



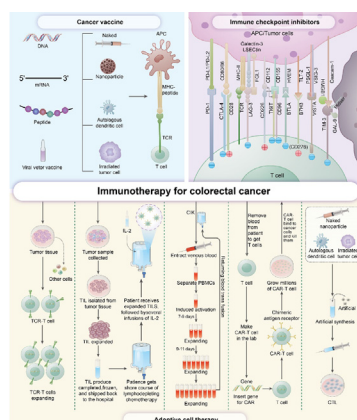
Recent research progress and clinical status of immunotherapy for colorectal cancer

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- Clinical application and research progress of immunotherapy on colorectal cancer.
- This review highlights the therapeutic potential of immunotherapy.
- The shortcomings of immunotherapy have yet to be addressed.
- Future research directions of immunotherapy.

Current immunotherapies for colorectal cancer are immunosuppressants, tumor vaccines, and over-the-counter cellular therapies.



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ABSTRACT

Background: The incidence of colorectal cancer (CRC) is increasing worldwide, particularly among younger adults. Consequently, developing novel treatment strategies for CRC is necessary. As a recently developed therapeutic modality, immunotherapy has attracted substantial attention from researchers worldwide. Immunotherapy, in which a patient's immune system is modified to identify and kill cancer cells, is more targeted and less harmful than chemotherapy, radiotherapy, and surgery. It has demonstrated promising therapeutic efficacy in metastatic and recurrent CRC. Immune checkpoint inhibitors (ICIs), such as pembrolizumab, nivolumab, and ipilimumab, have been approved for the treatment of advanced CRC in clinical practice. In addition, immunotherapies such as cancer vaccines and adoptive cell therapy (ACT) have received considerable interest. Although immunotherapy improves the prognosis and quality of life of patients, heterogeneous treatment outcomes may occur owing to differences in individual characteristics and tumor types.

Aim of review: This review aimed to summarize the recent research progress and clinical status of immunotherapy in CRC and explore future research directions in this field.

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Key scientific concepts of review: Immunotherapy strategies, such as immune checkpoint inhibitors, cancer vaccines, and ACT, target and eliminate tumor cells through the human immune system. In this review, we highlight the research progress, applications, and future perspectives of immunotherapy, providing novel avenues for enhancing its therapeutic efficacy in CRC in clinical settings.

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Introduction

More than 1.9 million new cases of colorectal cancer (CRC) and 904,000 CRC-related deaths were reported worldwide in 2022, representing approximately 10 % of the global cancer incidence and cancer-related deaths [1]. Surgery is the primary treatment for CRC; other treatment options include chemotherapy and radiation

therapy. Although the development of laparoscopic and robotic surgical techniques has improved treatment outcomes, local tumor recurrence remains a major concern postoperatively [2,3]. Therefore, surgical resection is frequently combined with chemotherapy or radiation therapy, with the combination of surgery and chemotherapy being the most common in clinical practice. Preoperative chemotherapy is aimed at reducing tumor size, decreasing

the clinical stage, and improving the success rate of surgery [4–6]; however, chemotherapy resistance poses a challenge to successful treatment. Neoadjuvant radiotherapy results in a notable decrease in tumor volume and recurrence rates, enhancing the efficacy of surgical resection [6]; however, it may increase the risk of toxic reactions [7]. The limitations of radiotherapy can be overcome by using radiochemotherapy, in which chemotherapy is administered concurrently with radiotherapy. This strategy targets cellular or molecular interactions that sensitize cancer cells to radiation, offering unique advantages over other therapeutic modalities [8]. However, some patients may relapse despite undergoing radiochemotherapy. In these cases, immunotherapy can be used to improve the prognosis [9,10].

Immunotherapy defends against tumors by acting on the immune system, such as stimulating T cells to identify endogenous tumor antigens at the tumor initiation site [11,12]. It enhances tumor-specific immunity, reduces immunosuppression, and triggers immune responses, activating the immune system to identify and eliminate cancer cells. Compared with conventional chemotherapy (e.g., FOLFOX), immunotherapy is more precisely targeted, has longer-lasting efficacy and fewer side effects, and can be combined with other therapies [13]. Although immunotherapy is an important treatment modality for CRC, its reliance on biomarkers and drug resistance limit its application in clinical settings [14,15].

Immunotherapy has shown promising efficacy in the treatment of CRC. Immune checkpoint inhibitors (ICIs) such as pembrolizumab, nivolumab, and ipilimumab have been used to treat patients with CRC with deficient mismatch repair (dMMR)/high microsatellite instability (MSI-H) [16,17]. Approximately 15 % of these patients may benefit from ICIs. However, the frequency of MSI and MMR testing remains low in clinical practice, and the efficacy of ICIs is limited in patients with CRC with proficient mismatch repair (pMMR) and low microsatellite instability (MSI-L) [18,19]. To date, studies have reported three mechanisms involved in the development and progression of CRC, namely, chromosomal instability (CIN), DNA methylation and MSI [20,21]. Among these mechanisms, MSI is an essential tool for classifying CRC and guiding the development of effective treatments [22]. Defects in MMR-related proteins lead to dMMR, which impedes the correction of genetic errors. These errors are permanently incorporated into tumor cell DNA, resulting in MSI-H [23].

In addition to ICIs, other immunotherapy approaches, have attracted considerable attention. Several trials have investigated the therapeutic potential of these approaches in cancer. Adoptive cell therapy (ACT) for CRC is in the preliminary stage of research and has some limitations. ACT has shown beneficial effects in hematological cancers; however, its effectiveness in solid tumors, such as CRC, remains inconclusive [24]. It is a promising treatment strategy for patients with metastatic CRC (mCRC) and those with unsuccessful outcomes from conventional therapies. A CRC vaccine named OncoVax has been extensively investigated since the 1980s when early-phase trials showed promising results [25]. However, validating the potential utility of CRC vaccines such as OncoVax is necessary to implement their widespread use.

To date, the Food and Drug Administration (FDA) has approved numerous single- and multi-agent immunotherapies for treating various cancers, including CRC. The immunotherapies approved for treating CRC are shown in Fig. 1. In this review, we present an overview of the research advances, applications, and future perspectives of immunotherapy in CRC, providing novel approaches to improving its applicability and effectiveness in clinical settings.

ICIs and their classification

CD4+ and CD8+ T cells, key components involved in anti-tumor immunity, interact with tumor antigens to regulate immune

responses through diverse inhibitory and costimulatory signals [26,27]. T cells can identify peptide antigens that are intracellularly generated and appear on major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs). Based on the expression of the CD4 or CD8 co-receptor, T cells are categorized into two types with unique functions. CD4+ T cells mediate direct immune responses through proinflammatory factors, whereas CD8+ T cells eliminate infected or tumor cells. CD4+CD25+ regulatory T cells (Tregs) are a specific subset of CD4+ T cells that can inhibit the immune system after an immune attack [28]. The activity and survival of these cells, which are crucial for the effectiveness of immunotherapy, are regulated by immune checkpoints. These checkpoints influence downstream signalling pathways by binding to corresponding ligands, triggering either immune stimulation or immune inhibition. Dysregulation of these checkpoints may lead to overactivation of immune responses, leading to autoimmune disorders, or suppression of immune responses, leading to tumor growth. Under physiological conditions, immune checkpoints suppress immune responses to prevent autoimmunity. However, disruption of their binding to corresponding ligands can restore or amplify antitumor responses and inhibit tumor cell proliferation [29].

The advent of immune checkpoint blockade (ICB) has remarkably improved the management of mCRC, locally advanced CRC (LACRC), and CRC with MSI-H/dMMR [30]. However, ICB has low effectiveness in the treatment of CRC with pMMR/microsatellite stability (MSS). Therefore, distinguishing immune subtypes can enhance the understanding of the complexity of CRC, which may help optimize existing immunotherapies [31–33].

To date, ICIs including anti-programmed cell death-1 (PD-1), anti-programmed cell death-ligand 1 (PD-L1), anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), and anti-T cell immunoglobulin and ITIM domain (TIGIT) monoclonal antibodies (mAbs) have received FDA approval for the treatment of various cancers [34].

ICIs

PD-1 inhibitors

PD-1 is expressed on the surface of dendritic cells (DCs), monocytes, and T and B cells. The binding of PD-1 to PD-L1 results in the inhibition of cytokine production and T-cell activation [35,36]. Tumor cells can identify PD-1 on T cells and upregulate the expression of PD-L1 upon interferon-gamma (IFN- γ) stimulation, leading to T-cell death [37]. In addition, it reduces CD4+/CD8+ T-cell infiltration in the tumor microenvironment (TME) and suppresses the production of cytokines facilitating the immune evasion of tumor cells [38,39]. PD-1 inhibitors disrupt its interaction with PD-L1, promoting the proliferation and infiltration of T cells in the TME and inducing anti-tumor responses [40–42]. A body of research has demonstrated that anti-PD-1/PD-L1 therapy has the potential to prolong survival in patients with advanced CRC and dMMR/MSI-H [43].

The PD-1 inhibitors currently used in clinical practice are nivolumab, pembrolizumab, tislelizumab, penpulimab, zimberelimab, serplulimab, toripalimab, sintilimab, and camrelizumab. The combined use of nivolumab and ipilimumab has shown promising efficacy in the treatment of mCRC with dMMR/MSI-H [44]. Nivolumab blocks the interactions between PD-1 and PD-L1/PD-L2, thereby promoting T-cell proliferation and cytokine release and amplifying the immune response. A study showed that compared with chemotherapy, pembrolizumab used as an initial therapy for mCRC with dMMR/MSI-H remarkably prolonged progression-free survival and decreased the incidence of treatment-associated complications [45]. Pembrolizumab is indicated for unresectable or metastatic solid tumors with dMMR/MSI-H that are resistant to standard therapy in both adult and pediatric patients. Tislelizumab

