Immunotherapy and immunoevasion of colorectal cancer

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The tremendous success of immunotherapy in clinical trials has led to its establishment as a new pillar of cancer therapy. However, little clinical efficacy has been achieved in microsatellite stable colorectal cancer (MSS-CRC), which constitutes most CRC tumors. Here, we discuss the molecular and genetic heterogeneity of CRC. We review the immune escape mechanisms, and focus on the latest advances in immunotherapy as a treatment modality for CRC. By providing a better understanding of the tumor microenvironment (TME) and the molecular mechanisms underlying immunoevasion, this review offers an insight into developing therapeutic strategies that are effective for patients with various subsets of CRC.

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Introduction

CRC is the third most common cancer worldwide following breast and lung cancers, with an incidence rate of 1.93 million in 2020.[1] Moreover, it is the second leading cause of cancer-related deaths worldwide, accounting for nearly 916 000 deaths in the same year.[1] The incidence rate of CRC among individuals younger than 50 years has been increasing in recent years.[2] Despite a screening-associated reduction in CRC mortality, ~25% of patients still present with metastatic disease at time of diagnosis, and 25–50% of patients presenting with early-stage disease will develop metastasis during the course of their disease.[3] The clinical outcomes of CRC largely depend on the tumor stage at time of diagnosis. According to Surveillance, Epidemiology, and End Results (SEER) staging, the 5-year survival rate in CRC decreases from 90% when cancer is localized, to 72% when regional, to 15% when distant.[4].

As with other cancers, chemotherapy, radiotherapy, and surgery have been the main modalities of CRC management. Treatment recommendations vary depending on disease localization and progression. Surgery is the first-line treatment for localized colon cancer (stage I–III).[5] For locally advanced CRC, patients who are node-positive (stage III) also receive adjuvant chemotherapy to eradicate remaining cancer cells, thus reducing the likelihood of recurrence.[6] However, the use of adjuvant chemotherapy in node-negative patients (stage II) remains controversial, and is assessed on an individual basis by weighing benefits and risks. Neoadjuvant chemoradiotherapy, with or without systemic chemotherapy, is an accepted therapeutic approach for patients with locally advanced rectal cancer (LARC).[7] In metastatic CRC (mCRC), systemic chemotherapy has proven to improve survival.[8] Surgery also provides a potentially curative option in selected patients with oligometastatic disease.[5] Despite treatment, some patients can experience disease recurrence. Moreover, systemic toxicity and low selectivity of conventional treatments have imposed the need for developing new therapeutic strategies to treat CRC.

A better understanding of the molecular basis of tumorigenesis has contributed significantly to the development of targeted therapies. These therapies have become an attractive approach by virtue of their ability to selectively target cancer-specific pathways or evoke potent anti-tumor immune responses.[9] One targeted therapy approach that provokes the host's immune system to combat cancer cells is immunotherapy. By specifically targeting tumor-associated cell surface antigens, cancer immunotherapeutic approaches spare normal cells, thus exhibiting superiority over the less-selective chemotherapy or radiotherapy.[10] This paradigm shift in cancer treatment is due, in no small part, to the durable response and promising clinical responses elicited in subsets of hematological and solid tumors.[11] Among the various cancer immunotherapeutic approaches, monoclonal antibodies (mAbs) targeting immune checkpoints (ICs) elicited promising results in the treatment of solid tumors with high mutational burden.[12,13] As a consequence, the US Food and Drug Administration (FDA) approved the use of IC inhibitors (ICIs) for the treatment of microsatellite instability (MSI)-high (MSI-H) and mismatch repair (MMR)-deficient (dMMR) solid tumors, including CRC.[14] However, the efficacy of ICIs is notably low in CRC subtypes that are MMR proficient (pMMR) and MSS or have low MSI (MSI-L).[15] Unfortunately, these subtypes constitute most CRC tumors (85%), leaving large room for improvement. In this review, we discuss the key mechanisms of immune escape in CRC. We focus on the molecular and histopathological classifications of CRC, and provide an overview of the latest advancements in immunotherapy directed against CRC.

CRC classification

Although a small percentage of CRC (2–5%) occurs in the frame of inherited diseases, such as Lynch Syndrome,[16] most emerge sporadically. In this regards, CRC has always been described as a multifactorial digestive pathology by which multiple associated risk factors augment one's susceptibility to CRC acquisition.[17] Such a factorial plurality translates, at the molecular level, into an extensive diversity of tumor phenotypes. This notion has raised the need for classification of CRC based on molecular phenotypes and histopathological features.[18] Importantly, combining immune and molecular stratifications could provide insight into the choice of immunotherapy to be offered, on an individualized level, to ameliorate treatment prognosis.

The Cancer Genome Atlas classification

Extensive genomic analysis of CRC samples allows for effective molecular CRC categorization models. One comprehensive study identified two radical CRC categories that are differentiated mainly according to the mutation rate or methylation state.[19] These categories are the nonhypermutated and the hypermutated groups. The Cancer Genome Atlas (TCGA) revealed that most sporadic CRC cases (84%) belong to the nonhypermutated group, which arise from chromosomal instability (CIN). These are characterized by a high frequency of DNA somatic copy number alterations (SCNA), microsatellite stability (MSS) and low mutation rate (<8/Mb). By contrast, ~16% of sporadic CRC cases fall into the hypermutated group, most of which are caused by acquired hypermethylation and the consequent silencing of *MLH1*. This eventually results in dMMR pathways, thereby leading to MSI and a high mutation rate.[20] Such a rate is associated with increased neoantigen burden and an ensuing higher tumor immunogenicity. Indeed, the median number of mutation-derived neoantigen is 20 times higher in MSI-H versus MSS tumors. Around 3% of hypermutated cases are classified as ultra-mutated tumors (>40 mutations/Mb) because of mutations in the exonuclease domain of DNA polymerase epsilon or delta 1 (POLE or POLD1). These enzymes become defective, introducing incorrect nucleotides during DNA replication.[19] Analysis of the microsatellite instability status of CRCs is warranted in patients in whom Lynch syndrome-related CRC is suspected. The MSI-H phenotype is a hallmark of such patients.[21] Moreover, MSI status can predict prognosis in patients with early-stage CRC, in which MSI-H tumors are associated with better prognosis and improved survival.[22] In patients with stage IV CRC, TCGA categorization was implemented beneficially in 2017 upon FDA approval of the utilization of ICI immunotherapy for CRC. Tumors with

dMMR and high MSI (dMMR-MSI-H) display a good response to ICI therapy compared with MMR-proficient (pMMR) tumors with MSS or low MSI.[15]

Consensus molecular subtype classification

Owing to the substantial role of gene expression profiling in cancer molecular classification, protracted research work in this field has resulted in fundamental advancements in CRC subtyping.[23] Many studies have reported considerable findings, especially regarding the histological features of CRC tumors.[18] In 2015, Guinney *et al.* formed an international expert consortium to integrate pooled data from all cohorts on gene expression-based CRC subtyping algorithms and developed a detailed and inclusive classification framework for CRC.[24] The framework characterized four main consensus molecular subtypes (CMS) and a fifth group that reveals mixed features from the previously detailed subtypes in agreement with the genetic mutational phenotype, mRNA expression, and intratumoral pathological aspects, thus portraying an intermediary profile or intratumoral heterogeneity (Figure 1).

The CMS1 subtype constitutes ~14% of CRC. It is distinguished on the basis of its high MSI (in 76% of CMS1 tumors), hypermethylated CpG island sites (CIMP) and mutated *BRAF*.[24] Deficiency in the MMR machinery because of hypermethylation-mediated MLH1 silencing results in an elevated tumor mutational burden (TMB) (>20 mutations/Mb in 80%) in this subtype of tumors.[20] A special feature of this group is the active expression of genes involved in immune cell chemotaxis generating high immune activation in the TME.[25] Consequently, CMS1 is entitled as 'MSI-immune' subtype, although MSI phenotype has been identified in a few of the other CMS groups. With respect to the infiltrating leukocyte subsets, CMS1 displays a high infiltration status with white blood cells of the innate immunity [natural killer (NK) cells, M1 macrophages, mast cells, activated dendritic cells (DCs), and neutrophils) and of the adaptive immunity [cytotoxic T lymphocytes (CTLs) and memory helper T (Th) cells]. This high immunogenicity is enhanced by diminution of regulatory T cells (Tregs).[26] However, augmented co-inhibitory signaling remains a pathognomonic molecular feature of this subtype. Expression of IC molecules, such as programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1) or cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), opposes the antitumor immunity of CMS1 by fleeing the immune oversight.[24,26] The 5-year survival for all stages of CMS1 CRC is 73%.[27] In fact, the MSI-H status of CMS1 conveys favorable prognostic outcomes in non-metastatic CRC.[28] However, a recent systematic review and meta-analysis found that mCRC CMS1 has the poorest outcomes compared with the other molecular subtypes.[29] This is in line with previous work demonstrating that MSI-H and BRAF are predictors of poor prognosis in mCRC.[30] Interestingly, the poor prognostic impact of MSI-H CRC was only evident when associated with *BRAF* mutation, which is a characteristic feature of CMS1.[31]

The CMS2 subtype predominates among patients with CRC, accounting for 37% of all cases. A characteristic molecular alteration of CMS2 is the abnormal activation of the Wnt/β-catenin pathway (canonical pathway), hence its name 'canonical'.[32] Particularities of this subtype include aberrant WNT and MYC signaling, few MSI tumors, and impotent immune cell activation and infiltration.[24] Specificities of CMS2 are the expression of epithelial differentiation markers and depletion of leukocyte infiltration. Unlike CMS1, this subtype is referred to as 'immune desert' because of the low number of infiltrating lymphocytes and monocytes, explained by minimal transcription of leukocyte chemotaxis and activation genes.[24,26] Nevertheless, few memory B and Th cells and naive Th cells were found in these tumors, but without the ability to mount an antitumor response.[26] Moreover, low-level expression of PD-1 and PD-L1 also characterizes the CMS2 molecular phenotype. Despite being immunologically cold tumors, CMS2 have the highest 5-year survival rate (77%) among all CMS subtypes.[27]

