Immunol Genet J, Vol. 8, No. 1, 2025, pp.22-38 DOI: https://doi.org/10.18502/igj.v8i1.17991

Review Article

Effectiveness of Immune Checkpoint Inhibitors in the Treatment of Colon Adenocarcinoma

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Received: 13 July 2024; Accepted: 19 October 2024

Abstract
Recently, immunotherapy has emerged as an innovative approach for the management of various cancers, particularly those in advanced stages. Among these novel interventions, immune checkpoint inhibitors have gained significant attention. Given the observed efficacy of these pharmaceutical agents in treating a range of solid tumors, such as metastatic melanoma, they present a promising therapeutic strategy for managing metastatic and advanced colorectal cancer (CRC). The high mortality and incidence rates associated with CRC, coupled with its substantial societal burden, underscore the critical need to assess the effectiveness and safety of this emerging treatment modality. The objective of this research is to evaluate the impact of diverse immune checkpoint inhibitors on the mortality and survival rates of patients with advanced CRC.

Keywords: Colon Neoplasm; Immune Checkpoint Inhibitors; Immunotherapy; Programmed Cell Death 1 Receptor; PD-1

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How to cite this article
Ghannadzadeh Kermani Pour R, Razi S. Effectiveness of Immune Checkpoint Inhibitors in the Treatment of Colon Adenocarcinoma. Immunol Genet J, 2025; 8(1): 22-38. DOI: https://doi.org/10.18502/igj.v8i1.17991

Introduction

Colorectal cancer (CRC) ranks as the second and third most prevalent cancer in women and men, respectively. Considering both genders, it is the third most common cancer, constituting 9.7% of all cancer cases except non-melanoma skin cancer (1). Although CRC was relatively rare as of the 1950s, it now accounts for about 10 percent of cancer-related deaths (1). The rise in CRC incidence can be attributed to factors such as smoking, insufficient physical activity, obesity, and unhealthy diets in Western nations (1). This shift in incidence and mortality rate is evident not only in sporadic cases but also in certain hereditary cancer syndromes. In fact, with the significant decline in Helicobacter pylori infection rates, colorectal cancer has become the predominant manifestation of Lynch syndrome, which previously mainly affected carriers, through gastric cancer (2, 3).

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Currently, the primary approaches to treat CRC are surgery, chemotherapy, radiation therapy, and targeted molecular therapy (4, 5). Despite recent advancements in various treatment methods for CRC (6-8), the annual death toll has remained about one million, constituting 9.4% of all cancer-related fatalities and making it the second leading cause of cancer deaths globally (9). Recently, immunotherapy has experienced swift progress and increased attention in clinical use due to its effective cancer-fighting characteristics, offering further motivation for CRC treatment (10, 11). Unlike conventional therapies, immunotherapy eliminates cancer cells by stimulating the body's antitumor immune response and specifically targets cancer antigens, thereby protecting healthy cells from harm (12-14).

The immune checkpoint (IC) pathways are responsible for regulating T cell responses to maintain immune homeostasis and prevent autoimmunity. However, tumors can exploit these immune-inhibitory pathways to evade immune responses against tumor antigen-specific T cells (15). In the tumor microenvironment of different cancers, ICs and their ligands are frequently overexposed. This overexpression and its role in tumor immune evasion has led to the development of some medications that block interactions between ICs and their ligands, thereby commencing effective antitumor responses. The two most prominent ICs are cytotoxic-T-lymphocyte-associated protein 4 (CTLA-4 or CD152), programmed cell death 1 (PD-1 or CD279) and its ligand (programmed cell death ligand 1 (PD-L1 or CD274 or B7 homolog 1))(16). Several immune checkpoint inhibitors (ICIs), as a type of immunotherapy, have been created and have demonstrated favorable outcomes in some malignancies (15, 17). For instance, it has been proven that the application of ICIs improves the prognosis and survival of patients suffering from non-small-cell lung cancer (18, 19), melanoma (20), and head and neck squamous cell carcinoma (21).

Based on the mutation pattern, CRC is divided into two groups: deficiency mismatch repair/ high levels of microsatellite instability (dMMR/ MSI-H) and proficient mismatch repair/ non-high levels of microsatellite instability (pMMR/ non-MSI-H)(22, 23). Numerous clinical trials have indicated that ICIs display potent and consistent treatment outcomes applying for dMMR/ MSI-H CRC patients. Consequently, the US Food and Drug Administration has authorized various medications, such as Nivolumab (PD-1 blocking agent) and Pembrolizumab (PD-1 blocking agent) for the treatment of these patients (24-26).

In this article, the effectiveness and also adverse effects of various ICIs in the management of CRC are explained. Furthermore, the distinction in efficacy between conventional therapies and ICIs as an alternative or adjuvant treatment is discussed. Finally, the optimal approach for utilizing this medication in the management of CRC, specifically assessing whether it is more advantageous as a monotherapy or in conjunction with adjuvant therapy is presented.

Understanding Colorectal Cancer (CRC): A brief Overview

Epidemiology

The ongoing decrease in cancer mortality rate since 1991 has led to a 29% overall reduction, equating to around 2.9 million fewer cancer-related deaths. This consistent improvement is primarily attributed to the decline in smoking and breakthroughs in treatment, leading to a substantial drop in lung cancer and hematopoietic and lymphoid malignancies. However, the pace of progress for cancers that can be detected early via screening, such as breast cancer, prostate cancer, and CRC, has been slowing down (27).

Following the implementation of population-based CRC screening in the 1990s, the overall CRC incidence has declined by over 35% (28). However, while older adults have experienced significant decreases, the incidence of CRC in younger adults has nearly doubled during the same period (29). In the US, incidence rates for individuals aged 20-49 years have increased rapidly from 8.6 per 100,000 in 1992 to 13.1 per 100,000 in 2016, with the largest increase observed in adults aged 40-49 years (28). Despite significant reductions in mortality rates for older adults due to screening and treatment advancements (30, 31), the mortality rate among younger adults has remained unchanged at 2.8 per 100,000 (32). Even with the general population aging, by 2030, around 11% of colon cancers are expected to affect adults under 50 years of age (33).

Racial and ethnic minorities are more affected by early-onset CRC than non-Hispanic whites. While only 10% to 12% of CRC patients are under 50 years old, the percentage is almost twice as high among non-Hispanic blacks (16%) compared to non-Hispanic whites (9%)(34). Early-onset CRC rates have consistently been higher among blacks, but the difference with whites has recently decreased (35). Additionally, young Hispanics have experienced a rapid increase in incidence rates (36, 37).

The incidence rates of CRC vary geographically, with higher rates in the Mississippi Delta Region and Appalachia (around 14.0 per 100,000) and lower rates in western states (around 9.5 per 100,000)(28). It suggests that the incidence rate of CRC is largely affected by poverty and poor access to healthcare. According to this geographical distribution, certain factors have been linked to the occurrence of CRC, including environmental factors (such as industrial contamination and agricultural runoff)(38), lifestyle-related elements (like obesity and diet)(39) and occupational exposures (like trace elements and mineral dust) (40).

Pathogenesis and Risk Factors

Genetic Factors

Inherited mutations in specific genes are linked to a higher risk of CRC. Roughly, 10% of patients without prior selection possess inherited mutations in genes linked to high and moderate cancer susceptibility (41). Some of these mutations, such as DNA mismatch repair (*MLH1, MSH2, MSH6, PMS2, EPCAM*), *APC, MUTYH, SMAD4, BMPR1A, PTEN,* and *STK11* have been proven to play a noticeable role in CRC development, while the involvement of other genes like *BRCA1, BRCA2, PALB2, ATM, NBN, CHEK2, BARD1, BRIP1*, in CRC's pathogenesis remains uncertain (41-45).