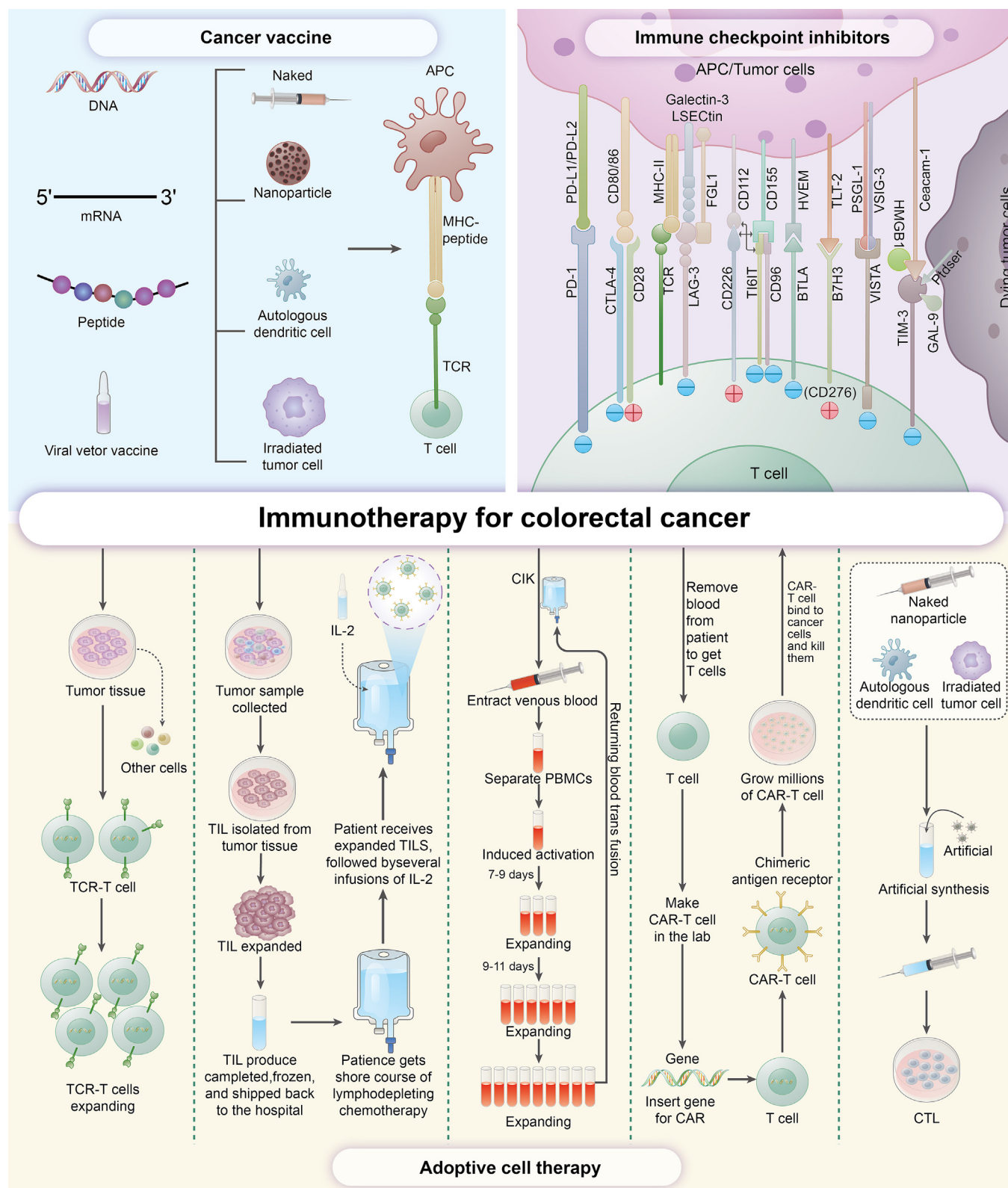


Fig. 1. Various immunotherapies for CRC: main classifications of cancer vaccines, investigational receptors for ICI therapies, and preparation of ACT effector cells. Based on the manufacturing method and mechanism, cancer vaccines are classified as cellular, viral, peptide, and nucleic acid vaccines. The main methods of ACT are TCR-T, TIL, CIK, CAR-T, and CTL. ACT require *in vitro* preparation. ICI has now identified a variety of target receptors on the basis of which novel drugs can be investigated.

is recommended for patients with previously treated, locally advanced, unresectable, or metastatic solid tumors with dMMR/MSI-H. Pucotenlimab has been demonstrated to be an effective treatment for adult patients diagnosed with dMMR/MSI-H solid

tumors who have undergone primary or secondary systemic therapy [30].

PD-1 inhibitors exhibit broad-spectrum antitumor activity irrespective of the tumor type, resulting in effective control of tumor

growth. The response of tumor cells to PD-1 inhibitors can be assessed based on various biomarkers. For instance, PD-L1 methylation is an effective indicator of the response to PD-1 inhibitors. In a study on patients with CRC, methylated PD-L1 (mPD-L1) was negatively correlated with PD-L1 mRNA expression and was associated with shorter overall and relapse-free survival. Therefore, mPD-L1 should be further investigated as a predictive biomarker of the response to PD-1/PD-L1 blockade therapy [46]. However, hypermethylation at K162 in PD-L1 has been associated with a poor treatment response [47]. In CRC, the correlation between prognosis and mtDNA copy number fluctuates with the MMR status, indicating that mtDNA copy number can be used as a prognostic biomarker and a target of ICIs [48]. Silencing of H3K9 through methylation (an epigenetic modification) can lead to resistance to ICB. Given that RTP4 expression is a promising indicator of the response to ICB, it may help enhance the effectiveness of epigenetic drugs and ICIs in CRC with dMMR/MSI-H [49]. Furthermore, evaluation of intratumoral tertiary lymphoid structures (TLSs) in CRC can effectively reflect the response to PD-1 inhibitors [50]. TLSs are prevalent in non-metastatic CRC with MSI-H and are positively correlated with survival, irrespective of the TNM stage [51]. Identification of novel biomarkers may facilitate the development of more effective PD-1 inhibitors.

PD-L1 inhibitors

PD-L1, a transmembrane glycoprotein of the B7 family that serves as a key ligand of PD-1, is found in activated T cells, B cells, and non-hematopoietic cells [52]. PD-L1, commonly examined alongside PD-1, functions as a negative regulator of T-cell activation. It is selectively expressed in specific macrophage subsets and is rapidly upregulated in various normal and tumor cells upon stimulation by IFN- γ and other proinflammatory cytokines. Its interaction with CD80 on activated T cells influences the outcomes and immune-related adverse reactions of PD-1/PD-L1 blockade therapy [53]. In addition, its binding to PD-1 inhibits the antitumor activity of T cells [54].

The PD-L1 inhibitors currently used in clinical practice or under investigation are durvalumab (drug I), atezolizumab (drug T), envafolelimab, sugemalimab, and adebrelimab. The combined use of PD-L1 and CTLA-4 inhibitors is the primary treatment strategy for advanced or metastatic CRC with MSI-H; however, some patients develop drug resistance and progress to advanced disease [55]. Envafolelimab has demonstrated promising efficacy in treating adult patients with irreversible or metastatic solid tumors with dMMR/MSI-H [56]. The combination of durvalumab with other drugs has shown some efficacy and manageable toxicity in the treatment of CRC with MSS [57–59]. In a clinical trial, the addition of atezolizumab to capecitabine plus bevacizumab therapy resulted in no significant clinical benefit in patients with mCRC [60]. However, some other combination therapies have shown clinical benefit in patients with mCRC [61,62].

CTLA-4 inhibitors

CTLA-4 is a T-cell co-receptor that has a high binding affinity for B7 family proteins expressed on APCs [63]. Its biological characteristics remain elusive, especially in the context of cancer. However, CTLA-4 inhibitors have been reported to increase the survival of patients with metastatic or non-metastatic CRC with dMMR/MSI-H [64].

The CTLA-4 inhibitors currently available for clinical use include ibrutinomab (Y drug), tremelimumab, cadonilimab, belatacept, and abatacept.

Ipilimumab is used to treat CRC with dMMR/MSI-H in patients aged ≥ 12 years. The combination of ipilimumab and nivolumab is used to treat patients with CRC who progress to advanced disease

after fluoropyrimidine plus oxaliplatin therapy. This strategy has demonstrated sustained, substantial clinical benefits while maintaining favorable tolerability during initial therapy [65].

TIGIT inhibitors

TIGIT, a poliovirus receptor (PVR)/nectin family protein and a CD28 family member, is expressed on activated T and NK cells and exhibits preferential binding to CD155 on APCs in various cancers, including blood cancer. It manifests in NK and T cells, functioning as a “buddy” to restrain activity solely for active or memory Th follicular cells and Treg subsets [66]. The interaction between TIGIT and CD155 is a promising prognostic biomarker and immunotherapeutic target for cancer.

Several TIGIT inhibitors are being evaluated for their therapeutic efficacy in various solid tumors and leukemia. The more advanced inhibitors are tiragolumab, ociperlimab, and domvanalimab [67]. The human anti-TIGIT IgG1/kappa mAb tiragolumab has an intact Fc region that prevents TIGIT from binding to CD155 [68]. mAbs exhibit potent antitumor activity through CD8 + T-cell-mediated immune responses and Fc-dependent function, resulting in the suppression of Treg infiltration in tumors. TIGIT and PD-1 are co-inhibitory receptors that are frequently co-expressed on tumor-infiltrating lymphocytes (TILs) with depleted function. High TIGIT expression has been associated with T-cell depletion, early recurrence, and poor overall survival (OS) in patients with CRC [69]. A study showed that the combination of atezolizumab and tiragolumab restored T-cell function in 46 % of patients with MSS CRC, whereas atezolizumab monotherapy improved T-cell function only in patients with MSI CRC [70]. Therefore, TIGIT-Fc therapy, used either as monotherapy or in combination with other ICIs, represents an effective approach to cancer treatment [71].

Studies have shown that TIGIT inhibits tumor growth and attenuates the resistance to ICIs in animal models [72,73]. Among the TIGIT inhibitors under investigation, vibostolimab has progressed to phase-II trials, whereas tiragolumab is being evaluated in phase-I trials. Patients with advanced solid tumors have shown good tolerance to ociperlimab and tislelizumab in a trial. In addition, a study showed that the combined use of ociperlimab and an anti-PD-1 mAb effectively activated the immune system, highlighting the therapeutic potential of this combination therapy against solid tumors [74]. Tregs have been demonstrated to play a pivotal role in the development and progression of tumors by constraining the capacity of adaptive immunity [75]. Studies have shown that anti-TIGIT therapy can suppress tumor growth by targeting FoxP3 Tregs [76]. Unlike anti-TIGIT antibodies, depletion of intratumoral Tregs or any other cell population expressing TIGIT does not exert antitumor effects. Anti-TIGIT antibodies trigger an antitumor response via the macrophage Fc γ receptors, resulting in the release of chemokines and cytokines in the CRC microenvironment [77]. The combined use of anti-TIGIT mAbs and other antitumor drugs has shown enhanced cytotoxicity in several clinical trials [78]. Future studies on TIGIT blockade therapy should focus on the identification of new predictive biomarkers or targets of TIGIT [79].

Other ICIs

Despite some success during the initial stages of therapy, approximately half of the patients receiving ICIs develop drug resistance. Therefore, identifying novel therapeutic targets is necessary. Studies have shown that lymphocyte activation gene-3 (LAG-3) [80,81], V-domain Ig suppressor of T-cell activation (VISTA) [82], T cell immunoglobulin and mucin-domain containing-3 (TIM-3) [83], B and T cell lymphocyte attenuator (BTLA) [84], and B7 homolog 3 protein (B7H3) [85] hold promise

Table 1
Clinical trials of major ICIs in CRC.

