New Treatment Strategies for Metastatic Colorectal Cancer

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New Treatment Strategies for Metastatic Colorectal Cancer

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About the Activity

The CME activity is based on the information learned from reading this monograph, New Treatment Strategies for Metastatic Colorectal Cancer. It was developed from an identified educational need for information about practical management issues in the practice of medical, surgical, and radiation oncology.

This activity has been developed and approved under the direction of the CME LLC.

Activity Learning Objectives

After reading New Treatment Strategies for Metastatic Colorectal Cancer, participants should be able to:

• Use treatments for metastatic colorectal cancer that are tailored to individual situations and therapeutic goals.
• Demonstrate the advantages and disadvantages of currently used biologics (in combination with chemotherapy) based on the latest trial data.
• Incorporate and appraise the variables that go into making a first-line treatment choice, which will affect second- and third-line treatment choices.
• Use an algorithmic methodology in determining first-, second-, and third-line treatment choices.
• Incorporate and appraise the variables that go into treating liver-limited disease.
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Edward Chu, M.D.
Colorectal cancer (CRC) is a major public health problem in the United States and throughout the world. Each year, this disease affects nearly 150,000 new patients, and it is the second leading cause of mortality, accounting for almost 50,000 deaths in the United States. Worldwide, CRC afflicts nearly 800,000 individuals and is associated with 500,000 deaths. Over the past 10 years, significant advances have been made in the screening and early detection of CRC as well as in the different treatment approaches, including surgery, radiation therapy, and chemotherapy, which are now available for patients with early-stage CRC and more advanced stages of the disease. Without question, CRC has now become a highly preventable and curable disease through regular screenings and early detection, and highly treatable when diagnosed at an even more advanced stage.

In this book, we have condensed and summarized a wealth of information on the treatment approaches for patients with advanced, metastatic CRC and present essential information in a practical and readable format. It is critical that a team of physicians from various specialties, including surgery, radiation oncology, medical oncology, pathology, and radiology, provide their special expertise in developing individual treatment plans for patients with CRC. The first chapter focuses on the biology of CRC and the key pathways involved in cancer formation, whereas the last chapter provides a nice overview of the role of prognostic and predictive biomarkers that are now being developed for this disease. The three middle chapters are devoted to an update on the role of cytotoxic chemotherapy, the integration of biologic agents in treatment regimens, and the role of a combined modality approach to treat patients with liver-limited metastatic disease. These chapters provide a timely review of the most up-to-date information presently available.
My hope is that this book will serve as a source of practical information that can be used by physicians and other health care professionals actively involved in the daily care of patients with CRC. This book should be viewed as a work in progress, and our hope would be to update it in the future and to incorporate new drugs and treatment strategies that reflect the rapid advances in the field of CRC.

Edward Chu, M.D.
Colorectal cancer (CRC) is a major public health problem in the United States and throughout the world. In the United States, it is third in cancer incidence and the second leading cause of cancer mortality. In 2008, there will be an estimated 52,000 deaths associated with this disease (1). Worldwide, there will be nearly 1 million new cases diagnosed in 2008, resulting in approximately 500,000 deaths.

Significant advances have been made in the understanding of the biology of CRC. It is now clear that CRC arises as a consequence of the progressive accumulation of genetic and epigenetic alterations that drive the transformation and progression of normal colonic epithelial cells to true cancer. Vogelstein and colleagues have played a central role in developing a genetic model for this disease, as seen in Figure 1 (2). There are several key features of this model, including the following: (a) mutational activation of oncogenes along with mutational inactivation of key tumor suppressor genes plays a critical role in the development of CRC; (b) mutations in at least four to five genes are required for tumor formation to occur; (c) the total accumulation of genetic mutations as opposed to their specific order with respect to one another is the more critical event; and (d) mutant tumor suppressor genes have been shown to exert a biologic effect even when present in the heterozygote state.
The process of colorectal tumorigenesis has been termed the polyp-carcinoma sequence, and it generally takes place over an 8- to 11-year time frame. There appears to be acceleration of this process in familial adenomatous polyposis and hereditary nonpolyposis CRC (HNPCC), the two major forms of hereditary CRC. Of note, familial adenomatous polyposis makes up 1%–2% of hereditary CRC, and it arises from genetic mutations in the adenomatous polyposis coli (APC) gene, whose protein end product plays a key role in the Wnt/β-catenin signaling pathway. HNPCC accounts for approximately 5%–8% of the hereditary forms of CRC, and it is caused by genetic mutations in the family of mismatch repair (MMR) genes, which include MSH1, MSH2, MSH6, and PMS2 (3). In fact, germline mutations in one of these four MMR genes have been identified in up to 80% of affected families, with 50% of the mutations affecting MSH1, 40% involving MSH2, and 10% involving MSH6. Mutations in PMS2 account for less than 5% of all HNPCC cases. The MMR system functions to preserve genomic integrity, and it is therefore not surprising that defects in this critical DNA repair function lead to the development of CRC and other solid tumors. Another key hallmark of MMR is its ability to mediate DNA damage-induced cell death. There is a large body of evidence documenting that CRC tumors with defective MMR are resistant to a broad range of cytotoxic agents, including the fluoropyrimidines, the platinum analogs cisplatin and carboplatin, the thiopurines, and several alkylating agents. As such, MMR-defective CRC tumors would be expected to have a selective growth advantage when compared with MMR-intact tumors.

In contrast to hereditary CRC, which makes up approximately 8%–15% of all cases of CRC, sporadic CRC accounts for nearly 85%–90%
of cases. The significant insights gained from studying inherited CRC, however, have contributed greatly to the current understanding of sporadic disease. Specifically, the same genetic alterations leading to dysregulation of key cellular signaling pathways in hereditary CRC have been implicated in the pathogenesis of sporadic CRC, and they include the Wnt/β-catenin, transforming growth factor (TGF)-β receptor, Notch, and Hedgehog (Hh) signaling pathways (3). Other important signaling pathways are critical for the regulation of colonic epithelial growth, including the epidermal growth factor (EGF) receptor, RAS/RAF/mitogen-activated protein kinase (MAPK) cascade, and phosphoinositide 3'-kinase (PI3K)/Akt signaling pathways. This chapter reviews the key role these signaling pathways play in the development of CRC.

**Wnt/β-Catenin**

The Wnt signaling pathway plays a critical role in embryonic development and maintenance of homeostasis in mature tissues, in particular intestinal epithelial regeneration (4). The Wnt family of proteins is secreted extracellular glycoproteins, and their target receptors are Frizzled (Fz), a transmembrane receptor protein, and low-density lipoprotein receptor–related protein 5 or 6 (LRP5 or LRP6). Binding of Wnt to its cognate receptor activates four different downstream signal transduction pathways: (a) Wnt/β-catenin pathway; (b) planar cell polarity pathway; (c) Wnt/calcium pathway; and (d) Wnt/protein kinase A pathway.

The major component of the Wnt/β-catenin pathway is the β-catenin destruction complex; this complex is composed of a tumor suppressor protein encoded by the APC gene, Axin, CKI, and GSK3. In the absence of receptor binding, the destruction complex binds to newly synthesized β-catenin protein, which is then rapidly degraded by the ubiquitin-proteasome pathway. In contrast, receptor binding by Wnt ligands inactivates the β-catenin destruction complex, resulting in accumulation of β-catenin. In this scenario, β-catenin is translocated into the nucleus to form a complex with TCF/LEF, a transcription factor, leading to transcriptional activation of certain target genes including c-Myc and Cyclin D.

Dysregulation of the Wnt/β-catenin signaling pathway has been observed in several cancers (5). For example, overexpression of Wnt ligands has been reported in various solid tumors, including CRC. The APC gene is mutated in both sporadic and familial CRC. The APC gene mutation gives rise to a truncated protein, resulting in a defective β-catenin destruction box, which is then no longer able to bind to β-catenin.
This mutation in the APC gene leads to decreased degradation of β-catenin and abnormal accumulation of β-catenin in the nucleus, with subsequent constitutive activation of Wnt target genes.

Mutations in the β-catenin gene have also been identified in several tumors, including CRC. Destruction of β-catenin by the proteasome pathway requires phosphorylation of the serine/threonine-rich region of the β-catenin. Mutations at these putative phosphorylation sites prevent the destruction of the β-catenin by the proteasome pathway, and the accumulated β-catenin protein induces constitutive activation of Wnt signaling.

**Transforming Growth Factor-β/SMAD**

The TGF-β signaling pathway plays a critical role in several essential biologic processes, including cell proliferation, differentiation, migration, and apoptosis (6). TGF-β signaling is initiated by binding of TGF-β ligands, of which there are three isoforms, to type II TGF-β receptors (TGFBR2). Upon ligand binding, TGFBR2 recruits and phosphorlates the type I TGF-β receptor (TGFBR1), which then phosphorlates two downstream transcription factors, SMAD2 and SMAD3. Phosphorylation of SMAD2 and SMAD3 leads to the formation of a hetero-oligomeric complex with SMAD4, and the resulting complex then translocates into the nucleus to interact with a broad range of transcription factors in a cell-specific manner, including c-jun; p300/CBP; c-myc; as well as cyclin-associated proteins cyclin D1, cdk4, p21, p27, p15, and Rb.

Several of the key downstream targets of TGF-β signaling are key cell-cycle checkpoint genes, such as p21, p27, and p15, and their activation leads to growth arrest. In this scenario, TGF-β would appear to play a critical role as a tumor suppressor. However, TGF-β signaling has also been shown to directly stimulate the production of several mitogenic growth factors, such as TGF-α, fibroblast growth factor, and EGF. In addition, SMAD-independent pathways, including RAS/RAF/MAPK, JNK, and PI3K/Akt, can be activated, all of which can drive the carcinogenic process. Finally, TGF-β has been shown to promote angiogenesis as well as regulate cell adhesion, motility, and the extracellular matrix, and these various processes collectively can lead to enhanced tumor invasion and metastasis. Presently, the precise mechanism(s) by which TGF-β can go from a tumor suppressor to a tumor promoter remains to be characterized but no doubt depends on the particular cellular context and milieu.

The gene encoding the TGFBR2 has repeats of A nucleotides in exon 3 and repeats of GT nucleotides in exons 5 and 7. These nucleo-
tide repeats are prone to DNA replication errors and frameshift muta-
tion, especially in the presence of DNA MMR gene inactivation.
Frameshift mutations in the TGFBR2 are present in up to 80% of CRC
with microsatellite instability (7). The overall incidence of this particu-
lar mutation in patients with sporadic CRC is approximately 30%, and
to date, this is the most common mechanism that leads to alterations in
TGF-β signaling (8).

Mutations in the TGFBR1 gene appear to be relatively rare in CRC.
The TGFBR1 *6A polymorphism has been reported to be related to
increased risk of CRC (9). However, the biologic significance of this
polymorphism remains to be established, as there does not appear to be
any significant difference in protein sequence of TGFBR1 between the
mutant TGFBR1 *6A and the wild-type gene.

Alterations in SMAD genes, either through deletion or mutation, can
also impair TGF-β signaling. SMAD4 was originally discovered as a
tumor-suppressor gene that was deleted in pancreatic cancer (DPC4,
deleted in pancreatic cancer 4) and is mutated in up to 25% of patients
with CRC (10). Alterations in SMAD2 have been found in less than
10% of cases. Interestingly, both SMAD2 and SMAD4 are localized on
chromosome 18q, a region that is commonly deleted in CRC. Together,
mutations in SMAD2 and SMAD4 are observed in up to 10%–25% of
CRC. In contrast, mutations in SMAD3 are a relatively rare event in
human CRC.

There is now growing evidence that alterations in TGF-β signaling
lead to the development of CRC. Although further work is required to
more carefully dissect the role of each component, this signaling path-
way is emerging as an attractive target for cancer drug development.
Such research should then provide the basis for designing novel and
rational therapeutic approaches.

**Notch Signaling Pathway**

The Notch signaling pathway plays a critical role in the proliferation of
intestinal epithelium. Five membrane-bound Notch ligands have been
identified: Jagged1, Jagged2, Delta-like (Dll) 1, Dll 3, and Dll 4 (11).
Under physiologic conditions, binding of Notch ligands to their cognate
transmembrane receptors (Notch 1–4) initiates proteolytic cleavage of
the receptors by α-secretase and γ-secretase to release the intracellular
domain of the Notch receptor. The cleaved Notch receptors (NICD)
translocate into the nucleus and form complexes with RBP-jκ (CSL or
CBF-1) and induce transcriptional activation of Notch-target genes. One
such Notch-target gene is hairy/enhancer of split (Hes1), a basic helix-loop-helix transcription factor, which activates downstream target genes (12). Activation of Notch signaling results in several important physiologic functions, which include maintenance of stem cells, determination of cell fate, regulation of differentiation, and oncogenesis.

Notch signaling has been shown to be constitutively activated in a broad range of cancers as a result of several genetic alterations via chromosomal translocation, point mutations, gene amplification, and other epigenetic events (13). Chromosomal translocations have been identified in T-cell acute lymphoblastic leukemia and non–small-cell lung cancer; gene amplification has been observed in ovarian cancer and breast cancer. Increased expression of Notch ligands is present in several solid tumors, including pancreatic cancer and CRC. Moreover, there appears to be intimate cross-talk between the Notch and RAS signaling pathways, where RAS-activating mutations have been shown to activate Notch signaling, and Notch activation is required for RAS-mediated transformation.

Hedgehog Signaling Pathway

The Hh signaling pathway was initially identified in Drosophila melanogaster and was found to play an important role in regulating proliferation, in establishing cell fate in flies, and in embryonic development (12,14). The Hh pathway has also been identified in humans, and it is critical for normal development and patterning of various organs, including the gut epithelium. There are three Hh homologues in humans: Indian (Ihh), Sonic (Shh), and Desert (Dhh). The receptor for Hh ligands is the Patched protein (Ptch), which suppresses the activity of Smoothened (Smoh), a G protein–coupled receptor-like receptor. Binding of Hh ligands to PTCH1 activates Smoh-mediated activation of GLI transcription factors, and this interaction then regulates the expression of several Hh target genes.

Abnormal activation of Hh signaling pathway has been associated with enhanced cell proliferation and the development of cancer (12,15). The Hh signaling pathway appears to play an essential role in certain types of solid tumors, especially basal cell skin cancer and medulloblastoma. Of note, the Hh signaling pathway is a key player in the development and repair of colonic epithelial cells, and studies have shown it to be activated in CRC. Various investigators have reported upregulation of levels of the Hh ligand Shh, the Hh receptor Ptch, and the Hh-associated transmembrane receptor Smoh in hyperplastic polyps, adenomas,
and adenocarcinomas of the colon. Interestingly, exogenous Shh is capable of promoting the growth of primary murine colonocytes, a finding that suggests that the signal triggered by Shh may facilitate CRC progression. Finally, it has been shown that colon cancer cells express significantly higher levels of Shh mRNA when compared with normal colon cells (16).

RAS

RAS is a member of the monomeric small G protein family (guanine nucleotide-binding proteins); the RAS superfamily of proteins plays a critical role in transmitting key extracellular signals, such as EGFs into intracellular signal transduction cascades (Figure 2) (17). More than 100 small monomeric G proteins in the RAS superfamily have been identified to date. RAS proteins possess guanosine diphosphate (GDP)/guanosine triphosphate (GTP)-binding and intrinsic GTPase activities, allowing them to switch between active (GTP-bound) and inactive (GDP-bound) conformations.

The RAS family of proteins contains a CAAX motif in the C-terminus, which serves as a substrate for post-translational lipid modification, including the covalent attachment of farnesyl pyrophosphate or geranylgeranyl pyrophosphate to the cysteine residue of the CAAX motif by prenylation (farnesylation or geranylgeranylation) (18). After lipid modification through palmitoylation, RAS proteins are transferred and attached to the plasma membrane through their farnesyl and palmitoyl moieties. RAS proteins activate the RAF/M EK/ERK signaling cascade, which then mediates cell growth and cell cycle entry via phosphorylation of key transcription factors such as c-FOS and M YC, phosphorylation of the RSK (ribosomal protein S6 kinase) and M N K (M APK-interacting serine/threonine kinase), and activation of the PI3K/A kt pathway.

The role of RAS in cancer is well-established (19). Approximately 15%–20% of all human cancers carry mutations in the RAS gene. Three RAS genes have been identified, which are translated into four RAS proteins: H RAS, N RAS, K RAS4A, and K RAS4B. Each of the RAS subtypes is activated in different types of cancers. In particular, K RAS is primarily activated in pancreatic cancer, CRC, non-small-cell lung cancer, and seminoma. In pancreatic cancer, the frequency of mutations may be as high as 90%. The main hot spots for these activating mutations are located near the bound nucleotide, in proximity to the nucleotide phosphate groups. Although naturally occurring mutations have been identified at residues 12, 59, and 61, the most commonly affected residues are
Figure 2. RAS signaling cascade. IGF-1R = insulin-like growth factor receptor 1.
at position 12 and 61. Each of these RAS gene mutations allows for stabilization of the RAS protein. The end result is a RAS protein that is mainly in the GTP-bound form, making it constitutively active and independent of stimulation by exogenous growth factors, such as EGF.

With respect to CRC, mutations in the KRAS gene occur in up to 30%–40% of all patients. As mentioned earlier, KRAS protein is a pivotal downstream component of the EGF receptor (EGFR) signaling cascade. As such, mutant KRAS proteins that are in a constitutively active conformation would presumably render tumor cells resistant to anti-EGFR agents, such as cetuximab or panitumumab. In support of this possibility, there are now a growing number of clinical studies that have shown that anti-EGFR antibody therapies are essentially inactive in patients with metastatic CRC whose tumors express mutant KRAS (20). This rapidly evolving area of clinical research is discussed in more detail in Chapter 5.

### Epidermal Growth Factor Receptor

The EGFR is a member of the HER (human EGFR) family, and includes HER1 (EGFR, ErbB-1), HER2 (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4) (21). The natural ligands for EGFR include EGF, TGF-α, amphiregulin, heregulin, heparin-binding EGF, and β-cellulin (Figure 3). On ligand binding, the EGFR can either undergo receptor dimerization by binding to a second EGFR molecule or preferentially forms a heterodimer with other members of the HER family, with the greatest affinity to ErbB-2. This process then mediates activation of a complex signaling network that regulates cell growth, proliferation, survival, invasion and migration, and even angiogenesis (22). Moreover, when this signaling pathway is activated, a scenario is set up in which tumor cells are resistant to chemotherapy as well as radiation therapy.

EGFR is differentially expressed in normal, premalignant, and malignant tissues, and overexpression of EGFR has been documented in up to nearly 90% of cases of metastatic CRC (21,23). In addition, EGFR is overexpressed in a broad range of solid tumors and is involved in their growth and proliferation through various mechanisms. Given the documented role of EGFR in the development and progression of cancers, this receptor-signaling pathway represents a rational target for drug development.

Cetuximab and panitumumab are anti-EGFR monoclonal antibodies presently approved by the U.S. Food and Drug Administration for the treatment of metastatic CRC; each of these agents is discussed in greater detail in Chapters 2 and 3. These antibodies bind with higher affinity to the EGFR than its natural ligands, and competitively inhibit binding of
Figure 3. Epidermal growth factor receptor (EGFR) signaling pathway. Binding of epidermal growth factor (EGF) or ligands of the EGF family, such as amphiregulin (AREG), epiregulin (EREG), and transforming growth factor (TGF), to EGFR induces homodimerization/heterodimerization of the receptor and phosphorylation of specific tyrosine residues (P). This leads to activation of downstream RAS/RAF/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways and expression of genes related to cell survival, proliferation, angiogenesis, metastasis, and resistance to chemotherapy and radiotherapy. PI3K/Akt signal transduction is negatively regulated by the oncoprotein PTEN. Loss of PTEN results in constitutive activation of Akt, stimulating cell survival.
the natural ligands to EGFR, thereby blocking receptor phosphorylation and downstream growth signaling, inducing receptor internalization, and reducing the level of EGFR expression on the cell surface (24). They have been shown to exert antitumor effects through inhibition of cell proliferation by inducing cell-cycle arrest and apoptosis and inhibiting tumor angiogenesis. In contrast to anti-EGFR antibodies, small-molecule inhibitors of the tyrosine kinase domain of EGFR have not been shown to have clinical activity when used alone to treat metastatic CRC. However, the underlying reason(s) for why there is such a differential activity between antibodies and small molecules remains unclear at this time.

**PI3K/Akt**

The PI3K signaling cascade plays an integral role in regulating several key cellular processes required for tumorigenesis, including protein synthesis, glucose metabolism, cell survival and growth, proliferation, cell repair, cell migration, and angiogenesis (Figure 4) (25). The superfamily of PI3 kinases is made up of 12 members; at the structural level, PI3K is composed of a 110-kDa catalytic subunit and an 85-kDa adaptor subunit. Signaling is modulated in multiple ways, including via growth factors (EGF, insulin-like growth factor 1, fibroblast growth factor), hormones (estrogen, thyroid hormones), vitamins, integrins, intracellular calcium, and the RAS-dependent MAPK pathway. On activation, the p85 subunit is recruited to the intracellular part of the growth factor receptor. Subsequent dimerization with the p110 subunit then leads to full enzymatic activity of PI3K, with subsequent generation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3), a lipid “second messenger” that has the capacity of binding and activating proteins with PH domains, localizing them to the cell membrane. PH domain proteins include 3-phosphoinositide–dependent protein kinase 1 (PDK1), which is responsible for the activation of Akt/PKB, a serine/threonine kinase (i.e., the best understood downstream effector of PI3K). PI3K is negatively regulated at the level of PIP3 by phospholipid phosphatases, such as the phosphatase and tensin homologue PTEN and the inositol 5' phosphatase 2 SHIP2. The underlying mechanism is dephosphorylation of PIP3 into its inactive form, PIP2.

There are several known effectors of PI3K, but the one most relevant for cell proliferation and cell survival is Akt (26). The Akt family of serine/threonine kinases consists of three members, Akt1, Akt2, and Akt3. Activation of Akt leads to enhanced cell growth and proliferation through downregulation of p21 and p27, through increased translation and stabi-
Figure 4. Phosphoinositide 3'-kinase (PI3K)/Akt signaling pathway. GSK3β = glycogen synthase 3β, NF-κB = nuclear factor-κB, PIP3 = phosphatidylinositol (3,4,5)-trisphosphate.
lization of cyclin D1, and through activation of the mTOR pathway. The process of cell survival is mediated through several different mechanisms, including inhibition of the pro-apoptotic Bcl-2 family member Bad, inhibition of the forkhead transcription factors that activate apoptosis-associated genes, and activation of nuclear factor-κB transcripational activity. In addition, Akt1 activity has been shown to enhance the secretion of matrix metalloproteinases and induce epithelial to mesenchymal transition, whereas increased expression of Akt2 appears to play an important role in upregulating the expression of β1 integrins and the process of cellular adhesion and motility. Taken together, these findings suggest that the Akt family members are important downstream mediators of cellular adhesion, motility, invasion, and metastasis.

