Colorectal cancer and tumor vaccines (Review)

JIANSHUANG MA, CONGMING XIANG and DONG XIA

Department of General Surgery, Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646000, P.R. China

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Abstract. Colorectal cancer (CRC), one of the most common malignant tumors of the digestive system worldwide, shows an annual upward trend in its incidence and mortality, posing a serious threat to human health and life. In recent years, with the rapid development of immunotherapeutic drugs and the continuous optimization of CRC treatment strategies, breakthrough progress has been achieved in CRC treatment, which has improved patients' prognosis to a certain extent. As an emerging immunotherapy, tumor vaccines can stimulate the body to produce specific immune responses against tumor antigens, activate cellular immunity, eliminate tumor cells and inhibit tumor growth. The present study reviewed different categories of CRC vaccines and their clinical applications in CRC treatment, analyzed the current challenges in CRC vaccine development and suggested future research directions to promote the further development and clinical application of tumor vaccines.

Contents

1. Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system worldwide, accounting for ~8.5% of total cancer-related mortality (1). According to the report from the World Cancer Research Fund, there were 1,926,425 newly diagnosed CRC cases and 904,019 deaths globally in 2022, with the age of onset gradually trending younger (2,3). Despite continuous advances in early screening and treatment methods for CRC, its high incidence and insidious nature still impose a heavy burden on global public health. Traditional CRC treatment primarily relies on surgical intervention, supplemented by radiotherapy and chemotherapy, yet patient prognosis remains unsatisfactory. In recent years, the emergence of immunotherapy has brought significant breakthroughs to CRC treatment. The U.S. Food and Drug Administration (FDA) has approved PD-1 inhibitors such as pembrolizumab and nivolumab for CRC patients with mismatch repair-deficient/high microsatellite instability (MSI-H) (4). However, this subtype accounts for only 15% of CRC cases. The remaining patients with mismatch repair-proficient (pMMR)/microsatellite stable (MSS) exhibit limited response to immune checkpoint inhibitors due to low immunogenicity and an immunosuppressive tumor microenvironment (TME), highlighting the urgent need for the development of novel immunotherapy strategies (5).

Therapeutic tumor vaccines are a type of biological agents that utilize tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs) to activate the patient's own immune system through *in vivo* presentation, inducing an active, specific and durable T-cell-mediated anti-tumor immune response. Compared with traditional CRC treatment methods, vaccine therapy is generally well-tolerated, with minimal dose-related toxicity and severe autoimmune reactions, demonstrating promising clinical application prospects (6). In 2010, the FDA approved the first tumor vaccine, sipuleucel-T, for the treatment of prostate cancer, officially marking the beginning of anti-tumor immunotherapeutic vaccines and attracting widespread attention in the oncology community (7). At present, a novel generation of vaccines targeting CRC is transitioning from the laboratory to clinical practice, offering the potential to open the door to long-term survival for patients with MSS tumors. The present review covered the types, research and development status, clinical application progress and current challenges of CRC vaccines, aiming to promote further research and application of tumor vaccines.

2. Types of CRC vaccines

Tumor vaccines are a form of biotherapy that activates the body's immune system through tumor antigens. Their

Correspondence to: Professor Dong Xia, Department of General Surgery, Affiliated Hospital of Southwest Medical University, 25 Taiping Street, Jiangyang, Luzhou, Sichuan 646000, P.R. China
E-mail: litterdevil@outlook.com

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mechanism involves introducing antigens such as tumor cells, peptides, or genes into the patient's body to induce cellular and humoral immune responses to eliminate tumors. Based on the different source of vaccine antigens, they can be classified into tumor cell vaccines, dendritic cell (DC) vaccines, nucleic acid vaccines, peptide/protein vaccines and viral vector vaccines (Tables I and II).

Tumor cell vaccines. Tumor cell vaccines are a category of vaccines prepared with tumor cells themselves as the core component (8). Their advantage lies in the ability to utilize the complete antigen profile carried by tumor cells. Through attenuation or inactivation techniques such as radiation or chemical drug treatment, the activity of tumor cells is eliminated, ultimately producing an immunogenic vaccine formulation (9). These vaccines encompass TSAs of CRC and are characterized by high immunogenicity. They can stimulate a broad-spectrum immune response against multiple tumor antigens, thereby reducing the risk of immune escape caused by the loss of a single antigen. However, tumor cell vaccines also contain TAAs, which are expressed not only on tumor cells but also in normal tissues, leading to immune tolerance that hinders the generation of a strong immune response against tumor cells (10). The resulting self-recognition blurs the attack boundaries of immune cells, not only weakening the killing effect on tumor cells but also potentially causing damage to normal tissues due to cross-reactivity. For patients with a history of allergies or pre-existing autoimmune diseases, this strategy is more likely to induce or exacerbate autoinflammation, thus limiting its application (11). In summary, tumor cell vaccines can provide a relatively comprehensive tumor antigen profile, covering unknown antigens and multiple epitopes, thereby reducing the risk of immune escape caused by the loss of a single antigen. However, their complex composition, challenges in individualized preparation and quality control, limited immunogenicity of unmodified tumor cells and the difficulty in manufacturing sufficient quantities of qualified vaccines remain obstacles to their application (12).

DC vaccines. DCs are one of the most potent antigen-presenting cells in the human body. The pivotal strategy of DC-based vaccines involves first inducing DC maturation using cytokines, followed by loading them with exogenous peptides or tumor lysates to enrich them with tumor antigen information before reinfusing them into the patients (13). Once the DCs home in on the lymph nodes, they present tumor antigens to $CD8^+$ and $CD4^+$ T cells, thereby initiating precise, specific and memory-driven anti-tumor immune responses (14). Consequently, DC vaccines offer distinct advantages, including well-defined targets, low off-target rates and a high degree of safety. However, DC vaccines still face several challenges, such as the complexity and high cost of personalized preparation, the limited efficiency of expanded DCs *in vitro* in migrating to lymph nodes *in vivo* and the potential suppression of vaccine-induced immune responses by immunosuppressive factors in the TME, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) (15). To overcome these hurdles, researchers have recently employed various strategies to enhance the efficacy of DC vaccines, including genetic modification to improve antigen presentation and migration capabilities, or combining cytokines [such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4] to optimize the *in vitro* culture microenvironment for DCs (16,17). Additionally, to improve DC vaccines, the functional differences among various DC subsets are gradually being deciphered: Monocyte-derived DCs are easily obtainable but have limited maturity; conventional DCs possess strong cross-presentation capabilities but are scarce in peripheral tissues; plasmacytoid DCs can secrete large amounts of type I interferon and play dual roles in activation and regulation (18). Additionally, future DC vaccine designs may utilize induced pluripotent stem cells to achieve standardized, editable 'super DCs' through directed differentiation, enabling them to exert optimal efficacy in tumor immune responses (19). In summary, DC vaccines leverage the potent capabilities of DCs for antigen uptake, processing and cross-presentation, effectively initiating anti-tumor T-cell immunity and making them suitable for personalized therapy. However, their complex *in vitro* isolation, loading, maturation and reinfusion processes are costly and clinical response rates are markedly influenced by DC quality, lymph node homing efficiency and the tumor immunosuppressive microenvironment (20).

