ISSN: 2157-2518
Journal of Carcinogenesis & Mutagenesis

OPEN ACCESS Freely available online

Review Article

Immunotherapy in Colorectal Cancer: A Review

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ABSTRACT

The results of the new randomized clinical trials show that immunotherapy is the preferred treatment for a small proportion of metastatic colorectal cancers. For Microsatellite Instability (MSI-H) metastatic Colorectal Cancer (mCRC), pembrolizumab, nivolumab, and ipilimumab are the currently authorized first and second-line immune checkpoints. But the problem instead concerns tumors with Microsatellite Stability (MSS or MSI-L) where the "cold" microenvironment does not allow immunotherapy to function properly. All efforts are now aimed at being able to make this microenvironment inflamed and "Hot". In this review, we examine all recent studies on immunotherapy for mCRC and assess novel drivers of immunotherapy therapeutic response.

Keywords: Colorectal cancer; Immunotherapy; Anti-PD-L1; Anti-PD-1

INTRODUCTION

Colorectal Cancer (CRC) is the second leading cause of cancer-related deaths and the third leading cause of cancer overall. It is a global health problem for treatment strategies [1]. Morbidity and mortality rates are declining thanks to screening. At diagnosis, 25% of patients with CRC have advanced disease, and 25% to 50% of patients with early-stage disease may have developed metastases [2-4]. The 5-year survival rate for patients with oligometastatic disease is 40% compared to patients with mCRC after tumor resection and chemotherapy [5-8]. Even if advantages have been obtained from the use of chemotherapy and targeted therapies, the 5-year prognosis is always poor and for this reason efforts are being made to develop new drugs. Immunotherapy treat cancer by stimulating the immune system. For patients with deficient Mismatch Repair (dMMR) or Microsatellite Instability-High (MSI-H), Immune Checkpoint Inhibitors (ICIs) have demonstrated remarkable effectiveness. By modifying the interaction between T cells, Antigen-Presenting Cells (APCs), and tumor cells, ICIs aim to reinvigorate suppressed immune responses.

Pembrolizumab and nivolumab (with or without ipilimumab) have gained approval from the U.S. Food and Drug Administration (FDA) as treatments for these patients. However, comprehending the potential benefits of immunotherapy for patients without Microsatellite Instability (MSS) poses a challenge [9]. Furthermore, this review outlines the present research endorsing the application of ICIs in Colorectal Cancer (CRC), emphasizes recent progress in the expanded use of ICIs in pMMR/MSS/MSI-L CRC cases, and sheds light on emerging biomarkers that could predict the response to immunotherapy.

LITERATURE REVIEW

We searched PubMed (www.ncbi.nlm.nih.gov/pubmed) for full-text articles from 2017 to May 31, 2023 using the keywords immunotherapy, cancer, colorectal cancer, anti PD-L1, and anti PD-1. The full-text articles found were carefully examined. In addition, all abstracts presented at international conferences between January 2020 and January 2023 were examined.

Biomarkers of reaction

DNA integrity relies on the essential function of Mismatch Repair (MMR) [10]. Immunohistochemical staining of MMR proteins, MLH1, MSH2, MSH6, or PMS2, allows the Categorization of Colorectal Cancers (CRCs) into two groups:

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Received: 05-Aug-2023, Manuscript No. JCM-23-22479; **Editor assigned:** 07-Aug-2023, Pre QC No. JCM-23-22479(PQ); **Reviewed:** 21-Aug-2023, QC No. JCM-23-22479; **Revised:** 28-Aug-2023, Manuscript No. JCM-23-22479(R); **Published:** 05-Sep-2023, DOI: 10.35248/2157-2518.23.S40.001

Citation: Colombo A, Porretto CM (2023) Immunotherapy in Colorectal Cancer: A Review. J Carcinog Mutagen. S40:001.

Those with deficient Mismatch Repair (dMMR) and those with proficient Mismatch Repair (pMMR) [11]. Microsatellite Instability (MSI) can be detected by PCR or next-generation sequencing and may result from insertions or deletions [11].

