Curr. Treat. Options in Oncol. (2016) 17: 41
DOI 10.1007/s11864-016-0414-4

Lower Gastrointestinal Cancers (AB Benson, Section Editor)

Deficient Mismatch Repair and the Role of Immunotherapy in Metastatic Colorectal Cancer

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Published online: 17 June 2016

This article is part of the Topical Collection on *Lower Gastrointestinal Cancers*

Keywords Immunotherapy · Colorectal cancer · Mismatch repair · Microsatellite instability · Immune checkpoint · Tumor microenvironment

Opinion statement

Division of colorectal cancers (CRCs) into molecular subsets yields important consequences for prognosis and therapeutic response. The microsatellite instability (MSI) immune subgroup, accounting for 15 % of early-stage and 3 % of metastatic CRCs, are a result of deficient cellular DNA mismatch repair (dMMR) mechanisms. dMMR CRCs are notable for greater survivability, yet lack of benefit from fluoropyrimidine-based therapy in early-stage disease as compared to proficient DNA mismatch repair (pMMR) CRCs but are substantially lethal when metastatic. The surging interest in cancer immunotherapy, particularly checkpoint blockade, has further led to a focus on MSI tumors, which are notable for their substantial T cell infiltrate. In this review, we will discuss the biologic underpinnings for the immunogenicity of dMMR CRC and the preclinical development of therapies intended to modulate this immune response. Next, we will discuss the previous and ongoing clinical trials specifically designed to evaluate immunotherapeutic treatment of dMMR CRCs. Building on the success of the early immune checkpoint inhibitor clinical trials for dMMR CRC, combinations with other antitumor immunotherapies may provide an even more robust response, thereby,

creating an alternative treatment regimen for those who have failed standard therapies or possibly resulting in prophylactic therapies for patients with highly oncogenic hereditary mismatch repair deficiencies.

Introduction

Worldwide, colorectal cancer is the second most common cancer in women and third most common in men, with ~1.4 million new cases diagnosed every year [1]. While increased screening and early intervention have reduced the rate of late-stage disease presentation, colorectal cancer (CRC) still claims the lives of almost 700,000 people annually worldwide [1, 2]. Accruing molecular insights into the pathogenesis of CRC have led to the proposal for new consensus molecular subtypes (microsatellite instability immune, canonical, metabolic, and mesenchymal) with the goal of stratifying clinical prognosis and providing a basis for development of focused therapies [3•]. Specifically, the microsatellite instability (MSI) immune subgroup comprises ~15 % of all CRCs and results from one or more deficient DNA mismatch repair (dMMR) proteins within the tumor cells [3•, 4].

The molecular pathogenesis of dMMR CRC arises from either mutational or epigenetic silencing of DNA repair genes [5, 6]. In sporadic dMMR CRC, the most common defect is hypermethylation of the promoter region of the MLH1 gene [6], however, mismatch repair-associated mutations in the MSH2, MSH6, MLH3, PMS2, and EXO1 genes also can play a role in dMMR CRC development [7–10]. Non-MLH1 gene mutations are particularly likely to be involved in the CRC pathogenesis when they are inherited at the germline level, such as seen with hereditary non-polyposis colorectal cancers (Lynch syndrome) [11]. The presence of dysfunctional mismatch repair, manifested as an increase in microsatellite size variability throughout the patient's genome, ultimately leads to further DNA mutations. Multiple resulting frameshift mutations have been directly linked to the development of dMMR CRC, including those affecting tumor-suppressor genes such as TGF-βRII and BAX [12–14].