Nucleic acid vaccines. DNA vaccines are a type of nucleic acid vaccine that deliver DNA fragments encoding TSAs into human cells via gene editing technology (21). These fragments utilize the host's ribosomes to synthesize the corresponding antigens, which then activate the immune system through antigen-presenting cells (22). Unlike tumor cell vaccines, the pivotal characteristic of DNA vaccines lies in leveraging the host cell's own expression system to produce tumor antigens, rather than relying on artificially injecting tumor antigens. Compared with the traditional 'exogenous antigen delivery' model, DNA vaccines eliminate the need for antigen carriers, bypass complex purification processes and cold chain logistics and can be stored long-term at room temperature. They also offer advantages such as easy purification, high stability and avoidance of complications associated with viral vectors (23). However, these nucleic acid vaccines exhibit relatively low antigen presentation efficiency and immunogenicity, often requiring the use of lipid nanoparticles to enhance delivery efficiency and adjuvants to boost immune responses (24). Additionally, DNA vaccines carry the risk of random integration into the host genome, potentially disrupting cellular genes. While this risk has not been confirmed in clinical trials to date, longer follow-up periods and more sensitive integration site monitoring technologies are still needed to fully verify their safety. In summary, DNA vaccines offer advantages such as ease of construction, good stability, relatively low cost, high safety and the ability to encode multiple antigens or immunomodulatory molecules, while also inducing major histocompatibility complex (MHC)-I/II-related cellular and humoral immunity. However, they require entry into the nucleus for expression, have relatively limited delivery efficiency and immunogenicity in humans and their clinical efficacy is often constrained by the tumor immunosuppressive microenvironment (25).

Similar to DNA vaccines, RNA vaccines are another type of nucleic acid vaccine that deliver RNA fragments encoding specific antigens into host cells, enabling autonomous translation into tumor antigens and subsequently triggering specific immune

Table I. Advantages and disadvantages of various types of vaccines.

Type	Advantages	Disadvantages
Tumor cell vaccines	High immunogenicity; low risk of escape	Prone to immune tolerance; autoimmune diseases
Dendritic cell vaccines	Clear target; low off-target rate; high safety	Complex process; high cost; high immunosuppression
DNA vaccines	Easy purification; high vaccine stability; avoidance of viral vector complications	Low antigen presentation efficiency and immunogenicity; risk of gene integration
RNA vaccines	Avoids the risk of genome integration; short production cycle, easy for large-scale production	Poor stability; low immunogenicity
Peptide/protein vaccines	High safety; easy to produce; low cost	Prone to immune tolerance; low immunogenicity
Viral vector vaccine	Higher transduction efficiency and immunogenicity	Prone to causing viral toxicity events; complex production process

responses (21). After target cells internalize mRNA, the translated tumor antigens are presented by MHC class I molecules, thereby activating CD8+ T cell-mediated anti-tumor immune responses. Recent studies have also shown that adding specific signal peptides to mRNA sequences encoding tumor antigens can simultaneously promote antigen presentation by MHC class II molecules and stimulate CD4+ T cell responses (26-28). Compared with DNA vaccines, RNA vaccines do not need to enter the nucleus to express antigens, which avoids the risk of genomic integration and results in relatively higher safety. Additionally, RNA vaccines have shorter preparation cycles, are easy to manufacture on a large scale and can rapidly respond to novel antigens caused by genetic mutations (29). For example, the application of BNT162b2, the world's first approved RNA vaccine developed by Pfizer during the COVID-19 pandemic, demonstrated the advantage of RNA vaccines in achieving high efficacy against diseases with high mutation rates (30). However, RNA vaccines have poor stability and require low-temperature storage, imposing higher demands on transportation and storage conditions. Furthermore, due to their short survival time and susceptibility to degradation in the body, RNA vaccines cannot continuously stimulate the body to produce immune responses. To address this, nucleotide modifications or optimized sequence design are needed to enhance their stability and translation efficiency. Moreover, encapsulation with lipid nanoparticles can effectively promote cellular uptake and markedly improve their immunogenicity (31). In summary, RNA vaccines, particularly mRNA vaccines, offer advantages such as rapid design, strong adaptability for individualization, no need to enter the cell nucleus, no risk of genomic integration and the ability to encode full-length or multiple antigens while inducing a broad T-cell response. However, RNA is prone to degradation, its stability and *in vivo* delivery rely on carriers like lipid nanoparticles and challenges remain in antigen selection, targeted delivery and optimization of immune responses (32).

Peptide/protein vaccines. Peptide and protein vaccines are vaccines with specific peptide fragments or genetically engineered recombinant proteins as core antigen components (33). They can activate T cells and induce long-lasting antigen-specific immune memory. Due to the limited immunogenicity of peptide vaccines being restricted by the polymorphism of MHC molecules, different individuals may produce varying immune responses to the same peptide segment, which restricts the applicable population of the vaccine (34). Consequently, adjuvants are often required to enhance their immunogenicity and stimulate the infiltration of immune cells such as CD8+ T cells in the TME, thereby achieving the desired clinical effects (35). Protein vaccines generally contain complete antigenic proteins or their functional domains, providing more abundant antigen information and potentially activating more comprehensive immune response, including both humoral and cellular immunity (36). Although protein vaccines can partially overcome some MHC restriction issues, macromolecular proteins are susceptible to degradation by endogenous enzymes and may trigger non-specific immune responses, which affect the targeting and safety of the vaccine. Despite these drawbacks, the advantages of such vaccines lie in their well-defined composition, high safety, large-scale production via chemical synthesis or recombinant technology, low costs and the ability to precisely target specific antigenic epitopes, thereby reducing cross-reactivity with normal cells. For example, peptide vaccines designed against antigens highly expressed in CRC, such as carcinoembryonic antigen (CEA) and survivin, have demonstrated the potential to induce specific T cell responses in preclinical studies (37). Currently, researchers are committed to enhance the immunogenicity and clinical efficacy of peptide/protein vaccines by optimizing antigen sequences, developing novel adjuvants (such as Toll-like receptor agonists and nanoparticle carriers) and combining immune checkpoint inhibitors. Some candidate vaccines have entered early-phase clinical trials, providing new directions for the immunotherapy of CRC (38). In summary, peptide vaccines have well-defined components, high safety profiles and relatively easy synthesis and quality control, enabling them to induce immune responses against specific tumor epitopes (39). However, they are markedly limited by human leukocyte antigen (HLA) polymorphism, have short half-lives and weak immunogenicity and often require adjuvants, $CD4^+$ T cell assistance, or long peptide designs to enhance effector T cell responses (40).

Table II. Vaccine technology platforms.

Platform Type	Design principles	Advantages	Disadvantages	Specific applications
DNA vaccines	i) Traditional plasmid DNA-TAA platform: Plasmids encode tumor-associated antigens such as CEA, MUC1, HER2, PSA/PAP, gp100, TRP2, among others.	Simple preparation, clear antigenicity and easy for standardized production.	TAA are mostly self-antigens, which are prone to limitations such as immune tolerance, antigen heterogeneity and low-affinity T cells.	Early research on CEA DNA vaccines has been conducted in CRC, such as CEA66 DNA combined with cyclophosphamide and GM-CSF; studies on TAA DNA vaccines have also been carried out for melanoma, prostate cancer, breast cancer and other types of cancer.
	ii) Personalized Neoantigen DNA Platform: Based on tumor/normal tissue WES, RNA-seq and HLA prediction, construct multi-epitope personalized DNA plasmids.	The target exhibits strong tumor specificity, theoretically less affected by self-tolerance; it can simultaneously cover multiple clonal/subclonal neoantigens.	Rely on high-quality samples, sequencing, algorithmic predictions and GMP individualized production; manufacturing cycle and cost are relatively high; clinical benefits still require validation through randomized studies.	GNOS-PV02 can encode up to 40 neoantigens and is combined with an IL-12 plasmid and pembrolizumab; neoantigen-specific T-cell responses were also observed in the Phase I study of the individualized neoantigen DNA vaccine for TNBC.
RNA vaccines	i) Personalized neoantigen linear mRNA: Sequence patient tumor and normal samples, predict HLA-presented neoantigens and synthesize patient-specific mRNA.	Strong tumor specificity, theoretically low off-target effects, capable of inducing CD4/CD8 T cells with multiple epitopes.	Complex manufacturing chain, high cycle time and cost; requires sufficient tumor samples, robust algorithms and rapid GMP. manufacturing	For high-risk melanoma post-surgery to prevent recurrence, V940 + pembrolizumab is used in combination. In the 5-year follow-up, compared with pembrolizumab monotherapy, it reduces the risk of recurrence or death by 49%. Enrollment for the adjuvant melanoma Phase III study has been completed.
	ii) Traditional mRNA vaccines: Pre-encoded with common tumor-associated antigens or viral oncoproteins for specific cancer types.	Scalable, low-cost, fast drug delivery	The target is not entirely tumor-specific; immune tolerance and tumor heterogeneity are the bottlenecks.	As in second-line/later-line combination immunotherapy for advanced melanoma. BNT111 + cemiplimab met the primary endpoint in a Phase II study, with ORR showing significant improvement compared with historical controls.
Peptide vaccines	i) Traditional peptide vaccines: Target tumor-specific driver mutations shared by multiple patients, such as KRAS G12D/G12R/G12V, IDH1 R132H, EGFRvIII, among others.	Combining 'off-the-shelf' and 'tumor-specific' features; no need for full customization for each patient; theoretically less prone to antigen loss when targeting driver mutations.	Only suitable for patients with specific mutations; HLA presentation still needs to be confirmed; tumors may undergo antigen-negative clonal expansion; some projects have experienced Phase III failures.	ELI-002 targets KRAS G12D/G12R or seven KRAS/NRAS mutations; IDH1-vac targets IDH1 R132H; rindopepimut targets EGFRvIII. ELI-002 2P demonstrated an 84% mKRAS-specific T-cell response in MRD^+ pancreatic/colorectal cancer, with improved RFS in high T-cell responders; the 7P version is undergoing a randomized phase 2 study in adjuvant PDAC treatment.

