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Checkpoint Inhibitors and Combination Strategies in Colorectal Cancer: an Overview

Anastasia V. Poznyak [1]*, Nikolay Konstantinovich Shakhpazyan [2], Aleksandra Sergeevna Utkina [3], Elizaveta Romanovna Korchagina [4], Olga Nikolaevna Maltseva[5], Alexander N. Orekhov [6]

[1]Institute for Atherosclerosis Research, Osennyaya 4-1-207, 121609 Moscow, Russia

[2]Institute of Human Morphology, Petrovsky Russian National Center of Surgery, 2 Abrikosovsky Lane, 119991 Moscow, Russia;

Email ID : nshakhpazyan@gmail.com

[3]Department of Commodity Expertise and Customs Business, Plekhanov Russian University of Economics, 36, Stremyanny Lane, 115054 Moscow, Russia

[4]Institute of Experimental Medicine, 12, Academician Pavlov Street Street, 197022, Saint Petersburg, Russia

[5]Institute of General Pathology and Pathophysiology, 8 Baltiyskaya Street, 125315 Moscow, Russia

[6]Faculty of Biology and Biotechnology, National Research University Higher School of Economics

***Corresponding Author:**

Email ID : tehhy_85@mail.ru

ABSTRACT

Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality worldwide, intensifying the urgency for innovative therapeutic strategies. Current treatment paradigms primarily rely on cytotoxic chemotherapy; however, their efficacy is often compromised by systemic toxicity, resistance, and limited response rates—particularly in metastatic settings. Recent advancements in targeted therapies and immunotherapy, particularly immune checkpoint inhibitors (ICIs), offer promising alternative approaches, specifically for microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) CRC. ICIs, such as anti-PD-1 and anti-CTLA-4 monoclonal antibodies, have demonstrated significant clinical benefits, particularly in the context of MSI-H/dMMR CRC, achieving improved ORRs and progression-free survival compared to traditional therapies.

Despite these advancements, approximately 95% of CRC cases remain microsatellite stable (MSS) or low MSI (MSI-L), where ICIs have shown limited effectiveness. The complex tumor microenvironment and mechanisms driving immune evasion in MSS CRC underscore the need for further research into combination therapies targeting multiple immune checkpoints, such as LAG-3 and TIM-3, that may enhance antitumor responses in these patients. Moreover, ongoing clinical trials are exploring the role of novel immunotherapeutic strategies to broaden the applicability of ICIs in CRC treatment, aiming to identify biomarkers predictive of response and resistance.

This review presents a comprehensive overview of the current landscape of CRC immunotherapy, emphasizing the critical distinction between MSI-H and MSS tumors and the robust potential of innovative combination therapies to improve outcomes for a broader spectrum of CRC patients, while addressing the persistent challenges in managing this heterogeneous disease

Keywords: *Tumor, cytostatics, immune checkpoint inhibitors, colorectal cancer, microsatellite instability, dMMR, combination therapies*

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1. INTRODUCTION

CRC is the fourth leading cause of cancer-related mortality globally, accounting for 10% of all cancer diagnoses each year. It ranks as the second most common cancer among women and the third among men, with projections indicating that the number of cases will surpass 2.5 million annually by 2035. Alarmingly, monitoring efforts fail to identify 25% of new

CRC cases at early stages of the disease [1-4]. Chemotherapy has long been the cornerstone of treatment for metastatic CRC, utilizing agents such as fluorouracil (5-FU/F), oxaliplatin, irinotecan, and capecitabine, whether used alone or in combination. Nevertheless, the high risk of systemic toxicity, suboptimal response rates, and limited efficacy have driven the quest for more effective treatment options that offer greater tumor specificity, particularly for patients with advanced-stage disease [5,6].

Targeted therapies present an alternative strategy for managing metastatic CRC. These treatments inhibit specific molecules involved in cancer progression and metastasis. In the past two decades, various targets have been investigated in CRC [7-9]. Key among these are the inhibition of the epidermal growth factor receptor (EGFR) and the disruption of the Ras–Raf–MEK–ERK signaling pathway, which is critical for cellular growth, proliferation, and survival. Another important target is vascular endothelial growth factor-A (VEGF-A), a major driver of tumor angiogenesis [10-12]. The first monoclonal antibody against VEGF-A, bevacizumab, was approved in 2004. Since then, the Food and Drug Administration (FDA) has also authorized aflibercept (a VEGF-A inhibitor), ramucirumab (a fully humanized monoclonal antibody targeting VEGFR-2), and regorafenib (a VEGFR-2 inhibitor) for the treatment of CRC. Despite these advancements, the challenge of treatment resistance persists, highlighting the need for novel therapeutic strategies [13-17].

Recently, ICIs have been incorporated into the treatment landscape for CRC. These immunotherapeutic agents are monoclonal antibodies (mAbs) that target regulators of T cell receptor (TCR) activation, specifically programmed cell death-1 (PD-1), programmed cell death ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) on T cells and antigen-presenting cells [18-21]. ICIs have gained traction in treating various cancers, including melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), and hepatocellular carcinoma. In metastatic CRC (mCRC), ICIs have demonstrated promising clinical outcomes, particularly in patients with MSI-H resulting from defects in mismatch repair (MMR) genes such as MutL homolog 1 (MLH1), MutS homologs 2 and 6 (MSH2, MSH6), PMS2, and tumor-associated calcium signal transducer 1/epithelial cell adhesion molecule (TACSTD1/EPCAM) [22-25]. However, MSI-H CRC constitutes only 5% of all CRC cases, with the remaining 95% classified as MSS or low MSI-L, a group where the effectiveness of ICIs remains to be fully determined [26,27].

Background of Immunotherapy in CRC

Currently, the standard treatment for non-metastatic CRC typically involves surgical intervention, which may be followed by adjuvant chemotherapy. However, these strategies often yield suboptimal results, especially in advanced cases of CRC. Despite substantial progress in CRC research and the development of new therapies, two major obstacles—drug resistance and relapse—persistently impede effective treatment. Reports indicate that the objective response rate (ORR) to 5-FU therapy in CRC patients is approximately 40-50%. This underscores an urgent need for more potent treatment strategies, particularly for mCRC [28-31].

In recent years, advancements in gene discovery and immunotherapy have opened new avenues for CRC treatment. Unlike conventional chemotherapy and targeted therapies, immunotherapy does not directly attack tumor cells. Instead, it enhances the immune system's ability to recognize and eliminate cancer cells by identifying them as "foreign." The immune response is primarily mediated by TCRs on T cells, which engage with peptide-major histocompatibility complex (MHC) class I molecules displayed on various cells, including tumor cells [32-34]. This interaction helps the immune system differentiate between foreign agents and the body’s own cells. However, mere binding of TCRs to peptide-MHC class I complexes is insufficient for full T cell activation; this process is tightly regulated by co-stimulatory and co-inhibitory signals. ICIs have emerged as critical components in the field of tumor immunotherapy [35-38]. ICIs regulate the immune system by interacting with ligands and receptors to prevent it from mistakenly attacking healthy cells. Unfortunately, cancer cells can exploit this mechanism to evade immune detection, facilitating unregulated growth. Currently, inhibitors targeting cytotoxic CTLA-4 and PD-1 or its ligand PD-L1 have demonstrated promising antitumor effects across various studies, making them a focal point for ongoing research [39-42]. PD-1 is a significant immunosuppressive transmembrane protein located on T cells, primarily binding to two ligands: PD-L1 (B7-H1) and PD-L2 (B7-DC), with PD-L1 being more commonly expressed in the tumor microenvironment. Tumor cells can produce either PD-L1 or PD-L2, but PD-L1 is predominant. When PD-1 on T cells binds to PD-L1 on cancer cells, T cells are rendered ineffective in destroying the tumor [43-46]. However, blocking the interaction between PD-1 and PD-L1 with anti-PD-1 or anti-PD-L1 antibodies can restore T cell activity against tumor cells, resulting in significant antitumor effects [47-49].

