Novel Therapies in Development for Metastatic Colorectal Cancer

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ABSTRACT

Colorectal cancer (CRC) is the second most common cause of cancer mortality in the United States. Despite advances in therapy, metastatic CRC remains lethal, and further improvements in therapy are needed. Growing understanding of cancer biology, particularly in growth factor signaling, angiogenesis, and cancer immunology, has translated into many novel therapies under investigation. Patients are increasingly selected for clinical trials rationally on the basis of integral biomarkers. This review discusses several promising agents in development for metastatic CRC.

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Colorectal cancer (CRC) is a major cause of morbidity and mortality in the United States, the fourth most common malignancy, and the second most common cause of cancer death.[1] Therapy for metastatic (m)CRC has improved outcomes and survival starting in the middle of the first decade of the millennium,[2] attributable at least in part to the development and increase in clinical use of more effective cytotoxic chemotherapies, such as oxaliplatin and irinotecan, and of novel targeted therapies affecting angiogenesis and growth factor signaling, including bevacizumab, cetuximab, panitumumab, ziv-aflibercept, and regorafenib. Median overall survival with mCRC is now 29.0–29.9 months, as seen in the CALGB/SWOG 80405 study,[3] reflecting improvements in outcomes with the advances in treatment options for patients with mCRC. However, despite the advances in therapies, unresectable mCRC remains incurable, and novel treatments are necessary to further improve survival. There are a myriad of agents under investigation, and this review will highlight several promising drugs or drug combinations under development.

REFINEMENTS IN TARGETING EPIDERMAL GROWTH FACTOR RECEPTOR

Epidermal growth factor receptor (EGFR) is the molecular target of the monoclonal antibodies cetuximab and panitumumab and serves an important role in propagating mitogenic signals driving cell proliferation and growth. The RAS/RAF/MEK (mitogen-activated protein kinase kinase) pathway is activated upon the binding of growth factor ligands to EGFR. Consequently, activating mutations in *KRAS* or *NRAS* are predictive biomarkers of resistance to the anti-EGFR antibodies.[4–8] Although cetuximab and panitumumab improve progression-free survival (PFS) in RAS wild-type mCRC, the single-agent response rate is 13–17%, and most patients do not respond.[6,9] Thus, there is a need to improve on existing methods of targeting EGFR.

Sym004 is a mixture of 2 different anti-EGFR antibodies that target distinct epitopes in the extracellular domain of EGFR, synergistically promoting EGFR internalization and degradation.[10] A Phase I study of Sym004 enrolled 29 patients with mCRC, *KRAS* wild-type, who previously had clinical benefit from anti-EGFR therapy before progressing, and found a 3.3-month PFS and a 40% rate of any tumor shrinkage in the 17 subjects in the higher dose cohort.[11] Consequently, a randomized phase II study is ongoing, with the goal of enrolling 240 patients with anti-EGFR refractory, *KRAS* wild-type mCRC to receive either of 2 doses of Sym004 or investigator's choice of fluoropyrimidine or best supportive care, with a primary end point of overall survival (NCT02083653; descriptions of all trials are available at clinicaltrials.gov).

MEHD7945A is a human IgG1 antibody that is a dual inhibitor of EGFR and human epidermal growth factor receptor (HER)-3.[12] HER3 is a kinase-inactive coreceptor that can form heterodimers with EGFR, can bind a different range of ligands, including heregulin,[13] and is more effective at activating alternative cell signaling pathways, such as phosphatidylinositol 3-kinase (PI3K).[14] Activation of HER3 may bypass EGFR inhibition and facilitate resistance to anti-EGFR antibodies, suggesting that optimal sensitivity to EGFR inhibition also requires HER3 inhibition.[15] A Phase I study of MEHD7945A in 36 patients with refractory solid tumors included 12 patients with mCRC and found that 33% had stable disease for at least 8 weeks.[16] A randomized Phase II study is now under way, with the goal of randomizing 120 patients with mCRC, *KRAS* wild-type, to receive second-line FOLFIRI (leucovorin, 5-fluorouracil, and irinotecan)/cetuximab or FOLFIRI/MEHD7945A, with the primary end point of PFS (NCT01652482).

