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Wafik S El-Deiry *Editor*

Impact of Genetic Targets on Cancer Therapy

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Forward

In the last two decades, great strides have been made in unraveling molecular biology, immunology, genetics, and in some cases epigenetics as they apply to the cellular and organ pathology of cancer. This is hugely enabling in many ways as hematologists and oncologists throughout the world strive to develop the best and most personalized and often innovative recommendations to help their patients. A reasonable foundation for this and path forward is to understand what has been learned about various human tumors and to try to integrate this knowledge into medical practice. This is a daunting task by any standards in 2012 because there is simply a vast amount of genetic and genomic information upon which taking action remains unsupported by clinical evidence. This applies particularly well to individual patients whose tumors are not only unique but which can be heterogeneous and otherwise complex three-dimensional organ-like structures with multiple interacting cell types and local microenvironments. This is further amplified by the extremely rapid rate at which new information is accumulating. It should be stated that this book does not intend to set medical practice guidelines. However, progress will be less elusive and perhaps quicker if the scientific and medical communities take different approaches to accomplishing what both clinicians and patients want which is to benefit from the available knowledge. Importantly for those who treat cancer patients, our patients sadly and very often cannot wait and deserve every chance to benefit from the latest available information. Anecdotally this may be of benefit to individual patients and may lead to new directions for clinical or basic studies to move the field forward.

The authors of the various chapters were asked to comment on current practice in terms of standard of care approaches, to describe the molecular genetics and current understanding of tumor progression for their particular cancer type or hematological malignancy including the various key driver pathway alterations, to comment on cancer stem cells and the tumor microenvironment, and to include, to the best of their abilities, the available information on therapeutics targeting the molecular alterations in specific tumor types. I think this volume brings significant clinical insights for basic scientists and significant basic science and molecular

understanding for clinicians. The chapters are presented by both basic scientist and clinician authors who are using and studying the therapeutics targeting the genetic changes thereby making this volume very unique. I believe it is a very useful resource for seasoned investigators as well as students of all ages who care and who want to learn more and do more about the problem of cancer and its therapy. It is particularly rewarding that many of my colleagues at the Penn State Hershey Medical Center and Penn State Hershey Cancer Institute both in the Hematology/Oncology Division and other departments who are working on and treating the various malignancies graciously agreed to provide their valuable contributions to this effort. Hopefully you will appreciate what this volume brings, will enjoy reading it and learning from it, and perhaps might be inspired and/or motivated to get involved in the fight against cancer in your own way. There are many opportunities for doing so along many fronts despite the many challenges facing the field in terms of funding for research, access to health care and clinical trials, and costs of medical care.

Hershey, PA, USA Wafik S. El-Deiry, MD, Ph.D., FACP

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Novel Antineoplastics Targeting Genetic Changes in Colorectal Cancer

Jamal Joudeh, Joshua E. Allen, Avisnata Das, Varun Prabhu, Michael Farbaniec, Jeffrey Adler, and Wafik S. El-Deiry

Abstract Cytotoxic chemotherapy remains the mainstay of the medical management of colorectal cancer (CRC). Research over the last two decades has led to a molecular understanding of the oncogenic mechanisms involved in CRC and has contributed to the rational development of antineoplastics that target these mechanisms. During carcinogenesis, genetic changes often occur in molecules that play key functional roles in cancer such as cell proliferation, angiogenesis, apoptosis, cell death and immune-mediated destruction of cancer cells. Here, we review novel antineoplastics that are approved or in development for CRC that target molecules associated with genetic aberrations in CRC. Some of these targeted antineoplastics have proven effective against other solid tumors and hold promise in treating CRC whereas others are now routinely used in combination with cytotoxic agents. This article reviews antineoplastics that target genetic changes in CRC, their antitumor mechanisms, and their stage of development.

Key words Colon cancer • Colorectal cancer • Clinical trial • Targeted agents • Cancer therapy • Cancer genetics

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Introduction

Colon cancer has the third highest incidence and mortality among cancers in both men and women in the United States. There has been a recent decline in CRC mortality in developed countries because of increasingly better early detection methods and improved therapeutic options. Screening colonoscopy has decreased the mortality rate by 50% in CRC in United States [1]. Symptomatic patients usually present with hematochezia or melena, abdominal pain, unexplained iron deficiency anemia and/or a change in bowel habits. Left-sided cancer usually presents with changes in bowel movement while right-sided cancers often present with occult bleeding.

The majority of CRCs are adenocarcinomas with 70–90% being sporadic whereas less than 10% of patients have true inherited genetic factors linked to colorectal cancers. Most colorectal cancers progress from normal epithelium to invasive cancer via an intermediate precursor, the adenomatous polyp. This transformation was linked to a multistep process of specific genetic changes. Individuals with familial adenomatous polyposis (FAP) and individuals with hereditary nonpolyposis colon cancer syndrome (HNPCC, Lynch syndrome) were found to have an early single germline mutation while sporadic cancers result from the stepwise accumulation of multiple somatic mutations (Fig. 1). Studies showed that most colorectal cancers

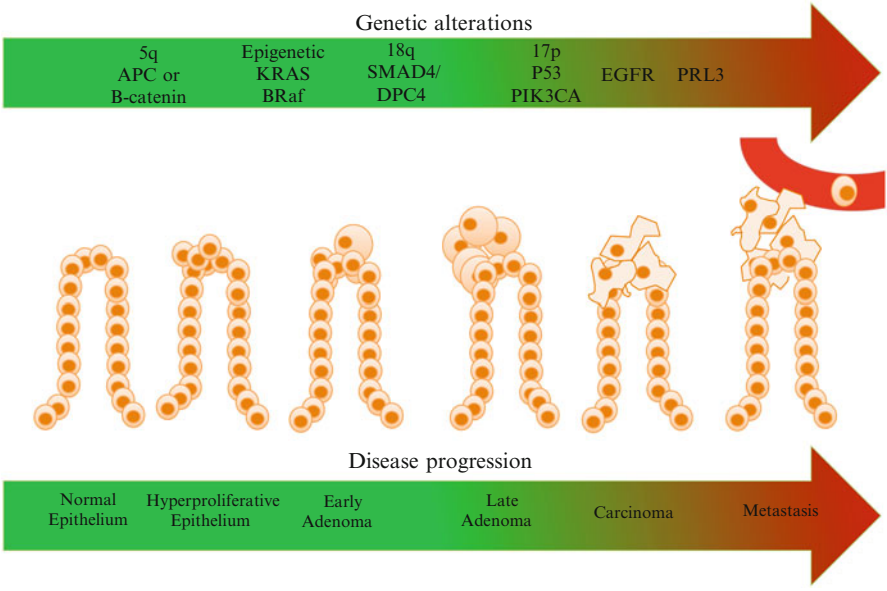

Fig. 1 Linear model of genetic changes that drive CRC. At the earliest stage of colon cancer genesis, normal colonic cells advance to a hyperproliferative state by mutations that inactivate either the APC gene on chromosome 5q or activate beta-catenin. Epigenetics and mutations in either KRAS or BRAF typically change the hyperproliferative cells into the early adenoma stage. SMAD4/DPC4 mutation on chromosome 18q then advances the mutant cells to a late adenoma stage. Finally, mutations in PIK3CA and p53 on chromosome 17p can transform late adenoma into carcinoma. Metastasis can occur during disease progression, which has been associated with PRL3 gene amplification

begin with inactivation (through a germline or sporadic mutation) of the APC gene. Chromosome 18 loss of heterozygosity (LOH), DCC deletion, KRAS oncogene mutation and p53 mutations were found to be a late event in colon carcinogenesis. The MSI-high (MSI-H) phenotype is associated with HNPCC syndrome but it is also found in 10–15% of sporadic colorectal cancers.

