Advances in Immunotherapy in the Treatment of Colorectal Cancer

Birendra KC, MD; Jimmy J. Hwang, MD; Carol J. Farhangfar, PhD, MBA; and S. Jean Chai, MD

Abstract

Colorectal cancer (CRC) remains the third most common cancer in the United States, with a high mortality rate. In the early 2000s, there was significant excitement with the introduction of targeted agents like bevacizumab and cetuximab into the treatment of metastatic CRC. However, over the last 15 years, treatment options have been static and remain fluorouracil-based cytotoxic chemotherapy in moderately toxic combinations such as FOLFOX and FOLFIRI. The advent of immunotherapies—in particular, checkpoint inhibitors—has opened a potential new avenue of treatment. As with other targeted approaches, there may be specific populations who are more responsive to immunotherapy. Patients with defective DNA mismatch repair system (MMR)/microsatellite instability (MSI-high) may have immunogenic potential. Investigators have shown durable responses with immune checkpoint inhibitors in patients with CRC in small clinical trials, with larger studies ongoing. Currently, the National Comprehensive Cancer Network recommends pembrolizumab and nivolumab in the treatment of metastatic CRC in the second- and third-line settings for patients with defective MMR/MSI-high. Furthermore, the FDA recently has granted accelerated approval to pembrolizumab for any cancer with MSI-high or MMR-deficiency that has progressed on standard therapy. We will discuss the underlying molecular mechanisms and review published and ongoing clinical trials with immunotherapy in the treatment of CRC.

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Introduction

Colorectal cancer (CRC) is a leading cause of cancer mortality in the United States with an estimated incidence of 135,430, and causing the deaths of an estimated 50,260 people in 2017. With increasing acceptance of screening strategies, incidence rates have declined by 3% per year from 2004 through 2013.[1-3] Nevertheless, the median survival of patients with metastatic CRC not amenable to surgery remains less than 3 years. Survival improves significantly with resectable metastatic disease to a 5-year survival rate of 26% to 40%.[4] There have been modest advances since 2004, when targeted agents like VEGF and EGFR inhibitors were introduced. However, immunotherapy provides a promising avenue of therapy.

Molecular Drivers of CRC

Vogelstein and colleagues theorized a predictable progression from adenoma to carcinoma. They proposed a stepwise accumulation of genetic and epigenetic events. This model provides insights into the role of "driver" alterations in tumor suppressor genes that confer selective growth advantages and give rise to cancer. Genes with mutations include: adenomatous polyposis coli (*APC*), *TP53*, SMAD family member 4 (*SMAD4*), *BRAF* V600E, and oncogenes such as *KRAS* and *PI3K* catalytic subunit α.[5,6] About 85% of CRCs develop as a result of chromosomal instability due to allelic losses, loss of heterozygosity, chromosomal amplifications, and translocations.[7] These abnormalities may be inherited or sporadic.

The remaining 15% of CRCs have defective DNA mismatch repair systems (MMR) caused by inactivation of mutL homologue 1 (*MLH1*), *MLH3*, mutS homologue 2 (*MSH2*), *MSH3*, *MSH6*, or PMS1 homologue 2 (*PMS2*). This may occur through inherited or sporadic mutations, or through epigenetic silencing. These dominant genomic features give rise to hypermutations and microsatellite instability (MSI).[7]

Recently, the consensus molecular subtypes (CMS) of CRC have been defined, which reflect these differing etiologies. The 4 proposed CMS are: CMS1 (MSI immune: 14%, hypermutated, microsatellite unstable, strong immune activation and *BRAF* mutations); CMS2 (canonical: 37%, epithelial, marked Wnt and MYC signaling activation); CMS3 (metabolic: 13%, epithelial, evident metabolic dysregulation and *KRAS* mutations); and CMS4 (mesenchymal: 23%, prominent transforming growth factor-β, stromal invasion, angiogenesis, and worse overall survival). Samples with mixed features (13%) possibly represent a transition phenotype or intratumoral heterogeneity.[8]

MMR and MSI: Predictors of Benefit for Immunotherapy

The MMR system has long been an area of active research in CRC. It is of pivotal importance for the rectification of DNA sequence mismatches during DNA replication. The main function of MMR

proteins is maintenance of genomic stability by correcting for single base nucleotide mismatches, insertions, or deletions that arise during DNA replication.[9] Deficient MMR can be secondary to germline mutations or sporadic hypermethylation, which silences DNA in MMR genes.