The PI3K signaling pathway is constitutively activated in a broad range of cancers, including CRC, breast cancer, prostate cancer, hematologic malignancies, glioblastoma multiforme, and lung cancer (27). PI3K activation can occur by several molecular mechanisms, including (a) activating mutations of PIK3CA, the gene encoding the 110-kDa catalytic subunit of PI3K; (b) gain-of-function mutations of oncogenes encoding positive regulators of PI3K (HER2, EGFR, RAS, c-src); (c) loss-of-function mutations affecting negative regulators of PI3K such as PTEN (i.e., loss of PTEN expression/function); (d) amplification/overexpression of receptor tyrosine kinases; and (e) mutations of genes encoding downstream effectors of the PI3K signaling cascade (e.g., PDK-1, Akt/PKB, RPS6KB1).

A large body of clinical evidence now shows that activation of components of the PI3K/Akt pathway has prognostic importance in several malignancies. In addition, PI3K activation has been identified as a clinically relevant mechanism of resistance to chemotherapy, hormonal therapy, and radiation therapy and to various therapies targeting certain signaling pathways, such as trastuzumab and lapatinib. Thus, the frequent activation of the PI3K/Akt pathway in human tumors and its potential role as a determinant of cellular drug resistance have made various individual components of this pathway attractive therapeutic targets for drug development (28).

Src Kinase

The Src family of nonreceptor protein-tyrosine kinases plays a central role in a wide range of cellular functions, including cell division, survival, motility, adhesion, invasion, and angiogenesis (29). There are nine members of the Src family: c-Src, c-Yes, Fyn, Lyn, Lck, Hck, Blk,
Fgr, and Yrk. These Src kinases are activated in response to various external cellular signals that promote proliferation, survival, motility, and invasion, through activation of cytokine receptors, receptor protein-tyrosine kinases, G protein-coupled receptors, and integrins. In addition to these key cellular functions, emerging data suggest that activation of Src kinase appears to play an important role in mediating chemoresistance.

c-Src is the best studied member of the Src family and the one that has been most often implicated in cancer progression. Of note, c-Src has been most widely investigated in CRC, in which it has been shown to be constitutively activated. However, c-Src has a similar role in other tumor types, including pancreatic cancer, breast cancer, lung cancer, head and neck cancer, and prostate cancer. The initial studies in CRC were largely correlative in nature, showing that c-Src expression at the protein level is increased in colon tumors when compared with normal colon mucosa. Subsequent studies have shown that c-Src expression is correlated with the malignant potential of cells and that adenomas with the greatest malignant potential showed the highest levels of kinase activity. In addition, the observation has been made that Src kinase activity is elevated in premalignant polyps, higher in primary colon tumors, and highest in metastatic liver lesions. These findings suggest that c-Src activity may contribute to the metastatic progression of CRC. Moreover, in one study, increased Src activity was an independent indicator of prognosis in patients with CRC and was correlated with poor prognosis (30).

Given the central role of Src in mediating key aspects of tumor progression and metastasis, Src kinases are attractive targets for drug development (31). This has become an active area of investigation, and several small-molecule inhibitors of Src kinase are presently undergoing preclinical and clinical testing. There are at least five agents in various stages of clinical development: dasatinib, AZD0530, bosutinib (SKI-606), BIBF 1120, INNO-406, and KX 01, and two small molecules in early preclinical testing.

Colon Cancer Stem Cells

Since the discovery of leukemic stem cells, significant efforts have focused on identifying the presence of cancer stem cells in solid tumors. Recent studies in breast cancer and brain cancer, respectively, showed that only a small fraction of cancer cells, on the order of approximately 1%, are able to initiate tumor growth (32,33). In general, a given tumor is composed of a mixture of cells with capacity for self-renewal, the can-
cer stem cell component, and proliferative cells with limited life span, which represent non-self-renewing cells. Recent work has shown that only a very small fraction of human colon cancer cells expressing epCAM\textsuperscript{high}, CD44\textsuperscript{+}, CD133\textsuperscript{+}, and CD166\textsuperscript{+} (colon-cancer initiating cells) were, in fact, capable of initiating tumor growth in immunodeficient mice (34,35).

CD133 is a transmembrane glycoprotein expressed in hematopoietic stem cells, endothelial progenitor cells, glioblastomas, and neuronal and glial stem cells (36). These cells remain undifferentiated and grow as spherical aggregates in the presence of serum or extracellular matrix and then differentiate once growth factors are removed. Flow cytometry of CD133\textsuperscript{+} colon tumor cells reveals a virtual absence of expression of the cytokeratin CK20, which is a marker associated with CRC differentiation. As expected, upon differentiation of this population of CD133\textsuperscript{+} cells, CK20 is expressed. However, once differentiated, CD133\textsuperscript{+} cells lose their tumorigenic potential.

There is now growing evidence that colon cancer stem cells (CSCs), as with normal stem cells, have specific survival mechanisms that allow them to be intrinsically resistant to the cytotoxic effects of chemotherapy as well as radiation therapy when compared with proliferating cells (37). For example, the multidrug resistance 1 transport protein (MDR1) and breast cancer resistance protein 1 (BCRP1) are expressed at high levels in hematopoietic stem cells and CSCs (38). Moreover, gene expression profiling has identified greater expression of DNA repair genes and antiapoptotic genes within the CD133\textsuperscript{+} population.

In addition, several important signaling pathways appear to play a critical role in stem cell self-renewal. In this regard, the Wnt/\(\beta\)-catenin pathway has drawn significant attention along with the Notch and Hh signaling pathways. In fact, these respective pathways may confer the property of resistance to chemotherapy and/or radiation therapy by protecting the CSC from exposure to various cytotoxic stresses. This protection appears to be mediated through downstream activation of several essential cell survival signals, such as PI3K/Akt, nuclear factor-\(\kappa\)B, Bcl-X\(_L\), and survivin.

Further research is ongoing to more precisely elucidate the underlying control mechanisms responsible for the continued growth of CSCs. It is clear that greater attention will need to focus on this specific population of cells, as they may be responsible for the development of cellular drug resistance and cancer recurrence. However, the Wnt/\(\beta\)-catenin, Notch, and Hh pathways, which all appear to be upregulated in CSCs, may serve as attractive and unique targets for the development of novel therapeutic strategies (39).
Figure 5. Network of cellular signaling proteins.
Conclusion

Significant advances have been made in the understanding of the basic biology of CRC. This chapter has reviewed several of the key signaling pathways that have been shown to play an important role in the growth and proliferation of CRC. However, colorectal tumors as well as other cancers, as a result of their inherent genomic instability, have tremendous redundancy in their ability to maintain growth through cross-talk interactions involving an intricate network of cellular signaling mechanisms (Figure 5). As such, this remarkable level of complexity makes successful treatment of CRC patients all the more challenging. Nevertheless, an enhanced knowledge of cancer biology has provided the rational basis for developing novel therapies that target specific growth factor receptors and critical signal transduction pathways. Regimens combining cytotoxic chemotherapy with these novel targeted agents are being designed in the first-, second-, and third-line settings for the treatment of CRC. In addition, intense efforts continue to focus on identifying critical molecular biomarkers that can be used to predict whether a particular signaling pathway is relevant for an individual patient and to then predict clinical response to chemotherapy and/or targeted therapies as well to identify which patients may be at increased risk for developing drug-specific side effects. The long-term goal of all of this work is to go from empiric treatment of patients to a more personalized approach with novel, truly targeted agents.

References


Colorectal cancer (CRC) is a major public health problem worldwide, and in Western countries, it is the second leading cause of cancer-related mortality. Approximately 50% of patients with CRC develop metastases, and most patients with metastatic disease eventually die of their disease. Presently, chemotherapy remains the cornerstone of therapy for patients with metastatic CRC (mCRC).

Cytotoxic Agents in Metastatic Colorectal Cancer

Benefit of Palliative Chemotherapy

In general, patients with mCRC who go untreated have a median survival of 5–6 months. Several randomized studies have shown that chemotherapy for mCRC prolongs survival and maintains and/or improves quality of life (1–3). In these early clinical trials, 5-fluorouracil (5-FU)-based chemotherapy regimens were used. The median survival of patients treated with fluoropyrimidine chemotherapy was in the range
of 11–12 months compared to 5–6 months for best supportive care (BSC) (1). The use of combination chemotherapy is superior to fluoropyrimidine monotherapy (Table 1) and has extended median survival to the 16- to 18-month range. In more recent trials, with the development of regimens that incorporate cytotoxic agents with biologic agents, median survival now approaches 20–24 months, and this important topic is reviewed in this chapter.

5-Fluorouracil Regimens

5-FU was synthesized in the late 1950s, and for almost 40 years, it was the only available agent to treat mCRC. This drug is inactive in its parent form and is converted within the cell to the cytotoxic metabolite fluorodeoxouridine monophosphate (FdUMP). FdUMP forms a ternary complex with the reduced folate 5,10-methylene-tetrahydrofolate and the enzyme thymidylate synthase (TS), which then leads to TS enzyme inhibition and subsequent inhibition of DNA synthesis and DNA repair. 5-FU can also be falsely incorporated into RNA and DNA, which leads to inhibition of mRNA translation and protein synthesis as well as inhibition of DNA synthesis and function, respectively.

For nearly 40 years, bolus 5-FU regimens were considered the standard treatment option. In general, the response rate (RR) to single-agent bolus 5-FU is approximately 10%. A meta-analysis comparing infusional 5-FU versus bolus 5-FU regimens found that infusional regimens yielded higher RRs (22% vs. 14%; \( P = .0002 \)) and improved overall survival (OS) (12.1 months vs. 11.3 months; \( P = .04 \)) (4).

Table 1. Impact of cytotoxic chemotherapy in the treatment of metastatic colorectal cancer

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median (mo)</th>
<th>1 yr (%)</th>
<th>2 yr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best supportive care</td>
<td>6</td>
<td>&lt;30</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5-FU + FA</td>
<td>11-12</td>
<td>45</td>
<td>20-30</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>12</td>
<td>50</td>
<td>20-30</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>18</td>
<td>60-70</td>
<td>30-40</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>18</td>
<td>60-70</td>
<td>30-40</td>
</tr>
</tbody>
</table>

FA = folinic acid, 5-FU = 5-fluorouracil.
Biochemical modulation of 5-FU clinical activity with a broad range of agents has been extensively studied, including leucovorin (LV), methotrexate, trimetrexate, interferon-α, interferon-γ, and N-phosphonacetyl-L-aspartate. The administration of LV increases the intracellular pool of key reduced folates, specifically 5,10-methylenetetrahydrofolate, and stabilizes FdUMP and TS in a ternary complex and leads to optimal inhibition of the TS enzyme. To date, LV has been the most successful biomodulatory agent to be combined with 5-FU. In fact, until 2000, the 5-FU/LV combination was considered the gold standard for the first-line therapy of patients with mCRC for 20–25 years. Studies have shown that the addition of LV to bolus 5-FU improves RRs (23% vs. 11%) compared with single-agent 5-FU (5). Of note, several studies have shown a higher RR and a lower toxicity for infusional 5-FU/LV regimens compared with bolus 5-FU/LV regimens.

The LV5FU2 regimen developed by de Gramont and colleagues used both an infusional schedule of administration and LV biomodulation to improve outcome. This regimen incorporates a 2-hour IV infusion of LV (200 mg/m²) followed by an IV bolus of 5-FU (400 mg/m²) and a 22-hour IV infusion of 5-FU (600 mg/m²). The bolus and intermittent infusion of 5-FU are repeated on day 2, and cycles are repeated every 14 days (6). Modifications of this regimen have been made by deleting the day 2 bolus or by completely eliminating bolus 5-FU. In other countries and in particular Germany, weekly infusional regimens of 5-FU/LV have been developed, but at the present time, they appear to be less popular.

In summary, infusional regimens are widely accepted as the optimal approach of administering 5-FU/LV, given the improved overall RR and time to progression (TTP) and improved safety profile, with a reduced incidence of mucositis and myelosuppression. As such, infusional 5-FU/LV now serves as the fluoropyrimidine backbone for combination regimens that incorporate other cytotoxic and biologic agents that are now discussed.

Irinotecan- and Oxaliplatin-Based Regimens

Irinotecan is a camptothecin analog that inhibits DNA topoisomerase I and induces single-strand DNA breaks and replication arrest. Oxaliplatin is a third-generation platinum analog that induces DNA cross-linkages and apoptotic cell death. Both irinotecan and oxaliplatin were initially studied in 5-FU refractory patients, with each showing clinical activity in this setting (7–10).
Based on this clinical activity in the disease refractory setting, studies were then designed to investigate the combination of 5-FU/LV plus irinotecan and of 5-FU/LV plus oxaliplatin in the front-line setting. Saltz et al. showed that weekly irinotecan in combination with the weekly 5-FU/LV Roswell Park regimen was significantly more active, with respect to RR and TTP, than 5-FU/LV in advanced CRC (11). Similar clinical benefit was observed when irinotecan was combined with the infusional LV5FU2 regimen.

In a randomized phase III study, de Gramont et al. (12) compared the FOLFOX4 regimen (oxaliplatin at a dose of 85 mg/m² as a 2-hour infusion on day 1, every 2 weeks, plus LV5FU2) with the de Gramont regimen of LV5FU2 alone in patients with previously untreated advanced CRC. This study set the stage for the future clinical development of FOLFOX, as this combination showed significantly longer median progression-free survival (PFS) times (9 vs. 6.2 months; P = .0003) and higher RRs (50.7% vs. 22.3%; P = .0001). Although the difference in median OS did not reach statistical significance (16.2 vs. 14.7 months; P = .12), this trial was not sufficiently powered to detect such a difference. Moreover, both treatment groups were able to receive active salvage therapies, thereby obscuring any potential survival difference.

Intergroup trial N9741 was a randomized phase III trial of the first-line therapy for mCRC with the bolus weekly IFL (irinotecan, 5-FU, and LV) regimen as the control arm (13). The two experimental arms of this trial included FOLFOX4 and a non–fluoropyrimidine-containing arm of irinotecan and oxaliplatin (IROX). This study showed that FOLFOX4 had significantly greater clinical efficacy than IFL in terms of RR (45% vs. 31%; P = .002), time to tumor progression (8.7 vs. 6.9 months; P = .0001), and median OS (19.5 vs. 14.8 months; P = .0001). In addition, when compared with IFL or IROX, FOLFOX4 was associated with a markedly lower incidence of febrile neutropenia and fewer gastrointestinal (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.

The N9741 trial demonstrated clear superiority of FOLFOX4 over the bolus weekly IFL regimen. However, the FOLFOX4 regimen used an 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 used. To address this issue, Tournigand et al.,
representing the GERCOR cooperative group in France, conducted a randomized, multicenter, open-label prospective phase III trial (14). This study used a simplified LV5FU2 regimen with a single 46-hour infusion rather than two 22-hour infusions on days 1 and 2, as had originally been developed by de Gramont and colleagues. In one arm, patients received FOLFIRI (folinic acid, 5-FU, and irinotecan), followed at progression by FOLFOX6; 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 TTP from initiation of first-line therapy to TTP after second-line treatment. The RRs for first-line FOLFIRI and FOLFOX6 were virtually identical at 56% and 54%, respectively. Median TTP was 14.4 months for FOLFIRI followed by FOLFOX6 and 11.5 months for FOLFOX6 followed by FOLFIRI, which was not statistically significant. Overall median survival for the two arms was also virtually identical—20.4 months and 21.5 months, respectively.

Both treatment arms were relatively well-tolerated, although 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 approximately 14% in both arms. Grade 3/4 myelosuppression was observed in a higher number of patients treated initially with FOLFOX6 (44% vs. 25%); however, the incidence of febrile neutropenia remained low in both arms, being less than 10%. Given the use of oxaliplatin, 34% of those treated with FOLFOX6 experienced grade 3/4 neurotoxicity.

The Tournigand study is important, as it documented the clinical equivalence of irinotecan and oxaliplatin in the first-line setting using the same infusional 5-FU/LV backbone. The second important point is that there does not appear to be an optimal sequence of regimens, as the OS at the end of two treatment arms was virtually identical. In support of the Tournigand trial is the Italian study conducted by Colucci et al., in which the clinical efficacy of FOLFOX and FOLFIRI was investigated (15). No significant differences were reported in RR between FOLFOX (34%) and FOLFIRI (31%). TTP (7.0 vs. 7.0 months), duration of response (9.0 vs. 10.0 months), and OS (14.0 vs. 15.0 months) were virtually identical between FOLFIRI and FOLFOX, respectively.

Taken together, these clinical trials have shown that the clinical activity of FOLFOX and FOLFIRI is equivalent in terms of RR, TTP, and OS. As such, they should be viewed as equivalent treatment options in the first-line setting. Both regimens are well-tolerated, with manageable safety profiles. However, given the different spectrum of toxicity with
each regimen, with peripheral sensory neuropathy being a significant issue with oxaliplatin and GI toxicity being potentially dose-limiting in patients with irinotecan, choices can be appropriately made as to which type of toxicity a given patient is willing to experience.

What about combining irinotecan and oxaliplatin together along with the 5-FU/LV base? Two randomized studies have addressed this interesting clinical issue. A Greek study failed to show a significant advantage in survival, TTP, and RR with the triplet FOLFOXIRI combination (folinic acid, 5-FU, oxaliplatin, and irinotecan) compared with the doublet of FOLFIRI in 283 patients (16). An Italian study, however, showed a higher RR, longer PFS, and improved OS in 244 patients treated with FOLFOXIRI compared with FOLFIRI (17). One of the potential limitations of this triplet regimen is the increased toxicity, primarily myelosuppression. In view of the limited data and the clear increased toxicity of the triplet combination, this combination cannot be recommended for routine clinical use today. However, there may be a role for this regimen in patients with liver-limited metastatic disease; this issue is further discussed in Chapter 4.

Oral versus Intravenous Fluoropyrimidine Therapy

Significant efforts have focused on determining whether the oral fluoropyrimidine capecitabine can be substituted for 5-FU/LV either as monotherapy or in combination with cytotoxic chemotherapy to yield equivalent clinical efficacy. The initial studies compared oral capecitabine with bolus IV 5-FU/LV in the first-line treatment of patients with mCRC in two phase III clinical trials. An integrated analysis of several trials showed that oral capecitabine is more active than 5-FU/LV in terms of RR and equivalent with respect to TTP and OS (18). Of note, the overall safety profile favored capecitabine therapy, as there were significantly reduced myelosuppression, mucositis, and alopecia and fewer hospitalizations.

In a randomized phase III trial conducted by the AIO cooperative group in Germany, Porschen et al. (19) compared the combination of capecitabine plus oxaliplatin (CAPOX) with a regimen consisting of weekly infusional 5-FU/LV/oxaliplatin (FUFOX). This particular CAPOX regimen was somewhat different from others previously reported, as oxaliplatin was administered on a day 1 and 8 schedule every 21 days as opposed to a single injection on day 1 every 21 days. Clinical efficacy was nearly equivalent between the CAPOX and FUFOX arms: RR (48% vs. 54%), PFS (7.1 vs. 8.0 months; P = .117), and OS (16.8
vs. 18.8 months; $P = .26$). In terms of safety profile, both arms were well-tolerated, with manageable side effects, although there was a higher incidence of grade 2/3 hand-foot syndrome in the CAPOX arm.

The NO16966 trial was initially an open-label randomized phase III trial comparing XELOX with FOLFOX4 in the first-line setting (20). The XELOX regimen consisted of oxaliplatin, 130 mg/m$^2$ on day 1, and capecitabine, 1,000 mg/m$^2$ PO bid on days 1–14, every 3 weeks. This trial was subsequently amended to include a second randomization to bevacizumab, resulting in a 2 × 2 placebo-controlled factorial design, once it was clear that the anti–vascular endothelial growth factor (VEGF) antibody bevacizumab was going to be approved for use in the first-line setting. The primary endpoint of this study was PFS and noninferiority of XELOX to FOLFOX4. In the analysis of the original two-arm study (N = 634), XELOX was found to be equivalent to FOLFOX4 in terms of PFS (7.3 vs. 7.7 months), and RR similar (37% vs. 39%). Of note, the incidence of grade 3/4 neutropenia (7% vs. 44%) and febrile neutropenia (1% vs. 5%) was significantly higher in patients treated with FOLFOX4 chemotherapy when compared with XELOX. In contrast, grade 3/4 GI toxicity in the form of diarrhea and hand-foot syndrome were higher in the XELOX arm.

In addition to the NO16966 study, there are several phase II trials, and a few underpowered randomized phase III trials, all of which confirm the equivalent clinical activity between the combination of capecitabine with oxaliplatin with FOLFOX/FUFOX regimens. Relatively less has been done in terms of investigating the combination of capecitabine and irinotecan. In Europe, the EORTC 40015 trial was a randomized phase III study comparing CAPIRI (capecitabine and irinotecan) with FOLFIRI (21). Because of increased toxicity and mortality associated with the CAPIRI arm, this study was terminated prematurely. In particular, there was a significant increase in GI toxicity in the form of diarrhea and dehydration associated with CAPIRI. In the United States, the BICC-C trial investigated three different irinotecan-based regimens—FOLFIRI, modified IFL, and CAPIRI (22). This study was later amended to include the FOLFIRI and modified IFL arms plus bevacizumab. Analysis of the first phase of this study revealed that the CAPIRI regimen had reduced clinical efficacy when compared with FOLFIRI, which was most likely related to increased GI toxicity. Of interest, the Dutch Colorectal Cancer Study Group recently reported the results of the CAIRO trial, which compared the sequential use of capecitabine followed by irinotecan and followed by the combination of capecitabine and oxaliplatin with combination regimens incorporating CAPIRI followed by CAPOX chemotherapy (23).
Although the CAPIRI regimen used the same dose and schedule of capecitabine and irinotecan as was used in the EORTC 40015 and BICC-C studies, this regimen was clinically active, with a manageable safety profile. It is clear that more work is required to define the optimal dose and schedule of capecitabine and irinotecan, especially in view of combining this cytotoxic regimen with the biologic agents, such as bevacizumab and cetuximab.