Clinicaltrials.gov Identifier or Reference	Phase	Study treatment groups	Enrollment	Primary Outcome Measures	Recruitment status
NCT05371197	II	Envafohimab	26	PCR	Recruiting
NCT04715633	II	Camrelizumab	53	CCR or PCR	Active, not recruiting
NCT02563002	III	Combined With Apatinib Pembrolizumab (MK-3475)	307	OS	Completed with results
NCT04008030	III	Nivolumab Alone, Nivolumab in Combination With Ipilimumab	831	PFS	Recruiting
NCT04730544	II	Two Combination Treatment Regimens of Nivolumab and Ipilimumab	96	AEs	Recruiting
NCT04357587	I	Pembrolizumab,in combination with SOC radiation and Capecitabine	6	PFS.	Completed
NCT04895722	II	co-formulated pembrolizumab/quavonlimab versus other treatments	320	ORR	Active, not recruiting
NCT05239741	III	Pembrolizumab (MK-3475) Versus Chemotherapy	100	PFS	Recruiting
NCT05328908	III	Relatlimab-nivolumab Fixed-dose Combination Versus Regorafenib or Trifluridine + Tipiracil (TAS-102)	720	OS	Active, not recruiting
NCT02460198	II	Pembrolizumab (MK-3475)	124	ORR	Completed
NCT05217446	II	Encorafenib Plus Cetuximab Taken Together With Pembrolizumab Compared to Pembrolizumab Alone	104	PFS	recruiting
NCT02997228	III	Combination Chemotherapy, Bevacizumab, and/or Atezolizumab	120	PFS	Suspended
NCT04751370	II	Neoadjuvant Nivolumab Plus Ipilimumab and Short-Course Radiation	31	Pathological complete response	

ICIs, Immune checkpoint inhibitors; CRC, colorectal cancer; ORR, objective response rate; AEs, adverse events; PFS, progression-free survival; OS, overall survival; pCR, pathological complete remission; cCR, clinical complete remission.

as effective targets of ICIs in clinical practice. Clinical trials aimed at evaluating the therapeutic value of these targets are summarized in Table 1.

Unaddressed challenges

Resistance to ICIs is classified as primary and secondary. Primary resistance refers to the lack of therapeutic effects, whereas secondary resistance refers to the emergence of treatment resistance that leads to tumor recurrence in responders [86,87]. Approximately 45 %–60 % of patients with mCRC with dMMR/MSI-H exhibit primary or secondary resistance to ICIs [88]. Although the pathways and mechanisms underlying the development of drug resistance remain unclear, they are reported to involve tumor-related, TME-related, and host factors [89]. The three intrinsic mechanisms involved in ICI resistance include impairment of antigen presentation, depletion of neoantigens, and dysfunction of IFN signaling. In addition, the TME, a complex ecosystem of tumor cells, stromal elements, immune cells, extracellular matrix, and cytokines, is influenced by the gut microbiome and diet [90].

Resistance to ICIs may be overcome through combination therapy. To date, various combinations of ICIs, chemotherapy, targeted therapy, radiation therapy, antiangiogenic agents, and off-label ACT have been investigated. Notably, chimeric antigen receptor T-cell (CAR-T cell) therapy has shown effectiveness in hematological cancers, yielding encouraging results in preclinical and clinical settings. The use of antiangiogenic drugs in combination with immunotherapy has shown synergistic effects [91], and similar interactions between ICIs and antiangiogenic drugs have been observed in murine models [92]. Nevertheless, additional research is necessary to formulate efficacious treatment strategies for CRC with dMMR/MSI-H.

ICIs have been observed to induce a unique set of adverse effects, with immune-related adverse events (irAEs) being reported in 70–90 % of patients [93,94]. The irAEs affect almost all vital organs, especially the skin, thyroid, lungs, colon, rectum, liver, and pituitary gland. Moreover, they may cause damage to the neural and cardiovascular systems, increasing the risk of mortality [95]. ICI-associated cardiotoxicity is rare but life-threatening, counteracting the survival benefit of ICIs [96]. irAEs may be attributed to cross-reactivity caused by the misidentification of tumor

antigens by the immune system. Overcoming these challenges is essential for the successful treatment of cancer with ICIs [97].

Adoptive cell therapy

In recent years, various types of immunotherapies have shown encouraging results in clinical trials. Among these therapies, ACT is particularly beneficial for the treatment of refractory and recurrent tumors. ACT involves modifying a patient's immune cells, *ex vivo* and re-injecting them into the patient to achieve immune activation and direct tumor cell killing.

Different types of ACTs include CAR T-cell [98], engineered T-cell receptor T-cell (TCR T-cell) [99], TIL [100],cytokine-induced killer (CIK) cell [101], lymphokine-activated killer (LAK) cell, cytotoxic lymphocyte (CTL), and dendritic cell (DC) therapies. Because CAR T-cell therapy recognizes surface antigens, it is more suitable for hematological malignancies and solid tumors with high expression of surface antigens. On the contrary, TCR T-cell therapy recognizes tumor cells through antigen presentation and may have broader therapeutic applications than CAR T-cell therapy.

Immunotherapy may not benefit some patients with CRC owing to tumor heterogeneity, immune evasion of tumor cells, or the restricted access of T cells to solid tumors. However, some clinical trials have shown that patients with advanced CRC appear to tolerate ACT well [102].

Types of ACTs

CAR T-cell therapy

In CAR T-cell therapy, peripheral blood T cells are isolated from patients or donors and genetically modified *in vitro* to express the chimeric antigen receptor (CAR), bypassing MHC restriction [103]. After genetic modification, the cells are extensively expanded *in vitro*. Patients undergo lymphocyte-depleting chemotherapy before being injected with CAR-expressing T cells. These cells specifically identify target antigens and rapidly proliferate, exerting anti-tumor effects *in vivo* [104].

The CAR T-cell therapies currently available for clinical use include Kymriah, Yescarta, Tecartus, Breyanzi, Abecma, Relmacel, and Carvykti. Most of these therapies primarily target hematological malignancies. On April 19, 2022, the FDA granted Fast Track

designation to Stansai Biotech's CAR T-cell therapy product GCC19CART for the treatment of relapsed or refractory mCRC. In June 2022, Stansai Biotech presented updated data on GCC19CART to the American Society of Clinical Oncology (ASCO). GCC19CART is an autologous CAR T-cell therapy that targets and destroys cancer cells that express the CRC marker guanylate cyclase C (GCC).

One of the biggest obstacles to improving the clinical applicability and safety of CAR T-cell therapy is maximizing effective antigen specificity. Existing CAR T-cell therapies primarily target carcinoembryonic antigen (CEA) and GCC/GUCY2C [106]. Epithelial cell adhesion molecule 9 (EpCAM), epidermal growth factor receptor (EGFR), NKG2D, human epidermal growth factor receptor 2 (HER-2), and CD133 are potential target antigens of CAR T-cell therapy in CRC [106]. Phase-I trials have evaluated the potential of CEA as a target of CAR T-cell therapy in advanced CRC; however, the results are yet to be published. A study showed that CEA-targeted CAR T-cell therapy was tolerated well among the treated patients, with therapeutic effects being observed in a majority of the patients [107]. Approximately 80 % of patients with mCRC have overexpression of GCC in tumor tissues [108], which suggests that GCC is a specific biomarker for mCRC [109].

The application of CAR T-cell therapy in CRC is limited by various factors, CAR T-cell transport, poor therapeutic efficacy against solid tumors, and high costs [110]. To avoid immune rejection, CAR T-cell therapy involves the use of a patient's T cells to target cancer cells in the patient's body. This autologous nature of CAR T-cell therapy increases the cost of production.

Artificial intelligence (AI) has become remarkably popular in medical science in recent years. The study utilizes an extensive array of large-scale proteomic, transcriptomic, and genomic data to identify specific targets for CAR T-cell therapy. Furthermore, it has the potential to streamline gene editing in CAR T cells by enhancing the efficacy of genome editing instruments. The design and execution of clinical trials for CAR T-cell therapy can be facilitated by the utilization of AI. This approach entails the determination of optimal dosages, treatment regimens, and combinations of therapies. Additionally, it encompasses the management and prediction of risk factors and treatment outcomes [110]. AI analyses patient data to determine drug toxicity and response and develop personalized treatment strategies. The combined use of CAR T-cell therapy and other immunotherapies may improve the prognosis of cancer.

TCR T-cell therapy

The TCR identifies antigens presented by MHC/HLA on target cells. In TCR T-cell therapy, the natural TCR sequence is modified to produce a TCR capable of recognizing tumor-specific antigens. These modified TCR-expressing T cells can recognize both tumor-specific and intracellular antigens. TCR T-cell therapy targeting NY-ESO-1 has shown promising safety and efficacy in the treatment of solid tumors in a clinical trial [111]. It is the most promising T-cell-based immunotherapy for solid tumors at present.

Various TCR T-cell therapies are under investigation in phase-I or -II clinical trials. These therapies are categorized based on their targets, which primarily include tumor-associated antigens (TAAs) such as NY-ESO-1 [112], MAGE family proteins [113], MART-1, and CEA [105] and neoantigens such as hepatitis B virus (HBV) and KRAS.

A personalized T-cell therapy named IMA101 has made substantial research progress in various solid tumors, including CRC [111].

TILs

In TIL therapy, CD4⁺ and CD8⁺ cells with potent anti-tumor effects are isolated from the tumor tissues of patients, stimulated and activated via interleukin 2 (IL-2) *in vitro*, expanded *in vitro*, and re-injected into the patients for treatment. This approach max-

imizes immune activation [114] and realizes effective treatment of primary and secondary tumors [115].

A TIL therapy called lifileucel, developed by Iovance Biotherapeutics, Inc., has recently received accelerated approval from the FDA, making it the world's first TIL therapy approved for the treatment of solid tumors. Lifileucel can be used to treat advanced melanoma that has progressed after PD-1 blockade therapy [116]. TIL therapy has certain unique advantages, such as multi-targeting ability, strong tumor-targeting and infiltration ability, and fewer side effects; therefore, it is an ideal treatment strategy for solid tumors [117].

Although the source of TILs varies across patients, the modified cells that are eventually reinjected into the patients are similar. A study demonstrated the effectiveness of TIL therapy in the treatment of KRAS-positive CRC [118]. In the study, a 50-year-old woman with mCRC was administered a single dose of 1.48×10^8 TILs, which contained approximately 75 % of CD8⁺ T cells targeting the KRAS G12D mutation. Treatment with these cells resulted in complete regression of metastases and a partial 9-month remission.

