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*REVIEW*

# Molecular insights into clinical trials for immune checkpoint inhibitors in colorectal cancer: Unravelling challenges and future directions

Samantha Sharma, Naresh Singh, Anita Ahmed Turk, Isabella Wan, Akshay Guttikonda, Julia Lily Dong, Xinna Zhang, Mateusz Opyrchal

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## Abstract

Colorectal cancer (CRC) is a complex disease with diverse etiologies and clinical outcomes. Despite considerable progress in development of CRC therapeutics, challenges remain regarding the diagnosis and management of advanced stage metastatic CRC (mCRC). In particular, the five-year survival rate is very low since mCRC is currently rarely curable. Over the past decade, cancer treatment has significantly improved with the introduction of cancer immunotherapies, specifically immune checkpoint inhibitors. Therapies aimed at blocking immune checkpoints such as PD-1, PD-L1, and CTLA-4 target inhibitory pathways of the immune system, and thereby enhance anti-tumor immunity. These therapies thus have shown promising results in many clinical trials alone or in combination. The efficacy and safety of immunotherapy, either alone or in combination with CRC, have been investigated in several clinical trials. Clinical trials, including KEYNOTE-164 and CheckMate 142, have led to Food and Drug Administration approval of the PD-1 inhibitors pembrolizumab and nivolumab, respectively, for the treatment of patients with unresectable or metastatic microsatellite instability-high or deficient mismatch repair CRC. Unfortunately, these drugs benefit only a small percentage of patients, with the benefits of immunotherapy remaining elusive for the vast majority of CRC patients. To this end, primary and secondary resistance to immunotherapy remains a significant issue, and further research is necessary to optimize the use of immunotherapy in CRC and identify biomarkers

to predict the response. This review provides a comprehensive overview of the clinical trials involving immune checkpoint inhibitors in CRC. The underlying rationale, challenges faced, and potential future steps to improve the prognosis and enhance the likelihood of successful trials in this field are discussed.

**Key Words:** Colorectal cancer; Immune checkpoint inhibitors; Clinical trials; Immunotherapy; Microsatellite instability; Microsatellite stability; DNA mismatch repair



**Core Tip:** Colorectal cancer (CRC) often eludes early detection, limiting the efficacy of existing chemotherapy and targeted therapies. This article delves into the realm of immune checkpoint inhibitors in CRC, dissecting their mechanisms and outcomes through a comprehensive review of clinical trials. It sheds light on the underlying rationale, challenges faced, and potential strategies to improve prognosis and trial success in this critical domain. Notably, while microsatellite instability-high CRC exhibits heightened responsiveness to checkpoint inhibitors, the article underscores potential breakthroughs in treating microsatellite stable CRC-the predominant cases-providing insights into bettering prognosis and trial outcomes in CRC treatment.

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## INTRODUCTION

Colorectal cancer (CRC) is a prevalent malignancy recognized worldwide for its intricate pathogenesis, diverse etiologies, and clinical outcomes[1,2]. Approximately 147950 new cases are expected to be diagnosed in 2023, along with an estimated number of 53200 deaths due to CRC[3]. Moreover, the incidence of early onset CRC is increasing as well[4]. CRC arises from the malignant transformation of epithelial cells lining the colon or rectum. The development of CRC is influenced by a multitude of risk factors, including advanced age, dietary choices, obesity, and inflammatory bowel disease[5-7]. The molecular pathogenesis of CRC is complex, with genetic and epigenetic alterations that drive tumorigenesis and contribute to disease progression[8-12]. These alterations intricately disrupt essential signaling pathways, such as WNT/β-catenin pathway, KRAS/BRAF/MEK/ERK pathway, and PI3K/AKT/mTOR pathway governing critical cellular processes, including cell proliferation, differentiation, and survival[8,9,13-15].

Currently, several approaches are employed for CRC treatment, including surgical procedures, chemotherapy, radiation therapy, targeted therapy, and immunotherapy[16]. However, following preliminary diagnosis, the 5-year survival rate of CRC patients is 65.0%, which significantly decreases to approximately 13% for metastatic CRC (mCRC)[17]. Treatment of advanced or mCRC is hindered by several challenges. Treatment options are particularly limited for patients who have exhausted multiple lines of treatment. Additionally, CRC tumors can develop resistance to chemotherapy, diminishing treatment efficacy over time[18,19]. The toxicity associated with chemotherapy and targeted therapies further complicates treatment and affects patients' quality of life. mCRC also has poor prognosis. Tumor heterogeneity adds another layer of complexity, contributing to treatment resistance and variability in patient responses[20,21]. To this end, immunotherapy targeting immune checkpoints such as PD-1/PD-L1 axis and CTLA-4 shows promise in treating advanced CRC, particularly in CRC tumors with microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR)[22]. Combination therapies, involving immunotherapy with chemotherapy, targeted therapies, and other immunomodulators, further offer the potential of synergistic effects and enhanced treatment efficacy[23,24]. Ongoing research efforts on predictive biomarkers, such as tumor mutational burden (TMB) and immune cell infiltration patterns, aim to identify patients most likely to benefit from immunotherapy[25,26]. Hence, immunotherapy holds promise as a transformative approach for the management of advanced or mCRC with durable responses and improved patient outcomes.

Immunotherapy using immune checkpoint inhibitions (ICIs) has recently emerged as a promising therapeutic approach for various cancers, including CRC. Understanding the immune infiltration patterns in CRC patients with microsatellite-stable (MSS) *vs* microsatellite-instability (MSI) phenotype is crucial for developing immunotherapeutic strategies. While MSI-H tumors may benefit from immunotherapy due to their higher immune infiltration and mutational load, MSS tumors may still require alternative or combination approaches to enhance the antitumor immune response and improve treatment outcomes[27,28]. To this end, ongoing research efforts aim to unravel the complexities of immune infiltration in different CRC subtypes to guide the development of more effective and personalized therapeutic interventions. Initial studies conducted between 2010 and 2013 showed limited clinical activity of ICIs in patients who were not selected based on specific biomarkers or treatment history[29-33]. Eventually, several promising findings have led to the approval of ICIs for MSI-H or dMMR CRC. Nonetheless, a low response to immunotherapy remains a

significant challenge in the treatment of MSS or proficient MMR (pMMR) CRC, highlighting the need for further research to enhance effectiveness and identify biomarkers to improve treatment outcomes[34,35]. Newer immunotherapeutic approaches have been investigated for CRC treatment, including cancer vaccines, adoptive T-cell therapy, and oncolytic viruses[36,37]. These approaches aim to stimulate the immune response against cancer cells by various means, including inducing antigen-specific T cell responses, genetically modifying T-cells to recognize and attack cancer cells, and using viruses to selectively target and destroy cancer cells[38,39]. A multitude of clinical trials, spanning both ongoing and concluding studies, have been conducted to explore the efficacy and safety of diverse drugs and combination therapies for CRC treatment. This review provides insights into the current landscape, challenges, and potential advancements in this field. CRC clinical trials involving ICIs and their mechanistic actions are outlined, treatment strategies and the future trajectory of CRC therapeutics are discussed.

## MOLECULAR INSIGHTS AND THERAPEUTIC PROGRESS IN CRC

Molecular characterization of CRC has identified two major subtypes, MSS and MSI CRC that account for approximately 85% and 15% of all CRC cases, respectively[40-42]. Clinical and pathological features of MSS CRC differ from those of MSI CRC[2]. Specifically, MSS CRC is typically associated with older age, male sex, and distal colon location, whereas MSI CRC is associated with younger age, female sex, proximal colon location, and better prognosis[41]. Furthermore, the MMR status and CRC are intricately linked due to their role in maintaining genome integrity and preventing the accumulation of mutations that can lead to cancer[43,44]. MSI-H CRC tends to have a dMMR status, a higher mutational load, and a distinct molecular profile compared to MSS CRC, which has a pMMR[43-45]. In particular, MSI and MMR status are predictive biomarkers for response to ICIs therapy[34,42]. The consensus molecular subtype (CMS) classification system divides CRC into four distinct subtypes based on gene expression profiles: CMS1 (immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal)[46]. Each CMS subtype has a distinct molecular signature and clinical phenotype. The system thus offers a clear biological understanding and serves as a foundation for future clinical stratification and targeted interventions based on specific subtypes.