The TNM staging system is the international standard for staging colorectal cancer. The pathologic features at diagnosis (depth of bowel wall penetration (T), number of locoregional lymph nodes involved (N), and presence of extra-colonic metastases (M)) remain the best indicators of long-term prognosis for both colon and rectal cancer. Surgical resection is the only curative treatment for locoregional disease (stage I–III) and may be a curative option for patients with limited metastatic disease involving the liver and/or lungs (stage IV).

Adjuvant chemotherapy is usually reserved for patients with high-risk stage II and stage III (node-positive) disease. In the MOSAIC trial, 5-Fluorouracil, leucovorin, and oxaliplatin (FOLFOX versus 5-Fluorouracil, leucovorin (5-FU/LV), there was a trend toward improved disease-free survival with FOLFOX in the subgroup of stage II patients with high-risk tumors (clinical T4, poorly differentiated, perforation, obstruction, or <10 nodes in the surgical specimen). Overall survival was essentially the same in both groups [2]. On the other hand, adjuvant chemotherapy was evaluated in patients with stage II colon cancer with poor prognostic features; it did not substantially improve overall survival in stage II patients. Patients in this study were unlikely treated with oxaliplatin [3]. ECOG 5202 was designed to evaluate adjuvant chemotherapy in patients with stage II colon cancer by stratifying the patients as having low or high risk of recurrence depending on their molecular marker analysis. Loss of heterozygosity at chromosome 18q (LOH18q) and the lack of microsatellite instability (MSI) are potential markers for aggressive clinical disease that were used in the study. Patients who were in the high-risk category were prospectively stratified to treatment with FOLFOX with or without the addition of bevacizumab whereas low-risk patients were assigned to surveillance alone. The study was criticized for not having an observation arm in the high-risk category since adjuvant treatment is not standard of care in this group of patients. This study is currently closed to enrollment as one of the arms is no longer standard of care in the adjuvant setting [4].

For patients with stage III colon cancer, adjuvant chemotherapy was shown to reduce individual 5-year risk of cancer recurrence and mortality by about 30%. The addition of oxaliplatin to 5-FU showed a significant improvement in 3-year disease-free survival for patients with stage III colon cancer in two large randomized trials (MOSAIC and NASBP-C07) [2, 5]. There was an update for the MOSAIC study in 2009 that showed no benefit in overall survival with FOLFOX versus 5-FU/LV for patients with stage III who were more than 65 years old [6].

Bevacizumab is a humanized monoclonal antibody to vascular endothelial growth factor (VEGF), that was added to oxaliplatin-based chemotherapy in the NSABP C-08 and AVANT trials in patients with stage II or III colon cancer. It did not prolong disease-free survival or overall survival when compared to chemotherapy alone [7, 8].

Cetuximab is monoclonal antibody that targets the epidermal growth factor receptor (EGFR); its benefit in the adjuvant setting in combination with chemotherapy was tested in the N0147 trial. This trial was closed prematurely because of lack of benefit.

Patients with mutant KRAS had a worse disease-free survival and a trend toward worse overall survival [9]. Hence monoclonal antibodies that target EGFR are not currently indicated in any group of patients with resected colon cancer, though cetuximab is used in other settings.

The treatment of metastatic colorectal cancer (mCRC or stage IV) usually involves chemotherapy alone except in patients who have limited metastatic disease in the liver and/or lungs who are candidates for surgical resection. Triplet combination represents a standard option for first-line therapy to treat metastatic colorectal cancer. Many oncologists use FOLFOX in the first-line setting and FOLFIRI regimen (Irinotecan + 5-FU + leucovorin) in the second-line setting after failure of initial oxaliplatin-based therapy. However, the FOLFIRI regimen could be considered initially in a patient with a relative contraindication to oxaliplatin. Selection of oxaliplatin or irinotecan as part of cytotoxic backbone upfront in metastatic disease is mainly dependent on toxicity profile. In 2012, FOLFIRI plus cetuximab was approved as an option in the first line setting to treat metastatic CRC and can be considered especially when the KRAS mutation status is wild-type. FOLFOX or FOLFIRI plus bevacizumab remains as the most reasonable first line option for mCRC in 2012 especially in patients without known KRAS mutation status.

Patients who progress on FOLFIRI regimen initial therapy could benefit from FOLFOX regimen. In a study that evaluated the two sequences of FOLFIRI followed by FOLFOX, and FOLFOX followed by FOLFIRI, both sequences had similarly impressive survival benefits. In a pooled analysis of cohorts of older patients (aged 65 years or older) from two randomized clinical trials evaluated the benefit of bevacizumab plus 5-FU-based chemotherapy in first-line treatment of mCRC [10]. The study showed that adding bevacizumab to 5-FU-based chemotherapy improved overall survival and progression-free survival in older and younger patients. Bevacizumab is also approved for second-line therapy combined with other chemotherapy if it was not used with the first-line chemotherapy. There are some data that suggests a possible benefit for continued bevacizumab beyond first progression, though data from a randomized trial is lacking to corroborate this observation [11].

Two EGFR-targeted monoclonal antibodies are approved for metastatic colorectal cancer, though these therapies should be given only to patients with wild-type KRAS tumors. The addition of cetuximab to irinotecan-based chemotherapy improved median time to progression and median survival after failure of prior irinotecan-based chemotherapy [12, 13]. The addition of cetuximab to first-line oxaliplatin regimen showed mixed results in contrast to panitumumab, which significantly improved PFS in patients with wild-type KRAS tumors when combined with first-line oxaliplatin regimen. The combination of anti-EGFR antibody therapy and bevacizumab is not advised outside of clinical trials. The addition of panitumumab to bevacizumab resulted in increased toxicity and decreased PFS [14].

Dasatinib, a small molecule BCR-ABL and Src inihbitor, was found to sensitize mutant KRAS colorectal tumors to cetuximab in CRC lines [15]. The combination of dasatinib and cetuximab was shown to decrease prosurvival signaling through the MAPK, mTOR, and STAT pathways compared to untreated or monotherapies in preclinical studies. The combination also resulted in decreased cell proliferation and a higher amount of apoptosis [15]. A retrospective study evaluated the role of

PTEN loss, Akt phosphorylation, and KRAS mutations on the activity of cetuximab plus irinotecan in patients with mCRC. This study concluded that PTEN loss may be predictive of resistance to cetuximab plus irinotecan. Patients with PTEN-positive metastases and wild-type KRAS had longer PFS compared to other patients [16].

KRAS mutations and overexpression of EGFR were found to be important independent predictive markers in mCRC patients treated with cetuximab plus chemotherapy [17]. This study showed that tumors expressing high levels of EGFR or have wild-type KRAS are more likely to have a better PFS and OS when treated with cetuximab plus chemotherapy. In patients with wild-type KRAS tumor status, EGFR expression was a predictor of clinical response. Non-activating KRAS mutant tumor had better PFS and OS than patients with activating KRAS mutants [17]. BRAF mutations are mutually exclusive with KRAS mutations that are found in about 5–10% of mCRC. BRAF mutations are associated with poor prognosis overall but should not be used as predictive factor for patients with wild-type KRAS. In 2012, the presence of either a codon 12 or a codon 13 mutation in KRAS predicts resistance to anti-EGFR targeted therapy. Acquired resistance through KRAS mutation or EGFR extracellular domain mutation has been observed.

Inhibition of the $BRAF^{V600E}$ oncoprotein by the small-molecule vemurafenib in melanoma was shown to be highly effective, likely secondary to the low level of EGFR in melanoma [18]. On the other hand, inhibition of $BRAF^{V600E}$ in preclinical colon cancer models led to rapid feedback activation of EGFR [18]. This preclinical study proposed the benefit of adding BRAF and EGFR inhibitors for complete blockade of EFGR cascade. The role of genetics in the genesis, prognosis, and therapeutic sensitivity of colon cancer and other tumors is becoming increasingly important as we enhance our understanding of the disease. This has potentiated the field of personalized medicine, which is being vetted as a future direction in oncology and is becoming increasingly feasible with improvements in technology and associated costs.