### Strategy with Cytotoxic Agents

Based on the currently available data, several conclusions can be made with respect to cytotoxic chemotherapy, including the following:

- Infusional regimens of 5-FU/LV are superior to bolus schedules of 5-FU/LV.
- Combination regimens incorporating two cytotoxic agents are more active in the first-line treatment of CRC than fluoropyrimidine monotherapy. At present, the use of a triplet combination (FOLFOXIRI) should not be viewed as standard of care.
- FOLFOX and FOLFIRI have similar clinical activity in the first-line treatment of mCRC but display a different safety profile.
- The oral fluoropyrimidine capecitabine is at least as active as IV 5-FU/LV. Combination studies have shown a similar activity of the combination of capecitabine and oxaliplatin (XELOX/CAPOX) compared with IV 5-FU/LV/oxaliplatin (FOLFOX). The optimal regimen of capecitabine plus irinotecan (XELIRI/CAPIRI) has yet to be identified.
- Second-line salvage treatment is indicated in good-performance patients.
- Exposure of patients with mCRC to all three available cytotoxic agents (fluoropyrimidines, irinotecan, and oxaliplatin) during their treatment course increases OS (24).

### Targeted Therapies for Metastatic Colorectal Cancer

Several “targeted” agents have recently entered the clinical arena for the treatment of mCRC. In particular, agents targeting two key cellular processes, including the epidermal growth factor receptor (EGFR) and the VEGF, have been shown to be active in metastatic disease.
Angiogenesis Inhibitors

Bevacizumab

Bevacizumab is a humanized monoclonal antibody that targets and binds to VEGF, thereby preventing binding of this growth factor to its cognate VEGF receptors (25). VEGF is a central component in the process of angiogenesis, which is critical for the growth of primary and metastatic tumors. Up to a size of 1-2 mm, tumor cells are able to obtain required nutrients and oxygen from surrounding fluids via diffusion. Once the tumor size begins to increase further, however, new blood vessels must be developed to support continued growth. VEGF is a key component in the signaling pathway used to facilitate the growth of these new vessels. Significant efforts are being placed on developing new compounds and strategies with the intention of disrupting this angiogenic process. However, to date, bevacizumab is the only such agent to have demonstrated substantial activity in mCRC and to be approved for use in the United States and Europe.

The first trial to suggest a potential role for bevacizumab in the management of CRC was a relatively modest-sized randomized phase II trial of two different doses of bevacizumab plus weekly 5-FU and high-dose LV (Roswell Park 5-FU/LV schedule). Patients were randomized to 5-FU/LV alone or to one of two different doses of bevacizumab, either 5 mg/kg or 10 mg/kg given every other week. The RR, TTP, and survival were superior in the 5 mg/kg (low-dose bevacizumab) arm, with the 10 mg/kg bevacizumab arm appearing modestly superior to chemotherapy alone, but inferior to the low-dose bevacizumab arm (26). Thrombosis was the most significant adverse event and was fatal in one patient. Hypertension, proteinuria, and epistaxis were the other main safety signals observed. Because the role of this small randomized phase II study was to “pick the winner” for further clinical development, the 5 mg/kg dose of bevacizumab was selected as the dose to be taken forward for phase III development.

In the pivotal randomized phase III study conducted by Hurwitz et al. (27), the addition of bevacizumab to IFL chemotherapy in the first-line setting showed significant improvement in RR (44.8% vs. 34.8%; P = .004), PFS (10.6 vs. 6.2 months; P < .00001), and OS (20.3 vs. 15.6 months; P = .00003). The only adverse event that occurred with greater frequency in the bevacizumab arm was grade 3 hypertension (11% vs. 2.3%), which was easily managed with antihypertensive therapy. Based on its highly significant and clinically meaningful improvement in clinical efficacy, bevacizumab was granted approval in February 2004 by the U.S. Food and Drug Administration as first-line treatment for mCRC in combination with any IV 5-FU-containing regimen.
In patients who were deemed not to be optimal candidates for irinotecan-based chemotherapy in the first-line setting, Kabbinavar et al. (28) investigated the addition of bevacizumab to bolus weekly 5-FU/LV chemotherapy in a randomized phase II study. They found that patients treated with 5-FU/LV/bevacizumab experienced improved RR (26% vs. 15.2%; \( P = .055 \)) and PFS (9.2 vs. 5.5 months; \( P = .0002 \)) when compared with 5-FU/LV/placebo. Although the median OS (16.6 vs. 12.9 months; \( P = .16 \)) was also higher in patients treated with bevacizumab, this difference did not reach statistical significance. It is important to note that this study enrolled only 210 patients and may not have been sufficiently powered to show a survival difference. Moreover, at the time of disease progression, patients were able to receive effective salvage therapies, thereby diluting any potential survival benefit.

The TREE-2 study evaluated the safety and efficacy of three different oxaliplatin-based regimens in combination with bevacizumab in the first-line setting (29). The oxaliplatin-based regimens included mFOLFOX6, bFOL, which used a bolus weekly schedule of 5-FU/LV with oxaliplatin, and XELOX. The addition of bevacizumab to each of these regimens significantly improved overall RR (\( P = .011 \), pooled logistic regression analysis). In terms of clinical efficacy, TTP and OS in the mFOLFOX6 plus bevacizumab arm were 9.9 and 26 months, respectively, which were nearly identical to those in the XELOX plus bevacizumab arm (TTP, 10.3 months; OS, 27 months). The addition of bevacizumab to each of the three oxaliplatin-based regimens was well-tolerated, and bevacizumab did not worsen the side effects typically associated with any of the oxaliplatin-based therapies. The TREE-2 trial was the first clinical study to document the clinical efficacy and safety profile of bevacizumab in combination with oxaliplatin-based regimens in the first-line setting.

As noted previously, the NO16966 study was amended to evaluate the safety and efficacy of FOLFOX4/XELOX chemotherapy in combination with bevacizumab in the first-line setting (30). The addition of bevacizumab to oxaliplatin-based chemotherapy significantly increased the median PFS from 8 to 9.4 months (\( P = .0023 \)) and median OS from 19.9 to 21.3 months, a difference that did not reach statistical significance. Of interest, the addition of bevacizumab did not improve the overall RR associated with FOLFOX/XELOX chemotherapy, a finding in sharp contrast to previously reported studies combining bevacizumab with IFL and 5-FU/LV chemotherapy, respectively. The safety profiles were similar to those from other trials with bevacizumab, with no new safety signals being observed. This study is important, as it is the first randomized trial documenting the true efficacy of bevacizumab in com-
bination with oxaliplatin-based chemotherapy and provides support for the use of this combination regimen in the first-line setting.

In contrast to other clinical studies in which the addition of bevacizumab improved PFS by up to nearly 5 months, a much smaller clinical benefit was observed in the NO16966 study. One potential explanation for this smaller-than-expected difference may be the fact that the treatment duration of patients in the bevacizumab group was not longer than in the placebo arm. Indeed, treatment with bevacizumab was not continued in this trial despite the absence of disease progression and despite the absence of severe toxicity that would normally lead to stopping the treatment. A possible explanation for stopping the treatment in the trial may be the perception of the “stop and go” strategy leading to interruption of chemotherapy before progression, at the moment the patients experienced even some minor toxicities. This leads to the issue as to the optimal treatment duration of chemotherapy with bevacizumab. Although not proven, this finding might suggest that bevacizumab should be continued until progression and/or toxicity. This hypothesis is also underscored by the in vivo findings in mouse xenograft models that have shown rapid tumor growth after stopping VEGF inhibition and by the rebound increase in serum VEGF levels after stopping a VEGF inhibitor in cancer patients. There are also intriguing data from the U.S. expanded access registry study (BRiTE) suggesting that post-progression continuation may improve OS as well as survival beyond first progression (31). In addition, the overall incidence of bevacizumab-associated adverse events was comparable in patients who did and did not receive bevacizumab beyond progression.

The BICC-C study was a randomized phase III study originally designed to compare the efficacy and toxicity of three irinotecan-based regimens in combination with different 5-FU regimens that included FOLFIRI, modified IFL (2 weeks on, 1 week off), and CAPIRI (capecitabine plus irinotecan) (21). However, when bevacizumab was approved for use in the first-line setting, this trial was amended to a randomized phase II trial and included only the FOLFIRI and modified IFL arms in combination with bevacizumab. A second randomization was incorporated into this study, such that all patients received the cyclooxygenase-2 inhibitor celecoxib or placebo. There were three main findings from the second phase of this study. First, FOLFIRI was superior to modified IFL and CAPIRI in terms of clinical efficacy and safety profile. Second, the addition of bevacizumab to FOLFIRI enhanced clinical benefit, yielding an overall RR of 54.4% and a PFS of 11.2 months, with no increased incidence of side effects. Finally, no clinical benefit was observed with the addition of celecoxib.
Current practice in the United States and Europe is to use cytotoxic regimens such as FOLFIRI, FOLFOX, or XELOX in the front-line setting in combination with bevacizumab. In support of this are the BRiTE and First BEAT studies, which are two observational cohort trials conducted in the United States and Europe, respectively. These studies investigated the clinical efficacy and toxicity of any standard cytotoxic chemotherapy plus bevacizumab in patients treated in the community setting. Nearly 2,000 patients were treated in each study, and the overall PFSs were remarkably similar, being 10.1 months in the BRiTE study and 10.9 months in the First BEAT study. When specifically looking at FOLFOX, FOLFIRI, and XELOX in combination with bevacizumab, the median PFS was in the 10- to 11-month range. With respect to safety profile, these community-based studies did not identify any new safety signals other than those that have already been reported.

The toxicities commonly associated with chemotherapy, such as nausea, vomiting, diarrhea, cytopenias, and asthenia, do not appear to be significant issues for bevacizumab. In contrast, the main safety signals associated with bevacizumab therapy include hypertension, proteinuria, arterial thrombosis, bleeding complications (mainly epistaxis), impaired wound healing, and a low chance of GI perforations (up to 1.5%) (Table 2). Although there does not appear to be an increased risk of venous thromboembolism, the risk of arterial thrombosis is increased especially in patients older than age 65 years and in patients with a history of arterial thrombosis (e.g., stroke, transient ischemic attack, angina, and myocardial infarction). It should be noted that bevacizumab has essentially no clinical activity as a single agent in mCRC, and as such, it must be given with standard cytotoxic chemotherapy regimens.

Table 2. Safety overview of bevacizumab

Bevacizumab does not increase chemotherapy-related toxicities.
Bevacizumab has specific side effects:
- Hypertension
- Proteinuria
- Thromboembolic events: arterial
- Bleeding: minor mucosal (epistaxis) and major hemorrhage (non-small-cell lung cancer)
- Gastrointestinal perforation
- Wound healing/postoperative bleeding
that do possess these toxicities. However, bevacizumab does not exacerbate the toxicities typically observed with cytotoxic chemotherapy. Significant efforts have focused on identifying clinical, biochemical, and molecular markers that would predict which patients would respond to bevacizumab therapy. No such predictive biomarker has yet to be identified, however. An analysis of predictive markers showed that indeed bevacizumab increased the activity of irinotecan plus 5-FU/LV regardless of the level of VEGF expression, thrombospondin expression, and microvessel density. Mutations of KRAS, b-raf, and p53 did not predict for prolonged survival on bevacizumab plus IFL, and bevacizumab was active regardless of KRAS status.

Other Anti–Vascular Endothelial Growth Factor Inhibitors
Several novel molecules are being developed that target various key aspects of the VEGF signaling pathway (Table 3). Vatalanib and axitinib are small-molecule inhibitors of VEGF-R1, VEGF-R2, and VEGF-R3. Two randomized studies were conducted in the first- (CONFIRM 1 trial) and second-line treatment of mCRC (CONFIRM 2 trial) in which vatalanib was combined with FOLFOX. However, both studies failed to show any benefit when vatalanib was combined with FOLFOX chemotherapy. Presently, studies are evaluating the role and activity of other VEGF receptor tyrosine kinase inhibitors in mCRC in combination with a standard cytotoxic chemotherapy. In the first-line setting, two ongoing phase III trials are investigating the combination of FOLFOX plus AZD2171 and the combination of FOLFIRI plus sunitinib. Evaluation (in a phase III study) of aflibercept (VEGF Trap), a humanized soluble VEGF receptor protein, in the second-line treatment of mCRC in combination with FOLFIRI is ongoing. Finally, studies are being planned to investigate axitinib in combination with chemotherapy in the front-line setting.

Anti–Epidermal Growth Factor Receptor Inhibitors

Cetuximab
Cetuximab is a chimeric immunoglobulin (Ig)G1 monoclonal antibody that binds selectively to the EGFR (33). By binding to the EGFR receptor binding site, cetuximab blocks the physiologic ligands from binding to and activating EGFR, preventing phosphorylation of the tyrosine kinase on the intracellular domain of the receptor and thereby preventing downstream signaling. Preclinical models indicated that cetuximab had modest in vitro and in vivo single-agent activity but had greater activity when combined with cytotoxic chemotherapy. With this as rationale, the first
<table>
<thead>
<tr>
<th>Compound</th>
<th>Monoclonal antibody</th>
<th>Clinical trials in metastatic colorectal cancer</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Cediranib</td>
<td>VEGFR 1, 2, and 3 (TKI)</td>
<td>Phase III for first and second line</td>
<td>Clinical development in non-small cell lung cancer was interrupted due to toxicity.</td>
</tr>
<tr>
<td>Vatalanib</td>
<td>VEGFR 1, 2, and 3 (TKI)</td>
<td>Phase III for first and second line</td>
<td>Phase III studies failed to show benefit (did not meet primary endpoint).</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Multi TKI</td>
<td>Phase III for first line</td>
<td>In phase II, 46% of patients had delay or dose modification due to toxicity.</td>
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<tr>
<td>Aflibercept</td>
<td>Targets VEGF-A</td>
<td>Phase III for second line</td>
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<td>(VEGF Trap)</td>
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<tr>
<td>Axitinib</td>
<td>VEGFR 1, 2, and 3 (TKI)</td>
<td>Phase III planned</td>
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<tr>
<td>Motesanib</td>
<td>Multi TKI</td>
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TKI = tyrosine kinase inhibitor, VEGFR = vascular endothelial growth factor receptor.
trial investigated the activity of cetuximab plus irinotecan in patients who had failed irinotecan-based chemotherapy and found that 22% of patients experienced an objective tumor response. In a second phase II trial, cetuximab monotherapy was investigated in irinotecan-refractory mCRC. Five of 57 patients (9%) achieved a partial response. A subsequent larger, multicenter trial (BOND trial) (Table 4) conducted in Europe provided strong confirmatory evidence of the activity of cetuximab in refractory mCRC (34). This trial randomized 329 patients with EGFR-expressing, irinotecan-refractory mCRC between cetuximab/irinotecan and cetuximab alone in a 2:1 schema. Overall RRs were 23% for cetuximab/irinotecan and 11% for cetuximab alone, respectively. Time to tumor progression in the BOND study was 4.0 months for the combination and 1.6 months for single-agent cetuximab. Survival in the two arms was not significantly different, but this was most likely due to the fact that crossover was allowed for patients who progressed on cetuximab monotherapy. Moreover, the trial was not sufficiently powered to show a survival difference. In a randomized trial of the Canadian and Australian groups, cetuximab monotherapy led to an improved survival compared with BSC in chemorefractory CRC (35). Further analysis

Table 4. Key clinical trials of anti–epidermal growth factor receptor antibodies in metastatic colorectal cancer

<table>
<thead>
<tr>
<th>Third line</th>
<th>BOND: cetuximab ± irinotecan</th>
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<tr>
<td></td>
<td>NCIC C0.17: cetuximab vs. best supportive care</td>
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<td></td>
<td>Panitumumab vs. best supportive care (KRAS wild type)</td>
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<tr>
<td>Second line</td>
<td>BOND: cetuximab ± irinotecan</td>
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<tr>
<td></td>
<td>EPIC: irinotecan ± cetuximab</td>
</tr>
<tr>
<td>First line</td>
<td>(Randomized) phase III studies</td>
</tr>
<tr>
<td></td>
<td>CRYSTAL: FOLFIRI ± cetuximab</td>
</tr>
<tr>
<td></td>
<td>PACCE: chemotherapy/bevacizumab ± panitumumab</td>
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<tr>
<td>Other phase III studies in progress</td>
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CRYSTAL = Cetuximab combined with irinotecan in first-line therapy for metastatic colorectal cancer, EPIC = Erbitux Plus Irinotecan in Colorectal Cancer, FOLFIRI = folinic acid, 5-fluorouracil, and irinotecan, NCIC = National Cancer Institute of Canada, PACCE = Panitumumab Advanced Colorectal Cancer Evaluation.
revealed that the effect of cetuximab was confined to patients whose tumors expressed wild-type KRAS. Patients with a KRAS mutant tumor had no benefit of cetuximab in the Canadian/Australian trial, nor in other retrospective trials in chemorefractory CRC.

The results of several phase II trials and one phase III trial of cetuximab in the first-line therapy of mCRC have been reported (see Table 4). A small phase II pilot trial of cetuximab plus weekly bolus IFL demonstrated a 44% RR. Several small phase II studies of cetuximab plus irinotecan and weekly infusional 5-FU/LV, FOLFIRI, FOLFOX, and weekly infusional 5-FU/LV plus oxaliplatin regimens have shown encouraging results. The largest experience to date comes from the CRYSTAL trial, a randomized phase III trial comparing FOLFIRI and FOLFIRI plus cetuximab. In this study, the median PFS was significantly longer for the combination of FOLFIRI plus cetuximab compared with FOLFIRI alone: a hazard ratio (HR) of 0.85 and a median PFS of 8.9 months compared with 8.0 months ($P = .0479$) (36). There was also a higher RR for the combination of FOLFIRI/cetuximab—39% vs. 47%—leading to higher resection rates of metastases in patients with initially unresectable metastases. In particular, the addition of cetuximab led to a higher R0 resection rate in patients with liver-limited disease. Of note, the effect of cetuximab was confined to patients with a KRAS wild-type tumor: the HR for PFS was 0.68 ($P = .017$) for the combination FOLFIRI/cetuximab. There was a 16% increase in RR (43% to 59%) in patients with a KRAS wild-type tumor. In patients with a KRAS mutant tumor, cetuximab therapy did not confer any clinical benefit to FOLFIRI chemotherapy. The data of the OPUS study, a large randomized phase II trial investigating cetuximab in combination with FOLFOX, provide further support to the concept that the benefit of cetuximab is confined only to wild-type KRAS patients. Patients with KRAS tumors did not benefit from cetuximab treatment, and although the patient numbers are small, there may even be a deleterious effect.

Panitumumab
Panitumumab, formally known as ABX-EGF, is a fully human IgG2 monoclonal antibody that also targets the EGFR. A phase II study of this agent in patients with mCRC demonstrated a 10% RR and 38% rate of stable disease. In terms of clinical efficacy, the median duration of response was 5.2 months (95% confidence interval [CI] of 4.5–7.5 months), the median PFS was 2.0 months (95% CI of 1.9–3.8 months), and the median survival was 7.9 months (95% CI of 5.7–9.9 months). The vast majority of patients experienced some degree of acneiform skin rash. Only one of the 148 patients treated experienced a dose-limiting
allergic reaction, suggesting that this agent may have a lower incidence of hypersensitivity allergic reactions than cetuximab. Although no formal randomized comparisons have been done with panitumumab and cetuximab, this agent appears to be quite similar to cetuximab, both in mode of action and in single-agent efficacy.

A randomized phase III trial of panitumumab plus BSC versus BSC in 463 patients with EGFR-expressing, oxaliplatin- and irinotecan-refractory mCRC showed a significantly longer PFS for panitumumab-treated patients compared to BSC alone. The median PFS was 8 weeks for panitumumab and 7.3 weeks for BSC (HR 0.54, 95% CI 0.44–0.66; P <0.0001) (37). Although median OS was not improved with panitumumab in this trial, this may have been due to a crossover effect, where patients treated with BSC at the time of progression could then receive panitumumab. The RR in the panitumumab patients was 10% and was confined only to patients with the wild-type KRAS (38).

Combination of Vascular Endothelial Growth Factor and Epidermal Growth Factor Receptor Inhibitors

Preclinical in vivo animal models have documented additive efficacy and even synergy when anti-EGFR inhibitors are used in combination with anti-VEGF targeted agents. In a randomized phase II study (BOND-2), irinotecan-refractory CRC patients received irinotecan at the same dose and schedule as per their last treatment administration before study plus cetuximab (400 mg/m^2 loading dose, then weekly at 250 mg/m^2) plus bevacizumab (5 mg/kg given every other week) versus cetuximab (400 mg/m^2 loading dose, then weekly at 250 mg/m^2) plus bevacizumab (5 mg/kg given every other week). The results of this study were striking, as the use of two antibodies plus irinotecan (N = 43) yielded an impressive 37% RR and TTP of 7.3 months, compared with an RR of 20% and 4.9 months TTP in patients treated with cetuximab plus bevacizumab alone (N = 40). Of note, no unexpected toxicities were encountered with the use of the dual biologic agents (39).

The first trial to investigate the use of dual biologics in the front-line treatment of mCRC was the large randomized PACCE phase III trial assessing the role of bevacizumab and panitumumab in combination with standard chemotherapy (FOLFOX or FOLFIRI) as first-line treatment for advanced disease (40). The combination of dual biologics was associated with significantly shorter PFS and OS and increased toxicity compared with bevacizumab alone when FOLFOX chemotherapy was the cytotoxic backbone. Although clinical efficacy was not compromised when bevacizumab and panitumumab were combined with FOLFIRI chemotherapy, there was significant worsening of the safety profile, with
a marked increase in the incidence of infections and grade 3/4 GI toxicity, in the form of diarrhea and dehydration.

CAIRO2 is a randomized phase III trial conducted by the Dutch Colorectal Cancer Study Group that investigated capecitabine, oxaliplatin, and bevacizumab in combination with cetuximab (41). Patients were randomized to one of two arms: arm A (capecitabine [1,000 mg/m² bid day 1-14 of 3-week cycle], oxaliplatin [130 mg/m² day 1], and bevacizumab [7.5 mg/kg day 1]) and arm B (capecitabine [1,000 mg/m² bid day 1-14 of 3-week cycle], oxaliplatin [130 mg/m² day 1], bevacizumab [7.5 mg/kg day 1], and cetuximab [250 mg/m² after initial 400 mg/m² dose]). Oxaliplatin was omitted after the sixth cycle, and the capecitabine dose was subsequently increased to 1,250 mg/m². The primary endpoint was PFS, and the secondary endpoints were OS, overall RR, and toxicity.