dMMR CRCs possess many unique characteristics that make them distinguishable from other CRCs. In terms of clinical presentation, they more commonly originate in the proximal colon, as opposed to CRCs with proficient mismatch repair (pMMR) mechanisms, which are more commonly found at distal sites [15–17]. Additionally, dMMR CRC patients have a greater inflammatory state as evidenced by higher C-reactive protein, neutrophil, and platelet counts than pMMR CRC patients, as well as worse prognostic inflammatory scores based on these variables [18]. Histological tumor comparisons reveal that dMMR CRCs are more likely to be expansile than infiltrative, lack heterogeneity and "dirty necrosis", appear to have a Crohn's-like inflammatory response and/or mucinous differentiation, and have an abundance of tumor-infiltrating lymphocytes (TILs) [19, 20]. Importantly, it has been established that dMMR CRC patients have overall superior survival outcomes and are less likely to have metastases than pMMR CRC patients [16, 21]. However, should a dMMR CRC metastasize or relapse following initial treatment, this advantage disappears and they fair no better, if not worse, than pMMR metastatic CRC patients [3•, 22, 23]. This prognostic improvement in early-stage disease is hypothesized to be correlated with the robust TIL response within dMMR CRCs, which is an induced reaction to the neoantigens generated by dMMR-related hypermutations [20, 24•]. The development of novel immune checkpoint inhibitors, vaccines, and other immunotherapeutics has opened the possibility to exploit the intrinsic immunogenicity of dMMR CRCs for an improved therapeutic outcome over current standard CRC therapies of metastatic disease. In this review, we

will focus on the intratumoral and systemic immunity related to dMMR CRCs and the role that immunotherapy may play in the treatment of these malignancies.

Immune-related genetics and cell biology of mismatch repair-deficient colorectal cancers

Neoantigen production

Aside from disabling tumor-suppressor genes, frameshift mutations produced within the genomes of dMMR CRC patients may also yield tumor-specific peptides. These peptides are referred to as neoantigens and can be processed and presented from patient MHC molecules. Almost 30 years ago, Bodmer et al. hypothesized that creation of these neoantigens might cause an increased rate of tumor immune recognition and, ultimately, be one of the means by which Lynch syndrome CRCs have an improved prognosis over other CRCs [25]. Utilization of software-predicted HLA motifs subsequently assisted in identifying potential frameshift peptides that could serve as immunogenic epitopes within dMMR CRCs [26–30], which eventually lead to the identification of neoantigen-specific cytotoxic T lymphocyte (CTL) responses against human dMMR CRC cells [31, 32].

dMMR CRC neoantigen-specific immunity is thought to be further promoted by their greater dendritic cell (DC) infiltrate, which allows for increased processing and presentation of neoantigen epitopes to CTLs [33]. Macrophages and mature DCs have also been found at higher concentrations within dMMR CRCs, which suggests that they may promote effector T cell proliferation and tumor site migration [33–35]. Further evidence of the immunogenicity of dMMR tumors includes observations that Lynch syndrome patients possess measureable serum antibodies against dMMR CRC-associated frameshift peptides [36]; however, the clinical implications of these findings are questionable as there is currently no evidence to suggest that the quantity or quality of healthy Lynch syndrome patients' antibody responses differ between those with a history of CRC. It should also be noted that dMMR CRC immunogenicity could be driven by the presence of other unique non-frameshift antigens dominant in a dMMR environment, such as antigens that are splice variants [37], virus-based [38], and constitutively overexpressed [39].

Effector T cells

In order for a tumor to co-exist with healthy tissues in a patient's body, it must sustain immunologic tolerance and evade T cell-mediated killing. When comparing dMMR to pMMR CRCs, it has not only been documented that dMMR CRCs possess a larger quantity of TILs, but also that the ratio of activated TILs is greater and that TIL concentration is positively correlated to tumor cell cytotoxicity [20, 40, 41]. Moreover, dMMR CRCs have been found to have lower

rates of TIL apoptosis than pMMR CRCs [42]. High CD8+ TIL density has been established as a good prognostic marker for most CRCs [43, 44], however, it has greater prognostic significance for dMMR than for pMMR CRC [45]. Multiple studies have also found increased densities of memory CD45RO+ TILs in dMMR over pMMR CRC tumors, a biomarker associated with decreased signs of metastatic invasion, lower tumor staging, and increased survival rates among all CRC patients [35, 44, 46].

Another characteristic of dMMR CRC that promotes immunogenicity is its diminished pathologic inflammatory response, thought to be mediated by its increased levels of circulating and intratumoral regulatory T cell (Treg) subsets [33, 47, 48]. Although Tregs can inhibit activation and function of effector T cells, NK cells, and other anti-tumor mediators and are generally associated with poor cancer prognoses [49], Tregs are counterintuitively associated with improved CRC outcomes [46, 50]. This finding is thought to be related to the inhibitory effects that colonic Tregs are believed to have against Th17-mediated inflammation [51]. When there is a Th17-dominant colonic microbiome, the environment promotes VEGF-directed angiogenesis and inhibition of DC maturation and differentiation [52–54], which in turn is associated a with poorer CRC prognosis [51, 55]. There is also support for dMMR CRC's sensitivity to inflammatory mediators in the retrospective observation that stage II/III dMMR CRC patients who received the anti-VEGF drug, bevacizumab, had improved disease-free and overall survival while their pMMR counterparts did not [56].