Microsatellite Instability (MSI) refers to changes in microsatellite length resulting from alterations in MMR status, known as dMMR-MSI-H. Within the cell surface, MHC class I-peptide complexes contain mutant peptides recognized as neoantigens, stimulating immune cell priming and infiltration. In the tumor microenvironment, Circulating T Helper 1 (TH1) $CD4^+$ T cells, macrophages, and $CD8^+$ Tumor-Infiltrating Lymphocytes (TILs) release IFNs, which exert antitumor effects. However, in dMMR-MSI-H tumor cells, immune evasion is facilitated by continuous upregulation of T cell inhibitory ligands such as B7 family members PD-L1, CD80, and CD86 [12-16]. dMMR-MSI-H CRCs account for less than 15% of all colorectal cancers, and their incidence correlates with the tumor stage [17]. Only 5% of stage IV patients have dMMR-MSI-H, compared to 11% of stage III and 5% of stage II patients [18]. As a predictive biomarker for patients at different stages, dMMR-MSI-H holds significance [18-21]. In stages II and III, patients with dMMR-MSI-H exhibit a more favorable prognosis than those with pMMR-MSI-L. However, intriguingly, even when treated with immune checkpoint inhibitors, stage IV dMMR-MSI-H patients still have a poor prognosis [22].

Second-line mcrc dmmr-msi-h and immunotherapy

As second-line treatment for mCRC patients with dMMR-MSI-H, pembrolizumab with or without nivolumab and ipilimumab was authorised in clinical studies in 2017. In the phase II KEYNOTE 016 study, pembrolizumab was used for patients with refractory mCRC [23]. Overall Response Rates (ORR) for pMMR-MSI-L and dMMR-MSI-H mCRC were 0% and 16%, respectively, while Disease Control Rates (DCR) were 50% and 89%. Nivolumab with or without ipilimumab was investigated in the phase II study CheckMate142 in patients with mCRC and dMMR-MSI-H [24]. At a median follow-up of 13.4 months, MSI H patients had an Overall Response Rate (ORR) and Disease Control Rate (DCR) of 55% and 80%, respectively, compared to 0% and 16% for MSS patients. Progression-Free Survival (PFS) and Overall Survival (OS) at 12 months were measured in the study's 119 participants. Progression-Free Survival (PFS) and Overall Survival (OS) at 12 months were 71% and 85%, respectively [25,26].

Immunotherapy in first line mCRC

Due to the favorable outcomes observed in second-line treatment of metastatic Colorectal Cancer (mCRC) with dMMR-MSI-H, there is a growing interest in utilizing immunotherapy as a first-line therapy. Several randomized clinical trials have drawn significant attention [27-29].

In a phase III trial called KEYNOTE177, which focused on first line mCRC with MSI-H, pembrolizumab monotherapy was compared to standard therapy [30]. The trial enrolled 852 screened patients, out of which 307 (36% of the total) were randomized to receive either chemotherapy or pembrolizumab (153 and 154 patients, respectively). Following disease progression, 60% of the patients switched from chemotherapy to anti-PD-1 therapy (56 to pembrolizumab, and 37 discontinued treatment). The median Overall Survival (OS) with pembrolizumab was not reached at the time of analysis, while it was 36.7 months (with a range of 27.6 is not reached) with chemotherapy. Although pembrolizumab did not demonstrate superiority over chemotherapy in overall survival due to the statistical significance threshold not met (prespecified error of 0.025), the median Progression-Free Survival (PFS) for pembrolizumab was 16 months (with a range of 5 to 38 months), compared to 8 months (with a range of 6 to 10 months) for chemotherapy. Pembrolizumab as a monotherapy for MSI-H is becoming the standard of care for first-line treatment of mCRC [31].

In the CheckMate142 trial, the combination of nivolumab and low-dose ipilimumab was evaluated for efficacy and safety as a first-line therapy for patients with MSI-H in mCRC [32]. After a median follow-up of 13.8 months, the Objective Response Rate (ORR) and Disease Control Rate (DCR) were 60% and 84%, respectively, with a Complete Response (CR) rate of 7%. The ORR increased to 69% and the CR rate to 13% at 29 months. The combination of ipilimumab and nivolumab showed superior efficacy and safety compared to pembrolizumab monotherapy.