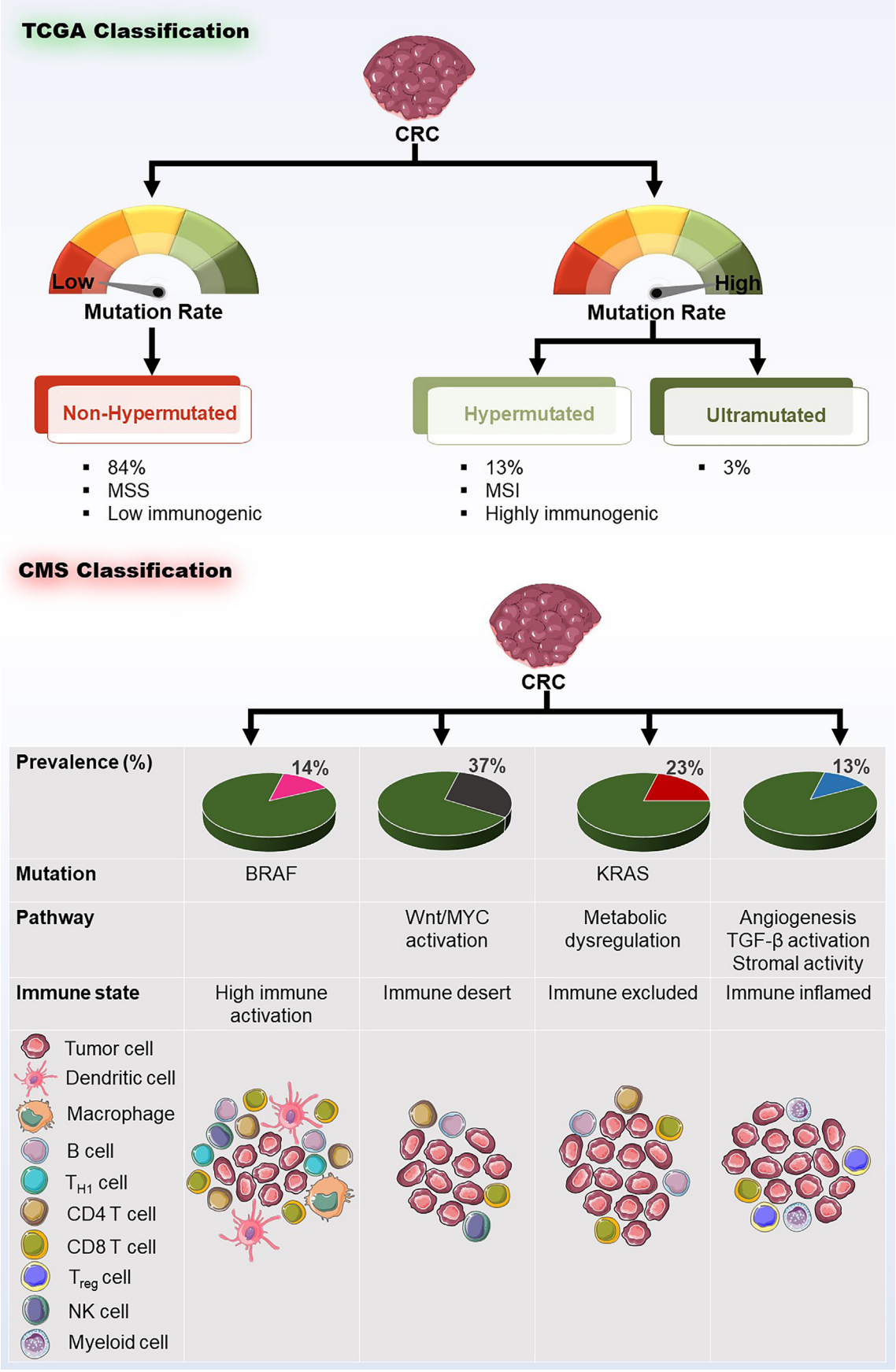
CMS3, a metabolic subtype, contributes to 13% of CRC cases, and is identified by prominent metabolic impairments. CMS3 depicts a genetic profile of KRAS mutations and, similar to CMS2, a quiescent tumor immune microenvironment.[24] Th17 and resting T cell infiltrations reinforce the immunological dormancy of this subtype and mark it as 'immune excluded'. Unlike CMS2, intratumoral cells of CMS3 tumors reveal PD-1 upregulation.[25,26] Even so, the CMS3 5-year survival rate shows only a slight reduction compared with CMS2 (75% versus 77%).[27]

CMS4 represents the exceptional hallmark of this consensus, because it is unique in integrating a mesenchymal phenotype of the tumor. Approximately 23% of CRC cases are classified as CMS4: mesenchymal with lineaments of epithelial–mesenchymal transition (EMT), tissue remodeling, angiogenesis because of TGF-β activation, and profound stromal activity.[24] Further attributes include a tumor-active inflammation status caused by high complement expression, in addition to few antitumor T cells and more Treg cells compared with CMS1.[25] Compared with other consensus molecular subtypes, CMS4 is considered the poor-prognosis CRC subtype, especially in relation to its histological features and markers that could drive its metastasis.[33] Substantial reduction in the 5-year survival rate (62%) provides a clinical evidence for the worse prognosis of CMS4.[27]

The CMS classification highlights the future of CRC clinical stratification by virtue of retrospective analyses, which confirmed the molecular association of CMS categories with clinical results and prognosis.[34] In congruence with the new era of immunotherapy research, Becht *et al.* emphasized the need to coalesce the TME profile with CMS classification to accomplish outbreaks in outlying appropriate CRC immunotherapy algorithms.[25]

Antitumor immunity in CRC

The interplay between the immune system and cancer cells shapes the prognosis of a tumor. The immune system can have a dual role in cancer development: (i) it can identify tumor cells and destroy them in a protective mechanism known as immune surveillance; and (ii) it can promote tumor growth and metastasis through immunosuppressive mechanisms. The dynamic function of the immune system is best expressed by the process of immunoediting, which has three phases: elimination, equilibrium, and escape. During the elimination phase, the host's



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immune system detects and eliminates tumor cells. If the elimination phase is incomplete, tumor cell variants that escaped this phase enter a state of equilibrium in which some tumor clones accumulate further changes and avoid elimination. Ultimately, the tumor evades immune elimination and develops progressively into a clinically detectable disease.[35] The mechanisms that promote tumor progression from immune surveillance toward evasion are diverse, and include formation of an immunosuppressive TME, downregulation of human leukocyte antigen (HLA) class I molecules, and upregulation of IC molecules.

Tumor immune microenvironment

The TME comprises tumor cells, tumor stroma (inflammatory cells, fibroblasts and vascular network), and the surrounding extracellular matrix. The non-malignant cells of the TME have an active role throughout the different steps of carcinogenesis and, thus, influence tumor behavior and response to therapy.[36] Different cells of the adaptive and innate immune system infiltrate CRC tumors, and interact with tumor cells by direct contact or through signaling molecules. These cells vary in proportion, activation status, and their role in promoting pro- or antitumor activity.

The innate immune system is the body's first line of defense against threats. Cells of the innate immune system (dendritic cells, macrophages, NK cells and neutrophils) have been well documented in the TME. Among these cells, NK cells infiltrating CRC tumors have been associated with good prognostic outcomes.[37] Functionally, these cells exert cytotoxic activity against virus-infected cells and tumor cells. Nonetheless, lower levels of NK cells were found in CRC tissues compared with those in healthy adjacent tissues, which might contribute, in part, to tumor escape from immune recognition.[38] Moreover, NK cell phenotype and function can be altered by the immunosuppressive TME; the characteristic phenotype of NK cells in patients with CRC was characterized by upregulation of inhibitory receptors and downregulation of activating receptors, leading to impaired cytotoxic activity.[39] DCs are other innate immune cells that infiltrate the TME. Given their antigen presentation capability, these cells have the ability to promote antitumoral immune responses. Consequently, it is reasonable to associate intratumoral DC infiltration with favorable outcomes.[40] However, the function of these cells can also be dysregulated by the complex interplay of molecules and cells within the TME, which results in DCs with impaired maturation and even reversed functions.[41] Studies regarding the association between tumor-infiltrating neutrophils (TINs) and survival outcomes in patients with CRC are conflicting. Some reports found that neutrophil infiltration enhances $CD8^+$ T cell functions,[42] and is associated with improved overall survival (OS) in patients with stage II CRC.[43] By contrast, high neutrophil:lymphocyte ratio (NLR) correlated with poor prognostic outcomes in patients with CRC.[44]

Unlike cells of the innate immune response, B and T cells mediating the adaptive immune response are associated with sustained antitumor immunity. In particular, high intratumoral infiltration of $CD8^+$ CTLs and $CD4^+$ Th1 cells was correlated with better prognosis in patients with CRC.[45] Indeed, naïve $CD4^+$ T cells recognize antigens presented on the DC surface and differentiate into Th1 lymphocytes upon exposure to IL-12. Th1 then release the antitumorigenic cytokines IFN-γ, TNF-α and IL-2, which promote tumor rejection, and recruitment and activation of effector cells of the immune system, including CTL.[46] By contrast, the role of B cells in the TME is not clear. Some studies demonstrated that B cells favor tumor development,[47] whereas others associated high intratumoral $CD20^+$ B cell infiltration with good prognosis.[48]

Despite adaptive immune response, the TME ultimately becomes immunosuppressive, and the tumor progresses from elimination toward evasion mode.[49] Three key effectors contributing to an immunosuppressive niche are myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs). MDSCs are heterogeneous group of regulatory cells that derive from the myeloid lineage. Their levels increase during states of chronic inflammation and tumor development, mainly because of dysregulated myelopoiesis in the bone marrow.[50] MDSCs include immature granulocytes, DCs, and macrophages, and are identified by distinct phenotypes, such as $CD14^+$ $CD11b^+$ $CD33^+$ HLA-$DR^{low/-}$. Increased production of MDSCs in patients with cancer has been correlated with low levels of antitumor immunity, and advanced tumor stage and metastasis.[51] Consistent with previous findings, patients with CRC had higher levels of circulating MDSCs compared with healthy subjects, and higher circulating and tumor-infiltrating levels of MDSCs in those patients correlated with tumor metastasis and progression.[52] Functionally, *in vitro* MDSCs were found to inhibit T cell antitumor activity,[52,53] and promote tumor progression by direct cell–cell contact with tumor cells.[53]

By contrast, macrophages are among the most abundant immune cells in the TME.[54] They are key effectors of the innate immune response, and can exhibit diverse and even opposing functions depending on their mode of activation. M1 and M2 subtypes represent the two extremes of the dynamic macrophage function: M1 macrophages promote a Th1 response and are associated with microbicidal and tumoricidal activities, whereas M2 macrophages promote a Th2 response and are associated with tis-

FIGURE 1

Classification of colorectal cancer (CRC) based on molecular and pathological phenotypes. The Cancer Genome Atlas (TCGA) classified CRC into two main groups: (1) hypermutated group, with a high mutation rate, hypermethylation, and microsatellite instability (MSI); and (2) nonhypermutated group with a low mutation rate, high frequency of somatic copy number alterations (SCNA), and MSI. By contrast, the genetic classification assorts CRC into four subtypes: (1) consensus molecular subtype (CMS)-1 (MSI-immune); (2) CMS2 (canonical); (3) CMS3 (metabolic); and (4) CMS4 (mesenchymal). CMS1 exhibits a high infiltration of innate immune cells [dendritic cells (DCs), macrophages, and natural killer (NK) cells] and adaptive immune cells [$CD4^+$ T cells, $CD8^+$ T cells, memory T helper (Th) cells, and B cells]. However, CMS2 and CMS3 subtypes are characterized by a low infiltration of immune cells. As for CMS4, it has an active inflammation status and is infiltrated by immunosuppressive cells, including regulatory T cells (Tregs) and myeloid cells. The prevalence, mutation profile, altered pathway and the immune state are indicated for each CMS group. Abbreviation: MSS, microsatellite stability.

