Recent Therapeutic Advances in the Treatment of Colorectal Cancer

Kristen K. Ciombor, Christina Wu, and Richard M. Goldberg

The Ohio State University Comprehensive Cancer Center, Columbus, Ohio 43210; email: Kristen.Ciombor@osumc.edu, Christina.Wu@osumc.edu, Richard.Goldberg@osumc.edu

Annu. Rev. Med. 2015. 66:83–95

First published online as a Review in Advance on October 9, 2014

The *Annual Review of Medicine* is online at med.annualreviews.org

This article's doi: 10.1146/annurev-med-051513-102539

Keywords

biologics, aflibercept, regorafenib, *RAS*, aspirin, immunotherapy

Abstract

Metastatic colorectal cancer is a prevalent disease for which novel targeted therapies and biologically based combinations are under development. Cytotoxic chemotherapy doublets (FOLFOX, FOLFIRI) and triplets (FOLFOXIRI) in combination with biologics are standard regimens, and efforts are ongoing to delineate the optimal sequence for each patient based on unique underlying tumor biology. Molecular profiling of metastatic colorectal cancer (including mutational analysis for *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and others) has become increasingly important for identification of prognostic and predictive biomarkers, as well as for insights into the biology that drives the tumor. Large comprehensive analyses such as that of The Cancer Genome Atlas have provided important clues into carcinogenesis and discerned potentially druggable targets for metastatic colorectal cancer. Novel therapeutic agents currently under investigation for subtypes of this disease include immunotherapies such as anti–programmed cell death receptor antibody, cancer stem cell inhibitors, targeted combinations such as *BRAF* and *PI3K* inhibitors, and the anti-*RAS* reovirus Reolysin®.

INTRODUCTION

Colorectal cancer (CRC) ranks as the fourth most common cancer in the United States and one of the most widespread cancers worldwide. There will be an estimated 136,830 new cases diagnosed in the United States in 2014 and an estimated 50,310 deaths (1). Screening practices with colonoscopies have been successful in identifying early CRC, enabling more patients to be candidates for curative therapies. However, >25% of patients are diagnosed with metastatic disease (2). There has been great progress in CRC therapy in the past five years, largely due to improvement in our understanding of carcinogenesis pathways from The Cancer Genome Atlas project and other efforts, discovery of new predictive biomarkers from clinical studies, and novel targeted agents introduced into clinical practice. Emerging data confirm that CRC, despite similar histology across cases, is a heterogeneous group of tumors that can be subdivided by their molecular features and treated differently; e.g., microsatellite instable (MSI) versus microsatellite stable (MSS), *RAS* mutated versus wildtype, *BRAF* mutated versus wildtype, and phosphatidylinositol-4,5-bisphosphate 3-kinase (*PI3K*) mutated versus wildtype. Clinical trials are now selecting for specific tumor features and targeting these tumors with novel agents and combinations.

CYTOTOXIC CHEMOTHERAPY FOR METASTATIC COLORECTAL CANCER (MCRC)

The survival for patients with untreated metastatic CRC (mCRC) is approximately six months, but overall survival is improved to greater than two years with the combination of cytotoxic chemotherapy and biologic agents. The backbone of chemotherapy in CRC is 5-fluorouracil (5-FU), a pyrimidine analog that disrupts DNA and RNA synthesis (**Table 1**). When 5-FU is administered as an intravenous bolus, it is given with leucovorin, a folate analog that stabilizes thymidylate synthetase and enhances the cytotoxicity of 5-FU. 5-FU is active as a single agent, but its efficacy and patients' survival are improved in the metastatic setting when it is combined with oxaliplatin, a platinum derivative and alkylating agent, or with irinotecan, a topoisomerase I inhibitor that affects DNA repair (**Table 1**). The 5-FU and irinotecan combination IFL has been shown to improve response rate (49% versus 31%, $p < 0.001$ for evaluable patients; 35% versus 22%, $p < 0.005$ by intention to treat) and median overall survival (17.4 versus 14.1 months, $p = 0.031$) when compared to 5-FU alone (3–5). The 5-FU and oxaliplatin combination FOLFOX has shown an improvement in median progression-free survival (9.0 versus 6.2 months; $p = 0.0003$)

Table 1 FDA-approved chemotherapy and biologic agents in the treatment of metastatic colorectal cancer

Therapeutic agent	Mechanism of action
5-Fluorouracil	Pyrimidine analog
Oxaliplatin	Platinum derivative, alkylating agent
Irinotecan	Topoisomerase I inhibitor
Regorafenib	Tyrosine kinase inhibitor of VEGFR1–3, TIE2, others
Bevacizumab	Monoclonal antibody to VEGF-A
Aflibercept	Recombinant protein, decoy receptor for VEGF-A, VEGF-B, and PlGF
Cetuximab	Monoclonal antibody to EGFR
Panitumumab	Monoclonal antibody to EGFR

Abbreviations: EGFR, epidermal growth factor receptor, FDA, US Food and Drug Administration; TIE2, tyrosine kinase with immunoglobulin and epidermal growth factor homology domain 2; VEGF, vascular endothelial growth factor.

and response rate (50.7% versus 22.3%; $p = 0.0001$), but not overall survival, when compared to 5-FU alone (6, 7). Clinical trials have optimized the administration of 5-FU (continuous infusion versus bolus, every two weeks versus weekly) with oxaliplatin and irinotecan. In addition, studies have shown that administering FOLFOX before FOLFIRI and vice versa show equivalent results provided patients receive all active agents during their treatment (8). In selected patients who have symptomatic tumor burden or need tumor reduction to be surgical candidates, a feasible treatment option may be a combination of all three agents (FOLFOXIRI). There is evidence for improved response rate and survival but at the cost of increased toxicity, including neurotoxicity and neutropenia. In a phase III clinical trial, patients were randomized to an irinotecan and 5-FU regimen versus FOLFOXIRI, and the response rate was 34% versus 60% ($p < 0.0001$); a higher R0 resection rate of metastases was seen in the FOLFOXIRI arm (6% versus 15%, $p = 0.033$) (9). Overall survival was improved with the triplet combination therapy arm [16.7 versus 22.6 months, hazard ratio (HR) 0.70, $p = 0.032$].

