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The Evolving Landscape of the Colorectal Cancer Vaccines: From Biological Mechanisms to Translational Therapeutics

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Abstract

Colorectal cancer (CRC) remains a major global health burden, and despite substantial advances in cancer immunotherapy, the clinical efficacy of therapeutic cancer vaccines in CRC has been limited. This review critically examines the biological, immunological, and translational factors that shape CRC vaccine development, with a particular focus on tumor immunopathology, antigen selection, vaccine platforms, and emerging combination strategies. We summarize current knowledge on CRC-associated tumor antigens, including selected tumor-associated antigens and neoantigen-based approaches, alongside major vaccine modalities evaluated in preclinical and early-phase clinical studies. Across the literature, vaccine-induced immunogenicity frequently exceeds demonstrated clinical benefit, highlighting a persistent translational gap. Synthesis of available evidence suggests that this gap is driven primarily by CRC-specific immune constraints, including immune exclusion, dominance of immunologically cold MSS/pMMR tumors, and tolerogenic pressures within metastatic niches, particularly the liver. We further discuss how rational combination strategies, especially those integrating cancer vaccines with immune checkpoint inhibitors (ICIs), may partially overcome these barriers. In addition, the review outlines the conceptual role of bioinformatics and immunoinformatics in supporting antigen prioritization, neoantigen discovery, and patient stratification in CRC vaccine research. Overall, this review emphasizes that future progress will depend on CRC-tailored antigen selection, mechanistically informed vaccine design, rational combination regimens, and rigorous clinical evaluation to define the realistic clinical role of therapeutic cancer vaccines in CRC.

1. Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide and remains a major cause of cancer-related mortality [1–3]. According to global cancer statistics published by the International Agency for Research on Cancer (IARC) in 2022, CRC ranks as the second leading cause of cancer-related deaths and the third most frequently diagnosed cancer globally. The global burden of CRC is projected to rise by nearly 60% by 2030, reaching more than 2.2 million new cases and approximately 1.1 million deaths annually [4, 5]. This rising incidence, particularly in developing countries, is strongly associated with lifestyle transitions toward Westernized behavioral patterns, including higher rates of obesity, physical inactivity, high consumption of red meat, alcohol, and tobacco, as well as high-calorie and low-fiber diets [6–12].

Despite notable progress in therapeutic modalities, clinical outcomes for CRC remain unsatisfactory, especially among patients with metastatic disease [3]. Although surgical resection, chemotherapy, radiotherapy, biological agents, immunotherapy, and various combination regimens are widely used and have received regulatory approval, their clinical performance remains constrained by therapeutic resistance, significant adverse effects, and limited long-term effectiveness. These shortcomings highlight the urgent need for more effective and durable therapeutic strategies [1, 13].

Immunotherapy has gained increasing prominence as a promising alternative to conventional cytotoxic chemotherapy due to its ability to restore and augment immune competence, which is often severely impaired in cancer patients undergoing standard treatments [14]. Immune checkpoint inhibitors (ICIs), adoptive cell therapy (ACT), and cancer vaccines represent some of the most widely investigated and impactful immunotherapeutic modalities to date [15]. However, the efficacy of ICIs and ACT remains confined to a subset of patients, with durable responses observed in only a minority [16]. Even though, monoclonal antibodies targeting ICIs have transformed the therapeutic landscape across a range of solid tumors and hematologic malignancies, their therapeutic activity is limited to specific patient subsets [17]. ICIs generally show limited efficacy in tumors lacking sufficient tumor-infiltrating lymphocytes (TILs) and are frequently associated with immune-related adverse events that may compromise patient quality of life and adherence to therapy [18]. Collectively, these limitations highlight the importance of therapeutic approaches that can facilitate the initiation or amplification of tumor-specific immune responses beyond reliance on pre-existing immunity alone.

Within this context, vaccine-based immunotherapy has emerged as a compelling therapeutic avenue because of their unique ability to prime and expand antigen-specific immune responses de novo. Vaccination remains one of the most successful medical interventions in human history, including within oncology [14]. Cancer vaccines offer unique advantages, particularly for patients who are refractory to other therapies, by inducing broad, antigen-specific, and durable T-cell responses targeting a diverse repertoire of tumor antigens [18, 19]. Unlike other treatment modalities, vaccines are designed to train the host immune system to recognize and eliminate tumor cells with heightened specificity [20–22].

Nevertheless, the success of immunotherapy continues to be hindered by the profoundly immunosuppressive nature of the tumor microenvironment (TME), which suppresses endogenous immunity and limits the efficacy of multiple immunotherapeutic platforms [23]. Immune responses elicited by therapeutic vaccines can be attenuated by immunoregulatory mechanisms within the TME [18]. In CRC, accumulating evidence demonstrates that key TME characteristics, most notably the density and functional competence of cytotoxic T lymphocyte (CTL) infiltration, serve as critical determinants of therapeutic outcomes. These observations underscore the need for innovative vaccine-based strategies capable of eliciting robust and sustained anti-tumor immune responses [24, 25]. Effective anti-tumor immunity plays a critical role in suppressing tumor initiation, growth, progression, and metastatic dissemination, thereby facilitating meaningful clinical responses [26].

Building upon the substantial advances achieved with ICIs, integrating these agents with therapeutic cancer vaccines offers a compelling scientific rationale for amplifying anti-tumor immunity [27, 28]. This rationale stems from the fundamentally distinct, yet highly synergistic, mechanisms through which cancer vaccines and ICIs modulate the cancer–immunity cycle. Therapeutic cancer vaccines function by priming and expanding antigen-specific T cells, thereby enhancing the activation and intratumoral infiltration of cytotoxic immune cells. In contrast, ICIs operate principally by overcoming inhibitory signals that suppress effector function, thus preventing or reversing the dysfunction and exhaustion of these effector T-cells within the TME. Although both modalities have demonstrated clinical activity as monotherapies, each faces substantial limitations, as previously discussed. The convergence of these complementary mechanisms provides a strong mechanistic foundation for therapeutic synergy. By driving potent antigen-specific immune priming while simultaneously relieving inhibitory signaling pathways, vaccine–ICI combinations have the potential to generate more durable, comprehensive, and clinically meaningful anti-tumor responses than either approach alone [18].

The review by Gallio et al. provides a valuable overview of CRC vaccine platforms, including recent updates in vaccine technologies, and highlights the emerging potential of combining cancer vaccines with ICIs. The authors appropriately recognize that such combination strategies may enhance therapeutic efficacy compared with vaccine or ICI monotherapy. However, the review does not provide a comprehensive mechanistic explanation of why vaccine–ICI combinations are biologically synergistic and how these combinations mechanistically overcome the immunological barriers that limit vaccine efficacy in CRC [29].

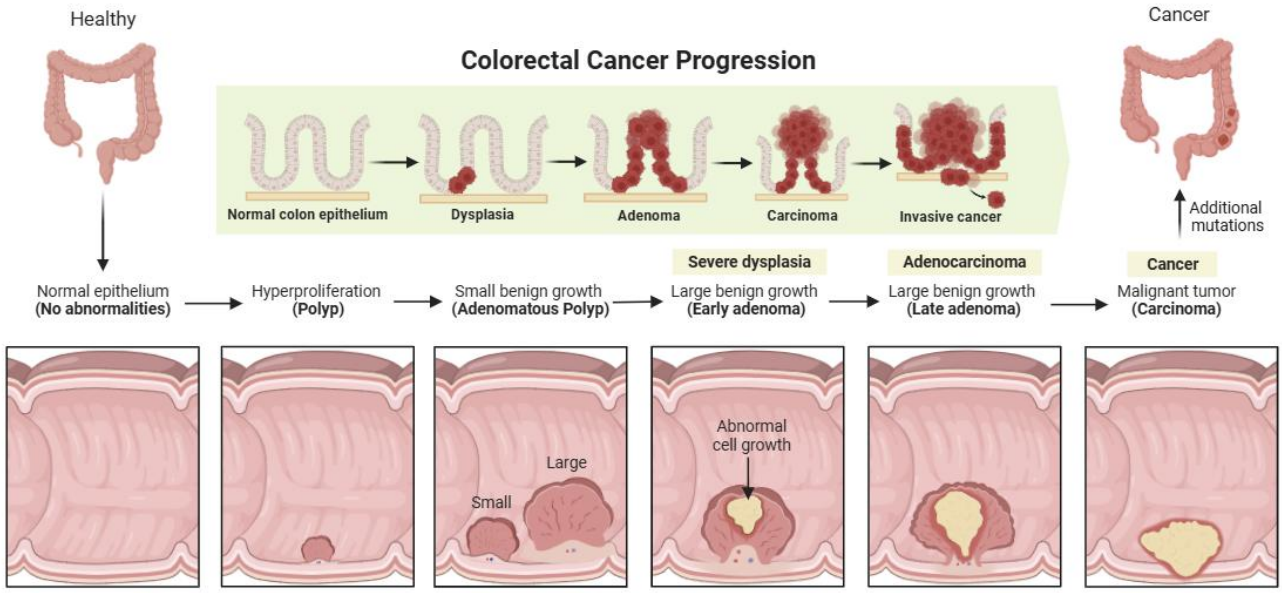

Figure 1. CRC progression and metastasis. CRC, Colorectal cancer.

In contrast, the present review is specifically designed to address these unresolved mechanistic gaps. This review aims to provide an overview of the evolution and current status of CRC vaccine development, highlighting recent advances in vaccine engineering, ongoing clinical challenges, and emerging strategies aimed at improving vaccine efficacy and accelerating translational potential. We also provide an in-depth analysis of the fundamental immunological mechanisms that underpin vaccine–ICI synergy, linking vaccine-induced antigen priming and T-cell expansion with checkpoint blockade–mediated reinvigoration of effector function within the TME. By integrating CRC-specific immunopathology with current knowledge of immune checkpoint biology, this review establishes a clearer biological rationale for rational vaccine–ICI combination strategies and offers a more mechanistically grounded framework to guide future translational and clinical development.

To clearly define its scope, this review focuses on therapeutic (rather than prophylactic) cancer vaccines for CRC, drawing primarily on preclinical and clinical (if available) studies without any restriction to the year of publication. We cover major vaccine platforms currently under investigation in CRC, including peptide-based, dendritic cell–based, nucleic acid–based, viral vector–based, and cancer cell-based vaccine.

2. Immunopathology of CRC

The development of CRC typically follows the classical adenoma–carcinoma–metastasis sequence. Aberrant crypt foci gradually evolve into benign adenomatous polyps, which may subsequently progress into sporadic CRC (Figure 1) [30]. As a multifactorial disease, CRC arises through a continuum of histopathological changes in the colonic epithelium, including hyperplasia, varying degrees of dysplasia (mild, moderate, or severe), and adenoma formation. These transitions are driven by the stepwise accumulation of structural DNA alterations induced by carcinogenic stimuli, ultimately culminating in malignant transformation [31]. Malignant transformation is generally characterized by three major stages: (1) acquisition of driver mutations within epithelial or stem cells, yielding genetic alterations that give rise to mutant cancer-initiating cells; (2) clonal fixation, during which mutant cells outcompete and replace their wild-type counterparts within the crypt; and (3) clonal expansion via crypt fission, enabling the spread and establishment of the mutant clone [30, 32]. At the molecular level, CRC arises through three principals, and often overlapping, genetic pathways: chromosomal instability (CIN), DNA mismatch repair (MMR) deficiency, and the CpG island methylator phenotype (CIMP) [31, 33–38].

As the second leading cause of cancer-related mortality worldwide, metastasis remains the predominant determinant of CRC lethality [39, 40]. Accumulating evidence demonstrates that signaling pathways within the TME critically shape both the progression and dissemination of CRC [41]. The TME constitutes a dynamic and highly interactive milieu that influences virtually all stages of tumor evolution, from initial tumor cell adhesion and early growth to mechanisms enabling immune evasion. It comprises a heterogeneous network of malignant cells and non-malignant constituents, including immune cells, fibroblasts, endothelial cells, and a complex extracellular matrix. Although each component possesses distinct biological functions, their

