

Advances in immune therapies for the treatment of microsatellite instability-high/deficient mismatch repair metastatic colorectal cancer (Review)

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Abstract. Microsatellite instability-high/deficient mismatch repair colorectal cancer (MSI-H/dMMR CRC) is a molecular subtype characterized by high-frequency mutations within DNA mismatch repair genes. Defects in the DNA mismatch repair machinery lead to subsequent frame-shift mutations, resulting in the generation of frame-shift peptides that serve as neoantigens. This has translated into exquisite sensitivity to immune checkpoint inhibitors (ICIs) and a significant clinical benefit from immune therapies in this patient population. The present article provides a comprehensive review of the advances in the field of immune therapies for MSI-H/dMMR metastatic CRC, with a focus on the major randomized clinical trials that led to Food and Drug Administration approval of specific ICIs for this population, a detailed review of the molecular background responsible for tumor response, as well as the mechanisms of resistance to ICI therapy. Finally, ongoing investigations of other immunotherapeutic strategies to address and overcome the challenges that currently limit response and long-term response to ICIs were presented.

Contents

1. Introduction
2. Immunotherapy in CRC: Molecular overview and rationale
3. Immunotherapy for MSI-H/dMMR mCRC: Review of clinical evidence and current recommendations
4. Mechanisms of resistance to ICIs in mCRC
5. Conclusions and future direction

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1. Introduction

According to the most recent global cancer statistics, colorectal cancer (CRC) is the second most common cause of cancer-associated death in males and females combined (1,2). In 2020, ~1.1 million new CRC cases were reported along with 576,858 deaths, accounting for nearly 10.0% of new cases and 5.8% of all cancer-associated deaths globally (1). While up to 10-15% of CRC patients carry one or more inherited pathogenic mutations associated with inherited syndromes (such as familial adenomatous polyposis or hereditary non-polyposis CRC) (3), 85% of CRC cases are sporadic and attributable to risk factors including age, race and sex, as well as modifiable risk factors including diet, tobacco use, diabetes and obesity (4). In the US, it is estimated that ~20% of newly diagnosed CRC patients have metastatic disease (mCRC) at the time of presentation (2), of which 3-5% harbor high microsatellite instability (MSI-H) and deficiency in mismatch repair mechanism (dMMR) of their genome (5,6).

MSI is the result of somatic or germline mutations in the DNA mismatch repair (MMR) genes MutL homolog 1, MutS homolog 2 (MSH2), MSH6, post-meiotic segregation homolog 2 and epithelial cell adhesion molecule. This defect leads to frame-shift mutations due to the accumulation of DNA replication errors in the microsatellites of DNA coding regions. As a result, tumors with MSI-H/dMMR molecular profile tend to accumulate multiple insertion/deletion mutations that translate into frame-shift peptides (FSPs) expressed on tumor cell surfaces as neoantigens and recognized by the immune system. This has made MSI-H/dMMR mCRC attractive targets for immunotherapies that enhance self-immunity against cancer via exploitation of these neoantigens (7). Prior to the development of immune checkpoint inhibitors (ICIs), the mainstay of first-line therapy for mCRC was combination chemotherapy plus an anti-vascular endothelial growth factor (VEGF) or anti-epidermal growth factor antibody, depending on tumor characteristics without accounting for MSI-H/dMMR molecular status (8); yet, most patients progressed within 1 year of treatment with these systemic regimens (9). The introduction of ICIs, on the other hand, has since revolutionized cancer therapy and

demonstrated impressive activity in patients with mCRC as well as other types of solid tumor that are MSI-H/dMMR (7,10,11). In the present review, the molecular rationale behind the use of immunotherapy in patients with MSI-H/dMMR mCRC is described, available clinical data supporting its use are discussed and possible approaches and future directions to overcome current therapeutic challenges immunotherapy is facing in this select patient population are highlighted.

2. Immunotherapy in CRC: Molecular overview and rationale

ICIs: Mechanism of action. ICIs rely on the ability of tumor cells to suppress the innate immune system by exploiting the interaction between major histocompatibility complex (MHC)-T-cell receptor (TCR) and key ligands on the surface of tumor cells, known as immune checkpoints. These up-regulated immune checkpoints include programmed death 1 (PD-1), PD-1 ligand (PD-L1) and cytotoxic T-lymphocytes-associated protein 4 (CTLA-4), all of which act by inducing anergy or 'dampening' of the immune system (12-14).

PD-1 is expressed on the surface of T-cells, B-cells, dendritic cells and natural killer (NK) cells and becomes overexpressed in inflammatory microenvironments such as the tumor microenvironment (TME) (12). When PD-1 binds to PD-L1 on tumor cells, an inhibitory signaling cascade is initiated and results in i) direct inhibition of tumor cell apoptosis, ii) conversion of effector T cells into regulatory T-cells and iii) kinase-dependent down-regulation of cytokine production required to stimulate proliferation and function of effector T cells (12,13). This is the main mechanism by which tumor cells escape immune surveillance (15). Similarly, CTLA-4 is another co-inhibitory molecule expressed on tumor cells that functions as an immune checkpoint by binding to B7-1 (CD80) and B7-2 (CD86) on antigen-presenting cells, resulting in down-regulation of tumor-reactive T-cell activation, expansion and anti-tumor effects (14).

ICIs specifically target these checkpoints by disrupting the interaction between tumor-expressed inhibitory signals and cells of the immune system. ICIs were initially indicated to improve survival of patients with metastatic melanoma and non-small cell lung cancer (NSCLC) leading to the approval of ipilimumab (anti-CTLA-4), pembrolizumab and nivolumab (anti-PD-1) (16-20). These initial results were further consolidated with long-term follow-up studies revealing prolonged survival (for ≥ 10 years) after treatment with ipilimumab (21). These robust responses have been explained by the high mutation prevalence commonly observed in both melanoma and NSCLC, suggesting that tumor cells with a high tumor mutation burden (TMB) generate more new peptides expressed as neoantigens on their MHC surface molecules; these neoantigens are recognized as non-self and result in priming of T-cell activation and cytotoxic killing (22,23). More recently, the US Food and Drug Administration (FDA) approved the use of pembrolizumab for all solid tumors with high TMB, further consolidating the concept of neoantigenicity promoting response to ICIs (24).

ICIs in MSI-H/dMMR CRC: Mechanisms of response. A schematic of the mechanisms discussed below is provided

in Fig. 1. In CRC specifically, the heterogeneous TME, which consists of immune cells, blood vessels, cytokines and growth factors, is rich in T-cells. Tumors with a greater T-cell infiltration have been associated with improved outcomes possibly via better immunologic control of tumor growth; in CRC, increased tumor-infiltrating lymphocytes (TILs) has been indicated to correlate with improved prognosis in terms of disease-free interval (25-27). However, the mere recognition of peptide-MHC class I complexes by the TCR alone is insufficient to efficiently activate T-cells without overcoming the co-inhibitory receptors discussed earlier and blocking the immune escape phenomenon. In fact, the use of ICI in patients with non-MSI-H/dMMR mCRC (95% of all mCRC cases), was indicated to offer little to no clinical benefit [reviewed in (28)]. By contrast, ICIs have demonstrated impressive potency in patients with mCRC as well as other types of solid tumor that are MSI-H/dMMR (7,10,11).

