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A New Hope? Combination Immunotherapy for Microsatellite Stable Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is one of the three most common malignancies globally while the mortality ranks second. Currently immunotherapy, like therapeutic monoclonal antibodies targeting immune checkpoints, have been found to have obvious benefit for patients who are DNA mismatch repair deficiency (dMMR)/high microsatellite instability (MSI-H) CRC, however the majority of patients are the DNA mismatch repair proficient (pMMR)/microsatellite stable (MSS) or low microsatellite instability (MSI-L), which are considered as the "cold tumors". The absent of tumor T cell infiltration, which is an essential feature of so-called "cold tumors," and thus contributes to the resistance to immune checkpoint inhibitors. In this article, we want to review the progress of immune combination therapy in MSS CRC (for example, anti-VEGF drugs, anti-EGFR drugs, MAPK signaling pathway target drugs, TGF-β antibodies, radiotherapy, bispecific antibodies, neoantigens vaccines and oncolytic viruses), and we hope to provide new sparks for the treatment of this group of patients.

Keywords: Colorectal cancer; Immunotherapy; Microsatellite stability; Combined therapy.

Introduction

Colorectal cancer (CRC) stands the third in the incidence of tumors in the world, which ranks the second in terms of mortality [1]. In 2018, there were 1.8 million confirmed cases of CRC and 880,792 people died from CRC worldwide. The incidence of CRC is rising among people who are under 50 [2]. The main genetic modification in CRC is according to the impairment of DNA mismatch repair (MMR) activity, which causes the microsatellite instability-high (MSI-H) in 15% as opposed to the microsatellite stable (MSS) or microsatellite instability-low (MSI-L) tumors without such impairment that share 85% in CRC [3,4]. Meanwhile, MSS metastatic CRC (mCRC) share about 95% in all mCRC cases, the survival rates of which remain undesirable at 14% approximately [5,6]. Currently, surgery, chemotherapy and radiotherapy are the standard conventional treatments for CRC, but due to the therapeutic limitations of these methods, immunotherapy is emerging. In recent years, the development of anti-PD-(L)1 antibodies has significantly bettered the treatment of several cancers, including non-small cell lung cancer, bladder cancer, melanoma, and others, which are considered to be representative immune checkpoint inhibitors (ICIs) [7,9]. Immunotherapy is also beneficial for patients with CRC, especially these with mismatch repair deficient (dMMR)/MSI-H CRC, who are more likely to be effective to ICIs compared to mismatch repair proficient (pMMR)/MSS or MSI-L CRC patients [10,11]. At present, the Food and Drug Administration (FDA) has given approval to two anti-PD-1 antibodies, pembrolizumab and nivolumab, as well as nivolumab combined with the anti-cytotoxic T lymphocyte-associated antigen-4 (anti-CTLA-4) monoclonal antibody, ipilimumab, for patients who are dMMR/MSI-H mCRC. However, MSS or MSI-L CRC is usually viewed as the "cold tumor" which has a poor response to immunotherapy [12]. Therefore, it is crucial to find out how to help these populations benefit from the immunotherapy. In the review, we want to summarize the efforts of immunotherapy-based combination therapy in MSS CRC, which may provide new therapeutic ideas in the clinic.

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Immunotherapy combined with other antitumor treatments