Overview of Genetic Alterations in Colorectal Cancer

In Western countries, death rates associated with CRC have steadily declined over the past few decades [20]. This is likely a result of several factors that include improved screening techniques and participation, changes in lifestyle, and improved therapies. Despite improvements in treatment options, cytotoxic chemotherapy along with surgery or radiotherapy remains the most frequently deployed strategy in the management of colorectal cancer. Nevertheless, refractory disease and systemic side effects of chemotherapy that often limit its dose and tolerability among patients has left physicians searching for alternatives. The last two decades have yielded an increased understanding of the molecular basis of cancer that has driven the development of antitumor agents that target critical signaling pathways that drive the genesis, maintenance, and/or progression of the disease.

The incidence of CRC appears to be linked to environmental factors and genetics. While modernized countries have benefited from declining death rates in CRC, their incidence rate is higher and is attributed to increased sedentary lifestyles and

obesity. The risk of developing CRC increases substantially with incidence in first-degree relatives and several syndromes that confer a substantially increased predisposition to CRC have been identified. These include familial adenomatous polyposis (FAP), MUTYH-associated polyposis, Lynch syndrome and other more rare syndromes (reviewed in [21]) that have been rationalized at the genetic level. For instance, FAP is directly linked to germline mutations in the APC gene, a tumor suppressor that is frequently inactivated in CRC.

The evolution of CRC is thought to be a progression of concomitant molecular and macroscopic events that convert normal colorectal epithelial to adenoma, followed by an adenoma to carcinoma transformation [22]. At the genetic level, CRC is comprised of several cumulative oncogenic alterations that include inactivating tumor suppressors and activating oncogenes. One of the earliest canonical events in CRC genesis is the inactivation of APC, which cooperates with the kinase GSK3-beta to complex with and negatively regulate the activity of the pro-proliferation transcription factor beta-catenin. Mutation of the oncogene KRAS has been proposed as a major step in CRC that advances the disease to the adenoma stage and has been found in approximately half of colorectal adenomas and carcinomas [23–25]. KRAS is a GTPase that mediates the signal transduction of several prosurvival receptors such as EGFR. G12V is one of the most common oncogenic mutations in the KRAS gene, which results in constitutively active pro-survival signaling that is normally controlled by upstream receptor-ligand complexes. Inactivation of the "gate keeper" tumor suppressor p53 is thought to be a late-stage event in CRC and is associated with transition from adenoma to carcinoma.

The traditional linear model of CRC development is useful to describe common oncogenic alterations that fit observations across a large population and may fit many typical cases, though cancer is clearly not a homogeneous and linear process. The progression of these genetic events to induce CRC may occur out of order, cooperate with other alterations, and may be accomplished by various mechanisms such as genomic instability or mutagens. Other genetic alterations can substitute with these canonical alterations by themselves or act in concert such as PTEN, STK11, SMAD4, IGF1, and COX2. Interestingly, some genetic events that act on the same signaling pathway can substitute for others such as the inactivation of beta-catenin in lieu of APC inactivation.

Numerous therapeutic targets have arisen by coupling the knowledge of the molecular events that drive CRC with other molecules that play an essential role in cancer. Significant insight has been gained regarding molecules that regulate key cellular processes conserved in cancer such as evading apoptosis, escaping immune surveillance, increasing cell proliferation through growth factor signaling, and angiogenesis (Fig. 2). These include molecules that are typically altered in CRC and other molecules that act on the same signaling pathway to drive the same phenotype. Novel targeted agents that inhibit the function or production of these key molecules are being pursued and have been approved in some cases such as bevacizumab, which inhibits angiogenesis by sequestering VEGF. Clinical trials are being pursued with these targeted agents as a monotherapy and in combination with standard of care therapies. Here, we review novel targeted agents that are currently being explored in CRC that exploit genetic alterations in cancer.

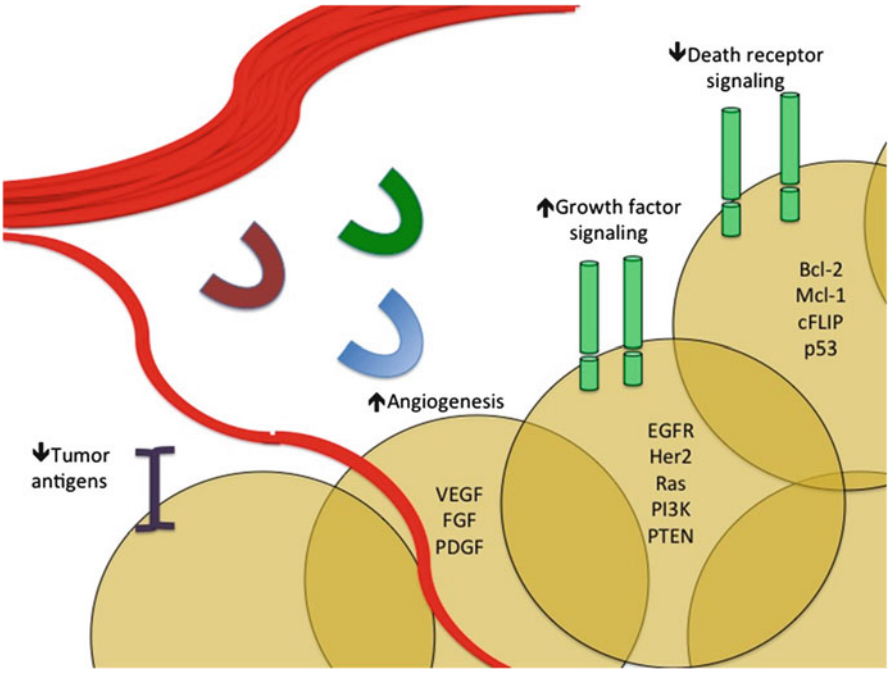

Fig. 2 Molecular targets that drive CRC tumor initiation and maintenance. Tumor cells downregulate death receptor signaling to avoid induction of apoptosis and upregulate growth factor signaling in order to divide more rapidly and in an unregulated manner. The increased proliferation rate of tumor cells requires an increased supply of oxygen and nutrients. This increased supply is provided by new blood vessels formed by upregulating cytokines involved in angiogenesis such as VEGF, PDGF, and FGF. Tumor cells also downregulate surface antigens that are recognized and attacked by the immune system so that the tumor can evade the immune surveillance of cancer

Targeting Cell Death Pathways

Agonistic TRAIL Death Receptor Antibodies

Apoptosis is a naturally occurring process that is necessary for homeostasis of multicellular organisms. Apoptosis occurs by the activation of effector caspases through either the intrinsic, mitochondria-dependent pathway or the extrinsic death pathway. Cancer cells can escape the cytotoxic effects of various conventional chemotherapies by bypassing the intrinsic apoptotic response to the DNA damage. Depending on the cell type, either the intrinsic or extrinsic death pathways can be initiated by binding of ligands or agonistic antibodies to specific death receptors on the cell surface. These death receptor-mediated pathways that induce apoptosis provide an alternative route to target cancer cell that become resistant to traditional chemotherapy (Fig. 3) [26].

