**Cancer Immunology Research**

# 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

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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 IFNγ-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, TGFβ 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

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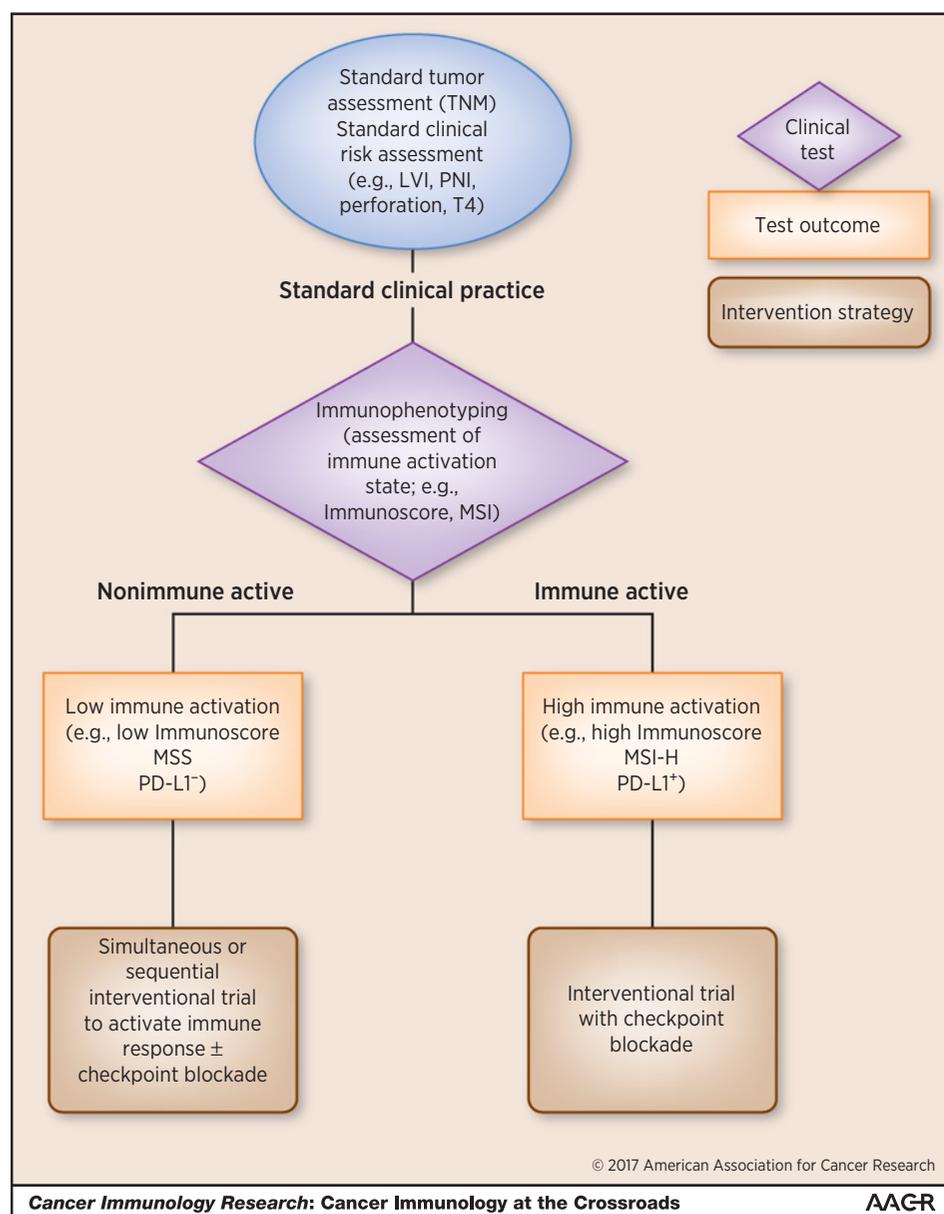