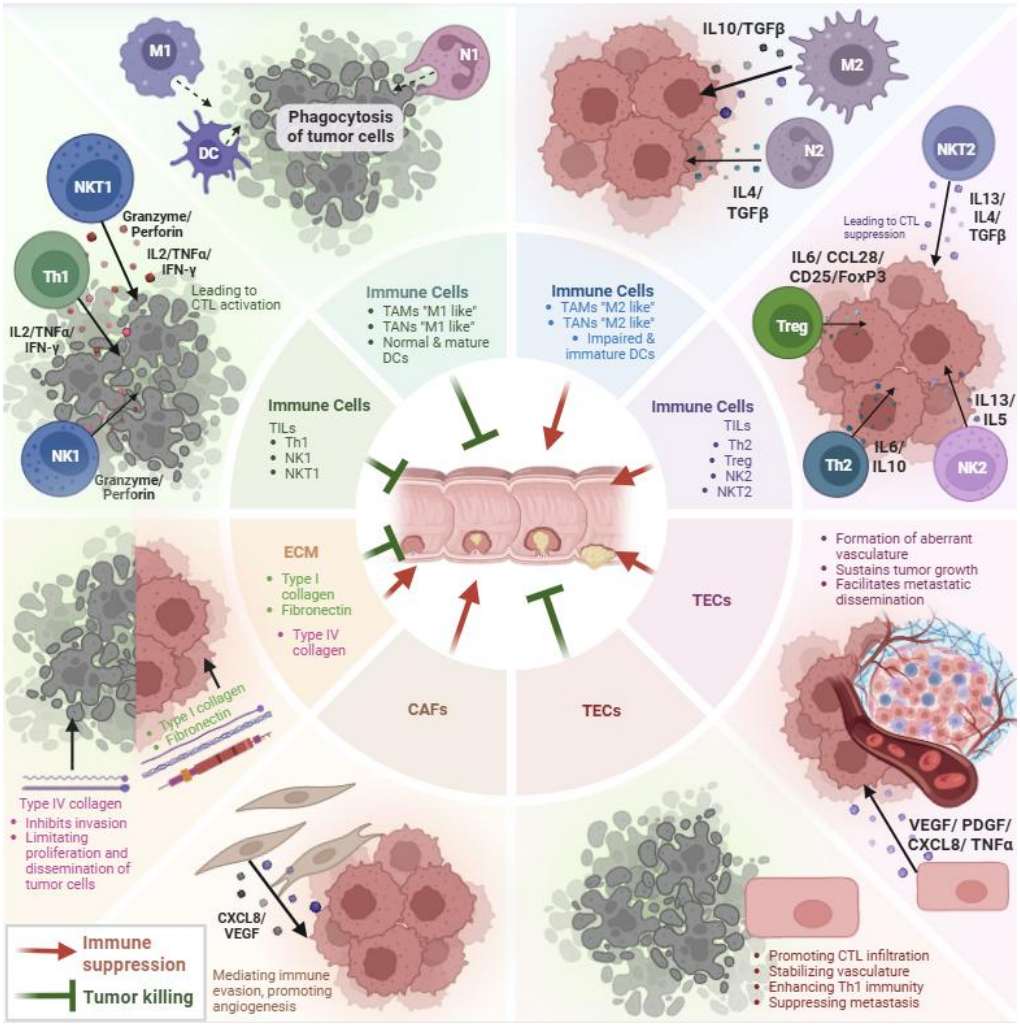

Figure 2. TME components and CRC. Both cellular and non-cellular elements of the TME may act as either promoters or suppressors of tumor progression, depending on the biological context. CAFs, Cancer-associated fibroblasts; CRC, Colorectal cancer; CTL, Cytotoxic T cell; DC, Dendritic cell; ECM, extracellular matrix; M; Macrophage; N, Neutrophil; NK, Natural killer; NKT, Natural killer T; TAMs, Tumor-associated macrophages; TANs, Tumor-associated neutrophils; TECs, Tumor-associated endothelial cells; Th, T-helper; TILs, Tumor-infiltrating lymphocytes; TME, Tumor microenvironment; Treg; T-regulator.

reciprocal interactions collectively orchestrate tumor progression, metastatic competence, and resistance to immune-mediated elimination (Figure 2). This intricate crosstalk underscores the profound complexity of CRC immunopathology and highlights the central role of the TME in dictating disease trajectory [40, 42].

Importantly, cancer progression is not solely dictated by tumor-intrinsic characteristics but is profoundly shaped by continuous interactions between malignant cells and host immune responses within the TME [43, 44]. Notably, the CRC TME exhibits pronounced immune spatial heterogeneity, whereby immune cell composition, density, and functional states vary across distinct tumor regions, including the tumor core (main concentration of tumor cells), tumor stroma, invasive margin (transition zone between tumor cells and normal cells), and tertiary lymphoid structures (TLS), thereby critically determines prognosis and therapeutic responsiveness. The tumor core contains the bulk of malignant cells, so that immune cells residing within tumor core display more direct and efficient interaction with tumor cells [45]. The tumor core tends to establish an immunosuppressive microenvironment characterized by hypoxia, dense extracellular matrix (ECM), and enrichment of regulatory immune populations, limit effective infiltration and function of CTLs. Surrounding this region is the invasive tumor margin represents a critical immunological interface in antitumor defense, thereby characterized by a markedly higher density of infiltrating immune cells, including T cells, B cells, natural killer (NK) cells, and dendritic cells (DCs), compared with other tumor regions [46]. In contrast, immune cells localized within the tumor stroma, located peripheral to the tumor core, are strongly associated with stromal remodeling and angiogenic processes, composed of abundant stromal elements that support tumor growth by providing structural support and metabolic resources, thereby

exerting a substantial impact on tumor growth, invasion, and metastatic potential [47, 48].

TLS are ectopic aggregates of immune cells that arise in non-lymphoid tissues, most prominently within the TME. Structurally analogous to secondary lymphoid organs, TLS displays organizational and functional features analogous to those of fully developed lymphoid organs, largely orchestrated by the coordinated interactions among T-/B-cells, DCs, germinal centers, and high endothelial venules (HEVs) within its architecture. TLS have been identified across multiple solid malignancies, including CRC, where their presence is frequently associated with favorable clinical outcomes [49–51]. Pathological investigations have demonstrated that tertiary lymphoid structures located within the mucosa of CRC patients display a high degree of structural organization, with well-defined T-cell and B-cell compartments, suggesting their capacity to sustain effective local immune responses [52]. The development is driven by chronic inflammation via cytokine- and chemokine-mediated signaling, particularly involving molecules such as CXCL13 and CCL21, along with the formation of high endothelial venules (HEVs). Through these coordinated processes, TLS facilitate localized aggregation and interaction of immune cells within the TME, enabling more efficient activation of adaptive immune responses compared with unstructured or diffuse immune cell infiltration [53, 54]. Lv et al. demonstrated that increased TLS density and a higher degree of structural maturation correlate with reduced recurrence rates and prolonged patient survival. Moreover, emerging data suggest a functional interplay between TLS and immune checkpoint inhibition, underscoring the therapeutic potential of targeting or leveraging TLS to enhance antitumor immunity in CRC [54].

Although multiple innate and adaptive immune populations actively participate in tumor–immune regulation, current American Joint Committee on Cancer (AJCC)/Union for Internation Cancer Control (UICC)-tumour, node and metastasis (TNM) staging follows a predominantly tumor-centric paradigm, in which prognostic assessment is based almost exclusively on anatomical and pathological features such as depth of invasion and lymph node involvement [55, 56]. TNM classification offers limited prognostic resolution, as clinical outcomes can differ markedly among patients classified within the same histopathological tumor stage, despite its considerable utility [57]. In contrast, immune effector cells, including CTLs, B cells, and macrophages, can mediate tumor control and elimination, whereas immunosuppressive populations such as Treg cells attenuate antitumor immunity and facilitate tumor progression and tissue invasion [58]. Studies demonstrated that the type, density, and spatial distribution of immune cells within CRC tumors provide superior prognostic value compared with conventional TNM staging. This immune contexture concept has been translated into a clinically applicable immune-based classification system, known as the Immunoscore [43, 59–61]. The Immunoscore quantitatively assesses intratumoral (tumor core) and invasive-margin T-cell infiltration and has been validated as a robust prognostic marker in CRC [43, 59, 62–64]. Importantly, the Immunoscore not only represents one of the strongest predictors of patient survival, but also offers a biological framework that is directly relevant to immunotherapeutic intervention [25]. The presence of naturally infiltrating T cells suggests a pre-existing immune response that may be further amplified by novel immunotherapy approaches.

Growing insights into the TME, including baseline antitumor immunity, and its critical role in CRC progression underscore substantial opportunities for TME-targeted therapeutic strategies, particularly in the management of metastatic CRC (mCRC). ICIs such as pembrolizumab and nivolumab, which block the PD-1 pathway, have been approved for use in patients with deficient mismatch repair (dMMR) or high microsatellite instability (MSI-H) [65, 66]. Despite their clinical success, only a minority of CRC patients derive meaningful benefit from these agents because MSI-H tumors account for approximately 15% of all CRC cases, whereas the remaining ~85% exhibit proficient mismatch repair (pMMR) or microsatellite-stable (MSS) phenotypes, which are characteristically unresponsive to current ICIs [67, 68]. This disparity in therapeutic responsiveness is largely attributed to distinct immunogenic profiles. MSI-H/dMMR tumors possess a high tumor mutational burden (TMB), generating abundant neoantigens that promote robust TIL recruitment and enhance intrinsic immunogenicity. Conversely, pMMR/MSS tumors exhibit substantially lower TMB, resulting in reduced neoantigen availability, weaker TIL infiltration, and consequently an attenuated antitumor immune response [69].

2.1. Immune Cells: Macrophages

Macrophages, a major myeloid cell subset, exert context-dependent effects in CRC. While they can suppress tumor cell proliferation, migration, invasion, and metastasis, macrophages may also promote tumor development and drive therapeutic resistance [70]. Tumor-associated macrophages (TAMs) are broadly categorized into classically activated “M1-like” and alternatively activated “M2-like” phenotypes [71]. Under physiological immune

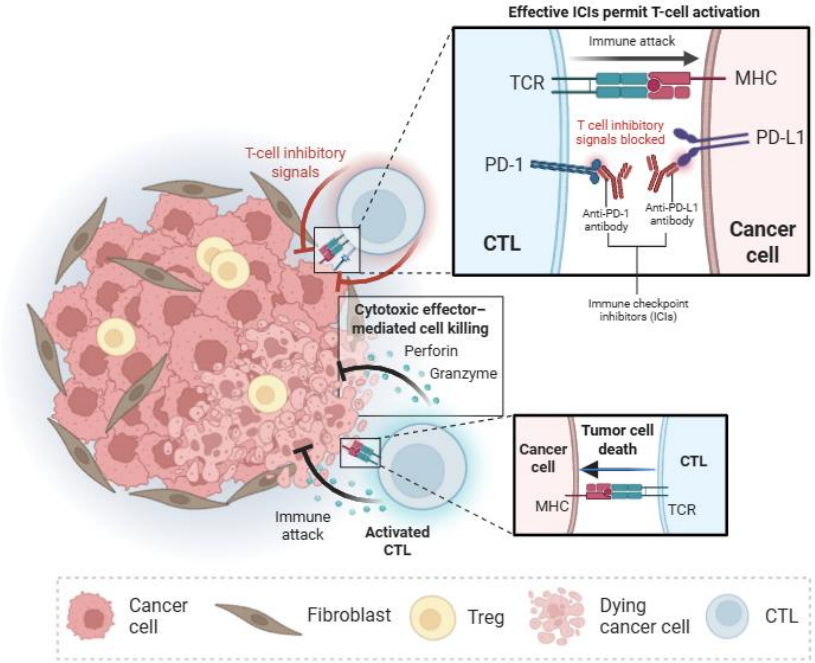

Figure 3. Immune evasion orchestrated by the PD-1/PD-L1 pathway in tumors. CRC upregulate PD-L1 to inhibit cytotoxic T-cell activity, but immune checkpoint inhibitors targeting PD-1/PD-L1 disrupt this suppressive interaction. Once released from inhibition, T cells regain effector function and discharge cytotoxic mediators such as perforin and granzymes, leading to targeted tumor cell elimination. CRC, Colorectal cancer; CTL, Cytotoxic T cell; ICIs, Immune checkpoint inhibitors; MHC, Major histocompatibility complex; PD; Programmed death; TCR, T-cell receptor; Treg, T-regulator.

conditions, macrophages predominantly adopt an M1 phenotype characterized by proinflammatory and immunostimulatory activity, which supports Th1 cytokine responses and inhibits CRC progression [72]. However, the anti-tumor role of M1 macrophages is not absolute. Chronic M1-driven inflammation, such as in colitis, has been linked to increased CRC risk, illustrating the dualistic nature of inflammation in colorectal tumorigenesis [73].

Sustained inflammation and/or infection contributes to an immunosuppressive TME by recruiting regulatory T cells (Tregs) and myeloid-derived suppressor cells or by skewing immune populations toward pro-tumorigenic states [74, 75]. Chronic inflammation further exacerbates genomic instability and induces the upregulation of immune checkpoints such as programmed death-ligand 1 (PD-L1), collectively dampening immune surveillance [76–80]. PD-L1 plays a pivotal role in restraining anti-tumor immunity by engaging the inhibitory receptor PD-1 on activated T cells, resulting in diminished proliferation, attenuated cytokine production, and impaired cytolytic activity, thereby enabling malignant cells to evade immune surveillance and defend themselves against immunological attack (Figure 3) [81–83]. These insights underscore the intricate interplay between inflammatory signaling and immunosuppression in CRC and reinforce the need for vaccines capable of generating robust immune responses while overcoming regulatory constraints.

Conversely, M2 macrophages promote malignant progression by: (1) secreting epidermal growth factor (EGF) and fibroblast growth factor-1 (FGF-1) to stimulate tumor cell proliferation; (2) releasing vascular endothelial growth factor A (VEGF-A) to enhance angiogenesis and suppress apoptosis; and (3) producing matrix metalloproteinases (MMPs) that facilitate invasion and metastasis [84–94]. M2 macrophages also induce epithelial–mesenchymal transition (EMT) through the IL-6/JAK2/STAT3 and PI3K/AKT pathways, increasing CRC cell motility and invasiveness [93, 95]. Through secretion of IL-10 and transforming growth factor-β (TGF-β), M2 macrophages suppress T-cell function, thereby accelerating tumor growth and contributing to aggressive CRC phenotypes [96, 97]. Increased immunosuppressive myeloid populations, including M2 macrophages, are a prominent feature of pMMR/MSS tumors and play a key role in driving immune exclusion and resistance to ICIs. In contrast, MSI-H/dMMR tumors are characterized by a high TMB, resulting in abundant neoantigen generation and a more “inflamed” TME enriched with infiltrating immune cells such as CTLs and M1-like macrophages, which are associated with improved anti-tumor immunity and better responses to ICIs [98–100].

Collectively, these immunopathological features highlight TAMs as critical regulators of immune suppression and therapeutic resistance in CRC, with important implications for cancer vaccine design. The

predominance of M2-like macrophages and their immunosuppressive mediators underscore the need for vaccines that elicit strong cytotoxic T-cell responses capable of overcoming macrophage-driven inhibition. Moreover, the central role of PD-L1 expression and macrophage-mediated T-cell suppression provides a strong mechanistic rationale for combining therapeutic cancer vaccines with ICIs or macrophage-modulating agents, either through the incorporation of immunostimulatory adjuvants or nanoparticle-based delivery systems. Another potential is to combine the vaccine with colony-stimulating factor-1 receptor (CSF-1R) inhibitors, which have been shown in preclinical CRC models to deplete M2 macrophages, enhance anti-tumor immunity by increasing CTLs, and enhance response to immunotherapy. The blockade of the CSF-1/CSF-1R pathway reprograms macrophages from M2 to a more immunostimulatory phenotype and enhances anti-PD-1 therapy efficacy in CRC mouse models [101–103]. Such combination strategies have the potential to simultaneously enhance antigen-specific T-cell priming while alleviating immunosuppressive barriers within the TME.