Recent advancements in immunotherapy have led to the development of innovative approaches. In 2019, Yost et al. conducted a study to explore whether PD-1 inhibitors exert their antitumor effects by "reactivating" tumor-infiltrating T cells or by recruiting new T cells to the tumor site [50]. The researchers analyzed T cells from tissue samples collected from 11 patients with basal cell carcinoma, both before and after anti-PD-1 therapy, employing RNA single-cell sequencing and TCR sequencing. Their findings indicated that the T cells responsible for the therapeutic effects of anti-PD-1 may not primarily be "resident T cells" or "memory T cells," but rather newly activated T cells. Another study revealed that effector-like T cells not only proliferated within tumors but also in adjacent healthy tissues and peripheral blood, with patients showing this clonal expansion responding best to PD-L1 inhibitor therapy. This suggests that identifying expanded T cell clones in peripheral blood could potentially serve as a predictor of clinical benefits from immunotherapy [51-54].

Furthermore, PD-1 is expressed not only on T cells but also on B cells, regulatory T cells, innate killer cells, and myeloid cells. Traditional understanding has primarily attributed the effects of PD-1-targeted immunotherapy to T cells, largely overlooking the significant roles played by other immune cells [55-57]. In January 2020, Strauss et al. found that PD-1 on myeloid cells might exert a more potent immunosuppressive effect than PD-1 on T cells, indicating that myeloid cells could significantly contribute to tumor immune evasion [58]. Additionally, Mayoux et al. highlighted dendritic cells as critical targets for PD-L1 inhibitors in generating an effective antitumor immune response [59]. Along with PD-1, PD-L1 can also bind to B7.1, a crucial co-stimulatory molecule presents on antigen-presenting cells like dendritic cells. When PD-L1 binds to B7.1 on dendritic cells, it inhibits B7.1's interaction with CD28 on T cells, thereby impairing T cell activation. However, when a PD-L1 antibody binds to PD-L1 on dendritic cells, it disrupts the PD-L1 and B7.1 interaction, allowing B7.1 to engage with CD28 on T cells, thereby enhancing T cell antitumor responses. In summary, while ICIs are recognized for inducing antitumor responses, the specific mechanisms involved require more in-depth investigation [60,61].

Presently, biomarkers such as microsatellite instability (MSI) or MMR status, tumor mutational burden (TMB), and the presence of tumor-infiltrating T cells are acknowledged as indicators of response to ICI therapy, regardless of the primary tumor site. CRC is classified into two categories based on its MSI or MMR status: MSI-H/dMMR, which accounts for about 15% of all CRC cases, and MSS/pMMR, which comprises approximately 85%. MSI-H/dMMR CRC typically displays higher TMB and increased T cell infiltration in the tumor microenvironment, leading to a more substantial antitumor immune response [62-65]. Initial studies have demonstrated significant therapeutic potential for ICIs in patients with advanced, treatment-resistant CRC, corroborated by findings from the KEYNOTE-016 trial. In 2015, this trial showed that among dMMR CRC patients treated with pembrolizumab (an anti-PD-1 inhibitor), the immune-related ORR was 40% (4 out of 10 patients), with a 20-week progression-free survival (PFS) rate of 78% (7 out of 9 patients) [66,67]. In 2017, pembrolizumab and nivolumab received FDA approval for treating patients with MSI-H/dMMR CRC.

The KEYNOTE-177 study further confirmed pembrolizumab's efficacy as a first-line treatment for MSI-H/dMMR patients, showing a considerable improvement in median PFS (16.5 vs. 8.2 months, HR 0.59, 95% CI 0.45–0.79, $p = 0.0002$) and fewer treatment-related adverse events compared to chemotherapy, either alone or in combination with a targeted agent [68]. Consequently, the National Comprehensive Cancer Network (NCCN) guidelines now recommend pembrolizumab as the standard primary treatment for advanced MSI-H/dMMR CRC, and MSI or MMR testing is now recommended for all patients with a history of CRC.

In contrast, MSS/pMMR CRC is characterized by low TMB and diminished immune cell infiltration within the tumor microenvironment compared to MSI-H/dMMR CRC. Unlike the encouraging responses seen with ICIs in MSI-H/dMMR advanced CRC, most MSS/pMMR CRC patients frequently exhibit resistance to immunotherapy, leading to their classification as "cold tumors" [69-72]. This resistance to immunotherapy presents a significant barrier to the broader implementation of ICIs in CRC treatment. To address these challenges and expand the reach of immunotherapy to a larger segment of CRC patients, researchers have initiated multiple clinical trials focusing on MSS/pMMR CRC. Encouragingly, some of these studies have identified a subset of MSS/pMMR CRC patients who show greater responsiveness to immunotherapy [73-74]. For instance, the NICHE study conducted in the Netherlands found that 4 out of 15 pMMR patients (27%) exhibited pathological responses, including 3 complete responses and 1 partial response [75]. Further analysis suggested that increased infiltration of CD8+ and PD-1-positive T cells may serve as predictors of immunotherapy success in pMMR patients. Given these findings, this review aims to provide an overview of the current state of CRC immunotherapy based on the MSI/MMR status of tumors, while also addressing the challenges and potential solutions in CRC treatment.

Table 1. **Mechanisms and Strategies of Immune Checkpoint Inhibitors in CRC Treatment**

Checkpoint Target	Mechanism of Action	Current Effects in CRC	Clinical Studies/Insights
PD-1	Inhibits T-cell activation by binding to PD-L1 on tumor cells	Promotes T-cell exhaustion; blockade revives T-cell function	Nivolumab and Pembrolizumab show high efficacy in MSI-H patients
PD-L1	Prevents T-cell activity by binding to PD-1; blocking restores immunity	Essential in mediating immune evasion	Atezolizumab demonstrates response rates in MSI mCRC patients

Checkpoint Target	Mechanism of Action	Current Effects in CRC	Clinical Studies/Insights
CTLA-4	Inhibitory receptor that dampens co-stimulatory signals to T cells	Enhances anti-tumor responses when blocked	Ipilimumab combined with Nivolumab yields robust effects in dMMR CRC
LAG-3	Suppresses T-cell proliferation; co-expressed with PD-1	Potential to overcome T-cell exhaustion	Trials ongoing with Relatlimab in combination with PD-1 inhibitors
TIM-3	Inhibitory receptor on T cells affecting immune tolerance	Associated with limiting T-cell response	Investigated in ongoing studies with MGB453 and Spartalizumab

Immunotherapy with Immune Checkpoint Inhibitors

Traditional cancer treatments, including surgery, radiation, and chemotherapy, are commonly employed across various cancer types. While these therapies can be quite effective in the early stages of cancer, they often lead to resistance and can produce significant side effects. Moreover, the ability of tumor cells to evade immune surveillance is a critical factor in the progression and metastasis of tumors in many patients [76-78]. Consequently, there is an urgent need for innovative strategies to overcome these challenges and enhance cancer treatment. One promising approach is immunotherapy using ICIs, which have demonstrated efficacy in the treatment of malignancies such as melanoma, NSCLC, and CRC. Given the essential role of T cells in targeting cancer cells, ICIs can be valuable by boosting T cell responses during the immune system's attack on tumors

Numerous studies investigating ICIs in colorectal cancer, whether as monotherapies or in combination with other treatments, have revealed encouraging results for CRC patients [79-82]. However, more research is necessary to fully evaluate their effectiveness. Over the past few decades, cancer immunotherapy utilizing mAbs that target immune checkpoints has emerged as a prominent strategy. By inhibiting immune checkpoints, T cells—especially cytotoxic T lymphocytes (CTLs)—are reactivated, enhancing their antitumor activity and bolstering the body's immune response against cancer. Targeting immune checkpoints has shown promise in improving outcomes across various cancers, including lung, liver, melanoma, ovarian, and prostate cancers [83-85].

The most recognized mAbs that target inhibitory checkpoints include Ipilimumab and Tremelimumab, which inhibit CTLA-4, as well as Nivolumab and Pembrolizumab, which target PD-1. Additionally, Atezolizumab and Durvalumab are anti-PD-L1 mAbs. This section examines the roles of anti-CTLA-4 and anti-PD-1/PD-L1 mAbs, as well as emerging immune checkpoints such as anti-LAG-3 and anti-TIM-3, in the treatment of CRC [86,87].