BIOLOGIC THERAPY IN COMBINATION WITH FIRST-LINE CYTOTOXIC CHEMOTHERAPY IN MCRC

The optimal role of biologics in the treatment of mCRC has yet to be defined, but recent clinical trials have shed light on the efficacy of such agents in various settings. In the first-line setting, both the vascular endothelial growth factor (VEGF)-A-targeted antibody bevacizumab and the epidermal growth factor receptor (EGFR)-targeted antibodies cetuximab and panitumumab have clear efficacy when combined with particular cytotoxic chemotherapeutic regimens, the latter group in *KRAS*-wildtype disease only (**Table 1**, **Figure 1**) (10–17). It was unclear, however, whether the addition of anti-EGFR therapy or the anti-VEGF therapy in combination with cytotoxic chemotherapy is a superior strategy.

The multicenter, phase III FIRE-3 trial randomized 592 patients with *KRAS*-wildtype mCRC to first-line FOLFIRI (5-FU bolus 400 mg/m^2, folinic acid 400 mg/m^2, irinotecan 180 mg/m^2, 5-FU infusion 2,400 mg/m^2 over 46 h) every two weeks plus either cetuximab (400 mg/m^2 on day 1, followed by 250 mg/m^2 weekly) or bevacizumab (5 mg/kg every two weeks) (18). The primary endpoint of overall response rate was similar between the cetuximab and bevacizumab groups in an intention-to-treat analysis (62.0% versus 58.0%, OR 1.18, 95% CI 0.85–1.64, $p = 0.183$), but the cetuximab arm demonstrated an improved overall response rate in patients assessable for efficacy (72.2% versus 63.1%, OR 1.52, 95% CI 1.05–2.19, $p = 0.017$). Median progression-free survival was not different between the cetuximab and bevacizumab groups (10.0 versus 10.3 months, HR 1.06, 95% CI 0.88–1.26, $p = 0.547$). Interestingly, however, the cetuximab arm demonstrated an overall survival advantage over the bevacizumab arm (28.7 versus 25.0 months, HR 0.77, 95% CI 0.62–0.96, $p = 0.017$). It is currently unclear why the cetuximab group in this trial had improved overall survival without progression-free survival improvement, and this is an active area of investigation. Potentially the explanation relates to the patient's drug treatment after transition off of first-line therapy.

In a similar manner, the phase III CALGB/SWOG 80405 trial evaluated the efficacy of the addition of cetuximab (400 mg/m^2 on day 1, followed by 250 mg/m^2 weekly) or bevacizumab (5 mg/kg every two weeks) to FOLFIRI (26.6% of overall patients analyzed) or mFOLFOX6 (73.4% of overall patients analyzed) in the treatment of patients with *KRAS*-wildtype mCRC (19, 20). Although initial accrual did not specify *KRAS* status, and a third treatment arm added cetuximab and bevacizumab to the cytotoxic regimens, this was amended to include only KRAS codons 12/13 wildtype patients, and the dual-biologic arm was stopped. In this study, the efficacy-futility boundary was crossed in early 2014. The primary endpoint of overall survival was similar

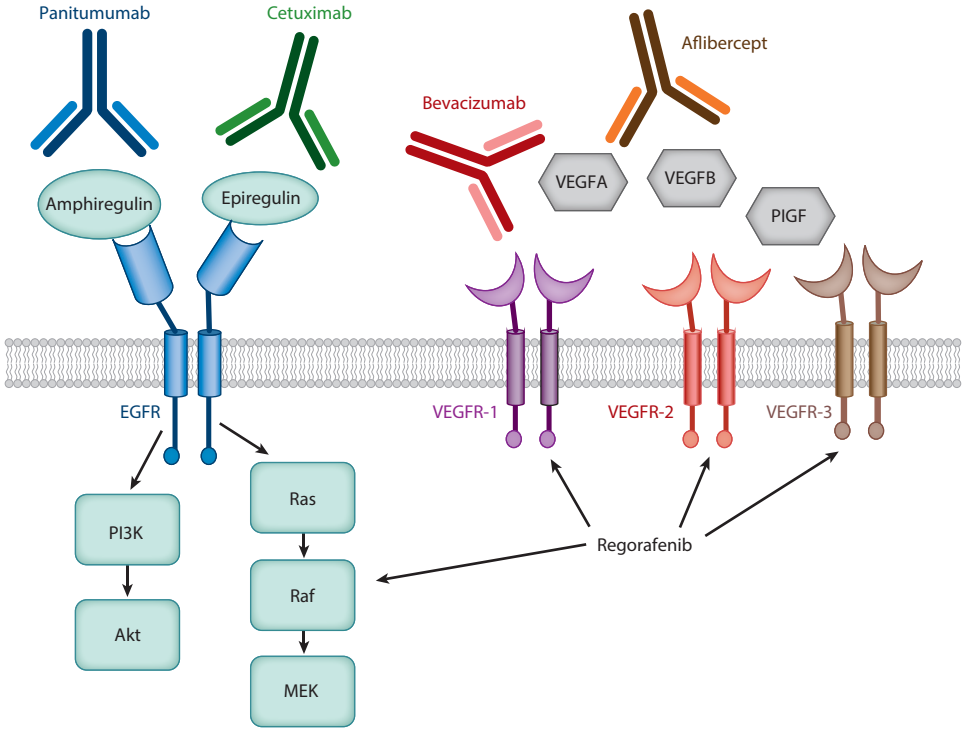

Figure 1

Signaling pathways targeted by biologic agents. Abbreviations: EGFR, epidermal growth factor receptor; MEK, mitogen-activated protein kinase kinase; PlGF, placental growth factor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

