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Progress of Immune Checkpoint Inhibitors Therapy for pMMR/MSS Metastatic Colorectal Cancer

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Abstract: Immunotherapy is one of the research hotspots in colorectal cancer field in recent years. The colorectal cancer patients with mismatch repair-deficient (dMMR) or high microsatellite instability (MSI-H) are the primary beneficiaries of immunotherapy. However, the vast majority of colorectal cancers are mismatch repair proficient (pMMR) or microsatellite stability (MSS), and their immune microenvironment is characterized by "cold tumors" that are generally insensitive to single immunotherapy based on immune checkpoint inhibitors (ICIs). Studies have shown that some pMMR/MSS colorectal cancer patients regulate the immune microenvironment by combining other treatments, such as multi-target tyrosine kinase inhibitors, anti-vascular endothelial growth factor (VEGF) monoclonal antibodies, chemotherapy, radiotherapy, anti-epithelial growth factor receptor (EGFR) monoclonal antibodies, and mitogen-activated protein kinase (MAPK) signaling pathway inhibitors and oncolytic viruses, etc. to transform "cold tumor" into "hot tumor", thereby improving the response to immunotherapy. In addition, screening for potential prognostic biomarkers can also enrich the population benefiting from immunotherapy for microsatellite stable colorectal cancer. Therefore, in pMMR or MSS metastatic colorectal cancer (mCRC), the optimization of immunotherapy regimens and the search for effective efficacy prediction biomarkers are currently important research directions. In this paper, we review the progress of efficacy of immunotherapy (mainly ICIs) in pMMR /MSS mCRC, challenges and potential markers, in order to provide research ideas for the development of immunotherapy for mCRC.

Keywords: immune checkpoint inhibitors, metastatic colorectal cancer, pMMR/MSS, combination immunotherapy, biomarkers

Background

Immune checkpoint inhibitors (ICIs) enhance anti-tumor immunity by regulating the interaction between T lymphocytes, antigen presenting cells (APC) and tumor cells, and its mechanism is to reactivate the immune response of T cells to tumor cells and inhibit tumor growth, which has brought long-term and lasting benefits to patients with multiple types of solid tumors.[1,2] The dMMR/MSI-H tumors are the main beneficiaries of immunotherapy due to their high immunogenicity, strong lymphocyte infiltration in tumor microenvironment, good prognosis, and insensitivity to conventional radiotherapy and chemotherapy.[3–5]

The results of KEYNOTE 016, reported by Professor Le's team at Hopkins Hospital in 2015, showed that dMMR metastatic colorectal cancer (mCRC) could benefit from pembrolizumab monotherapy, This study opened the door of immunotherapy for colorectal cancer and opened the era of immunotherapy for colorectal cancer.[6] Subsequently, data from the first phase of the CheckMate142 study, reported in 2016, suggested that recurrent dMMR/MSI-H mCRC could benefit from a combination of nivolumab and ipilimumab.[7] Long-term follow-up showed that the combination helped dMMR/MSI-H mCRC patients achieve 77% PFS and 83% OS at 1 year. Based on these data, the US Food and Drug Administration (FDA) has approved the combination of nivolumab and ipilimumab for previously treated dMMR/MSI-H mCRC patients.[8] The KEYNOTE-177 study, published in 2020, is the most important clinical trial in first-line therapy to date, which confirmed that pembrolizumab monotherapy can be used as the first-line standard treatment for dMMR/MSI-H colorectal cancer.[9,10] KEYNOTE-177 successfully rewrote the NCCN colorectal cancer treatment guidelines.[11]

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Studies on the mechanism have found that somatic mutations in MSI-H colorectal cancer produce a large number of nascent peptides, which can act as specific antigens of tumor cells.[11] The combination of these neoantigens with the major histocompatibility complex (MHC) can induce the immune response of CD4+ or CD8+ T cells, while attracting the infiltration of tumor infiltrating lymphocyte (TIL) in the tumor microenvironment, thus making the tumor "hot" and increasing the response of CRC to immunotherapy.[12,13]

Although dMMR/MSI-H patients belong to the "hot tumor" population that is sensitive to immunotherapy, the proportion of dMMR/MSI-H patients in all mCRC are only 4% to 5%,[10] and a larger proportion of pMMR /MSS patients belong to the "cold tumor" population that is insensitive to immunotherapy.[14] Studies have found that for pMMR /MSS colorectal cancer, the tumor lacks antigens that induce T cell initiation, and the tumor microenvironment lacks T cell infiltration, so the antitumor effect of ICIs in these "cold tumor" populations that are not sensitive to immunotherapy has certain limitations.[15–17] Existing studies have shown that the use of a single ICIs alone is essentially ineffective for pMMR/MSS mCRC.[6,18] How to break through this dilemma has always been the challenge. Most of the current treatment strategies use combination therapy to reverse the "cold tumor" into the "hot tumor" sensitive to immunotherapy, so as to improve the efficacy of immunotherapy in pMMR/MSS colorectal cancer, so that immunotherapy can benefit more colorectal cancer patients.[16,17,19,20] Although some combination treatment strategies have achieved good short-term efficacy and are promising in the treatment of patients with mCRC, most of them are single-arm small sample exploratory studies, and the research data are not completely stable and consistent (Table 1), and the safety and efficacy need to be verified by more prospective clinical trials with large samples.[14,21]

In addition, several studies are looking for other markers with potential to predict immunotherapy efficacy, not only for mismatch repair genes, but also for other molecular markers to enrich the population who can benefit from immunotherapy for pMMR/MSS colorectal cancer. Current studies have found that multiple molecular markers such as tumor mutation burden (TMB), polymerase ε and polymerase δ (POLE/POLD1) mutations, PD-L1 expression, tumor infiltrating lymphocytes, and gut microbiota have the potential and value to predict the efficacy of ICIs for pMMR/MSS colorectal cancer.[29,68–76] This article aims to review the research progress of ICIs combination therapy for pMMR/MSS mCRC and related studies on the predictive markers of ICIs efficacy, in order to provide references for finding the best immunotherapy strategy for pMMR/MSS mCRC patients.

ICIs Combined with Multi-Target Tyrosine Kinase Inhibitors

The mechanism of primary resistance to immunotherapy in pMMR/MSS colorectal cancer patients is complex and involves many factors in tumor cells and tumor microenvironment. The tumor microenvironment is composed of blood and lymphovascular networks, extracellular matrix (ECM), various stromal cells (including fibroblasts, mesenchymal stromal cells, and pericytes), and resident and infiltrating immune cells (including T lymphocytes, B lymphocytes, natural killer cells, and tumor-associated macrophages.[77,78] Tumor neovascularization plays an important role in tumor microenvironment. Previous studies have shown that overexpression of VEGF and the resulting over-activation of VEGF receptors stimulate endothelial cell proliferation, promote angiogenesis, and improve the availability of oxygen and nutrients in tumors.[79] Meanwhile, abnormal activation of receptor tyrosine kinase (RTK), including endothelial growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF), plays a key role in the development and progression of cancer. This makes abnormal RTK a potential therapeutic target for cancer therapy.[79,80]

Preclinical studies have shown that inhibitors targeting VEGF/VEGF receptor have both effective anti-angiogenesis and immunomodulatory effects, can induce tumor vascular degeneration, improve hypoxia and low pH in the tumor microenvironment, and increase the infiltration of immune T lymphocytes in the tumor microenvironment through tumor revascularization, then an inflammatory tumor microenvironment is generated;In addition, anti-VEGF therapy has a synergistic effect with immunotherapy by up-regulating the expression of PD-L1, reducing immunosuppressive cells [such as tumour-associated macrophages (TAMs) and regulatory T cells (Tregs)], and enhancing the anti-tumor activity of CD8+T cells.[81,82] Studies in mouse models have confirmed that the combination of anti-VEGFR2 and anti-PD-1 monoclonal antibodies can increase the production of tumor interferon-gamma, tumor necrosis factor-α and granzyme B, thereby enhancing the immune response of mice.[83]

Table 1 The Results of Clinical Trials Based on ICIs in Combination with Other Modalities in pMMR/MSS Metastatic Colorectal Cancer

Treatment	Study	Design	Result	Reference
ICIs combined with multi-target tyrosine kinase inhibitors	REGONIVO **NCT03406871**. Phase I b trial	Regorafenib plus nivolumab	Objective response rate (ORR) was 33.3% and the median progression free survival (PFS) was 7.9 months. The 1-year PFS rate and overall survival (OS) rate were 41.8% and 68.0%, respectively.	[22]
	NCT04126733. Phase 2 study, Single-arm,	Regorafenib plus nivolumab	ORR was only 7.1%, median PFS was 1.8 months, and median OS was 11.9 months.	[23]
	REGOMUNE **NCT03475953**. Phase II study	Regorafenib plus avelumab	Of the 40 evaluable patients, the best response was in 23 patients (57.5%) who were stable, with a median PFS and OS of 3.6 and 10.8 months, respectively.	[24,25]
	REGOTORI **NCT03946917**. Phase Ib/II trial	Regorafenib plus toripalimab	The ORR of 33 enrolled patients was 15.2%, and the median PFS and OS were 2.1 and 15.5 months, respectively.	[26]
	LEAP-005 **NCT03797326**. PhaseII trial	Lenvatinib plus pembrolizumab	The 32 MSS colorectal cancer patients had an ORR of 22% and a DCR of 47%; and the median PFS and OS were 2.3 months and 7.5 months, respectively.	[27]
	NCT03903705. Phase 1b study	Fruquintinib plus sintilimab	44 mCRC patients who received intermittent administration of Fruquintinib combined with sintilimab had ORR of 27.3% and mPFS of 6.8 months, respectively.	[28]
Combination therapy with two ICIs	CCTG CO.26 **NCT02870920**. PhaseII randomized study	Durvalumab plus tremelimumab and best supportive care (BSC) versus BSC alone	A total of 180 patients were included in the study, excluding patients with dMMR, and the results showed that the study met the primary endpoint, with the median OS increased from 4.1 months in the BSC group to 6.6 months in the dual immunotherapy group.	[29]
	NCT04362839. Phase I trial	Regorafenib plus ipilimumab plus nivolumab	ORR reached 31%, and the median PFS and OS were 4 months and 19.6 months, respectively, which were significantly improved compared with traditional third-line treatment for mCRC.	[30]
	NCT03860272. Phase 1A/B study	Botensilimab plus balstilimab (AGEN2034, a PD-1 inhibitor)	Results showed that dual immunotherapy had an ORR of 24% and a disease control rate (DCR) of 73% in 41 evaluable patients. At the same time, the safety status was good, and most treatment-related adverse events were grade 1–3.	[31]
	NCT02720068. Phase I study	Pembrolizumab plus anti-LAG-3 antibody MK4280 (favezelimab)	ORR reached 6.3% in 80 patients with pMMR/MSS mCRC who progressed after prior≥2 lines therapy. The median PFS and OS were 2.1 and 8.3 months, respectively.	[32]

(*Continued*)

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Table 1 (Continued).