At the 2008 Annual Meeting of the American Society of Clinical Oncology, Punt et al. (41) presented an updated interim analysis of this study on both the safety and clinical efficacy data. The median PFS was shorter in cetuximab-treated patients (10.7 vs. 9.6 months; \( P = .018 \)), albeit by only 1 month. Subgroup analyses indicated that patients whose tumors expressed mutated KRAS and who received cetuximab therapy experienced a shorter median PFS than patients who received no cetuximab. However, the addition of cetuximab did not significantly alter median OS (20.4 vs. 20.3; \( P = .21 \)), nor did it affect overall RR (44% vs. 44%; \( P = .88 \)). With respect to safety profile, this updated analysis confirmed that the dual biologic combination was relatively well-tolerated, with no increase in non-skin-related grade 3/4 toxicity, except for a significant increase in grade 3/4 diarrhea (26% vs. 19%; \( P = .026 \)).

Presently, it remains unclear as to why the use of cytotoxic chemotherapy plus dual biologic agents should lead to a worse safety profile and a reduced clinical activity. However, regardless of the underlying mechanism(s), these studies suggest that the use of dual biologics with an anti-EGFR and anti-VEGF antibodies should not be used as treatment for mCRC outside the context of a clinical trial. Moreover, they raise serious concerns as to the status of ongoing clinical trials designed to further assess their combined role in the front-line setting.

Toxicity of Anti–Epidermal Growth Factor Receptor Antibodies

Although more than 80% of patients suffer from an acniform-like rash after administration of the anti-EGFR monoclonal antibodies, the incidence of grade 3/4 skin rash is present in fewer than 10% of the patients. The rash is only rarely a reason to stop treatment (34,37). The association between rash and efficacy is proving to be quite intriguing. Retro-
spective analysis of the BOND data showed a clear association between higher grades of skin rash and clinical efficacy, as determined by RR and median TTP (34). This correlation also held true for OS, with the median OS increasing from 3 months in patients with no rash to 14 months in those who experienced grade 3 rash. The same relationship has been observed in the panitumumab registration study (37). The association between rash severity and survival seems to hold true across the range of clinical trials investigating cetuximab in CRC and other solid tumors. However, the fact that all of these analyses are retrospective suggests these data should be treated with caution. They should certainly not be made the basis of any decision by regulatory authorities to restrict continued dosing with EGFR inhibitors to patients showing a rash.

To more directly address the issue of cetuximab and skin rash, the EVEREST study was conducted (42). This was an interesting study conducted in Europe in which patients with no or slight rash were randomized between standard dose cetuximab/irinotecan and an escalating dose of cetuximab (combined with irinotecan) up to 500 mg/m$^2$/week. There was a trend toward increased response in the dose-escalating arm, where overall responses increased from 13% to 30%. These findings are intriguing in that this is the first prospective trial to demonstrate a correlation among cetuximab dose, biologic effect, and patient response. However, because this trial was relatively small and conducted at the phase II level, further validation of these findings in a larger prospective trial is warranted.

The management of skin rash and the associated post-inflammatory changes represents a significant issue. Although randomized trials on the optimal patient management are lacking, the experience with topical treatments (thus far of minimal value) and with oral antibiotics is growing, and experience-based treatment recommendations have now been published (43). In addition to skin rash, other adverse effects related to anti-EGFR antibody therapies are allergic reactions that are more frequent with the chimeric antibody cetuximab than with the human antibody panitumumab and hypomagnesemia, which results from renal wasting of magnesium (44). Careful evaluation of electrolyte status, including magnesium and calcium, is warranted, as some patients may require aggressive magnesium repletion.

**Conclusion**

Over the past 10 years, significant advances have been made in the treatment of advanced CRC. This progress has been made possible with the
introduction of three cytotoxic agents—capecitabine, irinotecan, and oxaliplatin—and with the recent approval of three biologic agents—bevacizumab, cetuximab, and panitumumab. During this time period, the median survival of patients with advanced metastatic disease has gone from 10–12 months to nearly 24 months.

Intense efforts have focused on identifying novel targeted therapies that target specific growth factor receptors, critical signal transduction pathways, and/or key pathways that mediate the process of angiogenesis. The recent clinical results with the anti-VEGF antibody bevacizumab, in combination with the IFL bolus weekly regimen, the weekly Roswell Park schedule of 5-FU/LV, the infusional FOLFIRI regimen, and the FOLFOX 4/XELOX regimens, validate the process of angiogenesis as an important chemotherapeutic target for CRC and suggest that this antibody can be safely and effectively used in combination with each of the active anticancer agents used in CRC. Similarly, the results from a series of phase II studies conducted in the United States and the randomized phase II BOND-1 study provide validation for the role of the EGFR signaling pathway as a key target for chemotherapy. Recent studies now suggest that this anti-EGFR antibody can be safely and effectively developed in combination with cytotoxic chemotherapy in the front-line setting as well as in the salvage setting. Taken together, these studies now provide the rationale for the treatment algorithm of mCRC, as outlined in Figure 1, which is now being widely adopted in the United States and in Europe.

Future Challenges with Anti–Vascular Endothelial Growth Factor and Anti–Epidermal Growth Factor Receptor Antibodies

There are several important questions and challenges relating to the use of the anti-VEGF and anti-EGFR antibodies in mCRC (44). Clearly, answers are needed to optimize the clinical outcome for patients and to provide for a more optimal use of medical resources. One of the most crucial challenges is to identify which patients are more likely to respond to bevacizumab-containing regimens and to the anti-EGFR antibodies cetuximab and panitumumab. Because it is now clear that anti-EGFR antibodies are active in wild-type KRAS tumors and not in mutant KRAS tumors, it will be critically important to identify and develop new drugs that are active in mutant KRAS tumors as well as in chemo- and bevacizumab-refractory patients. Moreover, it will be important to select additional biomarkers that can further select wild-type KRAS patients who would most benefit
Figure 1. Treatment algorithm for metastatic colorectal cancer. CT = chemotherapy, FA = folinic acid, FOLFIRI = 5-FU/leucovorin/irinotecan, FOLFOX = 5-FU/leucovorin/oxaliplatin, 5-FU = 5-fluorouracil, XELOX = oxaliplatin and capecitabine.

1 If no cardiovascular contraindications.
2 KRAS wild-type.
3 If intolerant of irinotecan: cetuximab or panitumumab mono-therapy.
from anti-EGFR therapy. Two potential candidates are the EGFR ligands amphiregulin and epiregulin.

A second important challenge relates to a series of key strategic questions on the best combination regimen, on the best sequence of treatments, and on the most optimal use of the different cytotoxic regimens in combination with biologic agents in CRC. Along these lines, a related issue focuses on whether bevacizumab should be continued even when disease progression has been documented.

An important issue revolves around identifying and understanding mechanisms by which tumors become resistant to cytotoxic chemotherapy, biologic agents, and their various combinations. To unravel the underlying causes, sequential tumor biopsies, serum, and plasma must be collected before, during, and after treatment to identify molecular markers that can explain the cause of acquired resistance to therapy.

The only option to cure patients with mCRC is the possibility of resection of metastatic disease. Resection of liver-only metastases has become a standard of care, with long-term survival in up to 25%–35% of selected patients (45). Patients with initially unresectable metastases that are downsized to resectable metastases by systemic treatment have a similar chance of long-term survival after resection (45,46). It is important to determine in this setting the optimal combination of cytotoxics and biologics (single agent or doublets) with the highest likelihood of regression that may lead to resection of the metastases. Indeed, several new studies focus on the subset of patients who will undergo resection after downsizing of initially unresectable liver metastases while treated with one or two biologics. Safety evaluation is very important in this setting because it is presently unclear as to what the potential impact of anti-angiogenesis inhibitors will be on postoperative complications and wound healing.

The hurdles for the demonstration of the activity of the new agents that are under development are increasing. This can probably be solved only through the precise understanding of the mechanisms of action and resistance of these drugs and especially through the selection of the patients with predictive molecular markers, who will be more likely to benefit from these new agents.

Another major challenge to patients and to the health care system involves the rapidly increasing financial costs associated with new treatment regimens for mCRC.

Finally, much work continues to focus on identifying the critical molecular and pharmacogenomic biomarkers that can be used to predict clinical response to treatment with chemotherapy and/or biologic agents or to identify which patients may be at increased risk for developing drug-specific side effects. The eventual goal of such translational studies...
is to facilitate the evolution of empiric chemotherapy to individually tailored treatments for patients with mCRC, which should enhance clinical benefit, reduce toxicity, improve quality of life, and improve medical resource use, leading to a reduction in overall financial costs.

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Second-Line Treatment of Metastatic Colorectal Cancer: Chemotherapy with Integration of Biologic Agents—Review of Current Data

Deirdre J. Cohen, MD, and Howard S. Hochster, MD

Colon cancer is currently one of the most prevalent malignancies diagnosed in the Western world. Despite improved detection methods, education, and early screening, it remains the second leading cause of cancer-related death. Approximately 20% of patients present with metastatic disease, and those who present with local, resectable disease have up to a 50%–60% risk of recurrence (1). With improved systemic therapy and surgical approaches, most patients with advanced disease are, at some point, candidates for “second-line” therapy during their course.

Until the late 1990s, the treatment of metastatic colon cancer was limited to only one effective drug, 5-fluorouracil (5-FU); this agent was shown to prolong both overall survival (OS) and improve quality of life (2). Since its synthesis in 1957, there have been numerous studies aiming to optimize treatment with 5-FU through biochemical modulation and changes in administration schedule. However, response rates (RRs) and OS have remained fairly constant, with overall RR in the 10%–20% range and OS on the order of 10–12 months, respectively (3).
During this era of single-agent fluoropyrimidine therapy, the notion of a standard second-line treatment for metastatic colon cancer did not exist. In general, the only option was to change the schedule of administration from bolus to infusion or vice versa. However, since 1997, several new agents have been developed, resulting in a significant evolution of treatment options for patients with advanced colorectal cancer. The availability of the chemotherapeutic agents irinotecan, oxaliplatin, and capecitabine as well as the biologic agents bevacizumab, cetuximab, and panitumumab has provided an expanded armamentarium against colon cancer that is both exciting and somewhat daunting. In this chapter, the available data for second-line treatment of metastatic colon cancer are reviewed, and a road map is provided on how best to integrate these agents after first-line failure.

Is Second-Line Treatment Beneficial?

Several studies address the essential issue of whether there is any value to additional chemotherapy after failure of first-line treatment in metastatic colorectal cancer. The mere availability of active drugs beyond 5-FU should not be assumed to yield an improved OS for patients who have progressed on prior treatment.

Irinotecan

Irinotecan is a camptothecin analog that functions through inhibition of DNA topoisomerase I, leading to single-strand DNA breaks and cell division arrest. Two pivotal randomized phase III studies demonstrated the clinical efficacy of irinotecan in second-line therapy (4,5). Cunningham et al. randomized patients who had progressed within 6 months of treatment with 5-FU to either irinotecan every 3 weeks with supportive care or supportive care alone (4). Median survival was significantly improved with irinotecan compared with supportive care (9.2 months vs. 6.5 months, respectively), and this effect was independent of performance status (PS). In addition, quality of life was significantly improved with second-line irinotecan, despite increased side effects and the well-known toxicities of this agent. The irinotecan-treated group had somewhat more grade 3 and 4 adverse events compared with the control arm (79% vs. 67%), specifically neutropenia, diarrhea, nausea, and vomiting. Rougier et al. randomized patients who had progressed while on 5-FU or within 3 months of the last 5-FU-based regimen to
either irinotecan every 3 weeks or additional 5-FU treatment on a different schedule according to investigator choice (5). Patients who received irinotecan had an increased median OS of 10.8 months compared with those treated with 5-FU, with a median OS of 8.5 months. Progression-free survival (PFS) was also improved with irinotecan (4.2 months) compared with 5-FU (2.9 months). There were more grade 3/4 treatment-related events in the irinotecan arm, but notably, there was no difference in the global health status score. Based on these two studies, single-agent irinotecan was proven to be an effective second-line treatment for metastatic colon cancer.

Oxaliplatin

Oxaliplatin is a third-generation platinum analog and is unique because of its retained diaminocyclohexane moiety when forming DNA adducts. Unlike cisplatin and carboplatin, the bulky side chain on oxaliplatin is thought to contribute to its different activity spectrum in colon cancer. Oxaliplatin was initially developed in France as a first-line therapy and approved in 1997. However, trials in the United States began at the time when the combination of irinotecan and 5-FU (IFL) was becoming standard therapy. Two large randomized phase III studies confirmed that first-line combined irinotecan and 5-FU was superior to 5-FU alone and thereby left a void for patients progressing on initial therapy (6,7). After the failure to obtain U.S. Food and Drug Administration (FDA) approval for first-line FOLFOX (oxaliplatin, leucovorin, and 5-FU) based on the European data, Rothenberg et al. performed a three-arm trial including patients who had progressed on IFL (8). A total of 463 patients were randomized to receive infusional 5-FU, single-agent oxaliplatin, or combined infusional 5-FU and oxaliplatin (FOLFOX4). The RR for patients treated with single-agent 5-FU or oxaliplatin was 0% and 1.3%, respectively. However, treatment with the combination resulted in a 9.9% RR, with an additional 50%–60% of patients maintaining stable disease for at least 2 months. Patients treated with FOLFOX also had a longer time to progression (TTP) of 4.6 months compared with 2.7 months with 5-FU and 1.6 months with oxaliplatin. Not surprisingly, overall toxicity was increased in the FOLFOX treatment arm when compared with either single agent. Grade 3/4 diarrhea, nausea, neutropenia, thrombocytopenia, and hand-foot syndrome were all significantly greater with FOLFOX. Both oxaliplatin-containing arms had higher rates of peripheral neuropathy when compared with 5-FU alone; however, cumulative neuropathy was less than 5%.
Despite increased toxicity, the FOLFOX regimen, which yielded greater clinical efficacy, also resulted in the largest improvement in tumor-related symptoms.

Sequence: Does It Matter?

As outlined in the previous section, both irinotecan and oxaliplatin are effective second-line agents in the treatment of metastatic colon cancer; however, it was still unclear after the previously described studies whether the sequence of administration of either agent had any effect on patient outcome. A well-designed, but underpowered study by the GERCOR Study Group was performed to help address this issue. A total of 226 previously untreated metastatic colon cancer patients were randomized to treatment with either FOLFIRI (folinic acid, 5-FU, and irinotecan) or FOLFOX (9). At time of progression, patients were then required to cross over to the other treatment arm, in essence substituting oxaliplatin for irinotecan or irinotecan for oxaliplatin. The primary endpoint was TTP after both regimens. The OS for both groups did not differ significantly, with a median survival of 21.5 months for the patients allocated to FOLFIRI then FOLFOX (arm A) versus 20.6 months for the patients allocated to FOLFOX then FOLFIRI (arm B) \( (P = .99) \). Median second PFS, the time duration from randomization until progression after second-line chemotherapy, also did not differ significantly and was 14.2 months in arm A and 10.9 months in arm B \( (P = .64) \). When RR and PFS were analyzed by first and second treatment lines, no differences were observed between the two treatment arms in first line; however, FOLFOX treatment in the second line (arm A) had a slightly higher RR (Table 1). One possible explanation for this finding is the fact that 74% of patients in arm A were able to receive second-line treatment, compared with only 62% in arm B, or higher rates of surgical resection in arm B. Another important point to be emphasized from the study is the different toxicity profiles of FOLFIRI and FOLFOX. There were significantly more episodes of thrombocytopenia, neutropenia, and sensory neuropathy with first-line FOLFOX, whereas FOLFIRI resulted in more nausea, mucositis, and alopecia. When compared with front-line treatment, second-line therapy with FOLFOX resulted in less frequent neutropenia and thrombocytopenia, but there remained persistently higher rates of grade 3 neurotoxicity. Apart from less frequent gastrointestinal toxicities, the side effect profile of second-line FOLFIRI treatment was comparable to that seen in the first-line setting.
Combination versus Sequential Therapy

The Tournigand study demonstrated that the sequence of combination therapy did not make a significant difference with respect to OS (9). However, given the substantial toxicity from combination therapy, with up to one-third of patients never receiving second-line treatment, the question of whether sequential single agents can provide a similar benefit with fewer adverse side effects is of great interest. Grothey et al. performed an interesting analysis of data from 11 phase III trials in patients with metastatic colon cancer treated first line with irinotecan- or oxaliplatin-based combinations (10). The objective of the review was to correlate the percentage of patients receiving second-line therapy and the percentage of patients receiving all three active chemotherapeutic agents with OS, based on the hypothesis that improvement in OS was dependent on the availability of all three drugs during the course of treatment (which largely depended on the time period the trial was conducted and when the agents were approved for routine use). They found that the percentage of patients treated with all three agents (5-FU, irinotecan, and oxaliplatin) at some point in their disease showed a statistically significant correlation with median OS (P <0.0001) (Figure 1) (11). However, consistent with results of the Tournigand study, there was no significant relationship between initial doublet therapy (i.e., irinotecan-
or oxaliplatin-based) and OS. Although univariate analysis demonstrated a survival advantage with first-line combination chemotherapy, multivariate analysis showed only that exposure to all three drugs, but not use of a specific doublet first line \((P = .69)\), was significantly associated with OS. Based on this review, it is clear that treatment after first-line progression is efficacious in prolonging survival; however, it could not definitively answer which, if any, sequence is superior.

Two studies were subsequently performed to answer the challenging sequencing question. The CAIRO trial sought to determine whether first-line combination treatment is better than sequential administration of the same drugs in terms of OS \((12)\). The MRC FOCUS study compared three different treatment strategies: sequential single agents, single agent followed by combination therapy, and first-line combination therapy \((13)\). In the CAIRO study, 810 patients were randomized to either (a) treatment with either single-agent capecitabine followed by irinote-
can on first progression and capecitabine plus oxaliplatin on second progression or (b) initial combination treatment with capecitabine plus irinotecan and then capecitabine plus oxaliplatin on progression. There was no significant difference in OS between the two treatment strategies, with median OS of 16.3 months versus 17.4 months with sequential and combination therapy, respectively. As expected, first-line combination therapy had an increased RR and prolonged PFS compared with monotherapy. Increased toxicity was also more frequently observed in the combination treatment group, with higher rates of diarrhea, nausea, vomiting, and neutropenia. The authors concluded that combination therapy was no more effective than sequential treatment in terms of OS, and in fact, was actually associated with significantly more toxicity. This study supports the model that the order in which drugs are given is not as important as receiving all three active agents during the course of disease, although the study is underpowered to detect anything but very large differences in survival.

The MRC FOCUS trial design was more complex and investigated different sequencing strategies (Figure 2). Using a three-arm design, the study examined the efficacy of sequential single agents, the value of continuing 5-FU on progression, the efficacy of deferred combination therapy, and the value of second-line oxaliplatin-versus irinotecan-based treatment. One of the main conclusions of the study was that there was not a superior sequence strategy in terms of OS, with median OS ranging from 13.9 months to 15.9 months. However, it must be highlighted that the patient population targeted were poor prognosis individuals and specifically included only patients who had inoperable metastatic disease for whom it was judged that even with a response to chemotherapy, surgery would remain unlikely. Deferred combination therapy was as effective as upfront combination chemotherapy and, not surprisingly, had significantly less initial toxicity. In addition, in second-line treatment, the combination therapies with 5-FU and either irinotecan or oxaliplatin both gave higher RRs than did single-agent irinotecan, although the rates of PFS were not significantly improved. Therefore, the usefulness of continuing 5-FU combined with irinotecan on progression remains questionable based on these results. In a comparison between irinotecan- versus oxaliplatin-based combination second-line treatment, there was no significant difference in OS, again confirming the findings from previous studies.

Based on current evidence, it is clear that the most effective strategy to prolong survival is using all three active chemotherapeutic agents during the course of the disease. The specific sequence of drugs does not appear to have a significant impact in patients with poor prognoses, who
have no chance for surgical resection. It appears that upfront combination therapy produces improved TTP, although studies are not able to demonstrate survival difference given efficacy of subsequent regimens. Improvement in TTP and symptom control favoring combination therapy should be considered in this setting, and in general, given this evidence, the choice of second-line treatment would depend on the patient’s comorbidities, PS, and goals of treatment.

**Biologics: Integration into Second-Line Treatment**

An enhanced understanding of the molecular and genetic basis of malignant behavior has led to the development of novel therapeutics that target multiple essential pathways for the cancer phenotype. Two pathways, in
Angiogenesis Inhibition

Sustained angiogenesis is one of the hallmarks of cancer, and as such, has been an important target in colon cancer. For tumors to grow beyond a certain size, they must be able to induce and maintain a vascular supply (14). The vascular endothelial growth factor (VEGF) and its receptors (VEGFR) have been shown to play a pivotal role in angiogenesis by increasing vascular permeability, stimulating endothelial cell proliferation and migration, and maintaining vascular integrity (15). Both VEGF and its receptors are overexpressed in colon cancer and correlate with a poor prognosis, making them a key target in treatment (16).

Drugs targeting the VEGF pathway can be divided into two broad categories: those that target the VEGF protein(s) and those that target its receptor(s). Presently, only one antiangiogenic agent is FDA-approved for use in colon cancer. Bevacizumab, a monoclonal antibody (MoAb) that binds VEGF, was initially approved for first-line therapy in metastatic colon cancer based on the pivotal trial of IFL plus bevacizumab or placebo (17). In this study, the addition of bevacizumab to a standard (at the time) first-line chemotherapy resulted in a 34% reduction in the risk of death and a 45% improvement in PFS, both highly statistically significant. Bevacizumab, combined with 5-FU-based chemotherapy, quickly became standard of care in first-line metastatic colon cancer.

The question of bevacizumab’s efficacy in the second-line setting was subsequently examined in the E3200 study (18). In this trial, 829 metastatic colon cancer patients previously treated with 5-FU and irinotecan, but no bevacizumab or oxaliplatin, were randomized in a 1:1:1 ratio to treatment with FOLFOX plus bevacizumab, FOLFOX alone, or bevacizumab alone. Based on an interim analysis, the bevacizumab alone arm was closed to accrual because of inferior outcome when compared with the chemotherapy-containing arms of the study. The addition of bevacizumab to FOLFOX resulted in a significant improvement in OS, with a 25% reduction in the risk of death. Median OS was 12.9 months with the addition of bevacizumab, compared with 10.8 months without (hazard ratio = 0.75; P = .11). Furthermore, the addition of bevacizumab also resulted in a gain in PFS compared with those treated with chemotherapy.
alone (7.3 vs. 4.7 months respectively, hazard ratio = 0.61; \( P < 0.001 \)). Although the addition of bevacizumab to FOLFOX in the second line improved OS and PFS, it also resulted in increased toxicity. Treatment with bevacizumab was associated with a 14% overall increase in rates of grade 3/4 toxicity, including neuropathy, hypertension, bleeding, and vomiting. Despite these safety signals, bevacizumab was approved for use in combination with FOLFOX in the second-line treatment of metastatic colorectal cancer, based on the significant benefit in OS. It is clear that bevacizumab, in combination with chemotherapy, is active in both first- and second-line treatment; however, in patients who have progressed on both irinotecan- and oxaliplatin-based chemotherapy regimens the benefit of bevacizumab may be less (19). There appears to be a less than 5% RR with the addition of bevacizumab to 5-FU in this heavily pretreated group, although benefit in improved TTP and disease stabilization was not able to be determined.