Combining anti-EGFR antibody therapy with a MEK inhibitor is another promising strategy for patients with *KRAS* mutations. Although *KRAS* status has traditionally been thought to be immutable,[17] recent research using more sensitive methods to

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Table 1. Results of Phase I clinical trials of BRAF inhibitors in *BRAF* mutant mCRC

Regimen	Number evaluable	Overall response rate (%)	Stable disease (%)	Disease control rate (%)
Vemurafenib [27]	21	5	30	35
Dabrafenib [26]	9	11	78	89
Dabrafenib+trametinib [33]	43	12	51	63
Dabrafenib+panitumumab [31]	15	13	73	87
Vemurafenib+cetuximab [32]	11	0	36	36
Encorafenib+cetuximab [30]	24	29	50	79
Encorafenib+cetuximab+BYL719 [30]	20	30	60	90
Dabrafenib+panitumumab+trametinib [31]	15	40	40	80
Vemurafenib+cetuximab+irinotecan [34]	8	50	50	100

detect the presence of *KRAS* mutations in circulating free tumor DNA has shown that there are increasing quantities of *KRAS* or *NRAS* mutant tumor DNA detectable upon treatment with anti-EGFR antibodies, rising more markedly soon before radiographic and clinical progression.[18,19] The combination of anti-EGFR antibody therapy and MEK inhibitor is undergoing investigation both in patients with acquired *KRAS* mutations after prior therapy with anti-EGFR therapy and in patients with de novo *KRAS* mutations. Early clinical trials testing MEK inhibitor monotherapies in mCRC proved to be ineffective.[20,21] However, the combination of anti-EGFR antibody and MEK inhibitor more effectively blocked reactivation of MEK and the downstream mitogen-activated protein kinase (MAPK) in vitro in cetuximab-resistant CRC cells with acquired RAS mutations, and caused tumor regressions in vivo in cell line xenografts and in patient-derived xenografts.[22,23] Thus, the clinical efficacy of a similar approach is also being investigated.

A Phase Ib/II basket trial has been initiated with a goal of treating 90 patients with mCRC with panitumumab and the MEK inhibitor MEK162 (binimetinib) (NCT01927341). This study will enroll 4 cohorts of patients with mCRC: 1) de novo RAS mutant without previous anti-EGFR antibody therapy; 2) RAS mutant acquired after prior anti-EGFR antibody therapy; 3) RAS wild-type after prior anti-EGFR antibody therapy; and 4) RAS wild-type without prior anti-EGFR antibody therapy. The primary end point for the Phase II portion of the trial is overall response rate. A Phase Ib study is also ongoing to combine MEHD7945A and the MEK inhibitor cobimetinib (GDC-0973) in *KRAS* mutant solid tumors, with a plan for an expansion cohort in mCRC with either acquired or de novo *KRAS* mutations (NCT01986166). These ongoing trials have capitalized on our growing understanding of innate and acquired resistance mechanisms to biologic targeted therapies and will be an important step in refining inhibition of mitogenic growth factor signaling.

COMBINATION THERAPIES IN *BRAF* MUTANT mCRC

Mutation in *BRAF* codon 600 is found in 8–10% of CRCs[24] and is a poor prognostic biomarker. Pooled analysis from 2 studies that randomized patients with mCRC, *KRAS* wild-type, to chemotherapy or chemotherapy with cetuximab found inferior survival in the *BRAF* mutant cohorts, with a median overall survival with chemotherapy alone of 9.9 months compared to 21.1 months in patients with *BRAF* wild-type and median overall survival with chemotherapy and cetuximab of 14.1 months compared to 24.8 months in those with *BRAF* wild-type.[25] Though the addition of cetuximab was associated with a nonsignificant trend toward improved survival in the *BRAF* mutant cohort (hazard ratio (HR), 0.62; 95% CI, 0.36–1.06; $P = .076$), the presence of the *BRAF* mutation is nevertheless a powerful, poor prognostic biomarker, and improvements in therapy are sorely needed for these patients.