CIK cell therapy

CIK cells are an allogeneic cell type developed by co-culturing human peripheral blood mononuclear cells with IL-2, anti-CD3 mAb (CD3McAb), IFN- γ , and IL-1 α for a predetermined duration *in vitro* [119]. As CIK cells are non-MHC-restricted, their activity is not limited by the tumor type and they can kill any type of tumor cell. However, they are most effective against cancer cells with high antigen expression. It is noteworthy that DCs co-cultured with CIK cells have strong, specific tumor cell-killing activity. CIK cell plus DC cell therapy is expected to become the first choice for radioimmunotherapy. In addition, CIK cell therapy can be combined with surgery and chemotherapy [120,121].

To date, only one CIK cell therapy product, named ImmunCell-LC, has received approval for the treatment of cancer worldwide. ImmunCell-LC is used as an adjuvant therapy after surgical resection in patients with hepatocellular carcinoma, brain tumors, and pancreatic cancer. A systematic evaluation of numerous clinical studies on patients with CRC demonstrated that CIK cell therapy substantially improved the prognosis [122]. Therefore, CIK cell therapy is a safe and effective adjuvant treatment for CRC after surgery [123].

NK cell therapy

In NK cell therapy, NK cells are harvested from a patient or a healthy donor, cultured in large quantities in a laboratory through activation and expansion, and reinjected into the patient. Existing strategies for NK cell therapy include *in vitro* autologous or allogeneic activation of NK cells, incorporation of mAbs on cells for antibody-specific cytotoxicity, and CAR NK-cell therapy. Adoptively transferred NK cells have demonstrated anti-tumor activity in xenograft mouse models of mCRC [124,125].

NK cells can be genetically modified to express receptors for antigens, such as CAR, increasing their specificity and effectiveness against cancer cells. Most pharmaceutical companies have adopted the approach of developing CAR-expressing NK cells, which can effectively identify and eliminate tumor cells. The efficacy of CAR NK-cell therapy is superior to that of CAR T-cell therapy in treating solid tumors. CAR NK-cell therapy has shown promising therapeutic effectiveness in mCRC, highlighting its potential as an alternative to CAR T-cell therapy [126]. NK cells can be treated with cytokines before reinjection to increase their *in vivo* activity and longevity [127].

Presently, no therapies involving NK cells have been approved for the treatment of CRC. However, these treatments are under investigation in numerous clinical trials and studies. According to a case report, NK cell therapy showed therapeutic efficacy in post-

Table 2
Clinical trials of major ACTs in CRC.

Clinicaltrials.gov Identifier or reference	Phase	Study treatment groups	Enrollment	Primary outcome measures	Recruitment status
NCT05248048	I	NKG2D CAR-T	9	DLT	Unknown status
NCT04513431	I	Anti-CEA-CAR T	18	MTD	Unknown status
NCT05240950	I	Anti-CEA-CAR T	18	AEs	Unknown status
				Security	Recruiting
				Effectiveness	
NCT05319314	I	GCC19CART	30	Efficacy	Recruiting
NCT05759728	III	CNA3103	45	AEs DLTs MTD	Recruiting
NCT03370198	I	NKR-2	1	Safety	Terminated
NCT03310008	I	NKR-2	36	Visit Day 43	Unknown status
NCT06530303	III	TIL	85	DLT · ORR	Not yet recruiting
				AEs	Recruiting
NCT03692429	I	CYAD-101	49	Occurrence of Dose Limiting Toxicities	Recruiting
NCT04991948	I	CYAD-101	34	DLT · ORR	Recruiting
NCT03904537	III	anti-PD-1 antibody-activated TILs	20	DFS	Unknown status
NCT06296056	I	Combined Immune Cell Therapy	20	AEs	Not yet recruiting
					Recruiting
NCT05676190	Not Applicable	CIK	80	DCR ORR	Recruiting
NCT01839539	II	DC-CIK	60	PFS	Unknown status
NCT02202928	II	DC-CIK	60	PFS	Unknown status
NCT02415699	IIIII	DC-CIK	100	DFS	Unknown status
NCT03524274	II	Activated CIK · CD3-MUC1 Bispecific Antibody	0	ORR	Withdrawn
NCT02487992	II	CIK · S-1 · Bevacizumab	1200	OS	Active, not recruiting
NCT01929499	II	Adjuvant Cytokine-induced Killer Cells	210	DFS	Unknown status
NCT03329664	III	CIK	20	PFS · TTP · Safety	Unknown status
NCT03084809	IV	CIK + FOLFOX4	46	Relapse Free Survival in 2 years	Completed
NCT02419677	IIIII	RFA + CIK	60	RFS	Completed
NCT02280278	III	CIK	550	DFS	Unknown status
NCT03220984	II	Immunocell-LC	28	DCR	Unknown status
NCT03431311	III	TCR-T	1	AEs	Terminated

ACT, Adoptive cell therapy; CRC, colorectal cancer; CAR-T, chimeric antigen receptor T; CEA, carcinoembryonic antigen; TILs, tumor-infiltrating lymphocytes; DLT, dose-limiting toxicity; MTD, maximal tolerable dose; ORR, objective response rate; AEs, adverse events; DFS, disease-free survival; DCR, disease control rate; CIK, cytokine-induced killer; DC, dendritic cell; PFS, progression-free survival; OS, overall survival; TTP, time to progression; RFS, recurrence-free survival; RFA, radiofrequency ablation; TCR, T-cell receptor.

operative recurrent CRC to a certain extent [128]. A study identified umbilical cord blood (UCB)-derived NK cells (eUCB-NK cells) as potent killers of CRC cells *in vitro*. These NK cells exhibited higher cytotoxicity than IL-2-activated NK cells and secreted large amounts of cytokines such as IFN- γ , TNF- α , GM-CSF, and CCL3. Notably, eUCB-NK cell therapy significantly inhibited tumor growth in HT29 xenograft models but not in LoVo xenograft models. This limited effectiveness may be attributed to insufficient NK cell recruitment. However, the combined use of eUCB-NK cell therapy and bevacizumab has been shown to enhance therapeutic efficacy [129].

Other types of ACTs

LAK cell therapy, an early ACT, was discontinued owing to the requirement of a high IL-2 dose, severe side effects, and substandard efficacy. Short peptide chains in DCs have been shown to reduce immunogenicity and tolerance, warranting further research [130]. CAR-M therapy is less effective in treating CRC owing to inadequate *in vivo* proliferation, migration, and target antigen expression [131]. CTLs, antigen-specific T cells that secrete cytokines and possess MHC molecules, are becoming obsolete in clinical practice owing to their limited tumor-killing activity.

Clinical limitations

ACT has some limitations in the treatment of CRC. First, an attenuated treatment response owing to drug resistance is a major barrier to successful ACT. Most patients receiving CAR T-cell ther-

apy have cancer cells that exhibit partial or complete deletion of the target antigen. The condition, which is referred to as antigen escape, has been demonstrated to have a deleterious effect on treatment outcomes [98,103,131]. Second, targeting CRC-related antigens is challenging because they are expressed at varying levels across healthy tissues. Therefore, antigen selection is paramount in the design of CARs, not only to enhance therapeutic efficacy but also to reduce off-target toxicity [98,131]. Third, CAR T-cell therapy in solid cancers, unlike in hematological malignancies, is hindered by the inability of CAR T cells to penetrate and infiltrate solid cancers owing to physical barriers such as the immunosuppressive TME and tumor stroma [98,103,131]. Fourth and last, CAR T cells are rapidly eliminated after metastasis, which challenges their survival, proliferation, and sustained antitumor effects [98,131]. Clinical trials focused on evaluating the therapeutic effectiveness of ACT in CRC are underway and may yield results that overcome the aforementioned limitations (Table 2).

Cancer vaccines and antigen presentation strategies

Cancer vaccines are aimed at improving the body's ability to induce targeted immune responses; activate cellular immunity; clear tumor cells; and suppress tumor progression by targeting tumor antigens presented as peptides, viruses, or nucleic acids [132]. Some cancer vaccines have shown promising therapeutic efficacy in solid tumors [133]. Based on the manufacturing method and mechanism, cancer vaccines are categorized as cellular, viral,

peptide, and nucleic acid vaccines. These vaccines comprise antigens, adjuvants, and formulation components, with the antigens being most important [133].

The effectiveness of cancer vaccines depends on the recognition of tumor antigens, either TAAs or tumor-specific antigens (TSAs), by T cells [133]. T cells trigger an immune response by recognizing these antigens presented via HLA on target cells [134]. Trials on TAA-targeted cancer vaccines have shown limited success [135]. The TAAs used to develop early CRC vaccines include CEA and melanoma-associated antigen (MAGE) [136], whereas those used to develop recent vaccines include mucin 1 (MUC-1), vascular endothelial growth factor receptors 1 and 2 (VEGFR1 and VEGFR2), EGFR, survivin, mitotic-colony-associated kinesin (MCAK), transmembrane 4 superfamily member 5 protein (TM4SF5), GUCY2C, and 5 T4 [137–139]. TSAs, also known as neoantigens, result from non-synonymous site modifications, insertions/deletions, coding alterations, splicing anomalies, and gene fusion events [140]. These mutations are commonly observed in the TP53, HT001, AIM2, TGF β R-II, and KRAS genes [141,142]. TSAs are unique proteins expressed on tumor cells and can stimulate potent tumor-specific T-cell responses [143].

Studies on CRC vaccines have shown favourable results in safety and feasibility testing. OncoVax and GVAX are key representatives of cell-based vaccines [144]. DC-based vaccines have shown promising therapeutic efficacy in melanoma and prostate cancer and are commonly used in CRC research [145]. The production of peptide-based vaccines is simple; however, combining them with strong adjuvants is necessary to enhance immunity. Studies have shown that peptide-based vaccines stimulate immune responses via TAAs or TSAs [146,147]. Clinical trials have shown that DNA vaccines are safe and induce robust immune responses; however, their therapeutic efficacy remains limited. Enhancing the effectiveness of these vaccines requires innovative approaches [148]. Compared with DNA vaccines, mRNA vaccines have higher potency, versatility, and production speed [134]. Viral vector vaccines, primarily involving the use of adenoviruses, poxviruses, and lentiviruses, are aimed at enhancing the expression of TAAs (such as CEA) and T-cell immunity [134]. CRC vaccines typically involve the use of cytokines, such as IFN- γ , and various TLR agonists as adjuvants [149,150]. Vaccines are usually administered through subcutaneous, intramuscular, or intradermal routes, as oral administration is challenging owing to the complexity of the gastrointestinal system [151]. Recently, nanovaccines have attracted increased attention owing to their potential in personalized cancer treatment. These vaccines are developed using lipids, polymers, inorganic carriers, or biological derivatives [152,153]. In addition, neoantigen-based vaccines represent another promising approach to CRC treatment [154].