Immune evasion mechanisms

Despite the robust immune response, dMMR CRCs persist through a variety of immune-evasive actions. For example, mutations in HLA class I genes and loss of the HLA class I expression appear to be more common in dMMR than pMMR CRCs [57]. Additionally, the presence of mutations in β2-microglobulin, a component of the class I complex, leads to HLA class I expression loss on dMMR CRC cells and is associated with higher disease staging [58]. Lack of HLA expression prevents presentation of neoantigens to CTLs, however, this may also leave malignant cells open to detection by other anti-tumor detecting cells (e.g., NK cells).

Furthermore, dMMR CRCs appear to have greater tumor infiltration of immune cells expressing the immune checkpoint protein PD-L1 [59••], which is known to repress effector T cell activation against tumor cells. While immune checkpoint proteins have been identified as promoters of cancer pathogenesis, it should also be noted that the amount of tumor and immune cell PD-L1 expression is often found to be positively correlated to anti-PD-1 treatment responsiveness [60]. These immunogenic attributes have driven several dMMR/pMMR CRC-stratified clinical trials in which the differential responses to immunotherapy among these groups have been studied.

Immunotherapy against mismatch repair-deficient colorectal cancers

Vaccines

Therapeutic cancer vaccines serve as an attractive method for the induction of a durable immunogenic response against tumor-associated antigens. A large

Table 1. Immunotherapy clinical trials treating mismatch repair-deficient colorectal cancer

ClinicalTrials.gov identifier and sponsor	Patient characteristics	Treatment regimen	Treatment duration	Phase and status
NCT01461148—Oryx GmbH & Co. KG	Stage III or IV dMMR/MSI CRC	Frameshift peptide vaccination (100 µg each of TAF1B(-1), HT001(-1), and AIM2(-1)) administered with a water-in-oil adjuvant emulsion	Weekly IV vaccinations for 4 consecutive weeks per cycle, for a total of 3 cycles	Phase I/II; completed
NCT01633970—Genentech, Inc.	Multiple cancer types, including dMMR/MSI CRC	Atezolizumab (800 or 1,200 mg) with chemotherapy (bevacizumab ± FOLFOX, nabpaclitaxel ± carboplatin, carboplatin and paclitaxel, or carboplatin and pemetrexed)	Atezolizumab: IV every 2 or 3 weeks Chemotherapy: varies by regimen[a]	Phase Ib; actively recruiting
NCT01876511—Sidney Kimmel Comprehensive Cancer Center	dMMR/MSI CRC, pMMR/MSS CRC, and dMMR/MSI non-CRC	Pembrolizumab (10 mg/kg)	IV infusions every 14 days	Phase II; actively recruiting
NCT01885702—Radboud University	dMMR/MSI CRC and healthy dMMR/MSI carriers with HLA-A2.1 phenotypes	Frameshift-derived neoantigen-loaded dendritic cell vaccination	Unspecified	Phase I/II; ongoing, not actively recruiting
NCT02060188—Bristol-Myers Squibb	dMMR/MSI and pMMR/MSS CRC	Nivolumab monotherapy (3 mg/kg) or dose escalation[b] of nivolumab (0.3, 1, or 3 mg/kg) and ipilimumab (1 or 3 mg/kg), followed by nivolumab monotherapy	Nivolumab monotherapy: IV every 2 weeks until DP Nivolumab/ipilimumab combo: every 3 weeks for 4 doses, or until DP	Phase I/II; actively recruiting
NCT02227667—Memorial Sloan Kettering Cancer Center	dMMR/MSI CRC and/or TIL-high CRC	Durvalumab	IV infusions for 12 months, until DP, or until alternative cancer therapy initiated	Phase II; actively recruiting
NCT02432963—City of Hope Medical Center	p53-overexpressing cancers, including dMMR/MSI CRC	Modified vaccinia virus Ankara vaccine expressing p53 with pembrolizumab	Vaccine: SC every 3 weeks for 3 doses Pembrolizumab: IV every 3 weeks for 3 7 doses	Phase I; ongoing, not actively recruiting
NCT02460198—Merck Sharp & Dohme Corp.	Stage IV dMMR/MSI CRC	Pembrolizumab (200mg)	IV infusions every 3 weeks for up to 2 years	Phase II; actively recruiting
NCT02460224—Novartis Pharmaceuticals	Multiple cancer types, including dMMR/MSI CRC Stage IV dMMR/MSI CRC	LAG3 inhibitor (LAG525) ± PD-1 inhibitor (PDR001)	Unspecified	Phase II; actively recruiting