Table II. Continued.

Platform Type	Design principles	Advantages	Disadvantages	Specific applications
	ii) Personalized neoantigen peptide vaccine: Perform WES/WGS/RNA-seq, HLA typing and neoantigen prediction on patient tumor and normal samples, synthesize patient-specific mutant peptides, typically multiple SLPs and combine them with adjuvants such as poly-ICLC and Montanide.	High tumor specificity with minimal expression in normal tissues, theoretically low off-target and autoimmune risks; capable of targeting patient-specific mutations; suitable for postoperative adjuvant therapy and minimal residual disease clearance.	Long preparation period and high cost; reliance on the quality of tumor samples, sequencing and prediction algorithms; not all predicted neoantigens are truly presented or highly immunogenic; difficulty in tumors with low mutation burden; challenging to rapidly apply in advanced patients with rapid progression.	NeoVax, PGV001, GEN-009, among others. NeoVax can include up to 20 personalized neoantigens derived from the patient's tumor; the PGV001 Phase I study demonstrated that personalized peptide neoantigen vaccines are feasible, safe and elicit immune responses in various high-risk recurrent tumors.
Tumor cell vaccines	i) Traditional tumor cell vaccines: Tumor cells are obtained from the patient or allogeneic tumor cells of the same cancer type are used, which are then irradiated, freeze-thawed, chemically treated, or inactivated and administered to the patient in combination with adjuvants such as BCG, GM-CSF and CpG.	Covers both known and unknown antigens; theoretically includes patient-specific neoantigens; does not require prior identification of target antigens. Suitable for development as universal products.	Long preparation cycle; significant batch-to-batch variation; difficulty in culturing certain tumor cells; limited waiting time for advanced-stage patients; weak immunogenicity.	OncoVAX: Autologous colon cancer cells + BCG, used for postoperative recurrence prevention research in colon cancer; Canvaxin: Allogeneic whole-cell melanoma vaccine, showed early signals, but subsequent Phase III melanoma studies failed to demonstrate survival benefits.
	ii) Personalized tumor cell vaccines: For example, adding immunomodulatory genes to tumor cells, such as expressing GM-CSF, while silencing immunosuppressive pathways like TGF-β and furin.	Broad antigen spectrum, with inherent local immune adjuvants; can transform the vaccination site into an 'antigen reservoir + DC recruitment site'; suitable for combination with low-dose cyclophosphamide, CTLA-4/PD-1 inhibitors and radiotherapy.	GM-CSF dosage and the local microenvironment are difficult to precisely control; high doses or sustained GM-CSF may promote myeloid-derived suppressor cells; multiple late-stage Phase III failures suggest that GM-CSF alone is insufficient.	GVAX pancreas: Irradiated pancreatic cancer cells are genetically modified to secrete GM-CSF, which attracts immune cells to recognize antigens on the surface of the irradiated tumor cells, with one of the goals being to eliminate microscopic lesions that may escape conventional treatment.
Dendritic cell vaccine	i) Traditional dendritic cell vaccines: DCs are incubated with known tumor antigen peptides or proteins, such as MART1, gp100, NY-ESO-1, WT1, CEA, HER2, MUC1, survivin, among others.	Clear antigen, relatively straightforward process; facilitates immune monitoring; suitable for validating the immunogenicity of a specific target.	HLA restriction is significant; single antigens are prone to escape; requires patient tumor expression of the target antigen; efficacy of monotherapy in advanced stages is limited.	Melanoma MART-1/gp100 DC vaccine, WT1 DC vaccine, CEA DC vaccine, HER2 DC vaccine, among others.
	ii) Personalized Neoantigen DC Vaccine:	High tumor specificity; low off-target risk;	Long preparation cycle and high cost; reliance on	Personalized peptide-DC, mRNA-DC and neoantigen

Table II. Continued.

Platform Type	Design principles	Advantages	Disadvantages	Specific applications
	Perform WES/RNA-seq/HLA typing on the patient's tumor, predict neoantigens and then load autologous DCs with neoantigen peptides or mRNA.	suitable for postoperative adjuvant therapy/ MRD; can be combined with PD-1.	sample quality and algorithm accuracy; predicted neoantigens may not be truly presented challenging for; tumors with low mutation burden.	DC vaccines for NSCLC, melanoma, glioma, ovarian cancer and other types of cancer.
Viral vector vaccines	i) Traditional viral vector vaccines: The vectors encode viral oncoproteins such as HPV16 E6/E7 and EBV LMP/EBNA. Viral oncoproteins are considered 'non-self' antigens, typically exhibiting stronger immunogenicity than common TAAs.	Strong correlation with tumor antigens; independent of tumor mutational burden; suitable for virus-associated tumors such as HPV and EBV.	Applicable only to virus-positive tumors; monotherapy for advanced solid tumors is generally insufficient and requires combination with PD-1/PD-L1 inhibitors, chemotherapy, or radiotherapy.	TG4001 expresses HPV16 E6/E7 + IL-2 using MVA; HB-200 is an arenavirus vector that expresses a non-oncogenic HPV16 E7/E6 fusion antigen, intended for HPV16-positive head and neck cancer and has been explored in combination with pembrolizumab.
	ii) Personalized neoantigen viral vector vaccine: After sequencing the patient's tumor and predicting neoantigens, multiple neoantigens are encoded into a viral vector, typically combined with ICI .	High tumor specificity, theoretically low off-target risk; viral vectors can enhance T-cell priming; suitable for postoperative adjuvant therapy, MRD, or low tumor burden scenarios.	Personalized manufacturing cycles and costs are high; neo-antigen prediction still has false positives; viral vector capacity is limited; rapid GMP manufacturing and strong bioinformatics capabilities are required.	TG4050: A personalized poxvirus neoantigen vaccine capable of delivering ~30 personalized neoantigens per patient. In an early adjuvant study for HPV-negative head and neck squamous cell carcinoma post-surgery, vaccinated patients exhibited neoantigen-specific T-cell responses and showed a lower early recurrence rate, though the sample size was small and follow-up was limited.