The increased sensitivity of MSI-H/dMMR mCRC tumors and their susceptibility to ICIs has been attributed to multiple immunologic, molecular and genetic factors. First, MSI-H/dMMR tumors have an abundance of TILs, specifically cytotoxic T lymphocytes, when compared with MMR proficient (pMMR) tumors (29). This observation has been linked to MSI-H tumors having a more favorable prognosis and disease course (30). While the mechanism behind this effect has remained to be fully elucidated, it has been hypothesized that the abundance of TILs creates an inflammatory TME that paradoxically does not eradicate the cancer but rather, triggers the up-regulation of several immune checkpoint molecules, including PD-1, PDL-1 and CTLA-4, in what appears an adaptive immune phenomenon that launches the immune escape mechanism discussed earlier (31). Part of this checkpoint-mediated immune escape adopted by cancer cells involves the increased conversion from effector T-cells to regulatory T-cells that secrete immunosuppressive molecules such as TGF- β , IFN- γ and IL-10, ultimately resulting in immune anergy (31). IFN- γ specifically has been indicated to up-regulate the expression of PD-L1, which is significantly higher in MSI-H tumors in comparison to microsatellite-stable (MSS) tumors (31). ICIs, by virtue of their mechanism of action, exploit these elevated levels of checkpoint inhibitors on cancer cells to disrupt the interaction between cancer cells and immune cells, ultimately 'blocking' the immune escape and reinvigorating the host's immune system. It is specifically the higher levels of checkpoint molecule expression in MSI-H tumors, originally adaptive to escape the host's immune system, that render this molecular subtype more sensitive and responsive to ICIs. Yet, high levels of PD-L1 would not solely explain this robust response to ICIs, as multiple studies have indicated that levels of PD-L1 expression alone are insufficient to predict response to ICIs, pointing towards the requirement for other molecular and genomic biomarkers [reviewed in (32)]. The TMB, which quantifies the total number of mutations present in a tumor specimen, has emerged as a promising quantitative genomic biomarker for response to ICIs, independent of the PD-L1 expression status [reviewed in (33)]. This becomes of unique relevance with MSI-H/dMMR mCRC that harbor a high level of somatic frame-shift mutations as a result of their dMMR mechanism. These molecular defects that translate into short

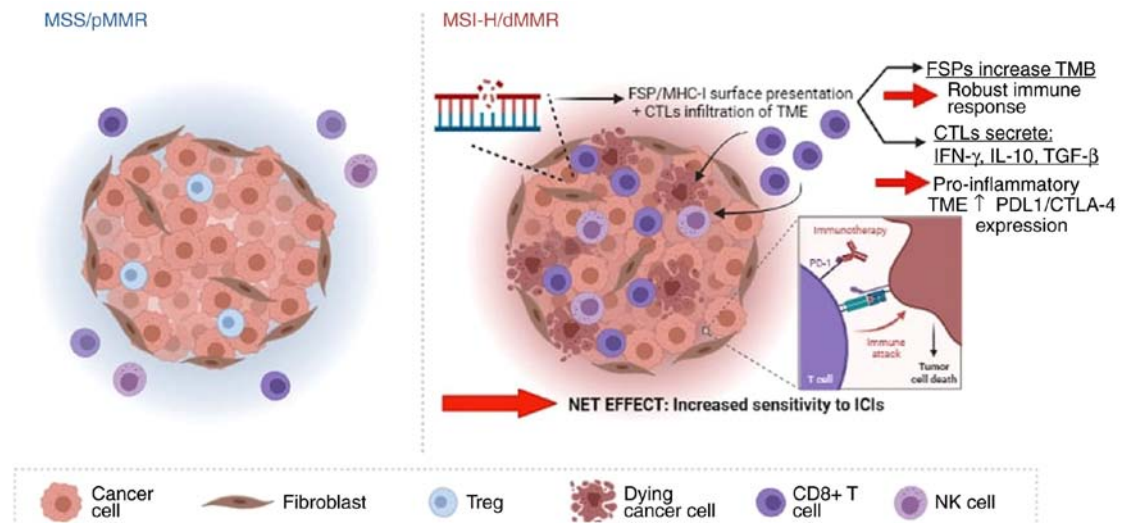


Figure 1. Mechanisms of sensitivity/response of MSI-H/dMMR CRC tumors to ICI therapy. dMMR results in the generation of FSPs that are presented as neoantigens via the MHC-1 system. Neoantigen recognition triggers the infiltration of CTLs and NK cells into the TME. CTLs secrete pro-inflammatory cytokines that promote the expression of PD-L1 and CTLA-4; these are subsequently recognized and inhibited by immune checkpoint inhibitors, leading to re-invigoration of T-cells that may attack and kill cancer cells. The presence of FSPs increases the TMB, resulting in a robust immune response to ICI. NK, natural killer; TMB, tumor mutational burden; TME, tumor microenvironment; FSPs, frame-shift peptides; CTLs, cytotoxic T lymphocytes; ICI, immune checkpoint inhibitor; MSI-H/dMMR CRC, microsatellite instability-high/deficient mismatch repair colorectal cancer; MSS, microsatellite-stable; pMMR, mismatch repair proficient; MHC, major histocompatibility complex; PD-L1, programmed death 1 ligand; CTLA-4, cytotoxic T-lymphocytes-associated protein 4; Treg, T-regulatory cell.

stretches of DNA (micro-satellites) serve as neoantigens (as FSPs), ultimately exposing cancer cells to the host's immune system, namely in the T-cell-infiltrated TME of MSI-H/dMMR tumors (7). The accumulation of neoantigens elicits a robust host immune response when recognized by the TILs (34), but also by involvement of macrophages and dendritic cells that serve as biologic immune intermediates for neoantigen presentation and delivery on the one hand, and as a pro-inflammatory vehicles that release inflammatory cytokines on the other hand, further enhancing the immunologic effect described earlier (35). In fact, MSI-H/dMMR tumors produce a significantly higher TMB in comparison to MSS tumors, in the realm of 10-fold or higher. Numerous types of these mutations are sequences of tri-nucleotide repeats within the introns of protein-coding sequences (36). Of these mutations, the majority result in altered amino acid sequences and neoantigen peptides. Thus, patients with MSI-H/dMMR tumors represent a unique population of patients with mCRC that have been indicated to benefit the most from immune-based therapies (37). In a recent study by Valero *et al* (38), patients with MSI-H/dMMR with high TMB levels were reported to have better overall survival (OS) compared to MSS and this survival benefit was further prolonged in patients treated with ICIs compared to non-ICI therapy.

Finally, the impact of the MSI-H/dMMR status on triggering an immune response and predicting a durable response to ICIs reaches beyond the mere quantitative load of neoantigens to the actual identity and genomic function of the neo-peptides. An early study of the mutations causing amino acid alterations suggested that, within the selected CRC samples, there were roughly 7 unique epitopes that were involved in tumorigenesis (39). Early identified FSPs included transforming growth factor- β receptor 2 (TGFBR2), phosphatase and tensin homolog, asteroid homolog 1, AIM2 and

caspace 5 (36,39-42) and these FSPs, along with others such as HT001, AIM2 and TAF1B, were detected at significantly higher frequencies in MSI-H CRC compared to MSS (41).

More recent studies using serologic and bio-informatics approaches have further identified a wider range of FSPs and certain FSPs have potential functional genetic implications (43-46). In general, mutations in coding exons that are generated as a result of deficient MMR in MSI-H CRC result in complete functional inactivation (47). While functional validation is still pending for numerous FSPs, these observations open ways for novel mechanisms that explain the exquisite sensitivity of MSI-H/dMMR mCRC to ICIs: Beyond their immunogenicity as FSPs, certain mutated genes may further exert a functional anti-tumor effect that amplifies the immune efficiency of ICI in MSI-H/dMMR mCRC. For instance, loss of TGFBR2 activity promotes an inflammatory response within the TME by inhibiting anti-inflammatory cytokines, increasing tumor-associated macrophage infiltration and NK T-cell activation (48). AIM2 on the other hand has been indicated to have tumor suppressive properties via promotion of an inflammatory response and inhibition of CRC cell proliferation and migration, similar to myristoylated alanine-rich protein kinase C substrate, another identified FSP that inhibits the proliferation of CRC (49-51). Inactivating mutation of these genes in MSI-H/dMMR mCRC may thus not result in promoting anti-tumor activity but may at least offer potential targets for precision therapy along with ICIs. The roles of other FSPs, such as TAF1B and ZNF294, remain to be identified (47,52) while others such as HT001 are known to be non-coding with a strict immunogenic function (53). The extent to which such mutations and their resulting FSPs have genomic implications in ICI therapy warrants further study.