Particularly, younger patients exhibit almost twice the prevalence of harmful inherited mutations, and about half of these mutations are found in DNA mismatch repair (MMR) genes linked to Lynch syndrome (42-44). While comprehensive testing of CRC tumors for MMR deficiency (MMRd) has been crucial in detecting individuals with Lynch syndrome, tumor phenotype can differ significantly, especially in patients carrying inherited mutations in *MSH6* and *PMS2* genes (46).

The growing prevalence of early-onset CRC is not well understood. Variations in clinical manifestations and tumor characteristics prompt the question of whether early-onset CRC is a distinct disease with unique pathogenesis mechanisms compared to CRC in older adults. While family history and hereditary cancer syndromes explain some early-onset CRC cases, lifestyle and environmental factors are likely to play a role as well. The increase in incidence has happened more quickly than can be explained by shifts in population genetics.

Various risk factors have been linked to CRC across all age groups. These factors include smoking (47, 48), alcohol consumption (49), obesity (50), red or processed meat intake (51), non-steroidal anti-inflammatory drugs (such as Aspirin which is thought to decrease risk of CRC)(52), micronutrients (like calcium and vitamin D, which are thought to be protective)(53), diet (54), physical activity (55) and chronic conditions (like diabetes and inflammatory bowel diseases)(56, 57). However, only a limited number of studies have investigated their impact on the risk of early-onset CRC.

Treatment and Prevention

Nowadays, plenty of cancers, including CRC, are being prevented by screening methods. According to a study that has been conducted, screening for CRC has proven to be beneficial for individuals over the age of 50 to reduce mortality and improve the long-term prognosis (58). To determine the most effective approach among all suggested approaches, plenty of factors must be taken into account, including the increase in life-years gained (LYG) as a result of screening, the financial burden, and the potential complications associated with screening. This study has revealed that there are a limited number of screening strategies that are suitable. These strategies include colonoscopy every 10 years, annual fecal immunochemical testing (FIT), sigmoidoscopy every 10 years with annual FIT, and computed tomographic colonography (CTC) every 5 years performed between the ages of 50 and 75. Based on the USPSTF's decision regarding CRC screening, all these models have exhibited comparable LYG

and a similar equilibrium between the advantages and the burden associated with screening (58). Besides, several studies have suggested that initiating the screening process from 45 instead of 50 meets a fair balance between benefits and complications (59, 60).

Roles of Immune Cells and Immune Mediators in the Pathogenesis of Colon Cancer

Individuals diagnosed with inflammatory bowel diseases, including ulcerative colitis and Crohn's disease exhibit an elevated propensity for colon cancer development, thereby substantiating the correlation between chronic inflammation and tumorigenesis (61). Furthermore, it is evident that inflammation also propels the progression of colon cancers that are not a consequence of inflammatory bowel disease. This is supported by studies demonstrating that consistent administration of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces mortality rates associated with sporadic colon cancer and induces regression of adenomas in familial adenomatous polyposis (FAP) patients who possess a mutation in the *Apc* gene (62). Consequently, colorectal cancer serves as a model for the association between inflammation and cancer.

Inflammation is propelled by soluble mediators, including cytokines and chemokines, which can either be secreted by the tumor cells themselves or, more commonly, by cells such as macrophages and mast cells that are recruited to the tumor microenvironment (63). Experimental depletion of mast cells or macrophages led to significant remission of Apc-initiated intestinal polyps in murine models, thereby validating the involvement of immune cells and their soluble mediators in both the onset and advancement of intestinal cancer (64, 65). Inflammatory cytokines and chemokines stimulate tumor cell proliferation, disrupt their differentiation, and enhance the survival of cancer cells.

Solid neoplasms are often permeated by various immune cells, including T cells, B cells, natural killer (NK) cells, mast cells, and macrophages (63). While both epidemiological studies and experimental data recommend that inflammation expedites tumor advancement, it's also recognized that immune cells can foster anti-tumor immunity (63). The type and quantity of infiltrating cells, along with the spectrum of soluble mediators they produce, balance the dichotomy between tumor-promoting inflammation and anti-tumor immunity (63). Examination of a substantial cohort of colorectal cancer (CRC) patients substantiated that the presence of T helper (Th)1 cells (and the expression of Th1-specific genes, such as *T-bet, IFNγ*) within tumors correlates with the absence of metastatic invasion, tumor recurrence, and improved prognosis (66, 67).

A high number of CD3 infiltrated cells within the tumor core or at the invasive tumor margin was noticeably associated with enhanced survival in CRC patients (66). Exploration of over 400 CRC patients approved that infiltration by CD8 T cells was associated with the absence of metastasis (67). Similarly, metastatic tumors exhibited a reduced number of CD8 T cells, and CRC with microsatellite instability (MSI), a factor associated with a favorable prognosis, demonstrated high infiltration of CD8 T cells (68). Lastly, colorectal cancer patients exhibiting high-grade inflammation demonstrated higher 5-year survival rates (69).

In line with the capacity of macrophages to regulate tumor growth, inhibit anti-tumor responses, stimulate angiogenesis, and facilitate tumor invasion and metastasis, an increased macrophage density is linked with unfavorable prognosis in breast, prostate, bladder, and cervical cancers (70-75). However, the prognostic implications of macrophage infiltration in colon cancer have been reported with varying conclusions (76-78). The latest investigation on the effect of macrophages on the tumor microenvironment reported that the proliferation of M1 macrophages led to tumor suppression. Unlike M1 macrophages, the M2 type was related to tumor progression (79). These findings indicate that the assessment of immune cell infiltration should be a crucial component in determining the prognosis of CRC (80).

The serum concentrations of various cytokines, including tumor necrosis factor (TNF)α, interleukin (IL)-8, IL-6, and vascular endothelial growth factor (VEGF), are found to be elevated in patients with CRC, and certain studies have proposed that elevated plasma levels of these cytokines may hold prognostic significance (81).

The invasiveness of cancer cells and tumor growth might be augmented by cytokines secreted from activated cancer stroma through activating carcinogenic signaling pathways within these cells (82). NF-κB could be activated by TNFα and IL-1β, and STAT3 by IL-6 (82). Epithelial cells express toll-like receptors (TLRs), which determine both commensal and pathogen-associated molecular patterns (PAMPs) from the gut microbiota. TLRs are supposed to function quite precisely. These receptors should be immunologically tolerant to commensal bacteria, while they should be responsive to pathogenic microorganisms. Therefore, an impaired function of TLR's immune response may lead to chronic inflammation and colon cancer (83). Various pathogens are able to stimulate TLR2 or TLR4 on cancer cells triggering the expression of a multitude of cytokines and chemokines that subsequently promote tumor cell growth (84). Activation of TLR signaling is associated with the stimulation of the NF-κB pathway, which can consequently lead to enhanced survival of tumor cells, resulting in chemoresistance (84). Besides, TLR4 has been demonstrated to improve colitis-associated colon cancer (84).

Elevated levels of IL-6 are observed in colorectal cancer patients in comparison to healthy individuals, and these IL-6 levels exhibit correlations with tumor stage, size, metastasis, and patient survival (81). Similarly, Crohn's disease patients also display increased serum levels of IL-6, which aligns with the heightened production of IL-6 by mononuclear cells located in lamina propria from individuals with inflammatory bowel disease (85, 86). The reliance of tumor cells on the inflammatory stroma suggests that targeting the tumor microenvironment holds promise for both preventive and therapeutic interventions. It has to be noted that based on the role of the immune system and mediators, including TNFα, IL-6, and IL-1β, as well as transcription factors necessary for their signaling, such as NF-κB and STATs, are novel targets for anti-tumor therapeutic strategies (63).