In contrast to the remarkable success seen with bevacizumab, a MoAb directed against VEGF, small-molecule inhibitors of VEGFR have not yet achieved the same level of clinical benefit when combined with cytotoxic chemotherapy. The CONFIRM 2 (Colorectal Oral Novel Therapy of Angiogenesis and Retardation of Metastases) trial was one of the largest second-line studies to incorporate a small-molecule inhibitor of VEGFR (20). Vatalanib, a multi-targeted tyrosine kinase inhibitor of the VEGFR kinase, was combined with FOLFOX chemotherapy. A total of 855 patients previously treated with 5-FU and irinotecan were randomized to treatment with FOLFOX plus vatalanib or placebo. The primary end-point of the study was not met, as there was no significant difference in OS with the addition of vatalanib. However, PFS was significantly prolonged with the addition of vatalanib (5.5 vs. 4.1 months). Furthermore, on subset analysis of patients with high lactate dehydrogenase levels, PFS was even longer when patients were treated with vatalanib (5.6 vs. 3.8 months; \( P < 0.001 \)). There are several potential reasons to explain why the addition of vatalanib did not improve OS, including incorrect dosing schedule, patient noncompliance, and a nonenriched patient population. Research continues with other small-molecule inhibitors, such as sunitinib and sorafenib, in combination with chemotherapy to determine their potential role in the treatment of advanced colorectal cancer.

Epidermal Growth Factor Pathway Inhibition

EGF receptor (EGFR or HER-1) is a member of the Erb (HER) family of receptors, a group that is abnormally activated in many epithelial malig-
nancies. It has been demonstrated that potentially 80% of colon cancers exploit EGFR for their pathogenesis, leading to unchecked and dysregulated growth (21). Because alterations in ErbB receptors have been correlated with more aggressive disease, lower rates of survival, and poor response to therapy, they have been a main target in colon cancer drug development. Similar to inhibitors of angiogenesis, two pharmacologic methods to block the EGFR signaling pathway have been developed—antibody targeting of EGFR and small-molecule inhibitors, which block the downstream effects of EGFR signaling. Of the different strategies, the MoAbs have shown much greater activity in colon cancer therapy, with two such agents, cetuximab and panitumumab, already approved by the FDA for use in refractory cases.

The first MoAb targeted against EGFR and approved for treatment of metastatic colon cancer was cetuximab, a chimeric antibody of the IgG1 type. Unlike bevacizumab, which initially was approved in first-line therapy in combination with 5-FU–based chemotherapy, cetuximab was initially approved in the advanced setting for use in irinotecan-refractory patients, either in combination with irinotecan or as a single agent. Cetuximab's approval was based on the results of the phase III BOND study, which replicated two prior U.S. single-arm studies (22). A total of 329 patients whose disease was resistant to irinotecan were randomized to receive either cetuximab plus irinotecan or cetuximab monotherapy. The majority of patients had received at least two prior treatments, all had received irinotecan, and 62.6% had received oxaliplatin. The overall RR was 22.9% in the combination group and 10.8% in the monotherapy group, suggesting that the addition of cetuximab could reverse irinotecan resistance. In patients who had received prior oxaliplatin, there was also a benefit to cetuximab treatment, with an RR of 22.2% in the combination group and 8.5% in the monotherapy group. TTP was significantly prolonged with combination therapy compared with monotherapy (4.1 and 1.5 months, respectively), whereas OS was not significantly prolonged (6.9 and 8.6 months, respectively). The most frequent toxicity observed in both arms was acneiform rash that occurred in about 80% of all patients, although grade 3/4 skin rash occurred in less than 10% of patients. Severe anaphylactic reactions to cetuximab developed in 1.2% of patients and were only seen in the monotherapy arm. As anticipated, there were more overall adverse events in the combination arm, with significantly greater rates of neutropenia and diarrhea. The results of this trial demonstrated that cetuximab is an effective agent in irinotecan-refractory patients irrespective of the number of previous agents received or prior use of oxaliplatin. Furthermore, cetuximab has the ability to reverse chemotherapy resistance, making it an attractive
Second-line treatment. A subsequent study examined the efficacy of single-agent cetuximab compared with best supportive care (BSC) (23). In this study, 572 patients previously treated with 5-FU, irinotecan, and oxaliplatin were randomized to treatment with cetuximab plus BSC or BSC alone, without any subsequent crossover available. The use of cetuximab in this heavily pretreated population (more than 80% with three or more prior regimens) resulted in a significant improvement in PFS that was translated into an improvement in OS—6.1 months in the cetuximab arm and 4.6 months in the BSC arm—although it was clear that only 40% of patients benefited. A large randomized trial, the Erbitux Plus Irinotecan in Colorectal Cancer (EPIC) trial, specifically examined the efficacy of cetuximab in the second-line setting (24). Nearly 1,300 patients previously treated with combined 5-FU and oxaliplatin were randomized to receive either cetuximab plus irinotecan or irinotecan alone. PFS was significantly increased with the addition of cetuximab (4.0 vs. 2.6 months). RR was also significantly improved by fourfold with the combination of cetuximab and irinotecan compared with irinotecan monotherapy (16% vs. 4%). Despite meeting the secondary endpoints of PFS and RR, the trial did not meet its primary endpoint of OS. There was no difference in OS for the two treatment groups (10 vs. 10.7 months) in spite of the large difference in PFS, a finding that can be explained by the fact that more than half of the control arm went on to receive cetuximab in subsequent treatment lines. Further supporting the value of cetuximab in second-line treatment, quality of life was better preserved with the addition of cetuximab (25). Based on current data, cetuximab benefits patients both in combination with chemotherapy and as monotherapy. It also seems to have similar absolute survival benefits independent of in which line of therapy it is used.

Panitumumab is a fully human MoAb that targets EGFR. In contrast to cetuximab, it is fully humanized and belongs to the immunoglobulin (Ig)G2 isotype. This molecule has been approved for use in refractory colorectal cancer patients based on a randomized trial comparing single-agent panitumumab with BSC (26). A total of 463 patients who had progressed on prior treatment with 5-FU, oxaliplatin, and irinotecan were randomized to treatment with panitumumab plus BSC or BSC alone. The difference in RR was significant, with 10% of patients receiving panitumumab responding versus 0% in the BSC arm. There was also a 46% reduction in the relative progression rate for patients receiving panitumumab compared with BSC. OS was not significantly different between the two arms because most patients crossed over to the treatment arm on progression. The major toxicity associated with panitumumab treatment was skin toxicity, which occurred in approximately 90% of patients, and
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only one patient discontinued treatment secondary to a hypersensitivity reaction. Recent data from this trial, with analysis of tumor samples in more than 90% of patients for molecular correlates, have shown that benefit in outcome is highly correlated with mutational status of KRAS protein, with all the benefit in wild-type group and no benefit in the mutated KRAS group (27). Panitumumab is currently approved as a single agent in refractory colon cancer and in Europe for the KRAS wild-type specifically. Further information on KRAS status in combination trials will steer future decisions on how these antibodies are used. Trials combining panitumumab with chemotherapy in the second-line setting are currently ongoing. Preliminary safety results have been reported from a trial comparing second-line FOLFIRI plus or minus panitumumab after progression on a single 5-FU-containing regimen, and no undue safety signals have been observed that might warrant cessation of the trial (28).

Similar to small-molecule inhibitors targeted against the VEGF pathway, those targeted against EGFR have not proved to be very effective in the treatment of colon cancer (reviewed in reference 29). Both gefitinib and erlotinib, receptor tyrosine kinase inhibitors of EGFR, have been evaluated in phase I and II settings, alone and in combination with chemotherapy. Neither agent has shown much activity as a single agent, and the combination with chemotherapy results in excessive toxicity.

Combined Blockade of Vascular Endothelial Growth Factor and Epidermal Growth Factor Receptor in Second-Line Therapy

Based on strong preclinical data demonstrating more effective growth inhibition, combined inhibition of VEGF and EGFR has been tested in clinical studies to determine its efficacy. One of the first trials to examine treatment outcomes with combined biotherapy was the National Cancer Institute-sponsored randomized phase II trial of bevacizumab/cetuximab with or without irinotecan (BOND-2) in 83 bevacizumab-naive, irinotecan-refractory patients (30). Although it is rare now to treat a patient who has not received bevacizumab front line, this was an important study to demonstrate tolerability of dual biologic therapy and positive outcomes. An improved RR and TTP were demonstrated for combined cetuximab/bevacizumab when compared with the prior BOND study in which irinotecan-refractory patients were randomized to receive cetuximab versus cetuximab/irinotecan. Specifically, in the BOND-2 trial, RR and TTP were 20% and 4.9 months for treatment with two antibodies versus 37%
and 7.3 months when these antibodies were combined with irinotecan (Table 2). These results are much better than those of the larger BOND trial (RR, 11% and 23%; TTP, 1.5 months and 4 months). It should be noted, however, that such cross-trial comparisons are problematic, and these are not completely comparable trials in terms of patient population, centers, and accruals. Safety analysis (the primary endpoint) revealed that combined cetuximab/bevacizumab/irinotecan was very well tolerated, without any unexpected grade 3 or 4 toxicities. Based on this study, the combination of dual antibodies and irinotecan is a promising second-line regimen in bevacizumab-naive, irinotecan-refractory patients. It offers the option of using combination antibody treatment without chemotherapy in the second-line setting, an attractive option for those patients intolerant of irinotecan. However, dual antibodies should be used with some caution, given a recent phase III study conducted in previously untreated patients with FOLFOX or FOLFIRI plus bevacizumab with or without panitumumab (31). An interim efficacy and toxicity analysis of those patients treated with FOLFOX plus the two antibodies demonstrated not only an increased incidence of grade 3 diarrhea, dehydration, infection, and pulmonary emboli with the addition of panitumumab, but also decreased PFS and OS. It remains to be elucidated whether these results

<table>
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|               | Irinotecan    | Bevacizumab  | Irinotecan +
|               | + cetuximab   | + bevacizumab+
|               |               | + cetuximab  |
| Overall RR    | 11%           | 23%          | 20%           | 37%           |
| Median TTP    | 1.5 mos       | 4.1 mos      | 4.9 mos       | 7.3 mos       |
| Median OS     | 6.9 mos       | 8.6 mos      | 11.4 mos      | 14.5 mos      |

OS = overall survival, RR = response rate, TTP = time to progression.
are a consequence of this specific four-drug combination or related to panitumumab itself.

Second-Line Treatment Road Map

As outlined previously, the various chemotherapeutic agents currently available have resulted in a staggering number of permutations with which a patient can be treated during the disease course. Therefore, it is important to focus on three key considerations before choosing a second-line regimen. First, it is crucial to know the type of therapy the patient received and the response, as prior treatment correlates with future treatment outcome. Second, a patient's comorbidities and PS are key in determining the choice of additional drug(s). Finally, it is crucial to define the goal(s) of therapy in terms of curative versus palliative intentions and for patient preferences.

Currently, FOLFOX plus bevacizumab has emerged as the most commonly used front-line therapy in the United States. After failure on this regimen, a number of second-line treatments can be administered (Figure 3). If the patient has maintained a good PS and has documented disease progression on therapy, but does not have a relative contraindication to irinotecan, such as an increased predisposition to gastrointestinal toxicity and specifically diarrhea, then either FOLFIRI (9) or FOLFIRI plus cetuximab (24) could be given. For those patients who are reaching oxaliplatin toxicity, oxaliplatin may be discontinued and a fluoropyrimidine-plus-bevacizumab therapy should be continued. Determining whether to combine cetuximab with FOLFIRI may depend on the goal of therapy and, as data emerge, KRAS mutation status. For instance, if a patient has limited liver or lung disease and surgical resection may be a future option if there is a response, then the addition of cetuximab at this point is warranted, as it has been shown to increase RR. If the goal of therapy is to prolong TTP and maintain quality of life, then FOLFIRI can be administered alone and cetuximab can be added after second progression without affecting OS. In contrast, if a patient progressing on FOLFOX plus bevacizumab has a poor PS and the goal of care is not curative, then irinotecan as a single agent can be used. At this point, panitumumab is not yet approved for second-line therapy, and studies support its use only as a single agent after second progression. However, trials are ongoing to determine if, like cetuximab, this antibody will also have a role earlier in the disease course.

For patients who receive front-line FOLFIRI plus bevacizumab, FOLFOX is an effective second-line regimen (32). For those patients
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with preexisting neuropathy, oxaliplatin is contraindicated, and in this setting, cetuximab plus irinotecan or cetuximab monotherapy could be offered based on the BOND study. In rare circumstances in which bevacizumab is not used up front, it can then be combined in the second-line setting with FOLFOX (18). It can also be combined with cetuximab alone or irinotecan and cetuximab based on the results of the BOND-2 study (30). Again, the usefulness of panitumumab in second line after FOLFIRI plus bevacizumab remains unknown. It is effective as a single agent in at least the third line and continues to be investigated earlier in treatment in combination with chemotherapy.

Figure 3. Algorithm for treatment of metastatic colon cancer. CapeOx = capecitabine and oxaliplatin, FOLFIRI = folinic acid, 5-fluorouracil, and irinotecan, FOLFOX = oxaliplatin, leucovorin, and 5-fluorouracil, 5-FU = 5-fluorouracil.
Unanswered Questions in Second-Line Treatment

Given that the most commonly used front-line regimens contain bevacizumab, the question of whether to continue this biologic agent beyond progression is of great interest. It is clear that bevacizumab has a role in second-line treatment for patients who have not yet received the drug, but it is unclear if it confers any benefit after first progression. Preclinical experiments demonstrating utility of bevacizumab on reducing interstitial pressure and rapid rebound of neovascularization support the concept of continued anti-angiogenesis. At this point, the only data addressing this issue are from a large observational study. The BRiTE study enrolled nearly 2,000 metastatic colon cancer patients who were to be treated with front-line bevacizumab and chemotherapy (33). On progression, second-line treatment was at the investigator’s discretion. The registry then looked at pre- and post-treatment-related factors, including the continuation of bevacizumab beyond progression. In a multivariate analysis, not only was the exposure to any second-line chemotherapy agent correlated with a significantly increased OS, but so was the continuation of bevacizumab after first progression. This finding may reflect selection bias, as patients living longer tend to eventually receive bevacizumab again. Although the findings are provocative, there remains no prospective randomized trial to document the value of bevacizumab continuation after failure on first-line treatment, or even a maintenance approach. It is hoped that the results from two trials that are currently accruing will definitively answer this question. The Intergroup IBET trial (S0600) randomizes patients who have progressed on FOLFOX or XELOX (oxaliplatin and capecitabine) plus bevacizumab to either FOLFIRI plus cetuximab or FOLFIRI, cetuximab, and bevacizumab. The AIO group trial randomizes patients who have progressed on a bevacizumab plus standard first-line chemotherapy regimen to a second-line regimen plus or minus bevacizumab. The results from these two studies are eagerly awaited, but at this point, there are insufficient data to recommend the continuation of bevacizumab after first-line failure.

The ability to choose treatment based on the molecular characteristics of a patient’s particular tumor and thereby predict response and toxicity is slowly becoming a reality. Tailored therapy is still in its infancy, but there have been some very promising observations, specifically with regard to the EGFR inhibitors cetuximab and panitumumab. Both of these drugs when used as monotherapy in the refractory setting lead to about a 10% RR overall. If there were a marker to identify those responders, it would enrich the population and thereby make targeted treatment with EGFR inhibition more effective. The KRAS oncogene
has emerged as a possible selection marker for treatment with EGFR inhibitors. It appears that KRAS mutations, which lead to a constitutively activated protein, confer resistance to EGFR inhibition. A large retrospective study examined the predictive role of KRAS in patients randomized to treatment with panitumumab versus BSC (27). The RR to panitumumab was 17% for wild-type KRAS patients and 0% in KRAS mutants, an almost doubling of the RR in this more selected population. Furthermore, the relative effect of panitumumab versus BSC on PFS was significantly greater among patients with wild-type KRAS (median PFS of 12.3 weeks for panitumumab vs. 7.3 weeks BSC) compared with patients with mutant KRAS, in whom no panitumumab benefit was seen (median PFS of 7.4 weeks for panitumumab vs. 7.3 weeks for BSC). Therefore, from these data, it appears that wild-type KRAS is necessary but not sufficient for panitumumab efficacy. Another retrospective study examined the predictive role of KRAS in patients randomized to panitumumab plus chemotherapy versus chemotherapy alone (34). Again, an increase in RR with panitumumab was seen only in KRAS wild-type patients, without any benefit and possibly even a deleterious effect on KRAS-mutant patients. At this point, using KRAS mutational status to determine whether or not to treat with EGFR inhibitors is premature. Additional data on combination trials in first and second line are expected to be presented at ASCO 2008 (see Chapter 2), which will give additional information on the relative weight of this effect compared with the chemotherapy platform. Prospective, randomized trials are awaited to confirm these observations; however, if confirmed, this information can be expected to be used in new treatment algorithms where KRAS status will direct therapeutic pathways similar to the use of HER-2 status in the present-day treatment of breast cancer.

Conclusion

The treatment of metastatic colon cancer no longer centers on the modulation of one active agent, 5-FU, but rather requires integration of several agents throughout a patient’s disease course. The development of the chemotherapeutics oxaliplatin, capecitabine, and irinotecan as well as the biologics bevacizumab, cetuximab, and panitumumab has resulted in a prolonged median OS, which currently exceeds 2 years. Given the improvement in systemic therapy, second-line therapy for metastatic colon cancer has now proved to significantly prolong survival and improve quality of life. To decide which second-line treatment is best for an individual patient, three key factors should be considered: (a) prior
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treatment and RR, (b) patient comorbidities and PS, and (c) goals of care,
including patient preferences. It is likely that in the very near future cer-
tain molecular markers, particularly KRAS mutation status, will play a
key role in deciding on therapeutic pathways and options in both first-
and second-line therapy. As a result of the incorporation of these key fac-
tors into the treatment algorithm combined with the many active avail-
able drugs, numerous second-line paths exist. In the future, it is hoped
that biomarkers, including genomics, molecular pathway biomarkers, and
intermediate endpoints, will help to better define the optimum treatment
regimen for each individual and streamline management decisions.

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   the reverse sequence in advanced colorectal cancer: a randomized GERCOR
    advanced colorectal cancer improves with the availability of fluorouracil-
    leucovorin, irinotecan, and oxaliplatin in the course of treatment. J Clin
11. Grothey A, Sargent D. Overall survival of patients with advanced colorectal
    cancer correlates with availability of fluorouracil, irinotecan, and oxaliplatin
regardless of whether doublet or single-agent therapy is used first line. J Clin Oncol 2005;23:9441–9442.


Although the incidence of colorectal cancer (CRC) has recently declined in some portions of the world, it remains one of the most important forms of cancer among both men and women. In the United States, it is estimated that approximately 149,000 new cases of CRC will develop in 2008, representing 10% of all newly diagnosed cancers (1). At the time of initial diagnosis, approximately 50%–55% of patients have stage II or III disease and 20% of patients have stage IV disease (2,3). Of those patients with stage II or III disease, approximately one-third develop recurrent disease after undergoing potentially curative therapy (4). For many patients who develop advanced disease, the spread of disease is frequently limited to a single site or organ of involvement. At presentation, 15%–25% of patients with an initial diagnosis of stage IV disease have liver metastases, and as many as 50% of patients who have a recurrence present with liver metastases (5–7). Approximately half of patients developing liver metastases have this as the only site of metastatic disease (8). This finding was demonstrated in an autopsy series in which nearly 50% of patients with metastatic CRC had the liver as the only site of metastatic disease (9). For patients with stage II or III disease who develop a recurrence, 30% have the liver as the sole site of recurrence.

The propensity toward single organ involvement in patients with CRC makes it different from many other cancers such as breast cancer, in which
there is more frequently wide dissemination early in the development of metastatic disease. The limited metastatic spread observed in a significant portion of patients with CRC holds out the possibility of aggressive and in some cases curative treatment despite this advanced stage of the disease. However, the success to such an approach is based on a coordinated team effort with surgery, medical oncology, and radiology.

**Surgery**

Surgery remains the only potentially curative therapy for patients with liver metastases from CRC, and there are now well-established clinical data to support its use. The first reports of resection of a liver metastasis began to appear in the medical literature in the early 1940s (10). The value of surgery was subsequently more clearly demonstrated in a series of 120 patients with liver metastases of CRC reported by Wilson and Adson (11). Among 60 of these patients who underwent hepatic resection, 5-year survival was 25%, and there were no 5-year survivors in 60 comparable patients who did not undergo surgery.

More modern surgical series have continued to report similar outcomes. In a review of 1,001 patients undergoing resection of hepatic metastases from colorectal carcinoma at the Memorial Sloan-Kettering Cancer Center, Fong et al. found that the median overall survival from the time of liver metastasis resection was 42 months (12). The actuarial survival at 5 and 10 years was 37% and 22%, respectively. In these earlier series, a number of factors, in particular, the number of liver metastases, appeared to be important in predicting survival. In an older multi-institutional review, Hughes et al. noted 5-year actuarial survivals of 37% for solitary metastases, 34% for two, 8% for three, and 18% for four or more lesions (13). Corresponding 5-year actuarial disease-free survivals (DFSs) were 36%, 32%, 0%, and 14%, respectively. When patients with three metastatic lesions are grouped together with patients who had four or more metastatic lesions, the actuarial 5-year survival was 14%, and DFS was 7%. More recent series suggest that outcomes have improved, with 5-year disease-specific survivals of 46%, 40%, and 29% for one, two to three, or four or more metastases, respectively (14). More impressively, the 10-year DFS for these same three groups was reported to be 35%, 29%, and 21%.