TAA, tumor-associated antigens; CRC, Colorectal cancer; FDA, Food and Drug Administration; MSI-H, microsatellite instability-high; MSS, microsatellite stable; pMMR, mismatch repair-proficient; TME, tumor microenvironment; TSAs, tumor-specific antigens; DC, dendritic cell; DCs, dendritic cell vaccines; Tregs, regulatory T cells; MDSCs, myeloid-derived suppressor cells; MHC, major histocompatibility complex; CEA, carcinoembryonic antigen; HLA, human leukocyte antigen; NKc, natural killer cells; CSF, colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; CpG, cytosine-phosphate-guanine; GBM, glioblastoma; MUC1, mucin-1; NSCLC, non-small cell lung cancer; PFS, progression-free survival; ICI, immune checkpoint inhibitor; LNP, lipid nanoparticle; LPX, lipoplex; CTL, cytotoxic t lymphocytes; DFS, disease-free survival; RFS, recurrence-free survival; OS, overall survival; CAFs, cancer-associated fibroblasts; TAMs, tumor-associated macrophages; TILs, tumor-infiltrating lymphocytes; TCR, t cell receptor; BCR, b cell receptor.

Viral vector vaccine. Viral vector vaccines are prepared by integrating the tumor antigens into the genome of a viral vector (41). After vaccination, the artificially modified viral vector cannot replicate due to genetic defects. Once the pathogen genes integrated into the viral genome are translated, the resulting pathogen protein can be transported outside the cell to stimulate an immune response. Viral vector vaccines feature mature technology and can be easily mass produced. They can induce long-lasting immune responses without the use of adjuvants (42). However, the widely existing anti-vector neutralizing antibodies in the general population may limit their repeated administration (43). Additionally, different types of vectors have varying requirements for the size of the inserted fragment, cytotoxicity and production complexity. Furthermore, the objective response rate of viral vector vaccines as monotherapy is limited. They need to be

used in combination with immune checkpoint inhibitors or chemotherapy to enhance efficacy. High doses of adenoviral vectors may also cause toxic events such as elevated liver enzymes and decreased platelets (44). In summary, viral vector vaccines offer high transduction efficiency and natural immunostimulatory capabilities, enabling efficient delivery of tumor antigens or immunoregulatory genes and eliciting strong cellular immune responses. However, the production processes are complex and may be affected by pre-existing or induced anti-vector immune responses, limitations in repeated dosing and potential safety concerns (45).

3. Development of CRC vaccines

Antigen type development. Antigens are essential substances that initiate specific immune responses in the body. At present, laboratories often use TAAs as a target for vaccine development. However, the expression of TAAs in tumor cells varies and certain TAAs are also endogenously expressed at low levels in normal tissues, which can lead to immune tolerance or trigger autoimmune responses. Therefore, researchers have developed various methods to modify tumor vaccines to overcome or mitigate such immune tolerance (46). For example, using CEA allosteric peptide ligands with higher HLA affinity can effectively activate specific cytotoxic T lymphocytes *in vitro* (47). Another approach to enhance specific T-cell activation is to develop DNA vaccines encoding CEA-derived peptides, cytokines, adjuvants, or helper T cell epitopes (48). In mouse models, such vaccines have demonstrated higher T cell activation compared with peptide-only vaccines (49). However, in clinical trials, the efficacy of these vaccines has generally been unsatisfactory, with clinical response rates not exceeding 17% (49). As a result, research on TSAs has gradually become a focus in recent years. TSAs are expressed exclusively by tumor cells and exhibits high tumor specificity, which can effectively avoid damage to normal tissues and markedly improve the safety and targeting of vaccine (50). Among these, neoantigens, as a special type of TSAs, are generated by tumor cell gene mutations, often originating from TGFBRII, KRAS point mutations and other frameshift mutations (51). They possess strong immunogenicity and individual specificity, making them ideal targets for the development of personalized tumor vaccines.

Recently, researchers have discovered that parasites can inhibit tumor growth, which challenges the absolute theory that parasites are the cause of tumors (52). For example, *Echinococcus granulosus* protein KI-1, *Toxoplasma gondii* protein GRA15II and *Trypanosoma cruzi* calreticulin have demonstrated the ability to suppress tumor growth, angiogenesis and metastasis (52). Parasites and their antigens can stimulate the MyD88 pathway, releasing potent pro-inflammatory cytokines such as IFN-γ, TNF-α, IL-1, IL-2 and IL-12, thereby creating an immune-stimulating environment unfavorable to tumor cells (52). These factors promote the differentiation of macrophages into the M1 phenotype, which in turn stimulates DCs, activates natural killer (NK) cells and promotes the differentiation of helper T cells toward the Th1 phenotype. Simultaneously, certain parasite molecules trigger apoptosis specifically in cancer cells without affecting normal cells (52). Consequently, parasites hold promise for contributing to the development of novel cancer immunotherapies, including immunotherapies based on highly immunogenic cancer vaccines, therapeutic antibodies and immunomodulators. They also offer opportunities for gene therapy, targeted therapy, synergistic combination therapy and combating tumor drug resistance. With the continuous advancement of antigen screening technologies, such as mass spectrometry and single-cell sequencing, more antigens with high immunogenicity and specificity will be identified, laying a solid foundation for the precise development of CRC vaccines.

Development of immunomodulatory adjuvants. Increasing clinical practice on vaccines has shown that the clinical efficacy of vaccine monotherapy is often unsatisfactory, which may be attributed to insufficient immunogenicity of the vaccine and the immunosuppressive TME (53). To address this challenge, scientists use vaccine adjuvants, substances that enhance the immunogenicity of antigens and modulate the type and intensity of immune responses, thereby improving the anti-tumor efficacy of vaccines. At present, common adjuvants used in CRC vaccine research include cytokines, CpG oligonucleotides, saponins (such as QS-21), aluminum adjuvants and novel nano-adjuvants. Cytokine adjuvants such as GM-CSF can recruit and activate DCs, promoting antigen uptake, processing and presentation, which in turn activates T cell immune responses. These findings have been widely validated in preclinical and clinical studies of various vaccine types (54). CpG oligonucleotides, such as Toll-like receptor 9 agonists, effectively stimulate innate immune cells (including DCs, B cells and NK cells) to produce pro-inflammatory cytokines and chemokines, enhancing Th1 responses (55). When combined with peptide or DNA vaccines, they markedly increase antigen-specific T cell responses and antibody levels (56). With advances in materials science, nano-adjuvants have become a research focus in recent years due to their unique physicochemical properties, such as high surface area, excellent biocompatibility and targeted delivery capabilities (57). Nanoparticles can encapsulate antigens and adjuvants to protect them from degradation and efficiently deliver them to antigen-presenting cells. Meanwhile, by modulating particle size, surface charge and functional group modifications, they can precisely regulate the direction and intensity of immune responses (58). For example, loading tumor antigens and adjuvants into liposomes, polymeric nanoparticles, or inorganic nanoparticles can markedly enhance the anti-tumor activity of vaccines. A preclinical study demonstrated that in a mouse tumor model, co-delivery of all-trans retinoic acid adjuvant and mRNA vaccine via lipid nanoparticles effectively stimulated intestinal mucosal immune responses (59). The addition of all-trans retinoic acid both improved the delivery efficiency of the nanoparticle vaccine and also induced activated T cells to highly express homing receptors, markedly increasing cytotoxic T cell infiltration, enhancing tumor suppression and prolonging animal survival (60). However, further clinical trials are still needed to validate its safety and efficacy (61). Additionally, appropriate adjuvants such as cytokines, galectin inhibitors, phosphorylated adjuvants, monoclonal antibodies and diprovocim, can be used in combination, which is expected to produce greater efficacy than when used alone (54).