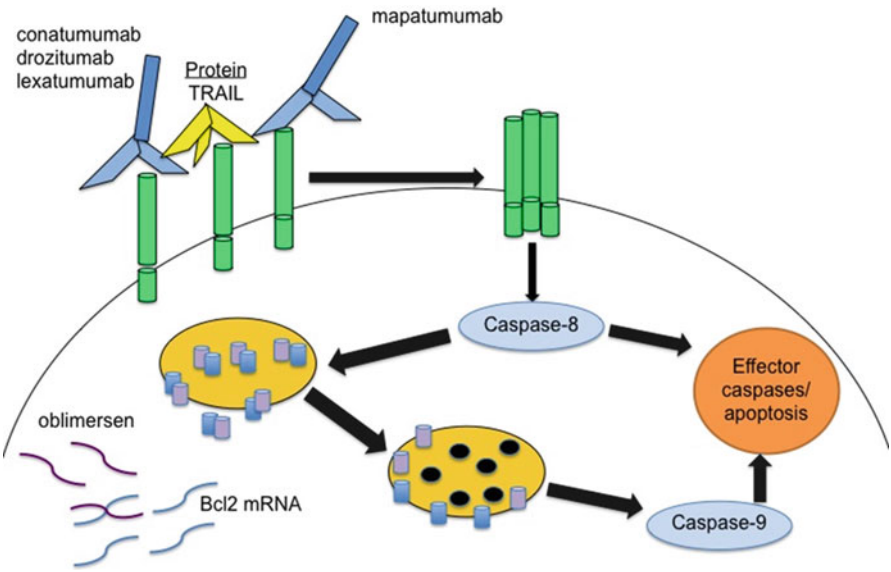

Fig. 3 Antineoplastics that target death receptor signaling in CRC. The pro-apoptotic TRAIL death receptors are engaged by several antibodies that are in clinical trials as antitumor agents. Death receptor 5 (DR5) is engaged by conatumumab, drozitumab, and lexatumumab. Death receptor 4 (DR4) is engaged by mapatumumab. Binding to these death receptors induces death receptor homotrimerization, which activates caspase-8 to trigger apoptosis through pathways that may or may not involve the permeabilization of mitochondria. The mitochondria permeabilization process is regulated by Bcl-2 family members, including Bcl-2 itself. Oblimersen is an antisense drug that targets the Bcl-2 transcript (mRNA) to prevent its translation and therefore downregulates its expression

While there are several death receptor ligands, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF receptor superfamily, is an attractive antitumor protein as it exerts differential cytotoxicity to cancer and normal cells. In most contexts, TRAIL binds two decoy receptors (DcR1 and DcR2) and two death receptors (DR4 or DR5), which results in the formation of the death-inducing signaling complex (DISC). DISC formation results in activation of the initiator caspase-8, which ultimately leads to activation of effector caspases-3, -6, and -7 (Fig. 3). Normal cells are thought to express higher levels of decoy receptors, which lack the intracellular death domains that, form the DISC and therefore do not initiate apoptosis [27]. Cancer cells evade cell death through a variety of resistance mechanisms such as loss of p53 function. The majority of GI cancers show alterations in the CD95 pathway molecules that impact on TRAIL sensitivity by alter the inhibitory effect of FLICE/caspase-8 inhibitory protein (c-FLIP or CFLAR) or the Bcl-2 family of proteins [28]. In addition to recombinant TRAIL, the death receptor pathway may also be accomplished by the agonistic activity of antibodies against DR4 or DR5.

Conatumumab is a fully humanized agonist antibody against DR5 that induces apoptosis via caspase activation in human tumor cell lines in vitro and demonstrated

anti-tumor efficacy in xenograft models of colon, lung, and pancreatic tumors. A link between the increase in serum caspase-3/7 activity and M30 level in the activation of the extrinsic apoptotic pathway by an anti-DR5 agonist antibody in a preclinical cancer model, which could be used as cell death biomarkers [29]. A phase Ib study of another DR5 agonist antibody, drozitumab, was conducted with first-line FOLFOX plus bevacizumab (BV) in patients with mCRC. The combination was well tolerated and no adverse interactions were found between drozitumab and the chemotherapy. This abstract was presented at 2011 Gastrointestinal Cancers Symposium [30]. In another phase Ib study, drozitumab was combined with cetuximab plus irinotecan or with FOLFIRI with or without bevacizumab in previously treated mCRC patients. This trial also reported no adverse interactions between drozitumab and the chemotherapy.

Lexatumumab (HGS-ETR2 developed by Human Genome Sciences) is another anti-DR5 agonist antibody that has been studied in a phase Ib trial. Lexatumumab was well tolerated and tumor regression was observed in two patients with CRC receiving lexatumumab in combination with folate, 5-FU and irinotecan. This study suggested that further evaluation of lexatumumab in combination with chemotherapeutic agents in phase II studies to evaluate efficacy is warranted [31]. Mapatumumab (HGS-ETR1) is the only DR4 antibody in clinical trials. Preclinically it showed cytotoxic activity against cancer cells but no objective response was found in a phase I study [32].

Most clinical studies showed that these antibodies are not effective when used as monotherapy in patients with gastrointestinal (GI) cancer. Combining TRAIL with other agents may overcome resistance mechanisms, such as combination of TRAIL-based therapies with c-FLIP inhibitors or the multi-kinase inhibitor sorafenib, which down regulates Bcl-2 and Mcl-1. Preclinical studies have shown that the TRAIL-DR5 pathway can cause hepatotoxicity and bile duct toxicity at high doses in mice treated with an anti-mouse DR5 monoclonal antibody.

Bcl-2

Oblimersen is an antisense agent that inhibits the translation of the anti-apoptotic Bcl-2. Oblimersen inhibits Bcl-2 protein production via providing a complementary genetic strand to the messenger RNA encoding for Bcl-2, which renders the cancer cell more sensitive to chemotherapy. In a phase I study the pharmacokinetic and biological effects of oblimersen were evaluated in combination with irinotecan in mCRC patients [33]. This combination was found to be safe and moderately active in patients with previously treated CRC. The recommended dose of oblimersen was determined to be 7 mg/kg/day for days 1–8 with irinotecan 280 mg/m^2/day on day 6 once every 3 weeks. Phase I/II studies with oblimersen are in progress in melanoma [34, 35], small cell lung cancer (SCLC) [36], prostate cancer, refractory acute leukemia and chronic lymphocytic leukemia (CLL). A phase I/II study is evaluating the effectiveness of combining oxaliplatin, fluorouracil, and leucovorin with oblimersen in patients with advanced CRC [37].

Targeting Growth Factor Signaling

The process of cell division is tightly controlled and normally requires stimuli. Growth factor signaling typically involves the binding of an extracellular ligand, such as EGF, to a receptor tyrosine kinase. Binding results in the homo- or hetero-oligomerization of the receptor and autophosphorylation events that activate downstream signaling molecules that lead to prosurvival effects (Fig. 4). Therefore it is unsurprising that cancers, including CRC, typically harbor genetic aberrations that