Ultimately, the ability to successfully treat CRC liver metastases with surgery requires that all of the metastases can be resected with negative margins (R0 resection) and with at least 20%–30% of the normal liver remaining after completion of the surgery. Criteria for resection have evolved over time (Table 1). Attempts have been made to develop guide-
Approach to Liver-Limited Metastatic Disease

Lines for resection of liver metastases but have met with limited success (15,16). In general, the ability to resect liver metastases is limited by the size, location, and number of the metastases. Unless there are absolute contraindications to surgery, such as inability to obtain an R0 resection while retaining adequate normal liver or the presence of portal lymph node involvement, the overall trend has been toward more inclusive criteria for resection. Liver resection should therefore be assessed as a potential treatment option by an experienced liver surgeon unless it is highly

<table>
<thead>
<tr>
<th>Traditional selection criterion</th>
<th>Current view</th>
</tr>
</thead>
<tbody>
<tr>
<td>No more than 3 metastases</td>
<td>Patients with &gt; 3 metastases have poorer prognoses but may still benefit from or be cured by resection despite the absolute number of metastases.</td>
</tr>
<tr>
<td>Unilobar disease</td>
<td>Benefit is now also seen for bilateral disease.</td>
</tr>
<tr>
<td>Small tumors (&lt; 5 cm in diameter)</td>
<td>Patients with large tumors &gt; 7 cm in diameter have poorer prognoses but may still benefit from or be cured by resection.</td>
</tr>
<tr>
<td>Metachronous detection of metastases</td>
<td>Synchronous detection is considered a contraindication to resectability.</td>
</tr>
<tr>
<td>Primary tumor staged as I or II only</td>
<td>Patients with a stage III primary have a poorer prognosis but may still benefit from or be cured by resection.</td>
</tr>
<tr>
<td>Resection margin &gt; 1 cm required</td>
<td>Radical (R0) resection achieving a negative margin even if only 1 mm.</td>
</tr>
<tr>
<td>No extrahepatic disease</td>
<td>Exceptions for isolated lung metastases, resectable local hepatic recurrence, direct diaphragmatic invasion.</td>
</tr>
<tr>
<td>Exclusion of patients &gt; 65 yrs</td>
<td>Patients &gt; 70 yrs may also benefit from resection.</td>
</tr>
<tr>
<td>No portal nodal involvement</td>
<td>Criterion remains valid.</td>
</tr>
</tbody>
</table>

unlikely that surgery would have any prospect of saving or significantly prolonging the patient's life.

Role of Chemotherapy

Although surgery has shown success in providing an opportunity for long-term survival, the majority of patients undergoing surgery eventually develop recurrent disease. The probability of recurrence at any site is estimated to be 65% at 5 years, with 80% of the recurrences occurring within 3 years of the liver resection (14). By 10 years, the risk of recurrence appears to end, with 20% of patients remaining disease-free (17). As noted previously, when the cancer recurs, the majority of these recurrences are in the liver. The use of chemotherapy provides a potential means of enhancing outcomes related to surgery. This approach also allows patients with initially unresectable disease to become potentially resectable (Figure 1). When chemotherapy is given after an R0 surgical resection, it is referred to as adjuvant therapy. It is referred to as neoadjuvant therapy when given before surgery for potentially resectable disease, and conversion therapy when given for initially unresectable disease.

Adjuvant Chemotherapy for Resected Liver Metastases

The potential benefit of adjuvant systemic chemotherapy for resected liver metastases has been assessed in a randomized multicenter phase III trial (18). Patients undergoing an R0 resection were assigned to either observation or chemotherapy. Patients with extrahepatic disease, including portal lymph nodes, were not eligible. Of the 171 evaluable patients, 85 were assigned to observation and 86 to receive adjuvant chemotherapy with leucovorin and bolus 5-fluorouracil (5-FU) daily for 5 days out of every 28 days for six cycles. Using DFS as the primary endpoint, 5-year DFS for patients in the observation arm was 26.7%; for those receiving leucovorin and 5-FU, the 5-year DFS was 33.5% (95% confidence interval [CI] 0.46–0.96, P = .028). Overall survival also showed a nonstatistical improvement in patients receiving chemotherapy compared with those in the observation arm (62.1 months vs. 46.4 months; 95% CI 0.48–1.10, P = .13). The role of potentially more active systemic regimens in the adjuvant setting has yet to be reported. Aside from this trial, the only other randomized trials to assess adjuvant therapy evaluated the potential benefit of hepatic artery infusion (HAI).
Whereas recent reports have focused on systemic therapy, prior trials assessed the potential benefit of liver-directed therapy. The rationale for liver-directed therapy builds on the observed pattern of recurrence after resection of liver metastases. A number of large retrospective surgical series have shown that approximately half of recurrences involve the liver (14,17,19). Clinical trials of HAI therapy after resection have reported improved survival as well as a decrease in hepatic recurrence compared with patients receiving systemic therapy.

Two randomized trials of HAI after surgical resection of hepatic metastases from CRC have been reported. In a trial from Memorial Sloan-Kettering Cancer Center, patients were randomized to systemic chemotherapy alone, with either bolus 5-FU and leucovorin or continuous infusion 5-FU, versus systemic chemotherapy alternating with HAI floxuridine (FUDR) (20). Seventy-four patients were randomized to combined therapy and 82 to systemic therapy. A significant benefit was seen in patients receiving combined therapy. With a median follow-up of more than 10 years, the median overall survival in the group receiving combined therapy was 68.4 months compared with 58.8 months for those receiving systemic therapy alone (21). At 2 years, the rate of sur-
vival free of hepatic recurrence was 90% in the combined therapy group compared with 60% in the systemic therapy-only group \( (P < .001) \). However, recurrence outside the liver appeared similar in both groups.

In a separate trial, patients with two to four resected hepatic metastases were randomized to resection alone versus HAI FUDR combined with systemic continuous infusion 5-FU (22). This trial also showed a marked decrease in hepatic recurrence with HAI as well as a significant improvement in recurrence-free survival (46% vs. 25%, \( P = .04 \)). A nonsignificant trend toward improvement in overall survival was observed in those receiving postoperative therapy (47 months vs. 34 months, \( P = .19 \)).

The current use of HAI therapy has declined with the introduction of more active systemic regimens. There was at least one attempt to compare systemic therapy with the combination of capecitabine and oxaliplatin (XELOX) to that same regimen alternating with HAI FUDR. However, this study failed to complete accrual.

Neoadjuvant and Perioperative Chemotherapy

No randomized trial of neoadjuvant chemotherapy for potentially resectable liver metastases has yet been reported. The potential benefit and feasibility of chemotherapy in this setting have been assessed in a phase II trial. In that trial, 20 patients with potentially resectable liver metastases received two to three cycles of oxaliplatin, leucovorin, and infusional 5-FU. Each cycle consisted of 6 weeks of treatment followed by a 2-week break. Using this approach, two patients had a clinical complete response (cCR), and 18 had a clinical partial response. A R0 resection was possible in 16 of the patients. With a median follow-up of 23 months, six of the patients undergoing R0 resection had developed recurrent disease, and one patient had a local recurrence of the primary tumor.

More information is presently available on the use of perioperative chemotherapy for potentially resectable liver metastases. In a phase II trial, preoperative chemotherapy with the combination of oxaliplatin, leucovorin, and infusional 5-FU (FOLFOX7) followed by surgery and then postoperative chemotherapy with irinotecan, leucovorin, and infusional 5-FU (FOLFIRI) was evaluated in 22 patients (23). Objective responses, including three patients with a cCR, were observed in 17 patients. Two patients had a pathologically confirmed complete response. All patients were able to undergo an R0 resection, although two patients were found to have peritoneal metastases at the time of surgery. The long-term outcome for these patients has not yet been reported.
The use of perioperative chemotherapy for potentially resectable liver metastases has also been evaluated in a randomized phase III trial (24). In this European Organisation for Research and Treatment of Cancer (EORTC)-sponsored trial (EORTC 40983, EPOC), patients with potentially resectable liver metastases were randomized to surgery alone or to six cycles of FOLFOX4 before surgery and six cycles after surgery. Based on all eligible patients, an 8.1% improvement was seen in 3-year progression-free survival for patients receiving perioperative chemotherapy. Although this result represented a significant improvement, it fell below the prespecified level of success for the trial. In follow-up to the EPOC trial, the EORTC is conducting a randomized phase II trial (EORTC 40051, BOS) of perioperative therapy in which patients are randomized to either FOLFOX6, bevacizumab, and cetuximab or FOLFOX6 and cetuximab. Given the potential importance of either neoadjuvant or perioperative therapy in improving the outcomes for patients with potentially resectable liver metastases, further work needs to be done to establish the appropriate duration of therapy and the potential complications of this approach.

The importance of obtaining a response while receiving neoadjuvant therapy has been assessed in several retrospective patient series. In a series of 106 patients from Memorial Sloan-Kettering Cancer Center with potentially resectable liver metastases, 52 received neoadjuvant therapy, and 54 underwent immediate surgery (25). A median of 8 months of neoadjuvant chemotherapy was given. Using this approach, 37% of patients had radiologic evidence of progression, 37% had stable disease, and 26% had evidence of disease regression. All patients were able to undergo resection. When compared with patients who did not receive neoadjuvant chemotherapy, a nonsignificant 5-year DFS was observed in those receiving neoadjuvant chemotherapy (38% to 52%, respectively). However, more important, patients with stable or responding disease had a significantly improved 5-year DFS of 85%.

Building on the observation of the potential prognostic importance of response to neoadjuvant therapy, the value of a complete response has been assessed. In a group of 38 patients with a cCR after initial chemotherapy, a total of 66 of 183 liver metastases disappeared on imaging (26). At the time of surgery with the use of intraoperative ultrasound, 46 of the 66 liver metastases could not be identified. Fifteen of the 46 metastases were surgically removed, and a pathologically confirmed complete response was documented in only three of the 15 metastases. This finding emphasizes the continued importance of surgery in the management of patients with liver metastases but also raises the concern that a complete response may hinder the ability to perform surgery.
Finally, the potential prognostic importance of progression of disease while receiving neoadjuvant therapy has been reported. Outcomes were assessed in a retrospective series of 131 patients (27). Using a combination of 5-FU, leucovorin, and either oxaliplatin or irinotecan, 44% of patients had evidence of tumor regression, 30% had stable disease, and 26% had progression of their metastases. All patients underwent surgery but only 118 had a potentially curative resection. Within these 118 patients, the 5-year overall survival was 37% for those with a partial response, 30% for those with stable disease, and 8% for those with progressive disease. Using this information, it has become clear that control of the metastatic disease appears to be an important determinant of the long-term outcome after surgery. Patients with evidence of progression while receiving neoadjuvant therapy may be better served by changing to a different neoadjuvant therapy rather than proceeding to surgery.

Chemotherapy for Initially Unresectable Liver-Limited Metastases

The majority of patients present with liver metastases that are initially deemed unresectable or not optimally resectable based on their size, number, or location. In this setting, chemotherapy has the potential to downsize the metastases and therefore allow an opportunity to perform surgery (Table 2). In the absence of surgery, patients with unresectable colorectal liver metastases of cancer have a poor prognosis. With best supportive care, survival is usually less than 1 year, and although chemotherapy may prolong survival, improve quality of life, and provide palliation, it is not curative. When 5-FU and leucovorin have been used, objective response

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Response rate (%)</th>
<th>Resection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Fluorouracil (5-FU)</td>
<td>15–20</td>
<td>Uncertain</td>
</tr>
<tr>
<td>FOLFOX (leucovorin, 5-FU, and oxaliplatin)</td>
<td>45–50</td>
<td>33</td>
</tr>
<tr>
<td>FOLFIRI (leucovorin, 5-FU, and irinotecan)</td>
<td>45–50</td>
<td>33</td>
</tr>
<tr>
<td>FOLFOXIRI (leucovorin, 5-FU, oxaliplatin, and irinotecan)</td>
<td>55–65</td>
<td>10–50</td>
</tr>
</tbody>
</table>
rates of 15%–20% are generally achieved (28). With the addition of the newer cytotoxic agents oxaliplatin or irinotecan onto the fluoropyrimidine backbone, improved response rates and survival have been observed (29).

Initial evidence of benefit from the use of preoperative chemotherapy for unresectable liver metastases came from retrospective single center patient series. The largest of these series was originally reported and subsequently updated by Adam et al. and Bismuth et al. from the Hôpital Paul Brousse in Paris (30,31). Excluding the 171 patients who were initially resectable, the use of preoperative FOLFOX permitted surgery to be performed in 14% of the remaining group of patients. With 5 years of follow-up after surgery, 34 patients were still alive, with 19 (22%) having no evidence of disease.

Similar results were reported in a retrospective study of 151 patients with metastatic CRC confined to the liver but unresectable at presentation (32). Treatment with chronomodulated chemotherapy using oxaliplatin, 5-FU, and leucovorin resulted in tumor shrinkage of more than 50% in 89 patients (59.6%) and permitted surgery to be performed in 77 patients (51%). Complete resection was achieved in 58 patients (38%). Among the 77 patients who underwent surgery, a median overall survival of 48.8 months was reported, and 5- and 7-year survivals were 50% and 30%, respectively.

This type of approach requires that patients be appropriately selected and that therapies with a high likelihood of efficacy be used. A review of published reports has shown that appropriate selection of patients remains challenging, given a large degree of variability between published studies (33). The selection of chemotherapy regimens is generally based on response rates seen in trials for patients with more generalized metastatic disease. Although high rates of response may be seen in this setting, the results do not always translate to a similar level of benefit when used in patients with metastases restricted to the liver.

Despite a relatively robust series of retrospective studies and secondary analyses from trials for metastatic CRC, only limited information is available from prospective clinical trials for patients with liver-limited disease. All of the trials reported to date have been phase II trials and generally included both high-risk but potentially resectable patients and patients with initially unresectable metastases. In a trial conducted by the North Central Cancer Treatment Group, 42 eligible patients received FOLFOX 4, with the intent to treat until their liver metastases became potentially resectable (34). A clinical response rate of 52% was observed, and ultimately 17 of the 42 patients underwent attempted resection. Fourteen of the patients (33%) were able to undergo an R0 resection. Median overall survival for patients undergoing resection was 35 months at the time of last follow-up. In a sim-
ilar trial using FOLFIRI, Pozzo et al. also reported a 33% rate of conversion that allowed a surgical resection to be performed (35).

Attempts to enhance the response rate have become the focus of more recent reports. Given the apparent benefit of both FOLFOX and FOLFIRI, response to a combination of these two regimens (FOLFOXIRI) was assessed in metastatic CRC as part of a phase III trial comparing FOLFOXIRI to FOLFIRI (36). In this trial, a planned secondary analysis of the rate of R0 resection was performed. Of the 244 patients enrolled, 33% had liver-only metastases. The rate of R0 resection after chemotherapy was 36% in the FOLFOXIRI arm, compared with 12% in the FOLFIRI arm (P = .017). Although patients in this trial were not randomized based on the presence of liver-only metastases, the increased ability for patients to undergo R0 surgical resection suggests potential benefit to this regimen. In general, the combination of FOLFOXIRI appears to be well tolerated. Neutropenia, diarrhea, and neurotoxicity were the primary toxicities reported. Only 9% of patients treated with FOLFOXIRI required treatment interruptions due to toxicity. The 60-day mortality rate was 1.6%, all due to rapidly progressive disease. A trial of FOLFOXIRI limited to patients with liver-only metastases has yet to be reported.

The likelihood of further advances with chemotherapy alone is small. Meaningful increases will likely instead come from the addition of targeted therapy to chemotherapy. The addition of either an anti-angiogenic or an epithelial growth factor receptor (EGFR) inhibitor to chemotherapy has enhanced response in metastatic CRC in general. Only limited information is available from clinical trials involving patients with liver-only metastases. A phase II trial of FOLFOX and the EGFR inhibitor cetuximab is currently under way in the North Central Cancer Treatment Group for patients with initially unresectable liver-only metastases. In a phase II trial of FOLFOX and cetuximab for patients with metastatic CRC, 37 of the 43 patients enrolled had liver involvement, 17 in whom the liver was the only site of involvement (37). Thirty-four of the 43 patients had an objective response. Ten of these patients underwent surgical resection of their metastases, including eight patients with liver metastases. In a retrospective series of cetuximab in 151 patients with liver metastases refractory to conventional chemotherapy, 27 (18%) had a response allowing resection (38).

Published information on the combination of chemotherapy and the anti-angiogenic inhibitor bevacizumab is more limited. Initial evidence of benefit came from a phase II trial of FOLFOX and bevacizumab for metastatic CRC involving 53 patients (39). Six of 34 patients with liver involvement had a response allowing a surgical resection. In a more
recent trial specifically for patients with liver metastases, neoadjuvant therapy with bevacizumab, capecitabine, and oxaliplatin was assessed (40). Although all of the patients in this trial had potentially resectable liver metastases, the trial demonstrated that bevacizumab can be given safely in the preoperative setting and that a high objective response rate of 73% can be achieved. Further evaluation of this combination in conversion therapy is indicated.

Liver Toxicity Secondary to Chemotherapy

Although neoadjuvant therapy is clearly providing benefit to selected patients, it has several potential limitations (41). With the increased use of neoadjuvant or conversion therapy, there has been a greater recognition of chemotherapy-associated liver injury. Both chemotherapy-associated steatohepatitis (CASH) and sinusoidal dilation have been reported. CASH has been more commonly reported with the use of irinotecan chemotherapy and presents histologically as severe steatosis, lobular inflammation, and ballooning (42,43). It also appears to occur more commonly in patients with higher body mass index. The development of CASH has been associated with a higher postoperative mortality rate related primarily to postoperative liver failure. In a series of 248 patients who had received chemotherapy before surgery compared with 158 who had not received any chemotherapy, 20.2% of patients receiving irinotecan had evidence of steatohepatitis in non–tumor-bearing liver, compared with 6.3% of patients who had received oxaliplatin, and 4.4% of those who had not received any chemotherapy (43). The 90-day mortality rate in those with evidence of steatohepatitis was 14.7% compared with 1.6% for those who did not.

Sinusoidal dilation has been associated with the use of oxaliplatin (43). Histologically, this toxicity presents as sinusoidal dilation with erythrocyte congestion. In more severe cases, it may evolve to periportal fibrosis and sinusoidal obstruction, similar to what is seen with venoocclusive disease. The development of sinusoidal dilation does not appear to have an increased risk of postoperative mortality. In a cohort of 303 patients who had undergone surgery for liver metastases, 92 were randomly selected for a detailed pathologic analysis of the resected liver (44). Twenty-three patients had received 5-FU and leucovorin alone; 52 oxaliplatin, 5-FU, and leucovorin; and 17 no chemotherapy before surgery. Vascular lesions in the non–tumor-bearing liver were commonly seen in patients who had received chemotherapy compared with those who had not (52% vs. 18%). This included higher rates of
sinusoidal dilatation and congestion, peliosis, hemorrhagic centrilobular necrosis, and regenerative nodular hyperplasia. However, no venoocclusive disease was seen. The presence of vascular changes was associated with a higher probability of receiving an intraoperative red blood cell transfusion.

Given the potential adverse effects of preoperative chemotherapy, the general recommendation has been to limit the duration of therapy before any planned surgery. This time frame has been somewhat arbitrarily set at 3–4 months of preoperative therapy for neoadjuvant therapy or to proceed to surgery when technically feasible in patients receiving conversion therapy. When patients are treated to best response, the risk of liver injury may increase, and subsequent surgery may become more difficult. If preoperative therapy leads to a complete response, it may become more difficult for the surgeon to precisely define the portion of the liver that should be resected. In some cases, a complete response could potentially make a patient inoperable. In this setting, some surgeons have allowed for regrowth of the liver lesion, which would then allow for easier visualization and subsequent surgical resection.

Conclusion

Beginning in the latter half of the twentieth century, progressive improvement in outcomes for patients with liver-only metastases from CRC has been observed. This advance has occurred through significant improvements in surgical techniques, in defining the patients who are eligible for liver-directed surgery, and the growing recognition that surgery is able to provide for long-term survival. In addition, the integration of chemotherapy has greatly enhanced the benefits gained from surgery in patients with initially resectable liver metastases as well as in those with initially unresectable disease. As patients with metastatic CRC increasingly receive chemotherapy with curative intent, treatment in a multidisciplinary environment will become central to achieving optimal outcomes. Without question, a medical oncologist, a radiologist, and a skilled hepatobiliary surgeon must be involved in deciding the best course of therapy and timing of surgery. To help guide these decisions, a computerized decision model (OncoSurge) has been developed and validated to aid decision making on a patient-by-patient basis in the management of liver metastases from CRC (16). With the progress in therapy that has occurred for CRC metastatic to the liver, the prospect of long-term survival is becoming a reality for an increasing proportion of patients.


The management of metastatic colorectal cancer (mCRC) has achieved significant therapeutic success with the introduction of more potent cytotoxic agents and molecular targeting agents. The current standard of care for patients with mCRC consists of a combination of the cytotoxic agent 5-fluorouracil (5-FU), leucovorin, and irinotecan (CPT-11) or oxaliplatin. The addition of molecular targeting agents bevacizumab and cetuximab has further increased the response rates (RRs) and improved progression-free survival (PFS) or overall survival (OS). However, a considerable percentage (40%–50%) of mCRC patients either do not experience clinical benefit in response to these therapeutic approaches or suffer from severe toxicities. Thus, reliable markers are needed to predict the efficacy of the available treatment modalities to develop therapeutic strategies for the individual patient. The overall goal of individualized therapeutic strategies is to maximize the therapeutic efficacy and to minimize toxicities. Furthermore, individual therapeutic strategies should reduce treatment expenses and improve overall pharmacoeconomics (1).

For the practicing clinician, it is important to distinguish between prognostic and predictive molecular markers. Prognostic markers reflect the natural course of the disease independent of any therapy. In contrast, predictive markers indicate the likelihood of response to a specific therapy and/or the potential for increased toxicity. Molecular markers may be expression levels

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Molecular Markers

Pharmacogenetic studies focus mostly on the identification of germ-line polymorphisms or intratumoral gene expression levels that are predictive for therapeutic outcome. Alterations in the genome or variations of gene expression levels of drug-metabolizing enzymes can also significantly change the pharmacokinetic and the pharmacodynamic behavior of chemotherapeutic agents, which can lead to increased toxicity and failure of therapeutic effects. The main goal of pharmacogenetic studies is to select patients who would benefit from a specific therapy and to identify those at risk for increased or even life-threatening toxicity (2).

