some solid tumor types. However, these early clinical results have not shown overwhelming evidence of clinical utility in CRC, for reasons that are as yet unexplained. Such results are modestly surprising given the abundant scientific evidence supporting the importance of this pathway in this disease. What are the potential explanations for these observations? One possibility is that the PI3K/Akt pathway is not as important in CRC as initially hypothesized. Given the growing body of evidence to date, we doubt that this is the case; although it is true that other genetic changes are also important in the development of CRC. Thus, although PI3K/Akt activation may still be a substantial step in the development of frankly invasive cancers, complete inhibition of this pathway in fully developed tumors may not be sufficient to reverse the malignant phenotype. It is more likely that combinations with other targeted agents and/or chemotherapy will be necessary to evaluate fully the potential benefits of this strategic approach. Such clinical trials are going.

2.2. Angiogenic Pathway Inhibitors: Integrin-Targeting Strategies

The success of bevacizumab and VEGF targeting has stimulated other strategies to block new blood vessel growth in tumors. The precise mechanism by which bevacizumab improves cancer chemotherapy in CRC is not known.

Originally, such strategies were designed to "starve" the tumor by blocking its blood supply; however, bevacizumab is most effective in combination with blood-borne chemotherapy and it has relatively modest activity as single agents. One alternative hypothesis is that VEGF targeting actually normalizes tumor vasculature and enhances drug delivery to the growing tumor cells *(28a)*. Nonetheless, this lack of a complete mechanistic understanding of VEGF-directed therapy this has not deterred other antiangiogenic strategies from entering clinical development.

One novel strategy has targeted a large and diverse family of cell adhesion proteins called integrins. These are a complex superfamily of transmembrane glycoproteins that exist as heterodimers composed of α and β chains *(29,30)*. Different heterodimers have different specificities of ligand binding and are associated with a variety of functions in a broad range of tissues. There are 18 known α integrin subunits and 8 β subunits, which associate to form at least 24 heterodimeric α/β pairs. Integrins have dual functions, serving as cell-surface adhesion structural proteins and as signaling receptors, although unlike receptor tyrosine kinases, they have no intrinsic enzyme activity. Integrins mediate cell–extracellular matrix and cell–cell interactions. Certain integrins can stimulate endothelial and tumor cell survival, whereas others promote cell aggregation and can regulate cell trafficking functions. They are postulated to have major but complex roles in tumor angiogenesis, invasion, and metastases *(31)*. Some integrin subfamilies are functionally interrelated to signaling via the PI3K/Akt pathway in some systems such as hormone resistant prostate cancer cells *(32)*. The various integrin heterodimers have different ligand-binding specificities. For example, the fibronectin protein binds to α4β1 and α5β1 integrins, where as lamin and collagen bind to α3β1, α2β1, and α1β1 integrins *(29,30)*.

A number of integrin targeting agents have entered clinical trials. One such agent is M200 (volociximab), a chimeric IgG4 anti-α5β1 antibody being developed by Protein Design Laboratories, Inc *(33)*. The α5β1 integrin is involved in cell adhesion, migration, and enhanced cell survival. It is present on tumor endothelial cells and it helps to regulate growth factor-activated endothelial cells. The *α5β1* integrin is also present on some tumor cells and on monocytes; however, it is not seen in endothelial cells from normal blood vessels. Inhibition of *α5β1* fibronectin binding leads to apoptosis of activated endothelial cells, inhibition of cell migration, and prevention of tumor-associated neoangiogenesis. The M200 antibody blocks binding of *α5β1* to fibronectin and it can inhibit tumor endothelial cell *α5β1*-mediated angiogenesis *(33)*. In laboratory studies it can inhibit human endothelial cell tube formation and it blocks choroidal neovascularization in primate models. It also actively blocks blood vessel growth in xenografts in chicken chorioallantoic membrane (CAM) assays. M200 cross reacts with chicken and primate *α5β1* but not with rodent integrins, making it inactive in standard rodent-based models. In a Phase I study of intravenously

administered M200 infused over 1 h weekly, it was well tolerated with no serious dose-limiting adverse events *(33)*. Pharmacokinetics were non-dose proportional with decreased clearance seen at higher dose levels consistent with possible saturation tissue kinetics. The overall plasma half-life was approx 16 d. In the first six evaluated patients, a partial response was observed in a patient with renal cell cancer and prolonged stable disease lasting longer than 4 mo was seen in patients with nonsmall cell lung cancer, melanoma, and parotid cancers. No disease stabilization was seen in the four patients with colon cancer enrolled in this study. Currently, M200 is in phase II studies in renal cell cancer.

