

A Blueprint to Advance Colorectal Cancer Immunotherapies



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Abstract

Immunotherapy is rapidly becoming a standard of care for many cancers. However, colorectal cancer had been generally resistant to immunotherapy, despite features in common with sensitive tumors. Observations of substantial clinical activity for checkpoint blockade in colorectal cancers with defective mismatch repair (microsatellite instability–high tumors) have reignited interest in the search for immunotherapies that could be extended to the larger microsatellite stable (MSS) population. The Cancer Research Institute and Fight Colorectal Cancer convened a group of scientists, clinicians, advocates, and industry experts in colorectal cancer and immunotherapy to compile ongoing research efforts, identify gaps in translational and clinical research, and provide a blueprint to advance immunotherapy. We identified lack of a T-cell inflamed phenotype (due to inadequate T-cell infiltration, inadequate T-cell activation, or T-cell

suppression) as a broad potential explanation for failure of checkpoint blockade in MSS. The specific cellular and molecular underpinnings for these various mechanisms are unclear. Whether biomarkers with prognostic value, such as the immunoscores and IFN signatures, would also predict benefit for immunotherapies in MSS colon cancer is unknown, but if so, these and other biomarkers for measuring the potential for an immune response in patients with colorectal cancer will need to be incorporated into clinical guidelines. We have proposed a framework for research to identify immunologic factors that may be modulated to improve immunotherapy for colorectal cancer patients, with the goal that the biomarkers and treatment strategies identified will become part of the routine management of colorectal cancer. *Cancer Immunol Res*; 5(11); 942–9. ©2017 AACR.

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Introduction

Colorectal cancer remains a substantial public health problem, currently ranking as the third leading cause of cancer-related deaths among men and women (1). Half of the patients with colorectal cancer either have metastatic disease at presentation or develop metastases subsequently. Although availability of chemotherapies and biologics has increased median survival in patients with metastatic disease, further treatment options are still needed (1). The efficacy of immunotherapy in colorectal cancer has been limited to the small percentage of patients with microsatellite instability–high (MSI-H) tumors, which prompted an approval of pembrolizumab (anti-PD-1) for MSI-H or mismatch repair–deficient solid tumors. To focus on colorectal cancer patients, the Cancer Research Institute and Fight Colorectal Cancer convened a broad group of scientists, clinicians, advocates, and industry experts to develop a blueprint for research, guideline development, and policy that would advance immunotherapy to the routine treatment of colorectal cancer.

Numerous immunotherapeutic modalities were tested in early-phase studies. However, few objective responses are seen in unselected colorectal cancer patients (2, 3). Observations that mutational load correlates with immune response to checkpoint blockade in many malignancies led to studies of checkpoint

blockade in patients with advanced colorectal cancer (4). In a phase II trial, administration of pembrolizumab resulted in a clinical benefit rate [objective response (OR) and stable disease (SD)] in 90% of MSI-H patients having OR or SD (by RECIST criteria). Whereas progression-free survival (PFS) at 6 months was 78% in the MSI-H group, the disease control rate (DCR) at 20 weeks was 11% in microsatellite stable (MSS) patients, and expression of PD-L1 was not significantly associated with either PFS or overall survival (OS). These data supported the approval for pembrolizumab in all MSI-H cancers.

Earlier studies revealed no objective clinical responses with nivolumab or anti-PD-L1 (BMS936559/MDX-1105) in unselected patients (5, 6). A study with nivolumab in MSI-H patients showed an objective response rate (ORR) of 26%, with 30% of subjects achieving SD (7), and nivolumab is now FDA approved for MSI-H colorectal cancer. These data suggest that successful immunologic targeting of MSS tumors may be achieved by attempting to alter their tumor microenvironment (TME) to be immune active (characterized by high T-cell infiltration, enhanced activation state, and an IFN γ -dominated cytokine milieu).