Over the years, substantial progress has been achieved in the development of CRC therapeutics, resulting in enhanced survival rates attributed to advancements in primary and adjuvant treatment modalities[47]. Notably, the inclusion of chemotherapy as a neoadjuvant or adjuvant intervention has emerged as a strategic approach aimed at mitigating the tumor burden, reduction, and stabilization[48-51]. Chemotherapy maintains its pivotal status in current treatment strategies. However, the utility of chemotherapy is curtailed by a restricted therapeutic range, significant adverse reactions, and the frequent occurrence of acquired resistance[52]. Several chemotherapy agents, radiotherapies, and other physical forces also induce destruction of cells and tissues, leading to death of immune cells and subsequently enhancing therapeutic outcomes[53-56]. Immunotherapy with checkpoint inhibitors has provided a significant improvement in cancer treatment, demonstrating high efficacy and manageable side effects in various tumor types[57-62]. However, the success of immunotherapy in CRC patients remains limited, with only a small subset of cases characterized by MSI-H or dMMR benefiting from treatment[63,64]. Thus, despite over 50 decades of research on immunotherapy for CRC treatment and major advancements, significant challenges remain in the diagnosis and management of CRC, particularly in the context of advanced or metastatic disease[27] (Figure 1).

Immuno-oncology is an emerging field of cancer treatment that involves harnessing the patient's immune system to recognize and eliminate cancer cells[64]. Immunotherapy can potentially improve treatment outcomes for patients with a wide range of malignancies[38]. Immunotherapy is often considered more beneficial than chemotherapy due to its ability to induce durable immune responses. Unlike chemotherapy, which primarily induces short-term cytotoxic effects on cancer cells and eliminates immunosuppressive cells[65-67], immunotherapy activates the immune system, particularly cytotoxic T-cells, to recognize and target cancer cells[38]. Consequently, immunotherapy offers the potential for sustained protection against cancer by maintaining an immunological "memory" that can detect and eliminate cancer cells in case of re-encounter[68,69]. Immunotherapy for cancer has thus brought about revolutionary transformations in the field of oncology, extending the survival of individuals diagnosed with aggressive life-threatening malignancies[57-61]. CRC patients with MSI-H or dMMR status show higher mutation rates, more neoantigens, and increased tumor-infiltrating lymphocytes (TILs), particularly cytotoxic T-cells[70], fostering a robust antitumor immune response. In contrast, MSS tumors have an immunosuppressive microenvironment with regulatory T-cells (Tregs) and other immunosuppressive cell types that hinder effector T cell activity[71,72]. Eventually, the prognostic value of the immunoscore was initially established in individuals with colon cancer, showing its ability to assess prognosis based on factors such as the density, type, and localization of infiltrating immune cells[73].

ICIs are drugs that blocks certain key proteins on the surface of immune cells, particularly T-cells, and cancer cells, and release the brakes on the immune response. The development of ICIs has been a breakthrough in the field of cancer immunotherapy[36,69,74-76]. These proteins, known as immune checkpoints, play crucial roles in regulating the immune response. By blocking these checkpoints, ICIs enhance the ability of the immune system to recognize and attack cancer cells, thereby boosting the body's natural anti-cancer response[38]. ICIs have become a cornerstone of cancer therapy, with a wide range of approved agents available for multiple malignancies, leading to increased utilization in various treatment settings, including (neo)adjuvant and maintenance therapy. Thus, ICIs are accessible to nearly half of metastatic cancer patients in economically developed countries[77-79].

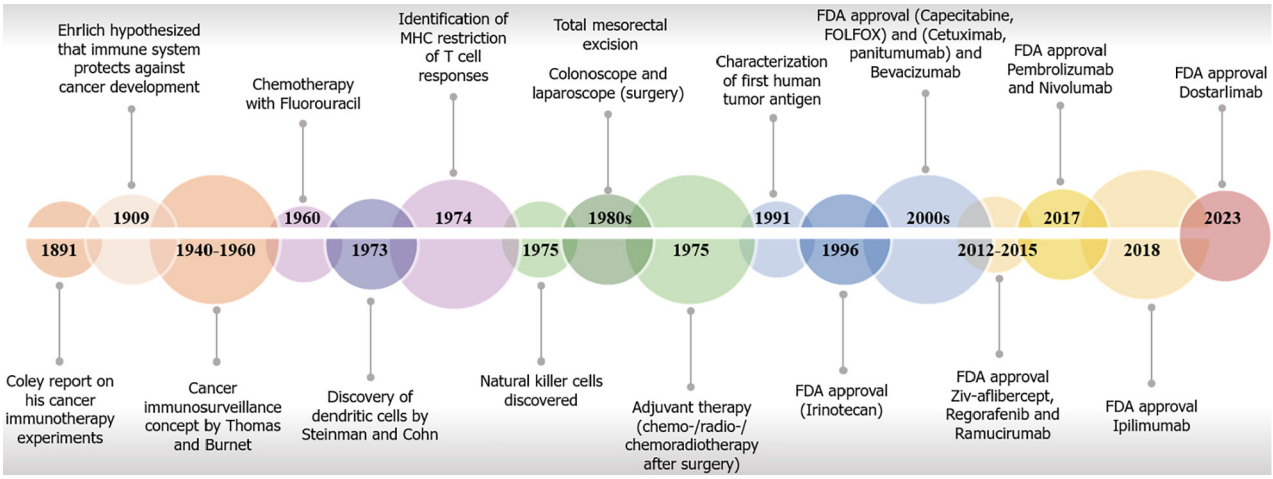


**Figure 1 Timeline with key milestones in immuno-oncology research and United States Food and Drug Administration–approved immune checkpoint inhibitors in colorectal cancer.**

## UNDERSTANDING THE MECHANISM OF IMMUNE CHECKPOINT INHIBITORS IN CRC

A solid understanding of the molecular drivers of CRC and identification of biomarkers of treatment response are essential for improving immunotherapy outcomes in patients with this disease[28,73,80-85]. A key element is the high TMB caused by genetic or sporadic mutations in MMR genes (such as MLH1, MSH2, MSH6, and PMS2), resulting in a deficiency of MMR proteins. This leads to an accumulation of genetic mutations in microsatellites[15,41], as observed in MSI-H or dMMR CRC tumors, compared to MSI-low (MSI-L) or pMMR. Consequently, enhanced immunogenicity is observed in such CRC cases, characterized by a higher count of neoantigens and substantial immune cell infiltration, including high numbers of tumor-infiltrating immune cells, such as CD8+ and CD4+ T-cells and macrophages[40,73,86-90]. Additionally, these tumors exhibit a microenvironment enriched with type I interferons, which distinguishes them from other CRC subtypes[87]. This immune-rich trait has been linked to improved rates of response to ICIs that block the PD-1/PD-L1 axis and T-cell activation[40,91]. In contrast, CRC tumors exhibiting pMMR along with MSS exhibit a low burden of mutations and low infiltration of CD4 and CD8 immune cells, resulting in evasion of the immune response.