**Figure 1.**
Proposed immunologic evaluation of patients with early-stage colorectal cancer. Standard tumor assessment and clinical assessment will lead to treatment via standard clinical practice for early-stage colorectal cancer patients. This could be supplemented by immunophenotyping consisting 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, immunopheno-typing 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).
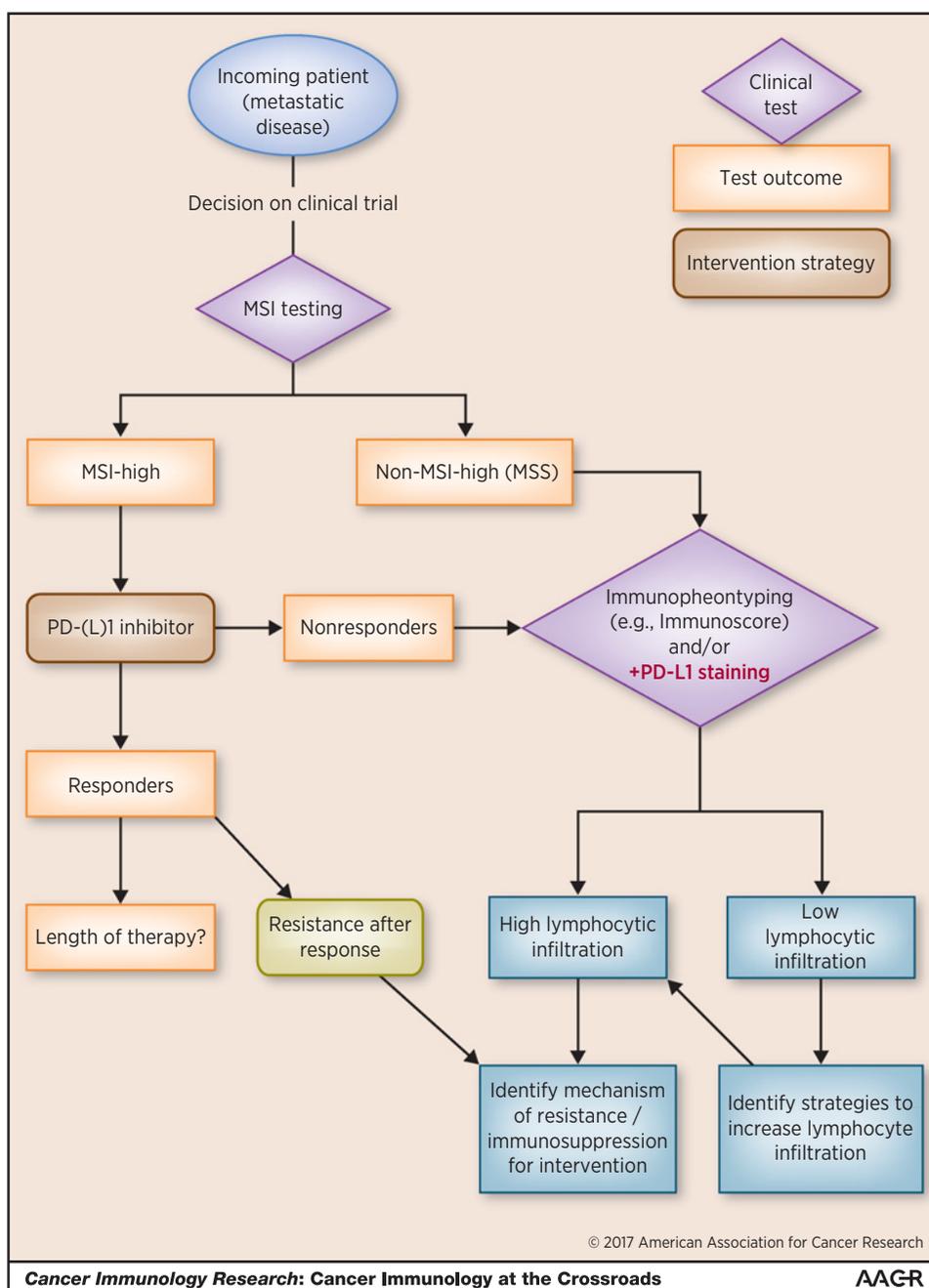
## Discussion

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.

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**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 | Yes | Yes |
| | | | | | TLRs | Yes | NA |
| | | | | | STING | NA | NA |
| | | | | | Epigenetic | Yes | Yes |
| Mesenchymal (VEGF, TGFB, galectin, COX-1) | RNA-seq | Poor (3, 13) | ↓ | ↓ Angiogenesis (VEGF blocking) | VEGF | Yes | Yes |
| | | | | ↓ Immunosuppression (TGFb inhibitor) | TGF | Yes | Yes |
| | | | | ↓ Immunosuppression (Galectin inhibitor) | Galectin | NA | NA |
| | | | | ↓ Immunosuppression (Cox inhibitor) | COX | Yes | Yes |
| | | | | ↓ Immunosuppression (epigenetic) | Epigenetic | 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 | Yes | Yes |
| | | | | | Chemo | Yes | Yes |
| | | | | | Neoantigens | Yes | NA |
| | | | | | Vaccines | Yes | 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 | Yes | Yes |
| | | | | | Epigenetic | Yes | Yes |
| | | | | | LAG3 | Yes | Yes |
| | | | | | TIM3 | Yes | Yes |
| | | | | | CEACAM | 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 | Yes | Yes |
| | | | | | Epigenetic | 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 disproportionally 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