Table 1. (continued)

ClinicalTrials.gov identifier and sponsor	Patient characteristics	Treatment regimen	Treatment duration	Phase and status
NCT02563002—Merck Sharp & Dohme Corp.		Pembrolizumab (200mg) or standard therapy (mFOLFOX6- or FOLFIRI-based ± bevacizumab or cetuximab)	Pembrolizumab: IV every 3 weeks for up to 35 doses Standard therapy: IV 2 week cycles[c]	Phase III; not yet open for enrollment
NCT02646748—Incyte Corp.	Historically anti-PD-1-responsive cancers (e.g., dMMR/MSI CRC)	Pembrolizumab with JAK1 inhibitor (INCB039110) or PI3K-δ inhibitor (INCB050465)	Pembrolizumab: IV every 3 weeks JAK1 and PI3K-δ inhibitors: PO daily	Phase Ib (Part 1b); actively recruiting

Legend: *DP* disease progression, *IV* intravenous, *PO* by mouth, *SC* subcutaneous

[a]Bevacizumab is given at 10 or 15 mg/kg IV every 3 weeks. FOLFOX consists of 2-week cycles of IV oxaliplatin (85 mg/m^2), leucovorin (400 mg/m^2), or levoleucovorin (200 mg/m^2), and 5-fluorouracil (5-FU; 400 mg/m^2 bolus). Carboplatin is administered to a target area under the curve (AUC) of 6 mg/mL IV every 3 weeks. Paclitaxel is given at 200 mg/m^2 IV every 3 weeks. Pemetrexed is given at 500 mg/m^2 IV every 3 weeks. Nab-paclitaxel is given at 100 mg/m^2 IV every week

[b]Four nivolumab/ipilimumab dose combinations will be tested: 0.3/1, 1/1, 1/3, and 3/1 mg/kg

[c]mFOLFOX6 consists of 2-week cycles with oxaliplatin (85 mg/m^2), leucovorin (400 mg/m^2) or levoleucovorin (200 mg/m^2), and 5-fluorouracil (5-FU; 400 mg/m^2 bolus) on the first day, then 5-FU (1200 mg/m^2) over two additional days. FOLFIRI is identical to mFOLFOX6, except that oxaliplatin is substituted for irinotecan (180 mg/m^2) on the first day. Bevacizumab (5 mg/kg) is given on the first day of each 2-week cycle. Cetuximab is given at 400 mg/m^2 over 2 h, then at 250 mg/m^2 over 1 h every 7 days of each 2-week cycle

number of CRC vaccine clinical trials have been initiated and completed, including those based on dendritic cells, autologous tumor cells, recombinant viral vectors, and/or peptides [61–66]. Despite the many various attempts, there have been mixed results produced from these studies. Nonetheless, a recent retrospective analysis of CRC patients treated with active specific immunotherapy (ASI) revealed there to be a possible therapeutic difference in dMMR/MSI CRC subjects [67••]. In this study, CRC patients who had resection of their primary tumor were randomly assigned to receive or not receive four rounds of intradermal injections containing a mixture of irradiated autologous tumor cells and Bacillus Calmette-Guérin bacteria (referred to as ASI) [68]). The investigators initially concluded that there was a recurrence-free survival benefit for Dukes B (stage II) ASI-treated patients, but no benefit for Dukes C (stage III) patients; a difference initially attributed to differences in tumor burden. After this initial study, the researchers revisited the patients' preserved tumor samples to compare 31 dMMR and 154 pMMR (Dukes B and C) CRC specimens [67••]. Recurrence-free 15-year survival of dMMR CRC patients (23/27; 85.2 %) was found to be significantly greater than that of pMMR CRC patients (99/154; 64.3 %), independent of treatment group. While this finding is congruent with previous reports of improved dMMR CRC survival, it was most profound that the entire dMMR CRC cohort had a significantly improved survival rate over almost all pMMR CRC groups (e.g., Dukes B patients without ASI, Dukes C patients with ASI, Dukes C patients without ASI). dMMR CRC patients had a greater percentage of patients with recurrence-free survival than the ASI-treated pMMR CRC Dukes B group, although this difference was not statistically significant.