Advances in the taxonomy of colorectal cancer have expanded our understanding of the pathways that are potential immunotherapeutic targets (8). Other immune active tumors include those arising due to acquired DNA hypermethylation that renders the mismatch repair pathway ineffective. Like MSI-H tumors, these are associated with T-cell infiltration resulting from gene expression of class II antigenic proteins, T cell-promoting chemokines, and inhibitory checkpoint receptors, such as PD-1, CTLA4, and LAG-3 (2, 9, 10). However, other colorectal cancers lack these features due to the adverse mesenchymal subtype that is associated with stromal infiltration, TGF β production, and angiogenesis. By identifying the positive and negative immunologic factors within these subtypes and developing strategies to augment or inhibit them, routine immune management of colorectal cancer may become a reality.

Features Associated with Improved Immune Responsiveness

Identifying immune-related prognostic and predictive features is the subject of ongoing research. Techniques for categorizing these features, including IHC staining and gene expression (2, 4–6, 8–12), have been extensively studied, but functional studies on tumors and infiltrating leukocytes are also of interest. In an initial retrospective IHC analysis evaluating the prognostic relationship between tumor-infiltrating lymphocytes and clinical outcomes in early-stage disease, Galon and colleagues identified a correlation between recurrence-free survival and the density of infiltrating CD3⁺ CD8⁺ lymphocytes at the tumor margin and center. Infiltration by specific subsets of T cells had a better correlation with clinical outcome than simply examining the density of lymphocytic infiltration around tumors (3, 12). This was confirmed in a prospective validation study, called Immunoscore, of 2,667 patients through a quality-controlled worldwide consortium that could be replicated across all involved sites (13). The score was generated by evaluating the CD3⁺ and CD8⁺ density and location in primary tumors, resulting in 4 scores for each patient. The mean quintile score was used to assign the patient into low, intermediate, or high immunoscore groups, which corresponded to high-, medium-, and low-risk related to clinical outcomes, respectively. Immunoscores were prognostic for time to recurrence, disease-