Analyses of tumor DNA and RNA not only help to identify predictive and prognostic markers, but may also reveal novel mechanisms of resistance and detect new potential therapeutic targets. Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) targeted therapies were developed based on the significant role of VEGF and EGFR pathways in tumor development and progression. The evaluation of the EGFR and VEGF pathways has revealed potential predictive and prognostic markers associated with clinical outcome to targeted therapies.

A variety of molecular techniques have been used in the search for novel molecular markers. Immunohistochemistry (IHC) is an easy and widely used method to determine protein expression in normal and tumor

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### Table 1. Technical approach for the identification of novel predictive molecular markers in normal and tumor tissue

<table>
<thead>
<tr>
<th>Type of change</th>
<th>Nature of change</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Mutations</td>
<td>Qualitative</td>
<td>PCR</td>
</tr>
<tr>
<td>Polymorphisms</td>
<td>Static</td>
<td>DNA sequencing</td>
</tr>
<tr>
<td>Methylation</td>
<td>Static</td>
<td>DNA sequencing</td>
</tr>
<tr>
<td>Allelic deletions (LOH)</td>
<td>Static</td>
<td>DNA sequencing</td>
</tr>
<tr>
<td>mRNA Gene expression</td>
<td>Quantitative</td>
<td>qRT-PCR</td>
</tr>
<tr>
<td>Protein Protein expression</td>
<td>Quantitative</td>
<td>Microarrays</td>
</tr>
<tr>
<td>Protein function</td>
<td>Dynamic</td>
<td>IHC</td>
</tr>
<tr>
<td>Protein function</td>
<td>Dynamic</td>
<td>HPLC</td>
</tr>
</tbody>
</table>

HPLC = high-performance liquid chromatography, IHC = immunohistochemistry, LOH = loss of heterozygosity, mRNA = messenger RNA, PCR = polymerase chain reaction, qRT-PCR = quantitative real-time PCR.
tissues. However, IHC is a semiquantitative method that is limited by the sensitivity of monoclonal antibodies used; issues related to tissue handling, storage, and stability; and differences in interpretation between reviewers. In CRC, none of the IHC biomarkers has yet proved to be a reliable predictor for therapeutic outcome. For example, IHC analysis of EGFR expression is still part of the package insert for cetuximab and panitumumab. However, no studies to date have identified an association between level of EGFR expression and clinical outcome in response to anti-EGFR therapy. Therefore, it is now accepted that the EGFR expression level, at least as assessed by IHC, is not an appropriate marker to select patients for EGFR-targeted therapy.

More advanced approaches in the search for molecular markers include DNA and RNA microarrays, allowing a genome-wide screening for DNA fingerprints and RNA expression profiles. Refined technical protocols in the polymerase chain reaction (PCR) enable further differentiation of the DNA fingerprints into single nucleotide polymorphism (SNP), chromosomal aberrations, microsatellite instability, and differential methylation status of DNA promoter regions (3). Quantitative real-time PCR (qRT-PCR) uses mRNA to detect specific gene expression levels in relative values (target gene–reference gene ratio). In the past, the use of PCR was limited by the necessity for fresh tissue samples. At present, DNA and RNA isolation can be successfully accomplished from paraffin-embedded tissue samples. Furthermore, the use of laser capture microdissection of tumor cells from paraffin-embedded tissue avoids contamination of normal tissue, which then greatly improves the specificity of the information on genes and their expression levels in the tumor (4).

The usefulness of molecular markers for clinical practice has been assessed by defining their levels of evidence. Level I evidence is considered definitive and is obtained from high-powered, prospective, randomized, controlled trials on molecular markers. Level II evidence includes prospectively performed clinical trials that study molecular markers as a secondary objective. Level III and IV evidence retrieve data from large or small retrospective clinical evaluations of molecular markers. Level V evidence is considered to possess the weakest evidence and is derived from single clinical cases. The available data on molecular markers in mCRC have reached level III evidence. However, at least level II evidence is required for the application of new molecular markers in routine clinical practice (5).

The aim of this chapter is to provide clinicians with an overview of the relevant molecular markers associated with outcome and toxicity to chemotherapeutic and targeted agents in mCRC patients. These markers may help to guide the selection of a specific treatment strategy for individual patients in the future. The development of individualized thera-
Molecular Markers of Fluoropyrimidines

The fluoropyrimidine 5-FU is the most frequently used chemotherapeutic drug in CRC. The efficacy of 5-FU–based chemotherapy has been associated with expression levels of several key genes, including thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD). These enzymes play a pivotal role in metabolism of 5-FU, and as such, they serve as potential targets of 5-FU action (Figure 1). The gene expression levels, polymorphisms, and protein expression levels have been assessed in tumor tissue samples and blood samples using PCR and IHC techniques. Their correlation with therapeutic response and survival in mCRC patients is discussed in the following section (Tables 2 and 3).

Thymidylate Synthase

TS is the primary target of the active metabolite of 5-FU, 5-fluorodeoxyuridine monophosphate (FdUMP). TS catalyses the reductive methylation of deoxyuridine monophosphate to yield deoxythymidine monophosphate, an essential precursor of deoxythymidine triphosphate that is required for DNA synthesis and repair. The active metabolite of 5-FU, FdUMP, forms a stable ternary complex with TS and 5,10-methylenetetrahydrofolate (CH₂THF), which blocks deoxythymidine monophosphate production, thereby inhibiting DNA synthesis and repair. Prolonged thymidylate depletion eventually leads to apoptosis.

Several clinical studies focused on the prognostic and predictive value of TS expression in 5-FU–based chemotherapy in CRC patients. This research was stimulated initially by findings in the preclinical setting that a major resistance mechanism to 5-FU and other fluoropyrimidines is increased expression of TS. Consequently, the presence of TS gene polymorphisms as well as TS gene expression levels has been suggested to predict therapeutic response and toxicity. Independent retrospective clinical studies identified a significant inverse relationship between TS expression levels and therapeutic response to 5-FU–based chemotherapy. TS gene expression levels, as assessed by RT-PCR, and TS protein levels, as determined by IHC, have consistently shown a strong association between low...
TS levels and improved median survival and RR to 5-FU as well as 5-FU/oxaliplatin treatment in mCRC (6,7).

TS expression is regulated, at least in part, by the TS promoter-enhancer region (TSER). Two polymorphisms have been identified within the 5'-untranslated region (5'-UTR) of the TSER. The first and most frequent polymorphism consists of a double (2R) or a triple (3R) tandem repeat of a 28-base pair sequence (8). Polymorphisms within the tandem repeats have been postulated to affect transcriptional and/or translational efficacy of the TS gene. In vitro and in vivo studies have revealed that the presence of the 3R sequence within the 5'-UTR of the TSER results in greater translation efficacy than with the 2R sequence. In
mCRC, a fourfold increase in TS mRNA levels was detected in patients homozygous for the 3R TS variant in comparison with patients homozygous for the 2R TS variant (9). Retrospective evaluation of the role of 2R/3R TS polymorphism in mCRC patients has shown that 3R/3R homozygous patients are less likely to respond to 5-FU–based chemotherapy compared with 2R/2R homozygous or 2R/3R heterozygous patients. This correlation is consistent with the fact that 3R/3R homozygous patients express higher TS mRNA levels in their tumors than those with the 2R sequence, which then leads to higher levels of TS protein and subsequently, lower RRs to 5-FU–based chemotherapy. In addition, a prospective study in mCRC patients treated with 5-FU–based chemotherapy demonstrated that the

Table 2. Germline polymorphisms and clinical significance in chemotherapy of metastatic colorectal cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Functional significance</th>
<th>Clinical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymidylate synthase (TS)</td>
<td>TSER 28-base pair double (2R)/triple (3R) tandem repeat 5'UTR</td>
<td>TS expression ↑</td>
<td>5-FU response ↓</td>
</tr>
<tr>
<td></td>
<td>TSER 3R G&gt;C SNP</td>
<td>TS expression ↓</td>
<td>5-FU response ↑</td>
</tr>
<tr>
<td>Dihydropyrimidine dehydrogenase (DPD)</td>
<td>G&gt;A SNP exon 14</td>
<td>DPD activity ↓</td>
<td>5-FU toxicity ↑↑</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>(TA)$_7$ repeat UGT1A*28</td>
<td>Glucuronidation SN-38 ↓</td>
<td>CPT-11 neutropenia ↑</td>
</tr>
<tr>
<td></td>
<td>G-3156A SNP</td>
<td>UGT1A1 transcription ↓</td>
<td>CPT-11 toxicity ↑</td>
</tr>
<tr>
<td>ERCC1</td>
<td>118C-T SNP</td>
<td>ERCC1 gene expression ↓</td>
<td>Oxaliplatin response ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oxaliplatin survival ↑</td>
</tr>
</tbody>
</table>

2R/2R TS genotype is associated with favorable median survival compared with the 2R/3R and 3R/3R TS genotype (19 months vs. 10 months and 14 months, respectively) (10).

In addition to predicting for therapeutic response, the presence of 2R/3R polymorphisms within the TSER appears to be significantly associated with fluoropyrimidine toxicity. Patients with the 3R/3R TS genotype experience less toxic side effects of 5-FU–based chemotherapy when compared with patients expressing the 2R/3R or 2R/2R TS genotype. One possible explanation of this association is that the higher TS gene expression level in the 3R/3R TS genotype results in incomplete TS enzyme inhibition. As a result, this would lead to reduced therapeutic efficacy in tumors and to reduced toxicity in normal tissues. For this reason, analysis of polymorphisms within the TS promoter gene may help to identify patients who would most benefit from 5-FU–based chemotherapy as well as identify those patients at increased risk for toxicity.

The described relation of TS gene expression levels and distinct TS gene polymorphisms applies to the majority of patients. However, approximately 25% of patients homozygous for the 3R/3R TS genotype were found to have low TS expression levels. These findings indicate that TS expression and therefore response to 5-FU–based chemotherapy is not only affected by the TS genotype. Further insights into the regulation of TS expression have identified a second polymorphism within the TS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Method</th>
<th>Predictive and prognostic significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymidylate synthase (TS)</td>
<td>qRT-PCR, mRNA expression</td>
<td>5-FU response</td>
</tr>
<tr>
<td>Dihydropyrimidine dehydrogenase (DPD)</td>
<td>qRT-PCR, mRNA expression</td>
<td>5-FU survival</td>
</tr>
<tr>
<td>Thymidine phosphorylase (TP)</td>
<td>qRT-PCR, mRNA expression</td>
<td>5-FU response</td>
</tr>
<tr>
<td>ERCC1</td>
<td>qRT-PCR, mRNA expression</td>
<td>Oxaliplatin survival</td>
</tr>
</tbody>
</table>

5-FU = 5-fluorouracil, mRNA = messenger RNA, qRT-PCR = quantitative real-time polymerase chain reaction.
promoter-enhancer UTR. Specifically, a G-to-C (G>C) SNP exists within the 3R variant of the TS gene, is referred to as the 3RC polymorphism, and was found to lead to significantly decreased TS expression compared with the 3RG variant. However, TS expression levels appear to be equivalent to the 2R genotype. Thus, depending on level of TS expression, the respective TS genotypes can be assigned into two defined groups: (a) high TS-expressing genotypes (3RG/3RG, 3RG/3RC, 2R/3RG) and (b) low TS-expressing genotypes (3RC/3RC, 2R/3RC, 2R/2R). These findings may explain, in part, the observation that patients with the 3R/3R polymorphism may have low TS gene expression levels. It is this particular subset of patients that might benefit from 5-FU-based chemotherapy.

It must be emphasized that TS genotype analysis is affected by the material (blood or tumor tissue) that is specifically used for DNA isolation. In human colon cancer, the TS locus at the short arm of chromosome 18 is frequently altered. In tumors, loss of heterozygosity at this locus leads to a different genotype (e.g., 2R/loss or 3R/loss) than in patients with the heterozygous genotype 2R/3R in peripheral blood. The differences between the TS genotype of tumor and normal tissue are of significant relevance in the assumption of the therapeutic efficacy of 5-FU-based chemotherapy. In mCRC patients, the 2R/loss genotype of tumor cells is linked to a significantly improved RR to 5-FU-based chemotherapy compared with the 3R/loss genotype. Hence, evaluation of TS polymorphisms should include the search for loss of heterozygosity of the short arm of chromosome 18 to predict therapeutic response to 5-FU-based chemotherapy.

Thymidine Phosphorylase

TP converts 5-FU to fluorodeoxyuridine (FUDR), which is then converted to the active metabolites FdUMP and 5-fluorodeoxyuridine triphosphate. In vitro studies have shown that increased TP expression correlated with increased sensitivity of tumor cells to 5-FU, most likely due to increased synthesis of the active metabolites FUDR, FdUMP, and 5-fluorodeoxyuridine triphosphate. However, analysis of TP mRNA expression in mCRC patients indicated that tumors with high TP were actually less likely to respond to IV 5-FU-based chemotherapy (11). One possible explanation for this observation may relate to the fact that TP is identical to platelet-derived endothelial cell growth factor, which is a potent angiogenic growth factor. Therefore, high TP expression may reflect a more invasive and malignant tumor phenotype that is less sensitive to chemotherapy in vivo. In the in vitro situation, however, sensitivity of tumor cells to chemotherapy does not depend on the angiogenic qualities of TP.
TP is also the key enzyme in the conversion of the oral fluoropyrimidine capecitabine to its active form, 5-FU. Therefore, elevated TP expression levels in a tumor would be predictive for increased efficacy of this therapy. Of note, in a phase II clinical trial of capecitabine in combination with irinotecan (XELIRI), a strong correlation was observed between level of TP expression in both primary and metastatic colorectal tumors, as assessed by IHC, and clinical benefit, as determined by response, time to progression, and OS (12). Thus, the results of this phase II clinical trial suggest that assessment of TP expression may help to better select patients for oral fluoropyrimidine capecitabine therapy.

Dihydropyrimidine Dehydrogenase

DPD is the rate-limiting enzyme in the catabolic metabolism of 5-FU. This enzyme inactivates more than 80% of an administered dose of 5-FU and therefore significantly limits the bioavailability of 5-FU. In human tumors, DPD is expressed at variable levels, which may in part explain the variable response to 5-FU (7).

Retrospective analyses have shown that low levels of DPD expression, as determined by qRT-PCR in biopsy specimens from CRC patients, favor increased RRs to 5-FU–based chemotherapy. In tumors responsive to 5-FU, DPD expression levels were relatively narrow (0.60 × 10^{-3} to 2.5 × 10^{-3}, 4.2-fold) compared with that of the nonresponding tumors (0.2 × 10^{-3} to 16 × 10^{-3}, 80-fold). Thus, a DPD expression level of less than 2.5 × 10^{-3} was shown to correspond with RRs of approximately 50%. Low expression levels with concomitant low enzymatic activity might then lead to accumulation of active metabolites of 5-FU, thereby resulting in improved bioavailability and therapeutic response.

Decreased enzymatic activity of DPD has been attributed to 17 different mutations within the DPD gene. The most common variant of the DPD gene is a G-to-A (G>A) SNP in the invariant GT splice donor site flanking exon 14. This G-to-A substitution leads to reduced translation of DPD mRNA and diminished DPD activity.

In addition to the effect of low DPD gene expression and DPD activity on therapeutic response, higher systemic levels of 5-FU and its active metabolites could potentially increase the risk of toxic side effects. Patients characterized by one or two non-functional DPD alleles have been shown to suffer from severe or even lethal toxicities of 5-FU–based chemotherapy (13). Therefore, screening for polymorphisms within the DPD gene can have a dramatic impact on patient selection for 5-FU–based chemotherapy with respect to therapeutic response and toxicity.
However, 40%–50% of patients with normal DPD enzyme activity suffer from severe toxicity to 5-FU–based chemotherapy, indicating that DPD is not solely responsible for altered 5-FU metabolism. More recently, genetic and epigenetic alterations of another enzyme involved in 5-FU catabolism, dihydropyrimidinase, have been identified, which may account for the increased toxicity in patients with normal DPD activity. Further studies are ongoing in a larger population of cancer patients to confirm the potential role of dihydropyrimidinase (14).

The combined analysis of gene expression levels of TS, TP, and DPD in CRC patients treated with 5-FU revealed a favorable RR in tumors characterized by expression values of all three genes below the nonresponsive cut-off values. An overall RR of 92% was found within this group of tumors. Those tumors that did not respond had high gene expression levels for at least one of the markers. These observations clearly highlight the need for testing multiple markers to improve the selection of patients to a distinct therapeutic strategy (7).

In conclusion, these molecular markers predict which fluoropyrimidine, 5-FU or capecitabine, is more effective and less toxic in individual patients. Furthermore, the search for novel molecular markers has led to the identification of new fluoropyrimidine derivatives with improved specificity and reduced toxicity profiles.

Molecular Markers of Irinotecan (CPT-11)

Irinotecan (CPT-11) is, in essence, a prodrug and requires conversion to the active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) by carboxylesterases, which are abundant in the liver but also present in other tissues. SN-38 exerts its cytotoxic effects by binding to the complex of topoisomerase I and DNA (15). Single-strand breaks induced by SN-38 are reversible after removal of the drug from the system. SN-38 is excreted in the bile and urine after glucuronidation to SN-38 glucuronide (SN-38G) by the uridine diphosphate glucuronosyltransferases (UGTs) (16). The hepatic UGT1A1 and UGT1A9, as well as the extrahepatic UGT1A7, are the key players involved in the detoxification and elimination of SN-38. UGT1A1 activity in glucuronidation of exogenous drugs and endogenous substrates (bilirubin, hormones) can be significantly altered by genetic polymorphisms (17).

CPT-11 is indicated in first-line therapy in combination with 5-FU and leucovorin and as monotherapy in the second-line setting for patients who progressed on oxaliplatin-based chemotherapy. The efficacy of CPT-11 in the treatment of different malignancies is limited by
its toxicity, which can include potentially life-threatening diarrhea and neutropenia. Attempts have been made to identify patients who are more sensitive to CPT-11 adverse events. In particular, the presence of the homozygous UGT1A1*28 polymorphism has been associated with increased risk of severe neutropenia (18).

Genetic variants of UGT1A1 have been identified within the TATA promoter region (see Table 2). The wild-type promoter UGT1A1*1 has six TA repeats. Variant alleles with five, seven, or eight TA repeats have been described. With increasing dinucleotide repeats within the TATA promoter region, the activity of UGT1A1 and detoxification of SN-38 decrease. The seven repeat (TA)$_7$ polymorphism UGT1A1*28 is the most common, with a homozygous (7/7) genotype found in 10% of North Americans. Prospective evaluation of patients with advanced malignancies, including 10 CRC patients, has revealed a significant association between the presence of the homozygous (7/7) UGT1A1*28 genotype and development of CPT-11-(300–350 mg/m$^2$ every 3 weeks)-induced severe neutropenia. Patients with a homozygous genotype were characterized by a 9.3-fold higher risk for severe neutropenia compared with the heterozygous (6/7) and wild-type (6/6) genotype. Grade 3/4 diarrhea was infrequently observed. This latter observation may be due to the reduced excretion of glucuronidated SN-38 (SN-38G) in patients with reduced UGT1A1 activity. After biliary excretion into the bowel, SN-38G can be reconverted by bacterial $\beta$-glucuronidases into its active form SN-38, which can then cause local toxic effects in the bowel (19).

Other genetic variations of UGT1A1 include a G-to-A SNP, G-3156A, which has been shown to be associated with decreased transcriptional activity of UGT1A1 and severe toxicity. Patients harboring both the UGT1A1*28 and UGT1A1 A/A genotypes appear to be at highest risk for severe and even lethal toxicity (19).

Predictive values for toxicity and therapeutic response have also been found for hepatic UGT1A9 and extrahepatic UGT1A7 in mCRC patients treated with CPT-11, 125 mg/m$^2$ weekly, and capecitabine, 900 or 1,000 mg/m$^2$ (20). Within this study population of 67 mCRC patients, low-activity alleles of UGT1A7 *2/*2 and UGT1A7 *3/*3 were significantly associated with improved therapeutic response and lack of severe gastrointestinal toxicity. The UGT1A9-118 (dT) (9/9) genotype was also significantly associated with reduced toxicity and increased therapeutic response. Interestingly, no significant association between the UGT1A1 genotype and toxicity and therapeutic response was observed within this study population. Furthermore, low-activity genotypes of UGT1A7 and UGT1A9 were not associated with increased risk for severe neutropenia as it is described for low-
activity UGT1A1. One possibility for these observations is that different treatment schedules of CPT-11 were used in the different studies (300–350 mg/m$^2$ every 3 weeks vs. 125 mg/m$^2$ weekly). In general, administration of CPT-11 using a weekly schedule results in higher rates of diarrhea, whereas the every-3-week schedule is usually associated with higher rates for neutropenia (21).

In summary, the toxicity profile of CPT-11 is related to its elimination pathways. Glucuronidation of SN-38 by UGTs enhances its elimination from serum. Hence, UGTs with low activity are associated with high serum levels of SN-38 and increased risk for severe neutropenia. High glucuronidation activity of UGTs results in enhanced excretion of SN-38G in the biliary and gastrointestinal tract and predisposes for an increased incidence of diarrhea.

The significant association of the UGT1A1*28 genotype with increased risk for severe neutropenia prompted the U.S. Food and Drug Administration (FDA) to change the labelling of CPT-11. According to the new labelling guidelines, an initial dose reduction for patients homozygous for UGT1A1*28 is recommended. An FDA-approved diagnostic blood test is available on the market specifically testing for the UGT1A1*1 (wild type) and the UGT1A1*28 genotype. Preliminary studies indicate an association between the homozygous UGT1A1*28 genotype and toxicity with the regimen of CPT-11 given at 300–350 mg/m$^2$ every 3 weeks. Although genotyping for UGT1A1*28 may assist clinicians in the decisions for CPT-11 therapy (16), further prospective clinical trials are warranted to evaluate the true predictive value of the UGT genotype as it relates to the different therapeutic regimens of CPT-11.

Bilirubin is an endogenous substrate of UGTs, and it has been suggested that this serum marker might serve as a surrogate marker to predict UGT activity. A strong association between high normal bilirubin values and increased risk of severe neutropenia has been described by investigators at the University of Chicago. However, the clinical use of serum bilirubin concentrations to select patients to different treatment strategies cannot be formally recommended. Liver metastases may affect the metabolism and excretion of bilirubin and therefore exclude general assumptions on the correlation of UGT activity and bilirubin level (22).

Several other molecular markers have been studied as predictive of therapeutic response and toxicity of CPT-11. Expression levels of the target enzyme topoisomerase I have been assessed using IHC and qRT-PCR. To date, no correlation can be made between topoisomerase I expression and response in mCRC patients. Of interest, preliminary results suggest that the expression profiles of the growth factor receptor EGFR and the DNA-repair factors excision repair cross-complementing-
group 1 (ERCC1) and glutathione S-transferase P1 may predict for improved therapeutic response. However, these findings need further validation in prospective clinical trials (23).