sue repair and tumor progression.[55] Several studies demonstrated that TAMs are M2 skewed, thus exhibiting protumoral functions and correlating with poor prognostic outcomes.[56] Indeed, TAMs were associated with increased angiogenesis, matrix remodeling, and depressed adaptive immunity.[57] In CRC, the role of TAMs remains controversial. High intratumoral macrophages was a predictor of good prognosis in some reports,[58] whereas, in other work, it was associated with EMT and tumor spread by secreting IL-6.[59] In addition, M1 macrophages were linked to favorable outcomes,[60] whereas M2 macrophages were associated with tumor progression and poor prognosis.[61] However, the exact opposite impact of M1 and M2 macrophages was found in other work.[62] These conflicting results could be explained, in part by, the lack of standardized protocols for differentiation of different macrophage subpopulations, plasticity of macrophages, and variation in the studied tumor types.[63]

Finally, Tregs are a subpopulation of $CD4^+$ T lymphocytes that recognizably express FoxP3, a regulatory protein that influence Treg gene expression[64]. Being characterized by their immunomodulatory functions, Tregs are crucial for immune homeostasis and maintenance of self-tolerance.[65] They also exhibit anti-inflammatory properties by suppressing T cell activity and secreting anti-inflammatory cytokines, such as IL-10 and TGF-β.[66] Treg elevation has been well documented in cancer,[67] but their prognostic significance remains controversial. On the one hand, Tregs can promote tumor progression by inhibiting antitumor immunity. On the other hand, they can prevent cancer development during inflammation-driven carcinogenesis, because of their anti-inflammatory properties.[68] In patients with CRC, the role of Tregs in tumor progression is inconsistent. One study reported that FoxP3 expression is correlated with tumor progression and metastasis.[69] However, other work showed that high intratumoral FoxP3+ Tregs are associated with better prognosis and improved survival.[70] Interestingly, Tregs might attenuate the tumor-promoting proinflammatory response elicited by Th17 against the gut microbiota.[71] Moreover, several subpopulations of Tregs with phenotypic heterogeneity have been described, including a FoxP3-negative Treg subpopulation.[72] Consequently, phenotypical characterization of Tregs based on FoxP3 alone might be misleading, and further evidence is required to establish the precise functions of distinct Treg subsets in patients with CRC.

HLA-1 downregulation

HLAs are cell surface proteins that display cellular peptides for recognition by immune cells. With regards to HLA-I expression, tumor cells pass through two phases. Initially, primary tumor lesions remain HLA-I positive, and expression of tumor-associated antigens on the surface of HLA-I induces a CTL response (permissive phase). Later, tumor cells become uniformly HLA-I negative (encapsulated phase). Between the two phases, cells are characterized by a heterogeneous pattern of MHC-I expression, in which HLA-I-negative tumor cells resist CTL killing.[73] Consequently, downregulation of HLA I is a potent mechanism of immune evasion.[74]

In CRC, downregulation of HLA I was reported in 73% of tumors.[75] The literature has identified two patterns of HLA I loss in CRC tumors: partial loss and complete loss. Partial loss of HLA I was associated with poor prognosis and decreased overall survival and disease-free survival.[76] However, tumors with complete loss of HLA I have better prognosis than those with partial loss, and similar prognosis to those with high HLA expression.[77] The complete loss of MHC I renders tumor cells susceptible to elimination by NK cells. However, cells with partial downregulation of HLA I escape both NK cells and CTL immune surveillance and, thus, are associated with poorer prognosis.[78] In addition, total HLA I loss is more frequently seen in MSI-H tumors, which are characterized by better prognosis compared with MSS tumors.[79]

Immune checkpoints

ICs are ligand–receptor complexes that regulate the immune response by exerting co-stimulatory or inhibitory signals.[80] In fact, T lymphocyte activation requires two signals: the first is provided by the interaction between antigenic MHC and T cell receptors (TCRs), and the second is an antigen-independent co-stimulatory signal.[81] A well-characterized co-stimulatory molecule is CD28, which interacts with B7 ligands (CD80 and CD86) on the surface of antigen-presenting cells (APCs). Once activated, T lymphocytes express co-inhibitory receptors, including CTLA4 and PD-1, which curtail the immune response. Consequently, the nature of signals relayed by the IC molecules determines the magnitude and type of immune response.[82] ICs are crucial for the maintenance of immune homeostasis and prevention of autoimmunity. However, their dysregulation is an important mechanism of immune evasion in tumors.[13] In fact, overexpression of ICs in cancer was associated with T cell exhaustion, resulting in dysfunctional T lymphocytes characterized by decreased effector function and reduced proliferation.[83]

Numerous tumor-associated ICs have been identified in the TME, including PD-1, CTLA-4, LAG-3, TIM-3, VISTA, and TIGIT.[84] PD-1 and CTLA4 are the more extensively studied IC molecules in patients with cancer, and their targeting has proved to be effective in activating antitumor immune responses.[12] PD-1 is expressed on the surface of activated immune cells, such as $CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, B lymphocytes, NK cells, DCs, and macrophages. In the TME, PD-1 is upregulated on the surface of tumor infiltrating lymphocytes (TILs), and is a marker of T cell exhaustion.[85] PD-1 has two ligands: PD-L1, which is expressed on the surface of immune cells constitutively, some nonhematopoeitic cells, such as epithelial cells, and, importantly, tumor cells; and PD-L2, expressed primarily by APCs, such as DCs and macrophages.[86] The aberrant expression of PD-L1 on cancer cells can be attributed to different mechanisms, including gene amplification, upregulation by oncogenic pathways and induction by pro-inflammatory cytokines (Figure 2). [87] Higher levels of PD-L1 where detected in metastatic CRC compared to primary tumors.[88] Consequently, tumor cells exploit the PD-1/PD-L1 pathway to escape immune surveillance, mainly by suppressing T cell responses in the TME. After binding to PD-L1, activated PD-1 accumulating near the TCR location recruits the phosphatase SHP2 into its cytosolic domain. SHP2 then dephosphorylates proximal TCR signaling molecules, resulting in decreased T cell proliferation, cytokine secretion

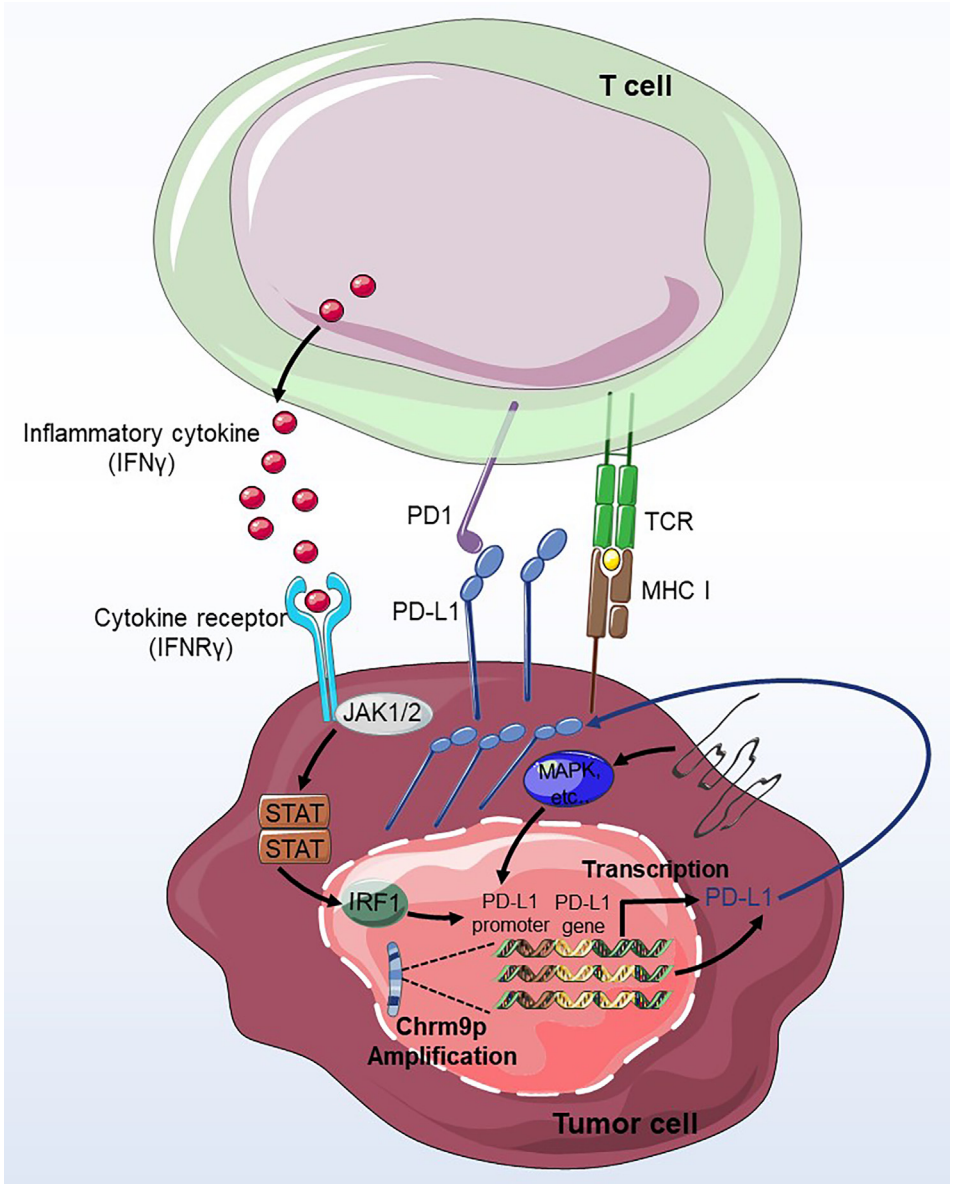

FIGURE 2

Mechanisms causing aberrant programmed death-ligand 1 (PD-L1) expression in cancer cells. Abbreviations: IFN-γ, interferon gamma; IFNGR, IFN-γ receptor; IRF1, interferon regulatory factor 1; JAK, Janus kinase; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; STAT, signal transducer and activator of transcription; TCR, T cell receptor.