Immunotherapy in combination with Anti-VEGF drugs and chemotherapy: The vascular endothelial growth factor (VEGF) has unique immunomodulatory properties. The tumor-associated immune deficiency could be caused by VEGF directly suppressing diverse immune cells in tumor microenvironment. For example, inhibiting the antigen presentation of DCs, inducing apoptotic in $CD8^+$ cytotoxic T lymphocytes (CTLs) and activating the T regulatory (Tregs) cells, may result in a pro-tumor microenvironment [13,14]. Thus, by blocking VEGF-VEGFR pathway, it may restore the immune mechanisms in the tumor microenvironment [15]. Blocking PD-1 and VEGF can recover the function of T cells, which may lead to the better control of MSS CRC [16]. Regorafenib plus nivolumab is being tested in REGONIVO (NCT03406871). Results showed that only one of the 25 Japanese patients had MSI-H/dMMR mCRC and the response rate was 33.3% after excluding this patient [17]. Results in the AtezoTRIBE (NCT03721653), which compared FOLFOXIRI and bevacizumab alone to FOLFOXIRI and bevacizumab mixed with atezolizumab, have been reported (199) of the (218) patients had advanced MSS CRC. In the combination group, the progression-free survival (PFS) was (12.9) months while it was (11.4) months in the control group [18]. A single-arm, phase II trial called REGOMUNE (NCT03475953) showed that regorafenib plus avelumab induced the antitumor immunity in part of MSS CRC patients [19]. Besides, an animal experiment found that fruquintinib plus sintilimab could prevent the tumor growth by regulating the tumor immune microenvironment, especially in MSS CRC [20]. According to the retrospective analysis of the safety and effectiveness of regorafenib combined with an anti-PD-1 antibody in MSS CRC, the best response was reported by 18 patients (78.3%), who had stable disease. Five other subjects had progressive disease (21.7%) and there was no evidence of a partial response (PR). The median PFS (mPFS) of 3.1 months and disease control rate (DCR) of 78.3% were recorded, which may need to be further evaluated in the future [21]. In the LEAP-005 (NCT03797326), patients treated by Pembrolizumab and Lenvatinib showed antitumor activity and had acceptable safety profile [22]. However, the latest phase 3 trial LEAP-017 (NCT04776148) in patients with unresectable or metastatic CRC that evaluated pembrolizumab and lenvatinib failed to achieve the primary goal of overall survival (OS). While there were improvements in OS, PFS, duration of response (DOR) and objective response rate (ORR), these trends were not obvious and statistics did not show any significance [23]. In order to evaluate the safety and effectiveness of SHR-1210 (one kind of anti-PD-1 antibodies) and apatinib in MSS/pMMR mCRC, Ren et al. conducted a phase II trial (NCT03912857). But results did not show strength in the treatment of MSS CRC [24]. In summary, the combination of immunotherapy and anti-VEGF drugs provides a novel idea for MSS CRC. However, the efficacy difference between various anti-angiogenic drugs and immunotherapies remains unknown. And it may be related to the multi-target action of the drugs, which may produce a stronger anti-angiogenic and immunotherapy synergy by inhibiting not the VEGF/VEGFR pathway but immunomodulatory targets such as angiopoietin receptors, colony stimulating factor receptors, and platelet-derived growth factor receptors [25,26], which require to explore further.

Immunotherapy in combination with Anti-EGFR drugs: One of the receptor tyrosine kinases (ErbBs) family, called the epidermal growth factor receptor (EGFR), is a significant role in cell proliferation, survival, differentiation and invasion in CRC [27]. Based on the studies, EGFR is overexpressed in about 50% of CRCs, which may lead to tumor invasion and other aggressive pathohistological features [28,29]. Currently, standard chemotherapy combined with the monoclonal EGFR-targeting antibodies, cetuximab + panitumumab, are utilized to treat patients with RAS and BRAF wild-type (WT) mCRC [30,31]. A fully human IgG2 mAb called panitumumab had high affinity for EGFR ligands, the combination of which may lead to the reduction of cell survival, proliferation, angiogenesis and inducing apoptosis [32]. A chimeric IgG1 antibody called cetuximab can generate antibody-dependent-cellular-toxicity (ADCC) and encourage the production of major histocompatibility complex (MHC) II molecules on dendritic cells (DCs), which stimulate the immune response [33]. The appearance of cetuximab or panitumumab has prolonged the survival of mCRC patients in 10-20% [34]. In the AVETUX trial (NCT03174405), new treatment responses were reported in the RAS and BRAF WT mCRC patients, 95% of which were MSS, with the DCR of 92% and the ORR of 79.5%. In this trial, the first-line treatment was cetuximab plus avelumab with FOLFOX [35]. A phase Ib/II trial is studying the efficacy of cetuximab combined with pembrolizumab in RAS WT CRC, in which 19 of 44 patients are MSS. Although the results were inactive, it had partial local immunologic efficacy [36]. It has been discovered that combining avelumab and cetuximab may have synergistic effects that improve the effectiveness of anti-EGFR drugs and anti-PD-L1 drugs [37]. The phase 2 multicenter trial (NCT04561336) of cetuximab + avelumab in 77 subjects showed that there was an 11.6-month median OS (mOS) and a 3.6-month median PFS, 92% of which were MSS CRC [38]. The aim of the CAVE 2 trial (NCT05291156) is to show that cetuximab alone results in a mOS of 10 months and cetuximab + avelumab lead to a mOS of 15 months, which represent a 33% improvement in mOS (hazard ratio of 0.67) [39]. To sum up, the joint of immunotherapy and anti-EGFR drugs has a synergistic effect on MSS CRC and can enhance the antitumor effect of the drugs.