2.2. Immune Cells: Lymphocytes

Tumor-infiltrating lymphocytes (TILs), comprising helper T lymphocytes (HTLs), CTLs, B cells, and NK cells, play pivotal roles in tumor recognition, immune surveillance, and immune escape [104]. Ropponen et al. demonstrated an inverse correlation between TIL density and tumor stage, noting significantly fewer TILs in late-stage (Dukes C–D) CRC compared to early-stage disease (Dukes A–B) [105]. Kuwahara et al. reported that elevated HTL infiltration correlates with improved survival in CRC, although HTL subsets exert divergent effects [106, 107]. Th1-polarized HTLs promote cytotoxic immunity and are associated with favorable prognosis, whereas Th2 responses tend to support tumorigenesis [108]. Elevated Th17 cell levels are linked to poorer outcomes, with Th17-derived cytokines (IL-17, IL-21, IL-22) driving CRC growth and progression [109, 110].

High intratumoral CTL density, representing the central effector population of the adaptive immune system and critical mediators of anti-tumor immunity, correlates strongly with decreased recurrence and enhanced survival [61, 111–113]. Nevertheless, CRC cells employ multiple mechanisms to evade CTL-mediated cytotoxicity. One prominent pathway involves TNF-α–driven upregulation of PD-L1, which suppresses CTL activation and diminishes the cytolytic capacity of granzyme-producing CTLs [114]. Elevated PD-L1 expression is consistently associated with advanced tumor stage, lymphatic involvement, distant metastasis, and poorer overall survival [115–117]. Moreover, additional TME-derived immunosuppressive factors, particularly TGF-β, further compromise CTL effector function by downregulating perforin, granzymes, and Fas ligand (FasL), collectively attenuating CTL-mediated tumor cell killing [118].

The accumulation of Tregs, central mediators of immunosuppression, are strongly associated with heightened metastasis and poorer clinical outcomes [119–121]. Tregs are characterized by high expression of CD25 and the transcription factor forkhead box P3 (FoxP3), both of which are essential for their suppressive activity on effector T cells [122–125]. Under physiological conditions, Tregs maintain immune homeostasis and prevent autoimmunity by suppressing IL-2 production, releasing adenosine, and secreting immunoregulatory cytokines such as TGF-β, IL-10, and IL-35 [126]. However, within the CRC TME, Treg-mediated effects are context-dependent, reflecting a functional and phenotypic heterogeneity that contributes to both pro- and anti-tumor outcomes [127–129]. During malignant transformation, TGF-β signaling undergoes changes that resulting in tumor promotor function rather than a suppressor [130]. Then, these aberrant TGF-β activation and signaling promote tumor progression through the induction of EMT, angiogenesis, cancer-associated fibroblasts (CAFs) activation, and immunosuppression within the TME [131, 132]. Not only EMT plays a central role in cancer cell migration and invasion, EMT has also been associated with resistance to PD-L1 blockade, targeted therapies, and conventional chemotherapy [133–135]. In addition, dysregulated TGF-β signaling promotes tumor angiogenesis through upregulation of pro-angiogenic mediators, leading to increased tumor vascularization and growth [136]. TGF-β further drives the differentiation of fibroblasts into CAFs, which contribute to therapeutic resistance and metastasis by remodeling the ECM, restricting T-cell infiltration, and secreting immunosuppressive and pro-tumorigenic factors. In the TME [135, 137], TGF-β drives phenotypic reprogramming of multiple immune cell populations, including DCs, macrophages, neutrophils, NK cells, Tregs, and CTLs, to a tolerogenic phenotype [131]. The ECM and TME populations, including CAFs, DCs, neutrophils, will be further explained in the next subsections.

Saito et al. delineated three Treg subsets in CRC: naïve Tregs (FoxP3^low/CD45RA^+), terminally suppressive Tregs (FoxP3^high/CD45RA^-), and a FoxP3^low/CD45RA^- population exhibiting pro-inflammatory characteristics. Notably, CRC tissues demonstrated a disproportionately higher frequency of FoxP3^low/CD45RA^- inflammatory Tregs compared

with other malignancies, such as melanoma. Based on these immunological profiles, CRC was further categorized into two immunological subtypes: "type A," characterized by low levels of inflammatory Tregs, and "type B," distinguished by abundant inflammatory Tregs. Type B tumors displayed marked upregulation of inflammation- and immunity-associated genes, including IL-12, TNF-β, and TGF-β, suggesting a more immunologically active microenvironment. Conversely, in type A CRC, high FOXP3 expression correlated with inferior clinical outcomes, underscoring the prognostic relevance of Treg phenotypic diversity in shaping disease behavior [138].

In addition to the cellular complexity described above, the molecular classification of CRC into MSI-H/dMMR and MSS/pMMR subtypes profoundly influences lymphocyte infiltration patterns, TME composition, and clinical responsiveness to immunotherapy as explained previously. Tumors with dMMR/MSI-H characterized by abundant TILs, including activated CTLs and Th1-polarized HTLs, making these tumors sensitive to treatment with ICIs while pMMR/MSS tumors are characterized by reduced immune cell infiltration. These distinct immune landscapes underscore the need for CRC vaccine strategies tailored to the molecular and immunological context of each subtype. This includes enhancing TIL recruitment and functional competence, amplifying intratumoral immune activation, and converting immunologically "cold" pMMR/MSS tumors into immune-reactive states, while leveraging the existing inflamed milieu in dMMR/MSI-H tumors to sustain effective antitumor immunity [139–142].

These immunopathological insights indicate that successful CRC vaccine strategies, including optimization of delivery platforms and synergistic combination regimens, should be guided by the functional composition and phenotypic quality of TILs, rather than by immune cell abundance alone. Preclinical evidences support dual targeting of TGF-β and immune checkpoint (PD-L1) as a strategy to overcome complementary immunosuppressive mechanisms in the TME, where simultaneous co-inhibition of these pathways has been shown to significantly enhanced antitumor immune responses by relieving TGF-β-mediated immune suppression and enhancing CTLs access and effector function beyond what is achieved with single agent alone [143, 144].

2.3 Immune Cells: APCs and Dendritic Cells

Antigen-presenting cells (APCs) are indispensable for initiating adaptive immunity by processing and presenting tumor antigens to T cells [145, 146]. Mature DCs, the most potent APC subset whose development is driven by Flt3L, are associated with favorable clinical outcomes when present in high numbers due to its central role to the initiation, coordination, and amplification of antitumor immune responses [146–150]. DCs promote the infiltration cytotoxic activity of Th1 cells, NK cells, and CTLs by facilitating their migration through CCR7 expression, upregulating costimulatory molecules such as CD80, CD83, and CD86, and secreting pro-inflammatory cytokines including IL-12, IL-6, TNF-α, and IL-1β [151–153].

DCs are broadly classified into two primary subsets: plasmacytoid DCs (pDCs) and classical DCs (cDCs) [154]. pDCs are characterized by their capacity to secrete large quantities of type I interferons (IFNs) upon viral stimulation and are critically involved in the regulation of immune tolerance, while cDCs are particularly efficient in antigen presentation and are further subdivided into $CD141^+$ cDC1 and $CD1c^+$ cDC2. $CD141^+$ cDC1 specialize in cross-presenting tumor antigens via major histocompatibility complex (MHC) class I to prime CTLs, whereas $CD1c^+$ cDC2 predominantly present antigens through MHC class II to activate HTLs [155, 156]. cDCs are pivotal to antitumor immune responses through their capacity to process and present TAAs to naïve T cells, but also function as central coordinators of antitumor immunity by engaging multiple immune cell populations, including T cells, NK cells, macrophages, and B cells [157]. Therefore, their capacity to prime T cells and facilitate the recruitment of additional immune effectors into the TME is critical for effective anticancer immune response [158]. Consequently, dynamic crosstalk between cDCs and other immune components within the TME plays a decisive role in shaping the efficacy of cancer vaccines, as effective antigen presentation and T-cell priming are prerequisites for translating vaccine-induced immunogenicity into durable antitumor responses [159].

cDCs counteract tumor immune evasion by processing and presenting tumor antigens and by priming CTLs that selectively eliminate malignant cells. In addition, cDCs regulate the equilibrium between immune tolerance and immune activation within the TME, a balance that is critical for effective antitumor immunity [158, 160]. Owing to their widespread distribution across peripheral blood, lymphoid tissues, and peripheral organs, cDCs are highly efficient at capturing TAAs, cross-presenting exogenous antigens via MHC class I, and migrating to draining lymph nodes to initiate interactions with naïve T cells and activate CTLs responses. These intrinsic properties enable cDCs to restore immune surveillance and overcome immunological tolerance within the TME [161, 162]. However, CRC cells can severely impair DC

differentiation, maturation, and function through various immunosuppressive mechanisms, including the production of TGF-β, thus leading to immune tolerance [145]. Within a TGF-β–enriched TME, DCs adopt a tolerogenic state characterized by impaired antigen presentation and diminished capacity to activate T cells [163]. Consistent with this immunosuppressive reprogramming, DCs in CRC patients have been reported to express CD85k, an inhibitory receptor associated with immune suppression and tolerance, indicating direct tumor-mediated modulation of the host immune landscape [164]. Collectively, these alterations underscore that reversing the tolerogenic programming of cDCs represents a critical strategy for restoring effective antitumor immune responses. Accordingly, cDC-based immunotherapeutic approaches in CRC hold considerable promise for enhancing vaccine-induced immunity, overcoming tumor immune evasion, and addressing tumor heterogeneity, while maintaining a favorable safety profile [165].

DC dysfunction in CRC arises from impaired precursor differentiation that reduces the overall DC population, phenotypic shifts toward tolerogenic profiles that promote immune suppression, and impaired maturation that diminishes their antigen-presenting and costimulatory capacities. As a consequence, dysfunctional DCs fail to effectively recognize antigens or deliver adequate activation signals required for robust T-cell priming [41]. Orsini et al. demonstrated that DCs from CRC patients exhibit reduced antigen-presenting capacity and lower expression of costimulatory molecules, accompanied by elevated immunosuppressive cytokine IL-10 and reduced IL-12/TNF-α production, cytokines essential for Th1 polarization and effective anti-tumor immunity [166]. Additionally, tumor-derived factor, such as Ese-3 downregulation in cancer cells, further impairs DC maturation and consequently facilitates tumor proliferation [167, 168]. Nagorsen et al. reported paradoxical findings in which S100+ DC infiltration predicts improved survival yet coincides with greater Treg accumulation, thereby supporting local immunosuppression and tumor growth [169]. Furthermore, Hsu et al. showed that CRC-associated DCs express high levels of CXCL1, a chemokine that promotes tumor cell migration, drives EMT, and enhances stemness features [170]. Together, these observations reveal that CRC-induced reprogramming transforms potent immunostimulatory DCs into dysfunctional, tolerogenic, and pro-invasive phenotypes resembling immature DCs. This phenotypic shift represents a major barrier to effective anti-tumor immunity and contributes significantly to immune evasion in CRC [164]. Consequently, DCs emerge as indispensable determinants of cancer vaccine immunogenicity and clinical efficacy.

Importantly, the extent and functional consequences of DC impairment are further shaped by CRC molecular subtypes. The distribution of pDCs population does not differ markedly between MSI-H/dMMR and MSS/pMMR CRC [171]. However, Ho et al. shows that the enrichment score for activated and immature DCs, particularly in the context of pMMR/MSS) CRC liver metastases, is lower than MSI-H/dMMR primary CRCs, indicating a profound deficiency in antigen presentation capacity and early immune priming within these tumors [172]. These findings suggest that effective vaccine strategies for pMMR/MSS CRC cannot rely solely on antigen delivery but must incorporate approaches that actively restore DC number and function, as explained previously. Rational vaccine design should therefore relieve immunosuppressive signaling within the TME and emphasize DC-targeted delivery systems, incorporation of potent innate immune adjuvants (e.g., Toll-like receptor (TLR) agonists), and combination strategies that promote DC expansion and maturation (such as granulocyte–macrophage colony-stimulating factor (GM-CSF)).

In order to create durable antitumor responses, therapeutic use of cDCs in CRC immunotherapy involves diverse strategies, such as ex vivo manipulation, DC-based vaccine platforms, and TME modulation aimed at improving DC activity. In ex vivo approaches, patient-derived cDCs are isolated, activated under controlled conditions, and loaded with tumor antigens before reinfusion, enabling personalized antigen presentation and robust T-cell priming while minimizing off-target toxicity. Controlled conditions during the activation of DCs maximizes their antigen-presenting capacity and ability to induce robust immune responses [173–176]. Also, loading cDCs with a diverse array of tumor antigens further helps address intratumoral heterogeneity and broadens immune recognition across distinct tumor cell subsets [177, 178]. Moreover, successful DC-based vaccines can promote durable immune protection through the induction of memory T cells while exhibiting a favorable safety profile compared with conventional anticancer therapies [165].