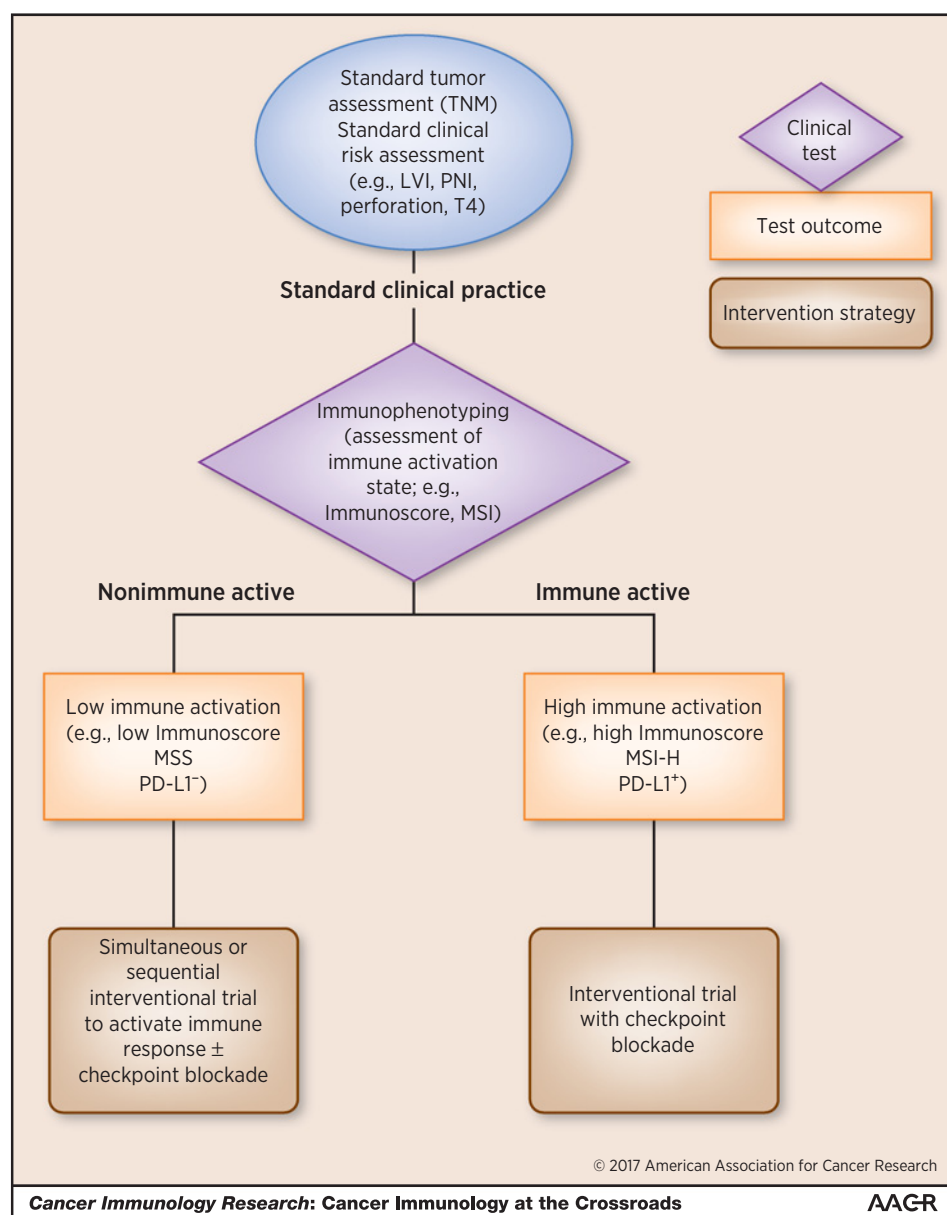
free survival (DFS), and OS in patients with stage I–III colorectal cancer. In patients with stage II colorectal cancer who also had low immunoscores (high-risk), about 21% (375/1,808) had an estimated 5-year relapse-free survival (RFS) of 76.8%, whereas 26% of patients in the same demographic group with high immunoscores (low-risk) had a 5-year RFS of 91.2%. Immunoscores were also a better prognostic for survival in newly diagnosed patients with localized colorectal cancer (14) and were individually prognostic of DFS in FOLFOX-treated patients (15). Approximately 20% of MSS patients with high immunoscores were found in a small (unvalidated) cohort of patients (14). The frequency of MSI-H status varied across different disease stages (stage II, 9%; stage III, 12%; and stage IV, 4%; ref. 16). In metastatic colorectal cancer, the immune contexture is grossly similar in primary and metastatic sites (17). These data support the future incorporation of immunoscores into the prognostic scoring systems for colorectal cancer. However, they also raise the question of whether immunoscores would be predictive of immunotherapy benefit in the adjuvant setting (Fig. 1).

Although MSI status and immunoscores are key features for separating patients into groups, other assays may also be valuable. RNA expression profiling elucidated a T cell–rich signature present in both MSI-H and some MSS colorectal cancer patients who had a better prognosis (18). High expression of lymphoid genes is associated with poor prognosis. Further analysis showed that this particular signature was associated with myeloid cells and fibroblasts with immunosuppressive mesenchymal markers (18) as well as a high proportion of endothelial cells and cancer-associated fibroblasts. This mesenchymal signature was identified as a marker of resistance in melanoma patients who did not respond to anti-PD-1 (19).

A signature consisting of an angiogenic and wound healing-related microenvironment has been documented (20–22). Understanding the immune context and the TME will play a vital role in designing strategies to improve immune function. The mesenchymal phenotype plays a role in disease outcome and can negatively impact immunotherapy treatment in a variety of cancers. Thus, further validation of this signature and its impact on checkpoint blockade is necessary to further delineate its contribution to disease progression and response to treatment. As the immunotherapy landscape moves toward therapeutic combinations, clinicians and scientists will need to understand the mechanisms by which combination agents impact colorectal cancer, which will require interrogation of biopsies before, during, and after treatment.