Eisai Pharmaceuticals has adopted a strategy of using small molecule inhibitors of integrins in the development of E7820, a novel oral sulfonamide agent that reduces the expression of α2, α3, α5, and β1 mRNA in tumor tissues *(34)*. E7820 blocks VEGF- and bFGF-induced human umbilical vascular endothelial cell tube formation in laboratory experiments and it effectively inhibits the growth of human breast and pancreas cancer xenografts. In a phase I study of daily oral E7820, the agent was well tolerated with dose-proportional pharmacokinetics. Major dose-limiting toxicities consisted of grade 3 thrombocytopenia and elevation of hepatic transaminases. Prolonged disease stabilization lasting longer than 6 mo was noted in four total patients, two with CRC and one with bladder cancer and malignant melanoma *(34)*. Current studies are examining E7820 in combination with irinotecan, cetuximab, and other targeted therapies. In preclinical in vitro and in vivo data, marked synergy was observed when E7820 was combined with other chemotherapeutic and targeted agents.

Other integrin-targeting agents in clinical development include MEDI522 (Vitaxin®) a humanized IgG1 anti-αvβ3 antibody developed by MedImmune *(35)*. It is in phase II trials against melanoma, prostate, leiomyosarcoma, and CRCs. Cilengitide (EMD121974) is a cycle peptide inhibitor of *αvβ3* and *αvβ5* developed by Merck KGaA *(36)*. This agent is in phase II studies in gliomas, melanoma, and prostate cancers. Finally, CNTO 95 is a fully human IgG1 anti-*αvβ3* and anti-*αvβ5* antibody that is being developed by Medarex/Centocor *(37)*. Thus, this remains an active area of clinical development.

2.3. Conventional Cytotoxic Agents

Despite the emphasis on developing targeted therapies as the wave of the future of anticancer drug discovery, conventional cancer chemotherapy may still provide substantial therapeutic advantages in the treatment of CRC. One promising example, DJ-927, is a microtubule-targeting taxane derivative with excellent oral bioavailability *(38)*. Preclinically, it is much more potent than standard taxanes such as docetaxel or paclitaxel in stabilizing microtubules, and it has excellent activity against taxane-resistant tumor cells that contain tubulin mutations or that over express P-glycoprotein (MDR-1). DJ-927 has a broad spectrum of antitumor activity in human and murine tumors and it

does not need Cremophore E or Tween-80 as a vehicle for oral administration. In phase I studies administering DJ-927 orally every 3 wk, the agent was well tolerated with the major dose-limiting toxicities being the expected neutropenia and myelosuppression *(38)*. Peripheral neuropathy was infrequent. Minor tumor responses were observed in patients with bladder and breast cancers. The pharmacokinetics were dose proportional with excellent and predictable systemic absorption. In preliminary phase II studies, an impressive number of preliminary objective responses have been observed in patients with colorectal and gastric cancers *(39)*. Further phase II studies with DJ-927 are ongoing.

3. CLINICAL IMPLICATIONS OF DEVELOPING TARGETED THERAPIES FOR CRC

To date, complete surgical resection remains the only curative strategy for CRC. However, two-thirds of patients undergoing resection experience local or distant recurrence, with 85% of those cases diagnosed within the first 2.5 yr after surgery. Therefore, identifying the patients who should receive systemic treatments (especially stage II patients) is an important consideration. Chemotherapy for CRC has three primary goals: adjuvant therapy to prevent local or metastatic recurrence; palliative therapy to prolong survival and improve quality of life; and neoadjuvant therapy to improve relapse-free survival time or enable secondary resection *(40)*. Nonetheless, the cold fact remains that a large proportion of patients undergoing systemic therapy for their disease do not obtain objective clinical benefit from their treatment. Alternatively, the benefit that they do obtain may be associated with substantial drug-related toxicities and morbidity.