(IFN-γ, IL-2, and TNF-α), altered effector functions, and reduced survival of activated T cells (Figure 3).[89]

By contrast, CTLA4 is another inhibitory checkpoint molecule that downregulates immune responses. It is expressed on the surface of activated T cells.[90] Sharing structural similarity with CD28, CTLA-4 binds to CD80 (B7-1) and CD86 (b7-2) but with higher affinity, thus outcompeting CD28 for binding to CD80 and CD86.[91] Consequently, CTLA-4 inhibits T cell activation by hindering the second activation signal and actively removing B7 ligands from APC surface, rendering the T cell anergic.[92] Furthermore, CTLA-4/CD28 ligation results in signaling-dependent T cell inhibition, by the activation of the phosphatases SHP2 and PP2A (Figure 3).[93] In contrast to activated T cells, CTLA-4 is constitutively expressed by Tregs, where it enhances their suppressive activity.[94] In this regard, high expression of CTLA-4 in patients with CRC was associated with poor prognosis,[95] and CTLA-4 blockade in murine colon cancer model improved antitumor immunity.[96]

Immune checkpoint inhibitors

ICIs are mAbs that block IC proteins expressed by immune cells, such as T cells, and by tumor cells, thus releasing the brakes on the immune system to eliminate cancer cells.[97] T cell reactivation is a cornerstone of ICI treatment, and T cell response to IC block-

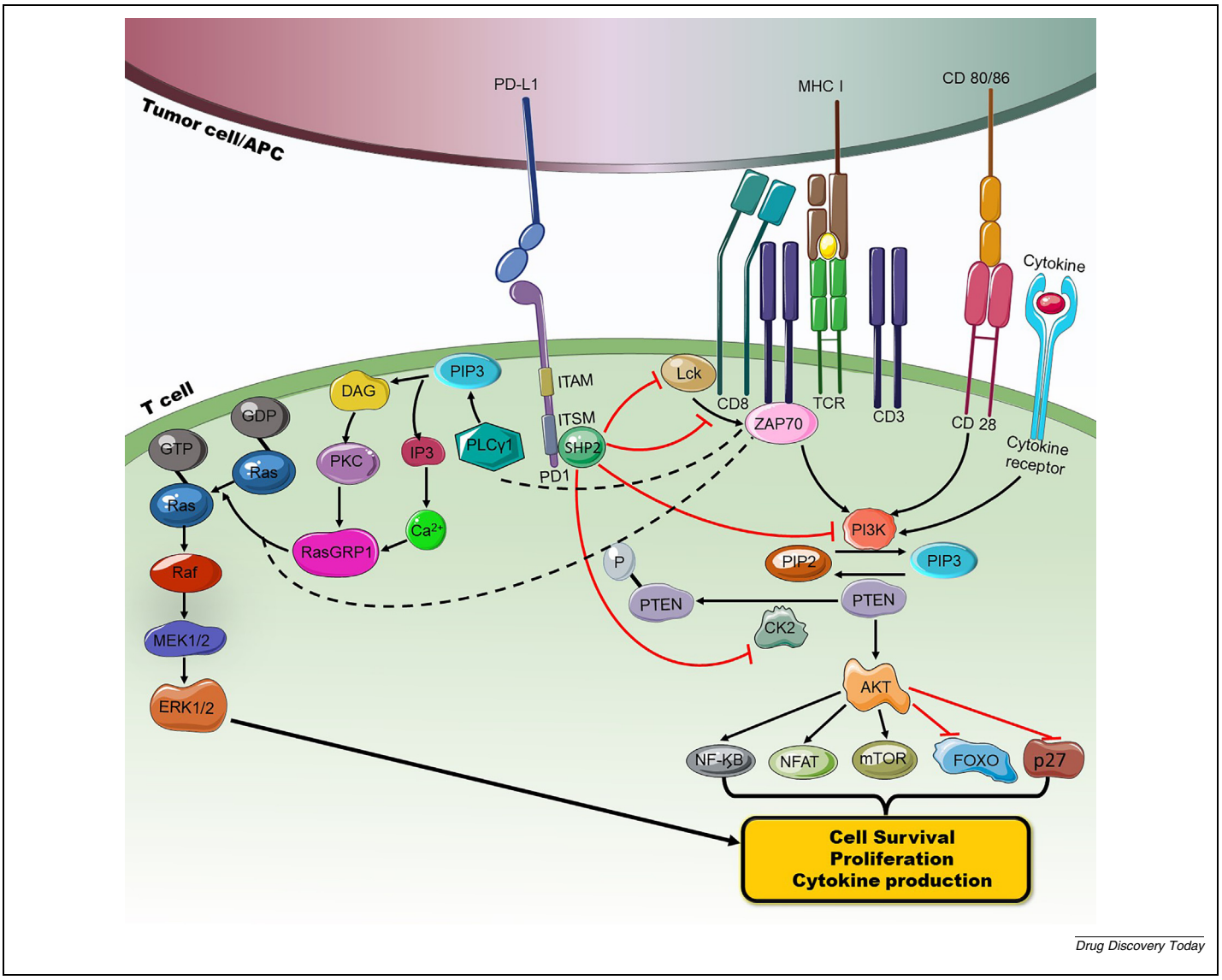

FIGURE 3

Programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) signaling pathways. Following T cell receptor (TCR) stimulation, TCR recruits zeta-chain-associated protein kinase-70 (ZAP70), which is activated by lymphocyte-specific protein tyrosine kinase (Lck). ZAP70 then initiates a series of phosphorylation events that ultimately lead to cellular activation. The PI3K-Akt and Raf-MEK-ERK pathways are among the signaling cascades triggered after TCR-MHC engagement. Activation of PD-1 leads to phosphorylation of its immunoreceptor tyrosine-based switch motif (ITSM) and immunoreceptor tyrosine-based inhibitory motif (ITIM). Phosphorylated ITSM, but not ITIM, recruits SH2 containing protein kinase phosphatase (SHP)-2 (and SHP1), which is involved in the dephosphorylation of proteins of different signaling pathways. Ultimately, the PD-1/PD-L1 interaction inhibits the PI3K-Akt pathway and disrupts the Raf-MEK-ERK pathway, resulting in decreased T cell survival, cytokine secretion, and effector function. Abbreviations: DAG, diacylglycerol; IP3, inositol triphosphate; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa B; NFAT, nuclear factor of activated T cells; PIP3, phosphatidylinositol 3; PKC, protein kinase C; PLCγ1, phospholipase C gamma 1.

ade can largely determine the outcome of therapy. However, increasing evidence suggests that ICIs also boost the tumoricidal activity of the host's innate immune system.[98] The clinical success of ICIs has revolutionized the field of immuno-oncology, leading to a paradigm shift in cancer treatment. Currently, nine checkpoint inhibitors have been approved by the FDA for the treatment of human malignancies. Of those, four are PD-1 inhibitors (pembrolizumab, nivolumab, cemiplimab, and dostarlimab), three are PD-L1 inhibitors (atezolizumab, durvalumab, and avelumab), one is a CTLA-4 inhibitor (ipilimumab) and one is a LAG-3 inhibitor (retatlimab) (Figure 4).[14]

The use of ICIs has shown remarkable success in the treatment of several human malignancies.[12,13] As for CRC, initial clinical trials targeting the PD-1/PD-L1 system have shown disappointing results. For example, in a Phase I clinical trial testing the safety and efficacy of nivolumab in patients with refractory solid tumors, only one patient out of 14 with CRC showed complete response.[99] Surprisingly, the MSI phenotype has been characterized In the CRC of the responsive patient.[100] Consecutive trials showed that response to PD-1 inhibitors depends on the MMR status of patients. In a Phase II clinical trial (NCT01876511), pembrolizumab was administered to patients with CRC who

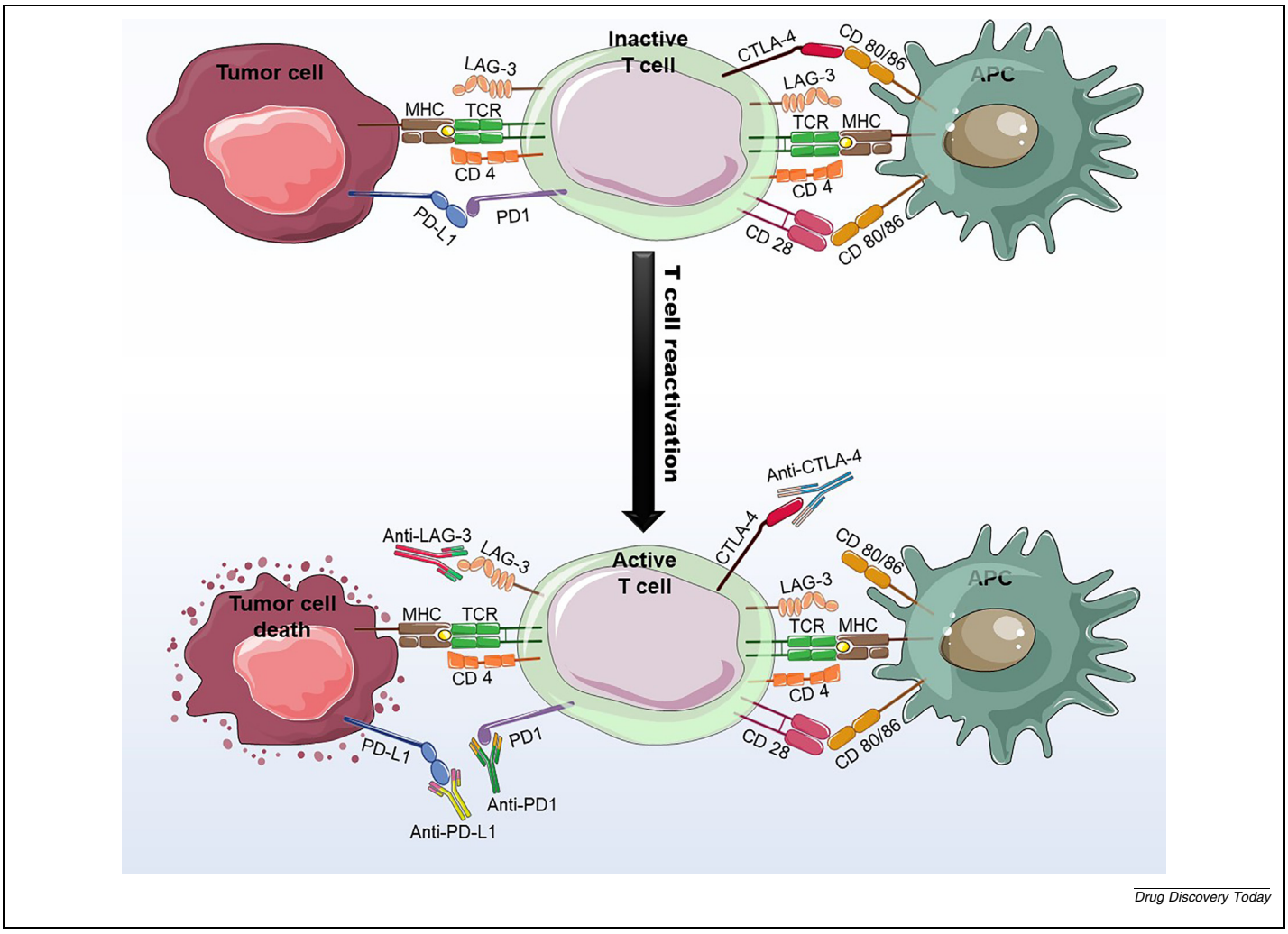

FIGURE 4

T cell activation by immune checkpoint blockade (ICB) in the tumor microenvironment (TME). T cell response to antigens is regulated by co-signaling molecules. CD28 is a co-stimulatory molecule that is constitutively expressed on the surface of T lymphocytes and is crucial for T cell activation. Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) outcompetes CD28 for binding to CD80 (B7-1) and CD86 (B7-2) on the surface of APCs, inhibiting T cell activation. Similarly, programmed cell death protein 1 (PD-1) on the surface of T lymphocytes interacts with programmed death-ligand 1 (PD-L1) on the surface of tumor cells, and inhibits T cell activation and survival. Lymphocyte activation gene-3 (LAG 3) is another immune checkpoint that interacts with MHC II and impairs its association with TCR and CD4, thus inhibiting T cell activation and proliferation. Monoclonal antibodies (mAbs) directed against immune checkpoints or their ligands restore the antitumor immune response by disrupting T cell co-inhibition. Anti-PD-1 mAbs (pembrolizumab, nivolumab, cemiplimab, and dostarlimab), anti-PD-L1mAbs (atezolizumab, durvalumab, and avelumab), anti-CTLA-4 mAbs (ipilimumab), and anti-LAG-3 mAbs (retatlimab) are the currently US Food and Drug Administration (FDA)-approved immune checkpoint inhibitors. Pembrolizumab, nivolumab, and the combination of nivolumab and ipilimumab are currently approved by the FDA for patients with colorectal cancer.