Framework for Assessment

The goal of the framework proposed here is to enhance personalized immunotherapy, which requires an improved understanding of the transcriptional and immune status of each patient's cancer. We propose an algorithm to assess the immunoreactivity of a patient's tumor for the development of an immunologically guided treatment plan, including standard of care (SOC) treatments and clinical trials. Patients with germline MSI-H tumors should undergo genetic counseling for themselves and family members and should be offered genetic testing for Lynch syndrome, if interested. All early-stage patients should undergo SOC tumor assessment staging, have MSI status documented, and, if available, use their immunoscore to determine immunoreactivity (Fig. 1). SOC is offered to patients with stage I/II

**Figure 1.**

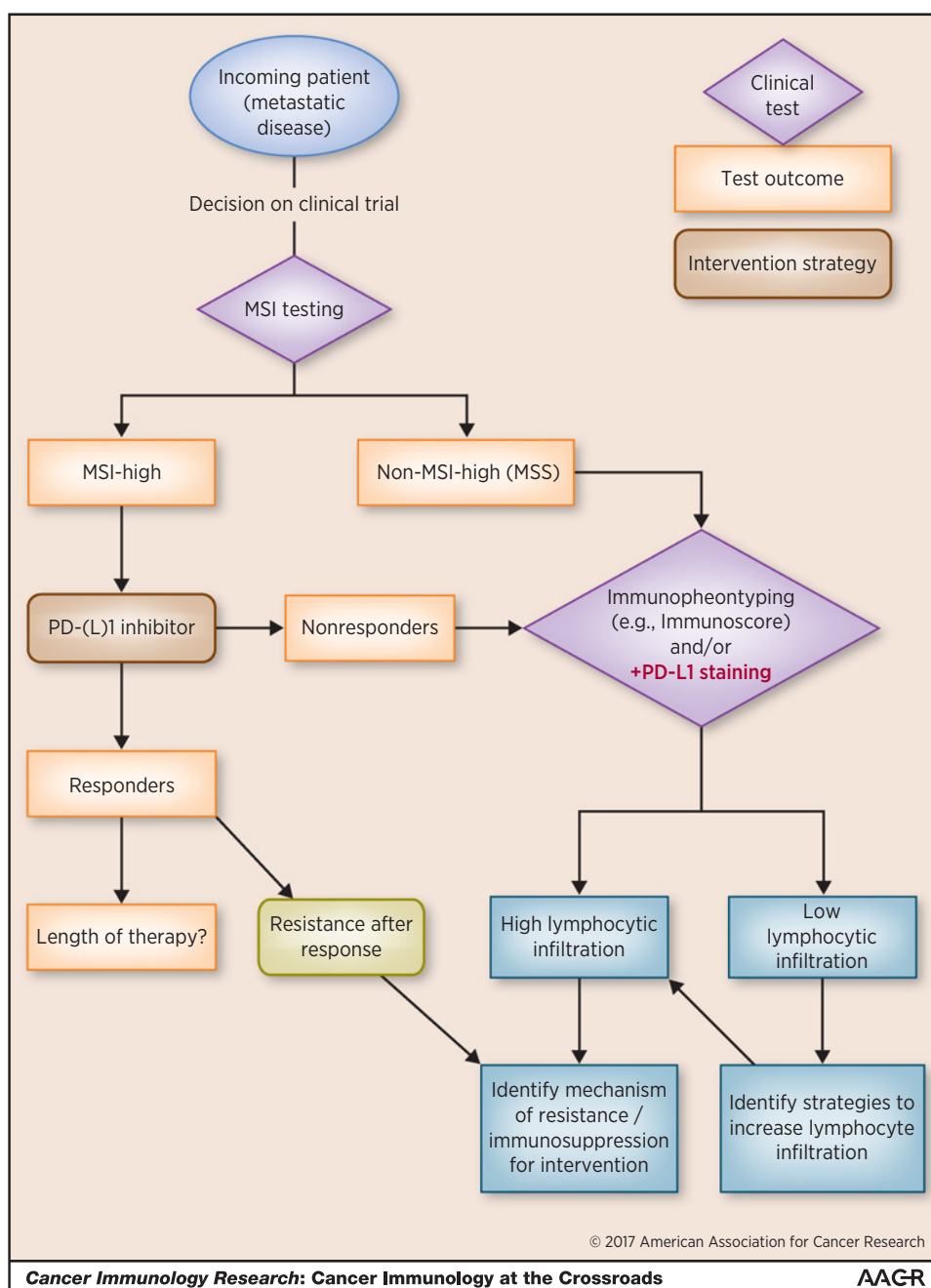
Proposed immunologic evaluation of patients with early-stage colorectal cancer. Standard tumor assessment and clinical assessment will lead to treatment via standard clinical practice for early-stage colorectal cancer patients. This could be supplemented by immunophenotyping consisting of immunoscore, immune-activation state, and MSI status. Patients with a low-density infiltration of CD3⁺ and CD8⁺ cells would be considered for simultaneous or sequential interventional trials to activate the immune response with and without checkpoint blockade in the presence or absence of agents targeting resistance mechanisms. Patients with high-density CD3⁺ and CD8⁺ cells with other high-risk clinical features could consider an interventional trial with standard therapy followed by or concurrent with checkpoint blockade.