Immunotherapy in combination with MAPK signaling pathway targeted drugs: Studies have revealed that changes in the mitogen-activated protein kinases (MAPK) pathway also have immunosuppressive effects, which is crucial for the progression of cancers [40]. Currently there are four known types of MAPK signaling pathways, of which the RAS/RAF/MEK/ERK cascade is the best characterized effector pathway. It suppresses cell growth, inhibits cell invasiveness, regulates apoptosis and induces angiogenesis [41]. RAS Inhibitions: Mutations in KRAS, one type of RAS series, are found in about 40% of CRCs [42]. Treated by sotorasib, ORRs of 32%, DCRs of 88%, and mPFS of 6.3 months were achieved in patients with KRAS G12C mutant tumors. While the study did not distinguish the microsatellite status, CRC patients showed 7.1% confirmed responses (3 patients) and 73.8% disease control (31 patients) [43].

MEK inhibitions: We have known that the double inhibition of MEK and PD-L1 can produce a synergistic antitumor effect [44]. An ORR of 8%, including 6 MSI-L/MSS patients, was observed in the phase I trial (NCT01988896), which appraised the efficacy of the combination of cobimetinib (a MEK inhibitor) and atezolizumab in 84 CRC patients who had chemotherapy failure [45]. In the IMblaze370 trial (NCT02788279), compared with regorafenib, atezolizumab did not improve OS in patients who were pMMR/MSS CRC obviously, with or without cobimetinib. PFS and ORR did not differ between arms [46]. RAF Inhibitions: BRAF is an important integral part of the RAS-RAF-

MEK-MAPK signaling pathway [47]. BRAF mutations occur in up to 15% of patients with CRC [48]. One of the most prevalent mutations is V600E, which causes the initiation of BRAF kinase and long-term RAS/RAF/MEK/ERK signaling [49]. In the trial (NCT03428126), trametinib + durvalumab in patients with MSS mCRC were studied for the immune-related response rate. The response rate of 3.4% overall was achieved by 29 of 48 patients with a confirmed PR of (9.3) months. There were seven stable disease (SD) patients and five patients showed a reduction in total carcinoembryonic antigen (1 PR and 4 SD) [50]. In 142 patients with $BRAF^{V600E}$ CRC, the clinical trial assessed the efficacy of dabrafenib (D) + panitumumab (P) ± trametinib (T). D+P, D+T+P, and T+P had the response rates of 10%, 21%, and 0%, respectively. For the 11 of 78 MSI-H/dMMR patients, the response rate was 46% (5 of 11) in comparison with 27% (18 of 67) in patients who were MSS/pMMR. And the only one patient achieved complete response (CR) was MSS/pMMR who were treated with D+P [51]. In the phase II trial (NCT03668431) which tested PD-1, BRAF and MEK inhibition in $BRAF^{V600E}$ CRC, the mPFS for patients with MSS $BRAF^{V600E}$ CRC was 5 months, cORR was 25%, and DCR was 75% [52]. These findings indicate that there is an intrinsic synergistic effect between MAPK pathway inhibitors and immunotherapy, which need to further explore.