Yet, and irrespective of their potential with regard to genomic function, the identification of these FSPs that are

present at higher frequencies in MSI-H/dMMR mCRC carries a significant potential for future vaccine development and other humoral FSP-based therapeutic strategies (42). With the mechanisms of response of MSI-H/dMMR to ICIs in mind, clinical evidence for the effectiveness of ICI for MSI has been provided in multiple studies, which is discussed in the following section.

3. Immunotherapy for MSI-H/dMMR mCRC: Review of clinical evidence and current recommendations

Evidence indicates that in patients with mCRC, response to ICIs is limited to cases with MSI-H/dMMR molecular status (54,55). In fact, the most recent practice guidelines across major National and International organizations recommend molecular testing for all newly diagnosed or recurrent cases of advanced and mCRC for MMR status and MSI markers (56-59). While those with pMMR are treated with systemic chemotherapy regimens, ICIs have been indicated for patients with MSI-H/dMMR who have failed systemic therapies and more recently, as an upfront therapy following an accelerated approval by the FDA as the 1st line therapy (60,61). In this section, evidence from key phase II and III trials supporting the use of ICIs in MSI-H/dMMR mCRC, as well as ongoing trials, are reviewed (Table I).

Search methodology. A systematic review was conducted according to the PRISMA guidelines with the last update of the search performed on March 31, 2021. The search was conducted in PubMed as well as major conference proceedings (American Society of Clinical Oncology; European Society of Medical Oncology) using the following query terms: (colon cancer OR rectal cancer OR colorectal cancer OR colorectal neoplasm cancer) AND (MSI-H OR dMMR OR MSI-H/dMMR) OR (immunotherapy OR ICI OR immune therapy OR anti-PD-1 OR anti-PD-L1 OR anti-CTLA-4). In addition, the clinical trials registry (clinicaltrials.gov) was searched to identify ongoing trials that have with so far unpublished reports. Studies were included if they evaluated checkpoint inhibitors as a monotherapy or in combination with any other agent in a clinical trial setting in patients with MSI-H/dMMR mCRC. Studies were excluded if they evaluated checkpoint inhibitor therapy or systemic therapies for localized CRC or patients with MSS/pMMR, if a study was a protocol-only publication without data or if it reported overlapping data. In the latter case, the study with the most recent and/or most comprehensive data was included. The initial search identified a total of 29,980 studies. After review by title, abstract and full-text review, 9 studies were included in the final review (Table I). Furthermore, 28 additional ongoing and unpublished studies were identified via clinicaltrials.gov (Table II).

Clinical evidence for anti-PDI therapy

i) Pembrolizumab. Pembrolizumab is currently approved for chemo-refractory MSI-H/dMMR mCRC and as a 1st-line agent for this population (61). It was first evaluated in a phase II study (NCT01876511) involving patients with mCRC who had at least two or more previous chemotherapy regimens and consisting of three cohorts: dMMR (n=11), pMMR (n=21)

and nine patients with non-CRC dMMR gastrointestinal cancers (7). The primary endpoint was objective response rate (ORR) evaluated by 'Response evaluation criteria in solid tumors' (RECIST) v1.1. In the dMMR mCRC cohort, ORR was 40% compared to 0% in the pMMR cohort. No complete response (CR) was observed but patients with dMMR status had a high disease control rate (DCR) of 90%, consisting of 40% partial responses (PR) and 50% of patients with stable disease (SD) when evaluated at 12 weeks. The median follow-up time was 36 weeks in the dMMR mCRC cohort and 20 weeks in the pMMR cohort, with a median progression-free survival (PFS) that was not reached in the dMMR group [vs. 2.2 months for the pMMR group; hazard ratio (HR)=0.10; P<0.001]. At week 20, PFS rates were 78 and 11%, respectively, and median OS was not reached in the dMMR group (vs. 5.0 months for pMMR mCRCs). In a follow-up study (KEYNOTE-016; NCT01876511) comprising 86 patients with different refractory dMMR cancers, 40 patients had dMMR mCRC (10). Compared to the previous study, the ORR was 52% but CR was achieved in 12% of patients with an average time to CR of 42 weeks. Neither median PFS nor OS were reached (median follow-up of 12.5 months) but later follow-up revealed a 2-year PFS of 59% and 2-year OS of 72% (10,62). In a subsequent phase II study (KEYNOTE-164; NCT02460198) involving cases of MSI-H/dMMR unresectable advanced or mCRC, pembrolizumab was administered to patients who had received at least two prior therapies including fluoropyrimidine, oxaliplatin and irinotecan (cohort A; n=61) or at least one prior therapy (cohort B; n=63) (62). The primary endpoint (ORR) was similar in both cohorts (33%) but patients with lesser prior treatment (cohort B) had higher CR rates at 7.9% (vs. 3.3%). It is worth noting that the ORR in this study (33%) was smaller than that of KEYNOTE-016 (52%) and this may be attributed to a smaller cohort size and the use of the immune-related RECIST (ir-RECIST) rather than RECIST in the latter study. Similarly, the median PFS was higher in cohort B (4.1 vs. 2.3 months) with an estimated 12-month PFS rate of 41% (vs. 34% in cohort A). As far as the median OS was concerned, it was not reached in the less pretreated cohort compared to 31.4 months in the group with more previous lines of treatment, with an estimated 1-year OS rate of 76 and 72%, respectively. Besides confirming prior findings of a durable clinical benefit in pretreated MSI-H/dMMR mCRC, the study indicated a potential benefit from using anti-PD-1 in earlier stages of the disease. Based on pooled data from both the KEYNOTE-016 and -0164 trials (n=90; pooled ORR: 36%) the FDA approved pembrolizumab for patients with pretreated MSI-H/dMMR mCRC (63).

As a first-line treatment, pembrolizumab was evaluated in a phase III trial, in which investigators evaluated the efficacy and safety of pembrolizumab (n=153) vs. investigator's choice of standard of care (SOC) chemotherapy (n=154) in dMMR mCRC (KEYNOTE-177; NCT02563002) (64). The primary endpoint of the study was median PFS with significantly longer intervals in the pembrolizumab cohort (16.5 vs. 8.2 months; HR=0.6 and P=0.0002) at the median follow-up (28.4 months). This was also clinically meaningful with close to half of the patients (48.3%) in the pembrolizumab arm without disease progression at 2 years, while patients experienced less drug-related adverse events compared to the SOC

Table I. Published phase II and III trials evaluating ICIs in patients with MSI-H/dMMR mCRC.