cancers, whereas FOLFOX chemotherapy is for stage III disease for those who are immune inactive. Future trials should evaluate simultaneous or sequential interventions to activate the immune response in the presence or absence of checkpoint blockade with and without TME-targeting agents. If the patient's tumor is immune active, they are candidates for standard therapy. In the metastatic setting, all patients' tumors should be assessed for MSI status (the first determinant of the type of standard or clinical trial intervention) in addition to standard genomic profiling (Fig. 2; ref. 23). If the patient is MSI-H and has progressed while on fluoropyrimidine, oxaliplatin, and/or irinotecan, they will likely receive a PD-1 inhibitor. If no response is seen, immunophenotyping should be performed. Patients with high lymphocytic infiltration may be candidates for trials combining PD-1/PD-L1 inhibition with agents specifically targeting potential mechanisms of resistance or immunosuppression (Table 1). For patients with

low lymphocytic infiltration, intervention strategies to improve lymphocyte infiltration are needed (Supplementary Table S1). However, many of these recommendations will require guideline development, validation, improved or enhanced clinical trial recruitment, and discussions surrounding reimbursement, which are implemented at different times during a study (Supplementary Table S2). Clinical tests, outcomes, and intervention strategies are outlined for early-stage colorectal cancer and metastatic disease (Figs. 1 and 2). In both clinical situations, guidelines should be developed and reimbursement strategies identified early on during the initial clinical testing phases, whereas the focus should be on patient recruitment and acquisition of research funding for clinical and mechanistic studies in later interventional stages. Data and methodology need validation throughout the clinical testing, outcome, and interventional stages.

Figure 2.

Research algorithm for immunologic evaluation of patients with metastatic colorectal cancer, describing an incoming patient with metastatic disease who would undergo MSI testing as a basis of whether or not to enter a clinical trial. MSI-high patients would receive a PD-1/PD-L1 inhibitor. Those patients that do not respond or become resistant after an initial response could lead to identification of mechanism(s) of resistance and immunosuppression in the TME, resulting in the use of therapeutic intervention(s) listed in Table 1. All non-MSI high (MSS) patients would undergo immunophenotyping, and depending on whether they have high or low lymphocytic infiltration, would enter an interventional trial, based on their immune status to answer research questions (Supplementary Table S1).



Cancer Immunology Research: Cancer Immunology at the Crossroads

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Discussion

Existing SOC therapies have immunologic manifestations and could be synergistic with immunotherapy. Radiotherapy, commonly used for neoadjuvant treatment of rectal cancer and locally advanced colorectal cancers, has broad effects on the immune system through the induction of immunogenic cell death (ICD), maturation of dendritic cells (DCs), and improved cross-presentation (24, 25). The abscopal effect (when local radiotherapy is associated with regression of metastatic cancer at a distant site) has been seen in the setting of checkpoint blockade (26, 27). The rationale for combining radiotherapy with immu-

notherapy is to induce an *in situ* vaccine effect, leading to antigenic spread, uptake of antigens, maturation of DCs, and activation of T cells (28). Like radiation, chemotherapy causes direct cell killing and induction of ICD (29). However, different types of chemotherapy exert different immunologic effects and should be further studied (30). Several hundred studies have investigated radiation or chemotherapy in colorectal cancer, and of these, a dozen have explored the effects of combining them with immunotherapy. Many research questions remain, including analyzing T-cell antigen recognition and phenotype. These questions may be answered by performing deep immune phenotyping as well as understanding whether ICD has occurred.