However, the efficacy of DC-based interventions is frequently constrained by the profoundly immunosuppressive CRC TME, which collectively impair DC maturation and T-cell activation [179]. Consequently, combination strategies aimed at reprogramming the TME have emerged as critical adjuncts to DC-based therapies. ICIs can alleviate T-cell exhaustion, enhance intratumoral T-cell infiltration, and synergize with DC-mediated

antigen presentations [180, 181]. In parallel, cytokine-based interventions such as GM-CSF promote DC maturation through activation of JAK/STAT5, MAPK, and NF-κB signaling pathways, leading to upregulation of MHC molecules, costimulatory ligands (CD80/CD86), and pro-inflammatory cytokines that collectively boost antigen presentation and amplify T-cell priming [182–184]. Additional approaches targeting suppressive immune populations, such as Tregs and TAMs, within the TME further improve DC efficacy by promoting anti-tumor immune responses [185, 186]. In preclinical models, the combination of cryoablation or radiofrequency ablation with immune checkpoint blockade and DC loading has been shown to restore immune equilibrium within the TME, confer protection against tumor recurrence, and enhance tumor-specific T-cell responses. The immunological effects of these approaches arise from the localized physical destruction of tumor tissue, via cold- or heat-induced ablation, which facilitates antigen release, precise tumor targeting, and subsequent activation of systemic antitumor immunity [187]. Collectively, these integrated strategies highlight the central role of cDCs as both antigen-presenting cells and immune orchestrators, underscoring the importance of combining DC-based approaches with TME-modulating interventions to maximize the therapeutic potential of CRC vaccines and immunotherapies.

2.4 Immune Cells: Neutrophils

Neutrophils, another major subset of the myeloid lineage, constitute the first line of defense against infection and acute inflammation. They exert antimicrobial activity through the release of lytic enzymes, phagocytosis, the formation of neutrophil extracellular traps (NETs), and the secretion of cytokines that sustain inflammatory responses and recruit additional immune cells [188, 189]. Within the context of cancer, tumor-associated neutrophils (TANs) have emerged as critical regulators of tumor biology. In CRC, TANs are strongly associated with patient prognosis and display both anti-tumor and pro-tumor activities depending on their phenotypic state and microenvironmental cues. Importantly, accumulating evidence indicates that neutrophils within the CRC TME can undergo phenotypic polarization toward pro-tumorigenic functions, thereby contributing to disease progression and immune evasion [189].

Triner et al. demonstrated that neutrophils suppress CRC progression and metastasis in mouse models by controlling tumor-associated microbiota. Conversely, neutrophil depletion disrupts intestinal flora, increases IL-17–producing bacteria, and accelerates CRC growth [190]. Other studies have shown that elevated neutrophil infiltration accelerates CRC progression via CXCL1/CXCR2 signaling, increased immunosuppressive gene expression, and matrix metalloproteinase-9 (MMP9)-mediated T-cell suppression via TGF-β activation [191, 192]. As mentioned before, TGF-β also suppresses IFN-γ production, a key cytokine required for macrophage activation and for sustaining the effector functions of NK cells and neutrophils, thereby reinforcing a broadly immunosuppressive tumor milieu [193, 194]. Consistent with these findings, Fridlender et al. reported that TGF-β within the CRC TME can polarize TANs from an anti-tumor "N1" phenotype to a tumor-promoting "N2" phenotype. N2 TANs enhance tumor growth by fostering angiogenesis and conferring resistance to anti-VEGF therapy, while simultaneously promoting a more invasive tumor phenotype [195]. Beyond their role in immune evasion, TANs further promote metastasis by degrading basement membrane components, thereby enabling tumor cell migration and extravasation. Collectively, these observations underscore the dual and context-dependent roles of TANs in CRC and highlight their substantial contribution to tumor progression, immune suppression, and metastatic spread [196].

dMMR/MSI-H tumors are characterized by elevated TMB and dense infiltration of CTLs, which limits the dominance of immunosuppressive neutrophil populations and contributes to improved responsiveness to ICIs. In contrast, pMMR/MSS tumors generally exhibit an effector T-cell infiltration and enrichment of immunosuppressive myeloid populations, including pro-tumorigenic N2-like TANs [99]. These subtype-specific immune landscapes underscore why TAN-driven immune suppression is particularly relevant in MSS CRC and highlight the need for vaccine and combination strategies that actively modulate neutrophil recruitment and polarization to remodel the TME and enable effective antitumor immunity in immunologically cold CRC.

Collectively, these findings highlight the functional plasticity of TANs and their substantial impact on CRC progression and immune suppression, specifically into pro-tumor N2 phenotypes under the influence of TGF-β and other tumor-derived signals. Anti–TGF-β exerts its suppressive effects on CRC through dual and complementary mechanisms, involving inhibiton of PI3K/AKT signaling in TANs and blockade of TGF-β/Smad signaling within tumor cells. Consistent with these effects, blockade of TGF-β signaling has been shown to repolarize TANs toward an anti-tumor N1 phenotype, thereby enhancing their cytotoxic activity against CRC cells while reducing the production of metastatic chemoattractants. Blockade of TGF-β signaling also enhances the expression of GM-CSF and IFN-γ, key

cytokines that regulate neutrophil differentiation within the TME [197]. These findings provide a strong mechanistic rationale for combination strategies aimed at alleviating TAN-mediated immune suppression, promoting T-cell infiltration, and ultimately improving overall therapeutic efficacy.

2.5 Cancer-Associated Fibroblasts

CAFs, the most abundant stromal component of the TME, play central roles in ECM maintenance and remodeling, desmoplasia, angiogenesis, tumor proliferation, immunosuppression, invasion, metastasis, and therapy resistance [198–200]. Within the TME, fibroblasts commonly acquire a chronically activated phenotype and differentiate into CAFs in response to tumor-derived growth factors, including TGF-β, epidermal growth factor, and bone morphogenetic proteins. TGF-β plays a crucial role in the transformation of fibroblasts into CAFs. CAFs are characterized by an elongated, spindle-like morphology, lack canonical markers of epithelial cells, endothelial cells, and leukocytes, and, importantly, do not harbor the oncogenic mutations observed in malignant cells [201]. Through ECM organization, CAFs regulate cancer cell behavior and increase tissue stiffness, thereby driving EMT and metastatic dissemination [202, 203]. Activin A secreted by CAFs has been shown to increase ECM stiffness and promote CRC progression [204].

CAFs also promote tumor growth by secreting a broad spectrum of chemokines and cytokines that engage in reciprocal crosstalk with cancer cells [200, 205]. Although the majority of CAFs arise from resident stromal fibroblasts, a significant subset originates from mesenchymal stem cells (MSCs) and mesothelial cells [198, 206]. MSCs, which possess pluripotent capacity, can be recruited to tumor sites, where tumor-derived signals drive their differentiation toward a CAF-like phenotype [207, 208]. Direct CAF–tumor cell interactions enhance tumor progression, invasiveness, and metastasis potential [209–211]. Mesothelial cells can similarly undergo mesenchymal transition, especially in highly invasive tumors, further contributing to CAF heterogeneity [212].

CAFs also mediate immune evasion [198, 213–215]. Zhang et al. reported that CXCL8 secreted by CAFs recruit monocytes into the CRC TME and induces M2 polarization through ICAM-1/VCAM-1 signaling or IL-8/CXCR2 pathways. The resulting M2-polarized macrophages synergize with CAFs to suppress immunosurveillance, amplifying TME immunosuppression [216, 217]. Consistent with these observations, high VCAM1 expression in CRC correlates with more aggressive tumor behavior and poor clinical outcomes, partly due to its ability to promote pseudopodia formation and enhance CRC cell transmigration across endothelial layers in vitro [218]. In addition to modulating immune escape, CAFs stimulate angiogenic processes by inducing endothelial cells to secrete VEGF. This increase in VEGF production is driven, at least in part, by CRC-mediated potentiation of IL-6 secretion by CAFs [219]. Collectively, these findings underscore the multifaceted pro-tumorigenic roles of CAFs in CRC biology.

Collectively, transforming CAFs into anti-tumor fibroblasts is essential for preserving ECM mechanical homeostasis. Current therapeutic strategies include therapeutic cancer vaccines targeting fibroblast activation protein (FAP), inhibition of TGF-β secretion, and targeting key signaling pathways [220].

2.6 Endothelial Cells

Tumor vascularization is essential for CRC invasion and metastasis. Tumor vasculature is primarily composed of endothelial cells, supported by pericytes, smooth muscle cells, and endothelial progenitor cells [221]. Endothelial cells, typically quiescent in healthy tissues, undergo rapid proliferation and structural remodeling in response to tumor-derived pro-angiogenic signals, culminating in the formation of new blood vessels (angiogenesis) [222]. Angiogenesis is driven by paracrine cues, including VEGF, platelet-derived growth factor (PDGF), CXCL8, and TNF-α, secreted by various components of the TME. These signals drive the formation of aberrant vasculature that sustains tumor expansion by supplying oxygen and nutrients, removing metabolic waste, and facilitating metastatic dissemination [223–225].

Tumor-associated endothelial cells (TECs) further potentiate CRC progression by upregulating multiple growth factor receptors, including VEGFR and EGFR, thereby amplifying angiogenic signaling [226]. Beyond their angiogenic role, TECs contribute to tumor aggressiveness through additional mechanisms, such as secreting soluble Jagged-1, which activates Notch signaling and promotes stem-like phenotypes in CRC cells [227]. TECs also express adhesion molecules such as E-selectin, which facilitates tumor cell adhesion, invasion, and metastasis, while simultaneously recruiting neutrophils that reinforce an immunosuppressive TME [228].

Analogous to CAFs, TECs also modulate immune responses within the TME. For example, TEC-expressed FasL can induce apoptosis in CTLs, thereby promoting immune evasion [229]. Transcriptomic analyses further reveal that TEC expression of SPARC, COL1A1, COL1A2, and IGFBP3 is strongly associated with immunoinhibitory cell infiltration and correlates with T-cell exhaustion

[230]. Nevertheless, a subset of TECs may exert antitumor effects by enhancing CTL infiltration, maintaining vascular normalization, fostering a Th1-dominant immune milieu, and attenuating tumor growth, migration, and neovascularization [231, 232].

Anti-angiogenic therapies, particularly those targeting VEGF/VEGFR signaling, can transiently normalize tumor vasculature, improve T-cell access to the tumor bed, and synergize with ICIs [233]. Together, these observations provide a strong rationale for rational combination strategies to overcome immune exclusion, optimize antigen delivery, and improve the magnitude and durability of antitumor immune responses in CRC.

2.7 Extracellular Matrix

Beyond serving as a structural scaffold, the ECM, composed of diverse cellular constituents and structural proteins, is a dynamic regulator of cell–cell interactions, paracrine signaling, proliferation, immune evasion, and metastasis, thereby represents as a critical component of the TME [234]. Accumulation and remodeling of ECM proteins increase matrix stiffness, a hallmark of EMT and a key driver of CRC progression and metastasis. Substantial evidence indicates that ECM components, including collagen and fibronectin, play essential roles in these processes [204, 235–237]. Collagen promotes stem-like traits and metastatic potential through activation of the integrin/PI3K/AKT/Snail signaling axis; notably, elevated COL5A2 expression has been linked to poorer clinical outcomes [237, 238]. Li et al. similarly demonstrated a positive correlation between type I collagen density and tumor stage [234]. Similarly, fibronectin contributes to CRC aggressiveness and portends unfavorable prognosis [239, 240].

Enhanced ECM stiffness creates a substantial physical barrier that restricts immune cell infiltration and limits the effective intratumoral delivery of immunotherapeutic agents. In addition, ECM stiffening can further promote immune escape by modulating the expression of immune checkpoint molecules, thereby diminishing the therapeutic benefit of immune checkpoint blockade. For example, increased collagen fiber density and the resulting matrix stiffening can upregulate PD-L1 expression, thereby facilitating tumor immune evasion [220]. ECM stiffness is also a critical determinant of tumor responsiveness to chemotherapy. Increased matrix rigidity has been shown to upregulate the activity of multidrug resistance protein 1 at the cell membrane, thereby enhancing drug efflux and promoting chemoresistance [241]. In addition, elevated tumor stiffness impairs intratumoral drug penetration, further reducing therapeutic efficacy [242].

Despite these pro-tumorigenic functions, certain ECM constituents exhibit antitumor properties. Type IV collagen, for instance, can inhibit cancer cell invasion, and the positioning of the ECM between the basement membrane and interstitial space functions as a physical barrier that restricts tumor proliferation, differentiation, and dissemination [234, 243, 244]. However, ECM remodeling, characterized by upregulated MMP-2 and MMP-9, disrupts this barrier by degrading type IV collagen, thereby facilitating tumor cell motility, invasion, lymphovascular infiltration, and progression to advanced stages. Such remodeling is a defining feature of the CRC TME and contributes significantly to poor clinical outcomes [245–249].

Therapeutic modulation of ECM architecture therefore represents a biologically plausible strategy to enhance vaccine delivery, remodel the TME, and improve the efficacy of therapeutic cancer vaccines. ECM-targeted vaccine approaches primarily aim to reduce matrix stiffness and density in order to facilitate T-cell infiltration and augment cytotoxic immune responses. Cancer vaccines encoding the *Spam1* gene express hyaluronidase, leading to ECM degradation, improved tumor perfusion, reduced hypoxia, and reprogramming of the immune milieu, ultimately enhancing antitumor activity and lowering recurrence rates [250]. Similarly, vaccines directed against the extra-domain A (EDA) of fibronectin (FN) have demonstrated efficacy in limiting metastatic dissemination [251], whereas those targeting the extra-domain B (EDB) of FN effectively reduce solid tumor burden [252]. In addition, early clinical studies of FAP-targeted cancer vaccines have reported favorable safety profiles and evidence of tumor engagement, accompanied by enhanced CTLsl–mediated antitumor immunity [253]. Moreover, vaccines expressing immature laminin receptor proteins have been associated with durable CTLs–dependent protective immune responses [254]. Additionally, reducing ECM stiffness by targeting collagen crosslinking can enhance T cell migration, thereby improving the efficacy of ICIs [255]. Similarly, small-molecule inhibitors of MMP-2/9 have been shown to enhance antitumor immune responses, decrease tumor burden, and improve survival, while simultaneously downregulating PD-L1 expression and potentiating the efficacy of ICIs–based immunotherapies [256]. Collectively, these findings highlight ECM stiffness as a critical modulator of immunotherapy responsiveness and underscore the therapeutic value of ECM remodeling in optimizing immunotherapeutic efficacy.