Molecular Markers of Oxaliplatin

Oxaliplatin is a third-generation platinum compound that is indicated for a wide variety of solid tumors, especially in patients with mCRC refractory to 5-FU/CPT-11 chemotherapy. As with the other platinum compounds, oxaliplatin interacts with DNA to form intrastrand cross-link DNA adducts, which leads to alterations in base pairing, replication, and transcription, ultimately resulting in cell death (24).

Resistance to oxaliplatin chemotherapy has been attributed to several different mechanisms such as decreased drug accumulation and increased drug inactivation, but most important to enhanced DNA repair capacity. Limited DNA repair capacity in malignant cells might have the desirable result of improved anticancer activity of oxaliplatin. However, impaired DNA repair within the normal tissue might simultaneously result in increased toxicity in response to platinating agents.

DNA intrastrand cross-links produced by oxaliplatin are identified and repaired by the nucleotide excision repair (NER) pathway. The ERCC1 is an essential member of the NER pathway. The association of ERCC1 with the endonuclease xeroderma pigmentosum complex group F (XPF) is critical for the stability and catalytic activity of XPF. The heterodimeric complex of ERCC1-XPF accounts for the cleavage and repair of DNA intrastrand cross-links (25).

ERCC1 gene expression levels in tumors have been shown to be predictive for therapeutic response and survival to oxaliplatin-based chemotherapy. Shirota et al. evaluated ERCC1 gene expression levels in mCRC refractory to 5-FU and CPT-11. Patients with low gene expression levels of ERCC1 (<4.9 × 10^3) experienced a significantly longer median survival compared with those patients with high gene expression levels for ERCC1 (≥4.9 × 10^3) in the tumor tissue (26). In this study population, no association was observed for ERCC1 gene expression levels and response to oxaliplatin chemotherapy (see Table 3).

Gene expression and enzymatic activity of ERCC1 are affected by SNPs located within ERCC1 codon 118 (see Table 2). The C-to-T SNP (SNP C-118T) leads to reduced ERCC1 expression at both the mRNA and protein level. Therefore, evaluating SNPs at ERCC1 codon 118 might help to predict response to oxaliplatin-based chemotherapy in mCRC patients. A retrospective analysis of normal and tumor tissue
samples from mCRC patients treated with 5-FU/oxaliplatin revealed a significantly improved RR in patients with the T/T genotype (RR 61.9%) compared with patients with the C/T (RR 42.3%) and the C/C genotype (RR 21.4%) (27). A separate study performed on blood samples from mCRC patients refractory to previous chemotherapy demonstrated that SNPs at ERCC1 codon 118 are related to survival and oxaliplatin-based therapy. This study identified the C/C genotype to be predictive for the most favorable median survival of mCRC patients (28).

Molecular Markers and Targeted Therapy

Tremendous focus has been placed on the development of targeted therapies, which focus on specific molecular pathways involved in tumor growth, proliferation, and angiogenesis. This targeted approach has led to the approval of three new biologic agents and resulted in significant progress in the management of mCRC. The recombinant human monoclonal immunoglobulin (Ig)G1 antibody bevacizumab targets the VEGF, whereas the chimeric monoclonal IgG1 antibody cetuximab and the fully human IgG2 antibody panitumumab target the EGFR. Bevacizumab has had a major impact on clinical efficacy when combined with chemotherapy and is presently approved for use in the first-line setting. Cetuximab and panitumumab are presently approved in the disease-refractory setting, but recent data suggest that cetuximab, in contrast to panitumumab, can also be safely and effectively combined with chemotherapy in the front-line setting. It is clear, however, that some patients do not benefit from these biologic agents and may experience undesirable side effects. Moreover, these targeted therapies are not inexpensive and contribute significantly to the overall high costs of treating patients with mCRC. Therefore, molecular markers are urgently needed to better select patients for VEGF- and EGFR-targeted therapy.

Vascular Endothelial Growth Factor–Targeted Therapy

To date, no validated molecular marker has been identified that can predict therapeutic efficacy for VEGF-targeted therapy. The data available from preclinical and clinical studies remain controversial. In preclinical studies, serum levels of VEGF and its receptor (VEGFR) correlate with aggressive tumor growth. However, serum levels of VEGF and VEGFR do not appear to be reliable predictors of therapeutic response and clinical outcome in patients treated with bevacizumab. The primary target of
anti-angiogenic therapy is the microcirculation of the tumor. Therefore, microvascular density has been evaluated in tumor tissue sections to assess the therapeutic response to bevacizumab. A retrospective analysis of mCRC patients treated with bevacizumab indeed showed a reduction in the microvascular density in tumor specimens. However, this observation did not correlate with therapeutic response or survival.

Preclinical studies revealed an impact of p53 mutation and KRAS mutation on VEGF signaling in tumor cells. These findings suggested that the genetic makeup of tumor cells might possess a significant impact on therapeutic response to anti-angiogenic therapy. Primary tumor tissue and metastatic tissue samples of mCRC patients treated with IFL (irinotecan, 5-FU, and leucovorin) plus bevacizumab or 5-FU/leucovorin plus bevacizumab were analyzed for mutation status of KRAS, b-raf, and p53 and their association with therapeutic response and survival (29). In this retrospective study, no association was observed between mutation status and therapeutic response to bevacizumab. Survival benefit from the addition of bevacizumab to first-line IFL was independent of KRAS, b-raf, or p53 mutation status. In general, patients with wild-type KRAS and b-raf experienced significantly improved median survival compared with patients characterized for mutant genotype (30). These findings require further confirmation in prospectively designed large clinical trials.

Preclinical studies and pilot clinical trials have suggested a strong association between the level of circulating endothelial cells (CEC) and circulating endothelial precursor cells (CEPC) and therapeutic response to anti-angiogenic therapy. Evaluation of CEC and CEPC in cancer patients might help to assess the most efficient dose for anti-angiogenic agents (optimal biologic dose) compared with the maximal tolerated dose used in standard chemotherapy. Further studies are needed to clarify the clinical significance of CEC and CEPC in anti-angiogenic therapy of mCRC patients (31).

**Epidermal Growth Factor Receptor–Targeted Therapy**

In advanced CRC, the EGFR-targeting monoclonal antibodies cetuximab and panitumumab possess significant therapeutic efficacy and are approved in second- and third-line therapy of these diseases. Clinical benefit is observed in approximately 10%–20% of mCRC patients. However, the remaining majority of patients do not respond to this specific targeted therapy. Therefore, significant efforts have focused on identifying reliable molecular markers that will predict which patients will respond to anti-EGFR targeting agents.
Overexpression of the EGFR and its ligands results in abnormal autocrine and paracrine stimulation of cell proliferation and angiogenesis. Potential predictive molecular markers might be identified by analyzing the expression profile of the EGFR, but also by looking at upstream and downstream effectors within the EGFR pathway (Figure 2). In addition, the clinical occurrence of an acne-like skin rash is strongly associated with favorable therapeutic response to cetuximab and panitumumab targeted therapy. Because the EGFR is most widely expressed in keratinocytes within the normal skin, the association of skin toxicity and therapeutic response implies that the genetic makeup of a patient influences the efficacy of EGFR-targeted therapy. Therefore, the genome of individual patients and tumors has been searched for potential predictive molecular markers (4).

Epidermal Growth Factor Receptor Expression
Cetuximab was initially approved for mCRC patients whose tumors overexpress EGFR as determined by IHC. Thus, the assessment of EGFR expression by IHC became the first molecular marker in targeted therapy to be approved by the FDA. However, it is now widely accepted that the analysis of the EGFR status by IHC is not an appropriate tool to select patients for EGFR-targeted therapy. Cetuximab has now been shown to be effective in patients irrespective of their EGFR expression. These observations reflect the inherent limitations of IHC sensitivity and the difficulty of establishing accurate detection of EGFR in multiple tumor sites.

Expression levels of the EGFR gene have been evaluated in tumor tissues using fluorescent in situ hybridization technique (FISH). Significantly improved RRs and longer PFS were observed in EGFR FISH-positive mCRC patients treated with cetuximab. The comparability of the results from different clinical studies is limited because of the differences in the cut-off values for EGFR gene copy numbers and differences in the detection technique of the EGFR gene using FISH. Thus, standardization is required in the assessment of the EGFR gene copy number by FISH to clarify its predictive value in EGFR-targeted therapy.

Epidermal Growth Factor Receptor Ligands
The EGFR ligands amphiregulin and epiregulin affect efficacy of EGFR-targeted therapy. Recently, high gene expression levels of amphiregulin (AREG) and epiregulin (EREG) in primary tumors were found to be predictive for longer PFS in mCRC patients treated with EGFR monoclonal antibodies (mAbs). AREG and EREG expression by tumors stimulate the EGFR pathway in an autocrine loop. As such, high gene expression levels of these ligands would reflect dependence of a tumor on the EGFR path-
Figure 2. Epidermal growth factor receptor (EGFR) signaling pathway. Binding of epidermal growth factor (EGF) or ligands of the EGF family, such as amphiregulin (AREG), epiregulin (EREG), and transforming growth factor (TGF), to EGFR induces homodimerization/heterodimerization of the receptor and phosphorylation of specific tyrosine residues (P). This leads to activation of downstream RAS/RAF/mitogen-activated protein kinase (MAPK) and phosphoinositide 3'-kinase (PI3K) pathways and expression of genes related to cell survival, proliferation, angiogenesis, metastasis, and resistance to chemotherapy and radiotherapy. PI3K/Akt signal transduction is negatively regulated by the oncoprotein PTEN. Loss of PTEN results in constitutive activation of Akt, stimulating cell survival.
way, which might then explain the increased sensitivity of the tumor to EGFR-targeted therapy (4).

**KRAS Mutation**

Downstream signaling of the EGFR depends on the activation of the small G protein RAS and protein kinase raf, which then triggers the MAP-kinase pathway (see Figure 2). The presence of mutations within the three mammalian ras isoforms (KRAS, HRAS, and NRAS) is among the earliest steps in CRC carcinogenesis. Activating KRAS mutations in the short arm of chromosome 12 at codon 12 are the most common mutations in human malignancies and are associated with aggressiveness and progression of diseases. In addition, the presence of KRAS mutations has been associated with tumor relapse in CRC patients. The frequency of KRAS mutations in adenomas and adenocarcinomas of the large bowel is reported to be 30%–40%. Because of the significance of KRAS in EGFR signaling and in carcinogenesis, KRAS mutation status has been suggested to be a promising predictive marker in EGFR-targeted therapy.

The association between the presence of KRAS mutation and therapeutic response and survival in mCRC patients treated with EGFR-targeting mAbs has now been evaluated in several retrospective clinical studies. The first results were elusive, revealing only a trend of KRAS mutations to be a negative predictor of response. The significance of KRAS mutation in EGFR-targeted therapy became clear in consecutive retrospective clinical studies. In independent studies, a prevalence of KRAS mutations of 27%–43% in mCRC patients was identified. A consistent significant association of favorable therapeutic response with KRAS wild-type with RRs of 17%–48% was found throughout the studies (Table 4). Two other studies found that OS was significantly higher for patients with wild-type KRAS compared with those with the mutated variant. Thus, the presence of KRAS mutation was significantly associated with nonresponding to cetuximab or panitumumab therapy. However, in one study, a small group of patients bearing KRAS mutations was identified to benefit from cetuximab therapy.

Based on these findings, the association of the KRAS mutation status with RR and PFS has been analyzed in mCRC patients enrolled in the ongoing randomized phase III CRYSTAL (FOLFIRI [folinic acid, 5-fluorouracil, and irinotecan] with or without cetuximab) study and randomized phase II OPUS (FOLFOX [oxaliplatin, leucovorin, and 5-FU] with or without cetuximab) study. In the CRYSTAL study, KRAS tumor mutation status was determined for 540 patients and treatment efficacy reanalyzed stratified by patient tumor KRAS mutation status. KRAS wild-type (N = 348) was significantly associated with improved RR (59% vs. 43%) and PFS (9.9 vs. 8.7 months) with the combination of FOLFIRI with cetux-
Table 4. KRAS mutation and its predictive significance in targeted therapy of metastatic colorectal cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Number of patients</th>
<th>KRAS WT:MT</th>
<th>Response</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Benvenuti et al. 2007 (34)</td>
<td>Cetuximab or panitumumab</td>
<td>48</td>
<td>32:16</td>
<td>10 (31%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Lievre et al. 2008 (35)</td>
<td>Cetuximab ± CPT-11 or FOLFIRI</td>
<td>114</td>
<td>78:36</td>
<td>34 (44%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>De Roock et al. 2007 (36)</td>
<td>Cetuximab ± CPT-11</td>
<td>113</td>
<td>67:46</td>
<td>27 (40%)</td>
<td>0 (0%)</td>
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<td>Di Fiore et al. 2007 (37)</td>
<td>Cetuximab + CPT-11 or oxaliplatin</td>
<td>59</td>
<td>43:16</td>
<td>12 (28%)</td>
<td>0 (0%)</td>
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<td>Khambata-Ford et al. 2007 (38)</td>
<td>Cetuximab</td>
<td>80</td>
<td>50:30</td>
<td>24 (48%)</td>
<td>3 (10%)</td>
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<td>Amado et al. 2008 (39)</td>
<td>Panitumumab</td>
<td>208</td>
<td>124:84</td>
<td>21 (17%)</td>
<td>0 (0%)</td>
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CPT-11 = irinotecan, FOLFIRI = folinic acid, 5-fluorouracil, and irinotecan, MT = mutant type, WT = wild type.
imab compared with patients treated with FOLFIRI alone. However, in the presence of KRAS mutations (N = 198), there was no significant difference in RR (40% vs. 36%) or PFS (8.1 vs. 7.6 months) between patients receiving FOLFIRI in combination with cetuximab or FOLFIRI alone (32). In the OPUS study, 233 patient samples were analyzed for KRAS mutation status and the efficacy of FOLFOX plus cetuximab versus FOLFOX alone. In KRAS wild-type patients (N = 134), the combination of FOLFOX plus cetuximab demonstrated a significant increase in tumor response and a significant improvement in PFS compared with FOLFOX alone. In contrast, in the KRAS mutant population, patients receiving cetuximab plus FOLFOX demonstrated no significant difference in overall RR or improvement in PFS compared to patients receiving FOLFOX alone (33).

Taken together, these clinical studies highlight a strong predictive value of KRAS mutations in EGFR-targeted therapy in mCRC patients. The large majority of mCRC patients with KRAS mutations do not respond to anti-EGFR mAbs. However, mCRC patients with known KRAS mutation should not be completely excluded from targeted therapy. There may be a subgroup of patients bearing KRAS mutation who might experience objective response to anti-EGFR mAbs. Large prospective clinical trials are warranted to clarify the role of KRAS mutation status in predicting therapeutic response to EGFR-targeted therapy in mCRC patients.

Germline Polymorphisms within the Epidermal Growth Factor Receptor Signaling Pathway

The association of skin toxicity and favorable therapeutic response in EGFR-targeted therapy has stimulated the search for distinct polymorphisms that might have a functional impact on the EGFR pathway. Several promising polymorphisms within genes of the EGFR and its downstream effectors have now been identified to be predictive markers in EGFR-targeted therapy. These results, however, should be viewed as preliminary due to the limited number of patients assessed and the retrospective nature of the studies. Presently, no specific recommendations can be made as to the role of these markers in the treatment decision-making process for mCRC patients (4).

The chimeric IgG1 mAb cetuximab is also able to induce antibody-dependent cell-mediated cytotoxicity (ADCC), which would direct natural killer cells of the innate immunity to kill antigen-expressing cancer cells. The significance of ADCC in cancer therapy has been previously documented, with the IgG1 mAbs trastuzumab targeting the EGFR 2 (HER2) and rituximab targeting the CD20 antigen in B-cell lymphoma. The killing function of immune cells is affected by two functional FCγ receptor (FCGR) gene polymorphisms, FCGR2A-H131R and FCGR3A-
V158F. There is now preliminary evidence suggesting that the presence of FCGR2A-H131R and FCGR3A-V158F polymorphisms are associated with clinical benefit, as determined by PFS in patients with mCRC. However, further studies are needed to determine the functional significance of FCGR3A polymorphisms and ADCC in mCRC patients treated with EGFR monoclonal antibodies.

Conclusion

Progress in cancer biology has expanded our understanding of the molecular and cellular mechanisms of cancer development, cancer metastasis, and resistance of cancer cells to chemotherapy and radiotherapy. Pharmacogenetic studies focus on the genetic variations in individual patients and attempt to correlate their association with response and survival to specific therapies. The genetic variations include functional relevant germline polymorphisms within the signaling pathways of the therapeutic target or the metabolism of the therapeutic agent itself. The identification of specific molecular markers to predict therapeutic response and/or toxicity will help to develop individualized therapeutic strategies for cancer patients.

Retrospective analyses of mCRC patients revealed promising molecular markers in the metabolism and stress response of standard chemotherapeutic agent 5-FU, CPT-11, and oxaliplatin. Polymorphisms and gene expression levels of TS, TP, and DPD have been the most widely investigated and significantly associated with response and survival in 5-FU-based chemotherapy. The identification of the association of the UGT1A1*28 polymorphism with impaired detoxification of CPT-11 and increased risk for severe neutropenia has caused the FDA to change the labelling of the drug indicating these findings. Finally, variations in the DNA repair capacity by the NER factor ERCC1 affect the therapeutic efficacy of oxaliplatin.

With the introduction of molecular targeting agents, the therapeutic abilities have further improved, but selection of patients to these specific therapies still remains controversial. To date, no markers have been identified that would predict the response to VEGF-targeting agent bevacizumab. In contrast, a variety of germline polymorphisms and oncogenic mutations have been associated with efficacy of EGFR-targeting agents cetuximab and panitumumab in mCRC patients. The presence of the KRAS wild-type genotype is the strongest predictor for beneficial therapeutic response to EGFR-targeted therapy compared with patients bearing a KRAS mutant genotype. Additional markers indiencing thera-
Molecular Markers

Despite intense research efforts, none of these promising molecular markers have reached access into routine clinical practice. Due to the limited number of patients and the retrospective design of the clinical studies, no specific recommendations can be made for the clinical application of molecular markers in directing treatment decisions. Furthermore, large and prospectively designed clinical trials are urgently needed to conclusively assess the significance of these molecular markers in the development of individualized therapeutic strategies in mCRC patients.

Author’s Disclosures of Potential Conflicts of Interest

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Honoraria: Heinz-Josef Lenz—Pfizer, Merck, Genentech, Roche, sanofi-aventis
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References


CME Post-Test

1. Germline mutations of which of the following mismatch repair genes is least commonly found among families affected by hereditary non-polyposis colorectal cancer?
   a) MLH1
   b) MSH2
   c) MSH6
   d) PMS2

2. Which of the following statements is true?
   a) Approximately 20%–25% of all human cancers carry mutations in one of six identified RAS genes.
   b) Activation of Src kinase apparently plays an important role in mediating chemoresistance.
   c) Epidermal growth factor overexpression has been documented in up to 75% of metastatic colorectal cancer (CRC) cases.
   d) The PI3K signaling pathway is constitutively activated in a broad range of cancers, including CRC, ovarian cancer, testicular cancer, seminoma, and lung cancer.

3. Combined use of irinotecan (Camptosar) and bolus 5-fluorouracil (5-FU) and leucovorin (LV) followed by a 22-hour infusion of 5-FU for 2 consecutive days every 14 days (LVFU2 regimen) in advanced CRC patients has imparted clinical benefit similar to that of weekly irinotecan used with the weekly 5-FU/LV Roswell Park regimen.
   a) True
   b) False

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4. Based on currently available data, a conclusion that may be made about cytotoxic chemotherapy against metastatic CRC is:
   a) Bolus regimens of 5-FU/LV are superior to infusional schedules of 5-FU/LV.
   b) Combination regimens incorporating three cytotoxic agents are more active in first-line treatment than is use of a fluoropyrimidine only and should be considered the standard of care.
   c) 5-FU/LV plus oxaliplatin (Eloxatin; FOLFOX) and 5-FU/LV plus irinotecan (FOLFIRI) have similar clinical activity and safety profiles when given as first-line treatment.
   d) Combined use of capecitabine and oxaliplatin (XELOX/CAPOX) has shown activity similar to that of intravenous FOLFOX.

5. Which of the following was a finding of the GERCOR Study Group, which randomized 226 previously untreated metastatic colon cancer patients to treatment with FOLFIRI or FOLFOX and then crossed patients over to the other treatment arm at time of progression?
   a) The overall survival for the groups did not differ significantly.
   b) The median progression-free survival for the groups did not differ significantly.
   c) Patients given FOLFIRI followed by FOLFOX had a slightly higher response rate.
   d) All of the above.

6. The results of the CAIRO trial of 810 metastatic CRC patients given either capecitabine alone followed by irinotecan on first progression and capecitabine/oxaliplatin on second progression or capecitabine/irinotecan followed by capecitabine/oxaliplatin on first progression showed that:
   a) First-line combination therapy yielded greater overall survival, toxicity, and safety than did first-line sequential therapy.
   b) First-line sequential therapy yielded greater progression-free survival than and similar toxicity as did first-line combination therapy.
   c) There was no significant difference in overall survival between the two treatment strategies, although combination therapy was associated with more toxicity.
   d) The trial was stopped when an unacceptable proportion of patients given combination therapy suffered grade 4 toxicities.

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7. Ultimately, the ability to successfully treat CRC liver metastases surgically requires that 95% of metastases be resected with negative margins (R0 resection) and with at least 30%–40% of normal liver remaining after completion of the surgery.
   a) True
   b) False

8. In 244 metastatic CRC patients, one-third of whom had liver-only metastases, combined use of FOLFOX and FOLFIRI (FOLFOXIRI) resulted in a ______ rate of R0 resection as compared with a 12% rate in patients treated with FOLFIRI.
   a) 6%
   b) 16%
   c) 26%
   d) 36%

9. Metastatic CRC patients having which of the following polymorphisms of the TS gene are less likely to respond to 5-FU–based chemotherapy?
   a) 2R/2R
   b) 2R/3R
   c) 3R/3R
   d) There is no difference among these groups

10. New labeling guidelines for irinotecan recommend an initial dose reduction for CRC patients homozygous for the __________ allele.
    a) UGT1A9*2
    b) UGT1A1*28
    c) UGT1A7*28
    d) UGT1A1*2

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