ICIs enhance the recognition and elimination of cancer cells by activating the immune system, resulting in a more potent and sustained anticancer immune response. Understanding the mechanisms of synchronization with the disease pathophysiology is crucial for optimizing the therapeutic potential of ICIs and improving patient outcomes. The main mechanisms of action of ICIs include blockade of the PD-1/PD-L1 pathway and CTLA-4, which regulates T-cell activity and is often upregulated in tumors to evade the immune system (Figure 2)[38]. Indeed, PD-1 and CTLA-4 serve as negative regulators of T-cell activation and exert their biological effects at specific anatomical locations and at various points throughout the lifespan of T-cells[92]. The varied and late-onset autoimmune manifestations observed in Pdcd1−/− mice differ significantly from those in Ctla4-/- animals, highlighting that the PD1 axis governs T-cell biology in a distinct manner compared to CTLA4[92,93].

The PD-1/PD-L1 axis plays a role in autoimmunity by negatively regulating T-cell activation[94]. Functional loss of PD1 protein results in splenomegaly in mice models[94]. Additionally, mouse models lacking the PD-1 gene exhibit cardiac inflammation, leading to dilated cardiomyopathy and accelerated type 1 diabetes mellitus[95,96]. The PD1 axis is crucial for regulating differentiated effectors in T-cells[93,97,98]. Upon binding to PD-L1, PD1 exerts inhibitory intracellular signaling, leading to T-cell exhaustion, and eventually suppressing the immune response[99-101]. In addition to its role in regulating conventional T-cells, PD-L1 on antigen presenting cells (APCs) also plays a role in controlling Treg differentiation and immunosuppressive activity[102]. Tumor cells upregulate PD-L1 surface expression to take advantage of the PD-1/PD-L1 axis and escape immune response.

Anti-PD-1 antibodies such as pembrolizumab and nivolumab are ICIs used in cancer immunotherapy. Anti-PD-1 antibodies not only enhance the activity of cytotoxic T-cells but also affect the overall tumor microenvironment (TME) as well. These antibodies can alter the balance of immune cell populations by reducing the number of immunosuppressive cells such as Tregs and myeloid-derived suppressor cells[103]. This shift contributes to a more favorable immunological milieu for anti-tumor responses[104]. Anti-PD-1 antibodies promote increased production of pro-inflammatory cytokines, such as IFN-γ. These cytokines play key roles in amplifying the anti-tumor immune response by activating other immune cells and enhancing the recognition and elimination of cancer cells. In contrast, anti-PD-L1 antibodies target PD-L1 ligands on cancer cells. By blocking the interaction between PD-L1 and PD-1 on T-cells, these antibodies disrupt a key immune evasion mechanism employed by cancer cells. Anti-PD-1/PD-L1 antibodies help overcome adaptive immune resistance by enabling T-cells to recognize and target cancer cells more effectively. This leads to continuous adaptation of the immune response against evolving tumor cells. Immunotherapeutic responses are often associated with the expression of specific immunological biomarkers[105-107]. For anti-PD-1/PD-L1 therapy, the expression of PD-L1 on tumor cells is a commonly used biomarker. Tumors with high PD-L1 expression may have a higher likelihood of responding to anti-PD-1 antibodies. The presence of TILs is also considered a positive prognostic indicator of immuno-

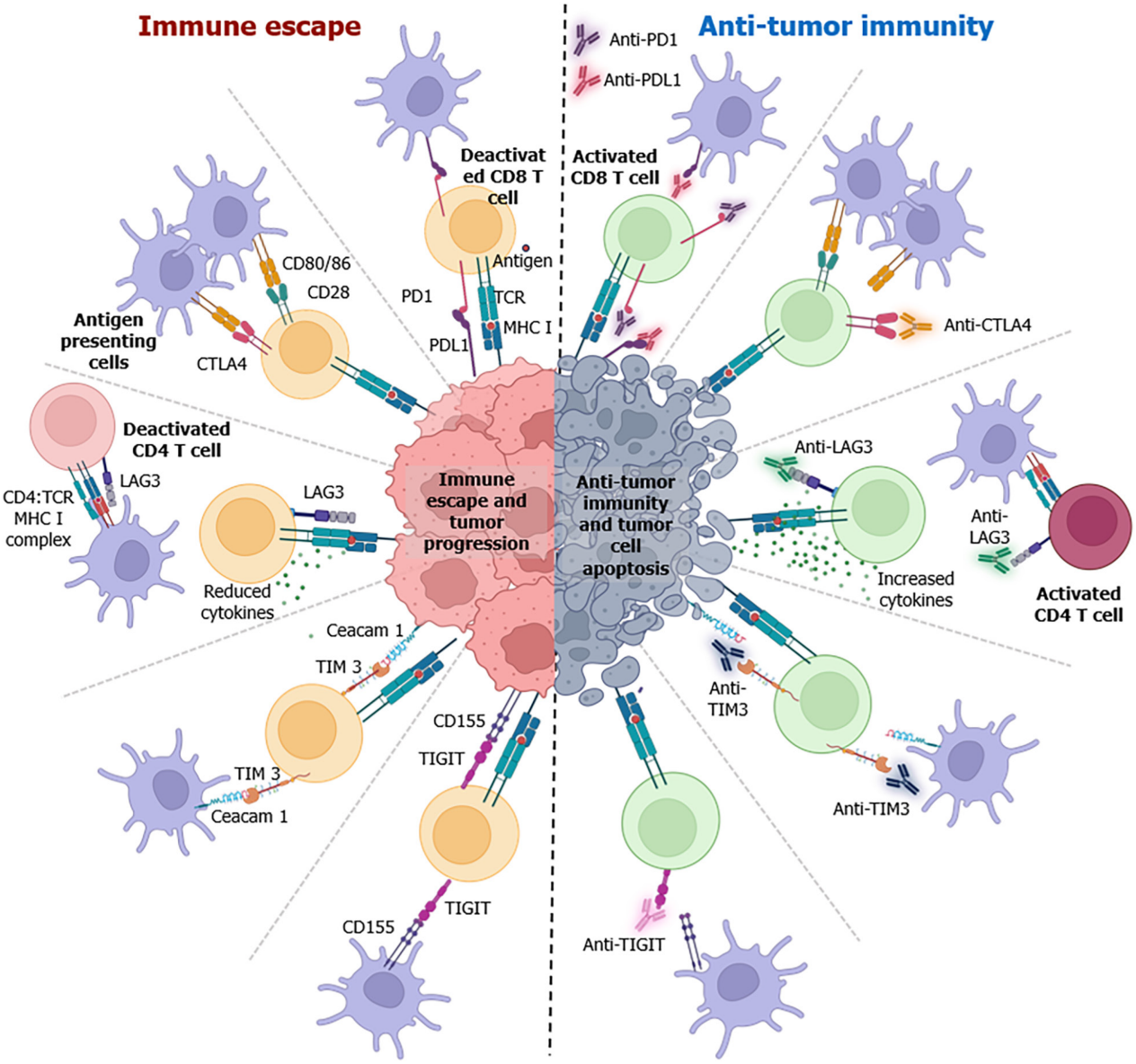


**Figure 2 This figure presents a schematic diagram of the intricate interplay between immune checkpoints, immune cells, and malignant cells.** It also elucidates the underlying molecular mechanisms employed in immune-checkpoint blockade. In the tumor microenvironment, the interaction of immune checkpoints leads to immune suppression and facilitates tumor progression (depicted on the left side of the diagram). Conversely, the administration of immune checkpoint inhibitions reverses the immune escape mechanism, fostering increased anti-tumor immunity and triggering tumor apoptosis (depicted on the right side of the diagram). FDA: Food and Drug Administration.

therapy response.