had received two or more prior chemotherapy regimens, and results were compared according to the MMR status of patients. A 20-week follow-up revealed that the objective response and progression-free survival rates were 40% and 78%, respectively, for patients with dMMR CRC compared with 0% and 11%, respectively for those with pMMR CRC.[101] The promising results of NCT01876511 have prompted further research to investigate the impact of pembrolizumab on MSI-H cancers. On May 23, 2017, the FDA granted accelerated approval to pembrolizumab for the treatment of patients with unresectable or metastatic CRC with MSI-H/dMMR who have progressed on prior treatment.[102] This decision was based on the data provided by five clinical trials, which demonstrated that pembrolizumab offers durable clinical benefit in the treatment of MSI-H cancers.[103] More recently, KEYNOTE-177, a randomized, open-label, multicenter trial, compared the efficacy of pembrolizumab versus standard chemotherapy in 307 patients with previously untreated stage IV MSI-H or dMMR CRC. Compared with chemotherapy, pembrolizumab was superior in terms of progression-free survival (median 16.5 months versus 8.2 months) and overall response rate (45.1% versus 33.1%).[104] Based on the results of this study, the FDA approved pembrolizumab as a first-line treatment for patients with MSI-H mCRC.[105] Investigators of CHECKMATE 142 assessed the efficacy of nivolumab in patients with metastatic dMMR/MSI-H CRC who had disease progression during or after, or were intolerant to, at least one previous line of

TABLE 1

FDA-approved ICIs for mCRC and their indications.

Trade name	International non-proprietary name	Strategy	FDA approval date	Indication	Recommended dose and regimen
Keytruda	Pembrolizumab	PD-1 inhibitor	May 23, 2017 (as second-line treatment) June 29, 2020 (as first-line treatment)	First-line treatment for patients with unresectable or metastatic, MSI-H, or dMMR CRC	200 mg for adults or 2 mg/kg (up to maximum of 200 mg) for children every 3 weeks or 400 mg every 6 weeks
Opdivo	Nivolumab	PD-1 inhibitor	July 31, 2017	Second-line treatment for patients with dMMR and MSI-H mCRC that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan	240 mg every 2 weeks
Yervoy	Ipilimumab	CTLA-4 inhibitor	July 10, 2018	Used in combination with nivolumab as second-line treatment for patients with MSI-H or dMMR mCRC that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan	Nivolumab 3 mg/kg IV followed by ipilimumab 1 mg/kg every 3 weeks for 4 doses, then nivolumab 240 mg every 2 weeks

treatment of metastatic disease. After a median follow-up of 12 months, the objective response rate was 31.1%, and 69% of patients experienced disease control for 12 weeks or longer.[106] In the same trial, the combination of nivolumab and low-dose ipilimumab as a first-line therapy in patients with dMMR/MSI-H mCRC resulted in a 69% overall response rate, a 84% disease control rate, and a 13% complete response rate.[107] The clinical trials discussed above provided evidence for the effectiveness of pembrolizumab, nivolumab, and nivolumab plus low-dose ipilimumab in the treatment of patients with dMMR/MSI-H CRC. Based on these findings, these ICIs have become the standard of care for patients with this subtype of CRC (Table 1).

Despite the impressive success of ICIs in the treatment of dMMR/MSI-H mCRC, their use in patients with non-dMMR/MSI-H mCRC (96% of patients with metastatic disease) has failed to demonstrate any clinical benefit or improved survival.[101,108] This can be attributed to different resistance mechanisms, such as the low TMB of pMMR/MSS tumors, shift toward an immunosuppressive TME, downregulation of MHC molecules on the tumor cell surface, and low TIL density in the tumor core (Figure 5).[109] The combination of ICIs with other therapies that have immunomodulatory properties might achieve clinical benefit in this subset of patients; however, novel approaches are still needed to improve the outcomes.

Other immunotherapies

Adoptive cell transfer therapy

Adoptive cell transfer (ACT) therapy is a passive immunotherapy approach in which tumor-specific cytotoxic cells are transferred to patients with cancer. The major effector cells used for the ACT modality are CTLs and NK cells, which are extracted from patients and donors, or differentiated from stem cells. These cells are then expanded and activated *in vitro*, and genetically modified by various gene-editing techniques to enhance their antitumor activity once infused into the patient. [110] ACT that uses T cells include TIL therapy and lymph node lymphocyte (LNL) therapy.[111] These two subsets of cells represent the host's natural immune response against cancer cells, through recognition and destruction of cells expressing TAA.[112] Alternatively, ACT cells can be derived from peripheral blood mononuclear cells (PBMCs) of patients or donors, and are then manipulated by various methods of gene modification and cytokine management to enhance their tumor-fighting abilities. Two major strategies of cellular manipulation in ACT are cytokine induction of killer cells and chimeric antigen receptor (CAR) engineering.[111] Cytokine-induced killer (CIK) cells are $CD3^{+}CD56^{+}$ antitumor cytotoxic cells that are obtained by culturing PBMCs in the presence of IFN γ, anti-CD3 antibodies, and IL-2. They are characterized by their ability to attack cancer cells in an MHC I-independent manner, without affecting normal tissues.[113] In this context, multiple studies have investigated the safety and efficacy of CIK therapy in patients with cancer. More recently, the combination of CIK cells with DCs has been extensively tested in patients with CRC, and increase overall survival and progression-free survival in these patients.[114] Moreover, postoperative adjuvant CIK cell therapy combined with chemotherapy was demonstrated to prevent disease recurrence and prolong survival in patients with CRC, particularly in those with advanced CRC.[115]

By contrast, CAR-T cells are genetically modified leukocytes that express TAA-specific chimeric receptors on their surface. These are typically made by combining the antigen-binding domain of an antibody with the signaling domains of a TCR, along with co-stimulatory molecules. CAR-T cells circumvent the MHC restriction of TCR by directly recognizing motifs expressed on the surface of target cells. This mechanism enables CAR-T cells to bypass immune evasion mechanisms used by some cancer cells, which downregulate MHC expression.[116] Recently, five adoptive CAR-T cell therapies were approved by the FDA for the treatment of cancer. Although CAR-T cell ther-

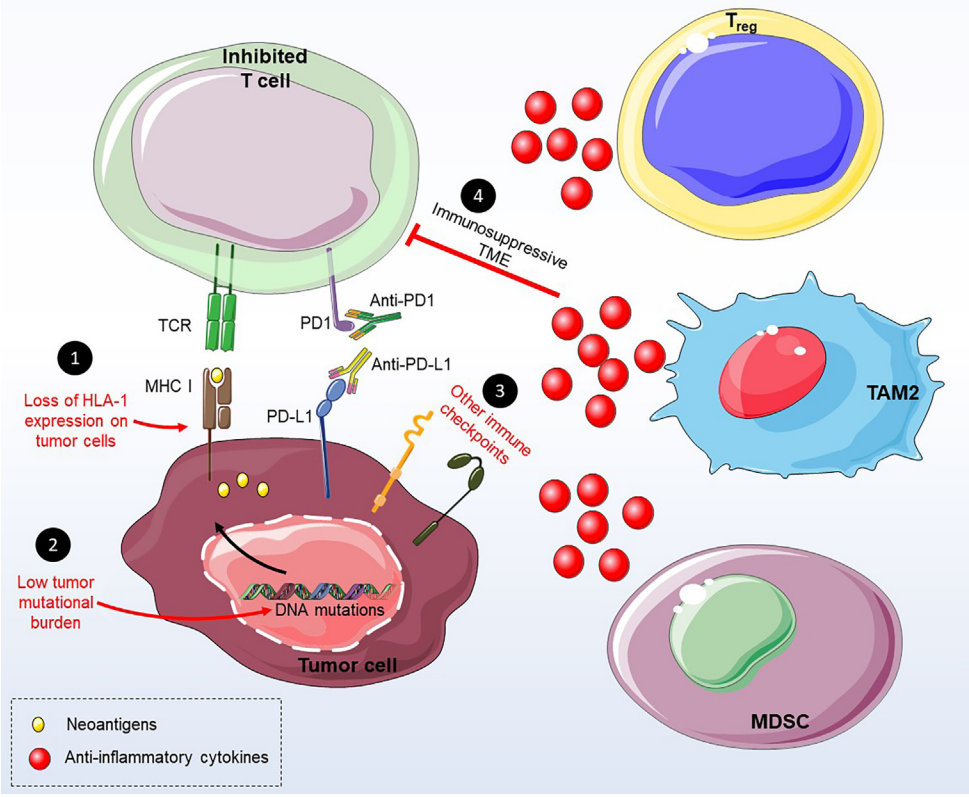

FIGURE 5

Mechanisms of resistance to immune checkpoint blockade in microsatellite instability-low (MSI-L)/microsatellite stable (MSS) colorectal cancer (CRC)**:** (1) Low tumor mutational burden that leads to low neoantigen load; (2) Loss of human leukocyte antigen (HLA) I expression on tumor cells; (3) presence of immunosuppressive cells that blunt T cell effector functions; (4) expression of various immune checkpoint molecules; and (5) immune-excluded tumors, where immune cells are concentrated in the outer invasive margin of the tumor. Abbreviations: MDSC, myeloid-derived suppressor cells; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TAM2: tumor-associated macrophage 2; TCR, T cell receptor; Treg: regulatory T cell.