Importantly, the immunosuppressive features of the CRC TME have direct implications for therapeutic vaccine design and rational combination strategies. The dominance of Tregs, M2-like macrophages, and so on limits effective antigen presentation and attenuates vaccine-induced T-cell priming, highlighting the need for vaccine platforms that incorporate potent adjuvants or DC–targeting strategies to enhance cross-presentation. Likewise, T-cell exhaustion driven by chronic antigen exposure and inhibitory signaling pathways (e.g., PD-1/PD-L1, CTLA-4) provides a strong mechanistic rationale for combining CRC vaccines with ICIs to restore effector function rather than relying on vaccination alone. In addition, stromal desmoplasia, aberrant vasculature, and metabolic constraints within the CRC TME impair T-cell trafficking and survival, supporting the integration of vaccines with agents that remodel the ECM, normalize tumor vasculature, or modulate tumor metabolism. Together, these observations underscore that effective CRC vaccine strategies must be explicitly designed to counteract dominant immunosuppressive mechanisms within the TME.

3. Immunological Basis of CRC Vaccines

Besides being one of the most prevalent forms of cancer and cancer-related deaths, CRC poses unique immunological challenges compared with many other solid tumors, such as melanoma, due to relatively low TMB and limited tumor antigens diversity for most of CRC cases, particularly MSS/pMMR, resulting in weak baseline immunogenicity and poor spontaneous T-cell priming [257]. Lower expression of tumor antigens hinders effective cross-priming and accelerates T cell dysfunction [258]. Moreover, CRC tumors are characterized by a highly immunosuppressive TME enriched in Tregs, M2-polarized macrophages, and pro-tumorigenic neutrophils, alongside dominant inhibitory signaling pathways such as PD-1/PD-L1 and TGF-β [259]. Collectively, these features promote immune exclusion, inappropriate antigen presentation, T-cell exhaustion, and resistance to immune-mediated tumor eradication, distinguishing CRC as a malignancy in which effective immunotherapy, and particularly vaccine-based approaches, must overcome multiple, layered barriers to achieve durable clinical benefit.

Because CRC represents a chronic pathological condition, effective and potentially curative antitumor immunity, unlike immune responses to acute infections, relies on the establishment of durable, long-lived T-cell immunity. Compared with short-lived effector T cells, memory T cells possess the capacity for long-term survival and functional activity across peripheral tissues and tumor sites, thereby representing a central benchmark of effective antitumor immune protection [260]. Effective antitumor immunity is sustained by antigen-specific memory T cells with strong proliferative potential [261]. In this context, vaccination represents a highly theoretically attractive therapeutic strategy due to its capacity to elicit highly specific anti-tumor immune responses with minimal adverse effects. Beyond acute immune activation, therapeutic CRC vaccines aim to establish durable immunological memory through the generation of long-lived memory T-cell subsets, including central memory T cells (T_{CM}), effector memory T cells (T_{EM}), and tissue-resident memory T cells (T_{RM}) [262]. Mizukoshi et al. showed that peptide vaccines can induce tumor-specific CTLs that persist for years and exhibit characteristics of long-lived memory, including self-renewal and sustained immune function without repeated vaccination [261]. In experimental models, cancer vaccines have also been shown to augment memory T-cell responses, and their combination with other immunotherapeutic modalities, such as bispecific antibodies, further enhances the durability and protective capacity of antitumor immune memory [263]. Collectively, these memory populations enable sustained immune surveillance and rapid recall responses upon antigen re-encounter, which are critical for long-term tumor control and prevention of disease recurrence [264]. By inducing robust antigen-specific priming and memory differentiation, cancer vaccines can enhance TIL density, support persistent effector function, and facilitate durable cytotoxic elimination of antigen-expressing CRC cells [262]. Compared with conventional therapeutic modalities, vaccines exhibit superior tolerability and are less likely to cause dose-dependent toxicities [265].

3.1 CRC-Antigen Processing and Presentation

Tumor antigens are broadly categorized into two groups: tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs), also referred to as neoantigens. TAAs are proteins markedly overexpressed on malignant cells relative to normal tissues, whereas TSAs arise exclusively from tumor cells and are absent in healthy tissues. Both antigen types, however, are capable of binding to human leukocyte antigen (HLA) molecules and being presented by MHC molecules to T cells, thereby initiating antitumor immune responses [178].

As illustrated in Figure 4, antigen processing begins with transcription of genomic loci (for TAAs) or mutated loci (for TSAs), followed by translation, protein degradation, and MHC loading, culminating in surface presentation of TAA/TSA peptides on cancer cells. Tumor recognition by

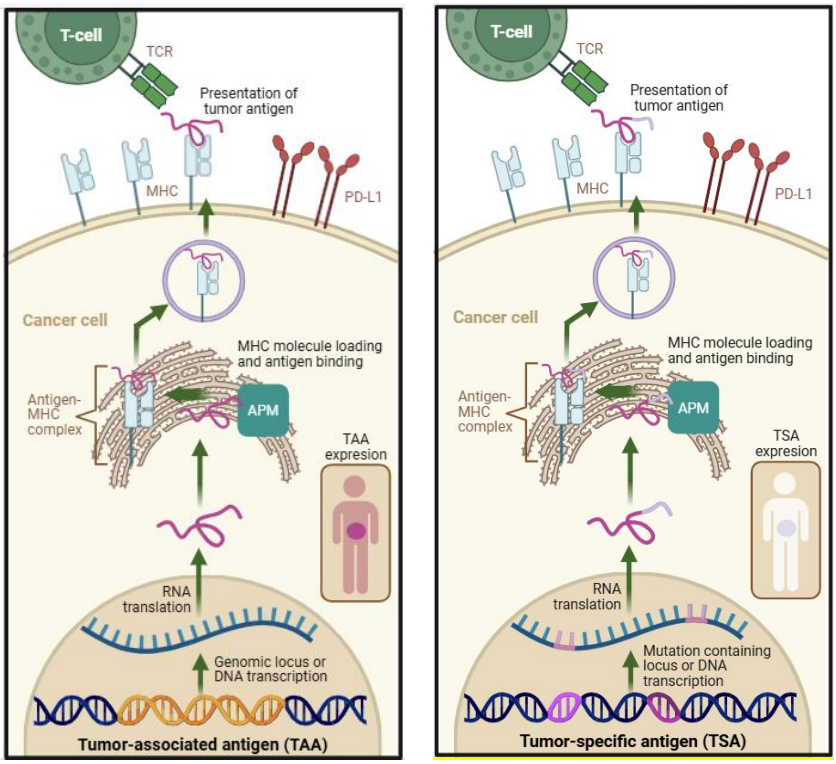

Figure 4. Key characteristics and processing pathways of tumor antigens. Antigen generation follows several steps: transcription of either the corresponding genomic locus (TAA; left) or the mutation-containing locus (TSA; right), followed by RNA translation, proteasomal degradation, loading of peptide fragments onto MHC molecules, and eventual presentation of the processed antigen–MHC complex on the tumor cell surface. TAA-derived proteins are highly expressed in tumors but remain detectable at low levels in normal tissues (pink patient; left), whereas the neoantigenic components of TSAs arise exclusively within tumor cells (cream patient; right). Tumor recognition by CTLs occurs through engagement of the TCR (green). Tumors may suppress this immune recognition by upregulating immune checkpoint molecules, including PD-L1 (red). APM, Antigen-presenting machinery; CTL, cytotoxic T lymphocyte; MHC, Major histocompatibility complex; TAA, Tumor-asssociated antigen; TCR, T-cell receptor; TSA, Tumor-specific antigen.

T cells occurs through the T-cell receptor (TCR). However, malignant cells frequently evade immune surveillance by upregulating immune checkpoint molecules such as PD-L1 [178]. Accordingly, immune checkpoint proteins have also emerged as auxiliary targets in vaccine-based immunotherapy, as their inhibition may enhance T-cell activation and mitigate immunosuppressive signaling [28]. Supporting this, Qi et al. demonstrated that checkpoint inhibition improves survival outcomes and yields early therapeutic signals therapeutic effects when combined with additional immunomodulatory strategies [30].

3.2 Mechanistic Basis of CRC Vaccine–Induced Immune Responses and Their Efficacy

The immune system comprises an intricate network of defense mechanisms that collectively protect the host from pathogens and foreign objects. It operates through two cooperative arms: innate and adaptive immunity [266]. Innate immunity responds rapidly, within hours, to pathogenic stimuli in a uniform and nonspecific manner, serving as the first line of defense. Conversely, adaptive immunity is highly specific and occurs when the innate immune system fails to destroy a pathogen or foreign object. However, its specific target requires the adaptive immune system to recognize the pathogen or foreign object invading the body first, resulting in a slower response. Although slower to develop, it is more precise and capable of generating immunological memory, thereby enabling accelerated and amplified responses upon re-exposure [266, 267].

Adaptive immunity encompasses humoral and cellular responses. Humoral immunity, the antibody-mediated arm of adaptive immunity, operates primarily within the extracellular space. It is initiated when plasma B cells, with assistance from HTLs (HTL-dependent activation), produce antigen-specific antibodies following engagement with MHC molecules or macrophage-derived signaling. Cellular immunity, on the other hand, is mediated by antigen-specific T lymphocytes that develop in the bone marrow, mature in the thymus, and become activated upon recognition of peptide antigens presented by APCs through MHC molecules. CTLs eliminate infected or malignant cells via apoptosis, whereas HTLs enhance B-cell antibody production, augment cytotoxic cell activity, and activate

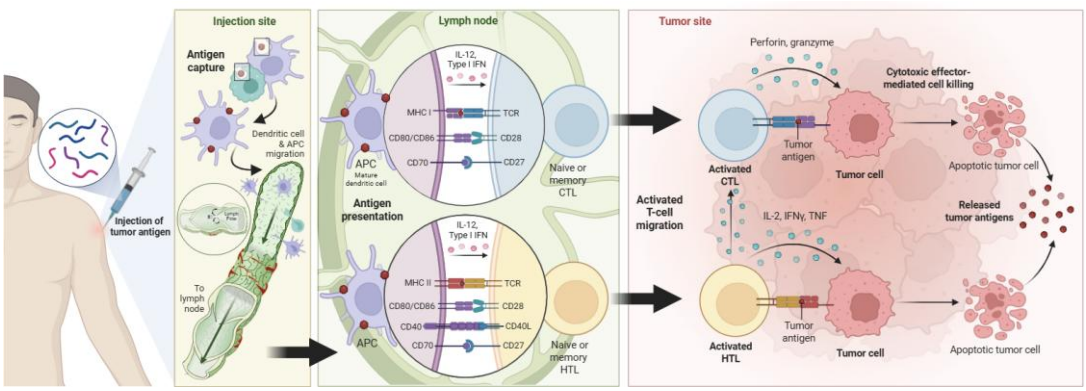

Figure 5. CRC vaccine immunological principle. APC, Antigen precenting cell; CTL, Cytotoxic T lymphocyte; HTL, Helper T lymphocyte; MHC, Major histocompatibility complex.

macrophages. Thus, HTLs function primarily as orchestrators of immune responses rather than direct cytotoxic effectors [268–270].

Like natural pathogens, vaccines are designed to initiate the immune activation cascade by engaging innate immunity. APCs, including DCs and macrophages, detect pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors (PRRs) such as TLRs [271–273]. Immature DCs possess high antigen-capturing capacity. Following antigen uptake, APCs upregulate MHC class I/II and costimulatory molecules in response to cytokines such as IL-12 and chemokine signaling (Figure 5). Antigen-loaded APCs then migrate to draining lymph nodes, where T-cell priming occurs [274].

Within the lymph nodes, captured antigens are processed into peptide epitopes, which are the essential immunogenic determinants recognized by T cells [271–273, 275, 276]. MHC molecules present only processed peptide fragments, not intact proteins [269, 277, 278]. The resulting epitopes are presented via MHC-I to activate CTLs or via MHC-II to activate Th1-type HTLs (Figure 5) [275, 279]. MHC-I–restricted presentation drives CTL proliferation and trafficking to the TME, where CTLs mediate cancer cell killing through perforin- and granzyme-dependent cytotoxicity and through cytokine release (e.g., IFN-γ, TNF-α) [271–273, 280, 281]. MHC-II presentation activates Th1-polarized HTLs, which further shape antitumor immunity by promoting CTL activation, supporting antibody production via B cells (including antibody-dependent cellular cytotoxicity, ADCC), and maintaining a pro-inflammatory milieu [271–273, 282].

Th1 cytokines generally potentiate antitumor immunity by activating both cellular and humoral immunity directed toward intracellular pathogens, whereas Th2-associated responses are primarily anti-inflammatory and less effective at controlling malignant cells. Th2 cells predominantly induce humoral immune responses against extracellular pathogens [283, 284]. Within the tumor setting, Th1-polarized HTLs play a pivotal role in strengthening antitumor immunity by activating APCs through CD40–CD40L costimulatory interactions, enhancing MHC class I expression on tumor cells via IFN-γ secretion, and coordinating multiple immune effector populations to establish a robust immunostimulatory environment [280, 281].

Damaged or stressed tumor cells release additional tumor antigens and damage-associated molecular patterns (DAMPs). These signals enhance antigen cross-presentation by APCs, broaden the spectrum of T-cell responses (polyclonality), and further amplify vaccine-induced antitumor immunity [285]. Collectively, CRC vaccines function by shifting the immune landscape from tolerance toward active antitumor immunity, thereby enabling more efficient progression of the cancer–immunity cycle [286].