Development of oral vaccines. Traditional vaccines are typically administered via subcutaneous or intramuscular injection, which primarily activates systemic immunity and exhibits limited efficacy against tumors occurring in mucosal tissues. By contrast, oral tumor vaccines are administered orally, leveraging the intestine, the 'largest immune organ' containing 70% of the body's lymphocytes, to activate both mucosal and systemic immune responses, enabling the recognition and attack of tumor cells for therapeutic or preventive purposes (62). Activated lymphocytes possess a natural 'homing' effect, allowing them to efficiently accumulate in mucosal lesions (63). This enhances the infiltration of immune cells in colorectal tumors and improves anti-tumor efficacy, thereby bringing significant clinical benefits. The injection-free administration also offers notable advantages, including high convenience, improved compliance and reduced adverse effects. However, oral vaccines face several challenges. For example, vaccines are prone to degradation by gastric acid and digestive enzymes in the gastrointestinal tract, leading to poor antigen stability and low bioavailability. Additionally, the immune tolerance mechanisms of the intestinal mucosa may also weaken the vaccine's immunogenicity (62). Therefore, the development of efficient delivery systems and immune adjuvants is essential to overcome these obstacles. Current research often employs nanocarriers such as microspheres and liposomes to encapsulate antigens, protecting the vaccine from degradation in the gastrointestinal environment and promoting uptake by intestinal mucosal cells (64). Concurrently, the combined use of mucosal adjuvants such as manganese ions and aluminum ions can effectively enhance antigen immunogenicity, eliciting potent mucosal and systemic immune responses (65).

In an animal experiment investigating oral mucosal immunotherapy for CRC, researchers utilized a manganese-enriched montmorillonite nanosheet vaccine (Mn-MMT@OVA). Following oral administration, the vaccine adhered to the intestinal mucosa to form a vaccine reservoir that gradually released antigens and manganese ions. These manganese ions activated mucosal immunity and eliminated immune tolerance via the cGAS-STING pathway. Simultaneously, they increased the infiltration of cytotoxic T cells into CRC tumors through the lymphocyte 'homing' effect, thereby markedly inhibiting tumor growth (66). Notably, the oral vaccine elicited strong antibody responses in the small intestine and colon and induced immune responses at distal mucosal sites such as the mammary and salivary glands. This suggests that Mn-MMT@OVA holds potential for providing broad immune protection across multiple mucosal surfaces and shows promise for cancer therapy. In the future, with continuous advances in delivery technology and adjuvant development, oral CRC vaccines are expected to evolve into a safe, effective and convenient prevention and treatment strategy.

4. Clinical applications of CRC vaccines

Tumor cell vaccines can be categorized into autologous and allogeneic vaccines based on the source of antigens (67). Autologous vaccines are derived from a patient's own tumor cells, providing a personalized antigen profile, while allogeneic vaccines are sourced from established tumor cell lines, offering advantages in production scalability and applicability (68). OncoVax, the most extensively studied autologous CRC vaccine, entered clinical trials in the 1980s and utilizes autologous tumor cells combined with the Bacillus Calmette-Guérin adjuvant (69). Although early clinical trials showed that OncoVax combined with surgical treatment did not markedly improve clinical efficacy, it markedly enhanced overall survival and disease-free survival in stage II CRC patients. Studies also indicate that OncoVax, when used in combination with 5-fluorouracil and calcium folinate, exhibits a favorable safety in CRC patients (70-73). GVAX vaccine is a transgenic allogeneic vaccine created by transferring the GM-CSF gene into tumor cells, which enhances immunogenicity through GM-CSF secretion (74). In a Phase II trial (NCT02981524) involving patients with advanced pMMR CRC, GVAX demonstrated a potent antitumor immune response (75). The trial included 17 patients who received pembrolizumab combined with cyclophosphamide on day 1 and GVAX on day 2 of a 21-day treatment cycle. Results showed the disease control rate was 18%, median progression-free survival was 82 days, median overall survival was 213 days and 41% of patients had a reduction in CEA levels of ≥30%. However, two patients experienced grade ≥3 treatment-related adverse events. Paired biopsy specimens before and after treatment revealed increased PD-L1 expression and tumor necrosis in some patients. Currently, tumor cell vaccines remain in the development stages, primarily in Phase II/III trials, with highly varying clinical outcomes. In addition to OncoVax and GVAX, platforms such as Vigil/gemogenovatucel-T, Canvaxin and belagenpumatucel-L have also accumulated clinical development records, but multiple late-stage studies have failed to consistently demonstrate superiority over standard therapies, although some programs continue to advance in biomarker-selected populations (76). The low clinical translation efficiency of tumor cell vaccines may be attributed to insufficient autologous tumor sources, long production cycles, significant batch-to-batch variation, insufficient antigen matching in allogeneic cell vaccines, altered immunogenicity caused by inactivation methods and the intrinsic immune evasion and immunosuppressive characteristics of tumor cells. Moreover, a high antigen load does not necessarily translate into a strong and effective T-cell response and the lack of clear potency assays and predictive biomarkers further limit clinical development. Although GVAX demonstrated limited clinical efficacy, the strategy of combining vaccines with immune checkpoint inhibitors still holds significant potential in CRC treatment.

APDC vaccine is China's first DC vaccine officially approved for clinical trials. A Phase I clinical trial was completed at Shanghai Changhai Hospital in 2003, followed by a Phase II clinical trial in 2004 to evaluate the efficacy and safety of the APDC vaccine combined with chemotherapy in treating metastatic CRC (77,78). The results showed that the combination therapy group achieved twice the treatment efficacy compared with the chemotherapy control group (78). Another Phase II clinical trial, which used autologous tumor lysate-loaded DC vaccines for refractory metastatic CRC, demonstrated that although tumor-specific T cells were induced and cytokine production increased, there was no improvement in survival outcomes compared with supportive

care alone (79). This highlights the necessity of combining DC vaccines with immunomodulatory therapies to enhance efficacy (80). A Phase I/II trial conducted by the Duke Cancer Institute investigated the effects of the CEARNA DC vaccine on 24 patients with metastatic CEA-expressing tumors at high risk of recurrence after tumor resection, most of whom had CRC. The results indicated that the vaccine was well-tolerated and could induce tumor-specific immune responses (81). Additionally, Rodriguez *et al* (82) performed a randomized controlled trial involving 15 patients with CRC with liver metastases who underwent surgery. The patients were randomly assigned to either a vaccine group or an observation group. The median disease-free survival times were 25.26 months and 9.53 months, respectively. At present, DC vaccines have achieved certain clinical successes, represented by the approved product sipuleucel-T for selected patients with metastatic castration-resistant prostate cancer (83). DCVax-L has completed a Phase III trial for glioblastoma and has submitted a marketing application in the UK, while ilixadencel, TLPO, DCVAC and TriMixDC-MEL remain primarily in the Phase II/III development stages. However, DC vaccine development is still limited by the need to collect patient cells, induce differentiation, maturation, antigen loading and reinfusion *in vitro*, making the process complex, costly and time-consuming. In addition, DC quality varies markedly among patients and it remains difficult to control whether DCs truly migrate to lymph nodes and effectively activate T cells. There is still no unified optimal strategy for antigen loading, maturation factors, administration routes and combination regimens. Clinical endpoints may also be influenced by subsequent therapies and cross-treatment, especially in diseases such as GBM, where the interpretation of external controls and long-term survival remains controversial. This highlights the necessity of combining DC vaccines with immunomodulatory therapies to enhance efficacy (80).