Though the development of novel mutant BRAF inhibitors like vemurafenib and dabrafenib spurred several studies, early-phase clinical trials of BRAF inhibitor monotherapy in *BRAF* mutant mCRC yielded only a 5–11% response rate.[26,27] Further preclinical research to determine mechanisms of resistance explaining this poor response rate found that the inhibition of mutant *BRAF* rapidly causes feedback activation of EGFR, allowing for reactivation of the RAS/RAF/MEK pathway and also of other pathways such as the PI3K pathway.[28,29] Consequently, the combination of anti-EGFR antibodies and BRAF inhibitors, potentially in addition to a third agent, is being actively investigated, with encouraging Phase I data hinting at efficacy (Table 1).

Several Phase I protocols have investigated the combination of anti-EGFR antibodies with a BRAF inhibitor such as vemurafenib, dabrafenib, or the investigational agent encorafenib, but the addition of a third agent improved response rates. The response rate with anti-EGFR and MEK inhibitor doublets has been as high as 30%.[30–32] In addition, the combination of BRAF inhibitor and MEK inhibitor has been studied in a Phase I expansion cohort of dabrafenib and trametinib in *BRAF* mutant mCRCs, with a response rate of 12%.[33] However, the addition of a third agent to the anti-EGFR antibody and MEK inhibitor further improves response rate to 30–50% in Phase I trials.[30,31,34] A Phase II study is continuing after the initial Phase I study investigating encorafenib, cetuximab, and the PI3K inhibitor BYL719, with the primary end point of PFS (NCT01719380). A randomized Phase II study has started to enroll patients to receive dabrafenib/panitumumab, dabrafenib/panitumumab/trametinib, or a chemotherapy comparator (NCT01750918). The UK Medical Research Council (MRC) is

commencing an umbrella trial, FOCUS4, for patients with mCRC who have stable or responding disease with standard chemotherapy after 16 weeks and will test whether maintenance therapy with standard chemotherapy, dabrafenib/panitumumab, or dabrafenib/panitumumab/trametinib will yield superior PFS in the *BRAF* mutant mCRC cohort. Finally, a randomized Phase II study, SWOG S1406, is ongoing to randomize 78 *BRAF* mutant mCRC patients to cetuximab/irinotecan or to vemurafenib/cetuximab/irinotecan, with a primary end point of PFS (NCT02164916). In sum, there are exciting, promising trials planned for *BRAF* mutant mCRC.

IMMUNOTHERAPY IN mCRC

Harnessing the ability of the immune system to mount a cytotoxic immune response against malignant cells is a strategy that may provide durable control of certain metastatic cancers. Cytotoxic T-lymphocyte antigen (CTLA)-4 is a negative regulator of the T-cell immune response, which is normally necessary to prevent autoimmunity.[35] Programmed cell death (PD)-1 is another negative regulator of T-cell response that may bind its ligand, programmed death-ligand 1 (PD-L1), which is often aberrantly expressed on tumor cells to evade immune detection.[36] Antibodies against CTLA-4, such as ipilimumab, and against PD-1, such as nivolumab or pembrolizumab, allow for increased cytotoxic T-cell activity, and many such agents were found to provide durable disease control in a sizable minority of patients with metastatic melanoma.[37–41] These drugs are now under investigation in several other malignancies. Whereas biomarkers predicting for response to the checkpoint inhibitors are not yet known, it is known that mutated proteins in tumors produce novel neoantigens that are presented to and targeted by the immune system.[42]