Ultimately, it should be noted that there was no significant difference in recurrence rates between the non-treated versus ASI-treated dMMR CRC cohorts; therefore, the researchers could not verify if ASI treatment was beneficial for these patients. Should the dMMR CRC group size have been larger, it might be suspected that the immunotherapy administered could induce at least an equal amount of benefit to that seen in the pMMR CRC group. Alternatively, the authors of this study point to the idea that the surgical tumor removal may induce enough of an inflammatory response in these neoantigen-rich cancers that they may already have reached a sort of anti-tumor immune response "limit" that would not benefit from further stimulation. In any case, this report shows the value of differentiating the clinical responses and outcomes that dMMR and pMMR CRCs yield following immunotherapy treatment.

In addition, a small phase I/II peptide vaccine clinical trial has also been conducted with dMMR CRC patients. The vaccine consisted of three frameshift neoantigens commonly associated with dMMR CRC (AIM2(−1), HT001(−1), and TAF1B(−1)) combined with Montanide® ISA-51 VG, a water-in-oil adjuvant emulsion used to promote vaccine immunogenicity (NCT01461148; Table 1) [69, 70]. The first results to be published out of this study described a single patient having detectable levels of both anti-HT001(−1) and anti-TAF1B(−1) antibodies [69]. These findings were expanded upon at the 2015 American Society for Clinical Oncology Annual Meeting, where preliminary results of this study were presented to show a favorable safety profile and found novel measurable induction of cell-mediated and humoral immunity against at least one frameshift peptide in all vaccinated patients [71]. As of this time, no results showing overall clinical outcomes of this patient cohort has been

published, although it was presented at the aforementioned meeting that one patient with stage IV disease had showed stable carcinoembryonic antigen (CEA) levels and disease for greater than 7 months after initiating the vaccination protocol.

A third vaccine trial is currently ongoing in which 5 dMMR CRC patients are being compared to 20 patients who have Lynch syndrome without any history of cancer (Table 1; NCT01885702). The administered vaccine consists of autologous dendritic cells that have been loaded with dMMR CRC-specific neoantigens, a method that has had some success in previous CRC clinical trials [63]. While these types of trials have yielded some intriguing findings, it is overtly clear that this data is far too limited to confirm therapeutic benefit of cancer vaccinations for dMMR CRC patients and there is a substantial need for further inclusion and identification of these patients within future vaccination trials.

Immune checkpoint inhibitors

Current immune checkpoint targeting therapies function by either inhibiting the T cell activation phase (blocking the interaction of T cell-expressed CTLA4 and antigen-presenting cell-expressed CD80/CD86) or the T cell effector phase (blocking the interaction of tumor or immune cell-expressed PD-L1/PD-L2 and T cell-expressed PD-1; Fig. 1). Limited data is available regarding the role of CTLA4 in CRC and whether anti-CTLA4 antibody therapy would be beneficial for dMMR CRC or any CRCs in general, although certain CTLA4