Treatment	Study	Design	Result	Reference
ICIs combined with chemotherapy	METIMMOX **NCT03388190.**	FLOX plus nivolumab versus FLOX alone	ORR rates in the combined immunotherapy group and chemotherapy group were 48% and 23%, CR rates were 16% and 0%, respectively, and the median PFS in the combined immunotherapy group and chemotherapy group were 6.6 months and 5.6 months, respectively.	[33]
	KEYNOTE-651: cohorts B and D **NCT03374254.**	Pembrolizumab plus mFOLFOX7 or FOLFIRI	Pembrolizumab combined with mFOLFOX7 had an ORR of 58% for first-line therapy and Pembrolizumab combined with FOLFIRI had an ORR of 16% for second-line therapy. Both combination regimens showed antitumor activity in pMMR/MSS mCRC patients.	[34]
	MAYA Trial **NCT03832621.**	Temozolomide Followed by Combination with Low-Dose Ipilimumab and Nivolumab	The results showed that the median PFS and OS were 7.1 months and 18.5 months, respectively, and the ORR was as high as 39%; the incidence of grade 3 and above adverse reactions is low.	[35,36]
	BACCI **NCT02873195**. PhaseII study	Capecitabine bevacizumab plus atezolizumab versus capecitabine bevacizumab plus placebo	The addition of atezolizumab failed to significantly improve PFS and OS.	[37]
	NCT03396926. Phase II study	Pembrolizumab plus capecitabine and bevacizumab	The ORR in the three-drug combination treatment group was 5%, which did not meet the predetermined target, but the median PFS and OS reached 4.3 and 9.6 months, respectively.	[38]
	MODUL cohort 2 **NCT02291289.**	Fluoropyrimidine (FP) + bevacizumab (BEV) + atezolizumab vs FP/BEV	In the updated analysis (median follow-up 18.7 months), PFS outcome was unchanged (HR = 0.96; 95% CI 0.77–1.20; p = 0.727) and OS with 51% of pts with an event was HR = 0.86; 95% CI 0.66–1.13; p = 0.28.	[39,40]
	CheckMate 9×8 **NCT03414983.**	Nivolumab+mFOLFOX6/bevacizumab (BEV) versus mFOLFOX6/BEV	The median PFS in both groups was 11.9 months, which did not meet the primary endpoint of PFS. After 12 months, the PFS rate in the NIVO + SOC group was higher than that in the SOC group.	[41]
	BBCAPX **NCT05171660** Phase 2 trial	Sintilimab plus bevacizumab, oxaliplatin and capecitabine	The results showed that ORR and DCR were 84.0% and 100.0%, respectively. This efficacy data shows that the addition of immunotherapy improves efficacy by about 20% compared to the existing standard regimen. The median PFS was 18.2 months.	[42]
	NIVACOR **NCT04072198**. Phase II study	Nivolumab plus FOLFOXIRI/bevacizumab	The results showed that ORR and DCR were 78.9% and 96.2%, but mPFS were only 9.8 months.	[43]
	AtezoTRIBE **NCT03721653**. Phase 2 trial	FOLFOXIRI plus bevacizumab with or without atezolizumab	The results show that adding atezolizumab to first-line FOLFOXIRI/bevacizumab is safe. The median PFS of 13.1 months in the altazomab group was better than that of 11.5 months in the control group.	[44]
	MEDETREME **NCT03202758**. Phaselb /II	Durvalumab plus tremelimumab combined with FOLFOX	ORR and CR were 62.5% and 25%, respectively, and 1-year PFS were 50%;48 patients were MSS type, with a median PFS of 8.2 months, showing good safety.	[45–47]

ICIs combined with Radiotherapy	ANAVA **NCT03854799**. Phase II study	Preoperative (PREOP) chemoradiotherapy (CTRT) plus avelumab (AVE)	The results showed that 22 cases (23%) achieved pathological complete response (pCR),59 cases (61.5%) achieved obvious pathological remission, the rates of non-immune and immune-related adverse reactions above grade 3 were only 8% and 4%.	[48]
	VOLTAGE-A **NCT02948348.**	Preoperative Chemoradiotherapy plus Nivolumab before Surgery	39 MSS LARC patients treated with nivolumab after the end of long-term concurrent chemoradiotherapy achieved 38% major pathological response (MPR) and 30% pathological complete response (pCR).	[49]
	NCT03104439. Phase II trial.	Nivolumab + ipilimumab + hypofractionated short-course radiotherapy	The results showed that the ORR and DCR were 10% and 25% respectively, and the mOS was 7.1 months.	[50]
	NCT03122509. Phase II trial.	Durvalumab plus Tremelimumab plus Concurrent Radiotherapy	Achieved an 8.3% response rate, which did not meet the preset ORR of at least 25%, despite a median OS of 11.4 months.	[51]
	ETCTN 10021 **NCT02888743**. Phase 2 study	PD-L1 and CTLA-4 inhibition plus low-dose fractionated RT (LDFRT) or hypofractionated RT (HFRT)	Best response was stable disease in one patient with an abscopal out-of-field response who developed new lesions after HFRT and 4 cycles with decreasing overall disease burden. Toxicity was, in general, consistent with PD-L1/CTLA-4 inhibition with no significant RT-related toxicities noted.	[52]
ICIs combined with targeted drug	**CAVE NCT04561336. Phase 2 Trial**	Cetuximab Rechallenge Plus Avelumab	The median PFS and OS of the population were 3.6 months and 11.6 months, respectively, but the overall objective response rate was not high, only about 7%.	[53,54]
	AVETUXIRI NCT03608046. Phase II trial	Avelumab Plus cetuximab and irinotecan	Cohort A was RAS wild type and cohort B was RAS mutant. The interim analysis results showed that the ORR of cohort A and cohort B were 30% and 0%, respectively, which seemed to be quite different. However, 6-month PFS rates were 40% and 38.5%, and 1-year OS rates were 50% and 46.2%, respectively.	[55]
	TEC **NCT05143099**. Phase II trial	Tislelizumab plus cetuximab and irinotecan	The results showed an ORR of 36.4%, exceeding the study's preset value of 30%, and a DCR of 78.8%, and mPFS has not yet been achieved.	[56]
	AVETUX **NCT03174405**. PhaseII trial	Avelumab Plus cetuximab and mFOLFOX6	Among the 41 MSS mCRC patients, the 12-month PFS rate was 40% and the median PFS was 11.1 months. The overall ORR and DCR were 81% and 89%, respectively.	[57]
	NCT03442569. Phase II study	Ipilimumab Plus nivolumab Plus panitumumab	ORR was 35%.	[58]
	NCT04017650. Phase I/II trial	Encorafenib Plus cetuximab Plus nivolumab	A total of 26 patients were enrolled, and the ORR and DCR of 24 patients that could be evaluated reached 50% and 96%, respectively, and the median PFS and OS were 7.4 months and 15.1 months, respectively.	[59]
	NCT01988896. Phase Ib study	Atezolizumab Plus cobimetinib	ORR and DCR was 17% and 39%, respectively. The 12-month PFS and OS rates were 11% and 43%, respectively.	[60,61]

(*Continued*)

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Table 1 (Continued).

Treatment	Study	Design	Result	Reference
	IMblaze370 **NCT02788279**. Phase3 trial	Atezolizumab with or without cobimetinib versus regorafenib	Results showed that atezolizumab alone or combined with cobimetinib achieved OS of 7.1 and 8.9 months, respectively, which was not significantly better than the standard treatment regorafenib monotherapy (OS of 8.5 months)	[62]
	NCT03428126. **P**hase II study	Durvalumab Plus trametinib (MEKi)	One of 29 pts had confirmed partial response (PR) lasting 9.3 months (mo) for an overall response rate of 3.4%. Seven pts had stable disease (SD) and Median progression-free survival was 3.2 mo (range 1.1–9.3 months).	[63]
	NCT03475004. Phase II study	Pembrolizumab Plus binimetinib and bevacizumab	ORR and DCR was 12% and 94%, respectively. The median PFS was 6.4 months and the adverse reactions were tolerable.	[64]
	NCT03668431. Phase 2 trial	Dabrafenib Plus trametinib Plus the anti-PD -1 drug sparatlizumab (PDR001)	In 32 patients with microsatellite stabilization, ORR and DCR reached 25% and 75%, respectively, and the combination regimen was well tolerated.	[65]
ICIs combined with novel development drugs	GEMCAD 1602 **NCT03152565**. **Phasel/II study**	Avelumab plus ADC vaccine	Combined therapy was safe and well tolerated. Median PFS was 3.1 months [2.1–5.3 months] and overall survival was 12.2 months [3.2–23.2 months].	[66]
	NCT02324257/ NCT02650713. Phasela/Ib studie	RO6958688 monotherapy (S1) or in combination with atezolizumab (S2)	Preliminary tumor size reduction (> −10% and < −30% [stable disease]) was observed in 4 (11%) additional pts in S1 and 5 (50%) in S2. The most common grade ≥ 3 (G3) related AEs were IRR (16.3%) and diarrhea (5%).	[67]

Abbreviations: ORR, overall response rate; PFS, median progression free survival; OS, overall survival; DCR, disease control rate; RT, Radiotherapy; MSS, microsatellite stability; mCRC, metastatic colorectal cancer; CR, complete response.

Regorafenib is the first FDA-approved multi-target TKI in metastatic colorectal cancer that has progressed after treatment with standard treatment, targeting angiogenesis (VEGFR1-3, TIE2), stroma (PDGFR-β) and cancer-causing receptor tyrosine kinases (KIT, RET and RAF).[84,85] Regorafenib has been shown to regulate anti-tumor immunity through different mechanisms. In a mouse hepatocellular carcinoma model, regorafenib normalizes tumor blood vessels by targeting VEGFR2/3, promotes the proliferation and activation of CD8+T cells, and improves the anti-tumor immunity of PD-1 blockers.[86]

In addition, Indoleamine 2,3-dioxygenase 1 (IDO1) overexpression has been shown to induce dendritic cells and regulatory T cells in a variety of cancers, leading to immunosuppressive effects, and therefore IDO1 is likely to be a potential target for anti-tumor immunotherapeutic intervention.[87,88] Studies have shown that regorafenib can simultaneously reduce the expression of PD-L1 and IDO1, reduce the TAM infiltration, and produce synergistic effects with anti-PD-1 monoclonal antibodies to significantly improve the anti-tumor effect.[89] Doleschel also found that regorafenib significantly reduced the infiltration of immunosuppressive TAM and Treg cells into the tumor microenvironment in his study of CRC mouse models developed after chemotherapy, while anti-PD-1 treatment significantly increased intratumoral IFN-γ levels, thus the two have a synergistic effect in regulating anti-tumor immune response, thus achieving sustained tumor suppression.[90] Based on the above theoretical basis, in recent years, the combination of multi-target receptor tyrosine kinase inhibitors, including regorafenib, and immune checkpoint inhibitors has been attempted in pMMR/MSS mCRC.