They encompass DNA vaccines, RNA vaccines, peptide vaccines, DC vaccines, and viral vector vaccines [155]. At present, the human papillomavirus (HPV) vaccine is used to prevent cervical cancer caused by HPV, the sipuleucel-T vaccine is used to treat metastatic prostate cancer [156], and the first-generation vaccine CIMAvax-EGFR and the second-generation vaccine Vaxira are used to treat non-small cell lung cancer.

Cell-based vaccines

Whole tumor cell-based vaccines

Whole tumor cell (WTC)-based vaccines are generally used in combination with an immune adjuvant. These vaccines involve the use of allogeneic or autologous tumor cells or cell lysates that lack tumorigenicity but exhibit immunogenicity. Tumor cell-based vaccines offer an ideal antigen reservoir owing to the high antigen content of tumor cells. However, these antigens must be released from tumor cells before being recognized and processed by APCs [157].

At present, OncoVax and GVAX are the main cell-based vaccines undergoing investigation for the treatment of CRC [144]. OncoVax, which is constructed using CRC cells from patients, is an autologous therapeutic vaccine used after surgical resection. Studies have shown that a three- or four-dose regimen of OncoVax after surgery is effective [158,159]. The combined use of OncoVax and 5-fluorouracil (5FU) or folinic acid has been shown to improve the outcomes of patients in phase-III trials [160]. OncoVax offers significant benefits in terms of overall survival, recurrence-free period, and recurrence-free survival in patients with stage-II CRC [161]. MUC-1, a transmembrane glycoprotein aberrantly upregulated in CRC, is a potential target antigen of peptide-, DC-, and WTC-based vaccines. In addition, it can be used in CRC cell/CRC stem cell (CCSC)-based immunotherapeutic vaccines [162]. GVAX is an allogeneic cellular vaccine. It produces granulocyte-macrophage colony-stimulating factor (GM-CSF). We found that GVAX improved antitumor immune responses in patients with advanced CRC with pMMR [144].

DC-based vaccines

As the most functional APCs, DCs are essential for initiating and regulating immune responses. A DC vaccine was found to delay recurrence in patients with CRC metastasized to the liver [163]. Sipuleucel-T (Provenge) is an FDA-approved immunotherapy based on DCs; however, it is not precisely a DC-based vaccine [164]. As sipuleucel-T has shown better therapeutic efficacy in patients with lower tumor burdens, its use as a prophylactic vaccine may be the optimal choice for patients at high risk of developing CRC [165].

Most clinical trials on CRC have reported the use of autologous tumor lysate-pulsed DC vaccines, as these vaccines consistently induce tumor-specific immune responses [166]. In these vaccines, DCs are loaded with exogenous tumor-associated peptides that are widely expressed in most patients with CRC [167]. Developing a DC-based vaccine is a delicate process that necessitates the use of cells collected from patients [168]. Studies have demonstrated that DC-based vaccines are successful in the treatment of CRC, with vaccinated patients having improved survival rates after surgery. The prolonged survival of patients may be associated with the increased abundance of T cells in CRC tissues [169,170].

Early-phase trials have indicated that CEA-targeted DC-based vaccines can safely generate anti-CEA responses in CRC; however, these trials have not shown evidence of prolonged OS or progression-free survival [171,172]. A DC-based vaccine loaded with CEA plus the transcribed neoantigens caspase-5 and TFG- β R-II is being investigated as a prophylactic vaccine for individuals with germline MMR mutations or as a therapy for patients with MSI-positive CRC. The CEA/DC/TT/IL-2 regimen has demonstrated safety and feasibility in patients with terminal CRC. In addition, vaccine adjuvants, such as tetanus toxoid, have been shown to stimulate non-specific immunity against tumors [173].

Molecular vaccines

Peptide vaccines

Peptide vaccines identify antigenic peptides or overexpressed proteins on the surface of tumor cells, demonstrating great adaptability and targeting capability for TAAs [174]. Peptide vaccines are usually less immunogenic and hence require adjuvants.

In our previous study, we developed a novel peptide vaccine named HSP105 that stimulated antitumor immunity and prolonged survival in patients with CRC [175]. In a randomized controlled trial, the peptide vaccine PolyPEP1018, which is composed of seven TAAs commonly observed in mCRC, demonstrated safety when used in combination with maintenance chemotherapy in patients with unresectable MSS mCRC. This vac-

cine effectively triggered specific immune responses and exerted antitumor effects [176].

Peptide vaccines have better tolerability and safety than traditional antitumor treatments such as chemotherapy and ICIs. They usually do not produce severe systemic AEs. Typical adverse reactions include grade-1 or -2 skin redness and small firm lumps at the vaccination site, which usually resolve quickly [177]. However, several peptide vaccines have shown unsuccessful results in clinical trials owing to immune evasion of cancer cells and the absence of relevant tumor markers [178]. The combination of radiotherapy and peptide vaccines has shown safety and effectiveness in the treatment of solid cancers [179]. In addition, the combined use of ICIs and peptide vaccines may enhance outcomes in some cancer cases [180,181]. Altogether, cancer vaccines based on peptides offer the benefits of safety, excellent tolerance, and potent immunity [178].

Owing to the MHC restriction of peptide vaccines, which primarily activate T cells, immune evasion may occur [168].

DNA vaccines

DNA vaccines, a novel type of cancer vaccine, involve the use of eukaryotic expression plasmids to introduce exogenous antigenic genes for the production of antigenic proteins in host cells. These proteins elicit an immune response, inducing autoimmunity and preventing diseases [148]. Some DNA vaccines have received approval for veterinary use. These vaccines can be injected directly into the body, triggering humoral and cellular immunity through DC-mediated production of MHC-I and MHC-II antigens [182]. However, to date, no DNA vaccines have received approval for use in humans [183].

DNA vaccines have therapeutic potential against metastatic tumors owing to their capacity to promote systemic immune responses. In addition, these vaccines can promote immune memory [184,185]. The limitations of DNA vaccines include suboptimal immunogenicity and their low potential for translation from pre-clinical to clinical trials [148]. The MelCancerVac vaccine uses DCs to specifically address overexpression of the TAA MAGE-A3 in CRC. Although the overall outcomes are less promising, some patients have experienced prolonged progression-free survival [186]. Most DNA vaccines developed for cancer treatment are under investigation in phase-I or -II clinical trials.

mRNA vaccines

mRNA vaccines introduce exogenous mRNAs encoding antigens into the host, allowing their translation in host cells to trigger a robust immune response [187]. Given their superior safety, efficacy, and easy synthesis, mRNA vaccines hold promise in the treatment of various human diseases, particularly malignant tumors. However, mRNA vaccines have drawbacks such as instability and low delivery efficiency *in vivo*. The use of nanotechnology in biomedical applications may help develop effective strategies for early detection and prompt treatment of cancer. A recent study showed that lipid nanoparticle (LNP)-based mRNA vaccines can overcome the problems of storage and inaccurate targeting. In pre-clinical trials, personalized mRNA vaccines have demonstrated therapeutic efficacy in multiple solid tumors, including CRC, highlighting the beginning of a new era of therapeutic cancer vaccines [188].

In a recent study, researchers successfully formulated an mRNA vaccine with liposome/pepsin-based delivery (CLPP) and demonstrated its safe and efficient transfection into the CRC cell line CT26. In addition, the vaccine induced potent immune responses in a CT26 xenograft mouse model of CRC [189].

RNA vaccines can elicit mild-to-moderate symptoms such as flu-like syndromes or injection-site reactions. Notably, up to 90 % of individuals who receive RNA vaccines experience some AEs,

including potentially severe conditions such as myocarditis and neurological inflammation [190].

Viral vector vaccines

A viral vector vaccine is composed of a viral vector encoding a pathogenic antigen and an adjuvant stimulatory molecule [191,192]. Upon vaccination, the encoded antigenic genes are translated to produce proteins that diffuse and induce immunity. The viral vector vaccines currently under investigation are technologically advanced, scalable, and induce durable immune responses without requiring an adjuvant [191].

Oncolytic virus (OV) therapy involves the use of OVs to promote immune responses and selectively destroy tumors [191]. Cowpox virus (VV) vectors, which can incorporate exogenously encoded antigens or immunomodulators, have shown efficacy in animal experiments and have been genetically modified to exhibit antitumor activity in many types of cancers, including CRC [193].

GA733 (also known as CO17-1A, KSI-4, Ep-CAM, or KSA), a CRC-specific antigen, is beneficial for both passive mAb therapy and induction of immune responses. It has been successfully cloned in baculovirus (BV), adenovirus (AV), and VV vectors. Notably, AV vectors encoding GA733 potentiated with IL-2 hold promise in the treatment of colon cancer [194]. Venezuelan equine encephalitis virus (VEEV)-based virus-like replicon particle (VRP)-CEA vaccine, which encodes a CEA (6D) epitope, can effectively stimulate CEA-targeted immune responses. A phase-I trial indicated the potential therapeutic efficacy of VRP-CEA in CRC [195]. Early-phase trials on the poxvirus vector vaccine PANVAC have demonstrated encouraging results in advanced CRC. This vaccine involves the use of two viral vectors incorporating tumor-specific epithelial mucin 1 and CEA, both aberrantly expressed in CRC. Clinical trials have validated the safety of PANVAC and its ability to stimulate T-cell responses, which amplify antitumor immune responses [196].

Adjuvants in cancer vaccines

When used alone, antigens induce weak immune responses; therefore, adjuvants are used to amplify the immune response by enhancing the function of APCs. Adjuvants can be categorized into immunostimulants and delivery systems. Immunostimulants target receptors on APCs to induce maturation and stimulation, thereby enhancing the activity of APCs [197]. Vaccine adjuvants include mineral salts, microbial extracts, emulsions, saponins, synthetic agonists, polymers, nanoparticles, and liposomes [198,199]. The commonly used vaccine adjuvants in cancer research include toll-like receptor (TLR) agonists, cytokines, lipid-based adjuvants, particulate adjuvants, and ICIs.

The clinical failure of peptide vaccines may be attributed to the inadequate activation of the immune response [200]. Despite the frequent use of incomplete Freund's adjuvant (IFA) in cancer vaccine trials, the objective response rate rarely exceeds 3 % [201]. Peptide vaccines containing IFA immobilize antigen-specific T cells upon injection, leading to their inactivation and depletion [202]. Therefore, the clinical efficacy of these vaccines is minimal.