Additionally, treatment-naïve mCRC patients with dMMR-MSI-H were included in the randomized Phase III COMMIT trial, where 347 patients were enrolled to receive mFOLFOX6/bevacizumab with or without atezolizumab. The primary endpoint of the trial was PFS, and secondary endpoints included OS, ORR, DCR, and frequency of adverse events [33].

Neoadjuvant and adjuvant therapy

Stage III CRC requires postoperative adjuvant therapy, and to explore the potential effectiveness of immunotherapy as adjuvant treatment, a phase III randomized controlled, ATOMIC study, enrolled 700 patients with stage III dMMR-MSI-H colon cancer [34,35]. The patients were divided into two groups, with one receiving 6 months of FOLFOX and the other receiving 6 months of FOLFOX plus atezolizumab, followed by 6 months of atezolizumab alone. The primary endpoint was Disease-Free Survival (DFS), while secondary endpoints included Overall Survival (OS) and the frequency of adverse events. Notably, neoadjuvant immunotherapy has shown promising results in early-stage CRC. An exploratory phase II trial called NICHE involved 40 patients with stage I and III colon cancer, out of which 21 had dMMR tumors and 20 had pMMR tumors [36,37]. The primary objectives were safety and survival, and patients with dMMR tumors who underwent successful surgery were treated with ipilimumab and nivolumab, resulting in a pathologic response in all 21 patients with dMMR tumors.

The NRG-GI002 randomized phase II trial assessed the efficacy of veliparib or pembrolizumab in combination with chemotherapy and radiation therapy in patients with Locally

Advanced Rectal Cancer (LARC) [38]. The primary endpoint is reduction in Neoadjuvant Rectal Cancer (NAR) score, and secondary endpoints include sphincter-sparing surgery, pathologic Complete Response (pCR), clinical Complete Response (cCR), Disease-Free Survival (DFS), toxicity, and Overall Survival (OS) [39]. In another trial, VOLTAGE, a phase Ib/II open-label, single-arm study, patients with locally advanced resectable rectal cancer underwent chemotherapy with capecitabine radiation therapy followed by sequential neoadjuvant immunotherapy [40]. The outcomes studied were pathologic complete response and major pathologic response, with 3 out of 5 patients with dMMR-MSI-H tumors achieving successful results [41]. These findings indicate that neoadjuvant immunotherapy may soon replace current treatment modalities for CRC with dMMR-MSI-H.

MSS/MSI-L CRC immunotherapy

As opposed to dMMR-MSI-H CRCs, which demonstrate a good response to Immune Checkpoint Inhibitors (ICI), pMMR-MSS/MSI-L tumors, accounting for approximately 95% of all metastatic Colorectal Cancers (mCRC), show poor efficacy with ICI treatment due to their low mutational load and limited recruitment of immune cells. To address the primary resistance to ICI, researchers are exploring new approaches and immunomodulatory techniques in pMMR-MSS/MSI-L CRCs, building on our increasing understanding of the tumor microenvironment in CRCs. Studies have shown that antiPD-1/PD-L1 and anti CTLA-4 antibodies have a synergistic effect [42]. In the CCTG CO.26 study, the efficacy and safety of combination ICI therapy were evaluated in patients with advanced refractory Colorectal Cancer (rCRC). This was a phase II trial that compared a combination of PD-L1 and CTLA-4 inhibitors, tremelimumab, and durvalumab, to Best Supportive Care (BSC) alone in pMMR-MSS/MSI-L CRCs [43]. At a median follow-up of 15 months, the experimental group had a median Overall Survival (OS) of 6 months, while the BSC group had a median OS of 4.1 months. This study was the first to indicate that the combination of anti CTLA-4 and anti PD-L1 could potentially improve OS in MSS mCRC. Preclinical models have suggested that reducing PGE2 production could enhance the anti-tumor effectiveness of ICIs [44]. In the NICHE phase Ib trial conducted in 2014, patients with pMMR tumors received preoperative treatment with ipilimumab and nivolumab, with or without celecoxib, and a pathologic response was observed in 4 out of 15 patients (27% response rate) [45]. In MSI-H tumors, CD8+PD-1+ T-cell infiltration was found to be predictive of response. For KRAS/NRAS/BRAF wild-type mCRC, panitumumab, an Epidermal Growth Factor Receptor (EGFR)-targeted monoclonal antibody, has been utilized. However, resistance to this treatment has been linked to increased expression of CTLA-4 and PD-L1 [46]. The LCCC1632 single-arm phase II clinical trial evaluated the safety and efficacy of combining nivolumab, ipilimumab, and panitumumab in patients with mCRC [47]. Among the 49 evaluable subjects, a median Progression-Free Survival (PFS) of 5.7 months and a 35% response rate at 12 weeks were observed. The trial continued to recruit participants after reaching the primary endpoint due to the favorable safety and efficacy outcomes, indicating that the combination of ICI and anti-EGFR therapy showed promise in treating MSS mCRC.