The REGONIVO study, reported in Japan in 2020, is a Phase I b trial that enrolled 24 patients with pMMR/MSS colorectal cancer after failure of standard treatment to evaluate the safety and efficacy of the combination of regorafenib and nivolumab. The results showed that the objective response rate (ORR) was 33.3% and the median progression free survival (PFS) was 7.9 months. The 1-year PFS rate and overall survival (OS) rate were 41.8% and 68.0%, respectively.[22] This study shows that some MSS mCRC patients may benefit from the combination of PD-1 inhibitors with multi-target TKI. However, this result was not replicated in the North American Phase II study, with an ORR of only 7.1%, median PFS of 1.8 months, and median OS (data immature) of 11.9 months. All responders had no liver metastases at baseline.[23] It is suggested that the benefit group should be screened in this treatment mode. The researchers subsequently conducted a retrospective exploratory analysis, and the results suggested that there was a certain correlation between some specific biomarkers and treatment response.[91]

The Phase II REGOMUNE study is evaluating the efficacy and safety of regorafenib in combination with Avelumab in treated MSS colorectal cancer. Of the 40 evaluable patients, the best response was in 23 patients (57.5%) who were stable, with a median PFS and OS of 3.6 and 10.8 months, respectively.[24,25] Studies have shown that PFS and OS obtained by this combination therapy are superior to historical data obtained by regorafenib alone.[92] The REGOTORI study, led by Professor Xu Ruihua, is a Phase Ib/ II clinical trial evaluating the efficacy of regorafenib combined with the PD-1 inhibitor toripalimab in treated MSS mCRC. The ORR of 33 enrolled patients was 15.2%, and the median PFS and OS were 2.1 and 15.5 months, respectively. Studies have also shown that patients with lung metastasis have a relatively higher effective rate, while patients with only liver metastasis have a relatively poor effect.[26]

Lenvatinib is another multi-target receptor TKI. In pre-clinical studies, it was found that Lenvatinib combined with pembrolizumab can activate CD8+ T cells by reducing TAM and activating interferon pathway, thus producing synergistic effects with anti-PD-1 antibodies and enhancing anti-tumor effects.[93] LEAP-005 (NCT03797326) is a Phase II study of lenvatinib and pembrolizumab in previously treated patients with advanced solid tumors of pMMR/MSS.[27] The 32 MSS colorectal cancer patients had an ORR of 22% and a disease control rate (DCR) of 47%; and the median PFS and OS were 2.3 months and 7.5 months, respectively. It showed good antitumor activity and a manageable safety profile. The Phase III LEAP 017 study (NCT04776148) is being recruited worldwide based on the results of pembrolizumab in combination with lenvatinib in treated pMMR/MSS mCRC patients.

Fruquintinib is a potent and highly selective small-molecule TKI against VEGFR1, 2, and 3,[94] and its survival benefits have been demonstrated in patients with mCRC in the Phase 3 FRESCO clinical trial.[95] Clinical trials of fruquintinib in combination with immunotherapy are also underway. Preliminary results from a Phase 1b/2 study of sintilimab combined with fruquintinib in the treatment of advanced colorectal cancer (NCT03903705) suggest that the combination of sintilimab and fruquintinib is effective and safe to tolerate in patients with mCRC who have failed standard therapy. In this study,44 mCRC patients with disease progression after receiving at least two-line chemotherapy

containing fluorouracil, oxaliplatin or irinotecan in the past were included. Patients who received intermittent administration of Fruquintinib combined with sintilimab had ORR of 23.8% and mPFS of 6.9 months, and mOS of 14.8 months respectively.[28]

It can be seen that despite the exciting results of the REGONIVO Phase Ib study, and ORR in MSS colorectal cancer patients reached 33%,[22] thus opening the paradigm of refractory MSS colorectal cancer with regorafenib combined with anti-PD-(L)1 monoclonal antibody. However, subsequent single-arm studies using immune checkpoint inhibitors in combination with different anti-angiogenesis targeting agents (tyrosine kinase inhibitors) as previously treated mCRC therapy have been reported, including the North American REGONIVO study,[23] REGOMUNE study (regorafenib combined with Avelumab),[25] REGOTORI study (regorafenib combined with toripalimab),[26] LEAP-005 colorectal cancer cohort (Lenvatinib combined with pembrolizumab).[27] Most studies did not repeat the results consistent with the Japanese REGONIVO study, but in general, the reported ORR of these studies ranged from 7% to 27%, DCR from 39% to 80%, and median OS from 7.5 to 15.5 months. Compared with previous studies on standard third-line monotherapy (regorafenib, fruquintinib or TAS-102), both ORR and OS were numerically improved.[92,95,96] However, the sample size of these studies is limited, so more high-level phase III randomized controlled trials are needed for validation, and the preliminary study supports the need to further explore the screening of advantageous populations in the future.

Combination Therapy with Two ICIs

Anti-PD-(L)1 Combined with Anti-CTLA-4 Therapy

Cytotoxic T lymphocyte antigen-4(CTLA-4) mainly inhibits T cell activation in the initial phase, thereby inhibiting T cell activity.[97] Anti-CTLA-4 monoclonal antibody can block the inhibitory signal transduction of CTLA-4, thereby promoting T cell activation and immune response to tumor cells; However, anti-PD-(L)1 monoclonal antibody removes the inhibition of the combination of PD-1 and PD-L1 on activated T cells and promotes the killing effect of T cells on tumor cells.[1,98] Therefore, the combination of the two can synergistically promote the anti-tumor immune response through the blocking complementary mechanism, thereby the acquired resistance to ICIs monotherapy is overcome[1,99] and has a good safety profile.[100] It has been observed that anti-PD (L)-1 antibody combined with anti-CTLA-4 antibody treatment has shown relatively excellent anti-tumor effects in a variety of malignant tumors (including melanoma, lung cancer, etc),[101,102] and has begun to be explored in colorectal cancer, although the exact mechanism of action is still unclear.[103]

The CCTG CO.26 randomized Phase II trial evaluated whether the combination of durvalumab and tremelimumab improved survival in patients with advanced refractory colorectal cancer compared to best supportive care (BSC). A total of 180 patients were included in the study, excluding patients with dMMR, and the results showed that the study met the primary endpoint, with the median OS increased from 4.1 months in the BSC group to 6.6 months in the dual immunotherapy group, while the incidence of grade 3 or 4 adverse events also increased significantly.[29] Although this study was the first to report positive results for PD-L1 monoclonal antibody combined with CTLA-4 monoclonal antibody in MSS mCRC, it was not widely accepted in the clinic, mainly because no patients in the study achieved complete response (CR). Only one patient with MSS type achieved partial response (PR). This study suggests the potential role of dual immunotherapy in MSS mCRC.[29]

In a clinical trial (NCT02754856), a combination of durvalumab and tremelimumab was administered to 21 pMMR patients with only liver metastasis before surgery, and evidence of extended relapse-free survival (RFS) and T cell activation was initially observed.[104] In addition, the Fakih team conducted a phase I study on nivolumab combined with ipilimumab and regorafenib in MSS mCRC patients who had not been treated with regorafenib and anti-PD (L) −1 monoclonal antibody,[30] and also achieved surprising results. ORR reached 31%, and the median PFS and OS were 4 months and 19.6 months, respectively, which were significantly improved compared with traditional third-line treatment for mCRC.[92,95,96]

Botensilimab (AGEN1181) is a new generation FC-enhanced CTLA-4 inhibitor that blocks the inhibitory function of CTLA-4 interactions with its ligands CD80 and CD86. A Phase 1A/B study by Professor Bullock et al explored the efficacy and safety of botensilimab in combination with balstilimab (AGEN2034, a PD-1 inhibitor) in treated MSS patients with mCRC. 34% of enrolled patients had previously received immunotherapy, and the median number of prior treatment lines was 4. Results showed that dual immunotherapy had an ORR of 24% and a disease control rate (DCR) of

73% in 41 evaluable patients. At the same time, the safety status was good, and most treatment-related adverse events were grade 1–3.[31] A randomized Phase II trial (NCT05608044) is about to start.

Anti- PD-(L)1 Combined with New Generation of Immune Checkpoint Inhibitors

To enable more patients to benefit from immunotherapy, there is growing interest in other immune checkpoints and new potential targets have been identified, such as LAG-3, TIGIT, TIM-3 or VISTA. These receptors, like PD-1 or CTLA-4, are physiologically classified as inhibitory immune checkpoint receptors that are induced after T cell activation to prevent excessive stimulation.[105–108] LAG-3,[109] TIGIT,[110] TIM-3,[105,111] and VISTA[112] are frequently co-expressed with PD-1 on tumor infiltrating lymphocytes (TILs) in a variety of cancers, including renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), ovarian cancer, and melanoma.

Lymphocyte activation Gene-3(LAG-3) is an inhibitory receptor on the surface of T cells and is often overexpressed in colorectal cancer.[113] LAG-3 molecule can negatively regulate T lymphocytes, and the combination of LAG-3 inhibitor and PD-(L)1 inhibitor can enhance anti-tumor activity.[114] As one of the new targets, LAG-3 has great potential in tumor immunotherapy. Targeting LAG-3 and its corresponding inhibitors may have a synergistic effect with anti-PD-(L)1 drugs to improve the immune response to cancer cells.[113]

The LAG-3 antibody favezelimab combined with pembrolizumab is currently in Phase I studies,[32] with an ORR of 6.3% in 89 patients with pMMR/MSS mCRC who progressed after prior ≥2line therapy. The median PFS and OS were 2.1 and 8.3 months, respectively. Meanwhile, the median OS (12.7 months) in Combined Positive Score (CPS) ≥1 group was better than that in CPS<1 group (only 6.7 months), suggesting that further screening according to the level of PD-L1 expression in pMMR/MSS patients can identify the advantaged patients receiving LAG-3 antibody combined with anti-PD -(L)1 therapy.

T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory domains (TIGIT) is an inhibitory receptor shared by T cells and natural killer (NK) cells. On the one hand, TIGIT can inhibit the function of NK cells, prevent their killing of tumor cells and release of tumor cell antigens. On the other hand, it can directly inhibit the function of CD8+T cells, thereby inhibiting the killing effect of CD8+T cells on tumors; Meanwhile, Tregs with high expression of TIGIT can inhibit a variety of immune cells in the tumor immune cycle.[115–118] Therefore, antibodies targeting TIGIT can restore the tumor killing ability of NK cells and CD8+T cells, and restore the anti-tumor immune response to normal.[118,119] Based on the inhibitory effect of TIGIT in anti-tumor immune response, it has become a new target of tumor immunotherapy with potential.

Some studies have shown that although blocking TIGIT or PD-1 alone can enhance the function of CD8+ T cells, blocking both receptors at the same time is more effective in enhancing anti-tumor immunity of CD8+ T cells.[120–122] Thibaudin et al found in vitro that Atezolizumab alone could only reactivate T cells from MSI-H tumors. The combination of Atezolizumab and anti-TIGIT monoclonal antibody tiragolumab reactivated T cells in 46% of MSS CRC samples, suggesting that the combination could initiate clinical trials in colorectal cancer patients with MSS status.[123]

GO30103 is the first human study to study the treatment of advanced solid tumors with anti-TIGIT monoclonal antibody tiragolumab.[124] In this non-randomized controlled trial, tiragolumab was well tolerated with or without atezolizumab and showed initial antitumor activity of the combination therapy. Later, some other anti- TIGIT monoclonal antibodies such as vibostolimab and etigilimab combined with anti-PD-1 monoclonal antibodies in the treatment of advanced solid tumors were also reported, and the results were basically consistent.[125,126] CITYSCAPE study is a phase II clinical study of TIGIT inhibitor tiragolumab combined with PD-L1 inhibitor Atezolizumab in the treatment of patients with PD-L1 positive metastatic NSCLC.[127] The combination of tiragolumab and Atezolizumab resulted in a significant improvement in PFS compared to Atezolizumab monotherapy, with no increased safety risk. However, unfortunately, the PFS of the subsequent Phase III SKYSCRAPER 01 clinical study on NSCLC patients with high PD-L1 expression did not reach positive results, and the OS data was not mature.