## Authors' Contributions

**Conception and design:** D.T. Le, V.M. Hubbard-Lucey, M.A. Morse, C.R. Heery, A. Dwyer, L.A. Diaz, S.R. Hamilton, F. Housseau, E.M. Jaffee, S.P. Kang, C.H. Lieu, W. Messersmith, N.H. Segal, R.A. Moss, J. O'Donnell-Tormey, N. Roach, A.B. Benson

## References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9–29.
2. Lal N, Beggs AD, Willcox BE, Middleton GW. An immunogenomic stratification of colorectal cancer: implications for development of targeted immunotherapy. Oncoimmunology 2015;4:e976052.
3. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012;12: 298–306.
4. Giannakis M, Mu XJ, Shukla SA, Qian ZR, Cohen O, Nishihara R, et al. Genomic correlates of immune-cell infiltrates in colorectal carcinoma. Cell Rep 2016 Apr 14. [Epub ahead of print].
5. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455–65.
6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443–54.
7. Overman M, Kpetz S, McDermott RS, Leach J, Lonardi S, Heinz-Josef L, et al. Nivolumab ± ipilimumab in treatment (tx) of patients (pts) with metastatic colorectal cancer (mCRC) with and without high microsatellite instability (MSI-H): CheckMate-142 interim results. J Clin Oncol 34: 15s, 2016(suppl; abstr 3501).
8. Guinney J, Dienstmann R, Wang X, de Reynies A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–6.
9. Xiao Y, Freeman GJ. The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. Cancer Discov 2015;5:16–8.
10. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov 2015;5:43–51.
11. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20.
12. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313: 1960–4.
13. Galon J, Mlecnik B, Marliot F, Ou F-S, Bifulco CB, Lugli A, et al. Validation of the Immunoscore (IM) as a prognostic marker in stage I/II/III colon cancer: Results of a worldwide consortium-based analysis of 1,336 patients. J Clin Oncol 34: 15s, 2016(suppl; abstr 3500).
14. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 2016;44:698–711.
15. Sinicrope F, Qian S, Hermitte F, Heying EN, Benson AB, Gill S, et al. Association of immune markers and Immunoscore with survival of stage III colon carcinoma (CC) patients (pts) treated with adjuvant FOLFOX: NCCTG N0147 (Alliance). J Clin Oncol 35: 15s, 2017(suppl; abstr 3579).
16. Fujiyoshi K, Yamamoto G, Takenoya T, Takahashi A, Arai Y, Yamada M, et al. Metastatic pattern of stage IV colorectal cancer with high-frequency microsatellite instability as a prognostic factor. Anticancer Res 2017;37: 239–47.

17. Fridman WH, Zitvogel L, Sautes-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol 2017 Jul 15. [Epub ahead of print].
18. Becht E, de Reynies A, Giraldo NA, Pilati C, Buttard B, Lacroix L, et al. Immune and stromal classification of colorectal cancer is associated with molecular subtypes and relevant for precision immunotherapy. Clin Cancer Res 2016;22:4057–66.
19. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell 2016;165:35–44.
20. Chen L, Gibbons DL, Goswami S, Cortez MA, Ahn YH, Byers LA, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat Commun 2014;5:5241.
21. Chen L, Heymach JV, Qin FX, Gibbons DL. The mutually regulatory loop of epithelial-mesenchymal transition and immunosuppression in cancer progression. Oncoimmunology 2015;4:e1002731.
22. Mak MP, Tong P, Diao L, Cardnell RJ, Gibbons DL, William WN, et al. A Patient-derived, pan-cancer EMT signature identifies global molecular alterations and immune target enrichment following epithelial-to-mesenchymal transition. Clin Cancer Res 2016;22:609–20.
23. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology. J Clin Oncol 2017;35: 1453–86.
24. Rovere P, Vallinoto C, Bondanza A, Crosti MC, Rescigno M, Ricciardi-Castagnoli P, et al. Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function. J Immunol 1998;161:4467–71.
25. Golden EB, Frances D, Pelliccotta I, Demaria S, Helen Barcellos-Hoff M, Formenti SC. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. Oncoimmunology 2014;3:e28518.
26. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012;366:925–31.
27. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res 2013;1:365–72.
28. Gulley JL, Arlen PM, Bastian A, Morin S, Marte J, Beetham P, et al. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. Clin Cancer Res 2005;11:3353–62.
29. Willett CG, Fung CY, Kaufman DS, Efird J, Shellito PC. Postoperative radiation therapy for high-risk colon carcinoma. J Clin Oncol 1993;11: 1112–7.
30. Tesniere A, Apetoh L, Ghiringhelli F, Joza N, Panaretakis T, Kepp O, et al. Immunogenic cancer cell death: a key-lock paradigm. Curr Opin Immunol 2008;20:504–11.
31. Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell 2014;25:846–59.
32. Holmgaard RB, Brachfeld A, Gasmi B, Jones DR, Mattar M, Doman T, et al. Timing of CSF-1/CSF-1R signaling blockade is critical to improving responses to CTLA-4 based immunotherapy. Oncoimmunology 2016;5: e1151595.

33. Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res 2014;74:5057–69.

34. Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, Sasada T, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. Cancer Immunol Res 2014;2:632–42.

35. Hochster H, Bendell JC, Cleary HM, Foster P, Zhang W, He X, et al. Efficacy and safety of atezolizumab (atezo) and bevacizumab (bev) in a phase Ib study of microsatellite instability (MSI)-high metastatic colorectal cancer (mCRC). J Clin Oncol 35: 4s, 2017(suppl; abstr 673).

36. Takamatsu M, Hirata A, Ohtaki H, Hoshi M, Ando T, Ito H, et al. Inhibition of indoleamine 2,3-dioxygenase 1 expression alters immune response in colon tumor microenvironment in mice. Cancer Sci 2015;106:1008–15.

37. Brandacher G, Perathoner A, Ladurner R, Schneeberger S, Obrist P, Winkler C, et al. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. Clin Cancer Res 2006; 12:1144–51.

38. Ernst PB, Garrison JC, Thompson LF. Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology. J Immunol 2010; 185:1993–8.

39. Zarek PE, Huang CT, Lutz ER, Kowalski J, Horton MR, Linden J, et al. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. Blood 2008;111:251–9.

40. Gessi S, Cattabriga E, Avitabile A, Gafa R, Lanza G, Cavazzini L, et al. Elevated expression of A3 adenosine receptors in human colorectal cancer is reflected in peripheral blood cells. Clin Cancer Res 2004;10:5895–901.

41. Ohta A. A metabolic immune checkpoint: adenosine in tumor microenvironment. Front Immunol 2016;7:109.

42. Allard B, Pommey S, Smyth MJ, Stagg J. Targeting CD73 enhances the antitumor activity of anti-PD-1 and anti-CTLA-4 mAbs. Clin Cancer Res 2013;19:5626–35.

43. Stagg J, Divisekera U, Duret H, Sparwasser T, Teng MW, Darcy PK, et al. CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. Cancer Res 2011;71:2892–900.

44. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, et al. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. Sci Transl Med 2015;7:279ra41.

45. Connolly K, Brungs D, Szeto E, Epstein RJ. Anticancer activity of combination targeted therapy using cetuximab plus vemurafenib for refractory BRAF (V600E)-mutant metastatic colorectal carcinoma. Curr Oncol 2014;21:e151–4.

46. Yaeger R, Cercek A, O'Reilly EM, Reidy DL, Kemeny N, Wolinsky T, et al. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. Clin Cancer Res 2015; 21:1313–20.

47. Bendell J, Kim TW, Goh BC, Wallin J, Oh DY, Han SW, et al. Clinical activity and safety of cobimetinib (cobi) and atezolizumab in colorectal cancer (CRC). J Clin Oncol 34: 15s, 2016(suppl; abstr 3502).

48. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature 2015;527:249–53.

49. Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. Proc Natl Acad Sci USA 2014;111: 11774–9.

50. Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell 2015;162:974–86.

51. Li H, Chiappinelli KB, Guzzetta AA, Easwaran H, Yen RW, Vatapalli R, et al. Immune regulation by low doses of the DNA methyltransferase inhibitor 5-azacitidine in common human epithelial cancers. Oncotarget 2014;5: 587–98.

52. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med 2015;372:2006–17.

53. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. Science 2010;327:291–5.

54. Zamarin D, Pesonen S. Replication-competent viruses as cancer immunotherapeutics: emerging clinical data. Hum Gene Ther 2015;26:538–49.

55. Romero P, Banchereau J, Bhardwaj N, Cockett M, Disis ML, Dranoff G, et al. The human vaccines project: a roadmap for cancer vaccine development. Sci Transl Med 2016;8:334ps9.

56. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015;348:69–74.

57. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 2015;348:803–8.

58. Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. Cell Host Microbe 2014;15:317–28.

59. Sears CL, Geis AL, Housseau F. *Bacteroidesfragilis* subverts mucosal biology: from symbiont to colon carcinogenesis. J Clin Invest 2014;124:4166–72.

60. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 2013;14:207–15.

61. Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res 2012;22:299–306.

62. Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. Front Microbiol 2015;6:20.

63. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, et al. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res 2012;22:292–8.

64. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacteriumnucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell Host Microbe 2013;14: 195–206.

65. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015;350:1079–84.

66. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013;342:967–70.

67. Daillere R, Vetizou M, Waldschmitt N, Yamazaki T, Isnard C, Poirier-Colame V, et al. Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced Therapeutic Immunomodulatory Effects. Immunity 2016;45:931–43.

68. Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. Nat Commun 2016;7:10391.

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