GM-CSF is a commonly used adjuvant in vaccine formulations [164]. The recruitment and activation of APCs are imperative for the induction of GM-CSF-stimulated antitumor T-cell responses [203]. In a clinical study, GM-CSF was found to prolong the survival of patients with breast cancer [204]; however, its use in CRC has been less successful. A study on a rodent model of CRC showed that GM-CSF prevented distant metastasis and mitigated tumor expansion, with minimal or no adverse reactions [205].

Alum, another common adjuvant, can enhance humoral immunity but not cellular immunity [206]. Upon activation, TLRs can effectively enhance antitumor immunity. TLR stimulation triggers

Table 3

Clinical trials of major cancer vaccine in CRC.

Clinicaltrials.gov Identifier	Phase	Patient Population	Intervention/ Treatment	Primary endpoints	Enrollment	Recruitment Status
NCT04147078	I	Post-operative	DC vaccine	Safety, feasibility	80	Recruiting
NCT03730948	I	CRC	mDC3 vaccine	Safety tolerability	4	Termination with results
NCT01885702	I/II	Lynch syndrome, MSI	mDC3 vaccine	Safety, feasibility	25	Active, not recruiting
NCT04912765	II	mCRC	DC Vaccine	Safety, feasibility	60	Recruiting
NCT05235607	I	CRC	Nivolumab	Safety, validity	60	Unknown
NCT06522919	II	pMMR MSS mCRC	DC vaccine	Safety, validity	36	Not yet recruited
NCT03185429	Not applicable	CRC	TSA-DC vaccine	Safety and tolerability	20	Unknown
NCT00558051	I	CRC	DC vaccine	Safety and immuno-efficacy	15	Completed
NCT00103142	II	CRC	PANVAC vaccine	Safety, validity	74	Termination with results
NCT00003433	I/II	Colon cancer	carcinoembryonic antigen RNA-pulsed DC cancer vaccine	Safety, validity	22	Completed
NCT00004604	I	Patients with metastatic malignant tumors	CEA RNA-pulsed DC cancer vaccine	therapeutic effect	24	Completed
NCT00027534	I	Advanced or metastatic malignant tumors expressing CEA	TRICOM-CEA	therapeutic effect	14	Completed
NCT00033748	II	mCRC	monoclonal antibody 11D10 anti-idiotypic ; monoclonal antibody 3H1 Alu Gel	therapeutic effect	56	Completed
NCT00228189	I/II	mCRC	CEA-loaded dendritic cell vaccine	Immune response, toxicity	30	Completed
NCT03391232	I/II	mCRC	PolyPEPI1018	Adverse events	11	Completed
NCT01461148	I/II	MSI-H CRC	FSP	Immune response	22	Completed
NCT03689192	I	mCRC	ARG1-18, 19, 10	Adverse events	13	Recruiting
NCT00641615	I	CRC	RNF 43–721	Safety	18	Completed
NCT04117087	I	MMR-p CRC	KRAS peptide vaccine	therapeutic effect	30	Recruiting
NCT05130060	I	mCRC	Nivolumab Ipilimumab CRC peptide vaccine PolyPEPI1018	Safety and tolerability	15	Completed
NCT06411691	II	MMR-p CRC	KRAS vaccine containing Poly-ICLC adjuvant	Efficacy and immune response	50	Completed
NCT04799431	I	MMR-p mCRC	New antigenic vaccine with Poly-ICLC adjuvant	Feasibility, safety and immune response	0	The research never started.
NCT01147965	I/II	Colon cancer	AD5[E1-, E2b-]-CEA(6D)	Safety	35	Completed
NCT01972737	I	Colon cancer	Ad5-hGCC-PADRE	Adverse events, antibody response	10	Completed
NCT00027354	I	mCRC	TRICOM-CEA(6D)	Safety, immune response		Completed
NCT01191684	I	CRC	Modified cowpox virus Ankara vaccine expressing p53	Safety and tolerability	12	Completed
NCT01972737	I	Colon cancer	Ad5-hGCC-PADRE vaccine	Adverse Reactions, Antibody Reactions	10	Completed
NCT06149481	I/II	mCRC	Therapeutic CEA, Brachyury and MUC1 TriAdeno Vaccine Platform	safety	60	Recruiting
NCT05078866	I/II	Lynch syndrome	Adenovirus tumor-specific neoantigen-initiated vaccine GAd-209-FSP	safety	60	Recruiting

CRC, colorectal cancer; mCRC, metastatic colorectal cancer; DC, dendritic cell; pMMR, proficient mismatch repair; MSS, microsatellite stability; TSA, tumor-specific antigen; CEA, carcinoembryonic antigen.

a transient state of tolerance known as TLR tolerance [207]. Personalized cancer vaccines using novel antigens induce high-affinity T-cell responses without causing autoimmunity, demonstrating good therapeutic potential [208]. Given that T-cell proliferation peaks around day 7, followed by the contraction and conversion of some effector T cells into memory T cells, the interval between vaccinations should be extended to 7 days [209].

Unaddressed challenges

The cancer vaccine technology is immature in several aspects; therefore, the efficacy of these therapeutic vaccines remains unsat-

isfactory. The relevant ongoing clinical trials are summarized in Table 3. Factors that challenge the development of effective cancer vaccines include the diversity of the TME, the presence of immunosuppressive cells, and potential tumor avoidance strategies [210]. Peptide vaccines can exert toxic effects on tumor cells but induce weak antitumor immune responses. Early-phase clinical trials have demonstrated that immune responses triggered by peptide vaccines weaken over time. However, vaccines targeting multiple TAAs may stimulate antigen-specific immune responses, potentially overcoming the resistance to peptide vaccines and suppressing the immune evasion of cancer cells. Standard mRNA vaccines often require numerous doses and substantial vaccine response strength

to induce adequate antitumor immune responses. Patients undergoing initial-stage adjuvant therapy may not respond optimally owing to the presence of a highly immunosuppressive TME [211]. The presence of preexisting immunity to viral vectors has been demonstrated to have the potential to reduce the efficacy of viral vector vaccines. Issues related to safety and the antigen-carrying capacity of specific viral vectors should be carefully examined to avoid adverse reactions after vaccination [212]. The common side effects of cancer vaccines include soreness at the injection site, headaches, flu-like symptoms, fever, nausea, diarrhea, rashes, reddening, itching, muscle aches, and respiratory complications. Less frequent but potentially severe adverse reactions include immune system disruption, psychiatric disorders, and pulmonary embolism. In addition, vaccines and their adjuvants may cause hyponatremia, anemia, and colitis and increase the levels of liver enzymes and creatinine [210].

Other factors affecting immunotherapy

Tumor immune microenvironment

The tumor immune microenvironment (TIME) mainly comprises tumor cells, immune cells, and cytokines. The interplay between these elements drives the immune response, resulting in either antitumor or protumor immune activity [213]. For instance, the immune system has the capacity to eliminate tumor cells through the cancer-immunity cycle. Conversely, cancer cells may evade immune surveillance by creating an immunosuppressive microenvironment. Therefore, remodeling the TIME represents an effective strategy for enhancing the tumor-killing ability of immune cells.

The TIME of CRC gradually weakens immunity by attenuating tumor-induced immune responses, facilitating the escape of tumor cells from the immune system. During the progression of CRC, Tregs and myeloid-derived suppressor cells (MDSCs) suppress antitumor immune activity, promoting tumor growth [214]. These immunosuppressive cells outnumber cytotoxic immune cells as the disease progresses. In addition, the immune checkpoints expressed on T cells [215,216], bind to their corresponding ligands expressed on cancer cells, respectively, attenuating immune responses and facilitating immune evasion.

The production of immunosuppressive cytokines by tumor cells facilitates the development of an immunosuppressive TME [217,218]. In addition, Tregs generate immunosuppressive cytokines to suppress the function of CTLs and maintain immunological tolerance [219,220]. As tumors evolve, immunosuppressors dominate over immune effector cells.

Inflammatory signaling pathways are another important component of the TIME. These pathways can promote carcinogenesis. For instance, local rectal inflammation promotes tumor development by activating proinflammatory signaling pathways, such as the NF- κ B and STAT3 pathways, which drive angiogenesis and suppress the tumor-killing activity of immune cells [221]. NF- κ B, an essential dimeric mediator that regulates genetic functions integral to immunity and inflammation, responds via its up- or down-regulation [222]. Upon activation, NF- κ B induces the release of proinflammatory cytokines, such as IL-1 and IL-6, stimulating cancer cell proliferation and survival. In addition, it can promote the expression of TNF and COX-2 [223,224]. TNF-induced NF- κ B signalling can accelerate tumor progression. Activated STAT3 is extensively expressed in CRC cells and contributes to the development of CRC [225].

Altogether, an immunosuppressive TME inhibits effective antitumor immune responses, promoting the progression of CRC. Therefore, regulating this TME may suppress protumor immune

responses while stimulating antitumor immunity [221]. Nanoparticles have promising applications in cancer immunotherapy. They can be used to deliver immunomodulatory agents specifically at the tumor site to modify the local TME [221]. Compared with traditional therapies, nanoparticle-based therapies induce robust antitumor immune responses with enhanced specificity and efficacy [226].

Gut microbiome

The gut microbiome is a crucial indicator of cancer owing to its influence on tumor progression and immune responses in various tumors, including CRC [227,228]. It hosts a diverse range of microbes, including bacteria, fungi, and viruses. To date, studies on the gut microbiome have focused primarily on bacterial interactions [227]. The gut microbiome contains bacteria that promote CRC development, such as *Clostridium nucleatum*, *Escherichia coli*, and *Bacteroides fragilis*; bacteria that suppress CRC development, such as *Bifidobacterium*, *Clostridium casei*, *Streptococcus thermophilus*, and *Lactocaseibacillus paracasei*; and several viruses and fungi that are important for the development and progression of CRC [229].

Gut microbes can alter the effectiveness of immunotherapy by modulating the TIME and secreting specific substances [230].

Remodelling of the TME by gut microbes to enhance the efficacy of ICIs

The gut microbiome has a substantial effect on the efficacy of anticancer therapies, and its composition can predict the response to ICIs [231,232]. Studies have shown that *Bifidobacterium pseudomallei*, *Actinobacillus mucilaginosa*, *Lactobacillus johnsonii*, and *Oslo-nella* strengthen the antitumor effects of ICIs. Notably, *Bifidobacterium pseudomallei* and *Allobaculum mucolyticum* act through the inosine-A2AR signaling pathway [233]. T-cell-targeted A2AR signaling may mediate the synergy between the gut microbiome and immunotherapy. In animal models, *Bifidobacterium bifidum* has been demonstrated to enhance the effectiveness of ICIs and mitigate adverse effects [234]. In addition, it has demonstrated prophylactic potential in patients with CRC. Clinical trials evaluating the therapeutic value of microbial agents combined with immunotherapy are underway. Future studies should investigate the effects of the combination of microbial agents and other anticancer therapies and the mechanisms through which gut microbes drive antitumor immunity [233].