apy has achieved breakthrough results in the treatment of hematological malignancies, treatment of solid tumors with cell therapy has yielded limited therapeutic benefits.[117] This limited clinical efficacy could be attributed to the existence of barriers that prevent T cells from penetrating the tumor site and functioning effectively. These barriers include abnormal vasculature, which hinders immune cells from reaching the tumor in sufficient numbers, a physical barrier characterized by the tumor stroma and protumor immune cells that prevent adequate infiltration of T cells, and an immunosuppressive TME that inhibits CAR-T cells from mounting an effective antitumor response. Additionally, a major challenge for CAR-T-based ACT in solid tumors is the identification of target antigens for these cells.[118]

By contrast, rigorous *in vitro* and *in vivo* experiments have been performed to provide novel treatment strategies for the recovery of patients with CRC (Table 2). To improve the outcome of ACT, a CAR was constructed by the fusion of the extracellular domain of the NK cell receptor NKG2D to DAP12, a key protein for signal transduction by NK cells receptors.[119] The CAR-NK cells therapy indulged an enhanced cytotoxicity and increased IFN-γ release, thus offering a new option to enhance the NK cell tumor response[119]. Carcinoembryonic antigen (CEA) might be a promising target of CAR in patients with CRC, and the use of CEA-targeted CAR-T cells in patients with mCRC refractory to conventional treatment was associated with stable disease in 70% of patients, without the occurrence of adverse effects.[120] Furthermore, CAR-T cells targeting the epithelial cell adhesion molecule EpCAM, overexpressed in various cancer tissues including CRC, provided a new target for CAR-T cell therapies in solid cancer treatment.[121] Moreover, combining EpCAM CAR-T cell therapy with the Wnt inhibitor hsBCL9CT-24 enhanced CAR-T cell therapy through inhibition of tumor growth and induction of tumor infiltration without affecting T cell survival, thus becoming an attractive combination regimen to improve efficacy and limit shortcomings of ACT.[122]

Vaccines for colorectal cancer

Cancer vaccines represent a novel form of active immunotherapy that stimulates the host's immune cells to mount an intense anti-cancer immune response after exposing them to cancer-specific antigens. These vaccines have been utilized to prevent relapse in patients with low tumor burden or to boost antitumor immune responses in advanced disease.[123] Despite the approval of some cancer vaccines, such as sipuleucel-T in 2010 for

TABLE 2

Clinical trials of ACT therapy for CRC.

NCT number	Study title	Adoptive cell therapy type	Trial Phase	Disease burden	Study treatment group
NCT02202928	Phase II Study of Autologous Tumor Lysate-pulsed DC-CIK Cell in Colorectal Cancer After Surgery	DC-CIK	II	CRC at stage I–III	Group A (received DC-CIK treatment, chemotherapy and radiotherapy) versus group B (received only chemotherapy and radiotherapy)
NCT02577588	Phase I Trial of Adoptive T Cell Therapy With Activated P53 Specific T Cells for Treatment of Advanced Colorectal Cancer	Autologous reactivated T cells against p53	I	Advanced colorectal cancer	Autologous T cell product at dose of 5×10^7 versus autologous T cell product at dose of 5×10^8
NCT04426669	A Phase I/II Trial in Patients With Metastatic Gastrointestinal Epithelial Cancer Administering Tumor-Infiltrating Lymphocytes in Which the Gene Encoding CISH Was Inactivated Using the CRISPR/Cas9 System	CISH CRISPR TIL	I/II	Metastatic gastrointestinal epithelial cancer	Phase 1: non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine + escalating doses of CISH inactivated TIL + high-dose aldesleukin Phase 2: non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine + MTD of CISH-inactivated TILs
NCT05194735	Phase I/II Study of Autologous T Cells Engineered Using the Sleeping Beauty System to Express T-Cell Receptors (TCRs) Reactive Against Cancer-specific Mutations in Subjects With Solid Tumors	TCT-T cell drug product	Phase I/II	Relapsed-refractory solid tumors, including CRC	TCR-T cell drug product versus TCT-cell drug product with aldesleukin (IL-2)
NCT04107142	A Phase I Dose-escalation Trial to Evaluate Haploidentical/Allogeneic Natural Killer Group 2D Ligand (NKG2DL)-Targeting Chimeric Antigen Receptor-grafted Gamma Delta (γδ) T Cells (CTM-N2D) in Subjects With Relapsed or Refractory Solid Tumour	NKG2DL-targetting CAR-grafted γδT cells	I	Relapsed-refractory solid tumors, including CRC	CAR-T Cell Therapy Group
NCT02757391	Pilot Study of Feasibility and Safety of Personalized Autologous CD81 T Cell Therapy Plus Anti-PD1 Antibody in Advanced Solid Malignancies	$CD8^+$ T cell therapy	I	Advanced gastrointestinal malignancies	CD8 1 T cell therapy, pembrolizumab
NCT02886897	A Phase II Study of Combinations of Dendritic Cells and Cytokine-induced Killer Cell (D-CIK) Immunotherapy And Anti-Programmed Death-1 In Refractory Solid Tumors	Autologous dendritic and cytokine-induced killer (D-CIK) cells	I/II	Treatment-refractory solid tumors	Autologous D-CIK cells with anti-PD-1 antibodies

castration-resistant metastatic prostate cancer, studies have failed to demonstrate a durable clinical benefit for these modalities. This could be attributed to the suppressive TME and the intrinsic ability of cancer cells to evade immune surveillance.[124] However, the ever-expanding knowledge of cancer immunology, along with the technological advances in bioinformatics, has resulted in a breakthrough in the development of cancer vaccines. Tumor-associated antigens (TAAs), which are proteins overexpressed by cancer cells, were the mainstay antigens in cancer vaccines. TAAs targeted for CRC vaccines include CEA, mucin 1 (MUC-1), and melanoma-associated antigen (MAGE), among others.[125] More recently, tumor-specific antigens (TSAs, or neoantigens), moieties exclusively expressed by cancer cells following mutations in coding regions of the DNA, have been shown to be a promising target for the development of personalized cancer vaccines. The development of a neoantigen vaccine involves identification of DNA mutations in a tumor tissue, evaluation of the binding affinity of the identified neoantigen to MHC molecules, and predicting the ability of the neoantigen to elicit T cell response. Finally, adequate neoepitopes are selected for vaccine generation, and delivered to patients.[126] Different delivery platforms have been utilized in cancer vaccines, including nucleic acid (DNA and mRNA), peptide, cell (cancer cells or lysates, and DCs loaded with neoantigens,) and vector (viral, bacterial, and yeast)-based vaccines.[127] Various CRC vaccines have been implemented in clinical trials, but none have achieved satisfactory results.[125] To combat the numerous mechanisms of immunoresistance, combinatorial therapy with checkpoint blockade or CAR-T cell adoptive transfer is being explored in clinical trials.[128] Nonetheless, further investigations are needed to optimize the perfect regimen for CRC treatment.

Combination therapy and its future prospects

With the ever-expanding range of treatment options, a significant challenge in CRC immunotherapy lies in identifying the optimal treatment strategy, which takes into account the patient's medical history, clinical characteristics of the cancer, as well as its immune and genetic landscape. The ultimate goal of immunotherapy is to achieve a robust and durable immune

response that prolongs the patient's survival. Recent research suggests that triggering immunogenic cell death (ICD), with the contaminant release of damage-associated molecular patterns (DAMPs), increases tumor immunogenicity and is associated with long-term success of anticancer therapies.[129] ICD has been found to transform the TME from an immune escape state to an immune elimination state.[130] Complementary to these findings, combining ICIs with chemotherapy, radiotherapy, and targeted therapies was demonstrated to potentiate the anticancer immune response.[131–135] Interestingly, a recent study showed that the use of temozolomide as an immune-sensitizing agent followed by a combination of low-dose ipilimumab and nivolumab in patients with MSS and O^6-methylguanine–DNA methyltransferase (MGMT)–silenced mCRC can induce a durable clinical benefit.[136] Moreover, the addition of nivolumab to the standard-of-care therapy (fluorouracil/leucovorin/oxaliplatin) for patients with mCRC is associated with higher progression-free survival and response rates.[137] New combinations that are being extensively investigated in preclinical and clinical studies include ICI combined with inhibitors of signaling molecules, such as BRAF V600E, KRAS G12C, and MEK inhibitors.[138,139] More recently, the gut microbiome has gained attention as a potential modulator of response to immunotherapies. Notably, a specific consortium comprising 11 bacterial strains was found to be associated with enhanced efficacy of ICIs in syngeneic tumor models.[140]

In the realm of ACT, preclinical investigations have shown that combining PD-L1 blockade with CAR-T cell therapy results in robust antitumor activity.[141] Furthermore, genetic modification of CAR-T cells to generate anti-CD30/CEA CAR-T cells has been found to confer superior immunity compared with monospecific anti-CEA CAR-T cells.[142] Several clinical trials are underway to evaluate the efficacy of combining ACT with other therapeutic approaches, including ICIs (NCT03904537), chemotherapeutic agents (NCT03950154), and targeted therapies (NCT02487992).

Concerning cancer vaccines, their combination with chemotherapeutic agents is a promising approach for overcoming limitations in cancer vaccine therapy.[143,144] Studies have shown that this combination can induce a CTL response, resulting in improved survival outcomes in patients with stage III CRC treated with the uracil-tegafur chemotherapy regimen.[145] In addition, ongoing clinical studies are investigating the potential of combining cancer vaccines with ICIs or CAR-T cells.[125] A promising development in the field of cancer vaccines is the combination with other immune modulators, such as IL-12.[146] This approach can transform cancer vaccines into synergistic immunomodulatory platforms that enhance their efficacy.

The aforementioned combination therapies have demonstrated promising outcomes in various clinical trials. However, there are significant challenges to overcome in determining the optimal treatment regimen and dosage that would result in clinically relevant immune responses. At present, the usage of combinational therapies in the management of CRC is in its nascent stage, and further research efforts are necessary to establish its place in disease management. Additionally, selecting patients who would benefit most from this approach necessitates additional clinical investigations. As a result, researchers must devote more attention to understanding the intricacies of combinational therapies to refine the selection process and improve patient outcomes.

Concluding remarks

The success of immunotherapy has revolutionized the treatment of several human malignancies. For CRC, the response was limited to a subset of patients with the dMMR-MSI-H phenotype. However, patients with pMMR-MSI-L or MSS phenotypes, which constitute the majority of patients with CRC, have shown little or no benefit from immunotherapeutic treatments. A better understanding of the TME and the tumor immune escape mechanisms, in addition to much-needed clinical trials using different combination modalities, will increase the likelihood of overcoming resistance and improving outcomes in patients with CRC. However, further research is warranted to improve the efficacy of treatment among patients with pMMR-MSI-L or MSS phenotypes, hoping for a shift in the treatment paradigm of CRC.

Data availability

No data was used for the research described in the article.