Differential Responses to Immune Checkpoint Inhibitors in MSS and MSI-H Tumors

The clinical efficacy of ICIs is significantly influenced by the microsatellite stability status of CRC tumors, fundamentally distinguishing the treatment outcomes for MSS and MSI-H tumors. Tumors exhibiting MSI-H typically harbor a mismatch repair deficiency, leading to the accumulation of numerous neoantigens [213]. This unique characteristic of MSI-H tumors enhances the immunogenicity of the cancer cells, facilitating a robust immune response when treated with ICIs, particularly those targeting the PD-1/PD-L1 and CTLA-4 pathways. In contrast, MSS tumors, which comprise the majority of CRC cases (approximately 95%), generally lack this immunogenic profile and often present a more immunosuppressive tumor microenvironment.

MSS tumors are often associated with an immunologically "cold" tumor microenvironment, featuring low levels of tumor-infiltrating lymphocytes (TILs) and reduced expression of MHC molecules [214,215]. This environment not only limits the activation of T cells but also may enable cancer cells to evade immune surveillance effectively. In contrast, MSI-H tumors frequently exhibit higher levels of immune cell infiltration, particularly activated cytotoxic T cells, which are precursors for effective anti-tumor immunity. The presence of these activated T cells, along with the upregulation of immune-related markers, renders MSI-H tumors more susceptible to the therapeutic effects of ICIs [216].

Clinical studies have consistently demonstrated that patients with MSI-H CRC experience significantly improved treatment outcomes with ICIs. For instance, trials involving anti-PD-1 agents such as pembrolizumab and nivolumab have shown objective response rates in MSI-H cohorts exceeding 40%, with substantial progression-free survival (PFS) benefits [217].

In contrast, MSS patients demonstrate minimal to no responses to ICIs, with response rates often reported as around 0% to 15%. This stark disparity in clinical outcomes underscores the necessity for patient stratification based on molecular characteristics prior to initiating ICI therapy [217].

Despite the lack of efficacy observed with ICIs in MSS tumors, emerging strategies are being explored to enhance their responsiveness. Combination therapies, integrating ICIs with conventional treatments, such as chemotherapy or targeted therapies, are under investigation. Preclinical models suggest that chemotherapy may enhance antigen presentation and improve the immunogenicity of MSS tumors, potentially converting them into a more responsive state [218]. Agents that stimulate immune responses, along with ICIs, might also be effective in overcoming resistance by modulating the tumor microenvironment. In Figure 4, we summarized the main strategies of overcoming the tumor resistance by modulating tumor microenvironment [219].

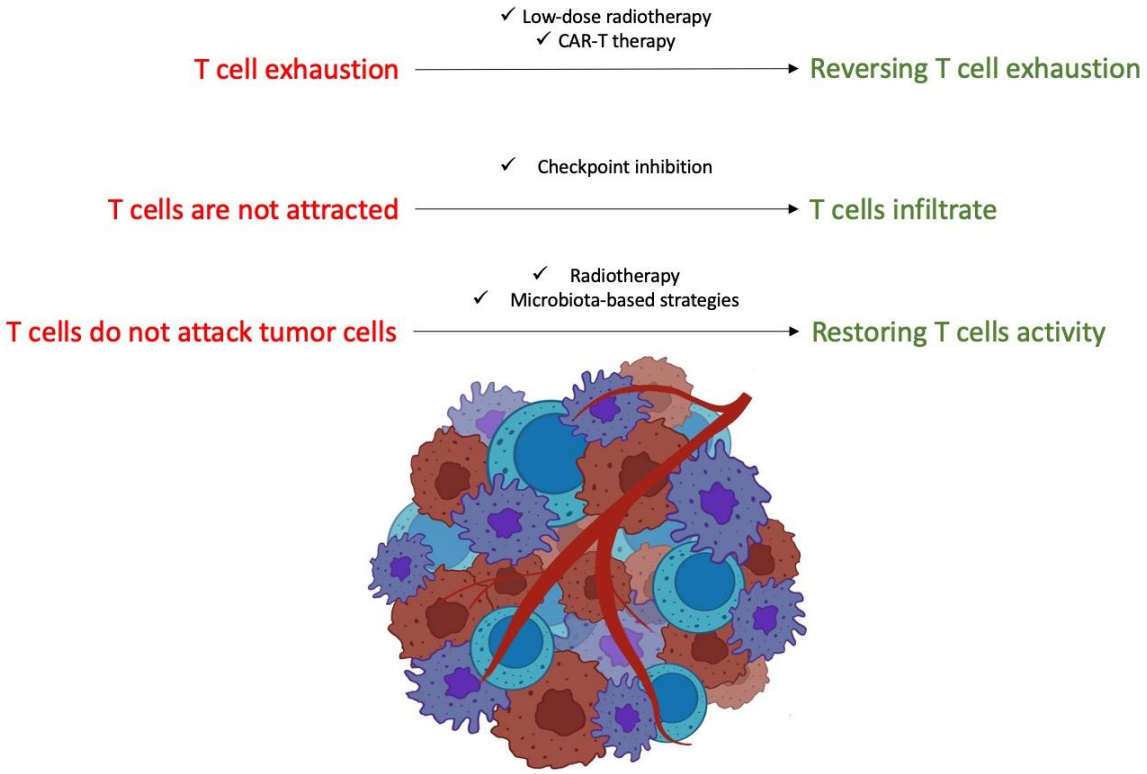

Figure 4. Strategies of modulating tumor microenvironment to overcome the tumor resistance.

Moreover, novel combinations targeting other immune checkpoints, such as LAG-3 and TIM-3, alongside traditional anti-PD-1 and anti-CTLA-4 therapies, hold promise for enhancing outcomes in MSS tumors. As research continues to unfold, identifying predictive biomarkers and developing effective combinatorial strategies will be vital for addressing the inherent resistance characteristics of MSS CRC [220].

In summary, the distinction between MSS and MSI-H tumor types is pivotal in understanding the differential responses to ICIs in CRC. While MSI-H tumors exhibit robust immunogenicity and favorable outcomes with ICIs, MSS tumors face significant challenges that require innovative therapeutic approaches. Ongoing research efforts aimed at enhancing immune responses in MSS CRC are critical to expanding the benefits of immunotherapy to a broader patient population [221].

Transforming the MSS Tumor Microenvironment: The Role of Combination Strategies

MSS tumors have historically presented significant challenges in the context of immunotherapy, particularly with ICIs, due to their immunologically "cold" tumor microenvironment. However, recent insights suggest that combinational strategies may effectively reshape this microenvironment, fostering greater responsiveness to ICIs [222].

Enhancing Antigen Presentation through Chemotherapy

One of the most promising avenues for augmenting the immunogenicity of MSS tumors is through the incorporation of chemotherapy. Standard chemotherapeutic agents, particularly those belonging to the classes of antimetabolites (e.g.,

fluorouracil, capecitabine) and alkylating agents (e.g., oxaliplatin), have been shown to directly enhance tumor antigen presentation by inducing immunogenic cell death (ICD). This process releases tumor antigens along with danger-associated molecular patterns (DAMPs), stimulating dendritic cell activation and promoting T cell priming in local lymph nodes [223].

By improving the cross-presentation of tumor-associated antigens and upregulating MHC molecules, chemotherapy can effectively convert a relatively "silent" tumor microenvironment into one that is rich in T cell activity. This transformation is crucial for overcoming the immune blockade typically observed in MSS tumors and bolstering patient responses to subsequent ICI therapy [224].

Synergistic Mechanisms with Targeted Therapies

In addition to chemotherapy, there is growing interest in combining ICIs with targeted therapies that can modulate the tumor microenvironment. For instance, agents that target the vascular endothelial growth factor (VEGF) pathway have demonstrated the ability to normalize aberrant tumor vasculature, leading to enhanced T cell infiltration and reduced immunosuppression within the tumor microenvironment. Combinations of anti-VEGF therapies with ICIs have shown promising preclinical results and are currently under investigation in clinical trials [225].