Discussion about Different Types of Immune Checkpoint Inhibitors and Their Diversity

Utilizing the body's capacity to initiate an immune reaction against cancerous cells has become a widely recognized approach to cancer treatment. The immune system's potential to aid in cancer therapy has been acknowledged for an extended period, but earlier efforts to employ this potential were not broadly adopted (87). In recent times, there has been a growing interest in this approach due to the remarkable achievements observed in melanoma, non-small-cell lung cancer, genitourinary cancers, and others (87). As the comprehension of the interplay between tumors and the immune system expands, innovative treatments with intricate modes of action are becoming standard care. Following the initial success of Ipilimumab in metastatic melanoma (88, 89), ICIs have transformed the landscape of systemic therapy for advanced conditions in numerous solid tumors (90-93).

IL-2, a cytokine influencing the cytotoxic function of T cells and the preservation of T-regulatory cells, was among the first immunotherapies applied in advanced disease (94). While the reaction rates were moderate, long-lasting responses were noted, indicating that certain patients might have been "cured (94)." However, the extensive application of IL-2 was restricted due to considerable toxicity. Ipilimumab, a fully humanized immunoglobulin G monoclonal antibody, was the first checkpoint inhibitor to show a survival advantage in metastatic melanoma (89). By blocking the CTLA-4 protein from connecting with its ligand, Ipilimumab ultimately inhibits its suppressive impact on T-cell activation.

Expanding on those findings, anti-PD-1 antibodies were created as the subsequent immune checkpoint inhibitors, and these agents have persistently enhanced outcomes while exhibiting reduced toxicity (95). The PD-1 receptor protein, present on T cells, B cells, and NK cells, connects with its ligand (PD-L1), which is expressed on stromal and tumor cells to suppress the immune response (87). Pembrolizumab and Nivolumab, both anti-PD-1 antibodies, have been linked to survival advantages in second-line treatment for melanoma and eventually, in first-line therapy as monotherapy (96-98).

The excessive synthesis of PD-L1 in a variety of neoplasms, including those located in the breast, lung, melanoma, liver, head and neck, and colon, may facilitate these malignant cells to suppress

the host's immune reaction against the neoplasm, frequently associated with a poor prognosis (99-101). The existence of PD-1 pathway constituents on malignant cells and their pivotal function in assisting the neoplasm in eluding the immune system renders this pathway an attractive objective for therapeutic intervention.

Pembrolizumab, a highly selective humanized immunoglobulin G4/κ monoclonal antibody, is engineered to directly impede the interaction between PD-1 and PD-L1/PD-L2 by adhering to PD-1. This pharmaceutical agent has demonstrated substantial antineoplastic activity and a favorable safety profile across a range of neoplasms and is presently sanctioned in over 60 nations for the management of one or more advanced malignancies. There have been reports of a correlation between the therapeutic response to PD-L1 blockade and the level of PD-L1 expression in the tumor prior to treatment (102). However, therapeutic responses have also been determined in patients with tumors that do not express PD-L1 (103).

Exploring the Effectiveness of Immune Checkpoint Inhibitors in the Treatment of Colon Cancer

In a localized setting, colorectal cancer can be managed through curative surgery followed by chemotherapy, often leading to a positive outcome (104). However, a significant number of individuals are first identified with metastatic CRC (mCRC) due to the lack of symptoms in the early stages (105). Regrettably, existing treatment modalities fail to deliver substantial therapeutic benefits, leading to a grim prognosis for the majority of mCRC patients (106). The 5-year survival rate stands at a mere 14% (24). Over the past ten years, the field of immunotherapy has seen rapid advancements and garnered significant interest due to its remarkable antitumor efficacy in clinical settings. This progress offers potential and optimism for patients battling advanced cancer and mCRC. Immunotherapy consistently targets antigens specific to cancer cells, thereby safeguarding normal cells from assault. Consequently, immunotherapy could represent a novel therapeutic option for mCRC (87, 107).

Inhibitors of PD-1/PD-L1 obstruct T cell dysfunction and apoptosis, instead promoting T cell activation. This offers a fresh therapeutic approach to the management of cancers, especially CRCs (108). In the context of mCRC, the infiltration of T cells into the tumor environment has been historically linked to positive outcomes, implying that PD-1/PD-L1 inhibitors could be potent against mCRC (7). As previously mentioned, CRC is divided into two categories based on the mutation pattern: tumors exhibiting mismatch-repair deficiency and high microsatellite instability (dMMR-MSI-H CRC) and tumors demonstrating proficient mismatch repair and low microsatellite instability (pMMR-MSI-L CRC)(109, 110). MSI, a condition resulting from the insufficiency of the MMR mechanism, is a key characteristic of certain tumors (111). These MSI/dMMR tumors exhibit a high mutational load, leading to the generation of highly immunogenic neoantigens due to frameshift mutations, and are accompanied by a significant infiltration of cytotoxic T lymphocytes. The tumor cells, in response, upregulate immune checkpoints to shield themselves from this adverse microenvironment (111). It is observed that sporadic MSI/MMR-deficient (dMMR) CRCs often co-occur with the *BRAF* V600E mutation, a factor associated with poor prognosis (112).

Approximately, 5% of mCRC exhibit MSI/dMMR, and about one-third of these are found to harbor the *BRAF* V600E mutation (112). dMMR-MSI-H CRC constitutes approximately 15% of colorectal cancer instances, and around 4-5% of mCRC patients present with this tumor type (110, 113-115). Numerous clinical trials have indicated that dMMR-MSI-H CRC may exhibit higher sensitivity to ICIs, including PD-1/PD-L1 inhibitors. Consequently, the Food and Drug Administration has approved immune checkpoint therapy as a therapeutic modality for dMMR-MSI-H CRC (114, 116, 117). Regarding the fact that MSI-H-dMMR mCRC has been detected in a minority of mCRC patients, PD-1/PD-L1 inhibitors have been used as an adjuvant therapy to yield the best therapeutic outcome (104).

Several studies such as randomized clinical trials, retrospective studies, prospective studies and case series studies have been conducted exploring the effectiveness and adverse effects of ICIs as a novel treatment for CRC. Most of these researches

focused on PD-1 inhibitors rather than PD-L1 inhibitors. Numerous investigations have examined the effectiveness of ICIs in mCRC. A study led by Overman MJ (116) demonstrated that Nivolumab, an anti PD-1, exhibits a significant and enduring response rate, extending survival beyond the expected median survival for patients with deficient DNA mismatch repair and high microsatellite instability (dMMR/MSI-H) mCRC. Noteworthy enhancements were noted in patient-reported outcomes, and the safety profile of Nivolumab was consistent with experiences in other tumor types (118-120), with no novel safety concerns identified. These findings propose Nivolumab as a novel therapeutic option for patients with dMMR/MSI-H mCRC. The study (116) also indicates that PD-L1 expression may not serve as a predictive biomarker in these patients. The study's results suggest that Nivolumab might demonstrate superior efficacy compared to conventional treatments in patients with BRAF-mutant tumors, who generally have a poor prognosis (116). The evidence from this study implies that MMR/MSI status could be utilized to pinpoint patients who would benefit from Nivolumab immunotherapy. These findings strongly support the notion that dMMR/MSI-H is a marker for response to PD-1 checkpoint inhibition in mCRC (116).

Nivolumab demonstrated a favorable tolerability profile in patients with dMMR/MSI-H mCRC (116), barring a small subset of patients who exhibited increased levels of lipase and amylase. This is in alignment with the established safety profile of Nivolumab in other solid tumors (118-120). The majority of adverse events (AEs) were manageable and resolved without incident, with no new safety concerns identified. The occurrence of gastrointestinal complications, such as diarrhea and colitis did not seem to be higher in this patient population compared to those with other solid tumors (120). A recent study by O'Neil BH *et al.* has further explored the impact of Pembrolizumab, an anti PD-1 agent, on the treatment of advanced CRC (121). The findings suggest that Pembrolizumab monotherapy was generally well-received in patients with advanced CRC who had undergone extensive prior treatment and exhibited PD-L1 positivity (121). The safety profile observed in this group was in line with prior experiences of Pembrolizumab usage in advanced solid tumors (122). The study noted antitumor activity in cases of microsatellite instability-high (MSI-H) CRC but not in microsatellite stable (MSS) CRC, even in patients preselected for PD-L1 expression, though not at equivalent levels (122). These findings align with those from the phase II KEYNOTE-016 study (123), which also involved Pembrolizumab treatment in patients with MSI-H CRC and non-CRC tumors. In summary, the data from this small patient group, part of the exploratory phase Ib KEYNOTE-028 trial (124), suggest that while Pembrolizumab monotherapy has a tolerable safety profile, its antitumor activity is limited in patients with heavily pretreated PD-L1–positive advanced CRC (121).