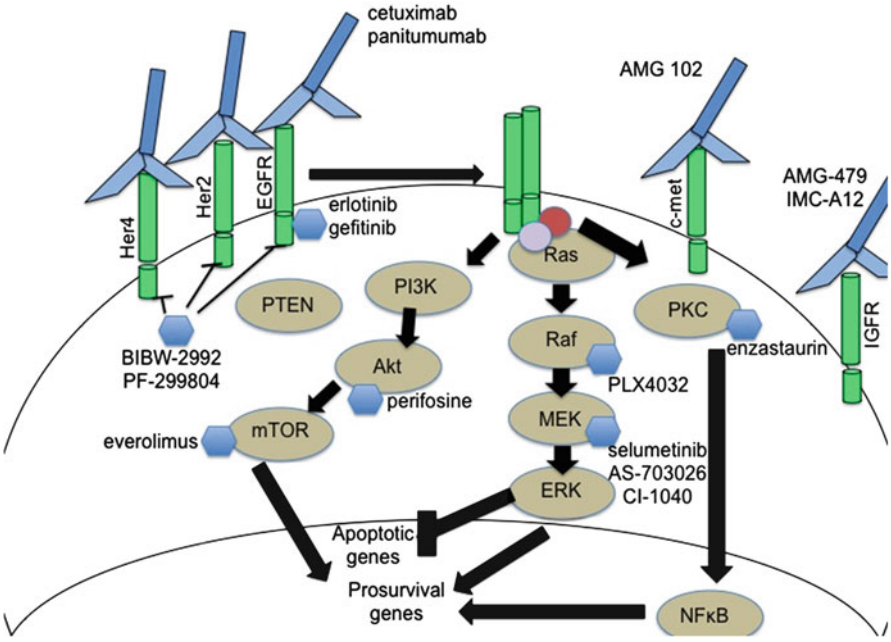

Fig. 4 Antineoplastics targeting growth factor signaling in CRC. Tumor cells require growth factor-independent signaling to increase their proliferation rate. Growth factor signaling typically involves a receptor kinase localize to the cell surface such as the EGFR family members, IGFR, or c-met. These receptors normally bind to secreted growth factors followed by events that turn on intracellular signaling. Several EGFR inhibitors have been developed including intracellular small molecule inhibitors such as erlotinib and gefitinib as well as antibodies such as cetuximab and panitumumab, which bind to EGFR to prevent ligand binding without turning on EGFR signaling. BIBW-2992 and PF-299804 are small molecules that inhibit multiple members of the EGFR family. AMG 102 binds and inhibits the c-met surface receptor. AMG479 and IMC-A12 are antibodies that bind to IGFR. Ligand-receptor complexes involved in growth factor signaling often activate the GTPase Ras, which activates PKC, PI3K/Akt/mTOR signaling, and the MAPK signaling pathway that involves the sequential phosphorylation of Raf, MEK, and ERK. The signaling pathways ultimately turn on genes that have oncogenic consequences such as upregulating prosurvival gene transcription and downregulating apoptotic genes. Enzastaurin is a small molecule inhibitor of PKC. Perifosine is a small molecule indirect inhibitor of Akt and everolimus is a small molecule mTOR inhibitor. Among the MAPK members, PLX4032 is a small molecule specific inhibitor of the V600E mutant form of BRAF where as MEK is inhibited by several small molecules such as selumetinib, AS-703026, and CI-1040

allow cancer cells to grow in the absence of such stimuli. This relationship has led to the development of several agents targeting growth factor signaling, which has generated the most successful targeted agents in terms of FDA approval to date.

The EGFR Family

The EGFR family has been a successful target for targeted cancer therapies. Increased EGFR signaling is particularly common in lung, breast, and CRCs through one or more of the family members, which includes HER1 (EGFR, ErbB-1), HER2 (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4) [38]. Agents that inhibit EGFR signaling have been approved by the FDA such as cetuximab in colon cancer and erlotinib and gefitinib in non-small cell lung cancer. However, responders to these inhibitors almost universally develop resistance through acquired mutations in these receptors after long-term use of EGFR inhibitors [39]. This has led to development of inhibitors to multiple EGFR family members such as BIBW-2992, which is an inhibitor of EGFR and HER2 as well as PF-299804, an inhibitor of EGFR, HER2, and HER4.

BIBW-2992 is an irreversible small molecule inhibitor of EGFR and HER2 that has efficacy against first generation EGFR inhibitor-resistant cancers in cell-based assays [40]. Increased efficacy was also noted in xenografts resistant to first generation EGFR inhibitors with significant regression as compared to erlotinib [41]. Other preclinical studies found significant tumor regressions in epidermoid carcinoma xenografts in mice. A Phase I trial of PF-299804, an EGFR, HER2, and HER4 inhibitor, studied 121 patients with advanced solid malignancies, 22 of which being mCRC. In this study, four patients with non-small cell lung cancer had a partial remission but no CRC patients experienced remission with the oral therapy. However, 44 patients of the 121 had stable disease that did not occur with previous treatment [42].

IGF Receptors

The insulin-like growth factor receptor (IGFR) is a family of receptor tyrosine kinases that bind insulin-like growth factors. Ligand binding activates two kinase cascades, the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway [43]. The MAPK pathway regulates cellular metabolism and is known to promote cell growth and survival whereas the PI3K/Akt pathway is involved in regulation proliferation and apoptosis. This receptor quickly became a cancer therapy target as many early studies found elevated receptor expression in colon carcinoma cell lines. One of the earliest studies in 1986 showed that 20% of colon cancer lines have a mild to moderate increase in IGF1 mRNA and 40% showed an increase in IGF2 mRNA relative to the surrounding normal colonic mucosa. There was a significant increase in IGF1 receptor (IGF1-R) staining in higher stage and metastatic colon carcinomas as compared to normal colonic cell

lines [44]. Based on some of this preclinical data, IGF1-R inhibitors are in development including monoclonal antibody antagonistic ligands that irreversibly bind the receptor to prevent downstream signaling.

One such monoclonal antibody is AMG-479, a fully human antibody produced by Amgen with an IC50 of 0.53 nanomolar against IGF1-R. A 2009 phase I trial with this antibody showed one complete response and one partial response in Ewing's sarcoma out of 15 patients with soft tissue sarcomas. Patients received dose escalations every 2 weeks with intravenous infusions of 1–20 mg/kg. After day 80 of treatment, one patient with Ewing's sarcoma had complete response of all pulmonary metastases and has maintained this remission. One of the five patients with neuroendocrine tumors had a partial response. While the four CRC patients enrolled in the trial did not respond, evidence in other cancers shows promise for IGF1-R monoclonal antibodies [45].

In 2010, a Phase II trial of the IGF1-R monoclonal antibody IMC-A12 compared cetuximab to the combination of cetuximab and this antibody in CRC patients refractory to cetuximab alone. In this study, none of the patients who received IMC-A12 monotherapy had a response. One out of 21 patients had a partial response to the combinatorial therapy that lasted approximately 6 months after treatment initiation. This patient was also noted to have KRAS wild type CRC [46]. One of the reasons why IGF1 receptor monoclonal antibodies seemed so promising in preclinical trials but in clinical trials with CRC have not been as successful could be the large amount of KRAS mutations found in late stage CRCs. KRAS is one of the downstream activators in the EGFR tyrosine kinase pathway and are found in 40–50% of CRCs [47], which confers resistance to IGF1R mAbs.

Hepatocyte Growth Factor (HGF)

Hepatocyte growth factor (HGF) has been shown to increase the motility of human colon cancer cells in vitro, which can be blocked by an anti-HGF antibody [48]. The HGF receptor is encoded by the c-Met proto-oncogene, which cross-talks with beta-catenin signaling to sustain and enhance CRC cell invasiveness [49]. A Phase Ib study of AMG 102, a fully human monoclonal antibody against HGF, in combination with bevacizumab found the combination to have an acceptable toxicity profile. Two of the 14 CRC patients in the study had a mean progression-free survival of approximately 36 weeks on the combination. Treatment-induced side effects were mild and included fatigue, nausea, constipation and peripheral edema and no anti-AMG antibodies were detected [50].

Mutant BRAF

KRAS mutations are present in 40–50% of the patients with mCRC, while the mutually exclusive BRAF activating mutation is present in up to 10% of mCRC and

confer a poor prognosis. BRAF mutations are associated with some response to treatment with monoclonal antibodies against EGFR. PLX4032/RG7204 is an oral small molecule inhibitor of mutant BRAF that has demonstrated efficacy in melanoma, thyroid cancer, and CRC among others. The activity of PLX4032 as monotherapy or in combination with capecitabine with or without bevacizumab was evaluated in a CRC xenograft model. Monotherapy was found to have superior activity to capecitabine or bevacizumab alone that was enhanced in combination with capecitabine ± bevacizumab [51].

In a phase I study, patients with mCRC with mutant BRAF were treated with PLX4032 at the previously determined maximum tolerated dose of 960 mg BID. As compared to the 81% response rate in metastatic melanoma, responses in this study were heterogeneous. The clinical activity was found to be more modest than previously seen in melanoma patients with mutant BRAF. This was rationalized by the increased heterogeneity of the biological consequences of BRAF activation in CRC patients compared to melanoma patients [52]. In 2012 it has become clear from preclinical studies that targeting EGFR may help with response of BRAF mutant colon cancer cells to BRAFtargeted agents.