Furthermore, targeted therapies that inhibit signaling pathways associated with tumor growth and survival, such as the PI3K/Akt/mTOR pathway, may also contribute to altering the tumor phenotype, enhancing the sensitivity of MSS tumors to immunotherapy. Preclinical studies suggest that inhibiting these pathways can lead to increased tumor cell susceptibility to immune-mediated attack while potentially restoring anti-tumor immunity [226].

Immune Modulators as Adjuncts to Therapy

Utilizing immune modulators, such as TLR agonists or immune-stimulating cytokines (e.g., IL-2, IL-15), represents another means to reprogram the MSS tumor microenvironment. These agents can enhance T cell activation and proliferation, while also promoting the infiltration of activated immune cells into the tumor bed. Such combinational approaches, particularly integrating these agents alongside ICIs, could feasibly boost the overall immune response and facilitate a more favorable tumor microenvironment for MSS tumors [215].

While significant strides have been made in understanding the potential of combinational strategies, ongoing clinical trials will be paramount in determining the most effective regimens for MSS tumors. Optimizing dosing schedules, timing of combination therapy, and patient selection based on molecular profiling will be essential for maximizing efficacy and minimizing toxicity [227].

Anti-CTLA-4

Blocking CTLA-4 with mAbs represents a promising anticancer approach as it enhances antitumor responses by promoting T cell activation. Anti-CTLA-4 antibodies bind to the CTLA-4/B7 receptors on T cells, amplifying their antitumor function by prolonging their activity [88-90]. Since regulatory T cells, known for their suppressive role within the immune system, also express CTLA-4, anti-CTLA-4 mAbs can further enhance antitumor responses by inhibiting the function of regulatory T cells. Immune checkpoint blockade is particularly promising as a therapeutic strategy for patients with dMMRor MSI-H mCRC.

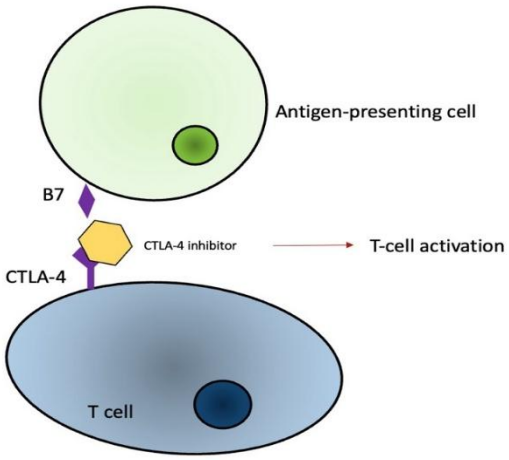

Figure 1. Schematic summary of CTLA-4 checkpoint inhibitor mechanism.

Ipilimumab, a fully human IgG1 anti-CTLA-4 mAb, was approved by the FDA in 2011 for the treatment of melanoma. As a specific CTLA-4 blocker, Ipilimumab boosts T cell antitumor activity by preventing CTLA-4 from binding to B7, thereby allowing CD28 to interact with B7 and resulting in sustained T cell activation [91-94]. When combined with Nivolumab, an anti-PD-1 mAb, this immune checkpoint blockade has exhibited a robust antitumor response in patients with dMMR/MSI-H mCRC.

Tremelimumab, a fully human IgG2 anti-CTLA-4 mAb, is currently under investigation for the treatment of solid tumors. In a phase II clinical trial, Tremelimumab alone did not demonstrate efficacy in patients with refractory mCRC. However, it has shown promising results in patients with advanced hepatocellular carcinoma [95-97]. Additionally, results from a phase II study indicated that combining Tremelimumab (anti-CTLA-4) with Durvalumab (anti-PD-L1) improved overall survival (OS) in patients with advanced refractory CRC. This suggests that combining anti-CTLA-4 with other ICIs such as anti-PD-L1 may be more effective than using anti-CTLA-4 as a standalone therapy in treating CRC [98-100].

Anti-PD-1

The PD-1/PD-L1 pathway serves as an inhibitory mechanism that plays a critical role in regulating T-cell activation and maintaining peripheral tolerance. By blocking this pathway with mAbs, it is possible to enhance the antitumor activity of T cells. Notably, PD-1 expression is heightened on the surface of CD8+ T cells within the tumor microenvironment of CRC. Consequently, PD-1 blockade emerges as a promising approach for CRC treatment [101,102].

The two FDA-approved anti-PD-1 mAbs are Nivolumab and Pembrolizumab. Nivolumab received its initial FDA approval in 2014 for treating advanced melanoma. It is a fully humanized immunoglobulin G4 (IgG4) anti-PD-1 antibody now authorized for multiple cancers, including melanoma, NSCLC, RCC, and Hodgkin's lymphoma [103,104]. A study assessing Nivolumab's efficacy in patients with dMMR/MSI-H mCRC revealed sustained responses in individuals previously treated for the disease. In this trial, patients received an intravenous dose of 3 mg/kg of Nivolumab once every two weeks, continuing treatment until disease progression, death, unacceptable toxicity, withdrawal of consent, or trial completion. Remarkably, 23 out of 74 patients (31%) achieved an objective response, while 51 patients (69%) demonstrated disease control for 12 months or longer, with a median follow-up of 12 months [105-107].

Additionally, phase I and II clinical trials have highlighted the positive impact of Nivolumab and other ICIs in the treatment of MSI-H mCRC. Pembrolizumab, another fully humanized IgG4 anti-PD-1 antibody approved by the FDA, has also been explored in combination with napabucasin in patients with MSI-H/MSS mCRC. Results from a phase I/II trial indicated the effectiveness of Pembrolizumab (200 mg every three weeks) alongside napabucasin (240–480 mg twice daily) for treating MSI-H/MSS mCRC [108-110]. Another study examined Pembrolizumab's effectiveness in CRC patients who expressed PD-L1, confirming its suitability for those with PD-L1-positive CRC. Furthermore, targeting PD-1 immune checkpoints in conjunction with anti-PD-1 (Nivolumab and low-dose Ipilimumab) presents a potentially promising treatment strategy for patients with previously treated MSI-H/dMMR mCRC [111,112].

Anti-PD-L1

PD-L1 is a crucial component of the PD-1/PD-L1 pathway, which inhibits T cell antitumor activity by binding to its receptor, PD-1. mAbs can target both PD-1 and PD-L1 to disrupt this inhibition of T cell signaling. Anti-PD-L1 mAbs, such as Atezolizumab, Durvalumab, and Avelumab, are frequently used in the treatment of melanoma, NSCLC, and RCC, respectively [113,114].

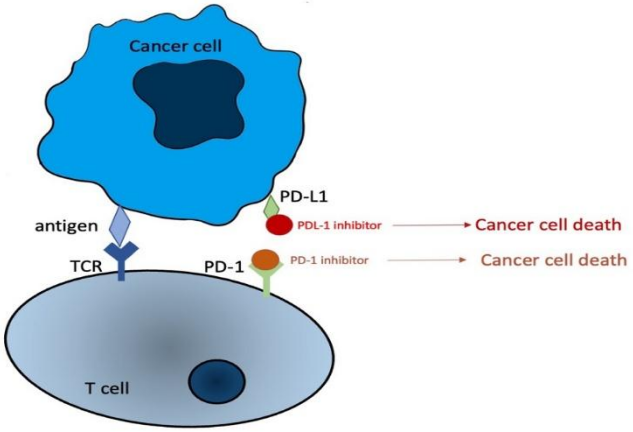

Figure 2. Schematic summary of PD-1 checkpoint inhibitor mechanism.

Atezolizumab, a humanized IgG1 mAb that targets PD-L1, has shown efficacy in various cancers, including metastatic urothelial cancer and lung cancer. In a phase Ib trial evaluating the combination of Atezolizumab and Bevacizumab (an anti-VEGF-A antibody) in 10 patients with MSI mCRC, the overall response rate (ORR) was reported at 30%, with a disease control rate of 90% and no unexpected adverse effects.