Mucin-1 (MUC1) vaccine is a peptide-based vaccine targeting cancer-associated MUC1, which utilizes its abnormally glycosylated core peptide to induce specific immune responses. In a clinical trial of MUC1 peptide vaccine (84), 53 participants vaccinated with MUC1 vaccine, while another 50 participants received a placebo. At week 12, 13 (25%) of the vaccinated individuals showed a ≥2-fold increase in MUC1 IgG levels, whereas no increase in antibody levels was observed in the placebo group. Among the 13 responders, 11 (84.6%) developed an immune response after a booster injection on week 52. Ultimately, tumor recurrence occurred in 31 out of 47 (66.0%) participants in the placebo group and 27 out of 48 (56.3%) in the trial group. Among the 11 individuals who developed an immune response at weeks 12 and 55, only three (27.3%) experienced tumor recurrence. There was no significant difference in the incidence of severe adverse events between the two groups. Currently, numerous peptide vaccine projects have entered clinical development, although most remain in Phase I/II or selected Phase III stages. For example, Tedopi/OSE2101 is advancing in a confirmatory Phase III trial for HLA-A2-positive, ICI-resistant non-small cell lung cancer (NSCLC); the Phase III trial of IO102-IO103 combined with pembrolizumab for melanoma did not achieve a statistically significant primary PFS endpoint; the KRAS peptide vaccine ELI-002 has completed early studies and is progressing to a randomized Phase II trial; and SurVaxM is in Phase IIb development for glioblastoma. Due to the frequent HLA restriction of short peptides, the applicable population is relatively narrow; when short peptides are presented by non-professional antigen-presenting cells, immune non-responsiveness or tolerance may occur. Although long peptides can induce more complete immune responses, their manufacturing and delivery are more complex and strong adjuvants as well as rational administration strategies are usually required. The most common failure pattern of peptide vaccines is the detection of immune responses without sufficient or durable clinical benefit (76). These findings indicate that MUC1 peptide vaccine can markedly induce specific immune responses and reduce tumor recurrence rates to some extent. However, its anti-tumor efficacy may be related to the strength of the immune response. Further studies need to combine it with adjuvants to further evaluate the clinical effects, while the future development of peptide vaccines may also rely on personalized long peptides and neoantigen peptide vaccines to improved align with precision medicine (76).

Tetchy is a plasmid DNA therapeutic vaccine targeting the transcription factor MYB, currently in Phase I/II clinical trials (MYPHISMO) (85). This clinical trial combines the TetMYB vaccine with PD-1 blockade, primarily exploring its safety and preliminary efficacy in CRC and adenoid cystic carcinoma. Although results have not yet been published, this study represents an innovative approach to personalized immunotherapy through combining transcription factor-targeted vaccination with PD-1 inhibition. In the broader field of solid tumors, most DNA vaccine projects are still in Phase I/II development. The most advanced direction is HPV-related precancerous lesions or cervical high-grade lesions, such as VGX-3100, which achieved its primary efficacy endpoint in a Phase III trial in China; however, this direction is closer to immunotherapy for precancerous lesions than treatment for invasive tumors. In invasive tumors, studies such as GX-188E combined with pembrolizumab for cervical cancer and GNOS-PV02 combined with IL-12/pembrolizumab for liver cancer remain in early to mid-stage development. Although DNA vaccines have advantages such as stability and relatively convenient manufacturing, they generally suffer from low immunogenicity and transfection efficiency (76). They require nuclear entry to express antigens, which raises theoretical regulatory concerns regarding genomic integration. In addition, naked DNA delivery *in vivo* is inefficient and often depends on electroporation, gene guns, microneedles, or other devices, which are inconvenient for clinical application. The strength and durability of induced T-cell responses are also insufficient and personalized neoantigen DNA vaccines face challenges in antigen prediction, production timelines and combination design with immune checkpoint inhibitors. mRNA-5671/V941 is a lipid nanoparticle-encapsulated mRNA neoantigen vaccine, specifically encoding tumor neoantigens for the four most common KRAS driver mutations (86). Its core objective is to break the body's natural tolerance to KRAS mutant proteins by simultaneously activating CD8+ and CD4+ T cell immune responses, achieving personalized immunotherapy for KRAS-mutant solid tumors such as advanced CRC and NSCLC (87). A Phase I clinical trial evaluated the efficacy of mRNA-5671/V941 as a monotherapy and in combination

with pembrolizumab for metastatic solid tumors (88). Despite promising preliminary results, the trial was terminated early due to commercial reasons. GRANITE is an mRNA vaccine based on tumor neoantigens. Results from a Phase I/II clinical trial using this vaccine in combination with the immune checkpoint inhibitors nivolumab and ipilimumab showed that among 13 enrolled patients with metastatic MSS CRC, six patients experienced a reduction of >30% in circulating tumor DNA levels, indicating that the therapy can help patients achieve molecular remission (89). The average tumor mutation burden in patients who achieved molecular remission was 2.9, compared with 3.6 in those who did not. The median overall survival for patients who did not achieve molecular remission was 7.8 months, while the median overall survival for the six patients who achieved molecular remission exceeded 22 months. Patients who achieved molecular remission demonstrated an improved survival advantage compared with those who did not. At present, RNA vaccines represent one of the most active platforms in late-stage clinical development. For example, the personalized neoantigen mRNA vaccine V940/intismeran, developed by Merck/Moderna in combination with pembrolizumab, reduced the risk of recurrence or death in Phase IIb follow-up of high-risk resected melanoma and has entered multiple Phase III or Phase II/III programs, including melanoma, NSCLC and cutaneous squamous cell carcinoma. BioNTech/Genentech's autogene cevumeran is being evaluated in randomized Phase II trials for pancreatic cancer, melanoma and CRC, while BNT111 has shown positive Phase II signals in advanced melanoma (90). Nevertheless, personalized mRNA vaccine manufacturing remains costly, time-consuming and technically complex, with demanding quality control requirements. Neoantigen prediction still has false positives and is affected by HLA type, tumor heterogeneity and antigen loss. mRNA is prone to degradation and relies on delivery systems such as LNP or LPX; excessive innate immune activation may inhibit mRNA translation and affect CTL responses. Moreover, Phase III trials still need to confirm whether improvements in disease-free survival (DFS) or recurrence-free survival (RFS) can consistently translate into overall survival (OS) benefits or higher cure rates. These issues constrain the development of the mRNA platform and future studies must balance stability, delivery efficiency, immunogenicity and safety (76).

Ad5-GUCY2C-PADRE is an adenovirus-vectored $CD8^+$ T cell-directed vaccine targeting the CRC highly expressed antigen GUCY2C. It is created by linking the transmembrane receptor GUCY2C, expressed in intestinal epithelial cells, with a universal helper T cell epitope that enhances immune responses. Results from Phase I/II clinical trial showed that among 10 CRC patients vaccinated, one patient had detectable antibody response against GUCY2C, four patients demonstrated GUCY2C-specific T cell responses and no adverse events above grade 1 were observed (91). Virus replicating RNA particles (VRP)-CEA is a therapeutic vaccine that uses Venezuelan equine encephalitis VRP as the vector and encodes the highly immunogenic CEA antigen. In a Phase I trial involving 12 CRC patients treated with the VRP-CEA vaccine, the 5-year recurrence-free survival rate was 75%, with no deaths reported during the follow-up period. All patients developed CEA-specific humoral immunity. After vaccination, 10 patients showed increased levels of CD8+ effector memory T cells and decreased levels of regulatory T cells, while nine patients exhibited elevated levels of CEA-specific CD8+ central memory T cells (92). At present, mainstream viral vector vaccines include non-replicating viral vectors and replicating oncolytic viruses. For example, T-VEC has been approved by the FDA for local treatment of selected patients with unresectable recurrent melanoma (93); nadofaragene firadenovec-vncg, an adenovirus vector-based IFNα gene therapy, has been approved by the FDA for BCG-unresponsive high-risk non-muscle-invasive bladder cancer; and oncolytic viruses such as H101 and DELYTACT have been approved in markets including China and Japan (94). However, traditional viral vector vaccines such as PROSTVAC showed early clinical signals but failed in Phase III trials (95). Although viral vector vaccines have significant development potential, pre-existing antiviral immunity and neutralizing antibodies may weaken repeated dosing and the immune system may preferentially target the vector rather than tumor antigens. Systemic administration faces challenges including clearance by the liver and spleen, toxicity and tumor selectivity, whereas intratumoral injection is limited by lesion accessibility. Viral replication, safety, shedding, environmental release and production titers also face regulatory pressures. These difficulties constrain the development of viral vector vaccines and their combination with PD-1 or CTLA-4 blockade does not necessarily translate into success in Phase III trials (96).