Gut microbial secretion products that enhance the efficacy of ICIs

Gut microbes modulate CRC development by interacting with host CRC and immune cells, releasing various metabolites, proteins, and macromolecules. Among these bacterial metabolites, primary bile acids and short-chain fatty acids are considered harmful, whereas ursodeoxycholic acid and butyric acid are considered protective [235]. Notably, *Roseburia intestinalis* suppresses colorectal carcinogenesis by producing butyrate, which enhances the efficacy of anti-PD-1 therapy by stimulating functional CD8⁺ T cells. Studies have indicated that secondary bile acids and tryptophan exert immunosuppressive effects [236]. Gut microbes degrade indigestible polysaccharides to acetate, propionate, and butyrate. These short-chain fatty acids directly affect CRC cells and immunity, controlling tumorigenesis and inflammation. In particular, they have been shown to inhibit the proliferation of CRC cells, induce cell cycle arrest, promote cell senescence and apoptosis, and exhibit antitumor activity [237,238]. Butyrate has been shown to enhance the antitumor effects of CD8⁺ T cells during chemotherapy [239]. The therapeutic efficacy of nivolumab and pembrolizumab combined with short-chain fatty acids has been investigated in solid tumors [240].

Gut microbes produce DNA-damaging toxins known as genotoxins. *B. fragilis* toxin (BFT) and *E. coli* toxin have been associated with CRC development [241,242]; however, their role in tumor inflammation remains unclear.

Treatment

Fecal microbiota transplantation (FMT) regulates antitumor immunity by altering the gut microbiota and affects the efficacy of immunotherapy, particularly ICIs [236]. Studies have highlighted the potential of FMT to increase the effectiveness of ICIs and attenuate drug resistance [243,244].

Probiotics have been demonstrated to be efficacious for the promotion of health in their host when administered in sufficient amounts [245]. Administration of probiotics such as *Bifidobacterium lactis* and *Lactobacillus acidophilus* has been shown to increase the proportion of butyrate producers in the tumor tissues, mucosa, and feces of patients with CRC (NCT03072641). In addition, a trial showed that administration of probiotics decreased the mRNA expression of the inflammatory cytokines IL-1b, IL-10, and IL-23A in the colon mucosa (NCT01895530). *Bifidobacterium bifidum* [232], *Akkermansia* [246], *Enterococcus*, *E. faecalis*, and *Ruminococcaceae* [247] have been identified as target gut microbes for enhancing the efficacy of ICIs.

Genetically engineered microbes with nutritional deficiencies and inducibility have demonstrated antitumor effects in preclinical trials [248–251], representing a promising approach to cancer treatment.

Drugs influencing the effectiveness of immunotherapy

Antibiotics

CRC is often accompanied by inflammatory and infectious conditions that require antibiotic (ATB) therapy. The gut microbiome regulates the response to immunotherapy [252]. Studies on rodent models have indicated that gut microbiota dysbiosis caused by either sterilization or ATB therapy severely diminishes the tumor-killing function of CD4⁺ T cells during ICI therapy [244]. A statistical compilation suggested that oral broad-spectrum ATB therapy increased the risk of colon cancer, particularly in the proximal region, but decreased the risk of rectal cancer, which may be attributed to changes in the gut microbiome and carcinogenesis [253].

Antacids

Proton pump inhibitors (PPIs) remarkably affect the gut microbiome by regulating gastric acidity and motility [254]. PPIs reduce the diversity of gut microbes, influencing the selection of favorable and detrimental bacterial species. In addition, PPIs have been shown to suppress an acidic TME and exhibit antitumor activity; however, these findings warrant validation in the context of CRC [255].

Opioids

Opioids affect receptors in not only the central nervous system but also the enteric nervous system, significantly affecting gastrointestinal function [256]. For instance, opioids can contribute to constipation and nausea. In addition, they can alter the composition of the gut microbiota and compromise intestinal barrier function, leading to gut bacterial translocation and subsequent inflammation [257]. The effects of opioids on cancer have been examined in limited studies, warranting further investigation.

Metformin

When metformin and PD-1 inhibitors are co-administered, they can improve intratumoral T-cell function and tumor clearance, increase IL-10 production, improve CD8⁺ T-cell function, and alter

the composition of gut microbes by influencing bacterial folate and serine/methionine metabolism [258,259]. However, metformin has not demonstrated noteworthy antitumor effects in clinical trials. Metformin decreases Yes-associated protein 1 (YAP1) expression, induces YAP1 phosphorylation, and blocks the entry of YAP1 into the nucleus to regulate PD-L1 expression in human CRC cells [260]. It stimulates CD8⁺ T-cell function in CRC by reprogramming tryptophan metabolism [261] and prevents hypoxia in the TME by inhibiting the oxygen consumption of tumor cells, leading to tumor cell clearance by T cells [259]. A liposomal oxaliplatin prodrug loaded with metformin has been shown to enhance the efficacy of immunotherapy in CRC [262]. Altogether, metformin has demonstrated therapeutic potential against CRC and may be used in immunotherapy as well as combination therapy.

Other factors influencing the effectiveness of immunotherapy

Age may influence the efficacy of immunotherapy. Research has demonstrated that PD-1 expression is elevated in the T cells of mature animals [263]. This upregulation may lead to overactive disease progression, a risk prevalent in geriatric patients, despite the use of PD-1/PD-L1 inhibitors [264].

Sex can influence not only the disease course of CRC but also the efficacy of immunotherapy. A study revealed a correlation between sex and the effectiveness of immunotherapy, with male patients having more therapeutic benefits in most cancers studied, including CRC [265]. Moreover, male patients with mCRC have been reported to outlive female patients with mCRC after ICI therapy [266].

The topical application of high-dose vitamin C shows promise as an adjuvant therapy. It regulates immune cell migration within the TME and suppresses tumor growth through a T-cell-based mechanism. This mechanism amplifies the cytotoxic function of CD8⁺ T cells and synergizes with ICIs, resulting in effective cancer treatment. Notably, the combination of vitamin C and ICIs can kill mismatch repair-deficient tumor cells with high mutation rates [267].

Challenges

Immunotherapy holds remarkable potential as a novel approach to cancer treatment. However, several issues associated with the use of immunotherapy in CRC remain to be addressed:

- (1) ICIs have shown good therapeutic efficacy in various CRC subtypes; however, their effectiveness in CRC characterized by pMMR/MSS or dMMR/MSI-L remains suboptimal [30]. This limited effectiveness can be attributed to two factors. First, CRC with pMMR/MSS is molecularly characterized by a low tumor mutation burden (TMB) and insufficient neoantigen production, which results in the inability of the immune system to recognize tumor cells. In addition, this CRC subtype has low immune cell infiltration and an imbalance of cytokines and signaling pathways in the TME. Second, combination therapy for CRC is in the preliminary stage of research and warrants several improvements. For example, the mechanisms underlying the synergistic effects of combination therapy remain unclear. Chemotherapy may enhance the immune response through immunogenic cell death (ICD); however, the dose and timing are insufficiently optimized, which leads to toxicity superimposition or unstable efficacy. In addition to MSS, certain biomarkers may help identify patients with different CRC subtypes. Strategies aimed at converting “cold tumors” to “hot tumors” are important for expanding the application of immunotherapy [268]. Table 4 summarizes the studies on CRC biomarkers

Table 4
Current and Potential Biological Markers of CRC.

Name and reference	Patient indications	Research progress
TMB [268]	Certain solid tumors	TMB has been shown to be an independent predictor of ICI treatment efficacy in several solid tumors, including CRC.
TILS [268]	CRC	TIL is associated with improved survival. The density and location of intertumor T cells have a high prognostic value in patients with CRC.
Cold/Hot [268]	Tumor	Hot tumors may be more sensitive to ICIs; therefore, patients with hot tumors may benefit more from immunotherapy. In addition, cold tumors can be converted to hot tumors by radiation and chemotherapy.
PD-L1 [268]	solid tumor	successful in the clinical management of CRC, but drug resistance is a major limitation of anti-PD-1 therapy in clinical practice.
B2M [268]	MSI-H CRC	The exact predictive role of B2M in CRC remains controversial. In CRC patients who have not received immunotherapy, up to 85 % of B2M-mutated tumors derive clinical benefit from immunotherapy, most notably in MSI-H tumors
B.pseudolongum [233,268]	multiple tumors	enhance the anti-tumor effect of ICIs therapy through Inosine-A2AR signaling
L.Johnsonii [233,268]	multiple tumors	Helps to enhance the anti-tumor effects of ICIs therapy.
Olsenella sp. [233,268]	multiple tumors	Helps to enhance the anti-tumor effects of ICIs therapy.
A.Muciniphila [268]	multiple tumors	enhance the anti-tumor effect of ICIs therapy through Inosine-A2AR signaling.
PLOD3 [269]	CRC	PLOD3 is highly expressed in many tumors including colorectal cancer. PLOD3 is upregulated in advanced CRCs and high PLOD3 expression is associated with poor survival. High PLOD3 expression is associated with low levels of B-cells, CD4 T-cells, M1 macrophages, CD8 T-cells, and a variety of immune-related features.
ferroptosis-related genes [270]	CRC	CRC patients with higher FRG scores showed poorer survival outcomes, higher immune dysfunction and lower response to immunotherapy.
SOC3 [271]	CRC	SOC3 may be involved in tumor immune evasion through immune cell infiltration or T cell rejection in different types of cancer
Hsa_circ_0066351 [272]	CRC	Expression of hsa_circ_0066351 is associated with better survival in CRC patients. Functional assays showed that hsa_circ_0066351 inhibited colorectal cancer cell proliferation.
Syntaxin-6 [273]	CRC	Knockdown of STX6 Suppresses Tumor Growth and Enhances Anti-PD-1 Efficacy in Mouse Models
AHCYL1 [274]	CRC	Tissues without AHCYL1 are less able to recruit natural killer (NK) cells, CD8 + T cells, and tumor-infiltrating lymphocytes (TILs) and are less responsive to immunotherapy.
YTHDF2 [275]	Most tumors	YTHDF2 may be involved in immune regulation by affecting immune checkpoint gene expression and immune cell infiltration in the tumor microenvironment
SCG2 [276]	CRC	The SCG2 high-expression subgroup is immunothermic and considered more amenable to immunotherapy
CHMP7 [277]	CRC	There is a strong correlation between CHMP7 and TME immune cell infiltration, which is involved in inhibitory TME formation and promotes tumor progression.
BST2 [278]	CRC	BST2 upregulation was significantly associated with Braf mutations, dMMR/MSI-H, CMS1 subtypes, and immune response, and is a potential biomarker for predicting immune checkpoint blockade therapy.
ULBP2 [279]	Colon cancer	ULBP2 is a target gene associated with immunity and prognosis in CC patients. CC patients with higher ULBP2 expression have a poorer prognosis.
PTCH1 mutation [280]	gastrointestinal cancer Colon cancer CRC	PTCH1 mutations may represent a potential biomarker for predicting the response to ICIs in GC.
SPEN Mutations [281]	CRC	SPEN mutations may improve the efficacy of ICI in CRC. SPEN mutations may serve as predictive biomarkers of ICI responsiveness
ADAMTSL2 [282]	Colorectal adenocarcinoma-predominant pan-cancer CRC	High expression of ADAMTSL2 is significantly associated with lower OS and immune infiltration in CRC
NIFK [283]	Colorectal adenocarcinoma-predominant pan-cancer	Correlation between NIFK and specific immune characteristics (e.g., immune cell infiltration, immune checkpoint genes, TMB, and MSI). NIFK expression was significantly up-regulated in tumor cells, and down-regulation of NIFK expression was found to inhibit the proliferation, migration and invasive ability of colorectal cancer cells.
Serum iron element [284]	Advanced Solid Tumors	Serum iron positively correlates with the efficacy of ICIs by enhancing innate immunity and cytokine release in advanced metastatic cancer
KRAS/NRAS [285]	Tumor	KRAS is mutated in about 40 % of CRC cases, and patients with CRC carrying KRAS mutations have a poor prognosis. NRAS is similar to KRAS but has been less well studied.
Soluble PD-L1 [286]	mCRC	Soluble PD-L1 is mainly composed of Secreted PD-L1, and its level is higher in patients with distant metastases.
MSI-H/dMMR [287]	CRC	MSI-H/dMMR may be correlated with a higher mutational load, which leads to neoantigen formation and activation of the body's immunity
Immunoscore [287]	solid tumor	Immunoscore, a biomarker that measures the density of CD3 + and CD8 + lymphocytes in the center of a tumor and its aggressive margins. Among other things, Immunoscore-IC is used to predict response to PD-1/PD-L1 checkpoint inhibitor immunotherapy in a variety of cancer indications.
POLE/POLD1 [288]	MSS or MSI-L CRC	Somatic or germline mutations in POLE and POLD1 contribute to the pathogenesis of CRC through DNA hypermutation phenotypes.
POLE P286R mutation [289]	CRC	Clinicopathologically, EOCRCs with POLE mutations are much more prevalent in the right colon than in the left, affecting men more commonly than women.
SLC11A1	CRC	SLC11A1 plays a crucial role in TME and may serve as a potential biomarker for poor CRC prognosis and poor immunotherapy outcome