Durvalumab, another human IgG1 mAb targeting PD-L1, works by inhibiting the interaction between PD-1 and PD-L1 [115-118]. Its efficacy and safety as a monotherapy were assessed in patients with MSI-H tumors, with a dosing regimen of 10 mg/kg administered intravenously every two weeks for 12 months. The trial results indicated an ORR of 23% in patients with MSI-H tumors and 22% in those with CRC, positioning Durvalumab as a viable treatment option for MSI-H tumors.

Avelumab is a fully human IgG1 monoclonal antibody that also targets PD-L1, disrupting its interaction with receptors and restoring immune functionalities, including T cell-mediated antitumor responses [119-121]. Research evaluating the optimal dosing of Avelumab in 53 patients with metastatic or locally advanced previously treated solid tumors, including CRC, indicated that the drug could be given at a dose of 20 mg/kg every two weeks, although further studies are ongoing.

Additionally, PD-L2, a different ligand for PD-1, is expressed in nearly 40% of CRC patients. Its increased presence in CRC has been linked to levels of IFNγ and glycosylation processes. Moreover, PD-L2 has been implicated in enhancing tumor cell invasion, making it a potential target for novel CRC therapies [122-124].

Anti-LAG-3

LAG-3 is an inhibitory immune checkpoint that plays a vital role in maintaining immune homeostasis by suppressing T cell proliferation and hindering cytokine release. Additionally, the co-expression of LAG-3 and PD-1 serves as a marker for CD8+ T cell exhaustion. Therefore, targeting LAG-3 in conjunction with other inhibitory immune checkpoints offers a promising therapeutic strategy for enhancing antitumor immune responses.

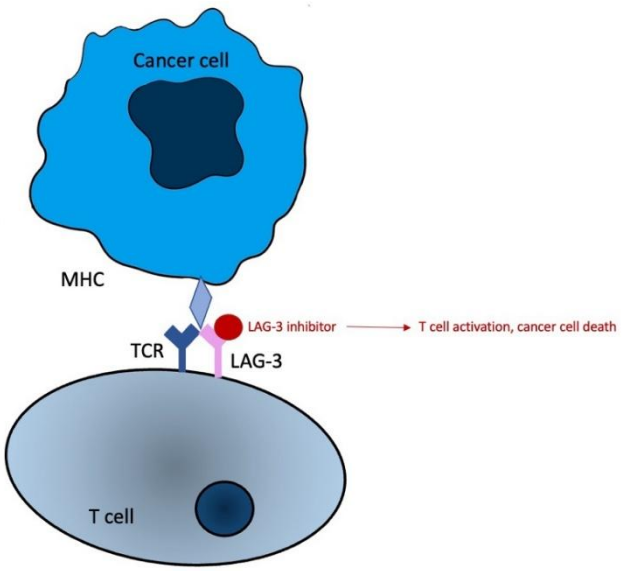

Figure 3. Schematic summary of LAG-3 checkpoint inhibitor mechanism.

Relatlimab is the first fully human IgG4 monoclonal antibody developed to target LAG-3 and is being investigated as a potential treatment for various solid tumors, including CRC [125-127]. A phase II study assessing the combination of Relatlimab with Nivolumab (an anti-PD-1 therapy) revealed antitumor activity in metastatic melanoma, with ongoing research to confirm these findings (NCT03743766). Other fully human IgG4 anti-LAG-3 monoclonal antibodies, such as LAG525 and MK-4280, are also undergoing clinical evaluation. LAG525, in combination with an anti-PD-1 agent, is currently being tested in a phase I/II study involving patients with a range of advanced solid tumors and hematologic malignancies [128-130]. Preliminary results from these trials indicate encouraging antitumor effects in neuroendocrine tumors, small-cell lung cancer, and diffuse large B-cell lymphoma.

MK-4280, an anti-LAG-3 monoclonal antibody, is being studied in a phase I/II trial (NCT03598608) in combination with Pembrolizumab for patients with hematologic malignancies, including classic Hodgkin's lymphoma and diffuse large B-cell lymphoma [131,132]. Additionally, a recent study investigated LBL-007, a novel anti-LAG-3 IgG4 antibody, in a

mouse model of CRC. Administered at a dose of 10 mg/kg twice weekly for three weeks, LBL-007 demonstrated a capability to inhibit tumor growth. The research also explored the combined effect of LBL-007 and anti-PD-1 antibodies, highlighting the potential for further investigation in solid tumors. However, given the limited clinical studies involving anti-LAG-3 therapies in CRC patients, further research is warranted in this area [133,134].

Anti-TIM-3

TIM-3 (T cell immunoglobulin and mucin domain-containing protein 3) is an inhibitory receptor present on T cells that plays an essential role in maintaining immune homeostasis by facilitating peripheral tolerance. By interacting with its ligand, galectin-9, this immune checkpoint induces apoptosis, thereby regulating T cell responses. Increased levels of galectin-9 have been detected in various solid tumors, including prostate cancer, cervical cancer, and melanoma [135-137].

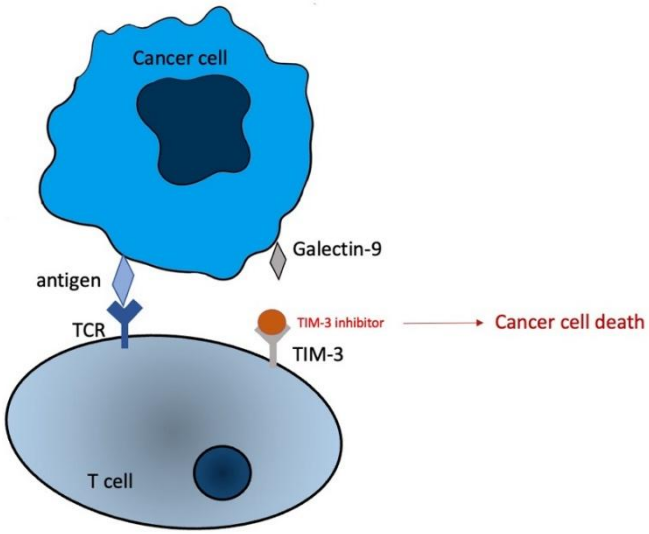

Figure 4. Schematic summary of TIM-3 checkpoint inhibitor mechanism.

Notably, existing anti-murine and anti-human TIM-3 antibodies, which have shown functional efficacy, act by disrupting TIM-3's interactions with phosphatidylserine and the adhesion molecule CEACAM1, while leaving its binding to galectin-9 unaffected. Two IgG4 human anti-TIM-3 mAbs, MGB453 and TSR-022, are currently being investigated as monotherapies and in combination with anti-PD-1 mAbs for the treatment of various advanced cancers [138,139].

For instance, a recent phase I/II trial evaluated the effectiveness of MBG453 both alone and in combination with Spartalizumab (an anti-PD-1 mAb) in patients with advanced conditions, including CRC. The results indicated that the combination of MBG453 and Spartalizumab was well-tolerated and demonstrated antitumor activity. Given that TIM-3 is overexpressed on CD8+ T cells in CRC patients, which may limit T cell activity, blocking TIM-3 in these individuals could enhance the anti-tumor immune response.

Overall, these findings provide compelling evidence that anti-TIM-3 mAbs are promising candidates for further investigation in CRC treatment, whether administered alone or in combination with other ICIs [140-142].

Double Blockade of Immune Checkpoints

Despite significant advancements in cancer treatment through immune checkpoint inhibitors, the efficacy of this approach has been less evident in certain cancers, such as CRC, compared to others. Research indicates that inhibiting a single immune checkpoint often results in the activation of alternative inhibitory checkpoints, which can diminish the therapeutic effectiveness of mAbs and lead to increased treatment resistance [143-146]. Consequently, combination therapies targeting multiple immune checkpoints have demonstrated greater potential in achieving more favorable therapeutic outcomes.

The combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapies has shown synergistic effects in patients with melanoma, RCC, and MSI-H mCRC. Specifically, concurrent blockade of CTLA-4 (via Ipilimumab) and PD-1 (via Nivolumab) has proven effective and is FDA-approved for patients with dMMR/MSI-H mCRC [147-149]. Preclinical studies using a murine colon cancer model (CT-26) demonstrated that dual inhibition of CTLA-4 and PD-L1 significantly enhanced tumor rejection and completely prevented liver metastasis. In contrast, blocking CTLA-4 or PD-L1 individually only reduced liver metastasis. Importantly, this research revealed that the combined blockade increased the presence of intratumoral CD8+ and CD4+ T cells while decreasing regulatory T (Treg) cell populations. Additionally, dual blockade resulted in

elevated expression of cytokines such as IFN-γ, IL-1α, IL-2, and IL-12 [150-152].

Further studies suggest that the MSI subset of CRC did not exhibit the expected response to PD-1 inhibition, indicating that combination immunotherapy targeting multiple checkpoints may offer a more effective treatment strategy for this CRC subset. Supporting this notion, the combination of Nivolumab and Ipilimumab showed a robust response in patients with MSI-H/dMMR mCRC, achieving a 55% ORR at 12 months and an OS rate of 85% in 119 patients treated with Nivolumab (3 mg/kg) in conjunction with Ipilimumab (1 mg/kg) every three weeks [153-156].

Similar findings were observed in a phase II trial that tested the combination of Durvalumab (an anti-PD-L1 antibody) and Tremelimumab (an anti-CTLA-4 antibody), which resulted in improved OS in patients with advanced refractory CRC. Investigations into combining various immune checkpoints have shown promising results; for instance, the combination of anti-LAG-3 and anti-PD-1 has exhibited potential in treating solid tumors. Another area of research involves MK-4280 (an anti-LAG-3 mAb) combined with Pembrolizumab (an anti-PD-1 mAb), which is currently being evaluated in a phase I/II trial for hematologic malignancies [157-159].

Furthermore, combining anti-TIM-3 with other ICIs, including anti-PD-1 mAbs, may yield favorable outcomes for patients. Therefore, the combination of anti-PD-1/PD-L1 and anti-TIM-3 is considered a viable treatment strategy for further exploration. Several ongoing studies are investigating the clinical efficacy of agents that block TIM-3 and PD-L1 either individually or in combination, including the use of LY3321367 (an anti-TIM-3 mAb) and LY3300054 (an anti-PD-L1 mAb) in patients with advanced solid tumors [160-162].

Clinical trials of ICIs in CRC

ICIs have shown significant effectiveness in patients with MSI-H or dMMR CRC. Among these, anti-PD-1 mAbs are the most commonly utilized, followed by anti-CTLA-4 monoclonal antibodies. Below is a summary of key drug interactions and ongoing clinical trials categorized by their target classifications [163-165].

PD-1

The FDA has approved two anti-PD-1 monoclonal antibodies: nivolumab and pembrolizumab. In the KEYNOTE-164 trial, which included 124 patients with MSI-H/dMMR CRC (61 in cohort A and 63 in cohort B), the median progression-free survival (PFS) was 31.3 months for cohort A and 24.2 months for cohort B (range: 0.1–27.1 months). Both cohorts had an ORR of 33% (95% CI: 21%-46% for cohort A and 22%-46% for cohort B) [166-168]. In a separate assessment of pembrolizumab's efficacy across various advanced dMMR cancer types, patients with MSI tumors and those with MSS tumors showed an ORR of 0% (95% CI: 0%-20%) and a PFS rate of 11% at 20 weeks. Another study involving patients with progressed, refractory PD-L1-positive colon or rectal cancer, regardless of MSI status, reported a median survival of 5.3 months. Most patients (n = 15, 65%) experienced disease progression, while one patient with MSI-H CRC (4%) demonstrated limited remission [169,170].

PD-1 + CTLA-4

Nivolumab, either alone or in combination with ipilimumab, is utilized for treating advanced MSI-H or dMMR cancers that have progressed following therapy with fluoropyrimidines, oxaliplatin, and irinotecan hydrochloride. In the CheckMate-142 study, 23 out of 74 patients receiving nivolumab (3 mg/kg biweekly) experienced an objective response, with 68.9% achieving disease control for 12 weeks or longer [171,172]. Pembrolizumab is also employed to treat MSI-H or dMMR cancers that have metastasized or are inoperable. For dMMR colorectal cancers, the immune-related ORR was 40% (4 out of 10 patients), and the immune-related PFS was 78% (7 out of 9 patients). In contrast, for pMMR colorectal cancers, the ORR was 0% (0 out of 18 patients), and the PFS was 11% (2 out of 18 patients) [173,174].

Additionally, a trial evaluating the combination of botensilimab and balstilimab reported a median follow-up of 6.4 months (range: 1.6–29.5). The ORR was 22% (95% CI: 12–35), and the disease control rate (DCR) was 73% (95% CI: 60–84), with the median duration of response not yet reached. The 12-month OS was 61% (95% CI: 42–75), while the median OS remains undetermined [175,176].

PD-1 + LAG3

Lymphocyte Activation Gene-3 (LAG-3), also recognized as CD223, primarily functions to negatively regulate T cell activity and is part of the immunoglobulin superfamily. LAG-3 suppresses T cell responses and plays a pivotal role in maintaining immune balance while promoting tumor immune evasion [177-179]. As a novel target, LAG-3 presents significant potential in cancer immunotherapy, although clinical trials are still in the early stages. In the NCT02720068 trial, the median follow-up was 5.8 months for the favicelizumab group and 6.2 months for the combination group. Another study combining BI 754111 and BI 754091 found that among 40 patients with advanced solid tumors and MSS mCRC, 3 (7.5%) achieved partial remission (PR), and 11 (27.5%) had stable disease (SD) [180-182].

PD-L1

The PD-L1 inhibitor atezolizumab demonstrated superior efficacy in the NCT02788279 trial, with a median follow-up of

7.10 months (range: 6.05–10.05). Additional research involving durvalumab included 30 cases of MSI-H/dMMR and 3 cases of POLE-mutant MSS CRC, reporting a median follow-up of 11.2 months (95% CI: 7.3–15.0) and an ORR of 42.4% (95% CI: 25.5–60.8) [183-185].

PD-L1 + CTLA-4

The single-arm Phase 1b/2 MEDITREME trial evaluated the safety and efficacy of durvalumab combined with tremelimumab and mFOLFOX6 chemotherapy as a first-line treatment in 57 patients with inoperable mCRC who had RAS mutations. The primary efficacy endpoint for patients with MSS tumors was met, demonstrating a 3-month PFS rate of 90.7% (95% CI: 79.2–96%) [186,187]. The response rate for secondary outcomes was 64.5%, while the median PFS was 8.2 months (95% CI: 5.9–8.6), and OS has not yet been reached for patients with MSS tumors.

Despite the clinical effectiveness of ICIs, their benefits are largely limited to a specific patient population, primarily those with MSI-H/dMMR tumors. Furthermore, challenges such as drug resistance and adverse events have restricted the broader application of ICIs [188-190].

Combination Treatment with Immune Checkpoint Inhibitors and Chemoradiotherapy in Locally Advanced Colorectal Cancer

The integration of immune checkpoint inhibitors (ICIs) with chemoradiotherapy represents an exciting and rapidly evolving field in the treatment of locally advanced colorectal cancer (LACRC), both in the neoadjuvant and adjuvant settings. This approach aims to leverage the strengths of both modalities to enhance therapeutic outcomes, particularly focusing on improving tumor regression rates and reducing recurrence while also addressing systemic disease control [228,229].

Neoadjuvant Therapy in LACRC

Neoadjuvant chemoradiotherapy (NACRT) or chemotherapy (NACT) is currently recognized as an optimal treatment paradigm for LACRC. These strategies have been shown to improve pathological response rates and reduce local-regional recurrence in locally advanced rectal cancer (LARC). However, a significant challenge remains in the form of distant metastasis, which continues to be a concern despite aggressive preoperative treatment. Interestingly, the total neoadjuvant therapy (TNT) strategy has emerged as a promising model, demonstrating enhanced tumor regression prior to surgery, yet it has not fully translated into improved overall survival benefits [230].