5. Challenges facing CRC vaccines

Tumor heterogeneity. Tumor heterogeneity refers to significant differences in cell morphology, gene expression, growth rate and immune microenvironment within the same tumor or among similar tumors in different patients (97). This heterogeneity leads to highly variable tumor antigen expression profiles, making it difficult for single-target vaccines to cover all tumor cell subpopulations and easily triggering immune escape, which is one of the pivotal challenges in the development of CRC vaccines. Using DNA sequencing technologies, researchers have shown that the mutation profile of CRC is extremely complex. Driver genes such as APC, KRAS and TP53 exhibit high heterogeneity across different patients and multiple clonal subpopulations compete and evolve within the same tumor, making it difficult for vaccines targeting a single antigen to achieve durable control (98). In the future, sequencing multiple spatially separated biopsy samples may be necessary to reveal the extent of intratumoral heterogeneity and achieve individualized precision therapy.

Notably, intratumoral heterogeneity evolves dynamically as the tumor progresses and their exposure to naturally occurring cellular stressors, such as changes in blood supply, local hypoxia, nutrient deprivation and associated metabolic dysregulation, reactive oxygen species production, the body's immune system, DNA damage and external stressors such as drug treatments (99). These dynamic changes not only drive competition and selection among subclones but may also promote the expansion of drug-resistant clones, leading to treatment failure. Tumor heterogeneity has been linked to poor patient prognosis and is regarded as a key determinant

of treatment resistance, therapeutic failure and reduced survival (100).

Therefore, to overcome the challenges posed by tumor heterogeneity, it is necessary to integrate multi-regional and multi-level molecular profiles with functional data and combine with single-cell sequencing, spatial transcriptomics and artificial intelligence models to analyze subclonal evolutionary trajectories and their microenvironment interactions. Only by dynamically capturing the evolution patterns of tumor heterogeneity across time and space can drug resistance pathways be predicted, to optimize the timing of combination therapies and ultimately achieve precise intervention from 'passive response' to 'active regulation', thereby improving long-term survival benefits.

TME. The TME is primarily composed of tumor cells, immune cells (including adaptive and innate immune cells), stromal cells (such as fibroblasts, endothelial cells and adipocytes) and non-cellular components (such as the extracellular matrix, chemokines, cytokines, growth factors and extracellular vesicles) (101). These components collectively form the environment in which tumor cells survive, not only supporting their proliferation, invasion and distant metastasis but also limiting immune cell infiltration and function through metabolic reprogramming, activation of hypoxia signaling pathways and the formation of physical barriers. Cancer-associated fibroblasts (CAFs) are the most abundant stromal cells in the TME. Through cell-to-cell contact, secretion of numerous immunomodulatory factors and extracellular vesicles, CAFs play a critical role in tumorigenesis, metastasis, angiogenesis, immune evasion and drug resistance, making them a potential therapeutic target in CRC (102). Meanwhile, the TME often contains abundant tumor-associated macrophages (TAMs), which secrete various pro-inflammatory cytokines, such as IL-1β, IL-6 and IL-23, promoting CRC tumor growth and progression (103). Additionally, TAMs not only directly suppress T cell activity by expressing high levels of immune checkpoint ligands, such as PD-L1, PD-L2 and VISTA, but also promote the expansion of Tregs in the TME, ultimately leading to T cell immunosuppression (104). The secretion of these immunosuppressive cells and factors can weaken vaccine-induced immune responses and reduce vaccine efficacy. Therefore, how to regulate the TME and enhance the infiltration and activity of anti-tumor immune cells is one of the major challenges in current vaccine development.

Vaccine efficacy and safety. The efficacy and safety of vaccines depend on their ability to precisely activate anti-tumor immune responses while avoiding systemic inflammation or autoimmune damage (105,106). An ideal tumor vaccine should utilize TSAs to activate T cells to recognize and eliminate cancer cells without attacking normal tissues. In vaccine design, it is crucial to balance immune stimulation intensity and safety. Excessively strong antigen immunogenicity may trigger autoimmune reactions, while weak immunogenicity may fail to elicit an effective anti-tumor response. Therefore, selecting neoantigens with high specificity and immunogenicity, combined with appropriate adjuvants and delivery systems, is key to enhancing vaccine effectiveness. The difficulty in forming immune memory after tumor vaccination limits its long-term protective effects. To improve durability, it is necessary to optimize vaccine delivery methods to promote effective activation of antigen-presenting cells and induce the generation of high-affinity memory T cells. Additionally, the efficacy of vaccine is markedly affected by the immunosuppressive state of the TME. Cells such as TAMs and CAFs hinder the infiltration and function of vaccine-induced effector T cells by secreting immunosuppressive factors and constructing physical barriers (107). Thus, combining strategies to reprogram or deplete TAMs and CAFs holds promise for improving vaccine efficacy. Furthermore, as an emerging immunotherapy, tumor vaccines have demonstrated certain anti-tumor potential in clinical trials. However, their development timeline is relatively short compared with traditional therapies, with insufficient clinical evidence, limited long-term follow-up data and certain vaccines failing to markedly extend overall survival in Phase III clinical trials (108). It is necessary to further optimization of antigen screening strategies and personalized treatment regimens in the future and to enrich clinical trial data to validate their safety and effectiveness.

Vaccination timing and methods. The timing and method of vaccination markedly influence the intensity and durability of immune responses (109-111). The design of tumor vaccination programs should be more personalized based on the patient's disease stage and treatment progress. However, there is currently a lack of uniform standards, though early intervention may be more conducive to activating effective anti-tumor immune responses. The majority of tumor vaccines currently serve as adjuvant therapies and can be administered after surgical resection or chemoradiotherapy to eliminate minimal residual disease. Vaccination routes typically include subcutaneous, intramuscular, intradermal, or intranodal injections, with different methods affecting antigen presentation efficiency and immune response patterns. For example, intradermal injections, due to their abundance of DCs, facilitate improved antigen uptake and presentation (112), while intranodal injections can directly activate T cell responses, demonstrating stronger immunogenicity (113). Additionally, age is an important factor influencing vaccine responses. Elderly patients often experience reduced T cell function and weakened antigen presentation capacity due to immunosenescence, thereby diminishing vaccine-induced immune responses (114). Since vaccines generally take a longer time to take effect, multiple doses combined with immune adjuvants are often required to enhance response intensity (115). By contrast, younger patients typically exhibit stronger immune response capabilities, allowing for appropriate adjustments in the number and timing of vaccinations. Therefore, differentiated vaccination strategies should be developed for different age groups. In the future, to determine scientific vaccination methods and timing, clinical research data should be enriched, while immune monitoring should be combined to dynamically assess individual response levels and explore the optimal timing and frequency of vaccination. It is also essential to comprehensively consider factors such as tumor type, stage and combination treatment regimens to

establish a vaccination system that integrates standardization with personalization, thereby maximizing immune activation and sustaining long-term immune memory.