3.3 Memory T-Cell Formation in CRC Vaccination

As explained previously, APCs secrete immunostimulatory cytokines, which enhance the expression of costimulatory molecules and promote effective T-cell activation [287]. T-cell responses to acute antigenic stimulation typically progress through three sequential phases: an initial phase of rapid clonal expansion, followed by contraction of the effector population, and culminating in the establishment of long-lived immunological memory [288]. CRC-specific T cells are activated and clonally expand through coordinated engagement of the TCR with peptide–MHC complexes and requisite costimulatory signals, subsequently differentiating into effector and long-lived memory T-cell populations [289]. After the elimination of vaccine antigens, effector CTLs enter a rapid contraction phase in which most antigen-specific cells undergo apoptosis, while a small fraction (approximately 5–10%) persist and differentiate into long-lived memory cells. Importantly,

this transition is not stochastic, as memory potential is unequally distributed among effector cells rather than being uniformly inherited. These memory cells have a distinguish feature to rapidly acquire effector functions and generate a robust secondary expansion of CTLs capable of swiftly controlling recurrences [264, 288]. The maintenance and differentiation of memory T-cell subsets are regulated by a combination of extrinsic cues and intrinsic transcriptional programs. During priming, cytokines such as IL-2 drive T-cell proliferation and are critical for effective CTL memory recall responses, whereas IL-7 and IL-15 support the survival, differentiation, and long-term maintenance of memory T cells [290, 291].

Clinical studies in CRC have demonstrated that vaccine-induced adaptive responses can persist over extended periods and are associated with sustained antigen-specific T-cell activity, retained resistance to subsequent tumor rechallenge, and improved long-term outcomes. This durable antitumor protection was shown to be dependent on CTL memory cells, as depletion of this population abolished the protective immune response [292, 293]. For example, vector-based VRP-CEA vaccination was associated with long-term survival and durable T-cell responses in stage III colon cancer patients [294], and GUCY2C-PADRE vaccines induced CTL memory responses in early-stage CRC patients that provided durable protection against metastases [295].

CTL memory cells persist as a heterogeneous compartment comprising both circulating and non-circulating subsets. Circulating memory populations include T_{CM}, T_{EM}, and stem cell–like memory T cells (T_{SCM}), whereas non-circulating cells are classified as tissue-resident memory T cells (T_{RM}), which reside within tissues and tumors and are largely excluded from the blood. T_{CM} and T_{SCM} cells express lymphoid homing receptors such as CD62L and CCR7, enabling their trafficking to secondary lymphoid organs and the bone marrow, while T_{EM} cells lack these receptors and preferentially circulate through the blood and peripheral tissues [296–298]. In contrast, T_{RM} cells are defined by the absence of circulating markers and the expression of tissue retention molecules, including CD103 and CD69 [299].

The diversification of memory T-cell subsets likely optimizes protective immunity through functional specialization. Upon antigen re-exposure, T_{EM} and T_{RM} cells provide rapid effector functions and frontline defense at sites of antigen encounter, while T_{CM} mount robust recall responses by undergoing self-renewal and rapidly differentiating into effector CTL, T_{EM}, and T_{RM} populations [260, 288]. The presence of TCM and TEM cells has been correlated with improved clinical outcomes in cancer patients. Consistent with this observation, ICIs immunotherapy has been shown in murine models of infection to enhance the generation and persistence of CTL memory cells, suggesting that similar mechanisms may contribute to the promotion of durable antitumor immune memory [300]. T_{SCM} similarly exhibit self-renewal capacity and highest degree of multipotency. Emerging evidence also indicates that locally reactivated T_{SCM} cells can transition into T_{CM}, T_{EM}, and effector CTL subsets within lymphoid tissues [260, 301]. In addition, locally reactivated T_{RM} cells can further contribute to immune recall by transitioning into T_{CM} and T_{EM} phenotypes, thereby reinforcing systemic and tissue-level antitumor immunity [260]. Because TRM are strategically positioned within peripheral tissues, they enable rapid local immune responses and represent attractive targets for vaccines aimed at enhancing protection at barrier sites and other organs [302].

3.4 Impact of the CRC TME on Vaccine-Induced Immunity and Rationale for CRC Vaccine-Based Combination Strategies

As detailed in Section 2, the CRC TME evolves in parallel with tumor progression and progressively dampens antitumor immune responses elicited during tumor development, thereby facilitating immune evasion [303]. This immunosuppressive milieu exerts profound suppressive effects on vaccine-induced immune responses. Immunosuppressive populations actively inhibit CTL infiltration, survival, and effector function [257, 304, 305]. In parallel, dominant inhibitory pathways such as PD-1/PD-L1 and TGF-β signaling promote T-cell exhaustion, exclusion, and functional impairment within the tumor bed. Consequently, although cancer vaccines can effectively prime antigen-specific T cells in peripheral lymphoid organs, these cells often fail to exert cytotoxic activity once they encounter the hostile CRC TME [135, 215]. This disparity between effective immune priming and intratumoral effector function represents a central obstacle to CRC vaccine efficacy and underscores the critical importance of elucidating CRC-specific immunosuppressive mechanisms for the rational design of effective immunotherapeutic strategies [303, 306].

Within this CRC-specific immunological framework, therapeutic CRC vaccines should be viewed as immune initiators rather than standalone effectors. In CRC, combination immunotherapy is designed to overcome the inherent limitations of single-agent immunotherapy and achieve improved therapeutic outcomes [257]. Accordingly, synergistic therapeutic benefits can be achieved through combination immunotherapy, in which complementary treatment modalities cooperatively enhance antitumor efficacy [307]. Vaccines provide the critical first step of expanding tumor-specific T-cell

repertoires and increasing tumor immunogenicity, while combination strategies, particularly ICIs, TGF-β blockade, and myeloid-modulating agents, are required to sustain and unleash these responses within the TME [308, 309]. ICIs or other immunomodulatory agents are administered in combination with cancer vaccines to potentiate vaccine-induced immune responses and enhance tumor cell eradication [310]. Such approaches modulate the TME by reprogramming immune-resistant T-cell populations, alleviating immunosuppressive signals, and promoting an inflamed, immunologically active milieu, thereby strengthening immune recognition and elimination of tumor cells [307, 311]. By enhancing antigen-specific priming while simultaneously relieving immune suppression and exclusion, rational vaccine-based combination approaches offer a biologically grounded strategy to convert immunologically "cold" CRC tumors into responsive, inflamed lesions capable of supporting durable antitumor immunity.

3.5 Determinants of Vaccine-Induced Memory T-Cell Quality in CRC Patients

The quality and durability of vaccine-induced immunological memory in CRC are influenced by host-related factors, particularly immunosenescence, which is prevalent in elderly CRC patients. Immunosenescence refers to the progressive decline in immune competence that occurs with advancing age, resulting in diminished responsiveness to new antigens and impaired vaccine efficacy in older adults. The immune system undergoes substantial age-related remodeling, resulting in immune responses that are strongly influenced by chronological age [312]. This age-related immune remodeling is characterized by a reduction in naïve T-cell output due to thymic involution [313], contraction of T-cell receptor diversity [314], and compromised generation of effective primary immune responses as illustrated by the poor vaccine response to hepatitis B [312, 315], while low-grade chronic inflammation and declines in regenerative capacity of hematopoietic lineages further exacerbate immune dysfunction [312, 316, 317]. Concurrent changes in hematopoietic stem cell (HSC) differentiation bias toward myeloid lineages at the expense of lymphoid precursor and declines in regenerative potential also contribute to decreased lymphoid cell production and repertoire diversity, which collectively undermine both innate and adaptive responses to vaccination [316].

Recent evidence suggests that age-related skewing of HSC differentiation toward the myeloid lineage is further intensified by telomeric stress [318]. Under conditions of telomerase insufficiency, the transcription factor basic leucine zipper transcription factor ATF-like (BATF) constrains the self-renewal capacity of lymphoid-biased HSCs, promoting obligatory lymphoid differentiation coupled with reduced progenitor maintenance. This dual effect is proposed to drive loss of HSC pluripotency and preferential expansion of myeloid-committed HSCs, a mechanism particularly relevant in humans where telomere erosion accompanies aging [319, 320]. Consistently, human HSC aging is characterized by increased DNA damage and elevated BATF expression, ultimately resulting in an age-dependent decline in early and committed B-cell progenitors expressing CD45RA, CD38, CD10, and CD19 [321].

A central functional hallmark of T-cell aging is the reduced production of IL-2 following T-cell activation, which compromises clonal expansion and memory differentiation; notably, supplementation with exogenous IL-2 has been shown to partially restore age-associated defects in T-cell activation [322]. In addition, age-related T-cell dysfunction can be ameliorated by pro-inflammatory cytokines or TLR ligands, providing a strong mechanistic rationale for the use of potent adjuvants in vaccine design to enhance immunogenicity in aged hosts [323]. Understanding these mechanisms is therefore critical for optimizing therapeutic cancer vaccine strategies, particularly in elderly CRC patients who represent the majority of the affected population.

Beyond age-related reductions in T-cell numbers and repertoire diversity, intrinsic functional impairments also arise within memory T cells during aging. Many of these defects stem from altered cellular resource allocation, in which enhanced investment in metabolic maintenance compromises the capacity of T cells to mount effective activation and effector responses to antigenic stimulation. Clinically, this deterioration of T-cell memory is exemplified by the reactivation of latent varicella–zoster virus (VZV) as herpes zoster and by reduced responsiveness to vaccines such as influenza in elderly individuals [324]. Furthermore, aging is associated with a loss of polyfunctional antigen-specific T cells, with a shift toward monofunctional populations, thereby further compromising effective T-cell–mediated immunity [325].

4. Tumor Antigens in CRC Vaccine Development

Vaccine efficacy is fundamentally determined by the precision with which target antigens are selected. Consequently, the identification of tumor antigens represents a critical first step in vaccine development. To identify CRC-associated antigens, Corulli et al. evaluated two principal characteristics: (1) overexpression of candidate proteins in malignant versus normal tissues, and (2) biological relevance to CRC pathogenesis and progression, including their association with adverse prognosis [326]. Aberrantly high expression under

malignant conditions is necessary, as elevated antigen levels enhance immunogenicity [327]. Additional criteria proposed by Boris-Minev for prostate cancer, also relevant to CRC, include: (1) high tumor-specific expression, (2) absent or minimal expression in normal tissues, and (3) exposure of extracellular amino acid domains accessible to immunologic targeting [328].

4.1 Candidate Tumor Antigens for CRC Vaccines

4.1.1 Carcinoembryonic Antigen

One of the earliest TAAs to be identified is carcinoembryonic antigen (CEA), a 200-kDa glycoprotein that is physiologically expressed only during embryonic development but aberrantly re-expressed at high levels in CRC [178, 329–331]. In addition to its association with several malignancies, CEA is strongly linked to gastrointestinal cancers and is implicated in anti-apoptotic signaling, tumor invasion, and metastatic dissemination [329, 331–335]. Multiple studies have demonstrated that CEA-targeted vaccines are capable of eliciting antigen-specific humoral responses, activating HTLs, and inducing CTL-mediated cytotoxicity in human subjects [3, 336, 337]. Early in vitro studies also demonstrated that DCs pulsed with CEA-derived peptides are capable of priming CEA-specific CTL responses [338]. However, as a TAA, CEA lacks strict tumor specificity and is also expressed at low levels by normal epithelial tissues, resulting in immune tolerance. To overcome this limitation, several strategies have been developed to enhance the immunogenicity of CEA-based vaccines. One approach involves the use of altered peptide ligands with increased HLA-binding affinity, which has been shown to more effectively activate CEA-specific CTLs in vitro [339]. Alternatively, DNA vaccines encoding CEA-derived epitopes in combination with immunostimulatory cytokines, adjuvants, or helper T-cell epitopes have been designed to augment antigen-specific T-cell responses. In murine models, these multicomponent vaccine platforms induced stronger T-cell activation compared with peptide-only vaccines [340, 341].

4.1.2 Cell Surface–Anchored CEA-Related Cell Adhesion Molecule 6

Cell surface–anchored CEA-related cell adhesion molecule 6 (CEACAM6) is a glycosylphosphatidylinositol (GPI)-anchored surface glycoprotein that is markedly overexpressed in CRC and functions as both a tumor marker and an independent prognostic factor [342–345]. Its elevated expression in hyperplastic polyps and adenomas suggests a role in early tumorigenesis. CEACAM6 contributes to CRC initiation, progression, and metastasis, positioning it as a biologically relevant therapeutic target [343–348].

4.1.3 Mucin-1

Mucin-1 (MUC-1) is a transmembrane protein with a molecular weight of 120–225 kDa, which can increase to approximately 250–500 kDa upon glycosylation [349]. MUC-1 is normally expressed on epithelial cells, where it is polarized to the apical surface and characterized by extensive glycosylation [350]. In contrast, neoplasia-associated MUC1 is markedly overexpressed and exhibits pronounced hypoglycosylation relative to its normal counterpart. This aberrant expression is upregulated in CRC, as well as in precursor colorectal adenomas, and is associated with its immunogenic properties, involvement in TCR and BCR epitopes, mediation of metastasis and chemoresistance, and correlation with poor prognosis and advanced disease stage in CRC patients [349–353]. Notably, elevated MUC-1 expression has been shown to correlate with increasing degrees of dysplasia [352]. These effects are partly attributable to the pro-apoptotic activity of MUC-1 toward activated human T cells, as it enhances the efficient lysis of cytotoxic T cells, thereby contributing to immune evasion, and inhibits human T-cell proliferation as well as interactions between cytotoxic T cells and their target cells [354].

A study by Zhang et al. further demonstrated that MUC-1 can indirectly upregulate PD-L1 expression on tumor cells through the recruitment of pro-inflammatory cytokines, such as IL-17A and IFN-γ, which drive increased PD-L1 expression in tumor cells and tumor-infiltrating myeloid cells. This process subsequently suppresses antitumor immune responses via the PD-L1/PD-1 signaling pathway, enabling tumor cells to evade immune surveillance [355]. MUC-1 is also expressed in both major colorectal tumorigenesis pathways, namely the adenomatous polyp and the serrated polyp pathway [356].