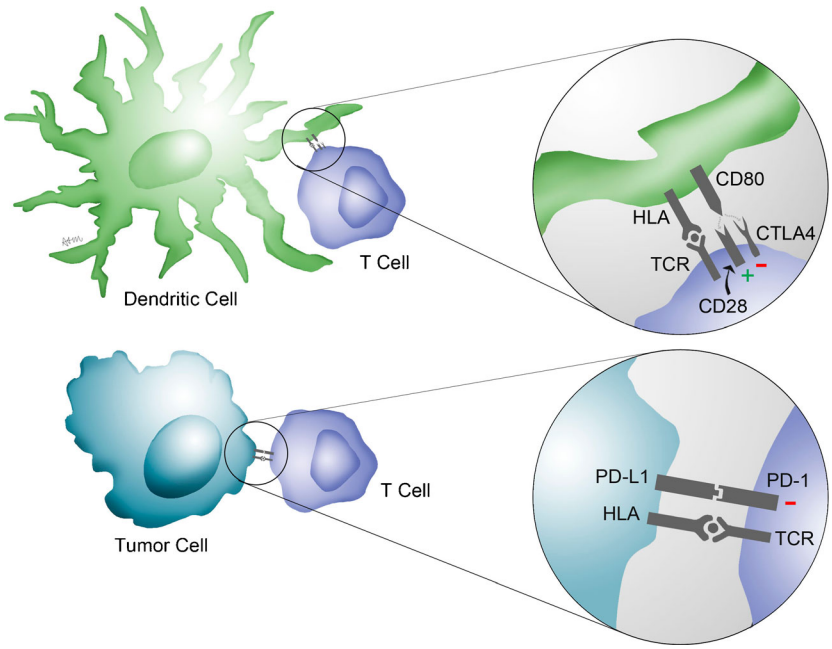

Fig. 1. (*Upper*) Diagram of dendritic cell/T cell interaction demonstrating the presentation of antigen by HLA molecules to the T cell receptor (TCR) and the activation of T cells by CD80 interacting with CD28 and the inhibition of T cells by the interaction of CD80 with CTLA4. (*Lower*) Diagram of tumor cell/T cell interaction demonstrating the presentation of antigen by HLA molecules to the T cell receptor (TCR) and the inhibition of T cells by the interaction of PD-L1 with PD-1. This illustration appears courtesy of Amber Morse.

polymorphisms have been suggested to be linked to poor CRC prognosis [72]. At this time, the only phase II trial studying the effects of an anti-CTLA-4 inhibitor (tremelimumab) on metastatic colorectal cancer reported only a 2 % response rate [73].

Alternatively, the targeting of PD-1/PD-L1 interactions has been found to produce greater treatment efficacy across a wide variety of malignancies. These cancers frequently demonstrate increased PD-1 expression, which historically has been associated with an immunologically tolerant tumor environment and the blockade of effector T cell activation [74]. Specifically, PD-L1 is expressed by >40 % of CRCs and has been correlated with increased tumor stage, poorer differentiation, and shorter overall survival [75]. In contrast to previous studies, early anti-PD-1 antibody clinical trials involving metastatic CRC patients found no substantial treatment benefit [76–79]. It appears that the most robust therapeutic responses to anti-PD-1 and anti-CTLA-4 drugs are seen in highly mutagenic malignancies (e.g., melanoma, non-small cell lung cancer), with the quantity of mutations present in each individual tumor also being positively correlated with the likelihood of immune checkpoint inhibitor treatment response [76, 80–82]; therefore, the necessity for re-evaluating the role of PD-L1/PD-1 interaction targeting in dMMR CRC became apparent [24•].

Based on the large neoantigen load, profuse T cell infiltration, and high PD-L1 expression, Le et al. hypothesized that dMMR/MSI CRC would have a significant clinical response to pembrolizumab (humanized anti-PD-1 antibody) treatment [59••]. This group conducted a phase II study in which stage IV CRC patients with or without dMMR tumors were administered 10 mg/kg pembrolizumab intravenously (IV) every 14 days and assessed for objective response, disease progression, and overall survival time (Table 1; NCT01876511). At the 20-week time point, only 1/10 dMMR CRC patients experienced progression of their disease, as compared to 11/18 pMMR CRC patients. Moreover, 40 % of the dMMR CRC patients had a radiographically objective response rate (as determined by Response Evaluation Criteria in Solid Tumors (RECIST) analyses) while none of the pMMR CRC patients achieved any response to pembrolizumab therapy. Progression-free and overall survival rates were also found to be significantly greater in the dMMR CRC group (hazard ratios 0.10 and 0.22, respectively) compared to the pMMR CRC group.