MEK

Aberrant expression of EGFR is common in human cancers, particularly in CRCs. EGFR family members signal by a pathway that is similar to IGFR signaling by acting through the Ras-Raf-MEK-ERK and PI3K signaling pathways, leading to cell proliferation and evasion of apoptosis. Due to this fact, the EGFR receptor has been a hotly pursued drug target for antineoplastics. Such drugs include cetuximab and panitumumab, which are monoclonal antibodies against the receptor [53, 54]. Unfortunately only about 8–23% of cancer patients respond to EGFR-targeting treatments due to activating mutations KRAS that cause resistance to EGFR monoclonal antibody therapy since Ras activation occurs downstream of the EGFR receptor as well IGF1R. These resistance mechanisms make therapies targeting activators downstream of Ras a priority. MAP kinase kinase (MEK) is an integral part of the Ras signaling pathway as a downstream signal transducer that has been pursued as cancer drug target.

A recent study described two highly potent small molecule inhibitors of MEK, selumetinib (AZD-6244) and AS-703026. In vitro studies demonstrated that both molecules reduced the proliferation of mutant KRAS cancer cells by 63–67%. As expected, there was no reduction in proliferation of mutant KRAS cells treated with cetuximab. In vivo studies using mouse models found that selumetinib decreased tumor size by 60–70% in mutant KRAS tumors [55]. Selumetinib may also increase radiation responsiveness of lung cancers and CRCs to two highly potent small molecule inhibitors by decreasing cellular response to hypoxia that induces therapeutic resistance. Tumor growth was delayed approximately 25 days more than controls in xenografts treated with both selumetinib and ionizing radiation, which is 15 more

days than radiation alone. There was also a significant decrease in the tumor density of blood vessels after 5 days of treatment with both selumetinib and radiation [55].

In 2009, a phase II trial was performed with selumetinib in CRC patients refractory to one or two previous therapies. In this study, the effects of oral selumetinib on disease progression were compared with that of capecitabine. There was no significant difference in disease-free survival between the two randomized groups receiving either therapy. There was one partial response out of the 35 patients in the capecitabine group and no responses in the selumetinib group. Unfortunately, approximately 80% of the patients experienced disease progression within the 2 year study while the others had stable disease.

Despite very promising preclinical data, several phase I and phase II trials of MEK inhibitors have been less than encouraging. A phase II trial of an oral MEK inhibitor, CI-1040, in non-small cell lung cancer, breast, colon, and pancreatic cancers was conducted in 2004. The oral therapy was well tolerated with minimal side effects; however this MEK inhibitor yielded no complete or partial responses [56]. A phase I trial of a MEK inhibitor was later attempted in the 2009 AS-703026 trial in advanced solid tumors. 78% of these patients had CRC and the other 22% had melanoma. There were two partial responses out of 15 previously treated advanced melanomas, however there was no documented response in CRC [57].

Akt

The PI3K/Akt pathway is a prosurvival signaling pathway downstream of many receptors that bind growth factors such as EGFR. Perifosine is a small molecule that inhibits the activation of Akt by a poorly understood mechanism and has been studied in melanoma, multiple myeloma, and sarcoma. In vitro effects on colon cancer cell lines have been reported [58]. Perifosine continues to be tested in clinical trials.

Mammalian Target of Rapamycin (mTOR)

mTOR is a substrate of Akt and the mTOR pathway is involved in several aspects of cancer cell survival and proliferation. Everolimus is a rapamycin analog that binds with a high affinity to FK-507 binding protein-12, which forms a complex that interacts with mTOR to block signaling by inhibiting the phosphorylation of S6K1 and 4E-BP1 by mTOR. Inhibiting the mTOR pathway impacts the expression of proteins involved in angiogenesis, cell growth and proliferation, and metabolism [59]. Everolimus is FDA approved for subependymal giant cell astrocytoma associated with tuberous sclerosis that cannot be surgically removed and advanced renal cell carcinoma after failure of sunitinib or sorafenib. It has been investigated in other solid tumors including CRC. A phase II trial of everolimus in combination with bevacizumab in refractory mCRC demonstrated a promising disease control rate [60].

Results showing safety and efficacy have been reported in a phase I trial of everolimus with irinotecan and cetuximab as second-line treatment in mCRC. A phase II study is planned [61].

Protein Kinase C (PKC)

PKC plays a role in the signaling of growth factor receptors that has cross talk with both Akt and mTOR. Enzastaurin was developed as an oral ATP-competitive selective inhibitor of the serine/threonine kinase protein kinase C-beta (PKC-beta) that was subsequently shown to inhibit multiple PKC isoforms, suppress the phosphorylation of Akt, GSK3β, and ribosomal protein S6. Enzastaruin has demonstrated pro-apoptotic and anti-proliferative effects on an array of cultured human tumor cells including CRC [62]. Several phase II trials failed to produce any promising signs of efficacy in solid tumors. The addition of enzastaurin to pemetrexed as second-line therapy in advanced non-small cell lung cancer failed to improve progression-free survival or overall survival [63]. The addition of enzastaurin to pemetrexed, carboplatin, and bevacizumab in stage IIIB/IV non-small cell lung cancer failed to improve progression-free survival [64]. Enzastaurin also failed to show sufficient single agent activity in recurrent high-grade gliomas. A trial investigating the addition of enzastaurin to capecitabine in metastatic or recurrent breast cancer after prior cytotoxic therapy was stopped early after finding no median overall survival benefit and shorter progressional-free survival in the enzastaurin arm [65, 66]. A phase III trial failed to show superior efficacy of enzastaurin compared to lomustin in recurrent intracranial glioblastoma [67]. On the other hand, enzastaurin has shown activity in prolonging freedom from progression in relapsed or refractory diffuse large B-cell lymphoma in a small subset of patients, and in relapsed or refractory mantle cell lymphoma [68, 69]. A 'window of opportunity' trial in chemonaïve asymptomatic mCRC patients showed that enzastaurin may have single agent activity [70]. However, a recent placebo controlled phase II trial of maintenance enzastaurin in combination with 5-FU, leucovorin, and bevacizumab following first-line chemotherapy in mCRC, failed to demonstrate a PFS advantage [71].

Targeting Angiogenesis

VEGF Receptors

In order for neoplasms to continue to propagate they require an adequate blood supply, which is accomplished by inducing angiogenesis (Fig. 5). One of the most important factors involved in angiogenesis is vascular endothelial growth factor (VEGF), which is sufficient in vitro to cause angiogenesis [72]. Due to the importance of angiogenesis in cancer, a number of therapies have been developed

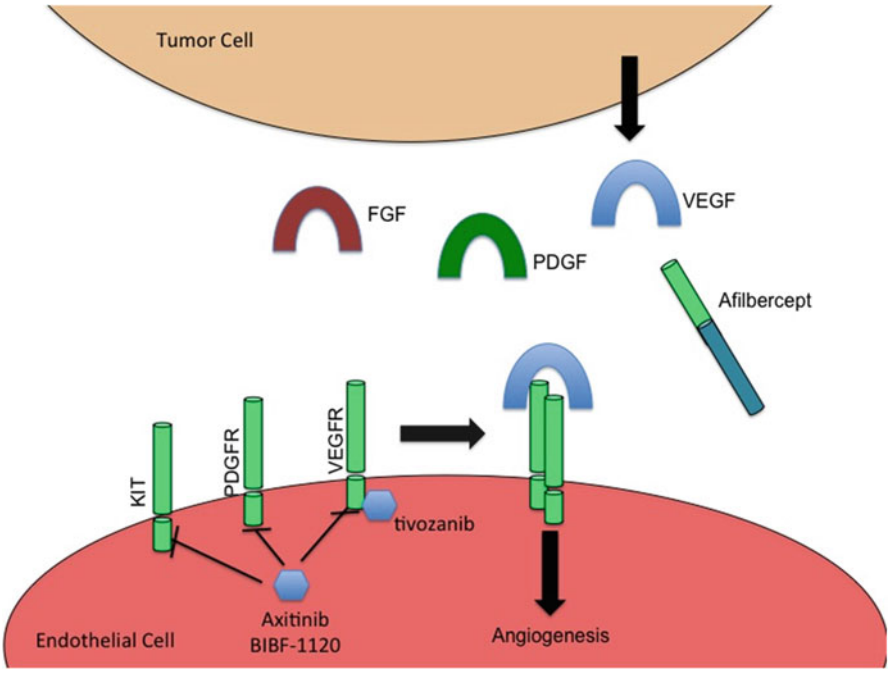

Fig. 5 Antineoplastics targeting angiogenesis in CRC. Several cytokines are secreted by tumor cells to induce angiogenesis. These cytokines such as VEGF are bound by surface receptor on endothelial cells that include KIT, PDGFR, and VEGFR. Aflibercept is a fusion protein that mimic two VEGF receptors. Tivozanib is a small molecule inhibitor of VEGFR and the small molecules axitinib and BIBF-1120 inhibit VEGFR, PDGFR, and KIT