T cell immunoglobulin and mucin domain 3 (TIM-3) are activation-induced inhibitory molecules involved in tumor immune tolerance and T cell depletion, while blocking TIM-3 pathway can promote the production of interferon-γ by T cells and enhance the immune function of tumor cells.[128] Studies have found that in colorectal cancer, tumor-infiltrating CD8+ T cells that express both PD-1 and TIM3 have significantly lower levels of IFN-γ production, indicating that PD-1+ TIM-3+ CD8+ T cells are more

dysfunctional than PD-1+ TIM-3-CD8 +T cells. Blocking TIM-3 may restore T cell response.[129] Therefore, targeting TIM-3 is a promising approach to further improve the effectiveness of current immunotherapy. Data from several I/II clinical trials indicate that the anti-TIM-3 antibodies LY3321367 and MBG453 are well tolerated as monotherapy and in combination with anti-PD-L1 LY3300054 or anti-PD-1 PDR001 monoclonal antibodies, respectively; and showed preliminary antitumor activity.[130,131] Encouragingly, partial responses were observed in 2 of the 6 patients with colorectal cancer who received the combination therapy.[130]

The immune regulation of colorectal cancer is a multi-link, multi-step and multi-mechanism process. These findings suggest that the use of single-agent anti-PD-1 monoclonal antibodies is not always sufficient to effectively restore T cell function, especially when these T cells co-express a variety of other immunosuppressive receptors. The results of the above clinical studies initially confirmed the effectiveness and safety of the combined treatment strategy of PD-L1 inhibitor and CTLA-4 inhibitor in MSS colorectal cancer, but most of the current studies are in the early stage, and more randomized controlled studies are still needed to continue to expand the sample for further verification. In addition, although the efficacy and safety of the combined treatment strategy of PD-L1 inhibitor and novel immune checkpoint inhibitors (LAG-3, TIGIT, TIM-3, VISTA, etc.) in MSS colorectal cancer has not yet been studied in clinical studies, according to the results of current clinical studies, They are expected to block multiple inhibitory signal pathways through combination therapy, thereby enhancing the anti-tumor immune response of the body to tumor cells, which is a promising anti-tumor immunotherapy strategy. In conclusion, combined immunotherapy strategies based on different mechanisms of action should be one of the models to continue to be explored in MSS colorectal cancer in the future.

ICIs Combined with Chemotherapy

ICIs Combined with Chemotherapy Alone

Chemotherapy-induced tumor cell death can promote the release and presentation of tumor antigens, increase tumor immunogenicity, and activate and improve the activity of CD8+ T lymphocytes, so immune checkpoint inhibitors combined with cytotoxic chemotherapy can theoretically improve the efficacy.[19,132–134] At present, basic chemotherapy drugs for colorectal cancer include platinum (such as oxaliplatin), topoisomerase inhibitors (such as irinotecan) and antimetabolic drugs (such as 5-fluorouracil).[135] It was found that both 5-fluorouracil and oxaliplatin were immunogenic. 5-fluorouracil (5FU) has selective cytotoxicity to myeloid-derived suppressor cells (MDSCs). In vivo, 5FU treatment of tumor-bearing mice resulted in a significant decrease in the number of MDSCs in the mice's spleen and tumor bed, but had no significant effect on T lymphocytes, natural killer cells, dendritic cells, or B lymphocytes.[136] Oxaliplatin can also induce immunogenic cell death and increase the immunogenicity of MSS tumors, thereby improving the efficacy of immunotherapy when combined with PD-(L)1 inhibitors.[137,138]

Studies of Dosset et al showed that 5-FU combined with oxaliplatin can improve anti-tumor immune response in MSS CRC mouse model, and then FOLFOX regimen can induce PD-L1 expression and high CD8+T lymphocyte infiltration in tumor microenvironment in tumor samples of colorectal cancer patients. Thus, the FOLFOX regimen has a synergistic effect with anti-PD-(L)1 monoclonal antibody.[139] These preclinical data suggest that the combination of a PD-(L)1 inhibitor with an inducer of immunogenic cell death can improve immunotherapy efficacy and support a therapeutic strategy of combining ICIs with chemotherapy such as FOLFOX regimens. Based on these data, a series of clinical studies were gradually carried out.

The METIMMOX study evaluated the efficacy of short-course repeat sequential oxaliplatin-based chemotherapy (FLOX) (oxaliplatin + fluorouracil) combined with nivolumab in first-line treatment of MSS mCRC. A total of 54 patients were enrolled, all of whom were RAS/BRAF mutation patients. ORR rates in the combined immunotherapy group and chemotherapy group were 48% and 23%, CR rates were 16% and 0%, respectively, indicating that immunotherapy combined chemotherapy could improve the chances of immunotherapy response for patients. However, the difference in median PFS between the two groups was not significant, 6.6 months and 5.6 months, respectively.[33] KEYNOTE-651 evaluated the efficacy and safety of Pembrolizumab in combination with mFOLFOX7 or FOLFIRI in mCRC.[34] The latest results showed that Pembrolizumab combined with mFOLFOX7 had an ORR of 61% for first-line therapy and Pembrolizumab combined with FOLFIRI had an ORR of 25% for second-line therapy. Both combination regimens showed antitumor activity in pMMR/MSS mCRC patients.

In addition, some studies have found that chemotherapy drugs related to DNA repair enzymes can produce better synergies with ICIs. O 6-methylguanine-DNA methyltransferase (MGMT) is designed to repair DNA damage caused by alkylating agents.[140] If the tumor has MGMT gene silence (promoter methylation and protein expression loss), it will enhance the sensitivity of tumor cells to alkylate agents such as temozolomide (TMZ),[141] and more secondary gene mutations may occur in the tumor after receiving TMZ, resulting in secondary high tumor mutation burden (TMB). The special synergistic mechanism makes TMZ a sensitizer for immunotherapy. Studies have reported that nearly 40% of mCRC patients have MGMT silencing.[142] The MAYA study is based on this theory to explore a TMZ induction chemotherapy followed by combined immunotherapy strategy in MSS mCRC. This Phase II study included MSS mCRC patients who failed to respond to multi-line therapy and had silent MGMT expression, and received 2 cycles of TMZ induction chemotherapy followed by nivolumab combined with low-dose ipilimumab. The results showed that the median PFS and OS were 7.1 months and 18.5 months, respectively, and the ORR was as high as 39%; the incidence of grade 3 and above adverse reactions is low.[35,36] This appears to be better than the results of a previous Phase II study of TMZ monotherapy in MGMT- silenced mCRC, which enrolled 41 patients with an ORR rate of only 10% and a median PFS and OS of only 1.9 and 5.1 months.[143]

ICIs Combined with Chemotherapy and Bevacizumab

The anti-angiogenic drug bevacizumab has been an important component of targeted therapy for mCRC and is approved for mCRC when used in combination with chemotherapy. By inhibiting the VEGF/VEGFR pathway, bevacizumab normalizes blood vessels, increases the infiltration of T lymphocytes, and activates immune effector cells by stimulating the maturation of dendritic cells and reducing the expansion of Tregs and MDSCs.[144–146] Therefore, ICIs combined with chemotherapy and anti-angiogenesis targeted therapy have been the focus of research.

A Phase II placebo-controlled BACCI study evaluated the clinical activity of capecitabine, bevacizumab combined with atezolizumab in advanced refractory MSS mCRC, showing that the addition of atezolizumab failed to significantly improve PFS and OS.[37] Another Phase II study evaluated the safety and tolerability of capecitabine, bevacizumab and pembrolizumab in the treatment of MSS mCRC. The ORR in the three-drug combination treatment group was 5%, which did not meet the predetermined target, but the median PFS and OS reached 4.3 and 9.6 months, respectively, and were tolerable.[38] Meanwhile, after FOLFOX combined with bevacizumab induction therapy in MSS mCRC patients in MODUL Cohort 2 studies, atezolizumab is added to Fluoropyrimidine (FP) + bevacizumab as first-line maintenance therapy. The results showed that although no new safety signals were found, there was no improvement in efficacy.[39,40]

ICIs in combination with standard chemotherapy and bevacizumab is also being explored as a first-line treatment. The researchers first evaluated the efficacy of atezolizumab combined with bevacizumab and FOLFOX in the first-line treatment of MSS mCRC patients, with an ORR of 52% and a median PFS of 14.1 months, showing no significant activity compared with standard treatment.[147] CHECKMATE-9X8 evaluated nivolumab combined with mFOLFOX6/ bevacizumab (NIVO+SOC) versus mFOLFOX6/ bevacizumab (SOC) for first-line treatment of mCRC. Most of the patients were MSS type. The ORR was 60% in the NIVO+SOC group and 46% in the SOC group, but the median PFS in both groups was 11.9 months, which did not meet the primary endpoint of PFS. However, after 12 months, the PFS rate in the NIVO + SOC group was higher than that in the SOC group.[41]

Two other clinical studies conducted in China have given some positive signals in terms of survival benefits. The Phase II BBCAPX study explored the efficacy of sintilimab combined with CAPEOX/bevacizumab in the first-line treatment of RAS mutation and MSS mCRC.[42] The results showed that ORR and DCR were 84.0% and 100.0%, respectively. This efficacy data shows that the addition of immunotherapy improves efficacy by about 20% compared to the existing standard regimen. The results of follow-up were published in 2023, suggesting that the ORR and DCR of the combination treatment regimen remained stable, with a median PFS of 18.2 months, and were generally well tolerated.[42] The study offers new hope for the up to 95% of MSS mCRC patients. However, the sample size of this study was small, only 25 people were included, and further large-scale studies are needed for verification. At present, the Phase III study of BBCAPX is in progress (NCT04194359). Another randomized, double-blind, phase 2 part of a phase 2/3 trial in China assessed the preliminary anti-tumor activity and safety of serplulimab plus HLX04 and XELOX as a treatment option for patients with MSS mCRC.[148] The results showed that the ORR reached 65.5% and the median PFS reached 17.2 months.

Although the median overall survival (OS) was not reached for either group, a trend of an OS benefit was observed for the serplulimab group (HR, 0.77; 95% CI, 0.41–1.45).