CTLA-4, a vital immune checkpoint, exhibits low basal levels in conventional T-cell. However, its expression is significantly induced after antigen activation. Activated T-cells expressing CTLA4 impede the interaction between B7-1 and B7-2 molecules on APCs and CD28, and thereby induce anergy and reduce T-cell activation[108-110]. TCR signaling studies affirm CTLA4's role in inhibiting T-cell activation and proliferation[111-113]. Ctla4-knockout mice were found to develop T-cell mediated autoimmune disease, which is mitigated by treatment with the CTLA4: Fc fusion protein (CTLA4Ig)[114-116]. Notably, CD4+ CD25+ Tregs, which are known for their immunosuppressive function, constitutively express CTLA4 and are necessary for the release of anti-inflammatory cytokines from Tregs[117,118]. These findings confirm that CTLA4 is a T-cell activation inhibitor with potential as a therapeutic agent against cancer[119]. Pre-clinical studies using anti-CTLA-4 antibodies aimed to prevent inhibitory signals, allowing for a more effective CD28 interaction with B7[120]. However, the results were found to depend on tissue specificity and tumor size[119,121]. Additionally, blocking CTLA4 enhances T-cell responses to tumor-associated neoantigens, and a high neoantigen burden predicts a positive response to anti-CTLA4 therapy.

Blocking CTLA-4 with anti-CTLA-4 antibodies promotes a sustained and enhanced T-cell activation. CD28 is a co-stimulator of T-cell activation that benefits from the increased availability of anti-CTLA-4 antibodies and facilitates enhanced binding to B7. This then amplifies co-stimulatory signals, promoting T-cell proliferation and function[120]. These antibodies also modify the TME by decreasing immunosuppressive cells, such as Tregs, creating a more favorable setting for anti-tumor immune responses[122,123]. Additionally, anti-CTLA-4 antibodies induce antibody-dependent cell-

mediated cytotoxicity, with immune cells, particularly natural killer cells, recognizing and eliminating target cells marked with therapeutic antibodies[124]. Often used in combination with other ICIs, such as anti-PD-1 or anti-PD-L1 antibodies, this approach targets multiple checkpoints simultaneously, enhancing the overall anti-tumor immune response. Anti-CTLA-4 therapy induces systemic immune activation, affecting not only the tumor site but also distant metastases, contributing to the potential for durable responses and efficacy.

ICIs can also enhance antigen presentation by dendritic cells (DCs), and thus facilitate the priming of T-cells to initiate a robust and targeted immune response against cancer[125,126]. Activated DCs present tumor antigens to T-cells effectively to promote T-cell activation and proliferation. Adaptive immune resistance involves a dynamic interplay between the immune system and cancer cells. Successful ICIs therapy is associated with memory T-cell generation. Memory T-cells contribute to long-term immune memory by enabling the immune system to respond rapidly to cancer cell recurrence. Understanding these immunological nuances provides insights into how ICIs contribute to unleashing and potentiating the ability of the immune system to recognize and eliminate cancer cells. Despite its success in MSI-H CRC, the clinical efficacy of immunotherapy in MSS CRC remains very limited[28]. Recent studies have found that characteristics such as high levels of TILs and expression of immune checkpoint molecules such as PD-L1 may help identify patients with MSS CRC who are more likely to benefit from ICI treatment.

## CLINICAL TRIALS INVOLVING CHECKPOINT INHIBITORS IN CRC

The emergence of checkpoint inhibitors has brought a remarkable shift in our approach to treatment of cancers, including CRC. Notably, these inhibitors have exhibited encouraging treatment outcomes in specific subsets of patients, such as those with MSI-H or dMMR tumors, which are characterized by augmented levels of TILs and heightened susceptibility to immune checkpoint blockade. This section focuses on noteworthy clinical studies on application, efficacy, and potential benefits of ICIs in CRC. In 2014, the Food and Drug Administration (FDA) approved Pembrolizumab, a PD-1 immune checkpoint inhibitor for melanoma treatment[127]. Tumor cells evade the immune system through the PD-1 pathway where PDL1 and PDL2 Ligands on tumor cells binds to the PD-1 receptors on T cells to inactivate T cells. Pembrolizumab binds to these PD-1 receptor and blocks their interaction with PDL1 and PDL2, thereby restoring the immune response [128]. Subsequently, in 2020, the FDA approved this drug for patients with unresectable or metastatic MSI-H or dMMR CRC, based on results from key clinical trials. Phase 2 open-label, multicenter trial (NCT01876511) was conducted to evaluate the safety, efficacy, and tolerability of pembrolizumab in MSI-H-positive patients[129]. Trials have shown no dose-limiting toxicities associated with pembrolizumab, with a promising disease control rate of 80% in patients with MSI-positive CRC, suggesting the potential of Pembrolizumab in CRC treatment. Subsequently, another trial (NCT02460198) postulated the efficacy of pembrolizumab in patients with unresectable tumors who underwent standard chemotherapy[130,131]. The results showed a promising overall progression response rate of 32 to 34 months in both cohorts. This study demonstrated the potential of pembrolizumab as an effective treatment option for patients with dMMR and MSI-H mCRC.

Chemotherapy has been used over the years for the treatment of patients with CRC to shrink tumor volume[132]. In 2015, a phase 3 clinical trial (NCT02563002) was conducted to test pembrolizumab as a first-line treatment in comparison with standard chemotherapy treatment in mCRC patients with MSI-H or dMMR tumors[133,134]. The results showed a significant improvement in the progression-free survival (PFS) rate of 16.5 months in comparison to the standard chemotherapy group at 8.2 months. The trial demonstrated pembrolizumab monotherapy to be superior to standard chemotherapy in terms of PFS and overall response rate (ORR) for patients with MSI-H or dMMR mCRC as a standard care option. The potential efficacy of pembrolizumab in these patients was demonstrated by increased production of neoantigens resulting from an elevated mutational burden. This, in turn, leads to a heightened recognition of tumor cells by cytotoxic T cells, which are primed by blocking the interaction between PD-1 and PD-L1. These results thus led to a paradigm shift in the treatment of this patient population.

The evolution of pembrolizumab has led to the development of more PD-1-targeting drugs whose efficacy and safety profiles were assessed. Nivolumab, another PD-1 monoclonal antibody, was approved in 2017 for use in mCRC treatment [28]. Both drugs exhibited similar modes of action in blocking PD1 and inducing increased CTLs cytotoxicity. However, these two antibodies also exhibited significant structural differences in their binding to PD-1[135]. The epitope region of pembrolizumab displayed a considerably larger overlap with the PD-L1 binding site compared to that of nivolumab. Notably, the binding sites of pembrolizumab and nivolumab on PD-1 showed almost no convergence[135]. A study published in 2017 compared the effectiveness of drugs with comparable effectiveness, which may potentially be interchangeable. The effectiveness of nivolumab has been studied in NCT02060188 MSI-H or dMMR mCRC patients with or without the CTLA-4 inhibitor drug Ipilimumab[136-138]. The treatment showed promising results, with a disease control rate of 80% with nivolumab alone. Combination treatment with a CTLA-4 inhibitor was effective in 51 of the 74 patients who achieved disease control for a minimum of 12 wk. However, further studies are still needed to determine the optimal treatment duration for pembrolizumab and to identify predictive biomarkers of response to immunotherapy in this population. Overall, Nivolumab plus ipilimumab combination therapy is a promising treatment with a better disease control rate and objective response.