between the cetuximab and bevacizumab arms (29.9 versus 29.0 months, HR 0.925, 95% CI 0.78–1.09, p = 0.34). Median progression-free survival was also similar between groups (10.4 versus 10.8 months, HR 1.04, 95% CI 0.91–1.17, p = 0.55). Further analysis is under way to determine whether expanded RAS testing will alter these outcomes, particularly in the cetuximab treatment arm. At the present time, however, the results of these two studies suggest that either bevacizumab or cetuximab in combination with cytotoxic chemotherapy is reasonable to consider in the first-line mCRC setting.

BIOLOGIC AGENTS AFTER FIRST-LINE CHEMOTHERAPY IN MCRC

In addition to its benefit in combination with chemotherapy in the first-line setting, the anti-VEGF agent bevacizumab is now known to be effective in second-line treatment, after progression of first-line treatment with bevacizumab. The phase III ML18137 trial randomized 820 patients to chemotherapy alone or in combination with bevacizumab, and the addition of bevacizumab improved overall survival (11.2 versus 9.8 months, HR 0.81, 95% CI 0.59–0.94, p = 0.0062) (21). Another biologic agent that targets the VEGF pathway is aflibercept, a recombinant protein with VEGF receptors 1 and 2 that targets VEGF-A, VEGF-B, and placental growth factor (PlGF) (**Table 1**, **Figure 1**). In the phase III VELOUR trial, 1,227 patients who had progressed on prior oxaliplatin-based therapy were randomized to FOLFIRI with or without aflibercept. Patients

who received aflibercept had an improved median overall survival (13.50 versus 12.06 months, HR 0.817, 95.34% CI 0.713–0.937, p = 0.0032) (22). Subgroup analysis of patients who received FOLFIRI and aflibercept showed that patients who had received prior bevacizumab in first-line therapy still benefited from aflibercept in the second-line setting (23). Both bevacizumab and aflibercept are suitable biologic agents that can be used in combination with second-line chemotherapy, and thus far there have not been any clinical trials to compare their efficacy.

The US Food and Drug Administration approved the novel agent regorafenib for chemotherapy-refractory mCRC in 2013. Regorafenib is an oral tyrosine kinase inhibitor that has multiple targets, including VEGF receptors 1, 2, and 3 (**Table 1**, **Figure 1**). In the phase IIII CORRECT trial, 760 patients were randomized to best supportive care (BSC) and placebo or BSC and regorafenib. Regorafenib and BSC provided patients with an improved overall survival of 6.4 versus 5 months (HR 0.77, 95% CI 0.64–0.94, one-sided p = 0.0052) (24). Interestingly, in this trial, stable disease and not tumor shrinkage was observed. Thus, analyses of serum and tumor samples collected from the patients on the trial have been undertaken in an attempt to find predictive biomarkers for this agent. Analysis of plasma biomarkers showed that high baseline IL-8 and PlGF were poor prognostic markers, but no clinically useful predictive biomarkers have yet been identified (25). Tumor samples have been tested for *KRAS*, *PIK3CA*, and *BRAF* mutations, but regorafenib was associated with clinical benefit with all subgroups (26).

MAINTENANCE BIOLOGIC THERAPY IN THE TREATMENT OF MCRC

Another setting in which the use of biologics is currently undefined is in maintenance chemotherapy (less intensive treatment meant to maintain a response after more intensive therapy) in the treatment of mCRC. Given the increasing survival of mCRC patients with improvements in chemotherapeutic agents, the question of maintenance therapy is an important one both for patient survival and for quality of life. The phase III CAIRO3 study randomized 558 mCRC patients with stable disease or better after induction chemotherapy with CAPOX-B (capecitabine 1,000 mg/m^2 twice daily days 1–14, oxaliplatin 130 mg/m^2 day 1, bevacizumab 7.5 mg/kg day 1) every three weeks for six cycles to either observation or maintenance treatment with capecitabine 625 mg/m^2 b.i.d. continuously and bevacizumab 7.5 mg/kg day 1 every three weeks (27, 28). Upon progression of disease, patients were reintroduced to CAPOX-B. The time from randomization to first progression, or PFS1, was superior in the maintenance arm as expected (8.5 versus 4.1 months, HR 0.44, 95% CI 0.37–0.53, p < 0.0001). The primary endpoint of PFS2, the time from randomization after induction chemotherapy to progression upon reintroduction of CAPOX-B, was also superior in the maintenance arm (11.5 versus 10.5 months, HR 0.81, 95% CI 0.67–0.98, p = 0.03); however, fewer patients in the maintenance arm received CAPOX-B reintroduction after first disease progression (47% versus 75%). Time to second progression of disease, or time from randomization to progression on any treatment given after PFS1 (TT2PD), was also superior in the maintenance arm (18.7 versus 14.1 months, HR 0.67, 95% CI 0.56–0.82, p < 0.0001), as was overall survival, although the latter was not statistically significant (21.7 versus 18.0 months, HR 0.87, 95% CI 0.71–1.06, p = 0.16). Overall quality of life was not significantly different between the two treatment arms. This study confirmed that maintenance therapy is a reasonable approach when compared to a chemotherapy holiday after induction chemotherapy, although no survival benefit was seen. Furthermore, the decision to pursue maintenance therapy or observation versus continuation of doublet chemotherapy plus a biologic is dependent on patient preference, treatment toxicity, disease burden, and the tempo of disease progression.