Table 1. Strategies to turn colorectal cancer tumors into immunoreactive MSS tumors

Cell type/pathway	Laboratory test	Association with prognosis	Goal level of expression	Interventions to turn CRC tumors into immunoreactive MSS	Currently in clinic?		
					Intervention	Monotherapy	Combination
Goal molecular pattern: IFN signature (CXCL9, CXCL10, CXCL16, IL15)	RNA-seq	Good (3, 11, 13)	↑	IFN-inducing agents (OV, TLRs, STING, epigenetic modifiers)	OV TLRs STING Epigenetic	Yes Yes NA Yes	Yes NA NA Yes
Mesenchymal (VEGF, TGFB, galectin, COX-1)	RNA-seq	Poor (3, 13)	↓	↓ Angiogenesis (VEGF blocking) ↓ Immunosuppression (TGFB inhibitor) ↓ Immunosuppression (Galectin inhibitor) ↓ Immunosuppression (Cox inhibitor) ↓ Immunosuppression (epigenetic)	VEGF TGF Galectin COX Epigenetic	Yes Yes NA Yes Yes	Yes Yes NA Yes Yes
MHC class-I molecules (MHC-I) and tumor antigen expression	RNA-seq	Good (3, 11, 13)	↑↑	IFN-inducing agents (OV, STING, TLRs, epigenetic modifiers), ↑ ags (radiation, chemo, vaccine, neoags), MEK inhibitors	XRT Chemo Neoantigens Vaccines	Yes Yes Yes Yes	Yes Yes NA Yes
CXCL12 (SDF-1)/CXCR4 interaction	RNA-seq	Poor (3, 13)	↓	Antagonists of CXCR4 or antibodies against CXCR4	CXCR4	Yes	NA
Monocytic signature (CCL2, CCL23, CSF1R)	RNA-seq	Poor (3, 13)	↓	↓ Monocytic cells (CSF1R-blocking, epigenetic modifiers)	CSF1R Epigenetic LAG3 TIM3 CEACAM	Yes Yes Yes Yes Yes	Yes Yes Yes Yes Yes
Checkpoint molecules	IHC	Poor	↓ (9)	↓ Negative regulation (other checkpoint inhibitors)	KIR	Yes	Yes
Targets of interventions: Cell Treg	IHC	Controversial (3)	↓ or ↑	Anti-CCR4 mAb, chemotherapy, CD25-directed immunotoxin	CCR4	Yes	Yes
Tumor-associated macrophages (M2; and MDSCs)	IHC	Poor (3, 13)	↓	↓ Monocytic cells (CSF1R blocking, epigenetic modifiers)	CSF1R Epigenetic	Yes Yes	Yes Yes
Endothelial cells	IHC	Poor (3, 13)	↓	↓ Endothelial cells (hyaluronidase) Anti-VEGF-Ab	Hyaluronidase	NA	NA NA

NOTE: Comparison of immunoreactive MSS with the mesenchymal transcriptional signature, its association with prognosis, treatment modalities to modulate the signature to the preferred immunoreactive state, and list of monotherapy and combination regimens that are currently in the clinic for colorectal cancer. Abbreviations: CAF, cancer-associated fibroblasts; CRC, colorectal cancer; XRT = radiation.

The TME consists of tumor stromal cells, myeloid-derived suppressor cells (MDSC), angiogenic factors, immunosuppressive cytokines, chemokines, and metabolic factors. These immunosuppressive cells directly suppress T-cell function and promote metastasis. Selective depletion of MDSCs with anti-CSF1R was shown to result in delayed tumor growth in mouse models of colorectal adenocarcinoma, increased intratumoral cytotoxic CD8⁺ T cells, and decreased regulatory T cells (Treg; ref. 31). Others demonstrated the combined efficacy of anti-CSF1R and either anti-PD-1 or anti-CTLA4 (32, 33). These data provide rationale for clinical trials combining this agent and checkpoint blockade.