Author, year (NCT-trial no.)	Phase	ICI/target	Line	Population/ cohorts	Median follow-up	i) Primary and ii) secondary endpoints	ORR	PFS	OS	(Refs.)
Le, <i>et al.</i> , 2015 (NCT01876511)	II	Pembrolizumab/ PDI	3+	C1: dMMR mCRC; C2: pMMR mCRC; C3: dMMR nonCRC	C1:36 wks;C2:20 wks;C3:21 wks	i) ORR; ii) PFS, OS	C1: 40% (vs. 0% in C2); 90% DCR in C1:-CR: 0; -PR: 40%; -SD: 50%	C1: 78% (vs. 11% in pMMR); mPFS: NR in C1 (vs. 2.2 mo in C2)	mOS: NR in C1 (vs. 5 mo in C2)	(7)
Le, <i>et al.</i> , 2017 (NCT01876511- KEYNOTE-016)	II	Pembrolizumab/ PDI	3+	86 patients with 12 different types; 40 had dMMR mCRC	12.5 mo	i) ORR; ii) PFS, OS	dMMR mCRC: 52% ORR; 82% DCR; -CR: 12%; -PR: 40%; -SD: 30%	mPFS: NR in initial assessment. At 2 years follow-up: 59%	mOS: NR in initial assessment. At 2 years follow- up: 72%	(10)
Le, <i>et al.</i> , 2020 (NCT02460198- KEYNOTE-164)	II	Pembrolizumab/ PDI	3+ 2+	MSI-H/dMMR advanced or mCRC; -C-A: 2 prior lines; -C-B: 1 prior line	31.3 mo	i) ORR; ii) DOR, PFS, OS	33% ORR; -CR: 7.9% in C-B (vs. 3.3% in C-A)	mPFS: 4.1 mo in C-B (vs. 2.3 mo in C-A); 1-year PFS: 41% in C-B (vs. 34% in C-A)	mOS: NR in C-B (vs. 31.4 mo in C-A); 1-year OS: 76% (C-B) and 72% (C-A)	(62)
Andre, <i>et al.</i> , 2021 (NCT02563002- KEYNOYE-177)	III	Pembrolizumab/ PDI	1st	MSI-H/dMMR mCRC: ICI vs. SOC	28.4 mo	i) mPFS; ii) ORR, DOR	43.8% in ICI (vs. 33.1%); mDOR: NR in ICI (vs. 10.6 mo)	16.5 mo in ICI (vs. 8.2 mo)	-	(64)
Overman, <i>et al.</i> , 2017 (NCT02060188- CheckMate-142)	II	Nivolumab/PDI	2+	MSI-H/dMMR mCRC; non-randomized	12 mo	i) ORR; ii) DCR, DOR	ORR: 32.4%; DCR: 63.5%; mDOR: NR; -CR: 2.7%; -PR: 29.7%	1-year PFS: 50.4%	1-year OS: 73.4%	(11)
Overman, <i>et al.</i> , 2018 (NCT02060188- CheckMate-142)	II	Nivolumab + Ipilimumab/PDI + CTLA-4	2+ or 1st	MSI-H/dMMR mCRC; non-randomized	13.4 mo	i) ORR; ii) DCR	ORR: 49% (4% CR; 45% PR); DCR: 79%	mPFS: NR; 1-year PFS: 71%	mOS: NR; 1-year OS: 85%	(82)
Chung, <i>et al.</i> , 2010	II	Tremelimumab/ CTLA-4	3+	Heavily pre-treated MSI-H/dMMR mCRC	2.3 mo	i) ORR; ii) DOR, PFS, OS	1 patient with objective response; SD DOR: 6 mo	1 Patient: 6 mo	1 Patient: 15 mo	(70)

Table I. Continued.

Author, year (NCT-trial no.)	Phase	ICI/target	Line	Population/ cohorts	Median follow-up	i) Primary and ii) secondary endpoints	ORR	PFS	OS	(Refs.)
Chalabi, <i>et al</i> , 2020 (NCT-03026140)	II	Nivolumab + Ipilimumab/PD1 + CTLA-4	1st	Early-stage disease (I, II or III); CRC neo-adjuvant setting. C1: dMMR; C2: pMMR	9.0 mo	i) pR ii) pR	pR: 100% in C1 (vs. 27% in C2); -CR: 60% (C1 vs. 13.3% in C2)	NR NR	NR	(71)
Eng, <i>et al</i> , 2019 (NCT02788279- IMblaze370)	III	Atezolizumab +/- Cobimetinib/ PD-L1, MEK1/2	3+	Unresectable advanced or mCRC. C1: Atezolizumab + Cobimetinib; C2: Atezolizumab; C3: SOC. -MSI-H/ dMMR: 5% of all cases	7.3 mo	i) OS; ii) PFS, ORR, DOR	No CR in any cohort	mPFS: 1.91 vs. 1.94 vs. 2.0 mo (C1 vs. C2 vs. C3)	mOS: 8.9 vs. 7.1 vs. 8.5 mo (C1 vs. C2 vs. C3)	(74)

Clinical trial details may be accessed at <https://www.clinicaltrials.gov/>. C, cohort; Wks, weeks; mo, months; NR, not reached; pR, pathologic response; MSI-H/dMMR mCRC, microsatellite instability-high/deficient mismatch repair metastatic colorectal cancer; pMMR, mismatch repair proficient; ORR, objective response rate; PFS, progression-free survival; mOS, median overall survival; DOR, duration of response; DCR, disease control rate; CR, complete response; ICI, immune checkpoint inhibitor; PR, partial response; pR, pathologic response; SD, stable disease; PD1, programmed death-1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4.

Table II. Ongoing/unpublished novel immune-based therapies in patients with microsatellite instability-high/deficient mismatch repair advanced or mCRC.

A, Checkpoint inhibitor-based therapies			
Trial identifier no.	Agent(s) (target)	Trial phase; cancer type	Treatment groups
NCT02912559	Atezolizumab (PD-L1)	Phase III; Stage 3 CRC	Adjuvant Atezolizumab + FOLFOX vs. FOLFOX alone
NCT02997228	Atezolizumab (PD-L1)	Phase III; 1st line mCRC	Atezolizumab vs. Atezolizumab + FOLFOX + bevacizumab vs. FOLFOX + bevacizumab
NCT03150706	Avelumab (PD-L1)	Phase III; mCRC, >1 prior therapy	Avelumab monotherapy
NCT02060188	Nivolumab (PD1) ± Ipilimumab (CTLA-4)	Phase III; Refractory CRC	Nivolumab ± - Ipilimumab, or - anti-LAG3 antibody
NCT01633970	Atezolizumab	Phase I; Locally advanced or metastatic solid tumors	Atezolizumab + bevacizumab Atezolizumab + bevacizumab + FOLFOX Atezolizumab + carboplatin + placlitaxel Atezolizumab + carboplatin + pemetrexed Atezolizumab + carboplatin + nab-placlitaxel Atezolizumab + nab-placlitaxel
NCT02817633	TSR022 (TIM3) ± Nivolumab (PD1)	Phase I; Advanced solid tumors	TSR022 dose escalation followed by combination with Nivolumab
NCT03099109	LY3321367 (TIM3) ± LY3300054 (PD-L1)	Phase I; Advanced solid tumors	LY3321367 dose escalation followed by combination with LY3300054
NCT02608268	MBG453 (TIM3) ± PDR001 (PD1)	Phase I/II; Advanced solid tumors	MBG453 dose escalation followed by combination with PDR001
NCT01968109	Relatlimab (LAG3) ± Nivolumab (PD1)	Phase I/II; Advanced solid tumors	Relatlimab dose escalation followed by combination with Nivolumab
NCT02966548			
NCT03250832	TSR033 (LAG3) ± anti-PD1	Phase I; Advanced solid tumors	TSR033 dose escalation followed by combination with anti-PD1
NCT03252936	IMP321 (LAG3)	Phase I; Advanced solid tumors	Dose escalation/safety study
NCT02676869			
NCT03005782	REGN3767 (LAG3) ± REGN2810 (PD1)	Phase I; Advanced solid tumors	REGN3767 dose escalation followed by combination with REGN2810
NCT03219268	MGD013 (LAG 3/PD1 bi-specific antibody)	Phase I; Advanced solid tumors	MGD013 dose escalation/safety study
NCT03119428	OMP313M32 (TIGIT)	Phase I; Advanced solid tumors	OMP313M32 dose escalation/safety study
NCT02794571	MTIG7192A (TIGIT) ± Atezolizumab (PD-L1)	Phase I; Advanced solid tumors	MTIG7192A dose escalation followed by combination with Atezolizumab
B, T-cell activation agonists			
Trial identifier no.	Agent(s) (target)	Trial type	Treatment groups
NCT01239134	TRX518 (GITR)	Phase I; Unresectable stage 3-4 solid tumors	TRX518 dose escalation/safety study
NCT02740270	GWN323 (GITR) ± PDR001 (PD1)	Phase I; Advanced solid tumors	GWN323 dose escalation followed by combination with PDR001
NCT02583165	MEDI1873 (GITR)	Phase I; Advanced solid tumors	MEDI1873 dose escalation/safety study
NCT03295942	OMP336B11 (GITR)	Phase I; Locally advanced or metastatic solid tumors	OMP336B11 dose escalation/safety study

Table II. Continued.