BEYOND *KRAS* EXON 2: RAS AS A PREDICTIVE BIOMARKER IN ANTI-EGFR THERAPY

A significant obstacle to further improvement in mCRC patient survival remains our inability to accurately predict which patients are going to benefit from particular chemotherapy or biologic regimens. This is especially challenging with respect to the biologic agents. For example, despite many attempts, predictive biomarkers for response to the well-known biologic bevacizumab are still lacking (29). Discovery of predictive markers for the anti-EGFR agents has been more successful; activating mutations in *KRAS* exon 2 (codons 12 and 13) are known to predict lack of response in patients treated with cetuximab and panitumumab (30, 31). Although *KRAS*-wildtype CRC is a necessary criterion for use of anti-EGFR agents, however, it is not sufficient to predict efficacy. In fact, response rates to anti-EGFR therapies for patients with *KRAS* exon 2 wildtype CRC are generally below 40% (30, 31). As a result, predictive biomarker discovery beyond *KRAS* exon 2 has been an important research aim of many investigators.

In a biomarker analysis of the PRIME trial, additional *RAS* mutations were found to predict lack of response to anti-EGFR therapy (32). In this study, tumors were tested for mutations in *KRAS* exons 2, 3, and 4, *NRAS* exons 2, 3, and 4, and *BRAF* exon 15. A total of 1,060 patients, or 90% of all randomized patients in PRIME, had their *RAS* status ascertained; 512, or 48%, had no *RAS* mutations. A total of 108 patients (17%) with nonmutated *KRAS* exon 2 had other *RAS* mutations. Of 619 patients without *KRAS* exon 2 mutations who could be evaluated for *BRAF*, 53 (9%) had *BRAF* V600E mutations. Interestingly, patients without *RAS* mutations treated with panitumumab plus FOLFOX4 had longer progression-free survival (10.1 versus 7.9 months, HR 0.72, 95% CI 0.58–0.90, $p = 0.004$) and overall survival (26.0 versus 20.2 months, HR 0.78, 95% CI 0.62–0.99, $p = 0.04$) than those treated with FOLFOX alone. Treatment effects differed between the subgroups of patients without *RAS* mutations and those without *KRAS* exon 2 mutations but with other RAS mutations. Such differences suggested that *RAS* mutations as a whole were negative predictive factors. Furthermore, there were no significant survival differences between treatment arms in patients with *BRAF* mutations, although *BRAF* mutations were associated with a poorer prognosis overall.

In a similar mutational analysis of the FIRE-3 study, the effect of *RAS* mutations on survival was tested in patients receiving FOLFIRI plus either cetuximab or bevacizumab (33). Of a total of 592 *KRAS* exon 2 wildtype patients, sequencing of all *RAS* mutations (*KRAS* 2, 3, 4; *NRAS* 2, 3, 4) was performed on 396 tumors. Overall response rate among all-*RAS* wildtype patients was not different between the cetuximab and bevacizumab arms (65.5% versus 59.6%, $p = 0.32$); however, median overall survival was improved for all-*RAS* wildtype patients treated with FOLFIRI plus cetuximab when compared with FOLFIRI plus bevacizumab (33.1 versus 25.6 months, $p = 0.011$). No difference in overall response rate (63.2% versus 42.9%, $p = 0.167$) or overall survival (12.9 versus 11.0 months, $p = 0.448$) between treatment arms was seen in *KRAS* exon 2 wildtype patients whose tumors harbored a *BRAF* V600E mutation.

As a result of these intriguing studies and others, we believe that it is now necessary to expand mutational testing in mCRC beyond *KRAS* exon 2 and that we will in the future need to plan for testing of as-yet-undiscovered biomarkers. A great deal of genomic investigation and discovery are needed for advancement in mCRC treatment and improved outcomes for patients.

ASPIRIN USE AND MOLECULAR PROFILING OF COLORECTAL CANCER

In addition to the recent emphasis of predictive biomarker discovery for chemotherapeutic agents in CRC, evidence is accumulating for the specific use of nonsteroidal anti-inflammatory drugs

such as aspirin for prevention of premalignant polyps, CRC onset and recurrence. Several randomized clinical trials have shown that aspirin use reduces the risk of CRC, particularly in patients with Lynch syndrome (34–36). This benefit has now been investigated by molecular profiling in several subsets of CRC patients. In a large observational study, aspirin use was associated with improved CRC-specific and overall survival in patients with *PIK3CA*-mutant CRC, but not *PIK3CA*-wildtype CRC (37). In an analysis from the VICTOR trial, regular aspirin use after CRC diagnosis was associated with a reduced rate of CRC recurrence in patients with *PIK3CA*-mutant CRC (38). A more recent, larger study showed that in metastatic *PIK3CA*-mutant CRC patients, regular aspirin use was significantly associated with improved overall and cancer-specific survival, but this survival advantage was not seen across cancer stages (39). The underlying mechanism behind the benefit of aspirin in this setting is unclear. *PI3K/AKT* activates *NF-κB* and *Wnt* signaling, and aspirin may work by inhibiting *NF-κB* (40), the *Wnt/β-catenin* pathway (41), *mTOR* (42), *AKT*, or *PDK1* (43); therefore, it has been hypothesized that patients with *PIK3CA*-mutant CRC may disproportionally benefit from this mechanism.