Le DT *et al.* have conducted further research into the impact of Pembrolizumab, an anti PD-1 agent, on the treatment of advanced CRC (125). The findings from the KEYNOTE-164 study (126) corroborate that Pembrolizumab yields sustained responses and possesses a manageable safety profile in patients with previously treated microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) advanced or metastatic CRC (125). Pembrolizumab has received approval for use in patients with previously treated MSI-H/dMMR CRC following treatment with Fluoropyrimidine, Oxaliplatin, and Irinotecan, as well as in patients with MSI-H/dMMR non-CRC solid tumors after one or more prior therapies, irrespective of tumor type or origin (125). In conclusion, the current study's data affirm the long-lasting clinical advantage of Pembrolizumab in patients with previously treated MSI-H/dMMR metastatic CRC, marking Pembrolizumab as a significant addition to the treatment alternatives for these patients (125). Additionally, a study led by Le DT *et al.* examined the effect of mismatch-repair deficiency on the response to PD-1 blockade (127).

The findings from this limited-scale phase 2 trial of Pembrolizumab for the treatment of both mismatch repair-deficient and proficient tumors lend credence to the theory that tumors deficient in mismatch repair exhibit a higher responsiveness to PD-1 blockade compared to those proficient in mismatch repair (127). Mismatch repair deficiency is a characteristic found in a wide array of cancers, including colorectal, uterine, gastric, biliary tract, pancreatic, ovarian, prostate, and small intestinal cancers (128-136). It is plausi-

ble that patients with mismatch repair-deficient tumors of these types might also derive benefit from anti-PD-1 therapy. Similarly, patients with tumors that harbor other DNA repair deficiencies, such as mutations in *POLD*, *POLE*, or *MYH* may also potentially benefit from this therapeutic approach (128, 137, 138). Several significant observations were made throughout the duration of this study. Firstly, alterations in serum levels of protein biomarkers, such as carcinoembryonic antigen (CEA) were found to correlate with clinical benefit following a single dose of therapy. Reductions in CEA levels were observed to precede objective radiographic evidence of treatment benefit by several months, suggesting that other biomarkers, such as circulating tumor DNA might also serve as effective alternative markers of early response (139, 140).

Secondly, the findings indicate that the analysis of tumor genomes can provide valuable guidance for immunotherapy. They bolster the perspective that the quantity and nature of alterations could be instrumental in assessing the potential efficacy of ICIs, even in cancers proficient in mismatch repair (136, 141, 142). Most crucially, the results demonstrate a strategy for treating a distinct category of tumors that is exclusively based on genetic status, irrespective of the underlying tumor type (127). In summary, the effect of ICIs on the treatment of advanced CRC depends on the status of mismatch repair mutations. According to the studies mentioned above, ICIs do not have a remarkable impact on pMMR-MSI-L cases, but a significant influence was demonstrated in dMMR-MSI-H patients. No critical and life-threatening adverse effect was detected secondary to the application of these novel therapies. A meta-analysis showed that PD-1 inhibitors were more effective than PD-L1 inhibitors, but it was postulated that some confounding biases may have affected this result (104). Moreover, this study concluded that PD-1 inhibitor monotherapy had fewer adverse events than either combination therapy or PD-L1 inhibitor monotherapy (104).

Comparison of Monotherapy by Immune Checkpoint Inhibitors and Combination Therapy

Neoplasms exhibiting MSI have been linked to potent reactions to PD-1 inhibitors (114, 116, 143, 144). However, microsatellite stable tumors, characterized by a low mutational load and minimal immune cell infiltration, continue to exhibit resistance to immunotherapeutic interventions (127, 145). Consequently, the pivotal challenge lies in devising innovative strategies to restructure the immunosuppressive microenvironment with the aim of targeting pMMR/MSS mCRC through either ICIs alone or in combination with other therapeutic modalities (146).

The combination of PD-1 blockade with VEGF inhibition has been explored in several clinical trials, but randomized studies in mCRC have not demonstrated a significant enhancement in progression-free survival (PFS) or overall survival (OS) with this combination (147, 148).

Regorafenib, an antiangiogenic multikinase inhibitor, has recently demonstrated immunomodulatory effects in combination with ICIs in a murine model of mCRC, potentially through aiming both the VEGF pathway and other immune-modulating molecules like colony-stimulating factor 1 receptor (CSF1R) (149). Some clinical studies suggest that the combination of ICIs with antiangiogenic drugs could augment the effectiveness of immunotherapy for neoplasms, such as melanoma (150) and renal cancer (151).

In a recent Japanese trial, the REGONIVO study (152), reported a potent response rate and extended PFS in Japanese patients with MSS mCRC, who were treated with the addition of Regorafenib, a tyrosine kinase inhibitor (TKI), to Nivolumab. However, the REGONIVO study was limited by its small sample size. In this study, the most common AEs of grade 3 or higher were rash (12%), proteinuria (12%), and palmar-plantar erythrodysesthesia (10%)(152). As a result, numerous studies are being conducted to investigate the potential of combination immunotherapies to transform MSS CRC into an immune-responsive malignancy. One such study was undertaken by Wang C *et al.* (153), with the aim of examining the effectiveness of a combination therapy involving Regorafenib and Nivolumab in patients with advanced CRC. However, this study reported no observable response in patients treated with this regimen (153).

The study suggested that the pattern of metastatic disease could influence the responsiveness

to PD-1/PD-L1 inhibitors (153). Previous clinical studies had indicated that patients with melanoma and non-small cell lung cancer who had liver metastases were less likely to respond and had a shorter survival time when treated with PD-1 inhibitors compared to patients with melanoma and non-small cell lung cancer without liver metastases (154).

Biopsies of tumors from these studies demonstrated a reduced infiltration of $CD8^+$ T cells in the primary tumor of the group with liver metastasis compared to the group without liver metastasis (153, 154). Furthermore, patients with liver metastases exhibited lower levels of $CD8^+$ T cells in extrahepatic distant metastases, implying that these patients may experience a weakened antitumor immune response and may be less likely to benefit from checkpoint inhibition (153). It is also plausible that liver metastases exert a systemic immunosuppressive effect, thereby diminishing the immune response both within and outside the liver in patients with solid tumors (153). The observation that liver allografts are accepted without the necessity for histocompatibility demonstrates the liver's ability to develop peripheral immune tolerance in immune-competent recipients (153, 155). Moreover, liver transplantation appears to increase the tolerance of liver recipients to transplants of other organs from the same donor, indicating that liver allografts may induce systematical suppression in the immune system (156, 157).

From a mechanistic perspective, this occurrence could be attributed to the elimination of activated $CD8^+$ T cells (158, 159), inadequate activation of $CD8^+$ and $CD4^+$ T cells (160, 161), and the stimulation of regulatory T cells induced by the liver (162). Consequently, this study proposes that liver metastases exploit the liver's immune tolerance, which induces systemic antitumor immune response suppression and reduces the effectiveness of PD-1 inhibitors (153). Additionally, notable racial differences exist between the REGONIVO trial and this study, as all participants in the REGONIVO trial were of Japanese descent (152, 153). In a separate investigation, in contrast to the Japanese REGONIVO study that demonstrated a high objective tumor response, no objective response was retrospectively identified with Regorafenib and anti-PD-1 antibody combination therapy in this study, indicating limited clinical efficacy in unselected Chinese patients with pMMR/MSS mCRC (146). However, this combination method showed significant benefits in terms of Disease Control Rate (DCR) and Progression-Free Survival (PFS) with a reasonable safety profile (146), a retrospective trial lately conducted in the USA by Wang C *et al*. reported a discouraging PFS and DCR (153).