Atezolizumab, a monoclonal antibody targeting PD-L1, was approved by the FDA in 2016 for the treatment of non-small cell lung cancer tumors[139]. The mechanism of action of Atezolizumab differs from those of pembrolizumab and nivolumab. Instead of binding to PD-1, atezolizumab binds to PD-L1 on tumor cells, and thereby provides a mode of action similar to that of the PD-1 antibody. A clinical trial (NCT02788279) was conducted to evaluate the efficacy of this drug alone and in combination with a MEK inhibitor, cobimetinib, compared to regorafenib (a multi-kinase inhibitor)

[140]. This combination is used as a therapeutic alternative to a MEK inhibitor that increases T-cell proliferation and CD8+ T cell infiltration, and PD-1 treatment to upregulate the PD-1/PD-L1 interaction, thereby downregulating the immunosuppressive TME. The results of the trial showed an improved PFS rate 1.91 of 2 months after cobimetinib treatment. In conclusion, combination of atezolizumab and cobimetinib improved PFS and ORR compared to regorafenib as a second-line treatment for patients with mCRC, yet did not significantly improve OS. These results underscore the potential of combination therapy and suggest improved investigations of combined immunotherapy drugs as promising targeted treatment approaches for mCRC. Combination therapy with MEK inhibition and PD-L1 blockade led to impressive long-term survival rates. MEK inhibitors act during the post-naïve stage of T-cell differentiation. MEK inhibition counteracts the expression of Nur77, which is associated with exhaustive T cell death induced by antigen-specific $CD8^+$ T cells, thereby rescuing T cell exhaustion[16].

Another PD-1 monoclonal antibody, dostarlimab, was approved in 2021 and tested in clinical trials (NCT04165772) in patients with MSI-H or dMMR CRC[141]. A high-resolution structure revealed that Dostarlimab binds to the flexible loops of PD-1, including the BC, C'D, and FG loops, in contrast to the binding modes of Pembrolizumab or Nivolumab [94]. The initial findings of the trials were published for 12 patients. Accordingly, all the patients (100%; 95%CI: 74-100) showed a complete clinical response[142]. The response was confirmed using magnetic resonance imaging, which showed no evidence of tumors. At that time, none of the patients had received chemoradiotherapy or undergone surgery. No cases of progression or recurrence were observed during follow-up (6–25 months). The study listed no grade 3 or higher AEs during the trial period. This study thus demonstrated the high sensitivity of dMMR locally advanced rectal cancer to single-agent PD-1 blockade. Despite these promising results, a longer follow-up period and a larger sample size are still needed to assess the duration of the response.

Following the improved success of ICIs in CRC patients with MSI-H or dMMR tumors, researchers have investigated their efficacy in MSS or pMMR CRC patients as well. These data suggested that more than 85% of CRC patients with MSS tumors show resistance to ICIs therapy. A clinical trial (NCT01876511) illustrating the potential of pembrolizumab in MSI-H or dMMR CRC patients has shown no measurable responses in any of the 18 patients with pMMR CRC, as defined by the RECIST criteria[129]. To overcome these limitations, combination treatments have been used to improve the drug responses. A clinical trial was conducted using PD-L1 with a multi kinase inhibitor in patients with MSS or pMMR CRC. Regorafenib targets stromal and oncogenic receptor tyrosine kinases, and shows anti-angiogenic activity due to its dual-target VEGFR2-TIE2 tyrosine kinase inhibition[143]. The NCT04126733 trial conducted between 2019 and 2022 included 94 CRC patients with MSS or pMMR[144]. The results showed an ORR of 7% and overall survival (OS) rate of 11.9%. The relatively reduced success observed in patients with MSS or pMMR CRC indicates the need for further advancement of drug efficacy to provide better outcomes in MSS tumors.

In 2016, a combination trial (NCT01988896) was performed in CRC patients with BRAF/KRAS mutations using cobimetinib and atezolizumab to study OS and PFS in MSS or pMMR CRCs patients[145]. These mutations are rarely identified but are more frequently found in patients with MSS CRC. BRAF and KRAS mutations are mutually exclusive, and BRAF-mt induces aberrant and inappropriate activation of the MAPK/ERK pathway, making it a good candidate for combination therapy with ICIs and kinases or MEK inhibitors[146]. As expected, the combination provided relatively better outcomes in patients with BRAF/KRAS mutations, with an OS rate of 43%. These findings provide compelling evidence that MAPK pathway blockade therapy combined with ICIs is promising for improving treatment efficacy in mCRC patients with MSS/pMMR BRAF mutations.

Recent developments have improved targeted immunotherapies using engineered ICIs to increase the success rate in patients with MSS or pMMR CRC[147]. A phase 1 clinical trial was conducted to categorize the adverse effects and dose-limiting toxicity of botensilimab, an Fc-engineered anti-CTLA-4 monoclonal antibody, in patients with MSS CRC. This Fc engineering promotes intratumoral Treg depletion and reduces complement fixation. This modification provides optimized T-cell priming, activation, and memory formation by strengthening antigen-presenting cell/T-cell co-engagement. The trial showed an ORR of 22% (95%CI: 12-35) and a disease control rate of 73% (95%CI: 42-75) for patients with non-hepatic disease in refractory CRC. The trial showed the efficacy of the anti-tumor activity in MSS CRC patients with active liver metastatic disease, and a phase 2 trial (NCT05608044) for MSS CRC has begun to study its potential in controlling tumor progression.

The success of ICIs in patients with MSI-H CRC has been hindered by their reduced potential in MSS CRC. The results from clinical trials in patients with MSS CRC undergoing immune checkpoint blockade immunotherapy suggest the need for the development of new pre-clinical mouse models to replicate the microenvironment of human CRC, and potentiate new targeted therapies to improve patient survival.

## LIMITATIONS OF USING IMMUNE CHECKPOINT INHIBITORS FOR IMMUNOTHERAPY

Although ICI therapy holds benefits, patients also often experience autoimmune-like effects known as immune-related adverse events (irAEs). This is likely the result of generalized, non-antigen-specific activation of the immune system following a checkpoint blockade. Inhibition of a naturally occurring central immune checkpoint releases potent immune effector mechanisms that may not adhere to the usual boundaries of immune tolerance towards self-tissues[148]. Human loss-of-function mutations in CTLA4 and its regulatory partner, LPS Responsive Beige-Like Anchor Protein, mimic the immune-related side effects of anti-CTLA4 therapy[149,150]. irAEs have been reported in 15%–90% of patients[57], with severe events requiring intervention being observed in 30% and 15% of patients treated with CTLA4 and PD1 axis inhibitors, respectively[151]. This immune checkpoint inhibitor leads to toxicity in naïve T cells and accumulation of memory T cells in peripheral organs[152,153]. Compared with PD-1, CTLA4 therapy possess with severe autoimmune

complications, as seen in pre-clinical and clinical trials[154].

Colitis is a frequent complication observed in ICIs therapy[155]. Anti-CTLA4 treatment resulted in a potentially higher colitis rate, ranging from 5.7% to 22% of patients, compared to 0.7% to 1.6% with anti–PD-1 agents[156]. The development of ICI-mediated colitis and diarrhea (IMC) may involve cytotoxic CD8+ T cells. Recent analysis of single-cell RNA sequences from patients with IMC revealed an expansion of tissue-resident memory CD8+ T cells into inflammatory populations within the colon tissue, suggesting that the activation or alteration of CD8+ T cell populations could be a potential mechanism for colitis induced by ICIs[157]. Therefore, with the potential use of immune checkpoint blockade, the current research should aim to identify potential predictive markers for organ-specific toxicities caused by immunotherapy.

## IMMUNE PROFILING OF MSI AND MSS CRC INFLUENCE ICIS SUCCESS

Patients comprising MSI-H or dMMR tumors have a significantly high overall mutation burden, with approximately 12 mutations per million DNA bases. In contrast, pMMR/MSI-L tumors have a relatively reduced tumor burden, with fewer than eight mutations per million DNA bases[158]. This phenomenon is primarily attributed to somatic defects in the function of MMR genes, with the most prevalent mechanism being hypermethylation of the MLH1 promoter, which serves as a prognostic marker. In MSI, frameshift mutations in protein-coding sequences can create diverse peptides that serve as potential neoepitopes, which are recognized as foreign by the immune system. Mutant peptides form complexes with major histocompatibility complex class I molecules and act as foreign neoantigens that initiate immune cell priming and infiltration. Within the TME, tumor-associated macrophages play a crucial role in influencing tumor growth and progression. Recent study has demonstrated a frameshift mutation in the TGFβRII producing an immunogenic peptide called p538[159]. This peptide is present in over 90% of tumors with dMMR, indicating its broad relevance in the field. These tumors exhibit robust infiltration by immune cells, particularly CD8+ TILs, Th1 CD4+ TILs, and macrophages[73]. Furthermore, the microenvironment of these tumors is notably enriched in type I interferons, which distinguishes them from other CRCs types.