As expected, dMMR CRCs possessed a greater mean amount of somatic mutations per tumor than pMMR CRCs (1782 and 73, respectively). Interestingly, it was found that the levels of somatic mutation in all tumors tested were positively correlated with progression-free survival times, suggesting that mutation load may serve as an important biomarker for dMMR CRC immunotherapy outcomes. This relationship has also been described in previous immune checkpoint inhibitor trials for other cancer types, however, while overall mutational/neoantigen load are positive outcome predictors, there have been no specific neoantigen peptides identified that can independently predict treatment response [81]. Another predictive correlate of dMMR CRC pembrolizumab response was seen in comparing patients who had germline mismatch repair mutations (e.g., Lynch syndrome) versus those who did not; all six patients without germline dMMR CRC had an objective response, whereas only 3/11 (27 %) patients with germline dMMR CRC had treatment responses. This could perhaps be due to the finding that germline dMMR CRCs generally average a lower number of frameshift

mutations than other dMMR CRC patients [83], but may also be related to the differing pathogeneses (e.g., methylation patterns) that these two dMMR CRC types have.

Adverse effects of the treatment were mostly similar in type and quantity with those described in previous pembrolizumab trials [84–86], which include rash/pruritus (24 %), diarrhea (24 %), and fatigue (32 %) [59••]. However, there was a significant report of asymptomatic pancreatitis (15 %) seen with these CRC patients that was not reported during melanoma or non-small cell lung cancer pembrolizumab trials, perhaps due to cancer localization. Another interesting finding is that while the amount of thyroid disorders seen in the pembrolizumab-treated CRC patients was not necessarily greater than that seen with other cancers, all thyroid issues were reported in dMMR, but not pMMR, CRC patients.

The limited activity for checkpoint blockade against pMMR CRC may explain the relatively poor response rate anti-PD-1 and anti-CTLA-4 inhibitors have elicited in prior CRC studies [73, 76–79]. While a larger dMMR CRC group size would have been desirable to study this effect, studies enrolling only metastatic cancer patients might demographically have fewer dMMR CRC cases; therefore, there is a strong need for further immune checkpoint inhibitor studies that continue to stratify cancers by CRC molecular subgroups, mismatch repair status, and/or mutation load to confirm therapeutic benefit. As of this time, the Le et al. group is continuing to enroll new CRC patients for this original study. In addition, two critical studies for stage IV dMMR CRC have been initiated: a phase II studying metastatic CRC patients that will all receive 200 mg IV pembrolizumab (every 3 weeks for three to seven doses; Table 1; NCT02460198) and a phase III for metastatic CRC patients that will receive either 200 mg IV pembrolizumab (every 3 weeks for up to 35 doses) or IV mFOLFOX6/FOLFIRI-based standard therapy (every 2 weeks; Table 1; NCT02563002) [87, 88]. Multiple clinical trials studying the response of dMMR/CRC patients to pembrolizumab combined with other therapies are also currently underway, including such treatments as p53 vaccines, JAK1 inhibitors, PI3K-δ inhibitors, and other immune checkpoint inhibitors (Table 1; NCT02432963, NCT02646748, NCT02460224).

Aside from pembrolizumab, other immune checkpoint inhibitors, such as the human anti-PD-L1 monoclonal antibody durvalumab, are being tested for efficacy against dMMR/MSI CRC (Table 1; NCT02227667). Another dMMR CRC study is administering a combination of standard chemotherapy with the PD-L1 inhibitor, atezolizumab (800 or 1,200 mg IV every 2–3 weeks; Table 1; NCT01633970). While there are no published findings on the efficacy of durvalumab or atezolizumab in CRC patients, it can be assumed that the researchers hope to find similar benefits in dMMR CRC patients as was seen in the pembrolizumab trial. Furthermore, a current study co-administering nivolumab (human anti-PD-1 monoclonal antibody) and ipilimumab (human anti-CTLA-4 monoclonal antibody) has been initiated for dMMR and pMMR CRC patients (Table 1; NCT02060188); a treatment regimen which has been found to be more efficacious than either agent alone in melanoma trials [89, 90]. It should be noted that past nivolumab/ipilimumab studies have reported an increased incidence of adverse effects following treatment; therefore, the CRC

researchers appear to be starting patients at low concentrations of these inhibitors before escalating their doses to historically efficacious levels.