Immunotherapy in combination with TGF-β antibodies: Transforming growth factor β (TGF-β) plays a critical role in the immunomodulation in MSS CRC [53]. TGF-β has dual effects, inhibiting the development of tumors by reducing cell proliferation and promoting apoptosis and increasing the survival of tumors by immune evasion. Researchers have found that regulating the TGF-β pathway could prevent TGF-β mediated immune evasion while promoting antitumor effect of tumor-infiltrating lymphocytes (TILs) at the same time [54,55]. The study found that the double block of PD-L1 and TGF-β could facilitate T cell infiltration into the tumor center and reduce TGF-β signal conduction in stromal cells, which produced powerful anti-tumor responses [56]. In the trial of a bifunctional fusion protein aiming at PD-L1 and TGF-β, M7824 (MSB0011359C), only 1 of 29 patients had a confirmed objective response, who was MSS CRC (NCT02517398) [57]. This may provide new thoughts for the treatment of MSS CRC.

Immunotherapy in combination with radiotherapy: One of the standard treatments of advanced CRC is chemoradiotherapy (CRT), whether in the neoadjuvant or adjuvant therapy [58]. Based on the studies, radiotherapy can improve the effect of immunotherapy via changing the tumor microenvironment. At the same time, immunotherapy can also promote the effect of radiotherapy by modulating different immune cells [59,60]. In a phase 2 trial (NCT03104439), which combined radiotherapy, ipilimumab and nivolumab in patients (n=40) who are MSS mCRC, results showed that the DCR in the CRC cohort was 37% and the ORR was 15% in an analysis of patients who received radiotherapy [61]. In the VOLTAGE trial (NCT02948348), the findings revealed that there was a 30% pathological complete response (pCR) in 11 cases in cohort 1; pCR rates are 75% (6/8) for MSS patients that underwent surgery; and pCR rates are 78% (7/9) in subjects with MSS, when samples experienced flow cytometry analysis. Additionally, subjects with MSS locally advanced rectal cancer (LARC) showed positive predictive values for PD-L1 expression and a higher $CD8^+$ T cell/effector regulatory T cell ratio [62]. Despite the mismatch repair status, a phase 2 trial which evaluated the short course radiotherapy (SCRT) in conjunction with CAPOX, capecitabine and oxaliplatin, plus camrelizumab for LARC demonstrated an excellent tolerability and a promising pCR rate in individuals with LARC (100% (1/1) in dMMR/MSI-H, 46% (12/26) in pMMR/MSS). Investigators are waiting the final results [63]. At present, based on the preliminary results, the clinical prognsis of patients who had pMMR/MSS mCRC may be improved by using PD-1 inhibitors in addition to radiotherapy and human granulocyte-macrophage colony stimulating factor (GM-CSF) [64]. The number of data available on how to use the approach in patients with MSS CRC, combing radiotherapy and immunotherapy, remains scarce. There is also a lot of researches going on (NCT04535024, NCT06045286). Also, it is still essential to investigate the timing of immunotherapy, which is referred as induction therapy or consolidation therapy, and radiotherapy.

Bispecific antibodies therapy: Bispecific antibodies stimulate the immune response by connecting tumor-rich antigens (such as the carcinoembryonic antigen (CEA), human epidermal growth factor receptor 2 (HER2), etc.) and immune cells, which are widely used in different kinds of tumors [65,66]. At present, bispecific antibodies in tumor immunotherapy are led via T cells. T-cell bispecific antibodies recruit and activate T cells while focusing on the surface receptors of tumor cells to mediate potent and selective cytotoxicity, while the recognition of tumor antigens presented by MHC molecules is not essential for T cell receptor mediated immune responses [67,68]. Moreover, researchers have found that the combination of bispecific antibodies with ICIs can increase the antitumor activity [69]. A T-cell bispecific antibody called Cibisatamab is able to direct T cells toward cancer cells that have CEA glycoprotein expressed on the cell surface, in spite of the specificity of T cell receptors [70]. Cibisatamab can connect to CD3 on T cell and also CEA on the surface of cancer cells, which may activate T cells and promote the emission of cytotoxic granules and cytokines [71]. In the clinical trials (NCT02324257, NCT02650713), patients with solid tumors that express CEA, including those with MSS mCRC, were treated with cibisatamab as a monotherapy or in conjunction with atezolizumab. Cibisatamab monotherapy in the setting of sustained dosage escalation showed evidence of antitumor activity. The combination with atezolizumab also enhanced the antitumor activity with a safe and controlled profile [72,73]. LY6G6D is expressed in CRC differently, with high expression in MSS CRC and MSI-L CRC while its expression is low in MSI-H CRC, so it may be a new target for the treatment of CRC. A full-length IgG1-based bispecific antibody is called LY6G6D-TDB that binds to the human LY6G6D antigen with one arm and the human CD3 on T cells with the other. Researches indicate that LY6G6D-TDB can kill colorectal cancer cells that exhibit high level of LY6G6D [74,75]. In addition to the two bispecific antibodies mentioned above, there are other bispecific antibodies ongoing for the treatment of CRC, such as anti-GPA33/anti-CD3 bispecific antibody, GUCY2C-CD3 bispecific construct (PF-07062119), VHH bispecific antibody, MGD007, MM-141 [76-79].