To date, there is little evidence of efficacy in unselected populations of patients with mCRC, but there are rare but tantalizing isolated cases of prolonged disease response with immunotherapy. A Phase II study of tremelimumab in mCRC showed responses in 1of 45 patients, with time to disease progression of 15 months in the patient who responded.[43] In a Phase I study of the anti-PD-L1 antibody BMS-936559, 0 of 18 patients responded.[44] A pilot Phase I study of the anti-PD-1 antibody nivolumab (BMS-936558/MDX-1106) showed 1 of 14 responses in mCRC, although the patient who responded had a durable complete response lasting for more than 21 months and had high microsatellite instability (MSI-H).[45] A subsequent larger Phase I study of nivolumab revealed that 0 of 19 mCRC patients responded.[46] Thus, even though responses were uncommon, they yielded a prolonged duration of PFS.

Further studies suggest that MSI-H is a predictive biomarker for response to immune checkpoint inhibition. CRCs with MSI-H have a high frequency of frameshift mutations, are associated with a higher number of tumor-infiltrating lymphocytes,[47] and thus may be more likely to respond to immunotherapy. The presence of PD-L1 expression on cancer cells is associated with response to nivolumab,[46,48] but only 1 in 8 unselected CRC tumor specimens in an early trial of nivolumab expressed PD-L1.[48] However, immunohistochemistry on 87 CRC samples showed that tumor cell PD-L1 was significantly more frequent in MSI-H CRCs than in microsatellite stable (MSS) CRCs (38% vs. 13%), and tumor infiltrating lymphocytes more frequently expressed PD-1 in MSI-H CRCs than in MSS CRCs (77% vs. 39%).[49] Further observation shows that PD-L1 is actually more commonly noted in tumor immune infiltrates (4/8 CRC tumors in an early nivolumab trial),[48] and MSI-H tumors, while having higher PD-L1 expression than MSS tumors, showed most of the PD-L1 expression in myeloid cells.[50] These MSI-H tumors have an immune microenvironment with a strong Th1 and cytotoxic T-lymphocyte component, unlike MSS tumors, and the high expression of inhibitory immune checkpoint receptors in MSI-H tumors demonstrates that these cancers are capable of evading the immune system.[50] Thus, whereas immunotherapy is unlikely to provide benefit to most patients with mCRC who have MSS tumors, patients with MSI-H mCRC may be more likely to respond, potentially with a durable response.

Several Phase II clinical trials are in development investigating immunotherapy in MSI-H mCRC. The PD-1 inhibitor pembrolizumab (MK-3475) is being investigated in a Phase II basket trial with an enrollment goal of 71 patients with MSI-H mCRC, MSS mCRC, or MSI-H non-CRC (NCT01876511). The CheckMate 142 trial is a Phase I/II trial of nivolumab monotherapy and of nivolumab with ipilimumab combination therapy in mCRC, with efficacy cohorts restricted to MSI-H patients (NCT02060188). The possibility of producing a long-lasting response with immunotherapy, even if in only a minority of patients, is exciting and merits further investigation.

TOLL-LIKE RECEPTOR AGONISTS IN CRC

The Toll-like receptor (TLR) family of transmembrane receptors transduce signals from inflammatory ligands, which may be derived from pathogens or from necrotic cells.[51] TLR9 is a member of the TLR family whose ligand is free DNA, and activation of TLR9 on plasmacytoid dendritic cells and B cells may provoke innate and acquired immune responses against tumor cells.[52]

Clinical trials of agents targeting TLR-9 are under way. MGN1703 is a synthetic covalently closed DNA loop that functions as a TLR-9 agonist. The IMPACT trial was a Phase II randomized, double-blind, placebo-controlled clinical trial that randomized 59 patients with mCRC to receive maintenance MGN1703 or placebo after attaining disease control with first-line chemotherapy. Notably, the enrollment goal was 129, but the trial was closed early due to slow accrual. MGN1703 had a trend toward improved PFS (HR, 0.56; $P = .070$), though median PFS was 2.8 vs. 2.7 months. There was no difference in overall survival (HR, 0.63; $P = .2886$), though only one-third of patients in the experimental arm had an event.[53] The IMPALA trial is now accruing in Europe and is a randomized Phase III clinical trial with an enrollment goal of 540 patients with mCRC who experience complete or partial response after standard-of-care front-line chemotherapy to receive either maintenance MGN1703 or investigator's choice of standard maintenance, with the primary end point of overall survival (NCT02077868).