References

1. WHO. Cancer. www.who.int/news-room/fact-sheets/detail/cancer. [Accessed June 8, 2023].
2. Siegel RL et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:177–193.
3. Garborg K, Holme Å, Løberg M, Kalager M, Adami HO, Bretthauer M. Current status of screening for colorectal cancer. *Ann Oncol*. 2013;24:1963–1972.
4. Colorectal Cancer Survival Rates | Colorectal Cancer Prognosis. www.cancer.org/cancer/colon-rectal-cancer/detection-diagnosis-staging/survival-rates.html [Accessed June 8, 2023].
5. Vogel JD, Eskicioglu C, Weiser MR, Feingold DL, Steele SR. The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Treatment of Colon Cancer. *Dis Colon Rectum*. 2017;60:999–1017.
6. Taieb J, Gallois C. Adjuvant chemotherapy for Stage III colon cancer. *Cancers*. 2020;12:2679.
7. Cercek A et al. Adoption of total neoadjuvant therapy for locally advanced rectal cancer. *JAMA Oncol*. 2018;4:e180071.
8. Cremolini C et al. First-line chemotherapy for mCRC—a review and evidence-based algorithm. *Nat Rev Clin Oncol*. 2015;12:607–619.
9. Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. *Signal Transduct Target Ther*. 2020;5:22.
10. Couzin-Frankel J. Cancer immunotherapy. *Science*. 2013;342:1432–1433.
11. Hoos A et al. Improved endpoints for cancer immunotherapy trials. *JNCI J Natl Cancer Inst*. 2010;102:1388–1397.
12. Khair DO et al. Combining immune checkpoint inhibitors: established and emerging targets and strategies to improve outcomes in melanoma. *Front Immunol*. 2019;10:453.
13. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. *Nat Commun*. 2020;11:3801.
14. Cancer Research Institute. Approval Timelines of Active Immunotherapies. www.cancerresearch.org/en-us/scientists/immuno-oncology-landscape/fda-approval-timeline-of-active-immunotherapies [Accessed June 8, 2023].
15. Kalyan A, Kircher S, Shah H, Mulcahy M, Benson A. Updates on immunotherapy for colorectal cancer. *J Gastrointest Oncol*. 2018;9:160–169.
16. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. *Gastroenterology*. 2010;138:2044–2058.
17. Johnson CM et al. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control*. 2013;24:1207–1222.

18. Müller MF, Ibrahim AEK, Arends MJ. Molecular pathological classification of colorectal cancer. *Virchows Arch*. 2016;469:125–134.
19. The Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487:330–337.
20. Chalmers ZR et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9:34.
21. Giardiello FM et al. Guidelines on genetic evaluation and management of Lynch syndrome: A Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology*. 2014;147:502–526.
22. Kang S, Na Y, Joung SY, Lee SI, Oh SC, Min BW. The significance of microsatellite instability in colorectal cancer after controlling for clinicopathological factors. *Medicine (Baltimore)*. 2018;97:e0019.
23. Marisa L et al. Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. *PLoS Med*. 2013;10:e1001453.
24. Guinney J et al. The consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21:1350–1356.
25. Becht E et al. Immune and stromal classification of colorectal cancer is associated with molecular subtypes and relevant for precision immunotherapy. *Clin Cancer Res*. 2016;22:4057–4066.
26. Karpinski P, Rossowska J, Sasiadek MM. Immunological landscape of consensus clusters in colorectal cancer. *Oncotarget*. 2017;8:105299–105311.
27. Thanki K et al. Consensus molecular subtypes of colorectal cancer and their clinical implications. *Int Biol Biomed J*. 2017;3:105–111.
28. Lochhead P et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. *JNCI J Natl Cancer Inst*. 2013;105:1151–1156.
29. ten Hoorn S, de Back TR, Sommeijer DW, Vermeulen L. Clinical value of consensus molecular subtypes in colorectal cancer: a systematic review and meta-analysis. *JNCI J Natl Cancer Inst*. 2022;114:503–516.
30. Tran B et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer*. 2011;117:4623–4632.
31. Venderbosch S 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;20:5322–5330.
32. Flores-Hernández E et al. Canonical and non-canonical Wnt signaling are simultaneously activated by Wnts in colon cancer cells. *Cell Signal*. 2020;72 109636.
33. Jackstadt R et al. Epithelial NOTCH signaling rewires the tumor microenvironment of colorectal cancer to drive poor-prognosis subtypes and metastasis. *Cancer Cell*. 2019;36:319–336.
34. Martini G et al. Molecular subtypes and the evolution of treatment management in metastatic colorectal cancer. *Ther Adv Med Oncol*. 2020;12 175883592093608.
35. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nat Rev Cancer*. 2021;21:298–312.
36. Wang M et al. Role of tumor microenvironment in tumorigenesis. *J Cancer*. 2017;8:761–773.
37. Bindea G et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity*. 2013;39:782–795.
38. Halama N et al. Natural killer cells are scarce in colorectal carcinoma tissue despite high levels of chemokines and cytokines. *Clin Cancer Res*. 2011;17:678–689.
39. Rocca YS et al. Altered phenotype in peripheral blood and tumor-associated NK cells from colorectal cancer patients. *Innate Immun*. 2013;19:76–85.
40. Gulubova MV, Ananiev JR, Vlaykova TI, Yovchev Y, Tsoneva V, Manolova IM. Role of dendritic cells in progression and clinical outcome of colon cancer. *Int J Colorectal Dis*. 2012;27:159–169.
41. Legitimo A, Consolini R, Failli A, Orsini G, Spisni R. Dendritic cell defects in the colorectal cancer. *Hum Vaccines Immunother*. 2014;10:3224–3235.
42. Governa V et al. The interplay between neutrophils and CD8+ T cells improves survival in human colorectal cancer. *Clin Cancer Res*. 2017;23:3847–3858.
43. Berry RS et al. High levels of tumor-associated oeutrophils are associated with improved overall survival in patients with stage II colorectal cancer. *PLoS ONE*. 2017;12:e0188799.
44. Mallappa S, Sinha A, Gupta S, Chadwick SJD. Preoperative neutrophil to lymphocyte ratio >5 is a prognostic factor for recurrent colorectal cancer. *Colorectal Dis*. 2013;15:323–328.
45. Tosolini M et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, Th2, Treg, Th17) in patients with colorectal cancer. *Cancer Res*. 2011;71:1263–1271.
46. Basu A et al. Differentiation and regulation of TH Cells: a balancing act for cancer immunotherapy. *Front Immunol*. 2021;12 669474.
47. Guo FF, Cui JW. The role of tumor-infiltrating B cells in tumor immunity. *J Oncol*. 2019;2019:e2592419.
48. Berntsson J, Nodin B, Eberhard J, Micke P, Jirström K. Prognostic impact of tumour-infiltrating B cells and plasma cells in colorectal cancer. *Int J Cancer*. 2016;139:1129–1139.
49. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol*. 2006;90:1–50.
50. Sica A, Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest*. 2007;117:1155–1166.
51. Umansky V, Blattner C, Gebhardt C, Utikal J. The role of myeloid-derived suppressor cells (MDSC) in cancer progression. *Vaccines*. 2016;4:36.
52. Zhang B et al. Circulating and tumor-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. *PLOS ONE*. 2013;8:e57114.
53. OuYang LY et al. Tumor-induced myeloi-derived suppressor cells promote tumor progression through oxidative metabolism in human colorectal cancer. *J Transl Med*. 2015;13:47.
54. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol*. 2013;14:1014–1022.
55. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep*. 2014;6:13.
56. Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers*. 2014;6:1670–1690.
57. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: The role of tumor-associated macrophages. *Crit Rev Oncol Hematol*. 2008;66:1–9.
58. Christofides A, Strauss L, Yeo A, Cao C, Charest A, Boussiotis VA. The complex role of tumor-infiltrating macrophages. *Nat Immunol*. 2022;23:1148–1156.
59. Wei C et al. Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. *Mol Cancer*. 2019;18:64.
60. Edin S, Wikberg ML, Oldenborg PA, Palmqvist R. Macrophages: good guys in colorectal cancer. *OncoImmunology*. 2013;2:2.
61. Pinto ML et al. The two faces of tumor-associated macrophages and their clinical significance in colorectal cancer. *Front Immunol*. 2019;10:1875.
62. Koelzer VH et al. Phenotyping of tumor-associated macrophages in colorectal cancer: Impact on single cell invasion (tumor budding) and clinicopathological outcome. *Oncoimmunology*. 2015;5:e1106677.
63. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol*. 2010;11:889–896.
64. Astarita JL et al. Treg specialization and functions beyond immune suppression. *Clin Exp Immunol*. 2023;211:176–183.
65. Kondělková K, Vokurková D, Krejsek J, Borská L, Fiala Z, Andrýs C. Regulatory T cells (Treg) and Their roles in immune system with respect to immunopathological disorders. *Acta Medica Hradec Kralove Czech Repub*. 2016;53:73–77.
66. Lei H, Schmidt-Bleek K, Dienelt A, Reinke P, Volk HD. Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners. *Front Pharmacol*. 2015;6:184.
67. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? *Cancer Sci*. 2019;110:2080–2089.
68. Wolf D, Sopper S, Pircher A, Gastl G, Wolf AM. Treg(s) in cancer: friends or foe? *J Cell Physiol*. 2015;230:2598–2605.
69. Liu Z et al. Presence of FOXP3(+)Treg cells is correlated with colorectal cancer progression. *Int J Clin Exp Med*. 2014;7:1781–1785.
70. Salama P et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. *J Clin Oncol*. 2009;27:186–192.
71. Ladoire S, Martin F, Ghiringhelli F. Prognostic role of FOXP3+ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. *Cancer Immunol Immunother CII*. 2011;60:909–918.
72. Shevyrev D, Tereshchenko V. Treg heterogeneity, function, and homeostasis. *Front Immunol*. 2020;10:3100.
73. Garrido F, Ruiz-Cabello F, Aptsiauri N. Rejection versus escape: the tumor MHC dilemma. *Cancer Immunol Immunother CII*. 2017;66:259–271.
74. Hazini A, Fisher K, Seymour L. Deregulation of HLA-I in cancer and its central importance for immunotherapy. *J Immunother Cancer*. 2021;9:e002899.
75. Anderson P, Aptsiauri N, Ruiz-Cabello F, Garrido F. HLA class I loss in colorectal cancer: implications for immune escape and immunotherapy. *Cell Mol Immunol*. 2021;18:556–565.
76. Speetjens FM et al. Clinical impact of HLA class I expression in rectal cancer. *Cancer Immunol Immunother*. 2008;57:601–609.