One of the great promises of the paradigm shift that is emerging in this new era of targeted therapy is the emphasis on developing more uniquely specific treatments tailored to the individual patient. A strict corollary to using specific targeted therapies to hit unique genetically defined aberrations in tumor cells is the associated strategy of individualizing treatments based upon a patient's own genetic background and tumor biology. The growing availability of new targeted therapies in clinical practice offers increasing choices for physicians. Thus, there is a growing need to develop well-validated biomarkers to select the optimal targeted therapy best suited to optimize therapeutic response. Understanding the variability of tumor biology of individual patients with the same diagnosis may not only allow for the stratification of high-risk patients into those who may or may not benefit from adjuvant therapy, but it may also provide insight into which therapies are likely to be most effective. Biomarkers can also be used to predict unwanted toxicities or side effects and may theoretically influence not only therapeutic selection but dosage as well.

Bringing predictive biomarkers for individualized patient therapy to the bedside requires several important considerations. Just as standardizing the width and size of railroad tracks helped pave the way for transcontinental travel in the United States, the first and perhaps most important consideration in biomarker development is identifying collection and analysis standards that permit data to be used and compared across laboratories and institutions. The source material from which biomarkers will be examined must also be considered. The source material can dramatically affect the robustness of an assay. Tumors are genetically unstable and are rarely pure populations of cells. Therefore, robust molecular assays that function reliably in a genetically diverse and impure population of cells are preferable. For this reason, DNA and antibody-based assays might be preferable to RNA expression-based biomarkers, which require more stringent collection and isolation procedures.

For validated biomarkers to be used in the clinic, they should easily integrate into existing standards of care. A minimally invasive procedure for sample collection or biomarker delivery is also preferred. For example, an assay that utilizes standard formalin-fixed paraffin-embedded tissue blocks would be preferable over those requiring snap-frozen core biopsies. Many of the biomarkers predicting drug sensitivity or toxicity based upon drug-transport or drug-metabolizing enzyme polymorphisms can be identified from readily accessible whole blood. Also desirable would be biomarker assays that can be delivered and viewed noninvasively through functional imaging technologies such as PET *(41)*, dynamic contrast-enhanced magnetic resonance imaging *(41)*, or thermoacoustic tomography (TAT) *(42)*. The advantage of bringing the "biomarkers to the patient" through functional imaging is that not only can the biomarkers be observed in their natural environment, but also their distribution within the tumor can more accurately be assessed. One limitation of many of these imaging technologies is the lack of ability to view concurrently multiple molecular markers from the same scan or image.

Ideally, molecular characterization of CRCs will not only provide insight into new targets for therapeutic intervention, but in the future, will form the basis for therapeutic selection *(43)*. It is important to remember that in most cases, standard treatment selections will not be single agents, but rather, therapeutic combinations. Therefore, clinical experiments designed to validate biomarkers that predict the potentially strong synergistic effects of combinatorial therapies should always be considered. Predicting drug synergism can be a very complex task that must also take into account dosage, timing, side effects, and toxicities.

Pathway-based therapeutic selection is one proposed strategy for rationally developing and selecting optimal combinations of drugs for patient care. This strategy works under the assumption that cancer is a progressive accumulation of nonrandom mutations. Within each deregulated pathway is a complex cascade

of regulatory oncogenes and tumor suppressors that represent potential targets for mutation, deregulation, and therapeutic intervention. The process of systematically screening patients for (1) polymorphisms predicting potential drug toxicity and (2) tumor-specific mutations driving deregulation of multiple proliferative pathways will someday provide the rational basis for selecting therapeutic strategies. For example, imagine a hypothetical CRC tumor that involves the EGFR as a primary site for deregulation. EGFR is a HER-family tyrosine kinase that initiates survival and proliferation signals through the Ras/Raf//MEK/ERK and PI3K/AKT pathways. In this example, EGFR can be activated via overexpression through an autocrine loop involving one or several of its ligands. EGFR might also be activated through direct mutation of its kinase domain. Alternatively, mutations might be focused downstream in the AKT and K-Ras pathway (*see* Fig. 1). In this example, hypothetical antibody-based treatment options can target the EGFR ligand, EGFR itself, or the downstream targets AKT and K-Ras. Conceptually, if an activating mutation of the EGFR kinase domain is driving the deregulation of this pathway, then selecting a therapy targeting an upstream event might prove ineffective, because limiting the availability of ligand to the cell does not overcome the constitutive activation of EGFR. Likewise, targeting a single downstream event like K-Ras or AKT alone might only provide partial response. It is important to note that the hypothetical example just presented is a gross oversimplification of the EGFR pathway in which substantial cross-talk and redundancy drive this pathway and our true knowledge of the genes regulating this pathway remains rudimentary. However, it does illustrate how knowledge of the molecular events driving the proliferation of CRC cells can prove important for therapeutic selection as more and more targeted treatment options become available to clinicians.