Approximately 15% of all CRCs exhibit MSI-H or dMMR characteristics[160]. Patients with MSI-H or dMMR tumors before ICIs therapy continue to have a poor prognosis. Cancers show significantly upregulated expression of PD1, PDL1, and CTLA4, rendering them potentially susceptible to ICIs[87]. In contrast, MSS or pMMR tumors lacking neoantigens are characterized by reduced T cell infiltration and elevated levels of immunosuppressive ligands. These characteristics offer insights into the disagreement between MSI-H or dMMR and MSS or pMMR CRCs in ICIs responses and could potentially serve as prognostic biomarkers for patient selection. As shown in previously described clinical trials, immunotherapy as a neoadjuvant approach has not shown any clinical benefit in patients with MSS or pMMR CRC, including individuals with mCRC.

Contrastingly, the MSS CRC, majorly referred to as an "immune cold" cancer type, are predisposed by various molecular factors contributing to the underlying resistance to immunotherapy[161]. MSS-CRC is characterized by larger chromosomal aberrations that mark the phenotype of MSS-CRC, resulting in a lower tumor mutation burden and reduced neoantigen configuration. This framework partially elucidates the disparate clinical responses to ICIs observed in these CRC subgroups. The MSS CRC TME hosts more tumor-associated macrophages, which have been associated with poor prognosis in most studies. Notably, a pioneer study identified that increased β-catenin activation (a downstream effect of APC mutation) resulted in reduced infiltration of CD8+ and CD103+ DCs, orchestrating an immune suppressive environment *via* T-cell exclusion[16]. The APC protein is mutated in more than 70% and 20% of MSS and MSI-H CRCs, respectively, driving the distinct oncogenesis mechanisms and subsequent “immune hot” and “immune cold” TME[162]. These differences are reflected in clinical trials with ICIs, where MSS tumors have very low response rates compared to MSI tumors (Table 1).

## IMPLICATION OF PRE-CLINICAL MOUSE MODELS OF CRC IN DRUG DEVELOPMENT AND IMMUNOTHERAPY

Mouse- and cell-based models have been used for decades to investigate the molecular origins of CRC, and more recently, to identify drug and immune responses in specific CRC types (Figure 1). These efforts have yielded tremendous improvement in our basic understanding of the disease and TME. However, despite recent approvals, the majority of patients continue to have limited immunotherapy options. A recurring challenge highlighted in the literature is the absence of a mouse model that precisely replicates the progression of human CRC from adenoma and adenocarcinoma to metastasis, including changes in the microenvironment. Initial mouse models lacked significant penetrance of the metastatic phenotype, often forming tumors in the small intestine rather than in the colon, unless induced by laparoscopy [163,164]. In 2013, the National Institute of Health formally concluded the Mouse Models in Human Cancer Consortium, leading researchers to explore alternative models, such as patient-derived xenografts and patient-derived organoids, to study the disease. The significance of the TME in metastasis remains a focus of current research, and the potential of checkpoint inhibitors and other immunological and inhibitor therapies are being explored. However, an ideal model still does not exist, highlighting the importance of an immunocompetent autochthonous model. Notably, single-cell RNA sequencing of mouse tumors to understand the mechanisms underlying immune-modulating therapies could help draw more impactful conclusions[165]. Despite these limitations, the diversity of the methods employed by researchers with

**Table 1 List of clinical trials with immune check point inhibition therapy**

| Trial identified (number of patients) | Treatment groups | Patients enrolled | Primary and secondary outcomes |
|---|---|---|---|
| Checkpoint inhibitor: Pembrolizumab | | | |
| NCT01876511 (*n* = 113) | Pembrolizumab | Cohort A: MSI positive (pMMR) CRC | Cohort A: ORR: 54.0% (95%CI: 37.0–69.0); PFS: 70% (95%CI: 57–86); OS: NA (95%CI: 151.86-NA) |
| | | Cohort B: MSI negative (dMMR) CRC | Cohort B: ORR: 0% (95%CI: 0.0–14.0); PFS: 16% (95%CI: 6–41); OS: 36.71 (95%CI: 21.29-69.43) |
| NCT02460198 (*n* = 124) | Pembrolizumab | mCRC with dMMR or MSI–H status Cohort A: Participants must have received prior treatment with standard therapies | Cohort A: ORR: 32.8 (95%CI: 21.3 to 46.0); PFS: 2.3 (95%CI: 2.1–8.1); OS: 31.4 (95%CI: 21.4–58) |
| | | Cohort B: Participants must have undergone at least one line of systemic standard of care therapy | Cohort B: ORR: 34.9 (95%CI: 23.3–48.0); PFS: 4.1 (95%CI: 2.1–18.9); OS: 47 (19.2–NA) |
| NCT02563002 (*n* = 307) | Arm A: Pembrolizumab | mCRC with high MSI–H or dMMR | Arm A: ORR: 45.1% (95%CI: 37.1–53.3); PFS: 16.5 (95%CI: 5.4–38.1); OS: NA (95%CI: 49.2–NA) |
| | Arm B: mFOLFOX6/FOLFIRI/Bevacizumab/Cetuximab/Pembrolizumab | | Arm B: ORR: 33.1% (95%CI: 25.8–41.1); PFS: 8.2 (95%CI: 6.1–10.2); OS: 27.6 (95%CI: 27.6–NA) |
| Checkpoint inhibitor: Nivolumab + Regorafenib | | | |
| NCT04126733 (*n* = 94) | Regorafenib and Nivolumab | Patients with pMMR or MSS CRC | ORR: 7% (95%CI: 2.4–15.9); PFS: 1.8 (95%CI: 1.8–2.4); OS: 11.9 (95%CI: 7.0 to NA) |
| Checkpoint inhibitor: Nivolumab + Ipilimumab | | | |
| NCT02060188 (*n* = 119) | Arm A: Nivolumab | MSI–H or dMMR mCRC | Arm A: No results posted |
| | Arm B: Nivolumab + Ipilimumab | | Arm B: ORR: 55% (95%CI: 45.2%–63.8%); PFS: 71% (95%CI: 61.4 to 78.7); OS: 85% (95%CI: 77.0 to 90.2) |
| | Arm C: Cobimetinib + Nivolumab + Ipilimumab | | Arm C: No results posted |
| | Arm D: Nivolumab + Daratumumab | | Arm D: No results posted |
| | Arm E: Nivolumab + BMS–986016 | | Arm E: No results posted |
| Checkpoint inhibitor: Atezolizumab | | | |
| NCT02788279 (*n* = 363) | Arm A: Atezolizumab | Patients with mCRC (MSI or MSS status unknown) | Arm A: PFS: 1.94 (95%CI: 1.91 to 2.10); OS: 7.10 (95%CI: 6.05–10.05) |
| | Arm B: Cobimetinib + Atezolizumab | | Arm B: PFS: 1.91 (95%CI: 1.87 to 1.97); OS: 8.87 (95%CI: 7.00–10.61) |
| | Arm C: Regorafenib | | Arm C: PFS: 2 (95%CI: 1.87–3.61); OS: 8.51 (95%CI: 6.41–10.71) |
| NCT01988896 (*n* = 84) | Atezolizumab + Cobimetinib | Patients having BRAF/KRAS mutation in mCRC | ORR: 8% (7/84) (6 patients: MSS, 1 patient: MSI) |
| NCT01633970 (*n* = 10) | Arm A: Atezolizumab + Bevacizumab | No results posted | No results posted |
| | Arm B: Atezolizumab + Bevacizumab + FOLFOX | | |
| | Arm C: Atezolizumab + Carboplatin + Paclitaxel | | |