B, T-cell activation agonists		Treatment groups	
Trial identifier no.	Agent(s) (target)	Trial phase; cancer type	Treatment groups
NCT02697591	INCAGN01876 (GITR) ± Nivolumab (PD1)	Phase I/II; Advanced solid tumors	INCAGN01876 dose escalation followed by combination with Nivolumab or Ipilimumab
NCT03126100	or Ipilimumab (CTLA-4)		
NCT01644968	Anti-OX40 + tetanus vaccine + keyhole limpet antigen	Phase I; Advanced cancer	Anti-OX40 + KLH vs. Tetanus vaccine + anti-OX40
NCT02559024	MEDI6469 (OX40)	Phase I; mCRC	MEDI6469 dose escalation/safety study
NCT02410512	MOXR0916 (OX40) ± Atezolizumab (PD-L1)	Phase Ib; Locally advanced or metastatic solid tumors	MOXR0916 dose escalation followed by combination with Atezolizumab
NCT02221960	MEDI6383 (OX40) + MEDI114736 (PD-L1)	Phase I; Recurrent and/or metastatic solid tumors	MEDI6383 vs. MEDI6383 + MEDI114736
NCT02318394	MEDI0562 (OX40) ± durvalumab (PD1) or tremelimumab (CTLA-4)	Phase I; Advanced solid tumors	MEDI0562 dose escalation followed by combination with durvalumab or tremelimumab
NCT02705482			
NCT02528357	GSK3174998 (OX40) ± Pembrolizumab (PD1)	Phase I; Advanced solid tumors	GSK3174998 dose escalation followed by combination with Pembrolizumab
NCT02923349	INCAGN01949 ± Nivolumab (PD1) and/or Ipilimumab (CTLA-4)	Phase I; Advanced solid tumors	INCAGN01949 dose escalation followed by combination with Nivolumab or Ipilimumab
NCT03241173			
NCT02335918	Varlilumab (CD27) + Nivolumab (PD1)	Phase I/II; Advanced solid tumors	Varlilumab + Nivolumab single arm: dose escalation/safety study

Clinical trial details may be accessed at <https://www.clinicaltrials.gov/>. mCRC, metastatic colorectal cancer; CTLA-4, cytotoxic T lymphocyte antigen 4; GITR, glucocorticoid-induced TNF receptor-related gene; LAG3, lymphocyte activation gene 3 protein; PD1, programmed cell death 1; PDL1, programmed cell death 1 ligand 1; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM3, T cell immunoglobulin mucin receptor 3.

group (grade 2-5 toxicities; 22 vs. 66% respectively). Based on these results, the FDA approved pembrolizumab as a first-line treatment option for patients with MSI-H/dMMR mCRC (61). An updated analysis of the results with the final PFS, PFS-2 (time from randomization to progression on the next line of therapy or any cause of death), as well as a health-related quality of life (HR-QoL), was recently published (65). At the median follow-up of 32.4 months (range, 24.0-48.3 months), pembrolizumab continued to be superior to SOC for PFS with a median PFS of 16.5 months (vs. 8.2 months; HR 0.60; $P=0.0002$), as well as 12- and 24-month PFS rates of 55.3 and 48.3% (vs. 37.3 and 18.6% with chemotherapy), respectively. The confirmed ORR was 43.8% (vs. 33.1%) for pembrolizumab with longer durations of response (DOR; median DOR not reached vs. 10.6 months in SOC). Similarly, PFS-2 was longer with pembrolizumab (median not reached vs. 23.5 months; HR=0.63) along with improved HR-QoL scores.

ii) Nivolumab. The clinical benefit of targeting PD-1 was also demonstrated with nivolumab. CheckMate-142 (NCT02060188) is a non-randomized phase II trial that evaluated nivolumab with or without ipilimumab (anti-CTLA-4) in MSI-H/dMMR mCRC patients that had been heavily pretreated (two or 3 previous lines of therapy) (11). In the initial part of the study, nivolumab was administered as monotherapy to 74 patients with ORR as a primary endpoint evaluated by RECIST v1.1. The results revealed an ORR of 32.4% with 2.7% CR and 29.7% PR (central review). The median DOR was not reached, 1-year PFS was 50.4% and 1-year OS was 73.4%. Disease control (defined as CR, PR or stable disease of 12 weeks or longer) was achieved by 63.5% of patients. This study was the first to demonstrate durable response and disease control with nivolumab in this patient population. Of note, the benefits of anti-PD-1 monotherapy were regardless of tumor PD-L1 expression, TILs status, mutational status (BRAF, KRAS) or germline dMMR status. Based on data from CheckMate-142, the FDA extended the approval of nivolumab for patients with MSI-H/dMMR mCRC who are refractory to 5-fluorouracil, oxaliplatin and irinotecan-based therapy (66).

In a second step of CheckMate-142, the efficacy of combining nivolumab with ipilimumab (anti-CTLA-4) was analyzed in 119 patients with dMMR mCRC who had received two or three lines of previous therapy (11). The rationale behind this combination is that both ICIs operate at different points of the immune response and when combined are able to act synergistically to promote the anti-tumor response (67). While the ORR as a primary endpoint reached 49% (4% CR; 45% PR), the median PFS and OS were not reached, with one-year estimates of 71 and 85%, respectively. The DCR for 12 weeks or longer and durable response for 12 months or longer was 79 and 83%, respectively. The analysis of this cohort revealed better efficacy in combining nivolumab and ipilimumab compared to nivolumab monotherapy. Finally, the third cohort in CheckMate-142 consisted of untreated patients with MSI-H/dMMR who received nivolumab plus low-dose ipilimumab and the results revealed an ORR of 60%, DCR of 84% and 12-month PFS of 77% (68). Those results further granted ipilimumab extended approval for use in patients with MSI-H/dMMR mCRC (69).

Clinical evidence for anti-CTLA-4 therapy. Unlike monotherapy with anti-PD1 agents, which has demonstrated clinical efficacy in the treatment of MSI-H/dMMR mCRC cases, treatment with single-agent anti-CTLA-4 monotherapy (ipilimumab or tremelimumab) failed to provide a clinical benefit in this population. In a single-arm phase II study of anti-CTLA-4 monotherapy with tremelimumab that involved 47 heavily pre-treated mCRC patients, 45 were considered response-evaluable, of which 44 did not reach the second dose of therapy (43 had progressive disease and 1 discontinued due to treatment-related adverse events) (70). Only one patient had stable disease for 6 months and the study did not demonstrate any clinically meaningful activity of single-agent tremelimumab. Thus, for now, anti-CTLA-4 therapy in MSI-H/dMMR mCRC is reserved for combined use with anti-PD-1 therapy, as discussed further above.