A preclinical MUC1 vaccine in a colon cancer model overcame tolerance and induced IFN-γ–producing HTLs and CTLs, resulting in tumor rejection or reduced burden [357]. Another, preclinical studies show that CRC stem cell (CCSC) vaccine with MUC1 antigen significantly activated NK, T, and B cells, inhibited tumor growth, and required MUC1 expression for full antitumor efficacy in murine CRC models [358, 359]. In advanced CRC patients, MUC-1 vaccination induced robust anti–MUC-1 IgG responses and durable immunological memory, indicating a potential role in CRC prevention [360]. In a multicenter, randomized, double-blind, placebo-controlled trial of a MUC1 peptide vaccine for the prevention of recurrent colorectal adenoma, vaccination elicited measurable immune responses but did not significantly reduce overall adenoma recurrence compared to placebo. Among participants who developed a ≥2-fold increase in anti-MUC1 IgG levels,

there was a trend toward lower adenoma recurrence and a 38 % absolute reduction in recurrence rates versus placebo, suggesting that immunogenic responders may derive clinical benefit. Importantly, the vaccine was immunogenic in a subset of recipients and was well tolerated without major safety concerns. These results indicate that while MUC1-targeted vaccination can induce antigen-specific immunity in high-risk individuals, boosting immunogenicity or combining with other immunomodulatory agents may be necessary to achieve meaningful preventive efficacy [361].

4.1.4 Epidermal Growth Factor Receptor

Epidermal growth factor receptor (EGFR) plays a central role in human tumorigenesis, and its dysregulation predominantly arises from receptor overexpression and mutations affecting distinct functional domains [362, 363]. Aberrant EGFR expression has been documented in a wide range of epithelial malignancies, including breast and triple-negative breast cancer, non–small cell lung cancer, head and neck cancers, CRC, and several other tumor types. Elevated EGFR expression in tumors is closely associated with increased tumor aggressiveness, unfavorable prognosis, reduced overall survival, diminished therapeutic responsiveness, and the development of treatment resistance [364]. Moreover, EGFR can form heterodimers with other members of the human epidermal growth factor receptor (HER) family, such as HER2, HER3, and HER4, leading to enhanced oncogenic signaling and the emergence of more aggressive cancer phenotypes with poorer clinical outcomes [365].

Foy et al. evaluated peptide vaccines targeting the EGFR as a tumor antigen platform. They developed immunogens corresponding to specific EGFR B-cell epitopes designed to elicit humoral and cellular anti-tumor immune responses. In preclinical models, vaccination with these EGFR peptide constructs significantly suppressed tumor growth and improved antitumor efficacy, demonstrating that antigen-specific immunization against EGFR can modulate tumor progression through enhanced immune recognition and effector activity. This work supports the feasibility of targeting EGFR not only with monoclonal antibodies but also through active immunization strategies that recruit both antibody-mediated and T-cell–dependent mechanisms of tumor control [364].

4.1.5 Survivin

Survivin, one of the most extensively studied members of the inhibitor of apoptosis protein (IAP) family, is overexpressed in malignant tumors and is strongly associated with a poor prognosis [366]. Beyond its canonical anti-apoptotic function, survivin also promotes tumor cell invasion and migration through activation of NF-κB–dependent signaling pathways, thereby contributing to metastatic dissemination [367]. Under normal physiological conditions, survivin expression is largely confined to proliferating fetal tissues and is absent from most differentiated adult cells [368]. In line with these findings, analysis of human transcriptomic datasets has identified survivin among a limited group of approximately 40 genes that are highly expressed in malignant tissues but show minimal or no expression in normal cells [369].

Sarela et al. have demonstrated marked upregulation of survivin across a wide range of human cancers, including CRC, where its expression is consistently associated with unfavorable clinical outcomes and poor prognosis [370]. Results from a meta-analysis encompassing 11 studies that conducted survival analyses demonstrated a significant association between survivin expression and poor prognosis in CRC patients. Furthermore, pooled analyses revealed that survivin expression was significantly associated with adverse pathological features, including lymph node metastasis and vascular invasion. Collectively, these findings indicate that survivin expression is linked to unfavorable clinical outcomes and a pro-metastatic phenotype in CRC, underscoring its potential utility as a candidate target for survivin-directed therapeutic strategies [371].

In the context of CRC immunotherapy, survivin has shown promise as a vaccine antigen. Clinical studies have demonstrated that CRC patients receiving DCs pulsed with survivin-derived peptides exhibited elevated frequencies of survivin-specific CTLs [372, 373]. In a subset of these patients, this immune activation was accompanied by reductions in tumor marker levels and, in some cases, measurable decreases in overall tumor burden [373].

4.1.6 Wilms' Tumor 1

Wild-type Wilms' Tumor 1 (WT1) protein is a TAA with broad expression across various malignancies, including colorectal adenocarcinomas, and play an important role in tumorigenesis of CRC, making it an attractive target for immunotherapy [374]. WT1 ranks at the top of a National Cancer Institute (NCI) prioritization list of cancer antigens based on criteria such as therapeutic relevance, immunogenicity, and potential for clinical benefit. WT1-targeted vaccines have been shown to induce specific immune responses in patients with both hematological and solid tumors, including measurable clinical activity [375]. In CRC tissues, WT1 expression is confirmed by immunohistochemistry alongside HLA class I and II

molecules, suggesting that WT1-derived peptides can be presented effectively to both CTLs and HTLs. A by Shimodaira et al. demonstrated that DC-based vaccine pulsed with WT1 is both safe, with only mild local and systemic reactions, and immunogenic in CRC patients and may contribute to durable antitumor immunity when integrated into multimodal treatment regimens [376].

4.1.7 Transmembrane 4 L Six Family Member 5

Transmembrane 4 L six family member 5 (TM4SF5), a tetraspanin-like cell surface glycoprotein with four transmembrane domains, is also overexpressed in CRC [377]. TM4SF5 promotes invasive behavior, enhances tumor cell motility, disrupts contact inhibition, and facilitates EMT [378–380]. Elevated TM4SF5 levels correlate with advanced disease and poor prognosis, highlighting its potential as a therapeutic target in CRC [3, 336, 337]. In a preclinical investigation of a peptide vaccine targeting TM4SF5 in a colon cancer model, Kwon et al. evaluated the therapeutic efficacy of a TM4SF5-specific peptide vaccine formulated with CpG-DNA and a liposome complex (Lipoplex) in tumor-bearing mice. Immunization with this vaccine elicited a robust production of TM4SF5-specific antibodies and was associated with significant suppression of tumor growth compared with control animals. The vaccinated mice also exhibited markedly reduced serum levels of VEGF, suggesting an anti-angiogenic effect contributing to tumor inhibition. These results demonstrate that a TM4SF5-targeting peptide vaccine can induce effective humoral immune responses and therapeutic antitumor activity in a murine model of colon cancer, supporting the feasibility of antigen-specific peptide vaccines in CRC immunotherapy research [381].

4.1.8 Protein Tyrosine Kinase 7

Protein tyrosine kinase 7 (PTK7) is a catalytically inactive transmembrane receptor that modulates key oncogenic pathways, including Wnt and VEGF signaling [382–393]. PTK7 is highly expressed in several malignancies, including CRC, and its expression correlates with poor differentiation, lymph node involvement, distant metastasis, and higher TNM stage [394–399]. PTK7 regulates tumor growth and dissemination and contributes to immune evasion through interactions with macrophage galactose-type lectin (MGL) [398, 400].

4.1.9 B7-H3

B7 Homolog 3 (B7-H3) is a transmembrane immune checkpoint molecule belonging to the B7 family of immunomodulatory proteins [401]. While minimally expressed in normal tissues, B7-H3 is markedly upregulated across multiple tumors. In CRC, elevated B7-H3 levels are associated with tumor progression, metastatic spread, resistance to apoptosis, and unfavorable clinical outcomes [402–416]. B7-H3 functions as a T-cell co-inhibitory molecule, enhances tumor aggressiveness via JAK2–STAT3 activation, and reduces T-cell infiltration within the TME, collectively contributing to immune evasion [417–423].

4.1.10 Kirsten Rat Sarcoma Virus

Kirsten rat sarcoma virus (KRAS) is a key regulator of intracellular signaling pathways and is mutated in over 40% of CRC cases. During tumorigenesis, somatic mutations can arise throughout coding regions of the genome, giving rise to neoantigens. In CRC, mutations in KRAS represent a prototypical source of such neoantigens [424, 425]. Early studies demonstrated that peptides derived from mutant KRAS can effectively stimulate CTLs in vitro [426, 427], as well as in patients with CRC [428]. Subsequent investigations further confirmed the cytolytic capacity of CTLs induced by mutant KRAS peptides through the lysis of HLA-A2–positive target cells pulsed with mutant KRAS peptides [429].

Clinical vaccination trials using mutant KRAS–derived peptides have yielded evidence of potential therapeutic benefit. While objective immune responses were observed in a limited proportion of vaccinated CRC patients, a subset remained disease-free following vaccination [430]. In a separate case report, adoptive transfer of activated T cells targeting the KRAS G12D mutation induced regression of multiple lung metastases in a CRC patient, with durable tumor control lasting several months before progression of a single lesion [431].

To further enhance immunogenicity, mutant KRAS peptide vaccines have been combined with cytokine-based adjuvants such as IL-2 or GM-CSF. The most robust immune responses were observed in patients receiving GM-CSF, characterized by increased frequencies of interferon-producing, antigen-specific T cells. However, despite strong immunological activation, no objective clinical responses were achieved, and disease progression occurred in all patients. This lack of efficacy was accompanied by a concurrent expansion of Tregs, suggesting immune suppression as a likely mechanism underlying the discordance between immune activation and clinical outcome [432].

4.1.11 p53

Mutations affecting the tumor suppressor gene TP53, or genes regulating p53 activity, are frequently observed across multiple malignancies, including CRC, re

sulting in abnormal p53 expression [433]. These somatic mutations can generate neoantigenic peptides that are presented by MHC molecules and recognized by the immune system, providing a rationale for targeting mutant p53 in cancer immunotherapy. Early clinical evidence supporting this concept was provided by a phase I/II trial employing a recombinant canarypoxvirus (ALVAC) vaccine encoding wild-type human p53 in patients with advanced CRC, which demonstrated that p53-directed vaccination was feasible, safe, and capable of inducing p53-specific cellular immune responses. In this study, IFN-γ–secreting p53-specific T cells were detected, particularly at higher vaccine doses, indicating preferential induction of Th1-polarized immunity despite robust anti-vector responses [434].

In a phase I/II clinical trial, a synthetic long peptide (SLP) vaccine targeting the tumor-associated self-antigen p53 in patients with mCRC demonstrated that the was well tolerated, with toxicity limited to mild (grade 1–2) local reactions. Importantly, vaccine-induced p53-specific T-cell responses were detected in the majority patients, thereby supporting the feasibility, safety, and immunogenic potential of p53-directed vaccination in CRC patients [435]. In a phase I/II clinical trial evaluating a p53-targeted SLP vaccine in combination with IFN-α in metastatic colorectal cancer patients, the addition of IFN-α significantly enhanced p53-specific cellular immunity compared with historical results from p53-SLP vaccination alone. Marked increases in IFN-γ–producing, p53-specific T cells, indicate a stronger type-1 immune response than that achieved with p53 peptide vaccination alone. The combination regimen was also well tolerated and safe [436].

4.1.12 Melanoma-Associated Antigen

Melanoma-associated antigen (MAGE), originally identified in melanoma, belongs to the cancer–testis antigen family, a subgroup of TAAs characterized by restricted expression in immune-privileged testicular tissue and malignant cells. Subsequent studies have demonstrated MAGE expression across a broad range of adenocarcinomas, including CRC. However, reported frequencies of MAGE expression in CRC vary substantially depending on the study and specific MAGE isoform, with positivity rates of approximately 14% for MAGE-A, 51% for MAGE-A1–6, and 28% for MAGE-A3 [437–439].

Clinical evidence supporting the therapeutic potential of MAGE-targeted vaccination remains limited. Takahashi et al. reported that vaccination with a synthetic long peptide containing a helper/killer hybrid epitope derived from MAGE-A4 elicited coordinated HTL and CTL responses, resulting in modest tumor growth suppression and disease stabilization in a single patient [440]. In contrast, a broader vaccination study evaluating multiple TAAs demonstrated enhanced antigen-specific CTL responses without translating into measurable clinical benefit [441].

Despite extensive efforts, the identification of optimal tumor antigens remains challenging, primarily due to the inherently low immunogenicity of many human cancers [442]. Consequently, additional immune stimulation is often required in designing vaccines based on unmodified tumor cells [443, 444]. Although single-peptide vaccines can induce robust immune activation, their impact on tumor regression is generally modest [178]. Current vaccine research therefore favors multi-epitope formulations composed of several multiple tumor-related peptides to achieve more durable and clinically meaningful outcomes.

5. Types of Vaccines and Advances in Clinical Trials and Therapeutic Outcomes

Figure 6 depicts various types of CRC vaccines, including peptide-based vaccine, DNA-based vaccine, RNA-/mRNA-based vaccine, cancer-cell based vaccine, dendritic cell vaccine, and virus- or vector-based vaccine. Each of which is discussed in detail in this section.

5.1 Molecular-Based Vaccines

Molecular-based CRC vaccines include peptide or full-length protein formulations as well as nucleic acid platforms such as RNA and DNA vaccines [286]. Peptide-based vaccines are engineered from immunogenic epitopes derived from tumor antigens, which are subsequently processed and presented through MHC class I and II pathways to activate T lymphocytes and establish durable antigen-specific immune memory against malignant cells [445]. These vaccines offer notable advantages, including high target specificity and relatively low manufacturing and storage costs [446, 447]. Despite their simplicity, affordability, and ease of production, no peptide-based cancer vaccine has yet achieved regulatory approval or commercialization. This is likely due to their inherently low immunogenicity of short peptide vaccine, HLA restriction that severely limits population coverage, limited capacity to induce sustained immune memory, and the requirement for booster dosing [28, 273]. Moreover, their clinical efficacy may be compromised by tumor-driven immune escape and antigen loss, both of which contribute to disease recurrence [448].