VEGF is a key mediator in angiogenesis, and overexpression has been associated with poor OS. Bevacizumab, which targets VEGF, is often added to first-line chemotherapy, and combination with immunotherapy is being tested clinically (34). Because VEGF inhibits DC maturation, anti-VEGF strategies may also enhance the induction and potency of immune responses. Data indicate that MSI-H tumors are responsive to bevacizumab-containing regimens, and early data show a DCR of 90% in MSI-H patients who received bevacizumab with atezolizumab (anti-PD-L1; ref. 35). Indolea-

mine 2,3-dioxygenase (IDO) is an enzyme that degrades the essential amino acid L-tryptophan and induces T-cell suppression (36). IDO contributes to disease progression and reduces OS in colorectal cancer patients (36, 37), and studies with an IDO inhibitor combined with anti-PD-1 are being pursued in colorectal cancer.

Adenosine is an ubiquitously expressed nucleoside released from metabolically active cells and has known immunosuppressive roles (38). The A2A adenosine receptor (A2AR) is highly expressed in the majority of immune cells. Stimulation leads to inhibition of T-cell and NKT cell proliferation, cytokine production, and proliferation of Tregs and MDSCs (39–41). Adenosine inhibitors are being tested in the clinic in combination with checkpoint inhibitors in colorectal cancer. CD73, an enzyme that performs the phosphohydrolysis of extracellular ATP into adenosine, can also be targeted. CD73-deficient mice are resistant to MC38-OVA colorectal cancer tumors, and anti-CD73 had activity as a monotherapy and was highly synergistic when given with checkpoint inhibitors (5, 42, 43). Many correlative questions need to be answered to understand whether these combination treatments may benefit patients.

The EGFR–RAS–RAF–MEK–ERK1/2 pathway is a critical target for panitumumab, cetuximab, as well as MEK inhibitors. MEK inhibitors can enhance T-cell function and improve checkpoint blockade as demonstrated in murine melanoma models (44). Clinical studies are investigating combinations between these inhibitors as well as with other modalities (45, 46). Data from a trial using atezolizumab in combination with the MEK inhibitor cobimetinib show the combination was well tolerated, and patients had an ORR of 17% (4 PR and 5 SD; ref. 47). This led to a randomized phase III trial comparing the combination with SOC in patients with third-line metastatic colorectal cancer. The combination of selumetinib with anti–PD-L1 in advanced solid tumors is being explored. Analysis of T-cell infiltration and changes in tumor HLA and antigen expression will be key to understanding the mechanisms of synergy for MEK inhibitors and checkpoint blockade.

Epigenetic agents have been used as therapies for cancer. The investigation of epigenetic drugs on the interaction of the immune system and tumors revealed multiple points of potential action (5, 48–51). In the CT26 colorectal cancer model, treatment with anti-CTLA4 and anti–PD-1 had low activity against large tumors, but adding the epigenetic-modulating drugs 5-azacytidine (a DNA methyltransferase inhibitor) and entinostat (a class I HDAC inhibitor) eradicated large tumors in most mice and improved survival (49). The combination of epigenetic modifiers and anti–PD-1/PD-L1 is being tested in multiple clinical trials for colorectal cancer.

Successes in the clinic with anti-CTLA4 and anti–PD-1 in melanoma have led to examining this combination in colorectal cancer (52). Preliminary data of nivolumab and ipilimumab in MSI and MSS colorectal cancer showed OR of 33% and 5%, respectively (7). Other checkpoint inhibitors as well as immune agonists should be studied further. In all cases, infiltrates need to be quantified and immune phenotyped to determine whether long-lasting T-cell memory is formed.

Stimulating the innate immune system can occur by activating pathogen-associated molecular patterns via Toll-like receptors (TLR) or through cyclic dinucleotides. This induces DC and proinflammatory macrophage activation, secretion of type I IFNs, and subsequent antigen presentation (53). Many innate immune agonists are being used in conjunction with therapeutic vaccines, and several trials for colorectal cancer combining TLR agonists with checkpoint blockade are underway.