Microsatellites are short DNA motifs of 1 to 6 bases that are repeated and distributed throughout the genome both in coding and noncoding regions. Owing to their repeated structures, microsatellites are particularly prone to replication errors that are normally repaired by the MMR system. Loss of function of 1 of the MMR proteins may lead to the accumulation of errors in microsatellites, resulting in genetic instability. Thus, defects in MMR lead to MSI, which may have oncogenic potential when errors occur in coding regions of crucial cellular functions and pathways.[10]

Many guidelines suggest universal screening of MSI to detect possible high risk for CRC. MSI can be tested by immunohistochemistry (IHC) and by polymerase chain reaction with excellent concordance, and most recently by next-generation sequencing.[11] CRCs can be classified as microsatellite instability-high (MSI-H), and microsatellite instability-low (MSI-L), depending on the percentage of loci with MSI. The MSI-H phenotype is defined by the presence of at least 2 unstable IHC markers among the 5 analyzed (or ≤30% of unstable markers if a larger panel is used). Patients who are MSI-H should be referred for further genetic testing and counseling for Lynch syndrome.

In addition to its utility in identifying patients and families who are at high risk for genetic cancers, MSI-H status in patients with stage II and III colon cancer has been shown to have prognostic impact. Ribic et al demonstrated that patients who were MSI-H had a better 5-year survival. Moreover, these patients did not have improvement in survival with the addition of adjuvant fluorouracil therapy, in part because their risk for relapse was lower than those who were MSI-L. The MSI-L population did benefit from adjuvant chemotherapy, as anticipated.[12] A meta-analysis confirmed a survival advantage in tumors with MSI-H (HR, 0.69; 95% CI, 0.56-0.85).[13]

MSI-H may be targeted for treatment using immunotherapy. In a phase I trial in 2012, Brahmer et al obtained a complete response in 1 patient with MMR-deficient CRC using the PD-1 inhibitor nivolumab. The response was durable for more than 21 months.[14] The authors suggested that MSI-H tumors are hypermutated and express numerous truncated proteins caused by frameshifts. These proteins act as neoantigens and elicit an immune response by tumor-infiltrating lymphocytes (TILs).[14,15] Thus, it was hypothesized that MSI-H tumors have a significant immunological response that is elicited by the neoepitopes created by increased DNA repair mistakes. These findings reinforced the practical importance of the MMR system not only in the development of cancer and as a prognostic marker, but also as a potential avenue in its treatment.

Immunotherapy in Colorectal Cancer

PD-1 is a transmembrane protein expressed on T cells, B cells, and natural killer cells. It is an inhibitory molecule that binds to PD-L1 and PD-L2. The PD-1/PD-L1/L2 interaction directly inhibits apoptosis of the tumor cell and promotes peripheral T-effector cell exhaustion and conversion of T effector cells to regulatory T (Treg) cells.[16] Blockade of this pathway with antibodies to PD-1 or its ligands have led to remarkable clinical responses in melanoma, non–small cell lung cancer, renal-cell carcinoma, bladder cancer, and Hodgkin lymphoma.

Two additional trials have suggested the activity of PD-1 blockade in metastatic CRC, and have led the National Comprehensive Cancer Center Network (NCCN) to recommend pembrolizumab and nivolumab for treatment of metastatic CRC in the second- and third-line setting.[17] In 2015, KEYNOTE-164[18] showed significant activity of pembrolizumab for second- or third-line treatment for MMR-deficient/MSI-H metastatic CRCs. Le et al conducted a phase II study of pembrolizumab (MK-3475), a PD-1 inhibitor, as monotherapy in a total of 41 patients with previously treated locally advanced unresectable or metastatic CRC with or without MMR deficiency. Pembrolizumab was administered intravenously at a dose of 10 mg/kg every 14 days to patients in 3 groups: those with 1) MMR-deficient CRCs (n = 11), 2) MMR-proficient CRCs (n = 21), and 3) MMR-deficient non-colorectal cancers (n = 9). The immune-related objective response rate (ORR) and 20-week progression-free survival (PFS) rate were 40% and 78%, respectively, for MMR-deficient CRCs compared with 0% and 11% for MMR-proficient CRCs. The median PFS and overall survival (OS) were not reached in patients with MMR-deficient CRC but were 2.2 and 5.0 months, respectively, for MMR-proficient CRC. A post hoc comparison of the cohorts with MMR-deficient and MMR-proficient colorectal cancers showed the HR for progression or death was 0.10 (P <.001), and HR for death was 0.22 (P = .05), respectively. Interestingly, patients with MMR-deficient non-CRC had responses smiliar to patients with MMR-deficient CRC (ORR, 71%; PFS, 67%). High somatic mutation loads were associated with prolonged PFS (P = .02). Whole-exome sequencing revealed a mean of 1782 somatic mutations per tumor in MMR-deficient tumors, as compared with 73 in MMR-proficient tumors (P = .007). Most common adverse events were fatigue (32%), rash or pruritis (24%), diarrhea (24%), abdominal pain (24%), constipation (20%), anemia/lymphopenia (20%), pancreatitis (15%), headache (17%), dyspnea (15%), arthralgia (17%) and hypothyroidism/thyroiditis (10%). Grade 3/4 adverse events included lymphopenia (20%), anemia (17%), hypoalbuminemia (10%), hyponatremia (7%), and diarrhea (5%).