Other potential biomarkers for the efficacy of aspirin in the prevention of cancer or its recurrence, both positive and negative, have also been investigated. Regular aspirin use was recently associated with lower *BRAF*-wildtype cancer risk, irrespective of *PIK3CA* or *KRAS* mutation, but not with lower risk of *BRAF*-mutant cancer (44). This may be partially explained by the fact that *BRAF*-mutant colon tumor cells are less sensitive to the effect of aspirin owing to upregulation of the *MAPK* pathway (44). In another study, aspirin use after a colon cancer diagnosis was associated with improved survival if the tumors expressed the human leukocyte antigen (HLA) class I antigen (45). Finally, regular aspirin use in the Nurses' Health Study and the Health Professionals Follow-Up Study was associated with a lower risk of CRC that developed within a background of colonic mucosa with high hydroxyprostaglandin dehydrogenase-15(nicotinamide adenine dinucleotide) (15-PGDH) expression (46). These intriguing findings provide a basis for further investigation and validation of various predictive biomarkers in the development and recurrence of CRC.

THE CANCER GENOME ATLAS AND COLORECTAL CANCER

In addition to biomarker discovery, a concerted effort to characterize the molecular genetics of CRC to better understand its underlying biological mechanisms was published in 2012. The Cancer Genome Atlas Network conducted a comprehensive integrative analysis of 224 CRC samples (47). Tumor samples were divided into nonhypermutated and hypermutated cancers, which were differentiated by degree of microsatellite instability and mutations in the DNA mismatch repair pathway. The hypermutated and nonhypermutated tumors, respectively, were found to have 15 and 17 somatic recurrently mutated and expressed genes. In the nonhypermutated group, these included *APC*, *TP53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, *TCF7L2*, *NRAS*, *CTNNB1*, *SMAD2*, *FAM123B*, and *SOX9*, among others. In the hypermutated group, these frequently mutated genes included *ACVR2A*, *APC*, *TGFBR2*, *MSH3*, *MSH6*, *SLC9A9*, *TCF7L2* and *BRAF*. The differences in mutational patterns and frequencies seen among these groups are intriguing and warrant further investigation to understand more clearly the biological sequence of genetic events in these varying tumors.

The analysis by The Cancer Genome Atlas also contributed to our understanding of altered pathways in CRC that may be helpful in our attempts to develop new therapeutics. Whole-exome sequencing and other integrative analysis of this comprehensive genomic data showed that recurrent alterations in the *WNT*, *MAPK*, *PI3K*, *TGF-β*, and *p53* pathways were common. As some of these pathways are currently or potentially druggable (e.g., with *WNT* inhibitors, *β-catenin* inhibitors, and *PI3K* inhibitors), these findings are of potentially great importance to the

future of experimental therapeutics in mCRC. Analyses such as these by The Cancer Genome Atlas, as well as those incorporating clinical outcomes for further prognostic and predictive impact, hold promise for unraveling the biological underpinnings and potential targets of this disease.

NEW THERAPEUTIC AGENTS FOR MCRC

Immunotherapy in CRC

Within oncology, perhaps the most exciting current trend in developmental therapeutics is immunotherapy. Promising clinical results of the anti–cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) monoclonal antibody ipilimumab and the anti–programmed cell death 1 (PD-1) receptor antibody nivolumab, among others, have been seen in tumor types such as melanoma and non–small cell lung cancer (48). Unfortunately, immunotherapy in unselected patients with CRC has been largely disappointing thus far. However, it may be that selected CRC patients will benefit from immunotherapy, and the challenge resides in discovering predictive biomarkers for this group of patients. For example, a patient with refractory CRC treated with BMS-936558/MDX-1106, a fully human immunoglobulin G4 (IgG4)–blocking monoclonal antibody now known as nivolumab, experienced a complete response maintained even off therapy for more than three years (49, 50). Further analysis demonstrated this patient's tumor manifested high microsatellite instability (MSI), with cell surface expression of PD-L1 by infiltrating macrophages and lymphocytes. Given that immune checkpoint inhibitors work best in tumors with high mutation burdens, it is hypothesized that tumors deficient in mismatch repair such as this patient's cancer may be particularly sensitive to immunotherapy given an ongoing accumulation of mutations at a high frequency. In addition, prominent lymphocyte infiltration is commonly seen in MSI-high tumors (51), much like in melanoma, arguing that perhaps baseline tumor-infiltrating lymphocytes may be a predictive biomarker for potential immune checkpoint blockade. As a result of these intriguing data, clinical trials of anti-PD-1 agents are currently under way in patients with MSI-high colorectal tumors.

Targeting Cancer Stem Cells in mCRC

Some cancer cells are able to perpetuate tumor proliferation and metastasis, and they are identified as cancer stem cells because of their qualities of self-renewal and differentiation (52). Due to these features, they are also more resistant to chemotherapy agents. BBI608 is an oral, first-in-class cancer stem cell inhibitor that targets multiple pathways known to drive carcinogenesis, including *STAT3* and *β-catenin*. In a phase I study in advanced solid tumors, 22 CRC patients with refractory disease were treated. Eight of twelve patients had stable disease, and the median overall survival was prolonged to 47 weeks (53). A phase II clinical trial is ongoing in the United States, in which CRC patients are treated with the combination of BBI608 and an anti-EGFR antibody (cetuximab or panitumumab) or capecitabine, the oral formulation of 5-FU. It remains to be seen whether this approach of inhibiting stem cells in tumors such as CRC will be effective, but the concept is certainly intriguing.