to target VEGF and its cognate receptor VEGFR. Aflibercept is a recombinant fusion protein consisting of the Fc portion of IgG1 combined with the third domain of VEGFR2 and the second domain of VEGFR1. This allows aflibercept to mimic VEGFR2 and VEGFR1 to prevent VEGF from binding to those receptors, thereby inhibiting angiogenesis. Preclinical studies have shown that aflibercept is an effective inhibitor of angiogenesis and tumor growth in animal models [73]. Aflibercept has shown tolerability in phase I trials in patients with solid tumors [74–77]. Aflibercept has shown clinical efficacy in recurrent platinum-resistant epithelial ovarian cancer and prolonged time to repeat paracentesis in advanced epithelial ovarian cancer with symptomatic malignant ascites [78, 79]. Clinical efficacy has also been shown in several other phase II trials including patients with platinum- or erlotinib-resistant locally advanced or metastatic non-small cell lung cancer, uterine leiomyosarcoma, inoperable stage II or IV melanoma, temozolomide-resistant recurrent glioblastoma, and anaplastic glioma at first relapse [80–83]. Limited clinical efficacy has been reported with aflibercept as a single agent in patients with recurrent metastatic urothelial cancer previously treated with a platinum-containing regimen [84]. A phase II trial showed efficacy in patients with

mCRC previously treated with bevacizumab and a recent phase I trial investigating aflibercept in combination with FOLFIRI in mCRC showed tolerability [85, 86]. The phase III VELOUR trial investigating aflibercept in combination with FOLFIRI as a second-line regimen in mCRC is anticipated to report its results during the second half of 2011. Similarly, the phase II AFFIRM investigating aflibercept in combination with FOLFOX as a first-line treatment for mCRC is also expected to have results by late 2011. In 2012, aflibercept was approved by the FDA in combination with FOLFIRI as a therapeutic option for patients with metastatic CRC, including about a 10% response rate in patients who were previously treated with Avastin in combination chemotherapy.

Tivozanib is an oral, ATP-competitive, small molecule inhibitor of VEGFR [87]. A phase II trial and subgroup analysis found that tivozanib as monotherapy achieved disease control for patients with different histological types of renal cell carcinoma (RCC), with longer PFS seen in patients with clear cell RCC compared to non-clear cell variants [88, 89]. Tivozanib has also been studied in phase Ib trials in combination with temsirolimus in metastatic RCC, in combination with paclitaxel in metastatic breast cancer, and as a monotherapy in non-small cell lung cancer [90–92]. A phase III randomized, controlled trial comparing tivozanib with sorafenib in patients with advanced RCC is pending results [93]. Recently presented in abstract form, an open-label phase Ib trial of tivozanib in combination with FOLFOX in patients with advanced gastrointestinal tumors showed safety and tolerability [94]. A phase Ib trial investigating tivozanib in combination with capecitabine for patients with advanced solid tumors including CRC is currently recruiting patients [94].

Axitinib (AG-013736) is an oral selective inhibitor of VEGF receptors [95, 96]. Axitinib inhibits the autophosphorylation of VEGF receptors (VEGFR) that normally occurs upon ligand binding, interferes with eNOS/AKT mediated signal transduction, decreases vascular permeability, and prevents VEGF-mediated endothelial cell survival. Axitinib demonstrates dose-dependent anti-tumor activity that is associated with a reduction in angiogenesis, tumor cell proliferation, and increased apoptosis. At higher concentrations, axitinib also has activity against PDGF receptors and KIT, which are also receptors involved in angiogenesis, may enhance its anti-tumor efficacy. However, it is likely that the principal effects of axitinib are mediated through the VEGF receptors when considering the pharmacokinetic/pharmacodynamic data where it has shown efficacy [97]. Several phase II studies have shown clinical efficacy in a variety of solid tumors including advanced non-small cell lung cancer, cytokine-refractory metastatic RCC, advanced thyroid cancer, advanced pancreatic cancer, and metastatic melanoma [98–101]. Axitinib has also shown activity in human breast cancer models in mice [102]. Recently, axitinib has been investigated as a second-line agent in mCRC. This open-label, randomized phase II trial compared axitinib to bevacizumab in combination with either FOLFOX or FOLFIRI. The study failed to show a difference between axitinib and bevacizumab with respect to either progression-free survival or median overall survival. However, a trend towards improved median overall survival was seen with axitinib in combination with FOLFOX in comparison to bevacizumab in combination with FOLFOX. Conversely, a trend towards reduced

median overall survival was seen with axitinib in combination with FOLFIRI in comparison to bevacizumab in combination with FOLFIRI [103].

Other Receptors That Mediate Angiogenesis

VEGF receptor inhibitors have proven to be effective targeted therapies. However, some tumors are still able to sustain angiogenesis by upregulating other vascular growth factors such as platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) [104]. This resistance mechanism led to the development of a triple angiokinase receptor inhibitor, BIBF-1120, which irreversibly inhibits VEGF, PDGF, and FGF receptors.

A phase I trial in 2009 investigated oral BIBF-1120 in 61 patients with advanced solid tumors, 30 of which were CRC. Of these patients, 56 had prior therapy with surgery or chemotherapy and continued to have disease progression. There were two partial responses, one in a patient with CRC and one in a patient with RCC. There was a complete response in a RCC patient, whose lung metastases disappeared 2 months post-treatment. BIBF-1120 was well tolerated when its MTD of 250 mg was split into twice daily dosing [105], though other phase I trials were not as successful. In a group of 21 patients with advanced solid tumors, there were no complete or partial responses but 16 patients had stabilization of disease for at least 56 days or a total of two cycles [106]. BIBF-1120 can also be combined with other common chemotherapeutic regimens for CRC. One study showed no additional adverse effects when BIBF-1120 was added to FOLFOX [107]. BIBF-1120 was also successfully added to the EGFR/HER2 inhibitor BIBW-2992 in alternating regimens with diarrhea and vomiting being the most common side effects. In this 2008 study, patients with advanced CRC that continued to progress on two to three therapies, including bevacizumab and cetuximab for 89% of patients, had alternating oral regimens of BIBF-1120 and BIBW-2992 and managed to have disease stabilization for at least 2 months. Unfortunately, there were no partial or complete responses in this patient population [108].