Neoantigens vaccines therapy: The immune system in patients is predominantly activated by tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) in tumor vaccines. The vaccine could induce not only specific cellular immunity but also humoral immunity, which prevent tumor growth and kill tumor cells [80]. There are many kinds of tumor vaccines that have been studied, like autologous vaccines, DC vaccines, viral vector vaccines, and peptide vaccines etc [81]. And only two FDA-approved vaccines, Hepatitis B virus and human papillomavirus, are available to prevent malignancy caused by viruses [82]. It is critical to choose the targeted antigens in any

tumor vaccines, which decide the efficacy of these vaccines. An ideal antigen should be exclusively present on cancer cells and not on healthy cells [83]. In tumor genomes, neoantigens arise from somatic mutations, which have key immunogenic potential. Neoantigen-based therapy can be identified by the immune system for their high affinity for the MHC, which would avoid the central and peripheral immune tolerance [84,85]. The latest study has discovered that the efficient inhibition of tumor growth in tumor-bearing mice has been achieved by adoptively transferring neoantigen-reactive T lymphocytes (NRTs) generated by vaccination with two mutant peptides. In the study, researchers also found that in vitro neoantigen stimulates enhanced NRT responses from patients with CRC, which may produce the more effective anti-tumor response [86]. Westcott PMK et al. found that low neoantigen-expressing models, such as MSS CRC, had low T cell infiltration and poor immune checkpoint blockade response, while one important factor influencing immunity is the expression of neoantigen. So, rescue of T cells may control tumors with low neoantigen expression [87]. Neoantigens arising from KRAS are frequently found in CRC, and in vaccine studies, vaccines made from mutant KRAS have demonstrated the antitumor activity [88]. Patients with CRC lung metastases displayed rapid tumor regression treated by activated CD8 T cells that can recognize G12D KRAS in one case report (NCT01174121) [89]. In another clinical experiment, two of seven participants had reactions after vaccine injection [90]. Based on a single-center, single-arm clinical trial, 66.67% (4/6) of the MSS CRC patients who were given the vaccines showed signs of neoantigen-specific immune responses. These patients also had obviously longer PFS than the patients who had no neoantigen reaction (19 months vs. 11 months) [91]. In an ongoing phase 1/2 research (NCT03639714), which evaluated the neoantigen vaccine combined with nivolumab and ipilimumab in patients with advanced metastatic solid tumors, results found that patients with MSS CRC had improved OS [92]. To assess the security and efficacy of peptide neoantigen-based vaccines in patients with dMMR CRC, Kloor M et al. carried out a phase I trial (NCT01461148). Results revealed that in all [22] patients with dMMR CRC, at least one of the peptide vaccinations generated both humoral and cellular immune responses without any significant adverse effects from the vaccine [93]. These findings mentioned above demonstrated that neoantigen vaccines show a lot of promise for treating of MSS CRC. Currently, some cancer vaccines are ongoing in the preclinical and clinical research and there is still a lot of work to do in the development of antigens and vaccine development platforms. There are also many experimental studies of neoantigen vaccines in combination with other treatment modalities in progress, including in MSS CRC.