Combination of ICI and radiation therapy

Preclinical investigations have revealed that Radiation Therapy (RT) can trigger Immunogenic Cell Death (ICD) and release Damage-Associated Molecular Patterns (DAMPs). Additionally, it can augment the antigen presentation by Antigen-Presenting Cells (APCs), activate T lymphocytes, and enhance the anti-cancer effects through abscopal effects [48]. DAMPs, which are characteristic of ICD, encompass immunogenic cell surface markers, inflammatory cytokines, and cancer-related neoantigens that are upregulated on tumor cells. In a single-arm phase II study [49], the combination of pembrolizumab and external radiation showed a response in only one out of 22 patients with pMMR/MSI-L CRC. However, more promising outcomes were observed when CTLA-4 and PD-1 inhibition were combined with RT in a phase II clinical trial (NCT03104439). In this trial, the Disease Control Rate (DCR) was 29.2 percent (7/24), and the Objective Response Rate (ORR) was 12.5 percent (3/24) [50].

Initial findings from the phase I/II VOLTAGE-A trial indicate that a comprehensive approach involving radical surgery, nivolumab, and neoadjuvant Chemoradiotherapy (CRT) could be an effective treatment for MSS patients with Locally Advanced Rectal Cancer (LARC) [41]. Among the patients in the study, one patient (3%) achieved a clinical Complete Response (CR) but opted out of radical surgery, while 11 out of 37 patients (30%) achieved a pathological Complete Response (pCR). Notably, 38% (14/37) experienced a major pathologic response, illustrating the potential of combining Immune Checkpoint Inhibitors (ICI) and Radiation Therapy (RT) in the treatment of cancer.

ICI and MEK inhibitor combination

The inhibition of Mek pathway, which is a downstream component of the RAS-MAPK system, leads to increased expression of MHC-I and PD-L1 within tumors. This, enhances the clonal expansion of T lymphocytes surrounding the tumor and improves the effectiveness of Immune Checkpoint Inhibitors (ICI) [51,52]. In a phase Ib trial, researchers evaluated a combination approach using the MEK inhibitor cobimetinib along with the PD-L1 inhibitor atezolizumab [53,54]. Preliminary results from a 2016 trial indicated that out of 23 patients with CRC, 4 (17%) showed partial responses. Among them, 3 had pMMR-MSI-L, and 1 had an unknown status. In the 2018 follow-up data, it was observed that a total of 7 out of 84 mCRC patients, comprising 6 with MSS/MSI-L and 1 with MSI-H, experienced manageable side effects and partial responses [55]. Despite the potential for synergy that had been established, a subsequent phase III trial, IMblaze 370, which compared atezolizumab versus atezolizumab alone versus regorafenib in rCRC patients with pMMR-MSI-L, did not confirm the anticipated synergistic effects [56]. Nevertheless, several studies have investigated the combination of MEK inhibitors and ICIs [28,57,58].

ICI and anti-VEGF

According to preclinical findings, anti-angiogenic drugs have the potential to enhance the infiltration of $CD8^+$ T cells into tumors. They can also boost the anti-tumor activity of $CD8^+$ T cells through various mechanisms, including the upregulation of PD-L1 expression, reduction of immunosuppressive cells like TAM and Treg, and improved interaction between Antigen-Presenting Cells (APCs and dendritic cells [59-61].