Multi-Targeted Agents

Sorafenib

Sorafenib is an oral multi-kinase inhibitor of VEGFR2, VEGFR3, Flt-3, PDGFR-β and c-KIT BRAF, RAF-1, and RET with demonstrable anti-angiogenic and anti-tumor activity. Sorafenib is FDA approved for use in advanced RCC and surgically unresectable hepatocellular carcinoma [109–111]. The utility of sorafenib in CRC is an active area of research. Recently published, the addition of sorafenib to

cetuximab in patients with mCRC improved overall survival by 2 months [112]. The combination of sorafenib and radiation has shown efficacy in human CRC xenografts and a phase I/II trial currently recruiting participants is investigating sorafenib, capecitabine, and external beam radiation in patients with locally advanced rectal cancer [113, 114]. The combination of sorafenib and bevacizumab as a salvage therapy in heavily pretreated mCRC patients showed promise of clinical activity that is still being evaluated in trials [115, 116]. A phase I/II trial of sorafenib in combination with cetuximab and irinotecan in patients with advanced mCRC has recently reported that the regimen was well-tolerated following amendment of the irinotecan dose/schedule; however, the phase II portion is unlikely to be opened due to limited responses [117]. A phase II trial of sorafenib in combination with FOLFIRI for patients with advanced CRC after failing treatment with oxaliplatin is currently recruiting participants [118]. Also currently ongoing, a trial investigating sorafenib in combination with irinotecan as second-line therapy in mCRC with mutant KRAS has reported favorable phase I results, and interim phase II reports showing evidence of disease control [119, 120]. A phase II efficacy assessment trial of sorafenib in combination with capecitabine in advanced pretreated CRC is currently recruiting patients [121]. Sorafenib is also being investigated in two phase II trials in combination with FOLFOX6 or FOLFIRI as second-line treatment in mCRC [122, 123]. A related kinase inhibitor called regorafenib was approved by the FDA in 2012 as single agent salvage therapy in metastatic CRC and was shown to benefit overall survival including in patients who had previously progressed on bevacizumab.

Sunitinib

Sunitinib is an oral tyrosine kinase inhibitor of VEGFR 1,2,3, PDGFRα, PDGFRβ, KIT, FLT3, RET, and the CSF1 receptor (CSF1R), that is approved for the treatment of advanced clear cell RCC and advanced GI stromal tumors after failure or intolerance to imatinib [124]. The role of sunitinib in the treatment and management of CRC is an active area of research. In human CRC xenograft models, sunitinib demonstrated single agent antitumor activity that synergized with TRAIL [125]. An early phase II trial of sunitinib in heavily pretreated mCRC patients failed to demonstrate a single-agent objective response rate. Subsequently, phase I results of sunitinib in combination with FOLFIRI in treatment-naïve mCRC showed tolerability and promising anti-tumor activity [126]. Sunitinib has also been investigated in combination with FOLFOX6 in mCRC as first-line treatment and is currently undergoing investigation in combination with FOLFOX in comparison to bevacizumab plus FOLFOX as first-line treatment in mCRC [127, 128]. Recently, a phase II study of sunitinib in combination with capecitabine in patients with mCRC refractory to prior treatment with 5-FU/irinotecan/oxaliplatin demonstrated feasibility and a high level of disease stability [129].

Dasatinib

Dasatinib is an oral ATP-competitive tyrosine kinase inhibitor of all members of the Src family of kinases as well as Abl, c-KIT, PDGFR, and EphA2 [130]. A phase I dose-escalation study of dasatinib in combination with capecitabine, oxaliplatin, and bevacizumab as first-line therapy in CRC identified a well-tolerated dose recommended for a phase II dose/schedule [131]. Recently reported was the premature termination of a phase II study of dasatinib in previously treated mCRC due to lack of efficacy [132]. A preclinical study showed that dasatinib sensitizes KRAS mutant CRC cells to cetuximab in vitro and in vivo [133]. Currently, a phase I study is recruiting patients for dasatinib and cetuximab as single agents or in combination for patients with CRC and resectable liver metastasis [134].

Harnessing the Immune System

Epithelial Cell Adhesion Molecule (EpCAM)

Catumaxomab is a trifunctional monoclonal antibody that recruits and activates different immune effector cells at the surface of tumor cells. Tripartite binding is accomplished by paratopes against CD3 to allow for binding to T-cells, an anti-EpCAM paratope to target tumor cells, and the Fc domain that is bound by Fc-receptor I-, IIa-, and III-positive antigen-presenting cells [135–137]. Catumaxomab has been studied in patients with malignant ascites due to peritoneal carcinomatosis. In one study of eight patients with peritoneal carcinomatosis of solid tumors including breast, ovarian, gastric, and one adenocarcinoma of unknown primary, patients were treated with intraperitoneal injections of either catumaxomab alone (4/8), another trifunctional antibody rexomun that targets Her2 instead of EpCAM alone (1/8), or a combination of the two antibodies (3/8). The therapy was found to be both well tolerated and clinically effective [138]. A phase I/II study of 23 women with malignant ascites due to ovarian cancer, showed that intraperitoneal administration of catumaxomab effectively induced tumor cell destruction, decreased ascites production, and reduced the necessity for repeat paracentesis [139]. In a recent study of patients with peritoneal carcinomatosis secondary to colon cancer, catumaxomab alone or in combination with chemotherapy was evaluated in comparison to cytoreductive surgery and hyperthermic chemoperfusion (HIPEC) with or without systemic chemotherapy and systemic chemotherapy alone. Their findings, which have been presented in abstract form, showed that catumaxomab had a preventative effect on the accumulation of malignant ascites, the development of intestinal obstruction, and conferred a survival benefit when compared to systemic chemotherapy alone [140]. A survival advantage when compared to paracentesis alone was shown in a recent study of catumaxomab in patients with malignant ascites due to GI cancers including colon, esophageal, pancreatic, gastric, and rectal cancers [141].

Toll-Like Receptor 9 (TLR9)

Toll-like receptors (TLR) are a family of specialized immune receptors that recognize pathogen-expressed molecules and elicit an immune response upon such pattern recognition. Each member of this family can detect one or more distinct pathogen-expressed molecules [142]. TLR 9 is exclusively expressed in human immune cells, B cells, and plasmacytoid dendritic cells. TLR9 detects unmethylated CpG dinucleotides, which are prevalent in bacterial and viral genomic DNAs but are uncommon in vertebrate genomes. TRL9 is stimulated by introducing synthetic oligodeoxynucleotides that contain unmethylated CpG dinucleotides [143]. Hence the novel idea of activating immune cells that express TLR9 was proposed in cancer therapy to enhance antigen-specific $CD4^+$ and $CD8^+$ T cells. Increased numbers of T cells with higher avidity are required in vivo as ineffective T cell triggering leads to much lower numbers of T cells that are less active killers and might tolerate the tumor [144].

Two types of TLR9 agonists were assessed in an in vitro study of CRC using a traditional CpG oligonucleotide and an immunomodulatory oligonucleotide [145]. This study showed that TLR agonists have antitumor activity regardless of p53, are cytotoxic in CRC cell lines, and synergize with radiation and chemotherapy. When TLR9 agonists were added to cetuximab or gefitinib, a small molecule EGFR inhibitor, the combination showed synergistic inhibition of tumor growth, downstream signaling proteins, and angiogenesis in colon cancer xenografts. The combination decreased resistance to cetuximab as well as to other EGFR inhibitors by decreasing the aberrant expression of downstream signaling proteins [146].

A33

The A33 antigen is a glycoprotein that was sequenced and cloned because of its significantly elevated expression in the epithelia of the lower GI tract in mCRC. A study conducted in 1996 found that 95% of mCRC had increased expression of the A33 antigen [147]. Although much has been learned about the antigen itself, its exact function remains unclear. A33 is a cell surface protein that appears to be internalized into cytoplasmic vesicles as determined by fluorescence microscopy [148]. A monoclonal humanized antibody against A33 was developed and was promising in preclinical studies targeting CRC cells and subsequent lysis with high expression of surface A33 [149]. A phase I trial of the A33 antibody was performed with eleven patients with advanced, chemotherapy-resistant CRC patients. Unfortunately, eight of the patients developed toxicity secondary to human anti-human antibody (HAHA) response. Of the three patients who tolerated the therapy, one achieved a partial response seen radiographically along with a significant reduction of carcinoembryonic antigen (CEA). Four of eleven patients had disease stabilization from 2 to 12 months with two cases having significant reduction in CEA [150]. Although the results from this phase I trial are promising, the significant toxicity of the antibody has limited its clinical use.