Of note, the nivolumab/ipilimumab combination has also been tested as a neo-adjuvant treatment for early CRC. In a recent phase Ib/II trial, nivolumab was administered in combination with ipilimumab to both dMMR (n=20) and pMMR (n=15) early-stage (I, II or III) CRC prior to surgery (71). In the dMMR cohort, 100% (20/20) achieved pathological response, with 60% CR and 95% major responses (defined as <10% residual tumor). Surprisingly, pMMR tumors also responded to the ICIs combination, albeit to a lesser extent (27% pathological response, 13.3% CR, 6.7% PR and 20% major responses). Regarding molecular markers, increased CD8⁺/PD-1⁺ T-cell infiltration was predictive of response in these tumors.

Clinical evidence for anti-PD-L1 therapy. Atezolizumab targets the PD-L1 immune checkpoint. To date, there has not been any completed large clinical study in MSI-H/dMMR mCRC; however, early-phase studies involving anti-PD-L1 agents have been reported: In a phase I dose-escalation study of atezolizumab, one of the patients with unselected (unknown MSI or MMR status) mCRC achieved a durable PR (72). Another anti-PD-L1 monoclonal antibody (BMS-936559) was evaluated in 207 patients with advanced solid tumors, including 18 with unselected mCRC but no clinical response was observed (73).

Atezolizumab has also been evaluated in combination with SOC therapies. In a phase III trial (IMblaze370; NCT 02788279) performed by Eng *et al* (74), 363 patients with unresectable, locally advanced CRC or mCRC were treated with a combination of atezolizumab and cobimetinib/MEK 1/2 inhibitor (cohort 1; n=183) or atezolizumab monotherapy (cohort 2; n=80) or SOC regoratinib (cohort 3; n=183). Patients with dMMR tumors constituted 5% of all CRC cases. The results revealed no CR in any cohort along with low rates of PR with no difference between the 3 different cohorts. Similarly, no difference in PFS (1.91 vs. 1.94 vs. 2.0 months, respectively) or OS (8.9 vs. 7.1 vs. 8.5 months, respectively) was observed.

Atezolizumab has also been evaluated in a phase II study as a first-line treatment in unresectable wild-type BRAF mCRC in combination with fluoropyrimidine-bevacizumab treatment (MODUL; NCT02291289) (75). Compared to chemotherapy only, there was no improvement in PFS (HR=0.96) or OS (HR=0.86).

Two large trials involving atezolizumab are currently underway: In the COMMIT trial (NCT02997228), a phase III study, patients newly diagnosed with mCRC (n=373, expected sample size) are randomly assigned to either receive FOLFOX with bevacizumab (control arm) with or without atezolizumab or atezolizumab monotherapy (76). The primary trial end-point was PFS, and secondary end-points included OS and ORR. This awaited study may potentially provide evidence for incorporating ICIs with SOC first-line agents in MSI-H/dMMR mCRC. Another ongoing phase III randomized trial, (ATOMIC ALLIANCE A021502; NCT02912559) is allocating stage III MSI-H/dMMR CRC to either SOC adjuvant chemotherapy monotherapy (FOLFOX for 6 months) or combined with atezolizumab, with an additional 6-months maintenance treatment with anti-PD-L1 (77). This study will investigate the ability of ICIs to eradicate any minimal residual disease in this patient population.

Patterns of response and safety profile of ICIs in MSI-H/dMMR mCRC. Beyond the marked efficacy in patients with MSI-H/dMMR mCRC, the use of ICIs in this patient population revealed certain unique patterns of response previously uncommonly observed with mCRC conventional therapy. Pseudo-progression is one unique striking pattern encountered in mCRC, also reported in melanoma, whereby an initial radiographic tumor enlargement is observed after ICI therapy is initiated, followed by measurable tumor regression months to years after therapy initiation (78,79). This phenomenon may be explained by a transient increase in immune-cell tumor infiltration and pro-inflammatory cytokine release that generate edema (79). As a consequence, RECIST was modified to account for pseudo-progression through the development of immune-related response criteria (ir-RECIST) to assess responses to ICIs (80). In the trials reviewed above, RECIST was used for response assessment except in KEYNOTE-016, where ir-RECIST was used for primary endpoint assessment (10). This may explain why higher response rates were observed in KEYNOTE-016 compared to KEYNOTE-164 (where a pseudo-progression would be considered a progression as per the RECIST criteria).

Another aspect particular to ICIs in MSI-H/dMMR mCRC is the rate of CRs achieved compared to chemotherapy. In this population, CR rates with combination chemotherapy have been reported to be 1-2% (81), in contrast to 3% with pembrolizumab [median follow-up, 12.6 months; KEYNOTE-164; (62)], 3% for nivolumab plus ipilimumab (median follow-up, 13.4 months; CheckMate-142) and 3% for nivolumab monotherapy (median follow-up, 13 months) with an increase to 9% at a median follow-up of 21 months, thus achieving a deepening response over time [CheckMate-142; (11,82)]. These radiologic responses further translated to a clinical benefit as patients in CheckMate-142 who had CR or PR to nivolumab achieved a 100% 2-year OS compared to 50% among those with stable disease (83).

In addition to improved radiologic and clinical responses, the use of ICIs in mCRC has been unique in terms of DOR; for instance, the median DOR was not reached in CheckMate-142 after a median follow-up of 21 months (82). This has translated into prolonged median efficacy outcomes in MSI-H/dMMR mCRC in terms of median OS and 1-year OS as discussed earlier.

In terms of safety, ICIs have demonstrated a distinct safety profile compared to chemotherapy with toxicities relating to the skin (rash, pruritis), the gastrointestinal system (colitis) and endocrinopathies (thyroiditis, hypophysitis and adrenal insufficiency) (84). These toxicities are most likely secondary to autoimmune-like T-cell-mediated toxicities induced by disruption of the checkpoint's function. In the trials reviewed above, the most common immune-related toxicities (seen in at least 5% of patients receiving ICIs) included the following: Rash, pruritis, dry skin, diarrhea, colitis, nausea and vomiting, pancreatitis, gastritis, hepatic transaminitis, hypo/hyperthyroidism, hypophysitis, adrenal insufficiency, acute kidney injury, pneumonitis and myocarditis (10,11,84). Most of these adverse events developed within 12 weeks of treatment initiation and resolved within 12 weeks of onset or were easily reversed by appropriate therapy. Compared to toxicities experienced with systemic therapy, ICIs offer not only a unique profile but also significantly better safety in terms of side effects.

Ongoing trials of novel immune modulators and immune-based combination therapies

Novel immune modulators. At present, PD1 and CTLA-4 blockade are the only FDA-approved ICIs for the treatment of MSI-H/dMMR mCRC. However, several other immune checkpoint interactions regulate T-cell activation in the TME. These include the following: T-cell immunoglobulin mucin receptor 3 (TIM3), lymphocyte activation gene 3 and T-cell Ig and ITIM domains; in pre-clinical studies, they have all been indicated to contribute to T-cell exhaustion and promotion of CRC progression (85,86). These immune checkpoints are currently being evaluated in phase I clinical trials to assess their safety profile either as single agents or in combination with PD-L1 blockade (Table II) (87). Other agents with a different mechanism of action are also being evaluated; they act as antibody agonists of co-stimulatory immune receptors to enhance the host's immune system response against tumor cells. These include CD27, OX40 (CD134), 4-1BB (CD137) and glucocorticoid-induced TNF receptor-related gene (GITR; CD357 and CD40) (88).

Immune-based combination therapies. The use of ICIs in the treatment of MSI-H/dMMR mCRC has also been explored in combination with other conventional CRC therapies, including chemotherapy, targeted monoclonal antibodies (mAbs), radiation therapy (RT) and small-molecule tyrosine kinase inhibitors (TKIs). The rationale of combining ICIs with cytotoxic chemotherapy relies on evidence suggesting that cytotoxic cell killing results in cellular fragmentation that is taken up and presented to T-cells by antigen-presenting cells (89). Furthermore, chemotherapy-induced bone marrow suppression was indicated to decrease immune-suppressive T-regulatory cells as well as induce proliferation of other T-cells, resulting in stimulation of the immune system (90). Trials evaluating these treatments are summarized in Table II. Similar combinations are also being explored in patients with MSS/unknown MSS status mCRC [reviewed in (91)].