Certain studies have indicated that the combination of anti-PD-1 antibody SHR-1210 and apatinib, a drug that targets VEGFR-2 and inhibits angiogenesis, had a significant safety and potency in treating invasive solid tumors (163, 164). Subsequently, a study was designed by Ren C *et al*. to investigate whether the combination of anti-PD-1 antibody SHR-1210 and Apatinib was effective and safe (165).

In summary, the combination of Apatinib and SHR-1210 did not demonstrate remarkable treatment efficacy for MSS mCRC and was associated with significant adverse effects (165). It is evident that the high dosage of Apatinib was the primary factor contributing to the severe AEs observed in this trial, as lower dosages were utilized in other studies (164, 165). According to the findings of this study, compared to SHR-1210 alone or Apatinib monotherapy, the combination therapy of SHR-1210 and Apatinib exhibited a higher incidence of AEs (163, 165).

Conclusion

In summary, ICIs represent an innovative treatment approach for advanced solid tumors, including mCRC. Numerous investigations have been undertaken to understand the impact and potential side effects of ICIs on advanced CRC. Traditional treatment methods have shown limited effectiveness, with survival rates remaining disappointingly low. However, research indicates that the use of ICIs as an adjunctive therapy can enhance survival duration and improve prognosis.

The efficacy of these drugs is contingent upon several factors, one of which is the mutation pattern of the tumor cells. CRC can be classified into two categories based on this mutation pattern: tumors with dMMR-MSI-H CRC, and tumors with pMMR-MSI-L CRC. Research suggests that due to the high expression of various antigens, such as PD-L1 on tumor cells in dM-

MR-MSI-H CRCs, these CRCs are heavily infiltrated by immune cells. However, the expression of immune checkpoints like PD-L1 allows these cells to evade the immune system. Consequently, the therapeutic effect of ICIs on advanced CRC is largely dependent on the status of mismatch repair mutations. Studies indicate that ICIs do not significantly affect pMMR-MSI-L cases, but they have a substantial impact on dMMR-MSI-H patients. This observation is not exclusive to CRC but is also evident in other dMMR-MSI-H solid cancers. Additionally, the pattern of metastatic organ involvement is considered critical. Tumors that involve the liver are resistant to ICIs due to peripheral immune tolerance induced by the liver, whereas tumors without liver metastasis show a better response. No severe or life-threatening side effects have been reported as a result of these innovative therapies. To enhance the effectiveness of ICIs in pMMR-MSI-L patients, a combination of ICIs with other drugs, such as VEGF and VEGF receptor inhibitors and TKIs, has been attempted, but the outcomes have been inconsistent and contradictory. Therefore, additional trials and experiments are required.

Conflict of Interest

The authors declare no competing interests.

References

1. Kuipers EJ, et al. Colorectal cancer. Nat Rev Dis Primers. 2015;1:15065.
2. Capelle LG, Van Grieken NC, Lingsma HF, Steyerberg EW, Klokman WJ, Bruno MJ, et al. Risk and epidemiological time trends of gastric cancer in Lynch syndrome carriers in the Netherlands. Gastroenterology. 2010;138(2):487–92.
3. Vasen HF, Tomlinson I, Castells A. Clinical management of hereditary colorectal cancer syndromes. Nat Rev Gastroenterol Hepatol. 2015;12(2):88–97.
4. Yang Z, Deng W, Zhang X, An Y, Liu Y, Yao H, et al. Opportunities and challenges of nanoparticles in digestive tumours as anti-angiogenic therapies. Front Oncol. 2022;11:5581.
5. Akgül Ö, Çetinkaya E, Ersöz Ş, Tez M. Role of surgery in colorectal cancer liver metastases. World J Gastroenterol. 2014;20(20):6113–28.
6. Frank MH, Wilson BJ, Gold JS, Frank NY. Clinical implications of colorectal cancer stem cells in the age of single-cell omics and targeted therapies. Gastroenterology. 2021;160(6):1947–60.
7. Ganesh K, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH, et al. Immunotherapy in colorectal cancer: rationale, challenges and potential. Nat Rev Gastroenterol Hepatol. 2019;16(6):361–75.
8. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. JAMA. 2021;325(7):669–85.
9. Qiu H, Cao S, Xu R. Cancer incidence, mortality, and burden in China: a time-trend analysis and comparison with the United States and United Kingdom based on global epidemiological data released in 2020. Cancer Commun. 2021;41(10):1037–48.
10. Du W, Frankel TL, Green M, Zou W. IFNγ signaling integrity in colorectal cancer immunity and immunotherapy. Cell Mol Immunol. 2022;19(1):23–32.
11. Schürch CM, Bhate SS, Barlow GL, Phillips DJ, Noti L, Zlobec I, et al. Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front. Cell. 2020;182(5):1341–59.e19.
12. Kalafati L, Kourtzelis I, Schulte-Schrepping J, Li X, Hatzioannou A, Grinenko T, et al. Innate immune training of granulopoiesis promotes anti-tumor activity. Cell. 2020;183(3):771–85.e12.
13. Sahin U, Türeci Ö. Personalized vaccines for cancer immunotherapy. Science. 2018;359(6382):1355–60.
14. Leko V, Rosenberg SA. Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors. Cancer Cell. 2020;38(4):454–72.
15. Toor SM, Nair VS, Decock J, Elkord E, editors. Immune checkpoints in the tumor microenvironment. Semin Cancer Biol. 2020; Elsevier.
16. Byun DJ, Wolchok JD, Rosenberg LM, Girotra M. Cancer immunotherapy—immune checkpoint blockade and associated endocrinopathies. Nat Rev Endocrinol. 2017;13(4):195–207.
17. Saleh R, Elkord E. Treg-mediated acquired resistance to immune checkpoint inhibitors. Cancer Lett. 2019;457:168–79.
18. Peng M, Li X, Lei G, Weng YM, Hu MX, Song QB. The efficacy and safety of immune checkpoint inhibitor combination therapy in lung cancer: a systematic review and meta-analysis. Onco Targets Ther. 2018:7369–83.
19. Meng LF, Huang JF, Luo PH, Huang SX, Wang HL. The efficacy and safety of immune checkpoint inhibitor plus chemotherapy in patients with advanced non-small-cell lung cancer: a meta-analysis. Invest New Drugs. 2022;40(4):810–7.
20. Eggermont AM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, et al. Adjuvant pembrolizum-