Immunogenicity and clinical efficacy. Although the majority of CRC vaccines can induce detectable antigen-specific immune responses, this immunogenicity has not consistently translated into objective remission or overall survival benefits, indicating a significant translational gap between peripheral immune activation and effective intratumoral killing (116). The reasons may involve multiple levels, including antigens, host immune status, tumor microenvironment and trial design. First, previous CRC vaccines have primarily targeted tumor-associated antigens such as CEA, MUC1 and 5T4. Although these antigens are highly expressed in tumor tissues, they are not entirely tumor-specific and are susceptible to factors such as self-immune tolerance, HLA restriction, antigen heterogeneity and antigen loss. Consequently, the antigen-specific T-cell expansion detected in peripheral blood may lack sufficient affinity, broad-spectrum activity and sustained killing capacity (117). Second, CRC, particularly the pMMR/MSS subtype, typically exhibits low mutation and neoantigen burdens and is often accompanied by insufficient T-cell infiltration, immune exclusion and an immunosuppressive tumor microenvironment (118). Tregs, MDSCs, tumor-associated macrophages, as well as signaling pathways involving TGF-β, VEGF and immune checkpoints, collectively restrict the migration, expansion and effector functions of vaccine-induced T cells to the tumor site (119). Therefore, positive results in peripheral blood ELISpot assays, cytokine release, HLA multimer positivity, or increased antibody titers primarily indicate that antigen presentation and peripheral lymphocyte activation have occurred but do not prove the establishment of effective cytotoxic immunity within the tumor tissue (120). Consistent with this, endpoint studies of cancer vaccines have shown that peripheral circulating immune responses do not always align with changes in tumor-infiltrating lymphocytes (TILs), while *in situ* immune indicators such as TILs/Immunoscore hold more direct biological significance in assessing prognosis and recurrence risk in colon cancer (121). Clinical research also supports this conclusion: although CEA-specific T-cell responses were detectable in PANVAC-related studies, the two-year recurrence-free survival rates were similar between the DC/PANVAC and PANVAC/GM-CSF groups; GVAX combined with cyclophosphamide and pembrolizumab did not produce objective responses in pMMR advanced CRC; and the ECOG E5283 autologous tumor cell-BCG vaccine study showed no significant improvement in DFS or OS in the overall population, although subgroup analysis of stage II patients suggested potential benefit signals. These results indicate that vaccine development cannot simply equate 'detectable immunogenicity' with 'clinical efficacy.' Future CRC vaccine research should shift from merely pursuing peripheral immune responses to validating a complete anti-tumor immune cycle, including the quality of antigen selection, T-cell clonal expansion and persistence, intratumoral infiltration and spatial distribution, antigen presentation integrity, ctDNA/MRD dynamic changes and the remodeling of the immunosuppressive microenvironment. Additionally, vaccines may be more suitable for postoperative adjuvant therapy, minimal residual disease, or populations with low tumor burden and should be combined with strategies such as PD-1/PD-L1 blockade, anti-angiogenic therapy, chemoradiotherapy, or innate immune agonists to bridge the gap between immunogenicity and clinical efficacy (122).

6. Future developmental directions

Using big data to predicts tumor-specific antigens. By integrating patients' genomic, transcriptomic and HLA genotyping data via high-throughput sequencing and bioinformatics technologies, neoantigen epitopes can be precisely predicted, enhancing the specificity and immunogenicity of vaccine targets (123). Combining machine learning models to analyze tumor mutation burden, clonal subpopulation evolution and antigen presentation pathway characteristics holds promise for intelligent and efficient antigen screening. For example, the NeoDisc pipeline, which integrates genomic, transcriptomic and mass spectrometry immunopeptidomics data, can directly detect the antigen profile presented on the surface of tumor cells, increasing neoantigen screening accuracy by over 50% (124). Cutting-edge research from China, through the integration of methylation-proteomics dual-omics, has improved the detection sensitivity of liver cancer from 63.2 to 89.5% and ovarian cancer from 71.4 to 85.7%. This closed-loop model of data-driven and experimental validation is becoming the gold standard for neoantigen discovery (125). Based on in-depth mining of multi-omics data and optimization of artificial intelligence algorithms, neoantigen prediction will progressively become more accurate and efficient, markedly shortening the vaccine development cycle. The preparation of personalized vaccines will no longer be limited to traditional models but will advance toward a highly customized intelligent production system.

Evaluation of antigen immunogenicity using gene sequencing technology. Currently, most vaccines still face the issue of insufficient immunogenicity during use, which limits the activation and expansion of T cells. In the past, adjuvants were commonly used to enhance their immunogenicity. However, with the advent of single-cell sequencing and TCR/BCR repertoire analysis, it is now possible to directly assess the response frequency and functional status of neoantigen-specific T cells, thereby quantifying immunogenicity (126). By combining HLA-peptide affinity prediction models with *in vitro* stimulation assays, high-response antigens can be more accurately screened, avoiding resource waste on invalid targets (127). In recent years, researchers have utilized methods such as next-generation sequencing to predict and screen for 9-amino-acid peptides with neoantigenic potential based on whole-exome sequencing results of tumor tissues and MHC class I molecule affinity predictions (128). These peptides are combined with CpG adjuvants *in vitro* to simulate the presentation of endogenous antigens by antigen-presenting cells (particularly DCs) to CD8+ T cells, thereby activating their immune response. However, when quantifying the immunogenicity of each antigen using enzyme-linked immunospot assays, researchers observed that the immunogenicity of neoantigens did not show a

positive correlation with changes in MHC class I molecule affinity (129). Among the neoantigens with the highest immunogenicity, changes in MHC affinity values mostly ranged between 1 and 4 and the HLA-I genotypes associated with the highest T-values primarily exhibited binding to the largest number of neoantigen peptides (128). This suggests that high affinity is not the sole determinant of immunogenicity. T cell receptor diversity and the dynamics of antigen processing and presentation are equally critical. Further integrating TCR clonal abundance with neoantigen binding stability data can help construct more precise immunogenicity scoring models, optimizing vaccine target selection. Technological advances have provided researchers with unprecedented analytical capabilities, shifting vaccine design from experience-driven to mechanism-driven approaches. This has prevented several ineffective candidate antigens from entering clinical trials, markedly improving research and development efficiency and resource utilization.

CRC vaccines combined with other immunotherapies. In several clinical trials, monotherapy with vaccines or immune checkpoint inhibitors often yield suboptimal efficacy. Multiple studies have shown that combining CRC vaccines with PD-1/PD-L1 inhibitors exhibits significant synergistic effects, markedly enhancing the activity and persistence of tumor-infiltrating T cells. According to the latest midterm data from the COVAC-3 trial released in 2025, the PFS in the combination therapy group was extended by 3.8 months compared with the monotherapy group, with a more pronounced response observed in patients with MSI-H status (130). Vaccine-induced specific T cell clones can persistently expand after immune checkpoint blockade and transform into a memory phenotype, thereby enhancing long-term immune surveillance. Additionally, combining tumor vaccines with CAR-T or tumor-infiltrating lymphocyte therapies has also demonstrated feasibility, where vaccines act as 'primers' to improve the TME and enhance the homing efficiency and survival duration of adoptive cell therapies (131). In the future, personalized combination strategies based on dynamic biomarker monitoring will become mainstream, achieving the optimal sequencing and combination of precise immune interventions.

7. Conclusions

In summary, tumor vaccines offer a novel approach for the treatment of CRC by inducing the body to generate effective anti-tumor immune responses. Meanwhile, combining these vaccines with immunotherapies such as immune checkpoint inhibitors can markedly enhance the breadth and depth of T cell responses. However, due to factors such as tumor heterogeneity and the immunosuppressive microenvironment, research on CRC vaccines still faces certain challenges. With advances in multi-omics and artificial intelligence technologies, neoantigen screening, immunogenicity prediction and antigen delivery will continue to be optimized, driving further breakthroughs and progress in CRC vaccines. In the future, CRC vaccines are expected to usher in an era of precision immunotherapy, markedly reducing postoperative recurrence, extending long-term patient survival and pioneering a new model of full-course management for CRC that is 'preventable, treatable and curable'.

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JM drafted the initial manuscript, restructured the manuscript's organization and ideas, created the tables and performed proofreading. CX and DX wrote the final draft and edited the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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