Therapies Directed at *BRAF*-Mutant CRC

BRAF mutations have been recognized as an important process in colon carcinogenesis. *BRAF* mutations are detected in serrated adenomas that develop into colon cancers, and they have been associated with CpG methylation (54). Although only 5–10% of CRC tumors carry the mutation,

it is recognized as a biomarker of poor prognosis and indicative of a more aggressive cancer (55). *BRAF* inhibitors such as vemurafenib have been very effective in melanomas that carry the V600E *BRAF* mutation, but the same efficacy with single-agent vemurafenib has not been seen in CRC. This may be largely due to the fact that they are biologically very different diseases, despite the same *BRAF* mutation. Preclinical studies with RNA-interference-based genetic screens have shown that *BRAF* inhibition activates the EGFR pathway and that there is synergy when *BRAF* inhibitors are used in combination with anti-EGFR tyrosine kinase inhibitor or antibody (56). Therefore, ongoing clinical trials have combined *BRAF* inhibitors with an anti-EGFR antibody in CRC. In addition, proteomic analysis of *BRAF*-mutant CRC cell lines shows that the *PI3K* pathway is upregulated and that the combination of a *BRAF* and a *PI3K* inhibitor synergizes inhibition of cell growth (57). The efficacy of *BRAF* and *PI3K* inhibitors has also been demonstrated in mouse models for *BRAF*-mutant CRC (58). It will be interesting to see if dual targeting of *BRAF* and EGFR pathways or *BRAF* and *PI3K* pathways will prove to be effective in this patient population.

Targeting *KRAS*-Mutant CRC with Reolysin®

KRAS mutation is present in ~40% of mCRC patients, and these patients are not eligible for anti-EGFR therapy, as discussed above. In addition, mCRC patients with *KRAS*-mutant tumors have a worse prognosis overall than do patients with *KRAS*-wildtype tumors. Thus, these patients are in need of specific effective therapy targeting their *KRAS*-mutant disease. Reolysin®, a reovirus subtype 3 Dearing, is a naturally occurring double-stranded RNA virus that specifically infects and lyses tumors that harbor an activated *RAS* pathway (59). The advantage of Reolysin® is that a majority of adults have already been infected by the virus, and it causes only mild respiratory and gastrointestinal side effects (60). A phase I clinical trial in advanced solid tumors has shown that Reolysin® is safe and tolerable in patients (61). A woman with metastatic *RAS*-mutant breast cancer had a partial response, and biopsy of her chest wall mass showed viral replication and tumor necrosis. Phase I/II clinical trials of Reolysin® in combination with chemotherapy for patients with *RAS*-mutant tumors are under way, and these safety and efficacy data are eagerly anticipated.

CONCLUSIONS

Treatment and outcomes for patients with mCRC have been steadily improving with the introduction of novel cytotoxic and targeted agents into the therapeutic arsenal. Remaining challenges include further personalizing our regimens to individual patients given the distinct biological underpinnings of each tumor, its aggressiveness, and the overall extent of disease, as well as the performance status and preferences of the patient. Advances in molecular profiling technologies will allow us to further classify tumors into molecular subsets, but much investigational work has yet to be done to understand how best to target these tumor subsets. Meticulous pathway dissection via preclinical investigation, as well as rational and innovative clinical trial design and analysis, will be imperative for the further advancement of the treatment of patients with mCRC.

FUTURE DIRECTIONS

Future successes in the treatment of mCRC will require biologically rational drug combinations and sequences. Optimal incorporation of anti-VEGF and anti-EGFR therapies, both current and novel, as well as other pathway-driven agents, will need to be defined. Although cytotoxic chemotherapeutic agents have shown utility in this field, novel biologics and immunotherapies now hold promise for further advances. Precise molecular profiling of tumors, discovery of