In a phase Ib trial that supported this concept; 9 patients with metastatic Colorectal Cancer (mCRC and pMMR/MSI-L showed Stable Disease (SD. Additionally, one patient who received Immune Checkpoint Inhibitor (ICI therapy combined with an anti-angiogenic agent (atezolizumab plus bevacizumab had an Objective Response (OR [62,63]. Recent studies have also demonstrated remarkable anti-tumor effectiveness with the combination of regorafenib and nivolumab [64]. To investigate the safety and efficacy of the combination of nivolumab and regorafenib, 25 metastatic colorectal cancer patients (24 pMMR-MSS and 1 dMMR-MSI-H were enrolled in the REGONIVO phase Ib/II trial. The results were intriguing, with an Objective Response Rate (ORR of 36% and median Progression-Free Survival (PFS of 7.9 months. The 1-year PFS and Overall Survival (OS rates in colorectal cancer were 41.8% and 68%, respectively. Given these favorable outcomes, larger cohort studies are warranted [64]. In another study, the combination of pembrolizumab and lenvatinib was assessed in patients with treatment-naive advanced non-MSI-H/pMMR colorectal cancer in the LEAP-005 trial, which was an open-label, randomized, phase II trial [65]. At a median follow-up of 10.6 months, the Objective Response Rate (ORR and Disease Control Rate (DCR for 32 patients were 22% and 47%, respectively. The median Progression-Free Survival (PFS and Overall Survival (OS were 2 and 3 months, respectively. The Duration of Response (DOR was still ongoing. Due to the excellent anti-tumor efficacy and manageable safety profile, the enrollment in the study was increased to 100 patients [65].

Drivers of immunotherapy

For improve the effectiveness of immunotherapy, it is crucial to investigate biomarkers that contribute to treatment response. Four main categories of biomarker development for CRC immunotherapy include PD-L1 expression, pre-existing immune responses, tumor mutations, and the microbiome. Tumor Mutation Burden (TMB quantifies the total number of somatic mutations per coding region of the tumor genome, encompassing all non-synonymous coding mutations in the tumor exome. Various malignancies, including CRC, have shown that TMB serves as an independent predictor of success in ICI treatments. Immunotherapy is likely to be more effective in tumors with high TMB due to the correlation between strong immunogenicity and elevated TMB. Notably, both MSI-H and MSS tumors can have increased TMB levels. Preliminary confirmation of immunotherapy efficacy was observed in patients with elevated TMB levels in MSS CRC. In the REGONIVO trial, an exploratory analysis of 23 patients with CRC evaluated TMB. The group with high TMB had a median Progression-Free Survival (PFS of 12.5 months, while the low TMB group had a median PFS of 7.9 months, with Objective Response Rates (ORRs of 50% and 35.3%, respectively. Additionally, the CCTG CO 26 trial utilized ctDNA analysis of blood samples to assess plasma TMB. In MSS CRC patients treated with PD-L1 and CTLA-4 inhibitors, improved Overall Survival (OS was associated with higher plasma TMB, with a threshold of 28 mutations per megabase. A plasma TMB of 28 was suggested as a potential biomarker to identify patients who could benefit from receiving durvalumab in combination with tremelimumab.

Role of POLE/POLD1

POLE/POLD1 plays a crucial role in DNA replication. In the context of CRC, the development of a hypermutation phenotype in DNA is linked to somatic or germline mutations in POLE and POLD1. These mutations are present in about 74% of tumors classified as MSS or MSI-L, affecting nearly 7.4% of all CRC cases. Notably, pMMR POLE mutant CRCs exhibit distinct characteristics compared to POLE wild-type CRCs. They are more likely to express effector cytokines, show infiltration of CD8+ lymphocytes, express cytotoxic T-cell markers, and have increased levels of PD-L1, PD-1, and CTLA-4. POLE has been found to be more immunogenic compared to other approved biomarkers like MMR and MSI, and it is expected to potentially join them as an important biomarker. The presence of Tumor-Infiltrating Lymphocytes (TILs), especially cytotoxic $CD8^+$ T cells, has been associated with improved survival in retrospective studies of CRC. The density and location of T-cells within the tumor may have greater predictive value for CRC patients compared to conventional TNM staging approaches. The Immunoscore, a scoring method that assesses the number of $CD3^+$ T cells and $CD8^+$ T cells at the tumor center and infiltrative margins using standardized parameters, is used to evaluate this aspect. Presently, a phase II multicenter trial is underway to evaluate the efficacy of Immune Checkpoint Inhibitors (ICI in combination with chemotherapy and angiogenesis inhibitors as primary therapy for pMMR-MSI-L mCRC with high Immunoscore. Based on the Immunoscore concept, tumors are classified as hot, transformed, or cool, depending on their immune response. Tumors with T-cell infiltration are referred to as hot tumors, while those with inflammation but lacking invasiveness are called transformed tumors, and noninvasive tumors are termed cool tumors. This classification considers not only the Immunoscore but also the immune signature and microenvironment of the tumor. Patients with hot tumors tend to respond better to ICI, suggesting they might benefit more from immunotherapy.