Immunotherapy in combination with oncolytic viruses: Oncolytic viruses (OVs) are multifaceted tumor killers that are novel strategies for oncology treatment. They can not only directly kill tumor cells while retaining normal cells, but also release antigens and cause inflammatory responses in tumor microenvironment that indirectly enhance the antitumor immunity [94]. Currently, a variety of viruses have been studied as a carrier for oncolytic viruses and there are undergoing clinical trials in the treatment of cancers, which are more common in melanoma and gastrointestinal tumors. These viruses include vaccinia virus, adenovirus, coxsackievirus, reovirus, measles virus, and herpes simplex virus [95]. What's exciting is that studies have found that OVs and ICIs can work in tandem [96]. According to the research, OVs can turn the "cold tumor" to the "hot tumor" by killing tumor cells and also moving immune cells (like T cells, NK cells) to the tumor microenvironment, which can increase the sensitivity of tumor cells to ICIs. Additionally, by increasing the expression of the immune checkpoints on the tumor cells, OVs can improve the targeted function of PD-1/PD-L1 inhibitors [97]. Studies have demonstrated that the triple therapy (OVs, CSF-1R, and anti-PD-1 antibodies) can increase T cell infiltration in tumors and enhance anti-tumor $CD8^+$ T cell activity, which also reprogram the immunosuppressive tumor microenvironment to promote antitumor immunity. Ultimately, the tumor growth is controlled and survival is prolonged in mice with colon cancer [98]. With the union of low-dose mitomycin C and oncolytic HSV-1, which transfer the $CD8^+$ and $CD4^+$ T cells to the tumor cell, this also improved the sensitivity of ICIs in the mice model with dMMR CRC [99]. Therefore, considering that oncolytic virus can increase the sensitivity of tumors to ICIs, the combination of oncolytic virus and ICIs can be a effective treatment strategy for the patients with MSS CRC.

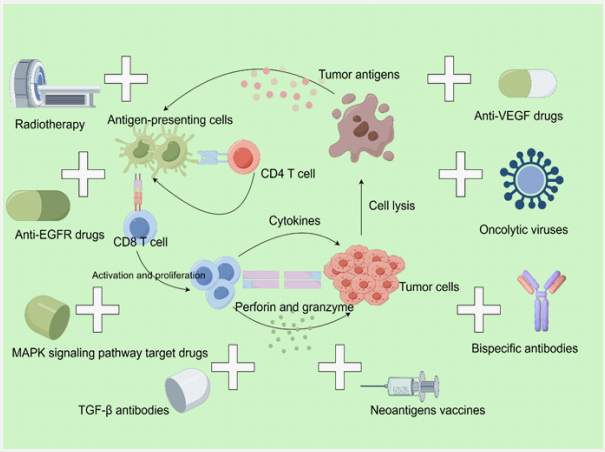

Figure 1: Combination immunotherapy in MSS CRC (By Fig draw.).

Table 1: Trials of combination immunotherapy in MSS CRC.

Identifier/reference	Study design	Population	Status	Trial description	Primary endpoints
NCT03406871	Phase I/II	Advanced or Metastatic Solid Tumors	Active, not recruiting	Regorafenib + nivolumab, single-arm dose excalation cohort	Recommended Dose (RD)/ Maximum Tolerated Dose (MTD)
NCT03721653	Phase II	First-line mCRC	Active, not recruiting	FOLFOXIRI + bevacizumab + atezolizumab vs FOLFOXIRI + bevacizumab, 1:2 randomized	PFS
NCT03475953	Phase I/II	Advanced or Metastatic Solid Tumors	Recruiting	Three dose levels of regorafenib in combination with avelumab (no dose escalation for Avelumab) in patients with advanced solid tumors followed by 17 phase II trials	Safety/clinical activity