CHECKMATE-142,[19] the third and largest trial to show the importance of immunotherapy in CRC, used nivolumab as second- or third-line treatment for MMR-deficient/MSI-H metastatic CRCs. Overman et al presented interim results of CHECKMATE-142 at the 2016 ASCO Annual Meeting. This phase II study used nivolumab with or without ipilimumab in treatment of patients with meta-

static CRC with and without high MSI-H. MSI-H patients received nivolumab (N) 3 mg/kg every 2 weeks (N3) or N 3 mg/kg + ipilimumab (I) 1 mg/kg every 3 weeks (N3+I1) x 4 doses followed by N3 until disease progression or other discontinuation. This was a small trial with 33 (N3) and 26 (N3+I1) MSI-H patients. There were 3 (N1+I1), 10 (N1+I3), and 10 (N3+I1) in the patients with non–MSI-H arm. The responses are shown in **Table 1**. Treatment-related adverse events (TRAEs) were in line with prior immunotherapy trials. These occurred in 26 (79%; N3) and 22 patients (85%; N3+I1); most common were diarrhea and fatigue (27% each; N3) and diarrhea (46%; N3+I1). The results were subsequently updated at the 2017 ASCO Gastrointestinal Cancers Symposium.[20] In the updated results, in MSI-H patients, the 74 patients who were treated with single-agent nivolumab had a centrally reviewed ORR of 27%, with stable disease in an additional 37.8%. The 12-month PFS rate was 48.9%, and the 12-month OS rate was 73.8%. Grade 3-4 TRAEs occurred in 20% of patients. TRAEs leading to discontinuation included acute kidney injury, increased alanine aminotransferase, colitis, and stomatitis (1 each). No treatment-related deaths occurred in this arm.

TABLE 1. Interim results of CHECKMATE-142.[19]

	MSI-H N	MSI-H N/I	MSS N1/I3	MSS N3/I1
ORR	12/47 (25.5%)	9/27 (33.3%)	1/10 (10%)	0/10 (0%)
Stable disease	29%	52%	N/A	N/A
Median PFS	5.3 months	NR	2.3 months	1.3 months
Median OS	17.1 months	NR	11.3 months	3.73 months

I indicates ipilimumab; MSI-H, microsatellite instability-high; MSS, microsatellite stable; N, nivolumab; N/A, not applicable; NR, not reached; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

Based on these data, the FDA went a step further and granted the first-ever indication for a biomarker, rather than cancer type. The FDA granted accelerated approval to pembrolizumab for patients with MSI-H or MMR-deficient cancer that has progressed following standard treatment. The most common types of cancers with MSI-H were colorectal, endometrial, and other gastrointestinal cancers. Other cancers with MSI-H and activity with pembrolizumab were breast, prostate, bladder, and thyroid cancers.[21]

Several other clinical trials, mostly phase I and phase II, are ongoing using immunotherapy in metastatic CRCs (mCRCs) (**Table 2**). Hochster et al presented updated efficacy and safety of atezolizumab (atezo, PD-L1 inhibitor) and bevacizumab (bev) in a phase Ib study of MSI-high metastatic CRC.[22] Patients were treated with atezo 1200 mg every 3 weeks plus bev 15 mg/kg every 3 weeks. Ten patients with MSI-H were enrolled, with an ORR of 30% (95% CI, 6.7%-65.3%); the disease control rate was 90%. The median OS has not been reached with a median follow-up of 11.1 months. Initial clinical activity was observed in heavily pretreated patients with MSI-high mCRC receiving atezo plus bev. This combination was well tolerated with expected toxicities.

Unfortunately, only a minority of patients, perhaps 5% to 15%, have MSI-H/MMR-deficient mCRC. These patients are the clearest potential beneficiaries of immunotherapy with checkpoint inhibitors. An area of active exploration is the potential use of checkpoint inhibitors in the broader population of patients with MSI-L or MSS. Bendell et al presented the interim results of the phase I clinical trial of cobimetinib (cobi) and atezo in CRC at the 2016 ASCO Annual Meeting.[23] As of October 12, 2015, 23 patients with CRC (22 *KRAS* mutant, 1 wild-type) were enrolled during escalation and expansion. No dose-limiting toxicities were observed and expansion occurred at atezo 800 mg twice a week and cobi 60 mg daily (21 days on/7 days off). Three responses were ongoing (range, 4.0 to 7.7 months at time of data cutoff). Interestingly, 3 responders were MMR-proficient, and 1 was unknown. ORR in *KRAS*-mutated patients was 20% and stable disease was achieved in 20%. Median PFS was 2.3 months, and the 6-month PFS rate was 25%.