In addition, ICIs combined with three-drug chemotherapy and bevacizumab have also been explored for first-line treatment, equivalent to the use of the strongest induction chemotherapy, in order to kill more tumor cells and produce more neoantigens, so as to enhance the effect of immunotherapy and improve the survival outcome of patients. Recently, two related clinical studies (NIVACOR study and AtezoTRIBE study[43,44] were published. Unfortunately, there was only some improvement in efficiency, but the survival benefit was not obviously. The single-arm Phase II NIVACOR study evaluated the efficacy of nivolumab combined with FOLFOXIRI/bevacizumab in first-line treatment of RAS/BRAF mutated MSS mCRC patients. The results showed that ORR and DCR were 78.9% and 96.2%, but mPFS were only 9.8 months.[43] The AtezoTRIBE study is a multicenter, open-label, randomized, controlled phase 2 clinical trial that included unresectable mCRC patients regardless of MMR status. The results show that adding atezolizumab to first-line FOLFOXIRI/bevacizumab is safe. The median PFS of 13.1 months in the altazomab group was better than that of 11.5 months in the control group.[44] At present, the OS data of AtezoTRIBE is still immature, and people are interested in further results of this study.

So far, immunotherapy combined with chemotherapy (including three drugs) and bevacizumab in the first-line treatment of MSS mCRC has shown very limited efficacy, and whether it can bring survival benefits to patients is still controversial. Further consideration should be given to the combination mode and related value of this treatment mode in the future. We look forward to the Phase III clinical study to obtain more data.

Dual ICIs Regimen Combined with Chemotherapy

The single-arm Phase 1b/2 MEDITREME trial evaluated whether the addition of durvalumab and tremelimumab to standard chemotherapy mFOLFOX6 improved therapeutic efficacy.[45,46] Preliminary results showed that ORR and CR were 62.5% and 25%, respectively, and 1-year PFS were 50%, indicating that the above regimen was initially effective in treating MSS mCRC patients.[47] In 2023, the results of the MEDETREME study were republished after long-term follow-up. Among them, 48 patients were MSS type, with a median PFS of 8.2 months, showing good safety. The study met its primary endpoint and achieved survival outcomes similar to those previously observed in bevacizumab combined chemotherapy regimens, which resulted in approximately 8 months of PFS.[149] This study showed that the dual immunotherapy combined with standard chemotherapy was significantly better than chemotherapy alone or immunotherapy alone for MSS mCRC on two key efficacy indicators of ORR and PFS. Researchers have also explored predictive biomarkers, and the relevant information is expected to guide more accurate CRC combined immunotherapy strategies in the future.[46]

To sum up, the overall efficacy of ICIs combined with chemotherapy in MSS mCRC patients has been limited. Compared with previous phase III clinical studies of first-line or second-line chemotherapy combined with targeted therapy, there seems to be no obvious advantage in ORR and survival. In the context of targeted therapy being the front-line standard treatment, In the future, more exploration directions will focus on the triple regimen such as immunotherapy combined with chemotherapy and anti-vascular targeted therapy, as well as the "AtezoTRIBE" model with three-drug chemotherapy, and double immunotherapy combined with chemotherapy. The optimal combined model is worthy of further exploration.

ICIs Combined with Radiotherapy

The combination of radiotherapy and immunotherapy may have synergistic effects based on several mechanisms. Radiotherapy can induce tumor cell death, release tumor antigens and activate immune signals, thus promoting systemic immune response.[150,151] Radiotherapy changed the tumor immune microenvironment and inhibited the immune escape of tumor cells, thus enhancing the killing ability of immune cells to tumor cells.[152–154] Radiotherapy can also promote the diversification of tumor antigen pool, promote the antigen presentation of dendritic cells and stimulate the body's specific immune response.[152] Radiotherapy can induce the aggregation of TILs and the up-regulation of PD-L1 expression, thereby increasing the sensitivity of immunotherapy.[155]

Preclinical data suggest that the combination of ICIs with an inducer of immunogenic cell death, such as radiotherapy, improves the efficacy of immunotherapy.[152,156] The synergistic effect of radiation therapy and ICIs has also been demonstrated in several studies of preclinical CRC tumor models.[157,158] These mechanisms provide a basis for the clinical study of radiotherapy combined with immunotherapy for mCRC patients.

The ANAVA study enrolled 101 patients with locally advanced rectal cancer (LARC), and 96 patients who were pathologically evaluable underwent preoperative chemoradiotherapy (capecitabine +conventional fractionated long-term radiotherapy + avelumab). The results showed that 22 cases (23%) achieved pathological complete response (pCR),59 cases (61.5%) achieved obvious pathological remission, the rates of non-immune and immune-related adverse reactions above grade 3 were only 8% and 4%. There were 39 cases of MSS rectal cancer in the study, suggesting that radiotherapy combined with immunotherapy may be a potentially effective program for patients with MSS rectal cancer.[48] The VOLTAGE-A study showed that 39 MSS LARC patients treated with nivolumab after the end of long-term concurrent chemoradiotherapy achieved 38% major pathological response (MPR) and 30% pathological complete response (pCR).[49]

A single-arm, non-randomized Phase II clinical trial (NCT03104439) enrolled 40 patients with MSS mCRC and administered nivolumab + ipilimumab + hypofractionated short-course radiotherapy. The results showed that the ORR and DCR were 10% and 25% respectively, and the mOS was 7.1 months, which indicated that radiotherapy combined with immunotherapy could improve the ICIs response rate and prolong the overall survival of MSS mCRC patients, providing a new treatment idea for MSS mCRC patients.[50] However, in a Phase II study, 24 patients with chemotherapy-refractory MSS mCRC who received durvalumab combined with tremelimumab and radiotherapy achieved an 8.3% response rate, which did not meet the preset ORR of at least 25%, despite a median OS of 11.4 months.[51] Another study explored the feasibility of Y90 liver radioembolization combined with durvalumab and tremelimumab for MSS mCRC and found that although the combination regimen was well tolerated by patients, there was no significant benefit.[159] Similarly, Monjazeb et al found that the addition of hypofractionated radiotherapy to PD-1 and CTLA-4 inhibitors had no significant clinical benefit for MSS mCRC patients resistant to first-line chemotherapy.[52] Therefore, the role of radiation therapy in overcoming therapeutic resistance to MSS mCRC remains to be further validated.

In summary, the preclinical model showed that combined radiotherapy on the basis of ICIs induced and enhanced systemic anti-tumor immune response. Relevant clinical studies have also confirmed that it can improve the pathological remission of MSS LARC patients, showing a relatively optimistic short-term efficacy. However, the safety and efficacy of this combination therapy model in MSS mCRC are not very consistent, and more large-scale prospective clinical trials are needed to further confirm it. At the same time, the choice of radiotherapy mode (including dose, segmentation, treatment order, etc.) is still inconclusive, and more studies are expected to further explore.

ICIs Combined with Targeted Drug

ICIs Combined with Anti-EGFR Monoclonal Antibody

Anti-EGFR therapy is the standard treatment for RAS wild-type mCRC. The Fc segment of cetuximab can bind to CD16 receptors on NK cells and dendritic cells, and direct killing of target cells by effector cells is mediated through antibody-dependent cell-mediated cytotoxicity (ADCC). Meanwhile, cetuximab can effectively improve the immune microenvironment and promote the infiltration of immune cells within the tumor.[160,161] In addition, cetuximab alone or in combination with chemotherapy can induce immunogenic cell death (ICD) of colorectal cancer cells, thereby activating an effective anti-tumor response.[162,163] Therefore, anti-EGFR monotherapy or combination chemotherapy and ICIs have a good synergistic mechanism basis.

Several studies have confirmed the initial efficacy of ICIs combined with anti-EGFR mab. The Phase II CAVE study included RAS wild-type mCRC patients treated with cetuximab, and 92% of enrolled patients had MSS status. When cetuximab and Avelumab were combined for third-line treatment, the median PFS and OS of the population were 3.6 months and 11.6 months, respectively, but the overall objective response rate was not high, only about 7%.[53] An updated long-term follow-up of the study found that the median OS was 18.6 months. At the same time, the significant effectiveness of cetuximab rechallenge combined with Avelumab was further verified in patients with RAS wild-type mCRC based on ctDNA cues.[54]

Another AVETUXIRI study was A Phase II study of Avelumab combined with cetuximab combined with irinotecan in the treatment of refractory MSS mCRC, in which cohort A was RAS wild type and cohort B was RAS mutant. The interim analysis results showed that the ORR of cohort A and cohort B were 30% and 0%, respectively, which seemed to be quite different. However, 6-month PFS rates were 40% and 38.5%, and 1-year OS

rates were 50% and 46.2%, respectively. Finally, the RAS wild-type cohort reached the primary efficacy endpoint, and the RAS mutant cohort also saw some survival benefits. These results also suggest that regardless of RAS status, ICIs combined with anti-EGFR mab can improve the outcome of immunotherapy to some extent.[55] The study is ongoing. A similar study evaluated the efficacy and safety of tislelizumab in combination with cetuximab and irinotecan in previously treated RAS wild-type mCRC patients in a single-arm Phase II TEC trial.[56] The results showed an ORR of 33%, exceeding the study's preset value of 30%, and a DCR of 79%, and the median PFS and OS were 7.3 and 17.4 months respectively.

Immunotherapy combined with anti-EGFR mab in first-line therapy is also being explored. A single-arm, multicenter Phase II AVETUX trial evaluated avelumab in combination with cetuximab and mFOLFOX6 in previously untreated RAS/BRAF wild-type, MSI-independent mCRC patients.[57] Among the 41 MSS mCRC patients, the 12-month PFS rate was 40% and the median PFS was 11.1 months. The overall ORR and DCR were 81% and 89%, respectively. This study preliminarily suggests that immunotherapy combined with anti-EGFR monoclonal antibody and chemotherapy as first-line treatment may further improve efficacy.

In addition, the Phase II AVETRIC study of cetuximab combined with Avelumab and mFOLFOXIRI three-drug chemotherapy regimen for first-line treatment of MSS RAS wild-type mCRC patients is ongoing and is expected to bring more tips on immunotherapy for advanced bowel tumors. There is also a phase II study that is changing ICIs from single to double immunotherapy, with a stronger combination therapy. In RAS wild-type and MSS type mCRC patients who had not been treated with anti-EGFR mab, the combination of panitumumab, ipilimumab, and nivolumab resulted in an ORR of 35%. This study showed that wild-type patients with RAS could have more choices and could try to adopt a combination regimen of anti-EGFR monoclonal antibody plus dual immunotherapy.[58]

In conclusion, the combination mode of anti-EGFR monoclonal antibody plus immunotherapy has a theoretical basis, and it also shows us better disease control. In particular, the effectiveness is more evident in the posterior treatment and in the RAS wild-type population, which is worthy of further exploration in the future.

ICIs Combined with Anti-EGFR Monoclonal Antibody and BRAF Inhibitor

Preclinical studies suggest possible synergies between BRAF inhibitors and PD-1/PD-L1 inhibitors. It has been reported that BRAF inhibitors combined with anti-EGFR mab can induce transient MSI-H phenotype in MSS colorectal cancer, suggesting that this combination model may bring better survival benefits for BRAF mutated MSS patients.[164]

A Phase I/II study (NCT04017650) evaluated the efficacy and safety of encorafenib (BRAF inhibitor) in combination with cetuximab and nivolumab in the treatment of BRAF V600E mutant MSS mCRC. A total of 26 patients were enrolled, and the ORR and DCR of 24 patients that could be evaluated reached 50% and 96%, respectively, and the median PFS and OS were 7.4 months and 15.1 months, respectively.[59] This is the best outcome for second-line and above treatment in patients with BRAF mutations, and it is well tolerated, and the randomized Phase II SWOG 2107 study is also ongoing.