additional druggable targets, and development of powerful predictive biomarkers will be critical to the successful treatment of patients with mCRC.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. National Cancer Institute Surveillance, Epidemiology, and End Results Program. SEER stat fact sheets: colon and rectum cancer. **http://seer.cancer.gov/statfacts/html/colorect.html**. Accessed May 4, 2014
2. Altekruse SF, Kosary CL, Krapcho M, et al. 2010. SEER cancer statistics review, 1975–2007, National Cancer Institute. **http://seer.cancer.gov/csr/1975_2007/**
3. Douillard JY, Cunninghan D, Roth AD, et al. 2000. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicenter randomised trial. *Lancet* 355:1041–47
4. Saltz LB, Cox JV, Blanke C, et al. 2000. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N. Engl. J. Med.* 343:905–14
5. Kohne CH, van Cutsem E, Wils J, et al. 2005. Phase III study of weekly high-dose infusional fluorouracil plus folinic acid with or without irinotecan in patients with metastatic colorectal cancer: European Organisation for Research and Treatment of Cancer Gastrointestinal Group Study 40986. *J. Clin. Oncol.* 23:4856–65
6. De Gramont A, Figer A, Seymour M, et al. 2000. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J. Clin. Oncol.* 18:2938–47
7. Giacchetti S, Perpoint B, Ziadani R, et al. 2000. Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. *J. Clin. Oncol.* 18:136–47
8. Tournigand C, Andre T, Achille E, et al. 2004. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J. Clin. Oncol.* 22:229–37
9. Falcone A, Ricci S, Brunetti I, et al. 2007. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. *J. Clin. Oncol.* 25:1670–76
10. Hurwitz H, Fehrenbacher L, Novotny W, et al. 2004. Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* 350(23):2335–42
11. Hochster HS, Hart LL, Ramanathan RK, et al. 2008. Safety and efficacy of oxaliplatin and fluoropyrimidine regimens with or without bevacizumab as first-line treatment of metastatic colorectal cancer: results of the TREE study. *J. Clin. Oncol.* 26(21):3523–29
12. Saltz LB, Clarke S, Diaz-Rubio E, et al. 2008. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J. Clin. Oncol.* 26(12):2013–19
13. Schmiegel W, Reinacher-Schick A, Arnold D, et al. 2013. Capecitabine/irinotecan or capecitabine/oxaliplatin in combination with bevacizumab is effective and safe as first-line therapy for metastatic colorectal cancer: a randomized phase II study of the AIO colorectal study group. *Ann. Oncol.* 24(6):1580–87
14. Van Cutsem E, Kohne CH, Hitre E, et al. 2009. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N. Engl. J. Med.* 360(14):1408–17
15. Van Cutsem E, Kohne CH, Lang I, et al. 2011. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J. Clin. Oncol.* 29(15):2011–19

16. Douillard JY, Siena S, Cassidy J, et al. 2010. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J. Clin. Oncol.* 28(31):4697–705
17. Douillard JY, Siena S, Tabernero J, et al. 2014. Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. *Ann. Oncol.* 25:1346–55
18. Heinemann V, von Weikersthal LF, Decker T, et al. 2013. *Randomized comparison of FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment of KRAS wild-type metastatic colorectal cancer: German AIO study KRK-0306* (*FIRE-3*). Presented at Am. Soc. Clin. Onocol. Annu. Meet., Chicago, IL, May 31–June 4
19. Venook AP, Blanke CD, Niedzwiecki D, et al. 2005. Cancer and Leukemia Group B/Southwest Oncology Group Trial 80405: a phase III trial of chemotherapy and biologic agents for patients with untreated advanced colorectal adenocarcinoma. *Clin. Colorectal Cancer* 5(4):292–94
20. Venook AP, Niedzwiecki D, Lenz HJ, et al. 2014. *CALGB/SWOG 80405: phase III trial of irinotecan/5-FU/leucovorin* (*FOLFIRI*) *or oxaliplatin/5-FU/leucovorin* (*mFOLFOX6*) *with bevacizumab or cetuximab for patients with KRAS wild-type untreated metastatic adenocarcinoma of the colon or rectum*. Presented at Am. Soc. Clin. Oncol. Annu. Meet., Chicago, IL, May 30–June 3
21. Bennouna J, Sastre J, Arnold D, et al. 2013. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. *Lancet Oncol.* 14:29–37
22. Van Cutsem E, Tabernero J, Lakomy R, et al. 2012. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J. Clin. Oncol.* 30:3499–506
23. Tabernero J, Van Cutsem, Lakomy R, et al. 2014. Aflibercept versus placebo in combination with fluorouracil, leucovorin and irinotecan in the treatment of previously treated metastatic colorectal cancer: prespecified subgroup analyses from the VELOUR trial. *Eur. J. Cancer* 50:320–31
24. Grothey A, Van Cutsem E, Sobrero A, et al. 2013. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicenter, randomised, placebo-controlled, phase 3 trial. *Lancet* 381:303–12
25. Heinz-Josef L, Van Cutsem E, Sobrero AF, et al. 2013. *Analysis of plasma protein biomarkers from the CORRECT phase III study of regorafenib for metastatic colorectal cancer*. Presented at Am. Soc. Clin. Oncol. Annu. Meet., Chicago, IL, May 30–June 3
26. Jeffers M, Van Cutsem E, Sobrero AF, et al. 2013. *Mutational analysis of biomarker samples from the CORRECT study: correlating mutation status with clinical response to regorafenib*. Presented at Am. Soc. Clin. Oncol. Gastrointestinal Cancers Symp., San Francisco, CA, Jan. 24–26
27. Koopman M, Simkins LHJ, Tije AJT, et al. 2013. *Maintenance treatment with capecitabine and bevacizumab versus observation after induction treatment with chemotherapy and bevacizumab in metastatic colorectal cancer: the phase III CAIRO3 study of the Dutch Colorectal Cancer Group*. Presented at Am. Soc. Clin. Oncol. Annu. Meet., Chicago, IL, May 30–June 3
28. Punt CJA, Simkens LHJ, May A, et al. 2013. *Updated results including quality of life of the phase III CAIRO3 study of the Dutch Colorectal Cancer Group: maintenance treatment with capecitabine and bevacizumab versus observation after induction treatment with chemotherapy and bevacizumab in metastatic colorectal cancer*. Presented at Eur. Cancer Congr., Amsterdam, Neth., Sep. 27–Oct. 1
29. Lambrechts D, Lenz HJ, de Haas S, et al. 2013. Markers of response for the antiangiogenic agent bevacizumab. *J. Clin. Oncol.* 31(9):1219–30
30. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. 2008. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N. Engl. J. Med.* 359(17):1757–65
31. Amado RG, Wolf M, Peeters M, et al. 2008. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 26(10):1626–34
32. Douillard JY, Oliner KS, Siena S, et al. 2013. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N. Engl. J. Med.* 369(11):1023–34