NOVEL ANTIANGIOGENIC SMALL-MOLECULE INHIBITORS

Antiangiogenic therapy is an important strategy in the treatment of mCRC, and the addition of bevacizumab, a monoclonal an-

tibody against vascular endothelial growth factor (VEGF)-A, to cytotoxic chemotherapy improves progression-free and overall survival in mCRC.[54,55] However, there are several mechanisms by which mCRC cells may develop resistance to bevacizumab, including increases in the levels of alternative circulating angiogenic factors besides VEGF-A, including placental growth factor (PlGF) and basic fibroblast growth factor (bFGF).[56] Ziv-aflibercept is a recombinant fusion protein of the VEGF-binding portions of human VEGF receptors (VEGFR)-1 and -2 fused to an IgG1 Fc region that sequesters VEGF-A, VEGF-B, and PlGF. The addition of ziv-aflibercept to FOLFIRI in second-line treatment of mCRC after progression through prior oxaliplatin-based chemotherapy improved PFS (HR, 0.758; 95% CI 0.661–0.869; $P < .0001$) and overall survival (13.50 vs. 12.06 months; HR, 0.817; $P = .0032$).[57] In the subgroup of 373 of 1226 patients who had undergone prior treatment with bevacizumab, the addition of ziv-aflibercept to second-line FOLFIRI yielded significant improvement in PFS (HR, 0.661; 95% CI 0.512–0.852), though overall survival was not significantly improved (HR, 0.862; HR, 0.673–1.104).[57]

Novel small-molecule inhibitors that inhibit proangiogenic signaling are being investigated in mCRC. The addition of small-molecule tyrosine kinase inhibitors against VEGFR to cytotoxic chemotherapy has not demonstrated efficacy to date. Cediranib is a tyrosine kinase inhibitor blocking activation of VEGFR1, -2, and V-3. However, it did not improve PFS or overall survival when added to mFOLFOX6 (modified folinic acid, 5-fluorouracil, and oxaliplatin-6) compared to bevacizumab+mFOLFOX6 in refractory mCRC[58] or in previously untreated mCRC.[59] Cediranib+FOLFOX or CAPOX (capecitabine and oxaliplatin) also failed to improve overall survival compared to FOLFOX or CAPOX alone in previously untreated mCRC.[60] Vatalanib (PTK787/ZK 222584) is a pan-VEGFR inhibitor that failed to improve PFS or overall survival when combined with FOLFOX4 in the first-line[61] or second-line[62] setting of mCRC therapy. Brivanib is an inhibitor of VEGF and FGF receptor (FGFR) that did not improve overall survival when added to cetuximab in *KRAS* wild-type refractory mCRC in the Phase III CO.20 study.[63] Nintedanib (BIBF 1120) is a novel inhibitor of VEGFRs, FGFRs, and platelet-derived growth factor (PDGF) receptors,[64] and the addition of nintedanib to mFOLFOX6 was not superior to bevacizumab+mFOLFOX6 in first-line mCRC in a Phase I/II trial.[65] The reasons for the failure of tyrosine kinase inhibitors combined with chemotherapy have not been clear, but hypotheses have included short half-lives of 8–24 hours for the various oral tyrosine kinase inhibitors and worse toxicity when combined with chemotherapy, limiting dose intensity.[66,67]