TABLE 2. Ongoing Checkpoint Inhibitors and Immune Modulators Phase II Clinical Trials in CRC.

Agent	Disease type	Study phase/ Estimated enrollment	Status	NCT number
Pembro	MSI-high mCRC	II/ 171	Recruiting	NCT01876511
Pembro + azacitidine	Chemorefractory mCRC	II/ 31	Ongoing; not recruiting	NCT02260440
Pembro + radiotherapy/ ablation	mCRC	II/ 48	Recruiting	NCT02437071
Pembro + mFOLFOX6	mCRC	II/ 30	Ongoing; not recruiting	NCT02375672
N and N combinations with I, cobi, daratumumab, anti-LAG-3 Ab	Recurrent and mCRC	II/ 340	Recruiting	NCT02060188
Durvalumab	mCRC	II/48	Recruiting	NCT02227667

Anti-LAG-3 ab indicates anti-lymphocyte activation gene-3 antibody; cobi, cobimetinib; I, ipilimumab; mCRC, metastatic colorectal cancer; mFOLFOX6, modified 5-fluorouracil, leucovorin, oxaliplatin; MSI, microsatellite instability; N, nivolumab; Pembro, pembrolizumab.

Conclusion

Immune checkpoint inhibition represents a breakthrough in cancer therapy, with durable responses and generally fewer adverse effects than conventional chemotherapy. However, immune-related adverse events (irAEs)

can be life-threatening, and include toxic epidermal necrolysis, colitis, hypophysitis, hepatitis, pancreatitis, iridocyclitis, neuropathies, and nephritis. Early recognition of irAEs and initiation of treatment are critical to reduce morbidity.

Predicting tumor responses to PD-1 blockade and selecting the optimal patient population remains a major challenge. A subset of patients with CRC who are MMR-deficient/MSI-high may be a target population for immunotherapy. Studies have demonstrated that the highest responses to PD-1/PD-L1 blockade occur in tumors with the highest mutational burden (melanoma, non–small cell lung cancer, squamous cell carcinoma of the head and neck, gastric cancer, and most recently urothelial cancer). Interestingly, MMR-deficient tumors were also noted to have high mutational burden and were associated with prolonged PFS.[18] In addition, identification of cytotoxic T-cell infiltration within tumors suggests pre-existing antitumor immunity and what has been found to predict response to PD-1/PD-L1 blockade. Identification of reliable biomarkers that will help identify the right patient population who would respond to immunotherapy needs further investigation.

Current success of immunotherapy is limited to only about 30% of MSI-H patients, which means only about 5% of all patients with CRC—a very small subset. Understanding why MSI-H tumors are responsive to immunotherapy will help develop better treatment options for all patients with CRC. One promising option would be to use immunotherapy in combination with agents that complement the cancer-immunity cycle. Using these agents in the right sequence could be a key to the success of immunotherapy. There has been a proposed stepwise immune response that occurs against tumors, which includes dendritic cell antigen presentation to T-cell priming and differentiation to effector and memory T cells. Throughout this process, T cells also must overcome tumor-derived immunosuppression including loss of *PTEN*, myeloid-derived suppressor cells, Treg cells, and tumor cell-secreted suppressive molecules. Combining therapies that enhance antigen presentation and boost T-cell priming—such as chemotherapy, ionizing radiation, and monoclonal antibodies—may help convert a cold (nonimmunogenic) tumor to a hot (immunogenic) tumor. At the same time, continuation of therapies that decrease tumor-derived immunosuppression (such as PI3K and BRAF inhibitors) throughout the treatment may further help lengthen immunotherapy's success.

Combination therapies may improve the outcomes in patients with CRC, but finding an effective combination for every patient will be a significant challenge. Additionally, combination treatments also have the potential for increased toxicity. Immunotherapies added to different targeted therapies, other immunomodulatory agents (eg, Wnt/β-catenin inhibitors), chemotherapy, and other modalities, such as radiation are being tested (**Table 2**). Better understanding of some important associated mutations like *KRAS*, *BRAF*, *PI3K*, *PTEN*, and β-catenin could help successful pairing of targeted therapies with immunotherapy.

Combination immunotherapy is a promising avenue of treatment for CRC. Its success will depend on identifying crucial molecular pathways and combining treatment modalities in the right sequence.

Author affiliations: All authors are with the Levine Cancer Institute, Carolinas Healthcare System, Charlotte, North Carolina.
Address correspondence to: S. Jean Chai, MD, Levine Cancer Institute, Carolinas Healthcare System, 1100 S. Tryon St., Suite 400, Charlotte, NC 28203. Tel: (980) 442-9100; Fax: (704) 446-4998; E-mail: Seungjean.Chai@carolinashealthcare.org.
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