33. Stintzing S, Jung A, Rossius L, et al. 2013. *Analysis of KRAS/NRAS and BRAF mutations in FIRE-3: a randomized phase III study of FOLFIRI plus cetuximab or bevacizumab as first-line treatment for wild-type KRAS* (*exon 2*) *metastatic colorectal cancer patients*. Presented at Eur. Cancer Congr., Amsterdam, Neth., Sep. 27–Oct. 1
34. Flossmann E, Rothwell PM. 2007. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet* 369(9573):1603–13
35. Rothwell PM, Wilson M, Elwin CE, et al. 2010. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* 376(9754):1741–50
36. Burn J, Gerdes AM, Macrae F, et al. 2011. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet* 378(9809):2081–87
37. Liao X, Lochhead P, Nishihara R, et al. 2012. Aspirin use, PIK3CA mutation, and colorectal-cancer survival. *N. Engl. J. Med.* 367(17):1596–606
38. Domingo E, Church DN, Sieber O, et al. 2013. Evaluation of PIK3CA mutation as a predictor of benefit from nonsteroidal anti-inflammatory drug therapy in colorectal cancer. *J. Clin. Oncol.* 31(34):4297–305
39. Kothari N, Kim RD, Gibbs P, et al. 2014. *Regular aspirin use and survival in patients with PIK3CA-mutated metastatic colorectal cancer*. Presented at Am. Soc. Clin. Oncol. Gastrointest. Cancers Symp., San Francisco, CA, Jan. 16–18
40. Williams JL, Ji P, Ouyang N, et al. 2008. NO-donating aspirin inhibits the activation of NF-kappaB in human cancer cell lines and Min mice. *Carcinogenesis* 29:390–97
41. Bos CL, Kodach LL, van den Brink GR, et al. 2006. Effect of aspirin on the Wnt/beta-catenin pathway is mediated via protein phosphatase 2A. *Oncogene* 25:6447–56
42. Din FV, Valanciute A, Houde VP, et al. 2012. Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. *Gastroenterology* 142:1504.e3–1515.e3
43. Zhu J, Huang JW, Tseng PH, et al. 2004. From the cyclooxygenase-2 inhibitor celecoxib to a novel class of 3-phosphoinositide-dependent protein kinase-1 inhibitors. *Cancer Res.* 64:4309–18
44. Nishihara R, Lochhead P, Kuchiba A, et al. 2013. Aspirin use and risk of colorectal cancer according to BRAF mutation status. *JAMA* 309(24):2563–71
45. Reimers MS, Bastiaannet E, Langley RE, et al. 2014. Expression of HLA class I antigen, aspirin use and survival after a diagnosis of colon cancer. *JAMA Intern. Med.* 174(5):732–39
46. Fink SP, Yamauchi M, Nishihara R, et al. 2014. Aspirin and the risk of colorectal cancer in relation to the expression of 15-hydroxyprostaglandin dehydrogenase. *Sci. Transl. Med.* 6:233re2
47. Cancer Genome Atlas Network. 2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 487(7407):330–37
48. Wolchok JD, Kluger H, Callahan MK, et al. 2013. Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* 369(2):122–33
49. Brahmer JR, Drake CG, Wollner I, et al. 2010. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J. Clin. Oncol.* 28(19):3167–75
50. Lipson EJ, Sharfman WH, Drake CG, et al. 2013. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. *Clin. Cancer Res.* 19:462–68
51. Smyrk TC, Watson P, Kaul K, et al. 2001. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. *Cancer* 91:2417–22
52. Lobo NA, Simono Y, Qian D, Clarke MF. 2007. The biology of cancer stem cells. *Annu. Rev. Cell Dev. Biol.* 23:675–99
53. Langleben A, Supko JG, Hotte SJ, et al. 2013. *A dose-escalation phase I study of a first-in-class cancer stemness inhibitor in patients with advanced malignancies*. Presented at Am. Soc. Clin. Oncol. Annu. Meet., Chicago, IL, May 30–June 3
54. De Roock W, Claes B, Bernasconi D, et al. 2010. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol.* 11:753–62

55. Van Cutsem E, Lang I, Folprecht G, et al. 2010. *Cetuximab plus FOLFIRI in the treatment of metastatic colorectal cancer* (*mCRC*)*: the influence of KRAS and BRAF biomarkers on outcome: updated data from the CRYSTAL trial*. Presented at Am. Soc. Clin. Oncol. Gastrointest. Cancers Symp., Orlando, FL, Jan. 22–24
56. Prahallad A, Sun C, Huang S, et al. 2012. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 483:100–3
57. Mao M, Tian F, Mariadason JM, et al. 2013. Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. *Clin. Cancer Res.* 19:657–67
58. Coffee EM, Faber AC, Roper J, et al. 2013. Concomitant BRAF and PI3K/mTOR blockade is required for effective treatment of BRAF(V600E) colorectal cancer. *Clin. Cancer Res.* 19:2688–98
59. Coffey MC, Strong JE, Forsyth PA, et al. 1998. Reovirus therapy of tumors with activated Ras pathway. *Science* 282(5392):1332–34
60. Minuk GY, Rascanin N, Paul RW, et al. 1987. Reovirus type 3 infection in patients with primary biliary cirrhosis and primary sclerosing cholangitis. *J. Hepatol.* 5(1):8–13
61. Gollamudi R, Ghalib MH, Desai KK, et al. 2010. Intravenous administration of Reolysin®, a live replication competent RNA virus is safe in patients with advanced solid tumors. *Invest. New. Drugs* 28:641–49