ICIs Combined with MAPK Signal Transduction Pathway Inhibitors

Overexpression and activation of mitogen-activated protein kinase (MAPK) pathways are often detected in mCRC, and dysregulation of these signal transduction pathways can be used as potential targets for cancer therapy.[165] RAS, RAF, MEK and ERK proteins are key factors in this pathway. Some studies have shown that RAS-MAPK pathway can directly promote tumor cell proliferation,[166] while BRAF mutations often lead to continuous activation of RAS/RAF/MEK/ERK pathway. Combined inhibition of upstream and downstream nodes of RAS/MAPK pathway is an important strategy to overcome drug resistance. Studies have found that the ORR of BRAF inhibitor monotherapy is only 0–5%,[167,168] and the co-inhibition of BRAF and MEK contributes to the inhibition of MAPK pathway. Therefore, the combination of anti-EGFR drugs with BRAF inhibitor and MEK inhibitor is one of the treatment options for BRAF V600E mutant mCRC. However, the therapeutic effect is still limited.[169–171]

Based on the available clinical evidence, the US Food and Drug Administration (FDA) approved BRAF inhibitor encorafenib in combination with cetuximab for BRAFV600E mCRC. However, with an ORR of only 20% and a median PFS of only 4.3 months, the clinical benefit was not long-lasting.[170,171]

In the era of immunotherapy, more and more studies have found that the activation of RAS-MAPK pathway is also related to the decrease of T lymphocyte infiltration in tumors, while inhibition of MEK can enhance T lymphocyte infiltration in tumors and synergistic effect with PD-1 inhibitors to enhance anti-tumor activity.[164,172,173] Therefore, on this basis, the exploration of clinical research has been carried out.

First, in a Phase IB study, Atezolizumab in combination with cobimetinib (a MEK inhibitor) achieved manageable safety and clinical activity in mCRC patients after standard treatment failure, with ORR and DCR of 17% and 39%, respectively. The 12-month PFS and OS rates were 11% and 43%, respectively.[60,61] Unfortunately, the IMblaze370 Phase III study of atezolizumab in combination with cobimetinib, published in the Lancet, yielded negative results. Patients who had previously failed multiple lines of therapy were enrolled, and regorafenib was used as the standard control. Results showed that the efficacy of the combination regimen and regorafenib standard third-line therapy was similar, and atezolizumab alone or combined with cobimetinib achieved OS of 7.1 and 8.9 months, respectively, which was not significantly better than the standard treatment regorafenib monotherapy (OS of 8.5 months).[62]

In addition, durvalumab plus the MEK inhibitor trametinib had a similar effect in MSS mCRC patients, showing acceptable tolerability, but no improvement in OS was seen.[63] A trial of another MEK inhibitor binimetinib combined with pembrolizumab combined with beizumab in mCRC patients who had failed multiline therapy showed that a total of 21 patients with pMMR/MSS mCRC were enrolled, with ORR and DCR of 12% and 94%, respectively. The median PFS was 6.4 months and the adverse reactions were tolerable.[64] Studies have shown initial clinical efficacy, but Phase III clinical trials are needed to confirm this. Therefore, whether MEK inhibitor combined with immunotherapy has a synergistic effect remains to be confirmed by further studies.

The co-inhibition of BRAF and MEK contributes to the inhibition of MAPK pathway, so the combination of BRAF inhibitor and MEK inhibitor is also one of the treatment options for BRAF V600E mutant mCRC, although the efficacy is limited.[169] Studies have shown that combining drugs targeting the RAS/MAPK pathway with immunotherapy may lead to an enhanced immune response, which may improve patient outcomes.[65] A single-arm phase 2 clinical trial of combined PD-1, BRAF and MEK inhibition with sparatlizumab (PDR001), dabrafenib and trametinib in 37 patients with BRAFV600E CRC, met its primary end point. In 32 patients with microsatellite stabilization, ORR and DCR reached 25% and 75%, respectively, and durability that were favorable relative to historical controls of BRAF-targeted combinations alone, and the combination regimen was well tolerated.[65]

Based on the regulatory effect of RAS/MAPK pathway targeting drugs on tumor immune microenvironment, continuing to explore new combined strategies of RAS/MAPK pathway targeting drugs and immunotherapy will likely bring lasting survival benefits for tumor patients with RAS/MAPK pathway mutations.

ICIs Combined with KRAS-G12C Inhibitors

Kirsten rat sarcoma virus oncogene homology (KRAS) mutation is an important genetic driver of colorectal cancer (CRC).[174,175] KRAS mutation can lead to activation of downstream signaling pathways, promote tumorigenesis and invasion, and thus affect the prognosis of colorectal cancer.[176,177]

Drug targeting of KRAS mutations is challenging. Although KRAS G12C inhibitors show significant clinical response after their emergence, the resistance mechanisms of KRAS G12C inhibitors are more complex and diverse. The main driving factors include secondary mutations of KRAS itself, reactivation of multiple nodes upstream and downstream of KRAS on the MAPK pathway, and immune deficiency.[178] Therefore, combination therapy may be an effective means to overcome drug resistance.

Preclinical trials of KRAS G12C inhibitors in combination with PD-1/PD-L1 inhibitors are underway. In 2017, British scholars found that KRAS signal could inhibit T lymphocytes' immune surveillance of tumor cells by up-regulating the protein expression of PD-L1 in tumor cells.[179] It has been demonstrated in preclinical models that the KRAS-G12C inhibitor sotorasib can promote anti-tumor immunity by inhibiting PD-L1 signaling in tumors, by increasing and activating T cell infiltration and making tumor microenvironment (TME) a highly sensitive to immune checkpoint inhibitors. Therefore, the combination of KRAS-G12C inhibitor and immune checkpoint inhibitor can produce durable activity.[180]

Another KRAS G12C inhibitor MRTX849, had similar results when used in combination with anti-PD-1 antibodies. MRTX849 reversed the immunosuppressed tumor microenvironment, thereby sensitizing the tumor to ICIs therapy. The results showed that in a genetically engineered mouse (GEM) model, the combination of MRTX849 and anti-PD-1 antibodies improved progression-free survival compared with either drug alone.[181]

In summary, preclinical studies have found that the combination of immune checkpoint inhibitors with anti-EGFR mab, BRAF inhibitors, MAPK signaling pathway inhibitors, etc., has a good mechanism basis. At the same time, some clinical studies have shown that immunotherapy combined with these targeted therapies has a certain effect in the treatment of MSS colorectal cancer, showing a certain therapeutic prospect. Unfortunately, most of these existing studies are single-arm small sample exploratory studies, and the data direction is not completely stable and consistent, and more research results and long-term follow-up are still needed to prove the efficacy of immunotherapy combined with targeted therapy in MSS colorectal cancer.

ICIs Combined with Novel Development Drugs

ICIs Combined with Cancer Vaccines

Cancer vaccines are considered an option for immunotherapy in most solid tumors, including colorectal cancer. Several cancer vaccines have been studied, including autologous vaccines, dendritic cell vaccines, viral vector vaccines, and peptide-based vaccines, which stimulate anti-tumor immunity by containing tumor antigens, nucleic acids, whole cells, and peptides.[182,183] These vaccines work by stimulating tumor antigen-specific cytotoxic T lymphocytes, which in turn recognize and potentially eliminate cancer cells in an antigen-specific manner.[183,184] These results provide a theoretical basis for the combination of cancer vaccine and ICIs. Therapies involving cancer vaccines in combination with conventional treatments or different immunotherapies have been shown to be beneficial in combating tumor resistance and improving clinical outcomes, but clinical trials are still in the early stages and more research is needed to demonstrate their therapeutic potential.[184–189]

Oncolytic viruses (OVs) are cancer vaccines that use viruses as carriers to selectively infect tumor cells with natural or genetically modified viruses with replication ability, then induce tumor cell lysis and death, release cytokines and cell antigens, and make the tumor change from “cold” to “hot”. Thus, the anti-tumor effect of ICIs can be improved.[190–194] Therefore, combining ICIs with OVs can enhance the anti-tumor ability of ICIs. OVs combined with PD-1/PD-L1 inhibitors has achieved good therapeutic effect in mouse animal models of colon cancer and ovarian cancer.[195]

The clinical trial of OVs drug PexaVec (a vaccinia virus) in combination with durvalumab for MSS colorectal cancer is ongoing, but it is still in the phase I/II study stage. Preliminary analysis shows that it is well tolerated, but the long-term curative effect has not been reported,[196] which needs continuous attention.

In addition, avelumab plus autologous dendritic cell vaccine has entered clinical studies and has been shown to be effective and well tolerated in chemotherapy treated MSS mCRC.[66] Although the primary endpoint of the study has not been met, the median PFS observed was 12.2 months, which was better than 5 months when anti-PD-1 monoclonal antibody was used as monotherapy.[6]

In conclusion, cancer vaccines may improve the efficacy of immunotherapy by altering the tumor microenvironment. To boost anti-tumor immunity, cancer vaccines in combination with currently used immunotherapy approaches such as ICIs are an attractive approach. This combination treatment approach can compensate for the limitations of each therapy when used alone, which also opens up the possibility for MSS metastatic colorectal cancer to benefit from immunotherapy.

ICIs Combined with Bispecific Antibody

Bispecific antibody (BsAb) is an artificial antibody that specifically binds to two antigens or epitopes at the same time, and can be prepared by cell fusion or recombinant DNA techniques. Due to its ability to simultaneously target two epitopes in tumor cells or TME, BsAb is gradually becoming an important component of the next generation of therapeutic antibodies.[197,198] BsAb stimulate immune response by binding tumor-rich antigens (such as CEA, HER2, etc.) and immune cells, and their efficacy is being explored in different types of solid tumors,[198,199] while BsAb in combination with ICIs can enhance anti-tumor activity.[198,200,201]

Cibisatamab is a T cell BsAb that targets both CEA on tumor cells and CD3 on T cells. Cibisatamab has shown effective antitumor activity in preclinical models, leading to increased intratumoral T cell infiltration and activation and upregulation of PD-1/PD-L1.[200] Therefore, cibisatamab combined with PD-1/PD-L1 inhibitors may enhance the anti-tumor effect. In vitro tests have shown that anti-CEA BsAb combined with PD-1/PD-L1 inhibitors can kill tumor cells to the maximum extent.[201] Considering that CEA is overexpressed in 80% of colorectal cancer and underexpressed in normal tissues, it can be used as an ideal tumor antigen for colorectal cancer. Clinical studies of BsAb for colorectal cancer are in the early stages. In two ongoing Phase I clinical studies, cibisatamab is being treated as a monotherapy or in combination with atezolizumab in patients with CEA expressing solid tumors (including MSS mCRC patients). Evidence of antitumor activity was observed with cibisatamab monotherapy at sustained dose escalation, and its combination with atezolizumab further enhanced antitumor activity with a manageable safety profile.[67]

Despite the potential therapeutic activity of BsAb in tumor therapy, its application still faces significant challenges, including tumor heterogeneity, difficult to control tumor microenvironment, insufficient co-stimulatory signaling to activate T cells, the need for continuous injection, fatal systemic side effects, and off-target toxic effects.