ab versus placebo in resected stage III melanoma. N Engl J Med. 2018;378(19):1789–801.
21. Cohen EE, Soulières D, Le Tourneau C, Dinis J, Licitra L, Ahn MJ, et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet. 2019;393(10167):156–67.
22. Wensink E, Bond M, Kucukkose E, May A, Vink G, Koopman M, et al. A review of the sensitivity of metastatic colorectal cancer patients with deficient mismatch repair to standard-of-care chemotherapy and monoclonal antibodies, with recommendations for future research. Cancer Treat Rev. 2021;95:102174.
23. Quiroga D, Lyerly HK, Morse MA. Deficient mismatch repair and the role of immunotherapy in metastatic colorectal cancer. Curr Treat Options Oncol. 2016;17(12):1–16.
24. Lichtenstern CR, Ngu RK, Shalapour S, Karin M. Immunotherapy, inflammation and colorectal cancer. Cells. 2020;9(3):618.
25. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab in microsatellite-instability–high advanced colorectal cancer. N Engl J Med. 2020;383(23):2207–18.
26. Casak SJ, Marcus L, Fashoyin-Aje L, Mushti SL, Cheng J, Shen YL, et al. FDA approval summary: pembrolizumab for the first-line treatment of patients with MSI-H/dMMR advanced unresectable or metastatic colorectal carcinoma. Clin Cancer Res. 2021;27(17):4680–4.
27. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7–34.
28. Stoffel EM, Murphy CC. Epidemiology and mechanisms of the increasing incidence of colon and rectal cancers in young adults. Gastroenterology. 2020;158(2):341–53.
29. Siegel RL, Fedewa SA, Anderson WF, Miller KD, Ma J, Rosenberg PS, et al. Colorectal cancer incidence patterns in the United States, 1974–2013. J Natl Cancer Inst. 2017;109(8):djw322.
30. Meester RG, Doubeni CA, Lansdorp-Vogelaar I, Goede SL, Levin TR, Quinn VP, et al. Colorectal cancer deaths attributable to nonuse of screening in the United States. Ann Epidemiol. 2015;25(3):208–13.e1.
31. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975–2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer. 2010;116(3):544–73.
32. Siegel RL, Miller KD, Jemal A. Colorectal cancer mortality rates in adults aged 20 to 54 years in the United States, 1970–2014. JAMA. 2017;318(6):572–4.
33. Bailey CE, Hu CY, You YN, Bednarski BK, Rodriguez-Bigas MA, Skibber JM, et al. Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975–2010. JAMA Surg. 2015;150(1):17–22.
34. Murphy CC, Sanoff HK, Stitzenberg KB, Baron JA, Lund JL, Sandler RS. Patterns of sociodemographic and clinicopathologic characteristics of stages II and III colorectal cancer patients by age: examining potential mechanisms of young-onset disease. J Cancer Epidemiol. 2017;2017:1–9.
35. Murphy CC, Wallace K, Sandler RS, Baron JA. Racial disparities in incidence of young-onset colorectal cancer and patient survival. Gastroenterology. 2019;156(4):958–65.
36. Garcia S, Pruitt SL, Singal AG, Murphy CC. Colorectal cancer incidence among Hispanics and non-Hispanic Whites in the United States. Cancer Causes Control. 2018;29(10):1039–46.
37. Wang DY, Thrift AP, Zarrin-Khameh N, Wichmann A, Armstrong GN, Thompson PA, et al. Rising incidence of colorectal cancer among young Hispanics in Texas. J Clin Gastroenterol. 2017;51(1):34–40.
38. James W, Jia C, Kedia S. Uneven magnitude of disparities in cancer risks from air toxics. Int J Environ Res Public Health. 2012;9(12):4365–85.
39. Zhang Z, Zhang L, Penman A, May W. Using small-area estimation method to calculate county-level prevalence of obesity in Mississippi, 2007–2009. Prev Chronic Dis. 2011;8(4):A85.
40. Shi X, Johnson N, Shelton BJ, Hopenhayn C, Tucker TT, Unrine JM, et al. Concentrations of arsenic, chromium, and nickel in toenail samples from Appalachian Kentucky residents. J Environ Pathol Toxicol Oncol. 2011;30(3):213–23.
41. Yurgelun MB, Kulke MH, Fuchs CS, Allen BA, Uno H, Hornick JL, et al. Cancer susceptibility gene mutations in individuals with colorectal cancer. J Clin Oncol. 2017;35(10):1086–95.
42. Stoffel EM, Koeppe E, Everett J, Ulintz P, Kiel M, Osborne J, et al. Germline genetic features of young individuals with colorectal cancer. Gastroenterology. 2018;154(4):897–905.e1.
43. Pearlman R, Frankel WL, Swanson B, Zhao W, Yilmaz A, Miller K, et al. Prevalence and spectrum of germline cancer susceptibility gene mutations among patients with early-onset colorectal cancer. JAMA Oncol. 2017;3(4):464–71.
44. Mork ME, You YN, Ying J, Bannon SA, Lynch PM, Rodriguez-Bigas MA, et al. High prevalence of

hereditary cancer syndromes in adolescents and young adults with colorectal cancer. J Clin Oncol. 2015;33(31):3544–50.
45. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol. 2015;110(2):223–62.
46. Durno CA, Holter S, Sherman PM, Gallinger S. The gastrointestinal phenotype of germline biallelic mismatch repair gene mutations. Am J Gastroenterol. 2010;105(11):2449–56.
47. Giovannucci E. An updated review of the epidemiological evidence that cigarette smoking increases risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2001;10(7):725–31.
48. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. JAMA. 2008;300(23):2765–78.
49. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose–response meta-analysis of published studies. Ann Oncol. 2011;22(9):1958–72.
50. Bardou M, Barkun AN, Martel M. Obesity and colorectal cancer. Gut. 2013;62(6):933–47.
51. Chan DS, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, et al. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. PLoS One. 2011;6(6):e20456.
52. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010;376(9754):1741–50.
53. Baron J, Beach M, Mandel J, Van Stolk R, Haile R, Sandler R, et al. Calcium supplements for the prevention of colorectal adenomas. N Engl J Med. 1999;340(2):101–7.
54. Fung T, Hu FB, Fuchs C, Giovannucci E, Hunter DJ, Stampfer MJ, et al. Major dietary patterns and the risk of colorectal cancer in women. Arch Intern Med. 2003;163(3):309–14.
55. Wolin KY, Yan Y, Colditz GA, Lee IM. Physical activity and colon cancer prevention: a meta-analysis. Br J Cancer. 2009;100(4):611–6.
56. Deng L, Gui Z, Zhao L, Wang J, Shen L. Diabetes mellitus and the incidence of colorectal cancer: an updated systematic review and meta-analysis. Dig Dis Sci. 2012;57(6):1576–85.
57. Bernstein CN, Blanchard JF, Kliewer E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer. 2001;91(4):854–62.
58. Knudsen AB, Zauber AG, Rutter CM, Naber SK, Doria-Rose VP, Pabiniak C, et al. Estimation of benefits, burden, and harms of colorectal cancer screening strategies: modeling study for the US Preventive Services Task Force. JAMA. 2016;315(23):2595–609.
59. Knudsen AB, Rutter CM, Peterse EF, Lietz AP, Seguin CL, Meester RG, et al. Colorectal cancer screening: an updated modeling study for the US Preventive Services Task Force. JAMA. 2021;325(19):1998–2011.
60. Wolf AM, Fontham ET, Church TR, Flowers CR, Guerra CE, LaMonte SJ, et al. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA Cancer J Clin. 2018;68(4):250–81.
61. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol. 2004;287(1):G7–G17.
62. Oshima M, Taketo MM. COX selectivity and animal models for colon cancer. Curr Pharm Des. 2002;8(12):1021–34.
63. Klampfer L. Cytokines, inflammation and colon cancer. Curr Cancer Drug Targets. 2011;11(4):451–64.
64. Gounaris E, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, et al. Mast cells are an essential hematopoietic component for polyp development. Proc Natl Acad Sci U S A. 2007;104(50):19977–82.
65. Oguma K, Oshima H, Aoki M, Uchio R, Naka K, Nakamura S, et al. Activated macrophages promote Wnt signalling through tumour necrosis factor-α in gastric tumour cells. EMBO J. 2008;27(12):1671–81.
66. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313(5795):1960–4.
67. Camus M, Tosolini M, Mlecnik B, Pages F, Kirilovsky A, Berger A, et al. Coordination of intratumoral immune reaction and human colorectal cancer recurrence. Cancer Res. 2009;69(6):2685–93.
68. Popat S, Hubner R, Houlston R. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005;23(3):609–18.
69. Klintrup K, Mäkinen JM, Kauppila S, Väre PO, Melkko J, Tuominen H, et al. Inflammation and prognosis in colorectal cancer. Eur J Cancer. 2005;41(17):2645–54.