The unique genetic alterations present in CRC represent the catalog of potential targets for future therapeutic advances in this disease. Currently, the list of genes recognized as contributing to cancer progression is growing as we start to understand better the molecular basis of cell cycle regulation, genomic stability/repair, apoptosis, angiogenesis, immortality/senescence, immune response, invasion/metastasis, and drug metabolism, transport, and resistance. Add to this the numerous mechanisms to activate or inactivate oncogenes and tumor suppressors including: insertions, amplifications, deletions, recombinations, translocations, transversion, missense, nonsense, and frameshift mutations, as well as epigenetic events, it seems amazing any mutational patterns with the potential for clinical utility in CRC emerge at all. And yet, surprisingly enough, mutational patterns do emerge and are well documented in CRC *(44)*. For example, 50% of CRCs and large polyps contain activating missense mutations of *K-Ras (45)*. Mutations in *BRAF*, a member of the Raf family of serine-threonine kinases, are found in 9–11% of CRCs. As mentioned previously, mutations in the catalytic subunit of PI3K (*PI3KCA*) are implicated in 32% of

CRCs, and 30% of CRCs have mutations in the tyrosine kinase family of genes including *FES*, *NTRK1-3, KDR, EPHA3*, and *MAP3K10*. Somatic mutations in CRC have also been reported in genes coding for *CTNNB1, BAX, FBXW7*, *SMAD2, TGFBR1–2, MLL, PTNP1*, and *PTNP11*. However, a major challenge for the years ahead is to better understand the functional consequences of these genetic patterns and to determine how best to exploit these differences in the clinic.

4. CONCLUSIONS

This superficial overview has described some of the more promising therapeutic targets and drug development strategies that offer the potential for improving future treatments for patients with CRC. We have also attempted to describe some of the shifting paradigms that will greatly alter both cancer drug development and the implementation and use of these new agents by practicing medical oncologists in the age of targeted therapies and molecular profiling of patients and their tumors. Undoubtedly, many of these highly promising experimental agents currently in clinical testing will fail, and some unexpected approaches will yield surprising clinical benefits, given the inherent uncertainties in cancer developmental therapeutics.

However, our current level of modest success raises a number of issues. Currently, phase III studies in CRC are complicated by the rapid increase in number of agents active in this disease. This level of progress also makes it difficult to anticipate what the standard of care for patients with advanced CRC will be in 2 or 3 yr from now. Paradoxically, this may inhibit CRC drug development because it complicates registration and clinical development planning for new agents in this disease, as clinical research scientists attempt to hit a "moving target." However, rather than being discouraged, as investigators in applied cancer research we find this perspective to be exhilarating. The level of science that is driving this process is unprecedented and while overexuberant expectations must always be tempered, we have no doubt that the next decade will herald many more therapeutic successes in the treatment of common solid tumors. Amazingly, CRC therapeutics, which was once a backwater of new drug development during the 35 yr when 5-FU reigned supreme as the only active drug for this disease, is now a model for the development of new targeted therapies for solid tumors.

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Evidence-Based Chemotherapy Strategies

Edited by
Leonard B. Saltz, MD
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Recent advances in the understanding of the biology of colorectal cancer have radically transformed the treatment options available to clinicians, leaving textbooks written only two years ago completely out of date. This evidence-based and data-driven guide presents reviews of cutting-edge therapies for colorectal cancer, all articulated by leading experts in the field. This integrated and focused consideration of colorectal management includes reviews of cytotoxic chemotherapy, targeted biologic therapies, as well as updates on adjuvant chemotherapy for colon cancer and combined modality management of rectal cancers. Attention is also focussed on the role of potentially curative multi-modality management of liver metastases, with considerations of the role of liver resection and discussions of non-surgical ablative therapies. Supportive issues are also considered, including contributions from experts in pain management and oncology nursing. The reviews presented here are concise, up-to-date, and are organized with the treating practitioner in mind.

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