With knowledge of distinct tumor biology, particularly the mismatch repair (MMR) status of tumors, we can identify subgroups of patients—specifically those with microsatellite instability-high (MSI-H) or deficient MMR (dMMR)—who may derive more significant benefits from ICIs than from traditional chemoradiotherapy. In this context, the NICHE trial has been pivotal, exploring neoadjuvant ICI treatment alongside chemotherapy, yielding favorable short-term outcomes without significant delays in surgical intervention [231].

Adjuvant Therapy Considerations

In the adjuvant setting, the application of ICIs post-surgery retains its potential, especially in patients exhibiting high-risk features like residual disease or aggressive tumor characteristics. However, there are considerable challenges, as postoperative immune system alterations can potentially hinder the optimal functioning of ICIs. Continued research in this area might lead to strategies that can identify the optimal patients and timing for introducing ICIs after surgical intervention [232].

The incorporation of ICIs into the neoadjuvant and adjuvant treatment regimens for locally advanced colorectal cancer signifies a leap towards personalized medicine that may significantly improve patient outcomes. A thorough understanding of tumor biology, coupled with ongoing clinical trials, will aid in refining these therapeutic strategies and optimizing patient selection. Ultimately, the goal will be to enhance both tumor regression rates and long-term survival, creating a more comprehensive approach to managing LACRC [233].

Combination of ICIs with Conventional Treatments

ICIs have shown considerable effectiveness across various cancers. When combined with conventional therapies such as radiotherapy and chemotherapy, they may enhance treatment outcomes compared to when used alone [191-193].

ICIs plus Radiotherapy

Radiotherapy is a traditional treatment modality that directly targets tumor cells, inducing immunogenic cell death and the release of tumor-associated antigens. This mechanism stimulates antigen-presenting cells and boosts the immune response by enhancing MHC I expression. Evidence suggests that the combination of radiotherapy and ICIs can produce synergistic effects, amplifying the antitumor response through the release of tumor-associated antigens and cytokines, as well as enhancing T cell immunity against tumor cells [194,195].

For instance, a systematic review and meta-analysis comparing the effects of ICIs combined with radiotherapy versus ICIs

alone in various models, including CNS melanoma metastases, NSCLC, and prostate cancer, found that this combination is both safe and promising for future clinical trials. A preclinical study incorporating radiotherapy (five daily fractions of 2 Gy) along with anti-PD-1 antibodies demonstrated a broad systemic antitumor effect and enhanced T cell responses in murine models using CT-26 and 4434 cell lines [196,197]. Additionally, a study involving a mouse model resistant to anti-PD-1 showed that radiotherapy could induce IFN-β production, increase MHC I expression, and ultimately enhance immune responses. Clinical data indicated that administering radiotherapy after Ipilimumab in patients with advanced melanoma resulted in abscopal responses and associated improved overall survival. Likewise, melanoma patients with brain metastases who received Ipilimumab after radiotherapy exhibited better median survival than those treated with Ipilimumab before radiotherapy [198-201].

These findings highlight the importance of determining the optimal sequence of radiotherapy and ICIs to maximize therapeutic efficacy. The chosen radiation dose is also critical for the success of combination treatment. In conclusion, while combining ICIs with radiotherapy shows promising synergistic effects, further research is necessary to validate this approach [202,203].

ICIs plus Chemotherapy

Chemotherapy is a commonly used cancer treatment that enhances tumor immunogenicity and induces immunogenic cell death. Cytotoxic chemotherapy works by disrupting DNA replication and transcription or interfering with mitotic spindle formation. Evidence suggests that certain chemotherapeutic agents can reduce circulating T regulatory cells and myeloid-derived suppressor cells, thereby enhancing anti-cancer effects. Combining chemotherapy with ICIs can also increase tumor cell sensitivity to ICI therapy [204-206].

The concurrent use of chemotherapy and ICIs has been explored in several solid tumors, including NSCLC and CRC. For example, the combination of chemotherapeutic agents like ixabepilone and gemcitabine with Ipilimumab showed a synergistic effect, leading to reduced tumor growth in animal models of CT-26 colon carcinoma. The pairing of 5-fluorouracil and oxaliplatin (FOLFOX) with anti-PD-1 antibodies resulted in effective tumor control in CRC mouse models. In CRC patients, FOLFOX chemotherapy was associated with high CD8+ T cell infiltration into the tumor microenvironment and increased PD-L1 expression, making it a promising strategy when used alongside ICIs [207,208]. This combination not only enhanced ICI efficacy but also improved CD8+ T cell functionality by reducing T cell exhaustion in CRC.

Moreover, the combination of decitabine and anti-PD-1 antibodies led to tumor growth inhibition and improved survival in a CT-26 mouse model, with decitabine augmenting the antitumor effect of anti-PD-1 therapy. Preclinical findings also indicated that combining oxaliplatin with ICIs improved the outcomes of ICI therapy in CRC mouse models by increasing immune cell infiltration within the tumor microenvironment [209,210]. Additionally, studies involving mouse models of breast and prostate cancer revealed that combining chemotherapy with ICIs can reduce resistance to chemotherapy. Building on these promising results, clinical trials are currently exploring the combined effects of ICIs and chemotherapy across various solid tumors [211,212].

Table 2. **Summary of Clinical Trials of Immune Checkpoint Inhibitors in CRC**

Trial Name	NCT number	Study design	Checkpoint Inhibitors	Patient Population	Key Outcomes
KEYNOTE-016	NCT01876511	Multi-center non-randomized, open-label phase 2 clinical trial	Pembrolizumab (anti-PD-1)	dMMR CRC patients (n=113)	Median follow-up time: 49.7 months ORR: 58% (23 PR, 28 CR) Disease control rate: 76% (16 patients with stable disease) Median PFS: 34.9 months (95% CI: 14.8-NR) Median OS: 80.8 months (95% CI: 33.2-NR) 3-, 5-, and 10-year OS

Trial Name	NCT number	Study design	Checkpoint Inhibitors	Patient Population	Key Outcomes
					rates: 55.1%, 53.7%, and 47.4% respectively
KEYNOTE-177	NCT02563002	phase 3, randomized, open-label study	Pembrolizumab (anti-PD-1)	MSI-H/dMMR patients (n=307)	Pembrolizumab superior to chemotherapy in PFS (16.5 vs. 8.2 months, HR 0.60, P=0.0002) Estimated restricted mean survival after 24 months: 13.7 months (pembrolizumab) vs. 10.8 months (chemotherapy) Overall response rate: 43.8% (pembrolizumab) vs. 33.1% (chemotherapy) Ongoing responses at 24 months: 83% (pembrolizumab) vs. 35% (chemotherapy) Treatment-related adverse events (grade 3+): 22% (pembrolizumab) vs. 66% (chemotherapy)
CheckMate-142	NCT02060188	open-label, multicentre, phase 2 study	Nivolumab (anti-PD-1) + Ipilimumab (anti-CTLA-4)	Advanced dMMR CRC (n=74)	31.1% of 74 patients achieved an objective response 69% of patients had disease control for 12 weeks or longer Median duration of response not yet reached, with 8 patients having responses lasting 12 months or longer Common grade 3/4 adverse events: increased lipase (8%) and amylase (3%) 31% of patients died, none of which were treatment-related.

Trial Name	NCT number	Study design	Checkpoint Inhibitors	Patient Population	Key Outcomes
NICHE Study	NCT03026140	phase 2, multi-center, open-label	Various ICIs	MSS/pMMR patients	pMMR cohort: 30% pathologic response rate (9/30 patients) dMMR cohort: 100% pathologic response rate (31/32 patients), with 69% achieving complete response No disease recurrence in dMMR cohort, 3 recurrences in pMMR cohort 12% grade 3 immune-related adverse events, no grade 4 or unexpected surgical complications.
NCT02720068	NCT02720068	Phase 1 Trial	LAG-3 and PD-1 inhibitors (Favicelizumab + Pembrolizumab)	Advanced solid tumors	6.3% confirmed ORR with fave + pembro, no responses with fave alone Common adverse events: fatigue (20%), nausea (15%) with fave, fatigue (16.9%) with fave + pembro No grade 5 TRAEs, 15% (Arm 1) and 20% (Arms 2C+5) grade ≥3 TRAEs.