Replication-competent oncolytic viruses (OV) selectively infect cancer cells, causing tumor cell lysis and activation of the innate immune system. OVs can be genetically modified to include immunomodulatory transgenes to enhance the immune system (54). Several OVs are being investigated in colorectal cancer as single agents and in combination with checkpoint inhibitors. Like innate agonists, OVs aim to augment IFN signaling and promote T-cell recruitment to tumors, making examination of infiltrates key to determining the effects of these agents.

Vaccines are effective public health interventions for infectious disease prevention. However, despite numerous studies, the efficacy of cancer vaccines remains inconclusive (55). Studies investigating neoantigens has prompted renewed excitement in the vaccine field (56). Data in a small clinical trial demonstrated enhancement of T-cell responses when utilizing neoepitopes in a DC vaccine in melanoma patients (57). Numerous studies are examining the effects of therapeutic

vaccination in colorectal cancer. Determining whether specific T-cell responses are engendered against the target antigen, causing increased infiltration into the tumor, and evaluation of the ongoing activation status of the infiltrating T cells are needed. With the addition of checkpoint inhibition and other TME modulators, therapeutic vaccines may have a renewed role in immunotherapy.

The intestinal microbiota form a symbiotic relationship with the host and have broad functions in immunity, inflammation, and disease (58). Numerous reports suggest that certain bacterial species have been disproportionately associated with colorectal cancer tumors and may contribute to disease pathogenesis (5, 59–64). The microbiome plays a role in immunotherapy treatment outcomes in mouse models and humans (5, 65–67). In melanoma patients treated with ipilimumab, the presence of the *Bacteroidetes* phylum in feces correlated with resistance to the development of checkpoint blockade–induced colitis (68). These results suggest that the microbiome could be used as a predictive biomarker during immunotherapy treatment and that, at least in mice, transfer of specific species could potentiate antitumor effects. Future clinical application will require fecal collection for microbiome analyses to assess whether microbiota dysbiosis occurs at different stages of disease and with different treatment regimens. This will improve our understanding of whether the microbiome is prognostic and/or predictive of treatment effects.

In summary, data show the promise of immunotherapy for colorectal cancer, in at least a subset of patients. Further studies that analyze the immune contexture and genomic profiles of these patients will aid in our understanding of why some colorectal cancer patients respond to immunotherapy while others do not. The goal of convening experts in colorectal cancer and immunotherapy was to fill the gaps in our understanding of this difficult-to-treat patient population and provide a platform for collaborative research to move immunotherapy into the SOC for colorectal cancer patients.

Disclosure of Potential Conflicts of Interest

M.A. Morse reports receiving commercial research support from Bristol-Myers Squibb and Merck. C.R. Heery is the chief medical officer at and has ownership interest in Bavarian Nordic. D.A. Deming is a consultant/advisory board member for Bristol-Myers Squibb. L.A. Diaz is on the board of directors at PGDx, has ownership interest in PapGene and PGDx, and is a consultant/advisory board member for Merck and PGDx. W.H. Fridman is a consultant/advisory board member for Adaptimmune and Novartis. E.M. Jaffee reports receiving commercial research support from Amgen and Bristol-Myers Squibb. C.L. Sears reports receiving a commercial research grant from and is a consultant/advisory board member for Bristol-Myers Squibb. N.H. Segal reports receiving commercial research support from Bristol-Myers Squibb, MedImmune/AstraZeneca, Merck, and Roche/Genentech and is a consultant/advisory board member for Bristol-Myers Squibb, MedImmune/AstraZeneca, Merck, Pfizer, and Roche/Genentech. R.A. Moss is the medical director at and has ownership interest (including patents) in Bristol-Myers Squibb. A.B. Benson reports receiving commercial research grants from Genentech and Merck and is a consultant/advisory board member for Bristol-Myers Squibb, Genentech, and Merck. No potential conflicts of interest were disclosed by the other authors.

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