ICIs Combined with Other Novel Target Drugs

At present, the combination of ICIs with some novel target drugs (such as IL-17 inhibitors and TGF-β1 receptor inhibitors, etc.) has shown certain efficacy in basic studies, and is being further promoted in subsequent clinical studies.[202–205]

Interleukin-17a (also known as IL-17) is mainly secreted by T-helper lymphocyte-17 (Th17)[206] and can up-regulate the expression of PD-L1 in multiple tumors. It induces the aggregation of MDSCs in the tumor, thereby leading to tumor immune escape and promoting tumor growth and invasion.[202,203,207,208] Therefore, targeting IL-17 has become a promising strategy to overcome immunosuppression and improve the sensitivity of anti-PD-1 therapy.[202,203] Inhibition of IL-17A has been observed to enhance tumor infiltration of lymphocytes in both colon and lung cancer tumor models in mice.[204,209,210] It is suggested that IL-17A may be used as a therapeutic target, making MSS CRC patients sensitive to ICI treatment.[204]

Basic studies have shown that transforming growth factor-β (TGF-β1) signaling pathway can promote tumor growth, drug resistance and metastasis by inhibiting host immune response.[211,212] At the same time, TGF-β1 was found to be the central mediator of immune tolerance in the tumor microenvironment of different cancer species, causing the immune escape of tumors,[205] suggesting that inhibition of TGF-β1 pathway can help enhance the effect of immunotherapy and provide a new treatment strategy for the immunotherapy of solid tumors. It is expected that the combination of these novel target drugs will bring new hope for the treatment of bowel cancer.

In addition, existing studies have found that environmental factors, especially gut microbiome, can influence response to cancer immunotherapy. The gut microbiome plays a crucial role in various basic physiological and pathological processes. It has been found that in malignant melanoma and lung cancer, gut microbiome affects the human response to ICIs treatment, and it is the diversity and composition of intestinal microbiota that led to the different anti-tumor responses of patients against pd-1 monoclonal antibodies.[213–216] Therefore, in order to improve the effectiveness of immunotherapy, the gut microbiome needs to be considered, especially in colorectal cancer, as it is closely related to the gut microbiome. These findings may provide a new therapeutic strategy for immunotherapy in patients with pMMR/MSS colorectal cancer.

To sum up, colorectal cancer is characterized by tumor complexity and heterogeneity, as well as the ability of tumor cells to evade immune surveillance through various means. Therefore, it is necessary to conduct personalized treatment targeting multiple targets and approaches to overcome the tumor evasion mechanism, so as to ensure satisfactory clinical results for different individuals. However, the development of these new therapeutic strategies, including cancer vaccines and specific double antibodies, has proven challenging, and these promising preclinical findings have not yet been translated into the clinic. Many factors contribute to this failure, including poor understanding of tumor biology and immunosuppressive tumor microenvironment, weak T cell response, and appropriate patient target selection.

Biomarkers for Predicting the Efficacy of ICIs in pMMR /MSS Colorectal Cancer

To date, dMMR/MSI-H status is an evidence-based biomarker for predictive ICIs benefit in mCRC.[3,7,9,217] Finding a biomarker to predict the efficacy of ICIs in MSS colorectal cancer could enable more patients to benefit from immunotherapy, but this remains a clinical challenge. Several biomarkers other than dMMR/MSI-H are currently being studied as positive predictors of ICIs benefit in mCRC. These include TMB, mutations of polymerase ε and polymerase δ (POLE/POLD1), PD-L1 expression, tumor-infiltrating lymphocytes and immune scores, gut microbiome, etc.[29,68–76]

Tumor Mutational Burden

TMB refers to the total number of somatic mutations in each coding region of the tumor genome, which can measure all non-synonymous coding mutations in the tumor exome.[218] TMB has been shown to be a molecular marker closely related to tumor immunotherapy efficacy in solid tumors, independent of MSI status and PD-1/PD-L1 expression.[218,219] It is currently believed that higher TMB is associated with greater immunogenicity, which may enhance the antitumor activity of immunotherapy.[220]

The KEYNOTE158 study evaluated the efficacy of pembrolizumab therapy on previously treated patients with advanced, incurable solid tumors, and found that patients with high TMB (≥10 mutations per megabase) had significantly higher ORR than patients with low TMB (29% vs 6%). High tissue TMB (tTMB-high) may be a new and useful biomarker for predicting the efficacy of ICIs.[221] Therefore, the US FDA has approved pembrolizumab for the treatment of refractory, advanced solid tumors with high TMB (≥10 mut/Mb) in tumor tissue.[68]

In colorectal cancer, only 16% of patients with high TMB colorectal cancer belong to MSI-H type, and the remaining 84% belong to MSS type. These patients with high TMB MSS colorectal cancer are likely to benefit from ICIs treatment.[222] Fabrizio et al found that 2.9% of colorectal cancer patients with MSS were identified as having high TMB, and PD-1 inhibitors were shown to be effective in cases with concurrent MSS/ high TMB. TMB is superior to MSI status in predicting the efficacy of ICIs in colorectal cancer treatment, and this subgroup may expand the group of CRC patients who may benefit from ICIs based treatment.[69]

In the REGONIVO study, it was found that pMMR/MSS colorectal cancer patients with high TMB were more likely to benefit than patients with low TMB after failure of standard treatment with Regorafenib combined with nivolumab.[22] In the follow-up molecular analysis of the CO.26 randomized Phase II trial, it was also found that MSS patients with TMB higher than 28 mts/Mb, the dual immunotherapy combination therapy can bring OS benefits, while patients with low TMB do not benefit. It was also found in the study that the proportion of plasma TMB higher than 28 mts/Mb in MSS patients was as high as 21%.[29]

However, the role of TMB in MSS mCRC has not been established, and there is currently no optimal mCRC-specific TMB cutoff to predict the benefit of ICIs-based treatment strategies. In the CO.26 study, if patients were divided into high and low TMB groups at a pre-specified cut-off point of 20 mts/Mb, the benefit of OS, PFS, or DCR could not be predicted.[29] Another study also showed that a TMB cutoff point of 10 mut/Mb does not predict the benefit of ICIs-based treatment of pMMR/MSS for mCRC.[223]

In summary, TMB is a promising biomarker, and high TMB status is associated with immunotherapy benefit in MSS colorectal cancer, a subpopulation that may expand the population benefiting from ICIs-based therapies. However, there are still problems such as limited sample size of existing studies, different TMB analysis methods, and different optimal cutoff values for high TMB (9–28 mut/Mb),[22,29,69] so the application of TMB as a biomarker to predict the efficacy of pMMR/MSS mCRC immunotherapy is still controversial.

Polymerase ε and Polymerase δ (POLE/POLD1) Mutations

DNA polymerase ε and polymerase δ (POLE/POLD1) genes encode the exonuclease domain of DNA polymerase, which is involved in DNA replication and proofreading. When the POLE/POLD1 gene is mutated, it may cause the dysfunction of the DNA damage repair system and lead to the increase of related neoantigens, making it have the characteristics of TMB-H.[70,71,224–226] Relevant studies have shown that POLE/POLD1 mutations can predict the efficacy of ICIs in the

treatment of non-small cell lung cancer, endometrial cancer and other solid tumors.[71,227] POLE/POLD1 gene mutation has gradually become an independent biomarker for predicting the efficacy of pan-cancer immunotherapy.[71]

Studies in colorectal cancer have shown that tumors with POLE/POLD1 gene mutations are basically pMMR/MSS types, POLE mutant tumors exhibit higher CD8+ T cell infiltration and up-regulation of PD-1/PD-L1 and CTLA4 expression compared with POLE wild-type tumors, which are similar to dMMR/MSI-H tumors and therefore may be sensitive to ICIs treatment.[70] However, this type of mutation only accounts for 1% of colorectal cancer patients.[16,70] In the treatment of MSS mCRC, the predictive value of POLE/POLD1 mutation on ICIs efficacy has also been confirmed in clinical studies, and pembrolizumab has a significant effect on MSS metastatic colorectal cancer with POLE/POLD1 mutation.[228,229] The combination of durvalumab and Avelumab has also shown promising clinical activity in POLE mutation mCRC patients. However, the clinical response to ICIs may be limited to patients with POLE exonuclease domain mutation (EDM).[230,231] Other studies have shown that not all POLE/POLD1 mutations cause TMB-H, and that patients with POLE/POLD1 mutations without TMB-H may not benefit from immunotherapy. Subsequent retrospective studies have shown that POLE pathogenic mutations may be a key factor in the benefit of immunotherapy.[232,233]

Expression of PD-L1

PD-1 is a transmembrane protein that is expressed on the surface of activated T cells, B cells and natural killer cells. PD-L1 is a ligand of PD-1, which is usually expressed on the surface of tumor cells.[234] In addition, PD-L1 is also expressed in lymphocytes, macrophages and stromal cells in the tumor immune microenvironment. The binding of PD-1 and PD-L1 can inhibit the proliferation and function of effector T cells, promote the transformation of effector T cells into regulatory T cells, and inhibit the apoptosis of tumor cells, thus escaping the surveillance and attack of the immune system.[235]

The high expression of PD-L1 in tumor tissues is correlated with the efficacy of immunotherapy in many solid tumors. In gastric cancer, non-small cell lung cancer and esophageal cancer, the PD-L-1 expression scoring system was used to predict the efficacy of ICIs.[236–239] However, prospective studies in advanced colorectal cancer have shown that the expression of PD-L1 is not predictive of immune efficacy.[240] At the same time, there is no correlation with dMMR/MSI-H.[241]

In a retrospective study, the association between PD-L1 expression and prognosis in patients with pMMR/MSS and dMMR/MSI-H CRC was investigated. Among 389 CRC patients, 68 were dMMR/MSI-H type, but PD-L1 expression was only confirmed to be associated with survival in the dMMR/MSI-H group, while no correlation was observed in the pMMR/MSS group.[72] Another single-arm, multicenter Phase II AVETUX trial evaluating avelumab in combination with cetuximab and mFOLFOX6 in previously untreated RAS/BRAF wild-type, MSS mCRC patients also did not observe any correlation between PD-L1 expression and PFS.[57]

In conclusion, although PD-L1 expression has an important predictive role in other gastrointestinal tumors (such as gastric and esophageal cancers) when using ICIs, there is no evidence to date that PD-L1 expression can be used as a predictive biomarker in pMMR/MSS mCRC patients. In addition, the expression of PD-L1 is a dynamic biomarker that can be changed under drug treatment. Dynamic monitoring techniques to detect the expression of PD-L1 by circulating tumor cells can be explored in the future, and more evidence is needed to support the role of PD-L1 expression in predicting the efficacy of ICIs in colorectal cancer.