Another strategy to enhance the host immune response includes combining ICIs with mAbs that block growth

factor receptors. In addition to this blockage, mAbs are thought to induce antibody-dependent cell-mediated cytotoxicity, hence justifying the above combination. However, current trials evaluating this combination are not specifically targeted to MSI-H/dMMR mCRC but rather serve as a potential strategy to sensitize the immune-insensitive MSS/pMMR CRC tumors. Small-molecule TKIs such as bevacizumab, an anti-VEGF molecule, have been evaluated in patients with MSI-H/dMMR mCRC. In preclinical studies, targeting VEGF has been demonstrated to offer potential therapeutic avenues for mCRC via suppression of tumor-associated macrophages, increase of interactions between dendritic cells and antigen-presenting cells, as well as augmenting endothelial vasculature to increase lymphocyte chemotaxis and T-cell tumor infiltration (92). This translated into a phase Ib clinical study that evaluated atezolizumab and bevacizumab combination in 10 pretreated patients with MSI-H/dMMR mCRC (93). At 11 months, the median OS had not been reached, the ORR was 30% and the median DOR was 7.8 months with 90% DCR. The ongoing COMMIT trial discussed earlier (NCT02997228) is investigating the combination of bevacizumab with chemo-immunotherapy (mFOLFOX6 and atezolizumab) as a first-line therapy in patients with MSI-H/dMMR mCRC (76).

Finally, the combination of ICIs with RT has also been considered in the treatment of mCRC based on the abscopal effect theory, whereby radiation of cancer cells is thought to induce immunogenic cell death (with increased neo-antigen exposure), resulting in immune activation against tumor cells at more distant sites (94). This theory remains anecdotal with pre-clinical evidence [reviewed in (95)], as well as clinical evidence of ipilimumab/RT combination in melanoma achieving marked tumor regression at non-irradiated metastatic sites (96). This synergistic treatment modality has not been previously studied or explored in patients with MSI-H/dMMR mCRC but has been evaluated as a way of sensitizing MSS/pMMR mCRC to immunotherapy. In a phase II study (NCT02437071), pembrolizumab was evaluated in combination with RT demonstrating good tolerance, but modest effects in terms of response to ICI monotherapy with only one patient achieving PR (ORR, 4.5%) (97). A subsequent study evaluated the effect of combining RT with dual ICIs: NSABP FC-9 (NCT02701400), a phase II single-arm study, evaluated the use of durvalumab (anti-PD-L1) plus tremelimumab (anti-CTLA-4) following hypofractionated RT in 20 patients with refractory MSS/pMMR mCRC (98). The initial results suggested a good overall safety profile and tolerance. Only two PRs were observed and lasted >44 weeks, along with 2 patients with stable disease for 12 and 16 weeks.

4. Mechanisms of resistance to ICIs in mCRC

Despite the marked responses to ICI compared to traditional therapies in patients with MSI-H/dMMR mCRC, up to 50% of patients ultimately acquire resistance to immunotherapy with subsequent progression and disease recurrence. While the MSI-H/dMMR status in itself, along with the TMB status, are powerful biomarkers of response to ICIs in mCRC, a

requirement remains to understand the mechanisms by which tumor cells develop resistance to ICIs and eventually manage to re-escape the host's immune surveillance mechanism.

Resistance to ICIs has been described as either innate, termed as 'immunological ignorance' pertaining to natural lack of immune response to developing tumors, or acquired following treatment with ICIs (99). Innate resistance is frequently observed in patients with systemic immune-suppression (e.g. HIV patients) or in those that express few molecular targets that are recognized by the immune system (e.g. non-virally-induced tumors with low TMB expression, referred to as 'cold'). This is not the case in patients with MSI-H/dMMR mCRC with high TMB and a TME rich in immune infiltrate. Those patients respond well to ICIs but eventually develop acquired resistance to immunotherapy. Acquired resistance has been looked at as a by-product of two elements: i) The tumor's transcriptional profile or gene signature, but more extensively ii) the molecular type of the TME as classified by O'Donnell *et al* (99).

As far as the genomic signature is concerned, gene-set enrichment analysis studies revealed that transcriptional profiles enriched with IFN- γ response genes are associated with better prognosis and response to anti-cancer immune therapies (100). Conversely, tumors with the innate anti-PD-1 resistance transcriptional profile lack any response to anti-PD-1 ICIs (101). In MSI-H/dMMR mCRC, IFN- γ response genes are enriched (31), accounting for the significant response to ICIs. Other potential genomic markers of response/resistance among this patient population have been investigated and preliminary results did not indicate any predictive impact of the RAS/RAF mutational status, PD-L1 expression or the origin of the MMR deficiency status (inherited Lynch syndrome vs. acquired/sporadic origin) (7,11,82,102). Numerous potential mechanisms of resistance are under investigation, including deleterious mutations in JAK, loss of MHC molecules or beta-2-microglobulin (B2M) loss-of-function mutations (103,104). Similarly, the amount of extracellular mucin (assessed by histopathology) has been suggested to be associated with resistance to ICI (105).

In terms of types of TME, MSI-H/dMMR mCRC tumors belong to type 1 TME which includes tumors with high TMB and an inflammatory gene signature. The three other types include different combinations of TMB levels along with presence/absence of inflammatory gene signature [reviewed in (99)]. The type of TME dictates the interaction between tumor cells and the immune system, and in type 1, high TMB levels and the presence of an inflammation gene signature are indicative of the existence of an ongoing but functionally suppressed immune response (106). Despite having the best probability of responding to the re-invigorative effect of ICIs, MSI-H/dMMR tumors exploit immune-suppressive/evasive strategies within the TME signaling pathway, which includes the following: Adaptive immune resistance pathways, loss of tumor antigen expression, insensitivity to interferons and cytokine/metabolite dysregulation (99). Understanding these mechanisms of resistance may provide additional potential targets to treat MSI-H/dMMR tumors. Table III summarizes the mechanisms of resistance to ICIs.

Adaptive immune resistance. As mentioned in prior sections, elevated levels of type I and II interferons as well as immunosuppressive cytokines (IL-6, IL-12, TGF- β) lead to up-regulation of PD-L1 on immune cells and potentiate immune escape. While this interaction between PD-1 and PD-L1 is successfully targeted and disrupted by ICIs, the actual expression of PD-L1 is regulated in a complex manner beyond cytokine release and this includes influences by genomic alterations, transcriptional control mechanisms, mRNA stability as well as oncogenic signaling and protein stability (107). These alternative regulators of PD-L1 expression potentially represent novel targets to augment the efficacy of ICIs and extend the DOR. Similarly, it was indicated that tumors are able to release exosomes that express PD-L1, further contributing to immune suppression (108). Other adaptive immune resistance pathways have also been characterized, including overexpression of CD155 in both tumor cells and tumor-infiltrating myeloid cells (109). Binding of CD155 to CD96 and TGIT on tumor cells results in inhibition of the TILs and conversely, binding to CD266 on T-cells and NK cells competes with the prior inhibitory interaction (110). Thus, therapies targeting CD96 and TGIT may present a possible way to overcome resistance with evidence from preclinical tumor models demonstrating efficacy and synergy when PD-1 inhibition is combined with TGIT targeting (109-111).

Finally, the adaptive immune resistance to PD-1 inhibition has been indicated to be mediated through T-cell induced production of macrophage colony-stimulating factor 1 (MCSF-1), suggesting co-treatment of patients with anti-PD1 and MCSF-1 inhibitors as a possible strategy (112).