Challenges and Future Perspectives

As the landscape of CRC treatment continues to evolve, the promising role of ICIs and combination strategies becomes increasingly apparent. While ICIs have transformed therapy for patients with MSI-H tumors, significant challenges remain in addressing the limitations of their efficacy, particularly in the MSS and MSI-L populations [235].

Limited Population Responsive to ICIs

ICIs have demonstrated significant efficacy in CRC patients with microsatellite instability-high (MSI-H) but exhibit minimal effectiveness in microsatellite stable (MSS) and MSI-low (MSI-L) populations. While around 20% of CRC patients are classified as deficient in mismatch repair (dMMR) and responsive to ICIs, the majority remain in the MSS/MSI-L category. For instance, in a phase II trial, pembrolizumab achieved an objective response rate (ORR) of 40% and a progression-free survival (PFS) of 78% in the MSI-H cohort, while the MSS/MSI-L cohort showed ORR of 0% and PFS of only 11%. This illustrates the stark contrast in efficacy and underscores the limited population that benefits from ICI therapy [229].

Additionally, even patients who initially respond to ICI treatment can develop acquired drug resistance, which contributes to disease progression. In essence, the durability of response to ICIs is restricted to a minority of patients, while a significant proportion develop resistance, highlighting the need for combination therapies or alternative strategies to enhance treatment efficacy [236]

Personalized Medicine and Biomarker Development

The identification and validation of reliable biomarkers are crucial for optimizing the use of ICIs in CRC. While MSI status and TMB are established indicators of potential response, further exploration into additional biomarkers is necessary. Emerging candidates, such as POLE mutations, TIL (tumor-infiltrating lymphocyte) profiles, and the gut microbiome, are garnering attention for their potential to predict the efficacy of ICIs. Implementing a biomarker-driven approach will facilitate the selection of appropriate candidates for immunotherapy, ultimately enhancing treatment outcomes [237].

Mechanisms of Acquired Drug Resistance

Acquired resistance to ICIs in CRC can be attributed largely to several critical factors, especially those stemming from the tumor microenvironment (TME):

Loss of Tumor Antigen Expression: The initial step of immune recognition relies on tumor antigen presentation. In CRC, mutations can disrupt the expression of MHC proteins, hindering antigen presentation, which leads to T cell resistance. This is particularly true for tumors with β2-microglobulin (β2M) mutations, commonly observed in MSI-H CRC. The inability to present antigens compromises immune recognition and response [238].

Reduced Response to IFN-γ: Interferon-gamma (IFN-γ) plays a crucial role in activating T cell responses. Mutations in the JAK1/JAK2 signaling pathways can inhibit the effects of IFN-γ, which are necessary for enhancing PD-L1 expression and fostering an anti-tumor environment. In CRC, approximately 10-12% of tumors may have JAK1 or JAK2 mutations, correlating with resistance to PD-1 blockade [239].

Cytokine and Metabolite Dysregulation: Various immunosuppressive factors within the TME, such as adenosine generated by hypoxic conditions, inhibit T cell function. The overexpression of immunosuppressive cytokines like TGF-β and VEGF can further hinder anti-tumor immune responses, implying that targeting these factors, perhaps by combining anti-angiogenic therapies, might enhance ICI effectiveness [240].

Exploiting Combination Strategies

Given the complexities of immune response in CRC, combination strategies have emerged as a promising avenue. The combination of ICIs with cytotoxic chemotherapy has shown potential, as in studies that demonstrate improved outcomes when traditional therapies are employed alongside ICIs. For instance, combining oxaliplatin-based chemotherapy with nivolumab resulted in a median PFS of 6.6 months and a substantial ORR in MSS mCRC patients.

Additionally, dual checkpoint blockade strategies, such as using nivolumab with ipilimumab, show significantly higher PFS and ORS rates in MSI-H populations, indicating that leveraging multiple immune pathways may be beneficial [241].

Moreover, targeted therapies like TGF-β inhibitors are gaining traction, with preclinical evidence suggesting they might assist in reversing immune resistance. Cancer vaccines also represent another interesting combination strategy, aiming to generate strong adaptive immune responses that could enhance the efficacy of ICIs [242].

Understanding the Tumor Microenvironment

A deeper understanding of the TME in CRC is essential for tailoring effective immunotherapy strategies. The interplay between immune cell populations, cytokine profiles, and metabolic pathways influences the response to ICIs. Future research should focus on elucidating the mechanisms of immune evasion and resistance within the TME, particularly in MSS tumors, to develop strategies that can “reeducate” the TME toward an immunogenic profile [243].

Innovations in Drug Delivery and Administration

Advancements in drug delivery systems, including nanotechnology and localized delivery methods, hold promise for improving the efficacy of ICIs in CRC. By enhancing the concentration of therapeutic agents in the tumor while minimizing systemic exposure, these innovative approaches could lead to improved patient tolerability and treatment response [244].

Clinical Trial Design and Implementation

As novel combination therapies and biomarkers are explored, refining clinical trial designs will be crucial. Adaptive trial designs that enable real-time modifications based on emerging data can facilitate the assessment of combination strategies and personalized approaches. Furthermore, collaborative efforts among researchers, clinicians, and industry stakeholders to share data will accelerate the development of effective treatment protocols [245].

Addressing Health Disparities

Finally, addressing health disparities in CRC treatment and outcomes is of paramount importance. Research should be directed toward understanding the impact of genetic, environmental, and socio-economic factors on response to ICIs, particularly among underserved populations. Ensuring equitable access to innovative therapies and conducting trials inclusive of diverse populations will be essential for advancing the field [246].

In conclusion, the future of checkpoint inhibitors in colorectal cancer hinges on the integration of personalized medicine,

innovative combination strategies, and a comprehensive understanding of the tumor microenvironment. By addressing existing challenges and fostering collaborative efforts in research and clinical practice, we can maximize the potential of ICIs and improve outcomes for CRC patients globally [247].

2. CONCUSION

The evolving landscape of CRC treatment emphasizes a significant shift towards immunotherapy, particularly with the introduction of ICIs. While notable advancements have been achieved, especially for patients with MSI-H or dMMR CRC, challenges remain in effectively treating the majority of patients with MSS disease. The limited effectiveness of ICIs in MSS CRC underscores the need for innovative strategies that can enhance immune responses and counteract resistance mechanisms.

The checkpoints found on the surfaces of cancer and immune cells play a crucial role in understanding how effective and appropriately applied checkpoint inhibitors can be in cancer treatment. These markers serve as valuable biomarkers that help identify patients most likely to benefit from specific therapies, such as PD-L1 expression, which is regularly tested in tumors before the administration of PD-1/PD-L1 inhibitors. Additionally, the presence of various immune checkpoints provides insights into the tumor microenvironment, revealing the immune status of T cells—whether they are active, exhausted, or absent. Furthermore, mechanisms that lead to the upregulation of these immune checkpoints may indicate potential resistance to therapy, and understanding these pathways can guide the development of combination therapies for improved outcomes. Overall, while checkpoint inhibitors have transformed cancer treatment, their effectiveness is closely linked to the specific mechanisms of immune checkpoints present in both cancer and immune cells. By selecting the right patients based on these key markers, we can significantly enhance therapeutic success.

Recent discoveries regarding the tumor microenvironment and the complex interactions of immune checkpoints have driven increased interest in combination therapies. By targeting multiple pathways—such as co-inhibitory receptors like LAG-3 and TIM-3, in conjunction with established ICIs—researchers aim to amplify antitumor responses and improve clinical outcomes for a wider patient population.

Moreover, there is a pressing demand for biomarkers that can refine patient stratification and customize immunotherapeutic approaches based on individual profiles, particularly for those with MSS CRC. Ongoing clinical trials and investigations into novel combinations represent promising opportunities to enhance treatment efficacy and address the unmet needs of CRC patients.

In conclusion, while the integration of immunotherapy has significantly transformed CRC treatment strategies, further research and innovation are crucial to realizing the full potential of these therapies. Leveraging insights into immunological mechanisms and patient-specific factors will be essential for developing more effective and personalized strategies in the fight against this challenging malignancy.

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