70. Eberhard A, Kahlert S, Goede V, Hemmerlein B, Plate KH, Augustin HG. Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for antiangiogenic tumor therapies. Cancer Res. 2000;60(5):1388–93.
71. Goede V, Brogelli L, Ziche M, Augustin HG. Induction of inflammatory angiogenesis by monocyte chemoattractant protein-1. Int J Cancer. 1999;82(5):765–70.
72. Goede V, Fleckenstein G, Dietrich M, Osmers R, Kuhn W, Augustin HG. Prognostic value of angiogenesis in mammary tumors. Anticancer Res. 1998;18(3C):2199–202.
73. Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y. Prognostic value of tumor-associated macrophage count in human bladder cancer. Int J Urol. 2000;7(7):263–9.
74. Lissbrant IF, Stattin P, Wikstrom P, Damber J, Egevad L, Bergh A. Tumor-associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. Int J Oncol. 2000;17(3):445–96.
75. Salvesen HB, Akslen LA. Significance of tumour-associated macrophages, vascular endothelial growth factor and thrombospondin-1 expression for tumour angiogenesis and prognosis in endometrial carcinomas. Int J Cancer. 1999;84(5):538–43.
76. Etoh T, Shibuta K, Barnard GF, Kitano S, Mori M. Angiogenin expression in human colorectal cancer: the role of focal macrophage infiltration. Clin Cancer Res. 2000;6(9):3545–51.
77. Forssell J, Öberg Å, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. Clin Cancer Res. 2007;13(5):1472–9.
78. Oosterling SJ, van der Bij GJ, Meijer GA, Tuk CW, van Garderen E, van Rooijen N, et al. Macrophages direct tumour histology and clinical outcome in a colon cancer model. J Pathol. 2005;207(2):147–55.
79. Zhang M, Li X, Zhang Q, Yang J, Liu G. Roles of macrophages on ulcerative colitis and colitis-associated colorectal cancer. Front Immunol. 2023;14:1103617.
80. Galon J, Dieu-Nosjean M, Tartour E, Sautes-Fridman C, Fridman W. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene. 2010;29(8):1093–102.
81. Knüpfer H, Preiss R. Serum interleukin-6 levels in colorectal cancer patients—a summary of published results. Int J Colorectal Dis. 2010;25:135–40.
82. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007;117(5):1175–83.
83. Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat Rev Immunol. 2010;10(2):131–44.
84. Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology. 2007;133(6):1869–79.e14.
85. Atreya R, Neurath MF. Involvement of IL-6 in the pathogenesis of inflammatory bowel disease and colon cancer. Clin Rev Allergy Immunol. 2005;28:187–95.
86. Reinecker HC, Steffen M, Witthoeft T, Pflueger I, Schreiber S, MacDermott R, et al. Enhanced secretion of tumour necrosis factor-α, IL-6, and IL-1β by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. Clin Exp Immunol. 1993;94(1):174-81.
87. Nixon N, Blais N, Ernst S, Kollmannsberger C, Bebb G, Butler M, et al. Current landscape of immunotherapy in the treatment of solid tumours, with future opportunities and challenges. Curr Oncol. 2018;25(5):373-84.
88. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-23.
89. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517-26.
90. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. N Engl J Med. 2016;375(19):1823-33.
91. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. N Engl J Med. 2015;373(2):123-35.
92. Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol. 2016;17(7):956-65.
93. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515(7528):558-62.

94. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, et al. High-dose recombinant interleukin-2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol. 1999;17(7):2105-05.
95. Zhang T, Xie J, Arai S, Wang L, Shi X, Shi N, et al. The efficacy and safety of anti-PD-1/PD-L1 antibodies for treatment of advanced or refractory cancers: a meta-analysis. Oncotarget. 2016;7(45):73068-79.
96. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372(26):2521-32.
97. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomized, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908-18.
98. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373(1):23-34.
99. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677-704.
100. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-64.
101. Disis ML. Immune regulation of cancer. J Clin Oncol. 2010;28(29):4531-38.
102. Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563-67.
103. Kerr KM, Tsao M-S, Nicholson AG, Yatabe Y, Wistuba II, Hirsch FR, et al. Programmed death-ligand 1 immunohistochemistry in lung cancer: in what state is this art? J Thorac Oncol. 2015;10(7):985-89.
104. Zhang X, Yang Z, An Y, Liu Y, Wei Q, Xu F, et al. Clinical benefits of PD-1/PD-L1 inhibitors in patients with metastatic colorectal cancer: a systematic review and meta-analysis. World J Surg Oncol. 2022;20(1):1-13.
105. Ciombor KK, Bekaii-Saab T. A comprehensive review of sequencing and combination strategies of targeted agents in metastatic colorectal cancer. Oncologist. 2018;23(1):25-34.
106. Modest D, Pant S, Sartore-Bianchi A. Treatment sequencing in metastatic colorectal cancer. Eur J Cancer. 2019;109:70-83.
107. Johdi NA, Sukor NF. Colorectal cancer immunotherapy: options and strategies. Front Immunol. 2020;11:1624.
108. Hudson K, Cross N, Jordan-Mahy N, Leyland R. The extrinsic and intrinsic roles of PD-L1 and its receptor PD-1: implications for immunotherapy treatment. Front Immunol. 2020;11:568931. https://doi.org/10.3389/fimmu.2020.568931
109. Evrard C, Tachon G, Randrian V, Karayan-Tapon L, Tougeron D. Microsatellite instability: diagnosis, heterogeneity, discordance, and clinical impact in colorectal cancer. Cancers (Basel). 2019;11(10):1567.
110. Lochhead P, Kuchiba A, Imamura Y, Liao X, Yamauchi M, Nishihara R, et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J Natl Cancer Inst. 2013;105(15):1151-56.
111. Cohen R, Rousseau B, Vidal J, Colle R, Diaz LA, André T. Immune checkpoint inhibition in colorectal cancer: microsatellite instability and beyond. Target Oncol. 2020;15:11-24.
112. Venderbosch S, Nagtegaal ID, Maughan TS, Smith CG, Cheadle JP, Fisher D, et al. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: A pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. Clin Cancer Res. 2014;20(20):5322-30.
113. Weiser MR. AJCC 8th edition: colorectal cancer. Ann Surg Oncol. 2018;25:1454-55.
114. Overman MJ, Lonardi S, Wong KYM, Lenz H-J, Gelsomino F, Aglietta M, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. J Clin Oncol. 2018;36(8):773-79.
115. Kok M, Chalabi M, Haanen J. How I treat MSI cancers with advanced disease. ESMO Open. 2019;4:e000511.
116. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz H-J, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18(9):1182-91.
117. Dudley JC, Lin M-T, Le DT, Eshleman JR. Microsatellite instability as a biomarker for PD-1 blockade. Clin Cancer Res. 2016;22(4):813-20.
118. Antonia SJ, López-Martin JA, Bendell J, Ott PA, Taylor M, Eder JP, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. Lancet Oncol. 2016;17(7):883-95.
119. Ferris RL, Blumenschein Jr G, Fayette J, Guigay