77. Watson NFS et al. Immunosurveillance is active in colorectal cancer as downregulation but not complete loss of MHC class I expression correlates with a poor prognosis. *Int J Cancer*. 2006;118:6–10.
78. Aptsiauri N, Garrido F. The challenges of HLA class I loss in cancer immunotherapy: facts and hopes. *Clin Cancer Res*. 2022;28:5021–5029.
79. Kloor M et al. Immunoselective pressure and human leukocyte antigen class I antigen machinery defects in microsatellite unstable colorectal cancers. *Cancer Res*. 2005;65:6418–6424.
80. Zhang Y, Zheng J. Functions of immune checkpoint molecules beyond immune evasion. *Adv Exp Med Biol*. 2020;1248:201–226.
81. O'Neill RE, Cao X. Co-stimulatory and co-inhibitory pathways in cancer immunotherapy. *Adv Cancer Res*. 2019;143:145–194.
82. Nandi D et al. T cell costimulation, checkpoint inhibitors and anti-tumor therapy. *J Biosci*. 2020;45:50.
83. Xia A, Zhang Y, Xu J, Yin T, Lu XJ. T cell dysfunction in cancer immunity and immunotherapy. *Front Immunol*. 2019;10:1719.
84. Toor SM, Sasidharan Nair V, Decock J, Elkord E. Immune checkpoints in the tumor microenvironment. *Semin Cancer Biol*. 2020;65:1–12.
85. Ahmadzadeh M et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood*. 2009;114:1537–1544.
86. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26:677–704.
87. Cha JH, Chan LC, Li CW, Hsu JL, Hung MC. Mechanisms controlling PD-L1 expression in cancer. *Mol Cell*. 2019;76:359–370.
88. Chen QY et al. Prognostic significance of immune checkpoints HLA-G/ILT-2/4 and PD-L1 in colorectal cancer. *Front Immunol*. 2021;12 679090.
89. Ai L, Xu A, Xu J. Roles of PD-1/PD-L1 pathway: signaling, cancer, and beyond. *Adv Exp Med Biol*. 2020;1248:33–59.
90. Van Coillie S, Wiernicki B, Xu J. Molecular and cellular functions of CTLA-4. *Adv Exp Med Biol*. 2020;1248:7–32.
91. Zhang H et al. Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. *J Exp Clin Cancer Res*. 2021;40:184.
92. Qureshi OS et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell extrinsic function of CTLA-4. *Science*. 2011;332:600–603.
93. Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. *Immunol Rev*. 2009;229:12–26.
94. Walker LSK. Treg and CTLA-4: two intertwining pathways to immune tolerance. *J Autoimmun*. 2013;45:49–57.
95. Omura Y et al. Prognostic impacts of tumoral expression and serum levels of PD-L1 and CTLA-4 in colorectal cancer patients. *Cancer Immunol Immunother*. 2020;69:2533–2546.
96. Grosso JF, Jure-Kunkel MN. CTLA-4 blockade in tumor models: an overview of preclinical and translational research. *Cancer Immun*. 2013;13:5.
97. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252–264.
98. Liu X, Hogg GD, DeNardo DG. Rethinking immune checkpoint blockade: 'beyond the T cell'. *J Immunother Cancer*. 2021;9:e001460.
99. Brahmer JR et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol*. 2010;28:3167–3175.
100. Lipson EJ et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. *Clin Cancer Res*. 2013;19:462–468.
101. Le DT et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509–2520.
102. FDA. *FDA Grants Accelerated Approval to Pembrolizumab for First Tissue/Site Agnostic Indication*. Silver Spring: FDA; 2019.
103. Casak SJ 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;27:4680–4684.
104. André T et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N Engl J Med*. 2020;383:2207–2218.
105. FDA. *FDA Approves First-Line Immunotherapy for Patients with MSI-H/dMMR Metastatic Colorectal Cancer*. Silver Spring: FDA; 2020.
106. Overman MJ et al. Nivolumab in patients with metastatic DNA mismatch repair deficient/microsatellite instability-high colorectal cancer (CheckMate 142): results of an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18:1182.
107. Lenz HJ et al. First-line nivolumab plus low-dose ipilimumab for microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: the Phase II CheckMate 142 study. *J Clin Oncol*. 2022;40:161–170.
108. Boland PM, Ma WW. Immunotherapy for colorectal cancer. *Cancers*. 2017;9:50.
109. Ghiringhelli F, Fumet JD. Is there a place for immunotherapy for metastatic microsatellite stable colorectal cancer? *Front Immunol*. 2019;10:1816.
110. Fan J, Shang D, Han B, Song J, Chen H, Yang JM. Adoptive cell transfer: is it a promising immunotherapy for colorectal cancer? *Theranostics*. 2018;8:5784–5800.
111. Kirtane K, Elmariah H, Chung CH, Abate-Daga D. Adoptive cellular therapy in solid tumor malignancies: review of the literature and challenges ahead. *J Immunother Cancer*. 2021;9:e002723.
112. Lin B, Du L, Li H, Zhu X, Cui L, Li X. Tumor-infiltrating lymphocytes: warriors fight against tumors powerfully. *Biomed Pharmacother*. 2020;132 110873.
113. Zhang Y, Schmidt-Wolf IGH. Ten-year update of the international registry on cytokine-induced killer cells in cancer immunotherapy. *J Cell Physiol*. 2020;235:9291–9303.
114. Du H, Yang J, Zhang Y. Cytokine-induced killer cell/dendritic cell combined with cytokine-induced killer cell immunotherapy for treating advanced gastrointestinal cancer. *BMC Cancer*. 2020;20:357.
115. Pan QZ et al. Efficacy of adjuvant cytokine–induced killer cell immunotherapy in patients with colorectal cancer after radical resection. *OncoImmunology*. 2020;9:1752563.
116. Marofi F et al. CAR T cells in solid tumors: challenges and opportunities. *Stem Cell Res Ther*. 2021;12:81.
117. Hao M et al. Combination of metabolic intervention and T cell therapy enhances solid tumor immunotherapy. *Sci Transl Med*. 2020;12:eaaz6667.
118. Yong CSM, Dardalhon V, Devaud C, Taylor N, Darcy PK, Kershaw MH. CAR T-cell therapy of solid tumors. *Immunol Cell Biol*. 2017;95:356–363.
119. Xiao L et al. Adoptive Transfer of NKG2D CAR mRNA-engineered natural killer cells in colorectal cancer patients. *Mol Ther*. 2019;27:1114–1125.
120. Zhang C et al. Phase I escalating-dose trial of CAR-T therapy targeting CEA+ metastatic colorectal cancers. *Mol Ther J Am Soc Gene Ther*. 2017;25:1248–1258.
121. Zhang BL et al. Preclinical evaluation of chimeric antigen receptor-modified T cells specific to epithelial cell adhesion molecule for treating colorectal cancer. *Hum Gene Ther*. 2019;30:402–412.
122. Li W et al. Targeting Wnt signaling in the tumor immune microenvironment to enhancing EpCAM CAR T-cell therapy. *Front Pharmacol*. 2021;12 724306.
123. Saxena M, van der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines. *Nat Rev Cancer*. 2021;21:360–378.
124. Roy S, Sethi TK, Taylor D, Kim YJ, Johnson DB. Breakthrough concepts in immune-oncology: cancer vaccines at the bedside. *J Leukoc Biol*. 2020;108:1455–1489.
125. Jia W et al. Colorectal cancer vaccines: the current scenario and future prospects. *Front Immunol*. 2022;13 942235.
126. Shemesh CS et al. Personalized cancer vaccines: clinical landscape, challenges, and opportunities. *Mol Ther*. 2021;29:555–570.
127. Shahnazari M, Samadi P, Pourjafar M, Jalali A. Therapeutic vaccines for colorectal cancer: the progress and future prospect. *Int Immunopharmacol*. 2020;88 106944.
128. Janssen E, Subtil B, Ortiz F de la J, Verheul HMW, Tauriello DVF. Combinatorial immunotherapies for metastatic colorectal cancer. *Cancers (Basel)*. 2020;12:1875.
129. Ahmed A, Tait SWG. Targeting immunogenic cell death in cancer. *Mol Oncol*. 2020;14:2994–3006.
130. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol*. 2013;31:51–72.
131. Kim R et al. 608P – Pembrolizumab (pembro) plus mFOLFOX or FOLFIRI in patients with metastatic colorectal cancer (mCRC): KEYNOTE-651 cohorts B and D. *Ann Oncol*. 2019;30:v229–v230.
132. Mettu NB et al. 533PD – 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.
133. Morris VK et al. Phase I/II trial of encorafenib, cetuximab, and nivolumab in patients with microsatellite stable, BRAFV600E metastatic colorectal cancer. *J Clin Oncol*. 2022;40:12.
134. Van Den Eynde M et al. Interim analysis of the AVETUXIRI Trial: avelumab combined with cetuximab and irinotecan for treatment of refractory microsatellite stable (MSS) metastatic colorectal cancer (mCRC)—a proof of concept, open-label, nonrandomized phase IIa study. *J Clin Oncol*. 2021;39:80.
135. Wang Y et al. Anti-PD-1/L1 lead-in before MAPK inhibitor combination maximizes antitumor immunity and efficacy. *Cancer Cell*. 2021;39:1375–1387.
136. Morano F et al. Temozolomide followed by combination with low-dose ipilimumab and nivolumab in patients with microsatellite-stable, O6-methylguanine–DNA methyltransferase–silenced metastatic colorectal cancer: the MAYA trial. *J Clin Oncol*. 2022;40:1562–1573.

137. Lenz HJ et al. Nivolumab (NIVO) + 5-fluorouracil/leucovorin/oxaliplatin (mFOLFOX6)/bevacizumab (BEV) versus mFOLFOX6/BEV for first-line (1L) treatment of metastatic colorectal cancer (mCRC): Phase 2 results from CheckMate 9X8. *J Clin Oncol*. 2022;40:8.
138. Ebert PJR et al. MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade. *Immunity*. 2016;44:609–621.
139. Liu L et al. The BRAF and MEK Inhibitors dabrafenib and trametinib: effects on immune function and in combination with immunomodulatory antibodies targeting PD-1, PD-L1, and CTLA-4. *Clin Cancer Res*. 2015;21:1639–1651.
140. Tanoue T et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature*. 2019;565:600–605.
141. Magee MS et al. GUCY2C-directed CAR–T cells oppose colorectal cancer metastases without autoimmunity. *OncoImmunology*. 2016;5:e1227897.
142. Hombach AA, Rappl G, Abken H. Blocking CD30 on T cells by a dual specific CAR for CD30 and colon cancer antigens improves the CAR T cell response against CD30− tumors. *Mol Ther*. 2019;27:1825–1835.
143. Okuno K et al. Phase I clinical trial of a novel peptide vaccine in combination with UFT/LV for metastatic colorectal cancer. *Exp Ther Med*. 2011;2:73–79.
144. Scurr M et al. Effect of modified *Vaccinia* Ankara-5T4 and low-dose cyclophosphamide on antitumor immunity in metastatic colorectal cancer: a randomized clinical trial. *JAMA Oncol*. 2017;3:e172579.
145. Kawamura J et al. Cytotoxic T lymphocyte response to peptide vaccination predicts survival in stage III colorectal cancer. *Cancer Sci*. 2018;109:1545–1551.
146. Haghighi-Najafabadi N, Roohvand F, Shams Nosrati MS, Teimoori-Toolabi L, Azadmanesh K. Oncolytic herpes simplex virus type-1 expressing IL-12 efficiently replicates and kills human colorectal cancer cells. *Microb Pathog*. 2021;160 105164.