| | | | |
|---|---|---|---|
| | Arm D: Atezolizumab + Carboplatin + Pemetrexed | | |
| | Arm E: Atezolizumab + Carboplatin + Nab–paclitaxel | | |
| | Arm F: Atezolizumab + Nab–paclitaxel | | |
| Checkpoint inhibitor: Durvalumab + Tremelimumab | | | |
| NCT03122509 (*n* = 25) | Arm A: Durvalumab + Tremelimumab + Radiotherapy | Metastatic Colorectal Cancer (MSI or MSS status unknown) | Arm A: ORR: 8%; Stable response: 12%; Progressive disease: 76% |
| | Arm B: Durvalumab + Tremelimumab + Ablation | | Arm B: No participants enrolled |
| Checkpoint inhibitor: Dostarlimab | | | |
| NCT04165772 (*n* = 200) | Arm A: Dostarlimab | Patients with dMMR rectal adenocarcinoma | Arm A: Complete response: 12 patients (100%; 95%CI: 74–100) |
| | Arm B: Dostarlimab + Capecitabine or 5–FU + IMRT | | Arm B: No participants enrolled |

CRC: Colorectal cancer; mCRC: Metastatic colorectal cancer; MSI: Microsatellite-instability; MSI-H: Microsatellite instability-high; pMMR: Proficient mismatch repair; dMMR: Deficient mismatch repair; MSS: Microsatellite-stable; 5–FU: 5-fluorouracil IMRT: Intensity modulated radiotherapy.

mice, the adaptability of the system, and the deductive formation of CRC images from diverse models remain impressive.

Various transplantation techniques have been introduced over the years to replicate complex TME in mouse models. The subcutaneous injection model has been widely used[166], yet it has limitations, such as creating an ectopic environment and lacking accuracy in mimicking the metastatic spread of cancer[167]. The orthotopic transplantation model has emerged as a promising alternative to address these issues. This involves a precise injection into the intestinal region, such as the cecal wall, colon, or rectum. Among these, the orthotopic CRC model using cecal wall injections has been widely adopted. However, it is essential to recognize that even this model has constraints as it does not faithfully replicate the anatomical location where tumors typically occur in humans and exhibits a microenvironment distinct from the rest of the colon[168].

Genetically engineered mouse models (GEMMs) are based on genetic engineering techniques, particularly the Cre recombinase lox-P system, to simulate tumorigenesis by modifying the structure of the genome[169]. GEMMs, including those incorporating mutations in genes, such as *APC*, *KRAS*, *p53*, and *MSH2*, provide valuable insights into the molecular mechanisms underlying CRC and play a pivotal role in the study of CRC development and therapeutic strategies. APC mutations activate Wnt/b-catenin, causing increased b-catenin levels and tumor development[170,171], restricting tumor growth within the intestines, and mimicking human CRC. In addition, compared with previous single-mutation GEMMs for CRC, transgenic mice established *via* combined APC/KRAS mutations have been shown to represent CRC initiation, progression, and metastasis more accurately into nearby tissues[163,172]. Advantages include specificity in mirroring human CRC growth, representation of TMEs, and the ability to visualize the CRC through colonoscopy. Immunotherapeutic studies using transgenic mice have revealed promising avenues for targeted treatments. However, limitations such as extended metastatic development time and limited colon-specific models warrant further refinement for comprehensive CRC research[173]. As a result, transgenic mice are often ineffective in representing the later stages of tumor development owing to highly variable metastasis[166]. Additionally, there are few current transgenic mouse models that lead to specific CRC development in the colon, as the majority of pre-existing models lead to CRC development within the small intestine or other nearby tissues, contributing to the development of familial cancers rather than specific GEMM mutation-derived CRC[174].

The observation that tumors in mice have a narrower phenotype than human tumors suggests that the mice themselves need to be subtyped before drawing comparisons with human subtypes. This recognition is crucial, especially considering that murine backgrounds are often congenic (with the same genetic makeup) and artificially altered for experimental purposes. In summary, the critical importance of selecting appropriate preclinical models in CRC research requires better understanding. Mouse models are indispensable tools for discovering effective therapeutic interventions. The evaluation of various transplantation methods, with particular emphasis on the orthotopic CRC model *via* cecal wall injection, provides a nuanced understanding of their utility while acknowledging the inherent limitations associated with this approach. This recognition is vital for refining experimental design and interpretation to better translate findings from preclinical studies to human clinical scenarios.

## FUTURE DIRECTIONS

Overall, immunotherapy, particularly with the use of ICIs like pembrolizumab and nivolumab has demonstrated significant clinical benefit in MSI-H CRC, while its efficacy in MSS CRC remains limited[91,130,133,138]. However, even in MSI-H tumors, the upregulation of immune checkpoint proteins, the presence of other immunosuppressive mechanisms within the TME, and the heterogeneity of MSI-H CRC tumors within the primary tumor and across

metastatic sites can contribute to varied responses to immunotherapy. Understanding these factors and further research on the mechanisms of immune resistance in patients with MSI-H CRC are essential to improve the outcomes of immunotherapy in this patient population.

In the field of ICI therapy for CRC, various research avenues to enhance treatment efficacy and broaden its scope of application are being actively pursued. Therefore, there is a need to identify response biomarkers and devise novel treatment approaches to address these challenges. One area of focus is the investigation of combination therapies in which ICIs are used in conjunction with chemotherapy, targeted therapies, and other immunotherapies. Similarly, combining immunotherapy with targeted therapy directed against specific signaling pathways, such as the MAPK pathway, may also improve treatment outcomes[136-138,140,175]. Here, the goal is to enhance the response rates and improve patient survival by leveraging the synergistic effects of different treatment modalities. Another important research avenue is the discovery of reliable biomarkers that can accurately predict patient response to ICIs. Although PD-L1 expression is currently used as a biomarker for some cancer types[176,177], its predictive value for CRC is limited[129,178]. For example, tumors with elevated PD-L1 expression may be more responsive to anti-PD-1/PD-L1 therapy, whereas tumors with low PD-L1 expression may require combination therapy to achieve a response. Similarly, tumors with specific genetic mutations such as BRAF V600E may require targeted therapy in addition to immunotherapy to achieve a response. Mutations in genes such as *JAK1*, *JAK2*, and *B2M* may contribute to treatment resistance[179-181]. Truncating mutations in B2M affect antigen presentation, and can lead to pembrolizumab resistance. Evidence indicates that a high somatic mutational load and neoantigen density are associated with an improved response to immune checkpoint blockade in various cancers. This is attributed to the increased presence of mutation-associated neoantigens, which contribute to greater T cell diversity[182]. Moreover, there are also ongoing research efforts on the development of novel immunotherapeutic agents such as bispecific antibodies, chimeric antigen receptor (CAR) T-cells, and vaccines, which may provide new treatment options for CRC patients[183-188]. Efforts are underway to identify additional biomarkers that assist patient selection and treatment decisions. These targeted therapies, when combined with combination therapies, have shown considerable potential in enhancing treatment efficacy and overcoming drug resistance. By simultaneously targeting different pathways implicated in CRC progression, these approaches aim to maximize therapeutic benefits while minimizing adverse effects. Future research should focus on identifying optimal drug combinations, elucidating synergistic interactions, and refining treatment regimens to improve patient response.