NCT03797326	Phase II	Pretreated Solid Tumors	Recruiting	Pembrolizumab + lenvatinib, 2 arms multi-cohort, parallel assignement	Safety/ORR
NCT04776148	Phase III	Colorectal Cancer	Active, not recruiting	Lenvatinib + pembrolizumab vs standard of care treatment (regorafenib or trifluridine and tipiracil hydrochloride)	OS
NCT03912857	Phase II	Advanced mCRC	Recruiting	Camrelizumab in combination with apatinib, one-arm, single-center, open-stage phase II clinical	ORR
NCT03174405	Phase II	First-line RAS/BRAF wt mCRC	Active, not recruiting	FOLFOX + avelumab + cetuximab, single arm	PFS
NCT04561336	Phase II	Pretreated RAS wt mCRC	Active, not recruiting	Avelumab + cetuximab, single arm	OS
NCT05291156	Phase II	Pretreated RAS/BRAF wt mCRC	Recruiting	Cetuximab + avelumab vs cetuximab only, 2:1 randomized	OS
NCT01988896	Phase Ib	Locally Advanced or Metastatic Solid Tumors	Completed	Atezolizumab + cobimetinib	Toxicity/Safety
NCT02788279	Phase III	Unresectable Locally Advanced or Metastatic Colorectal Adenocarcinoma	Completed	Cobimetinib plus atezolizumab and atezolizumab monotherapy vs. regorafenib	OS
NCT03428126	Phase II	MSS mCRC	Completed	Durvalumab + trametinib	ORR
NCT03668431	Phase II	BRAFV600E mCRC	Recruiting	Dabrafenib and trametinib in combination with PDR001	ORR/Adverse Events (AEs)
NCT02517398	Phase I	Metastatic or Locally Advanced Solid Tumors	Completed	MSB0011359C (M7824), open-label, multiple-ascending dose trial	AEs/Best Overall Response (BOR)/DCR
NCT03104439	Phase II	MSS and MSI-High Colorectal and Pancreatic Cancer	Recruiting	Nivolumab and ipilimumab and radiotherapy	DCR
NCT02948348	Phase Ib/II	Locally Advanced Resectable Rectal Cancer	Recruiting	Standard CRT (with capecitabine + radiotherapy) followed by sequential nivolumab therapy	Safety/ORR
NCT02324257	Phase I	Locally Advanced and/or Metastatic CEA (+) Solid Tumors	Completed	RO6958688 vs RO6958688 with/without obinutuzumab pretreatment	AEs/Dose-Limiting Toxicities (DLTs)
NCT02650713	Phase I	Locally Advanced and/or Metastatic CEA-Positive Solid Tumors	Completed	RO6958688 in combination with atezolizumab, an open-label, multicenter, dose escalation and expansion phase Ib study	AEs/DLTs/MTD
NCT01174121	Phase II	Metastatic Cancers	Recruiting	1/CD8+ Enriched TIL (CLOSED) vs. 2/Unselected TIL (CLOSED) vs. 3/Unselected TIL + Pembro Prior to Cells vs. 4/Unselected TIL + Pembro at POD	Response rate (RR)
NCT03639714	Phase I/II	Advanced Solid Tumors	Completed	GRT-C901/GRT-R902 + nivolumab + ipilimumab	AEs/ORR/DLTs
NCT01461148	Phase I/II	Advanced MSI-H Colorectal Cancer	Completed	FSP peptides	Immune response
NCT04535024	Phase II	MSS Oligometastatic Colorectal Cancer	Recruiting	Stereotactic Ablative Radiotherapy (SABR) +Sintilimab	ORR
NCT06045286	Phase I	MSS Colorectal Liver Metastases	Recruiting	High- and Low-dose radiotherapy +PD-1 Inhibitors	ORR

Conclusion

In summary, immunotherapy in combination with other antitumor treatments may overcome the deficiencies of immunotherapy in MSS/pMMR mCRC (Table 1, Figure 1). Although positive results have been found in some of these studies, further clinical trials are necessary to determine the most efficient combination therapy strategy to select the patients that can benefit from this method. The current molecular stratification guiding the treatment of CRC does not completely reflect the diverse genotypes and phenotypes of the disease. More information analysis is needed to provide more effective and personalized treatment for each subject. In the era of precision medicine, gut flora is another complex factor. Studies have noticed the role of the gut microbiota in the pathogenesis of CRC and in regulating the response to antitumor therapy [100]. Whether this can provide new sparks for the treatment of CRC remains to be explored further. In future, therefore, the clinical management of CRC patients will require a comprehensive integration of their oncogene mutations, gene and protein expression in the tumor and microenvironment, host immunity and its dynamics throughout the disease at the individual level, in order to achieve a true continuum of care through precision medicine.

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