PD-L1 levels

The most extensively studied biomarker assessed through immunohistochemistry is the co-inhibitory receptor ligand PD-L1. However, it has not been definitively established that the levels of PD-L1 expression are linked to the effectiveness of Immune Checkpoint Inhibitors (ICI in Colorectal Cancer (CRC. In the KEYNOTE016 phase II trial, which evaluated pembrolizumab in patients with refractory mCRC, Progression-Free Survival (PFS or Overall Survival (OS outcomes were

observed irrespective of the PD-L1 expression level. Similarly, in the Checkmate142 phase II trial comparing the efficacy of nivolumab monotherapy versus nivolumab in combination with ipilimumab, there was no significant correlation found between PD-L1 expression and Objective Response Rate (ORR).

Role of the microbiota

The gut microbiota plays a significant role in influencing the effectiveness of immunotherapy across various types of cancer. It is believed that the composition of the gut microbiota might serve as a predictor for the efficacy of Immune Checkpoint Inhibitors (ICI). Certain beneficial bacteria, such as *Lactobacillus johnsonii,* and Muciniphila, have been identified in this context. Moreover, Inosin-A2AR signaling was found to enhance the anti-tumor effects of ICI therapy when influenced by *Bifidobacterium pseudolongum* and *A. mucinifera.* A specific mechanism through which the gut microbiota positively interacts with immunotherapy involves T cell-specific A2AR signaling. However, further research is needed to fully comprehend the ways in which the gut microbiota regulates the host's anti-tumor immune response in the context of immunotherapy.

CONCLUSION

In recent years, immunotherapy has demonstrated significant improvements in the survival of a small subset of Colorectal Cancer (CRC) patients with the MSI-H phenotype. The FDA has approved pembrolizumab and nivolumab (with or without ipilimumab) as second line therapy for mCRC patients with dMMR-MSI-H based on strong evidence from two phase II. Furthermore, Pembrolizumab was approved as a first-line therapy for mCRC MSI-H in 2020, following the positive results from the KEYNOTE177 trial. Ongoing and upcoming clinical trials suggest that Immune Checkpoint Inhibitors (ICI) may also be beneficial as neoadjuvant therapy and for early dMMR-MSI-H CRC. However, the majority of mCRC patients with pMMR-MSI-L face challenges in overcoming primary immunotherapy resistance. To address this subgroup, various ICI-based strategies have been explored to modulate immune cells and enhance therapeutic efficacy. These include radiation therapy, combination therapy with antibodies that inhibit PD-1 or CTLA-4, combination therapy with small molecule TKIs like MEK inhibitors and ICIs, and the use of anti-angiogenic agents. Early phase clinical trials have shown promising results, but further research is necessary to establish the safety and efficacy of these approaches. As immunotherapy progresses, it is expected to transition towards biomarker-based therapies. Selection criteria will be crucial in identifying patients who will benefit the most from these therapies. While some biomarkers have already been identified, ongoing research aims to discover and validate highly sensitive and specific biomarkers.

With the expanding knowledge in this field, new combinations of therapies and biomarkers will guide clinicians towards more personalized and targeted treatment strategies for patients with CRC. This personalized approach holds promise for improving outcomes and enhancing the overall effectiveness of immunotherapy in CRC management.

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