A recent study revealed that immune cells form multicellular hubs in CRC samples that are spatially organized and functionally distinct from the surrounding immune cells[85]. The findings indicated that these immune hubs are composed of different cell types, including T-cells, B-cells, and myeloid cells, and are enriched in specific functional pathways related to the immune response and cell-cell communication. Researchers have also observed that the distribution and composition of immune hubs vary between patients and may be influenced by factors such as tumor stage and treatment history. Furthermore, the findings also demonstrated that the presence of immune hubs was associated with better clinical outcomes in CRC patients, suggesting a crucial role in the immune response to cancer. The authors proposed that targeting immune hubs could be a promising strategy for enhancing the efficacy of immunotherapy in CRC[85].

Additionally, there is a growing interest in exploring the use of ICIs in the early stages of CRC, such as adjuvant therapy following surgery. Early detection and intervention are pivotal for improving CRC outcomes. Emerging technologies, such as liquid biopsies and advanced imaging modalities, hold promise for the detection of CRC at earlier stages when treatment options are more effective[189-191]. Additionally, minimally invasive surgical techniques and organ-preserving approaches offer less invasive alternatives for managing early stage CRC, reducing morbidity, and improving the quality of life of patients[192].

Current research focuses on understanding the intricate mechanisms underlying drug resistance, including genetic mutations, TME interactions, and adaptive signaling pathways. Strategies for overcoming resistance include developing combination therapies that target multiple pathways, repurposing existing drugs, and developing novel agents to evade resistance mechanisms. Precision medicine approaches such as tumor molecular profiling and real-time monitoring facilitate the early detection of resistance mechanisms, allowing prompt adjustments to treatment strategies[193,194]. Furthermore, biomarker research in CRC is rapidly evolving with the aim of identifying molecular signatures crucial for diagnosis, prognosis, and treatment decisions. Biomarkers, such as mutations in genes such as KRAS and BRAF, not only influence tumor behavior, but also affect responses to targeted therapies, notably anti-EGFR antibodies[24]. Additionally, MSI status serves not only as a guide for immunotherapy but also as a valuable prognostic indicator as well. Liquid biopsies offer a noninvasive method to analyze circulating tumor DNA and to monitor disease progression and treatment responses[189]. Epigenetic alterations, such as DNA methylation patterns and microRNA expression profiles, are promising diagnostic and prognostic markers[24,195]. Traditional biomarkers, such as carcinoembryonic antigen, provide insights into tumor burden and treatment response, while gene expression signatures, such as Oncotype DX and ColoPrint, offer predictive value for treatment outcomes and recurrence risk assessment[196]. Integrating these diverse biomarkers into clinical practice can help personalize treatment strategies, optimize patient management, and ultimately enhance the survival outcomes for CRC patients. However, drug resistance remains a significant challenge, compromising the efficacy of chemotherapy, targeted therapies, and immunotherapy[132]. CRC cells develop resistance to chemotherapeutic drugs such as fluoropyrimidines and oxaliplatin through mechanisms such as altered drug metabolism and enhanced drug efflux[197,198]. Similarly, targeted therapies may encounter resistance due to secondary mutations or activation of alternative signaling pathways. Understanding and overcoming these mechanisms are crucial for advancing CRC treatment and improving patient prognosis. Finally, new targets and agents beyond the PD-1/PD-L1 pathway are being investigated. Promising preclinical data to have led to clinical trials of molecules targeting additional T cell checkpoint inhibitors, such as TIM3, LAG3, and TIGIT, in various advanced malignancies, including CRC (Figure 2)[94,199-202]. In addition to immune checkpoint blockade, molecules that enhance T-cell differentiation, survival, and prolif-

eration are being investigated as standalone treatments or in combination with checkpoint inhibitors. These molecules, including CD27, OX40, 4-1BB, and others, act as antibody agonists for the costimulatory group within the TNF receptor superfamily[203-209].

Personalized medicine has revolutionized CRC treatment by tailoring interventions to individual patient characteristics, including genetic and molecular factors. Over the years, personalized medicine has gained traction and treatment approaches have been tailored based on individual patient characteristics. This involves integrating tumor genetic profiling, immune profiling, and other personalized medicine strategies to identify the most effective treatment options for each patient[194,210,211]. Comprehensive genomic profiling and clinical data integration enable the identification of actionable targets and personalized treatment regimens. Artificial intelligence enhances data interpretation and improves the accuracy of treatment response prediction. Liquid biopsies provide a noninvasive method for monitoring disease progression and identifying therapeutic targets. Personalized medicine integrates liquid biopsy-based monitoring into treatment management, allowing for real-time therapy adjustments. This approach promises to optimize treatment strategies and improve the clinical outcomes for CRC patients with CRC. Advancements in biomarker research coupled with efforts to overcome drug resistance and implement personalized medicine offer a multifaceted approach to CRC management that holds great promise for enhancing patient care and outcomes in the future.

Moreover, additional novel strategies for CRC treatment such as mRNA vaccines, TILs therapy, CAR-T therapy, oncolytic virus therapy, bispecific T-cell engagers, and combination strategies aim to improve treatment outcomes by specifically considering metastatic location and TME regulation. Novel agents and therapeutic strategies are being developed to expand the range of options available for immune modulation of this disease. Accordingly, in addition to the development of new biomarkers and therapeutic strategies, there is also a need for better pre-clinical mouse models that can potentially or closely replicate the human CRC microenvironment, thus providing a better opportunity to unmask novel approaches for treatment. As the field of immunotherapy evolves, these directions hold great promise for advancing immune checkpoint inhibitor therapy and other immunotherapeutic approaches for CRC, ultimately resulting in improved patient outcomes.

## CONCLUSION

Immunotherapy has significantly reshaped the CRC treatment landscape, particularly for patients with MSI-H or dMMR tumors. Key accomplishments include the FDA approval of PD-1 inhibitors, such as pembrolizumab and nivolumab, for these patient subsets. Pembrolizumab has demonstrated promising outcomes both as a monotherapy and in combination with chemotherapy, surpassing standard treatments in terms of PFS and ORR[133,134]. Furthermore, combination therapies have shown promise, such as the use of nivolumab with ipilimumab (a CTLA-4 inhibitor), which has demonstrated improved disease control rates[136-138]. Additionally, atezolizumab in combination with Cobimetinib has shown enhanced PFS rates in second-line treatments, although further studies are needed to establish its effects on OS [145]. A recent study established Dostarlimab as a drug with 100% effectiveness against MSI-H or dMMR CRC tumors. Despite the preliminary success of immuno-oncology, challenges persist for CRC treatment, particularly those pertaining to the extension of immunotherapeutic benefits to MSS or pMMR tumors, which commonly exhibit resistance to ICIs. In this regard, irAEs associated with ICIs should be managed effectively, which requires identification of predictive biomarkers and the development of mitigation strategies. Combination therapies, as exemplified by the synergistic effects observed with nivolumab and ipilimumab, require further investigation to optimize their performance and to identify their underlying mechanisms. Exploring novel therapeutic targets beyond immune checkpoint blockade, including targeted therapies and engineered immunotherapies, holds promise for overcoming resistance mechanisms. Addressing these challenges requires interdisciplinary collaboration, ongoing preclinical and clinical research, and rigorous validation through well-controlled trials. By overcoming these obstacles, advancements in CRC treatment can be realized, leading to improved clinical outcomes and enhanced quality of life in affected patients. In conclusion, immunotherapy has revolutionized CRC treatment, resulting in improved outcomes and survival rates in MSI-H or dMMR patients. However, challenges persist in extending these benefits to patients with MSS or pMMR and in the management of irAEs. Future research should focus on optimizing combination therapies, identifying predictive biomarkers, and mitigating treatment-related toxicities to realize the full potential of immunotherapy in CRC management.

## FOOTNOTES

**Co-first authors:** Samantha Sharma and Naresh Singh.

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