Loss of tumor antigen expression. Another mechanism for acquired resistance is the loss of the ability to present neoantigens, mostly via mutations affecting the antigen-presenting machinery such as proteasome subunits or transporters associated with antigen processing, or proteins involved in folding and sub-cellular translocation of MHC molecules (113). For instance, loss-of-function mutations of B2M, a chaperone protein essential for the folding and transport of MHC molecules to the cell surface, limits the recognition of tumor antigens by T-cells (114). This was clinically associated with resistance to ICIs (101,104,115). Epigenetic events associated with tumor development and progression constitute another mechanism that alter human leukocyte antigen expression in tumor cells; unlike genetic alterations, epigenetic changes may potentially be reversed pharmacologically (113).

Insensitivity to IFNs. As indicated earlier, IFN- γ is a major regulator of anti-tumor immunity that may also promote tumor resistance. Exposure of tumors to IFN- γ in pre-clinical studies resulted in genetic instability in these tumors that translated into higher copy number variation associated with the DNA damage response and repair genes (116). However, IFN- γ was also reported to induce neoantigen loss/down-regulation, thus favoring tumor escape (116,117). Similarly, in tumor analysis from melanoma patients who responded poorly or acquired resistance to anti-CTLA-4 therapy, a higher frequency of mutations within genes

involved in the IFN- γ signaling pathway was noted and this included IFN- γ receptor 1 (IFNGR1), IFNGR2, JAK2 and IFN regulatory factor 1 (104,118). Similar observations of immune escape have also been made through loss of sensitivity to TNF (119). While the precise mechanisms behind this loss of sensitivity in both pathways have not been clearly defined, these observations suggest a potential benefit from genetic screening to identify new immunotherapy targets that operate in previously unknown pathways.

Cytokine and metabolite dysregulation. Hypoxia in TME promotes the accumulation of extra-cellular ATP that is metabolized to adenosine (120). One of the downstream effects of adenosine accumulation is the induction of strong immunosuppression within the TME: Adenosine signaling may impair effector T-cells and NK cells (120-122), and ADP-mediated immunosuppression through production of adenosine has been reported as a mechanism of tumor cell escape from PD-1/PD-L1 inhibition (123). Based on these observations, co-targeting enzymes involved in ATP degradation or blocking adenosine receptors may improve the efficacy of PD-1/PD-L1 inhibition (120). Tryptophan is another metabolite that mediates immunosuppression when catabolised in the TME, via upregulation of indoleamine 2,3-dioxygenase 1 (IDO1), an IFN- γ -inducible enzyme (124). Preclinical work in cancer mouse models indicated that IDO promotes immunosuppression and IDO1 inhibitors had synergistic effects with ICIs (125). Despite promising early-phase trials of IDO1 inhibitors, the first phase III trial of an IDO1 inhibitor in combination with an anti-PD-1 antibody in melanoma patients did not achieve its primary endpoint (NCT02752074). However, the study still demonstrated the importance of concurrent biomarker and target development along with ICI development. In addition to upregulation of immunosuppressive metabolites, tumor cells frequently overexpress immunosuppressive cytokines, including VEGF and TGF- β . VEGF promotes upregulation of PD-1, CTLA-4 and TIM3 on TILs, thus promoting T-cell dysfunction (126,127). Similarly, TGF- β was indicated to upregulate PD-L1 expression and to promote metastasis of lung tumors, thus suggesting the potential of co-targeting PD-L1 and TGF- β receptor (128).

5. Conclusions and future direction

Immunotherapy with checkpoint inhibitors has revolutionized the treatment of MSI-H/dMMR mCRC. This success is primarily based on the discovery of the immune escape phenomenon on the one hand and the advent of mAbs that block immune checkpoints and re-invigorate the immune system on the other hand. This strategy has proven successful in the MSI-H/dMMR population specifically based on this subtype's high mutation burden and its ability to present the FSP as neoantigens on MHC class I molecules to prime T-cells to recognize them as foreign. This has culminated in the FDA approval of anti-PD-L1 and anti-CTLA-4 ICIs for the treatment of MSI-H/dMMR mCRC. Despite this success, challenges have been encountered in primary non-responders and in those who develop acquired resistance to ICIs. As the current knowledge of the immune system and its intricacies continues to grow,

Table III. Mechanisms of resistance to immune checkpoint inhibitor therapy in microsatellite instability-high/deficient mismatch repair colorectal cancer.

Mechanism of resistance	Description
Adaptive immune resistance	- Immunosuppressive cytokine-mediated upregulation of PD-L1 - Release of PD-L1 expressing exosomes - CD155 up-regulation on tumor cells inhibits TILs - MCSF-1 mediated immune resistance
Loss of tumor antigen expression	- Loss-of-function mutations in antigen-presenting machinery (e.g. B2M) - Epigenetic modulation of HLA expression in tumor cells
IFN- γ insensitivity	- IFN- γ -mediated neo-antigen loss/down-regulation \rightarrow immune escape - Mutations in IFN- γ signaling pathway genes (IFNGR2, JAK2 and IRF1) \rightarrow poor response to anti-CTLA-4 therapy in melanoma
Cytokine/metabolite mediated immune dysregulation	
Adenosine-mediated immunosuppression	Hypoxia \rightarrow ATP to AMP conversion: AMP impair effector T-cells and NK cells \rightarrow escape from PD-1/PD-L1 inhibition
Tryptophan mediated immune suppression	IFN- γ \rightarrow \uparrow IDO1 expression \rightarrow \uparrow Tryptophan-mediated immune suppression in TME
Tumor expressed immunosuppressive cytokines	* VEGF \rightarrow \uparrow PD-1, CTLA-4 and TIM3 expression \rightarrow T-cell dysfunction * TGF- β \rightarrow \uparrow PD-L1 expression

CTLA-4, cytotoxic T lymphocyte antigen 4; PD1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; TIM3, T cell immunoglobulin mucin receptor 3; MCSF-1, macrophage colony-stimulating factor 1; HLA, human leukocyte antigen; IFN- γ , interferon- γ ; ATP, adenosine triphosphate; AMP, adenosine monophosphate; NK cell, natural killer cell; IDO1, indoleamine 2,3-dioxygenase 1; TME, tumor microenvironment; VEGF, vascular endothelial growth factor; TGF- β , transforming growth factor- β ; TILs, tumor-infiltrating lymphocytes; IFNGR2, IFN- γ receptor 2; IRF1, IFN regulatory factor 1.

a better understanding of the different mechanisms behind immune resistance is gradually being achieved, allowing for the development of novel therapies, therapeutic combinations and strategies to overcome resistance.

At present, new-generation ICIs are being evaluated alone or in combination, with the hope of augmenting initial host anti-tumor immune response and enhance therapeutic efficacy. Other strategies include combination of ICIs with conventional therapy (chemotherapy, radiation, TKIs), as well as with new immune modulators. Furthermore, and based on the high frequency of generated FSPs in MSI-H/dMMR mCRC populations, efforts have been directed towards exploiting these mechanisms to develop individualized therapies, either by developing epitope-based vaccines or through harnessing adoptive cellular therapies via *ex-vivo* T-cell manipulation and chimeric antigen receptor T-cell generation. Although still in their early phases of clinical research, these novel approaches in conjunction with the ongoing trials outlined in the present article offer promising avenues to further advance immunotherapy in patients with MSI-H/dMMR mCRC.

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AS and AB conceived and designed the structure of this manuscript. KC and MR reviewed the literature and collated data from major studies and clinical trials. KC, MR and RP provided the initial draft of the manuscript, including the tables. AS, AB, KC and RP revised the manuscript for important intellectual content. All authors read and approved the final manuscript. Data authentication is not applicable.

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