Tumor Infiltrating Lymphocytes and Immunoscore

There is usually infiltration of various lymphocytes in tumor parenchyma and stroma. These lymphocytes are collectively called TILs. TILs include T cells, B cells, NK cells, dendritic cells, macrophages and other subsets, and are an important part of tumor immune microenvironment. Existing studies have shown that anti- PD-1 /PD-L1 or anti-CTLA-4 treatment can induce increased CD8+ cytotoxic T lymphocyte infiltration in tumor, and these activated T cells may be related to tumor regression.[242–244]

Intratumor lymphocyte infiltration, especially the density of CD8+ T lymphocytes, is correlated with the prognosis of colorectal cancer.[245] Masucci's study showed that the infiltration of cytotoxic T cells and Th1 cells and the up-regulation of IFN-γ expression in colorectal cancer tissues could indicate the efficacy of ICIs, which also indicated that TILs could predict the efficacy of immunotherapy.[246] This is consistent with the findings of Loupakis et al. TILs and TMB were detected in 85 dMMR/MSI-H colorectal cancer samples treated with ICIs, and the results showed that PFS and OS were

significantly improved in the high TILs group, suggesting that TILs is a promising biomarker for predicting the efficacy of ICIs in dMMR/MSI-H mCRC.[247]

In recent years, the role of TILs in MSS colorectal cancer has been studied. Giannakis et al conducted whole exome sequencing on 619 colorectal cancers and found a significant association between high TILs and higher neoantigen load in MSS CRC, suggesting a possible sensitivity to ICIs treatment.[73] However, previous studies have suggested that MSS CRC has low TMB and little TILs, and is not sensitive to immunotherapy.[16] Interestingly, CD3+ CD8+ T lymphocytes were highly infiltrated in the microenvironment of approximately 45% of MSS CRC tumors,[74] suggesting that some specific populations of MSS patients may respond to immunotherapy.[16,248] NICOLE's study showed that pMMR/MSS CRC patients could benefit from nivolumab. Further immune microenvironment analysis showed that tumor-infiltrating CD3+ and CD8+T lymphocytes may be biomarkers of immune response in pMMR CRC.[249] Of course, unlike advanced CRC, the immune microenvironment in early CRC is relatively complete, and neoadjuvant immunotherapy may be able to better induce T lymphocyte proliferation and play a more significant curative effect.[250–252] In addition, increased CD8+ T cell infiltration was associated with better survival outcomes in MSS mCRC patients treated with Regorafenib and avelumab.[25]

In addition, researchers have found that the combination of CD8+T lymphocyte infiltration and PD-L1 expression can be used to predict the efficacy of ICIs in the treatment of various tumors.[253,254] Llosa et al found that MSS mCRC patients had a tumor immune microenvironment similar to dMMR/MSI-H patients when tumor tissue was infiltrated by highly expressed PD-L1 and highly expressed CD8+T cells, and patients could benefit from Pembrolizumab treatment. It is suggested that combined detection can predict the efficacy of ICIs in the treatment of MSS CRC patients.[255] According to the analysis of tumor samples before and after neoadjuvant therapy, the VOLTAGE-A study showed that the pCR rate of patients with PD-L1 tumor ratio ≥1% was 75%, and the pCR rate of patients with CD8+T cells/effector regulatory T cells (CD8+/eTreg) ratio ≥2.5 in tumor infiltrating lymphocytes was 78%. The pCR rate was 100% when the above two conditions were met simultaneously. The results show that PD-L1 expression and increased CD8+/eTreg ratio are effective predictors of the benefit of immunocombined chemoradiotherapy in MSS locally advanced rectal cancer (LARC)patients.[49]

These studies suggest the potential of TILs as a predictor of the efficacy of ICIs in MSS CRC. In order to more accurately predict the efficacy of ICIs, the researchers proposed an immune score based on the distribution of TILs, which graded the number of CD8+T cells, CD3+T cells, and CD45RO+ memory T cells located in the core and border areas of the tumor. The density of lymphocyte populations in the two regions was rated from low to high as 1 to 4 points.[256] The prognostic and predictive value of immune score based on the proportion of tumor immune cells in early and middle CRC is even higher than TNM stage and MSI status.[74] Meanwhile, the prognostic value of immune score on clinical outcome of mCRC patients has also been confirmed.[257] However, the predictive value of immunotherapy efficacy in mCRC has not yet been shown. At present, several studies have used immune scoring system to predict the efficacy of ICIs.[75,183,258] AtezoTRIBE Study This study uses an immune score. pMMR/MSS patients who may benefit from the combination regimen of immunization were screened by the immune score. Studies have shown that patients with higher immune scores have more OS benefits than patients with lower immune scores,[44] indicating that the immune score has the opportunity to help us screen out patients with pMMR/MSS CRC who may benefit from immunotherapy. More evidence is needed to confirm whether immune score can be used as a predictor of the efficacy of ICIs in MSS CRC.

Gut Microbiota, etc

Gut microbiota plays an important role in the maintenance and development of host immune system, and the efficacy of ICIs in the treatment of tumor is related to gut microbiota.[259,260] A study has shown that the ratio of Prevotella to Bacteroides in the gut of patients with gastrointestinal tumors is related to the efficacy of ICIs, and whether they can benefit from immunotherapy is likely to be related to the metabolites of the bacteria.[76] Another study showed that a microbiome containing 11 types of bacteria collected from the human gut was able to induce CD8+T lymphocytes capable of producing interferon gamma in the mouse gut, thereby enhancing the anti-tumor efficacy of ICIs.[261] Wang et al found that Clostridium in the baseline intestinal microflora could be used as a marker to predict the efficacy of ICIs.[26] In addition, it has been reported that in non-colorectal cancer tumors treated with ICIs, antibiotic use may alter the

number and function of immune cells in the gut, and alterations in the gut microbiota may negatively affect immunotherapy efficacy.[262,263]

In addition to the gut microbiota, the microbiota is also present within colorectal cancer tumor tissue. Among them, there are more studies on Fusobacterium nucleatum, a tumor resident bacteria in colorectal cancer. Related studies have shown that Fusobacterium nucleatum can activate interferon signaling pathway, induce PD-L1 expression and increase IFN-γ+CD8+TILs, thereby improving tumor sensitivity to ICIs. When there is a high level of Fusobacterium nucleatum in the tumor tissue, the treatment effect of ICIs in patients is good, and MSI status does not need to be detected,[264] suggesting that Fusobacterium nucleatum can predict the efficacy of ICIs in colorectal cancer, including MSS. In the future, we should gain a deeper understanding of the CRC gut microbiome and the biological mechanisms that influence colorectal cancer response to ICIs, which may guide us to recommend or avoid certain antibiotics during ICIs treatment for a tumor microenvironment with a better immune response.

In addition to the above markers, circulating tumor DNA (ctDNA) can better monitor the efficacy of ICIs in MSS colorectal cancer. Patients with low ctDNA levels are stable during treatment, while patients with elevated ctDNA levels often experience rapid progression.[265] In addition, a blood analysis of patients with chemotherapy-refractory mCRC receiving a combination of cetuximab and avelumab showed that a high neutrophil to lymphocyte ratio was a poor prognostic factor. Therefore, the ratio of neutrophils to lymphocytes can also be used as a predictive marker of immunotherapy efficacy.[266]

In summary, several studies are attempting to optimize or expand immune benefits population by screening ICIs efficacy predictor markers in MSS colorectal cancer, and some biomarkers have shown better potential and value. Among them, TMB and POLE/POLD1 genes have been shown to have a good predictive effect on ICIs efficacy in MSS colorectal cancer, but there is no consensus on the critical values of predictive markers such as TMB and TILs, while related markers such as PD-L1 expression, immune score and intestinal microbiota are being explored for predicting the efficacy of immunotherapy. The results of some studies are inconsistent, and the specific mechanisms and how to evaluate them need to be further explored. Considering that there are still few relevant studies to predict the efficacy of immunotherapy in colorectal cancer with MSS, more clinical studies are needed in the future to verify and explore high-value biomarkers in order to screen out patients who are more suitable for immunotherapy.

Summary and Outlook

Immunotherapy offers hope for long-term survival in patients with MSI-H colorectal cancer, but current studies have shown that immunotherapy, especially ICIs alone, is largely ineffective in treating patients with pMMR/MSS mCRC. How to improve the efficacy of immunotherapy in patients with MSS mCRC has always been one of the urgent problems to be solved. The key challenge is to transform the pMMR/MSS type of tumor into a highly immunogenic tumor. At present, the combination therapy strategy is a universal strategy to increase immunotherapy responsiveness.

From the perspective of mechanism, considering the possible synergistic effect of the combination of different treatment regiments, some ICIs combined with multi-target TKI, chemotherapy, radiotherapy, anti-VEGF /EGFR targeted therapy and other strategies have been carried out related clinical studies, and survival benefits have been initially seen. At the same time, new combined treatment strategies such as immunotherapy and cancer vaccines, BsAb, gut microbiota transplantation and targeting other targets are also being explored, which is expected to obtain more and better clinical data in the future, and increase the treatment options of pMMR/MSS colorectal cancer. At present, our preclinical studies are not deep enough, and the safety and effectiveness need to be verified by more prospective clinical trials with large samples. The immune mechanism is very complex, which makes pMMR/MSS immunotherapy for colorectal cancer face two problems: one is efficacy, and the other is safety. Considering that each treatment plan has its own adverse reactions, multiple treatments will inevitably bring about the superposition of adverse reactions, how to reduce the unnecessary toxic side effects caused by the combination of drugs and prolong the reaction time of immunotherapy is also the focus of clinical consideration. Therefore, we believe that the choice of regimen is very important in the clinical study of immune combination therapy for MSS colorectal cancer. More basic research should be conducted in the future to evaluate whether the combination regimen can activate the immune microenvironment of tumors to achieve synergies.

In addition, we need to find people who can really benefit from the combination immunotherapy strategy. This requires us to screen biomarkers and further refine the population to screen out patients who are more suitable for immunotherapy. In fact, the search for predictive biomarkers remains a major challenge in immunotherapy in pMMR/MSS colorectal cancer patients, not only to screen those who can benefit from immunotherapy, but also to avoid the unnecessary expense, hyperprogression and possible severe toxicity of treating non-responders. But unlike the genetic mutations of targeted drugs, the molecular markers of immunotherapy are more complex. It can be seen that some markers, such as TMB and POLE/POLD1 genes, have shown good potential and value in predicting ICIs efficacy in pMMR/MSS colorectal cancer patients. However, any single predictive biomarker has limitations, especially for highly heterogeneous colorectal cancer. It is often necessary to integrate a variety of different types of biomarkers for comprehensive judgment, so as to improve the ability to predict.

Through our study, we believe that for pMMR/MSS colorectal cancer patients, it is necessary to further elucidate the key mechanisms of immune escape and immune tolerance in the future, guide the development of more effective immunotherapy methods or drugs, and promote further basic and translational research to develop more accurate and effective combination therapy protocols. CONSIDERING that precision therapy based on molecular typing will inevitably become the premise and basis of future clinical practice and research design, it is expected to explore more dimensions of biomarkers and their combinations in the future, so as to more accurately enrich the benefit groups of immune therapy, and provide more accurate treatment for pMMR/MSS colorectal cancer patients. This will bring longer survival time and better quality of life for patients.

Data Sharing Statement

No additional data are available.

Author Contributions

The authors listed in this article have made significant contributions to the work of the report, whether in concept, research design, execution, data acquisition, analysis and interpretation, or in all of these areas, and meet the following criteria. All authors participated in drafting or writing, or substantially revised or critically reviewed the article, and have agreed on the journal in which the article will be submitted. Finally, all authors agree to take responsibility and be accountable for the contents of the article.

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