More recent trials have investigated monotherapy with tyrosine kinase inhibitors for multidrug refractory mCRC and may be more promising. Regorafenib has already been shown to be effective and is approved by the U.S. Food and Drug Administration (FDA) for refractory mCRC. Regorafenib is a promiscuous multikinase inhibitor targeting many kinases, including VEGFR1, VEGFR2, VEGFR3, TIE2 (tyrosine kinase with Ig-like and EGF-like domains-2), PDGFR, and FGFR.[68] In the Phase III CORRECT study, regorafenib monotherapy improved overall survival compared to placebo in refractory mCRC (6.4 vs. 5.0 months; HR, 0.77; 95% CI 0.64–0.94; 1-sided $P = .0052$).[69] Nintedanib is also under investigation as a monotherapy for multidrug-refractory mCRC and appears to be more promising in this context. In a Phase I study of nintedanib monotherapy in multidrug-refractory mCRC, 24 of 30 (80%) patients had stable disease lasting 8 weeks or more, with median time to progression of 2.4 months.[70] The LUME-COLON 1 study is an ongoing international Phase III study with an enrollment goal of 764 patients with mCRC refractory to fluoropyrimidine, oxaliplatin, irinotecan, bevacizumab or ziv-aflibercept, who have had anti-EGFR therapy, and will receive either nintedanib monotherapy or best supportive care, with primary end points of overall survival and PFS[71] (NCT02149108).

NOVEL ANTIMETABOLITES

TAS-102 is a novel oral nucleoside composed of a 2:1 molar ratio of trifluridine (FTD) and tipiracil hydrochloride (TPI). FTD is the active cytotoxic compound that inhibits thymidylate synthase as a monophosphate and is incorporated into DNA as a triphosphate. TPI increases the plasma half-life of FTD by inhibiting thymidine phosphorylase, which degrades FTD. A randomized Phase II trial of TAS-102 monotherapy vs. best supportive care was performed in 172 mCRC patients refractory to fluoropyrimidine, irinotecan, and oxaliplatin and revealed improvement in overall survival (9.0 vs. 6.6 months, HR, 0.56; 95% CI, 0.39–0.81; $P = .0011$).[72] The Phase III RECOURSE trial randomized 800 patients with mCRC refractory to fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab, and cetuximab or panitumumab of *KRAS* wild-type to either TAS-102 or placebo and found significant improvement in overall survival (7.1 vs. 5.3 months; HR, 0.68; 95% CI, 0.58–0.81; $P < .0001$) and PFS (2.0 vs. 1.7 months, HR, 0.48; 95% CI, 0.41–0.57; $P < .0001$).[73] On the basis of these positive results, TAS-102 was given Fast Track designation by the FDA, with the expectation of impending approval.

CONCLUSIONS

Significant advances have been made in therapy for mCRC, but further improvements are necessary to continue these advances. Ongoing trials point toward potential new therapies, with many incorporating integral biomarkers to allow for inclusion or to allocate patients to different arms of a basket trial. Randomized Phase II trials of novel anti-EGFR targeting antibodies are ongoing in *KRAS* wild-type mCRC, and additional trials investigating combinations of agents added to MEK inhibitors are in progress. Several combinatorial strategies including a BRAF inhibitor and an anti-EGFR antibody, are under investigation in *BRAF* mutant mCRC after encouraging Phase I data. Immunotherapy with checkpoint inhibitors is under investigation in MSI-H mCRC. Finally, in the multidrug-refractory setting, there is a randomized Phase III trial ongoing with nintedanib monotherapy (LUME-COLON 1),[71] and the completed Phase III trial of TAS-102 (RECOURSE) showed improvements in progression-free and overall survival.[73] Further correlative studies are needed, to distinguish potential biomarkers for response or resistance and to better identify the patients most likely to benefit from each of these investigational therapies.

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Disclosures of Potential Conflicts of Interest

Dr. Kopetz has served as Ad hoc consultant to Taiho, Amgen, BMS, Roche, Merrimark, Sysmex, Bayer, Agendia, Sanofi, Array Biopharma and GSK and has had sponsored research from Amgen, Roche, Sysmex, Agendia, Sanofi, GSK, Biocartis, and Guardant Health. Dr. Lee has indicated no potential conflicts of interest.