CRC, colorectal cancer; TMB, tumour mutational load; TIL, tumor-infiltrating lymphocyte; mCRC, metastatic colorectal cancer; MSI-H, high microsatellite instability; MMR, mismatch repair; ICIs, immune checkpoint inhibitors; PD-L1, anti-programmed cell death-ligand 1; MSS, microsatellite stability; MSI-L, low microsatellite instability; PD-1, anti-programmed cell death-1; TME, tumor microenvironment.

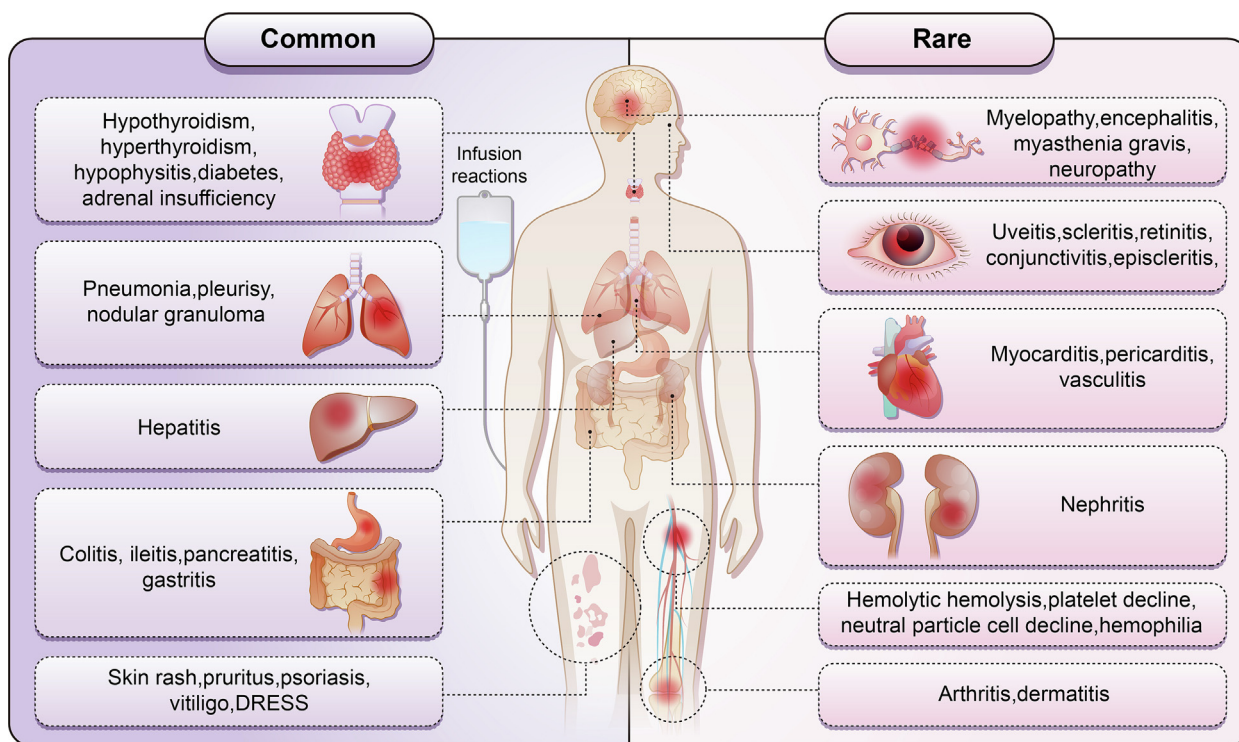


Fig. 2. Immune-related adverse events (irAEs) can affect almost any organ, and their manifestations range from minor side effects to life-threatening complications. Commonly, they include endocrine system adverse events, respiratory system adverse events, digestive system adverse events, skin adverse events and infusion reactions. Relatively rare are neurological adverse events, eye-related adverse events, heart-related adverse events, kidney-related adverse events, haematological adverse events and arthritis.

conducted in recent years [233,268–290]. The biomarkers that have been used to predict immunotherapy responses in CRC include PD-L1, MSI or dMMR, and TMB. However, the clinical translation of these biomarkers is hindered by various factors. For example, certain receptors, such as TIGIT/CD155, can competitively bind to ligands, and their mechanisms are complex and unclear [291]. Other factors include the lack of standardization in the assessment and reporting of biomarkers in preclinical studies [292,293], limited data on the predictive value of biomarkers in ICI therapy [292], and subjectivity resulting from the use of immunohistochemical (IHC) analysis.

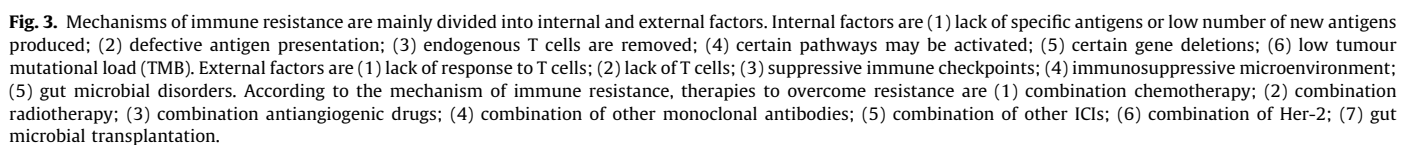
- (2) Despite a positive response to immunotherapy, improving therapeutic efficacy based on the TME, tumor type, and patient-specific factors is essential. Therefore, personalized therapy is required to enhance effectiveness [294].
- (3) Immunotherapy can cause side effects, which can progress to severe irAEs [93,94]. These irAEs can cause damage to multiple organs and systems (Fig. 2). Research has demonstrated that patients with CRC have a higher incidence (up to 27 %) of gastrointestinal irAEs than those with other cancers [295,296]. Different types of ICIs may lead to different degrees of immune activation. In addition, tissue-related factors may contribute to irAEs. irAEs may vary across ICI classes or tumor tissues. Moreover, the same ICI may cause varying irAEs in different tumor types, and this effect may be even more pronounced when these drugs are used in clinical practice [296,297]. Therefore, predictive tools and effective strategies should be developed to prevent and manage these effects.
- (4) Post-treatment drug resistance is possible, and the mechanisms through which tumor cells evade immune surveillance warrant further investigation. As shown in Fig. 3, the mechanisms involved in immunotherapy resistance are influenced by several factors.

To address the abovementioned challenges, immunotherapy can be combined with other strategies [88,98], such as improvement of the immunosuppressive TME; FMT; and use of immune adjuvants, OV vectors, and engineered bacteria.

Future perspectives

A majority of patients with CRC have the pMMR/MSS subtype and are insensitive to ICI-based immunotherapy [298]. In addition, patients with the dMMR/MSI-H subtype may also be resistant to immunotherapy. Autologous cell-based immunotherapies, such as CAR-T cell therapy, are subject to patient eligibility and have high costs. Moreover, strategies for metabolic reprogramming of the TME, signalling pathways of the immune system, mechanisms involved in antigen presentation and immune escape, and combinations of immunotherapies warrant further investigation.

To address the limitations of immunotherapy, it can be combined with other anticancer therapies to develop personalized treatment plans. TME remodeling, biomarker screening, and multidisciplinary integration are essential for the successful treatment of CRC. Owing to the heterogeneity of CRC and drug resistance, one type of immunotherapy is unlikely to eliminate all CRC cells. Therefore, the development of new combination therapies, such as the co-administration of two ICIs, is warranted. Recent studies have shown a strong correlation between gut microbes and immunotherapy, providing novel avenues for improving the treatment of pMMR/MSS-positive CRC. In addition, FMT and probiotic interventions have shown promise as adjunctive therapies. More biomarkers should be identified to predict the efficacy of immunotherapy and guide personalized treatment in CRC. Immunotherapy resistance is attributed to multiple mechanisms, such as immunosuppressive cell infiltration, mutations in key genes, gut microbial regulation, and metabolic abnormalities. Future studies should target cross-regulatory networks and use a



This article does not contain any studies with human or animal subjects.

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