Summary and future directions

Promising findings from the dMMR CRC pembrolizumab clinical trials has boosted the interest in immunomodulatory therapies for the targeted treatment of this important CRC subtype. Prior to the immunotherapy trials we have discussed, identification of dMMR CRC's unique genetic and pathological attributes had led to other investigations of specific therapies that could target this malignancy [91]. For example, it has been hypothesized that poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors could be part of an effective dMMR CRC treatment due to their efficacy in MSI CRC in vitro models and in human clinical trials against cancers with mutated double-stranded DNA (dsDNA) repair genes (e.g., BRCA-mutated breast cancer) [92, 93]. However, phase II clinical trials using olaparib, a PARP inhibitor, revealed no significant therapeutic benefit in either dMMR or pMMR CRCs [94, 95]. This is possibly due to the finding that PARP inhibitors induce dsDNA breaks and have worked best against tumors with defective dsDNA repair, which is mechanistically distinct from mismatch repair. Another suggested class of dMMR-targeted therapies are the mitotic inhibitors, which show significant efficacy against cancer cells with generally stable diploid chromosomes [91]. As dMMR CRC cells typically possess this type of chromosomal stability, it may be of interest to explore the use of currently available anti-microtubule medications for dMMR CRC.

An additional remarkable observation on the sensitivity of dMMR CRC to different chemotherapies with possibly immunologic basis comes from studies demonstrating that dMMR CRCs are insensitive to single agent fluorouracil, yet are sensitive to oxaliplatin-based regimens [96–98]. Intriguingly, cisplatin resistance is strongly associated with dMMR [99], despite its close chemical and mechanistic relation to oxaliplatin. These differences may perhaps be due to the finding that oxaliplatin cancer cell killing is dependent on a HMGB1/TLR4-dependent immune mechanism which [100], hypothetically, could be enhanced in an immunogenic dMMR CRC environment.

While a handful of immunotherapy clinical trials focusing on the treatment of dMMR CRC have been attempted in the past two decades, the first true breakthrough did not come until last year's discovery of the beneficial effect humanized anti-PD-1 antibody injections could have on dMMR CRC [59••]. This cancer is clearly not the only one that has had an exciting amount of success with immune checkpoint inhibitors; great strides in the treatment of melanoma, lung, and renal cell carcinomas have been also seen with anti-PD-1/PD-L1 and anti-CTLA-4 inhibitors [74, 76, 78, 81, 82]. It may also not have been a complete surprise that dMMR CRCs responded accordingly to pembrolizumab; its relative susceptibility to immune checkpoint inhibitors is congruent with the relationship that other high mutation rate (e.g., melanoma) cancers have to these treatments [76]. Furthermore, dMMR CRCs express many immune checkpoints (e.g., PD-1, PD-L1, CTLA-4, LAG-3, IDO) to a greater extent than pMMR CRCs, which likely makes them more amenable to these inhibitor treatments [24•]. The increased expression of non-PD-1 immune checkpoints is also suggestive that other immune checkpoint inhibitors can have favorable effects in the treatment of dMMR

CRC, either as monotherapies or combined with anti-PD-1 therapy.

Mismatch repair deficiencies are not only pathogenic drivers of CRC, but can also promote the development of gastric, ovarian, endometrial, prostate, and pancreatic cancers [101–105]; therefore, further elucidation of dMMR CRC-directed immunotherapy methods may be applicable to other malignancies as well. The importance of patient cohort size cannot be further emphasized as these valuable dMMR CRC immunotherapy clinical trials are pursued. While certain subtypes of cancers may be too rare to hope to study more than a small number of patients at a time, dMMR CRC makes up approximately 225,000 of the total new CRC cases per year worldwide. With the current guidelines for MSI screening in place, as well as other tumor genetic testing becoming more affordable and applicable, it can be hoped that mismatch repair status and other pathogenetic biomarkers will be readily integrated into immunotherapy research and clinical treatment.

Acknowledgments

This work was partially funded by a 2015 Conquer Cancer Foundation of the American Society of Clinical Oncology Medical Student Rotation award (D.Q.).

Compliance with Ethical Standards

Conflict of Interest

Dionisia Quiroga declares that she has no conflict of interest.

H. Kim Lyerly declares that he has no conflict of interest.

Michael A. Morse will be participating as a clinical investigator in the Keynote-177 trial sponsored by Merck Sharp & Dohme Corporation (NCT02563002), but will not be receiving any direct financial compensation. He has also received financial support through grants from Bristol-Myers Squibb, Merck, Aduro Biotech, AlphaVax, and Advaxis, and has received compensation from EMD Serono for service as a consultant.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by either of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

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