J, Colevas AD, Licitra L, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med. 2016;375:1856-67.
120. Food and Drug Administration. OPDIVO [package insert]. 2018.
121. O'Neil BH, Wallmark JM, Lorente D, Elez E, Raimbourg J, Gomez-Roca C, et al. Safety and antitumor activity of the anti–PD-1 antibody pembrolizumab in patients with advanced colorectal carcinoma. PLoS One. 2017;12(12):e0189848.
122. Deeks ED. Pembrolizumab: a review in advanced melanoma. Drugs. 2016;76(3):375-86.
123. Le DT, Yoshino T, Jäger D, Andre T, Bendell JC, Wang R, et al. KEYNOTE-164: Phase II study of pembrolizumab (MK-3475) for patients with previously treated, microsatellite instability-high advanced colorectal carcinoma. Am Soc Clin Oncol. 2016.
124. Ott PA, Elez E, Hiret S, Kim D-W, Morosky A, Saraf S, et al. Pembrolizumab in patients with extensive-stage small-cell lung cancer: results from the phase Ib KEYNOTE-028 study. J Clin Oncol. 2017;35(34):3823-29.
125. Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. J Clin Oncol. 2020;38(1):11.
126. Le DT, Kavan P, Kim TW, Burge ME, Van Cutsem E, Hara H, et al. KEYNOTE-164: Pembrolizumab for patients with advanced microsatellite instability high (MSI-H) colorectal cancer. Am Soc Clin Oncol. 2018.
127. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509-20.
128. Network CGA. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487(7407):330.
129. Maple JT, Smyrk TC, Boardman LA, Johnson RA, Thibodeau SN, Chari ST. Defective DNA mismatch repair in long-term (≥ 3 years) survivors with pancreatic cancer. Pancreatology. 2005;5(2-3):220-28.
130. Meltzer SJ, Yin J, Manin B, Rhyu M-G, Cottrell J, Hudson E, et al. Microsatellite instability occurs frequently and in both diploid and aneuploid cell populations of Barrett's-associated esophageal adenocarcinomas. Cancer Res. 1994;54(13):3379-82.
131. Nakata B, Qing Wang Y, Yashiro M, Nishioka N, Tanaka H, Ohira M, et al. Prognostic value of microsatellite instability in resectable pancreatic cancer. Clin Cancer Res. 2002;8(8):2536-40.
132. Network CGAR. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202.
133. Agaram NP, Shia J, Tang LH, Klimstra DS. DNA mismatch repair deficiency in ampullary carcinoma: a morphologic and immunohistochemical study of 54 cases. Am J Clin Pathol. 2010;133(5):772-80.
134. Levine DA, Mike CGA, Biggs B, Klesas CL, Heather W, Laird PW, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67-73.
135. Garg K, Leitao Jr MM, Kauff ND, Hansen J, Kosarin K, Shia J, et al. Selection of endometrial carcinomas for DNA mismatch repair protein immunohistochemistry using patient age and tumor morphology enhances detection of mismatch repair abnormalities. Am J Surg Pathol. 2009;33(6):925-33.
136. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):2189-99.
137. Jones S, Emmerson P, Maynard J, Best JM, Jordan S, Williams GT, et al. Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C→T:A mutations. Hum Mol Genet. 2002;11(23):2961-67.
138. Palles C, Cazier J-B, Howarth KM, Domingo E, Jones AM, Broderick P, et al. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nat Genet. 2013;45(2):136-44.
139. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008;14(9):985-90.
140. Diaz Jr LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014;32(6):579.
141. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science. 2015;348(6230):124-28.
142. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature. 2014;515(7528):572-76.
143. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade–based im-

munotherapy. Science. 2018;362(6411):eaar3593.
144. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord J-P, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair–deficient cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol. 2020;38(1):1.
145. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov. 2015;5(1):43-51.
146. Li J, Cong L, Liu J, Peng L, Wang J, Feng A, et al. The efficacy and safety of regorafenib in combination with anti-PD-1 antibody in refractory microsatellite stable metastatic colorectal cancer: a retrospective study. Front Oncol. 2020;10:594125.
147. Mettu N, Twohy E, Ou F-S, Halfdanarson T, Lenz H, Breakstone R, et al. BACCI: A phase II randomized, double-blind, multicenter, placebo-controlled study of capecitabine (C) bevacizumab (B) plus atezolizumab (A) or placebo (P) in refractory metastatic colorectal cancer (mCRC): An ACCRU network study. Ann Oncol. 2019;30:v203.
148. Grothey A, Tabernero J, Arnold D, De Gramont A, Ducreux M, O'Dwyer P, et al. Fluoropyrimidine (FP)+ bevacizumab (BEV)+ atezolizumab vs FP/BEV in BRAFwt metastatic colorectal cancer (mCRC): Findings from Cohort 2 of MODUL–a multicentre, randomized trial of biomarker-driven maintenance treatment following first-line induction therapy. Ann Oncol. 2018;29:viii714-viii15.
149. Hoff S, Grünewald S, Röse L, Zopf D. Immunomodulation by regorafenib alone and in combination with anti PD1 antibody on murine models of colorectal cancer. Ann Oncol. 2017;28:v423.
150. Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, Sasada T, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. Cancer Immunol Res. 2014;2(7):632-42.
151. McDermott DF, Huseni MA, Atkins MB, Motzer RJ, Rini BI, Escudier B, et al. Publisher Correction: Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nat Med. 2018;24(12):1941-41.
152. Fukuoka S, Hara H, Takahashi N, Kojima T, Kawazoe A, Asayama M, et al. Regorafenib plus nivolumab in patients with advanced gastric or colorectal cancer: an open-label, dose-escalation, and dose-expansion phase Ib trial (REGONIVO, EPOC1603). J Clin Oncol. 2020;38(18):2053-61.
153. Wang C, Chevalier D, Saluja J, Sandhu J, Lau CF, Akih M. Regorafenib and nivolumab or pembrolizumab combination and circulating tumor DNA response assessment in refractory microsatellite stable colorectal cancer. Oncologist. 2020;25(8):e1188-e94.
154. Tumeh PC, Hellmann MD, Hamid O, Tsai KK, Loo KL, Gubens MA, et al. Liver metastasis and treatment outcome with anti-PD-1 monoclonal antibody in patients with melanoma and NSCLC. Cancer Immunol Res. 2017;5(5):417-24.
155. Calne R. Mechanisms in the acceptance of organ grafts. Br Med Bull. 1976;32(2):107-12.
156. Calne R, Sells R, Pena J, Davis D, Millard P, Herbertson B, et al. Induction of immunological tolerance by porcine liver allografts. Nature. 1969;223(5205):472-76.
157. Qian S, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE. Murine liver allograft transplantation: tolerance and donor cell chimerism. Hepatology. 1994;19(4):916-24.
158. John BC, Crispe IN. Passive and active mechanisms trap activated CD8+ T cells in the liver. J Immunol. 2004;172(9):5222-29.
159. Qian S, Lu L, Fu F, Li Y, Li W, Starzl TE, et al. Apoptosis within spontaneously accepted mouse liver allografts: evidence for deletion of cytotoxic T cells and implications for tolerance induction. J Immunol. 1997;158(10):4654-61.
160. Limmer A, Ohl J, Kurts C, Ljunggren H-G, Reiss Y, Groettrup M, et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med. 2000;6(12):1348-54.
161. Wang J-C, Livingstone AM. Cutting edge: CD4+ T cell help can be essential for primary CD8+ T cell responses in vivo. J Immunol. 2003;171(12):6339-43.
162. Bamboat ZM, Stableford JA, Plitas G, Burt BM, Nguyen HM, Welles AP, et al. Human liver dendritic cells promote T cell hyporesponsiveness. J Immunol. 2009;182(4):1901-11.
163. Liang L, Wen Y, Hu R, Wang L, Xia Y, Hu C, et al. Safety and efficacy of PD-1 blockade-activated multiple antigen-specific cellular therapy alone or in combination with apatinib in patients with advanced solid tumors: a pooled analysis of two prospective trials. Cancer Immunol Immunother. 2019;68:1467-77.
164. Xu J, Zhang Y, Jia R, Yue C, Chang L, Liu R, et al. Anti-PD-1 antibody SHR-1210 combined with apatinib for advanced hepatocellular carcinoma, gastric, or esophagogastric junction cancer: an open-label, dose escalation and expansion study. Clin Cancer Res. 2019;25(2):515-23.

165. Ren C, Mai Z-J, Jin Y, He M-M, Wang Z-Q, Luo H-Y, et al. Anti-PD-1 antibody SHR-1210 plus apatinib for metastatic colorectal cancer: a prospective, single-arm, open-label, phase II trial. Am J Cancer Res. 2020;10(9):2946.