The use of minimal antigenic peptides necessitates further refinement, including precise epitope prioritization to ensure optimal targeting of CRC pathogenic pathways. Peptide vaccines also typically

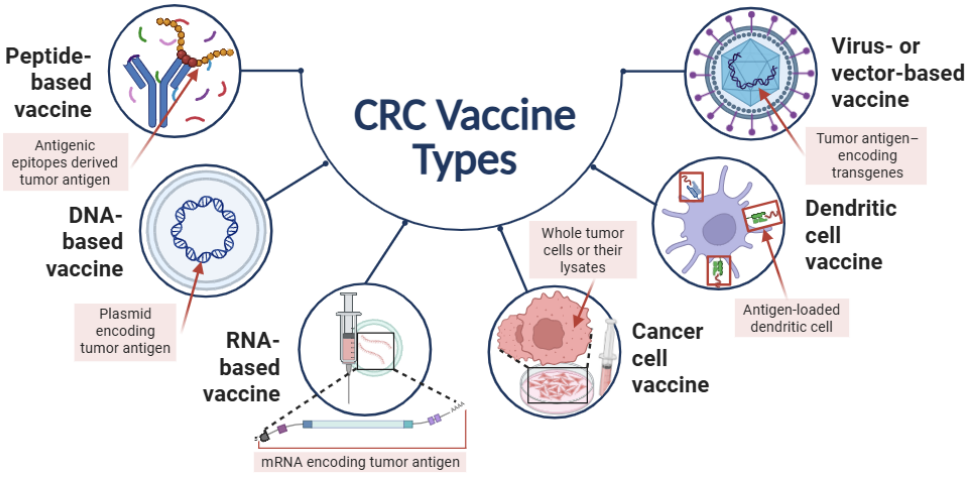

Figure 6. CRC vaccine types.

require potent adjuvants to augment immunogenicity [448]. Consequently, current clinical development efforts are increasingly focused on multi-antigen peptide vaccines formulated with strong adjuvants such as GM-CSF or Montanide, which enhance antigen presentation and T-cell priming [445, 446, 449–451]. Given the potential for antigen downregulation in CRC, combination immunotherapy, particularly with ICIs, has become an attractive strategy to counteract resistance mechanisms and amplify clinical benefit [27, 452, 453].

Although no peptide vaccines have reached global approval, multiple candidates have progressed into clinical development [273, 454]. A phase Ib trial evaluating PolyPEPI1018, a multi-epitope vaccine incorporating 12 peptides from seven mCRC-associated tumor antigens, demonstrated safety, favorable tolerability, and robust HTL and CTL activation [455]. Another candidate, ATP128, currently being evaluated in MSS/pMMR stage IV CRC, has been shown to activate dendritic cells via TLR2 and TLR4 signaling, stimulate cytokine release, and upregulate costimulatory molecules essential for effective T-cell priming [456].

DNA vaccines are plasmid-based platforms that encode one or more tumor antigens. Following delivery into host cells, these plasmids undergo transcription and translation to generate antigenic proteins, which are subsequently processed and presented via MHC class I and II pathways while concurrently activating innate immune sensors through cytosolic receptors [457, 458]. This mechanism distinguishes DNA vaccines from peptide- or protein-based formulations, which rely on antigen presentation through phagocytosis, and from cellular vaccines, in which APCs directly display antigens upon administration [459].

One of the aberrantly expressed oncoproteins in CRC, MYB, has been investigated as a DNA vaccine target [460, 461]. A MYB-encoded DNA vaccine demonstrated robust prophylactic and therapeutic activity in a transgenic mouse model, reflecting the biological relevance of MYB, which is implicated in promoting cell proliferation, suppressing differentiation, and enhancing resistance to apoptosis [460–462]. Despite these supportive preclinical findings and the theoretical advantages of DNA vaccine platforms, several limitations hinder clinical translation [463]. The major challenges include the lack of optimized delivery systems, suboptimal immunogenicity, intratumoral heterogeneity, and restricted nuclear entry of plasmid DNA, coupled with potential risks of plasmid integration into the host genome. Current strategies to improve efficacy focus on rational antigen selection, plasmid optimization, and combinatorial regimens aimed at reducing TME-mediated immunosuppression and enhancing immune effector function [286, 463, 464].

mRNA-based vaccines have similarly gained prominence as preventive and therapeutic modalities. These vaccines are synthesized in vitro and encode tumor antigens in the form of mRNA. Unlike DNA vaccines, mRNA requires no nuclear entry and undergoes immediate cytoplasmic translation, conferring advantages in efficiency, modifiability, and production speed [465, 466]. Once internalized, the mRNA is translated into antigenic proteins that are presented by APCs through MHC class I, inducing strong CTL responses [467].

Moderna Therapeutics has advanced several mRNA vaccine candidates for oncology, including CRC. In phase I/II trials, mRNA-4650 delivered intramuscularly elicited both CTL and HTL responses against neoantigens without significant adverse effects or tumor recurrence [468]. Another candidate, mRNA-4157, demonstrated safety, tolerability, and preliminary clinical activity in MSI-H CRC

when combined with ICIs in a phase I study, leading to its progression into phase II trials [469]. Additionally, mRNA-5671, designed to target KRAS mutations, is currently being evaluated in phase I trials in combination with ICIs among non–MSI-H patients (NCT03948763). Preclinical evidence indicates that mRNA-5671 can substantially enhance CTL responses, both as monotherapy and in combination strategies [470].

Despite the substantial promise of nucleic acid–based cancer vaccines, DNA and mRNA modalities are constrained by several key challenges. These include: (1) the high cost and extended timelines required for good manufacturing practice (GMP)-compliant production, and (2) stability issues, particularly with mRNA, which necessitates ultra-low–temperature storage and complicates broad clinical distribution [471]. Addressing these limitations is essential for enabling large-scale implementation and optimizing their therapeutic potential.

5.2 Cancer Cell Vaccines

Cancer cell vaccines employ whole tumor cells or their lysates as antigen sources and are designed to prime broad immune responses [472]. These vaccines may be autologous, offering individualized specificity by using patient-derived tumor cells, or allogeneic, derived from standardized cell lines that facilitate scalable production [442, 473]. Although whole-cell antigen presentation can reduce the likelihood of tumor immune escape, this approach carries an inherent risk of autoimmunity due to shared antigen expression in healthy tissues [286].

Among recent developments, OncoVax, an autologous tumor cell vaccine combined with bacille Calmette–Guérin (BCG), has demonstrated acceptable safety when administered alongside 5-fluorouracil (5-FU) and leucovorin. However, early trials integrating OncoVax with surgical resection failed to yield significant improvements in clinical outcomes [474]. Conversely, the allogeneic vaccine GVAX, engineered to secrete GM-CSF, has shown evidence of modulating anti-tumor immune responses in a phase II study involving patients with advanced pMMR CRC [475].

5.3 Dendritic Cell Vaccines

DC vaccines leverage the exceptional antigen-presenting capacity of DCs isolated via leukapheresis. Following ex vivo maturation with cytokines and pulsing with tumor peptides or lysates, these cells are reinfused to stimulate potent anti-tumor immunity. DC vaccines, which have shown notable clinical efficacy in melanoma and prostate cancer, have also been explored extensively in CRC [476].

A randomized controlled trial by Rodriguez et al. demonstrated that CRC patients receiving postoperative DC vaccination following chemotherapy exhibited a markedly prolonged mean disease-free survival (25.26 months) relative to controls (9.53 months) [477]. MelCancerVac, a DC vaccine pulsed with allogeneic melanoma lysate (DDM-1.13), showed modest median overall survival (7.4 months) in a phase II study of stage IV CRC patients; however, a subset of patients exhibited exceptional outcomes, including progression-free intervals exceeding 6 months, with two individuals achieving disease control for more than 27 and 37 months, respectively [478].

5.4 Vector-Based Vaccines

Vector-based vaccines utilize viruses, attenuated bacteria, or yeast as delivery vehicles to introduce tumor antigen–encoding transgenes and elicit vigorous immune responses. These vectors intrinsically contain PAMPs recognized by host PRRs, thereby activating innate immunity and facilitating robust adaptive responses. Recombinant viral vectors are particularly attractive due to their ability to co-deliver immunogenic TAAs and innate immune activators, enhancing the magnitude and specificity of anti-tumor immunity [479, 480].

Despite decades of research, no viral vector–based CRC vaccine has advanced to phase III trials or received regulatory approval. This translational failure reflects a convergence of biological, immunological, and clinical trial–related limitations rather than a lack of intrinsic vaccine potency. Key contributing factors include suboptimal trial design, inadequate patient stratification, and enrollment of heavily pretreated patients with advanced disease, in whom profound tumor-induced immunosuppression limits vaccine efficacy. Moreover, conventional response criteria and short-term tumor shrinkage endpoints are poorly suited to capture the delayed and cumulative benefits of immunotherapy, leading to premature classification of clinical failure despite evidence of vaccine-induced immune activation [481].

Dosing strategies and repeated vector administration have further constrained efficacy by inducing anti-vector immunity, thereby reducing antigen expression and limiting effective boosting. Importantly, immunological analyses have revealed clear differences between responders and non-responders, with clinical benefit associated with sustained, polyfunctional antigen-specific T-cell responses and memory formation, whereas non-responders exhibit transient activation, T-cell exhaustion, or dominance of immunosuppressive cell populations. Collectively, these lessons explain why

robust preclinical immunogenicity has not consistently translated into meaningful clinical benefit and underscore the need for optimized trial design, refined immune monitoring, rational patient selection, and combination strategies to overcome tumor-mediated immune resistance [481].

In a phase II trial, Redman et al. evaluated a modified adenovirus subtype 5 vector, Ad5 (E1–, E2b–)-CEA (AdCEA), in patients with MSS mCRC. When combined with standard-of-care therapy, AdCEA produced a modest improvement in progression-free survival (10.1 vs. 8.8 months) and elicited significantly stronger HTL and CTL responses compared with standard therapy alone (8/11 patients vs. 1/8 patients) [482]. The overall limited clinical efficacy likely reflects suboptimal induction of durable anti-tumor immunity when used as monotherapy, underscoring the need for rational combinatorial strategies to unlock the full therapeutic potential of vector-based vaccination in CRC [479].

6. Potentials for the Therapeutic Development of Cancer Vaccines

Vaccines have demonstrated substantial success in preventing virus-associated malignancies, such as hepatitis B virus–related hepatocellular carcinoma and human papillomavirus (HPV)–driven cervical cancer, by targeting viral proteins that directly contribute to oncogenesis. This success has extended into therapeutic settings, where HPV vaccines administered in combination with ICIs have shown superior clinical responses compared with ICI monotherapy. These findings strengthen the rationale for applying similar antigen-targeted vaccination strategies to CRC, particularly through the development of multi-antigen vaccines incorporating well-defined, biologically relevant, and immunogenic tumor proteins [483].

Therapeutic cancer vaccines are increasingly recognized as a valuable component of cancer management, as they include durable, antigen-specific immune responses capable of controlling minimal residual disease and preventing tumor recurrence. Although immunogenicity may be attenuated in advanced disease, evidence from phase III clinical trials demonstrates that therapeutic vaccines can improve disease-free survival and overall survival in malignancies such as prostate cancer, melanoma, and follicular lymphoma [484–486]. As a modality designed to augment antitumor immunity, therapeutic vaccines promote tumor regression and elimination while maintaining a highly favorable safety profile. Their antigen-specific activity also reduces the likelihood of off-target immune activation. By selectively delivering TAAs or TSAs, vaccines stimulate robust immune responses, enhance TIL accumulation, and activate cytotoxic mechanisms targeting antigen-expressing malignant cells [487]. When paired with immunostimulatory adjuvants, cancer vaccines can effectively potentiate T-cell-mediated immunity, a central determinant of tumor recognition and destruction [488–490].

In CRC, therapeutic cancer vaccines hold emerging promise, particularly as immunomodulatory agents capable of converting immunologically “cold” tumors into more inflamed and treatment-responsive lesions. Although CRC has historically shown limited responsiveness to vaccine monotherapy, multiple CRC-specific clinical and preclinical studies provide proof-of-concept that antigen-targeted vaccination can induce measurable antitumor immunity. Early-phase clinical trials using DC–based vaccines loaded with CRC-associated antigens such as survivin, WT1, CEA, and MUC-1 have consistently demonstrated safety and the induction of antigen-specific CTLs and HTLs responses in patients with advanced CRC or mCRC [373, 376, 491]. In some patients, vaccine-induced immune activation was accompanied by reductions in tumor markers or stabilization of disease [491].

Importantly, CRC-specific neoantigen vaccines targeting recurrent driver mutations such as KRAS and TP53 have provided further evidence of therapeutic potential. Peptide vaccines and adoptive transfer strategies targeting mutant KRAS have induced functional, mutation-specific T-cell responses in CRC patients, with isolated but striking clinical responses reported in metastatic disease [265, 431]. Similarly, p53-derived SLP vaccines have demonstrated favorable safety profiles and robust cellular immunogenicity in metastatic CRC patients, particularly when combined with immune adjuvants such as IFN-α, highlighting the feasibility of targeting shared tumor-associated antigens in CRC [434, 435].

Also, as explained in previous subsections, therapeutic vaccines alone may be insufficient in unselected CRC populations. They possess clear therapeutic potential when deployed in rational combination strategies, particularly alongside ICIs, chemotherapy, induced immunogenic cell death, or TME-modulating agents. Thus, CRC-directed cancer vaccines should be viewed not as standalone curative interventions, but as integral components of multimodal immunotherapy strategies tailored to tumor immunogenicity, molecular subtype, and host immune context.

Compared with classical drug-based therapies, therapeutic vaccines offer several key advantages:

(1) short treatment duration without daily dosing, (2) compatibility with ICIs to potentiate clinical outcomes, (3) the ability to induce durable immunological memory that prevents recurrence and can be boosted periodically, and (4) a highly favorable safety profile [265, 326]. Supporting this favorable safety profile, an immunoprevention study by Kimura et al. demonstrated that a single-antigen vaccine targeting the TAA MUC-1 elicited strong immunogenicity, inducing high titers of anti-MUC1 IgG and generating long-lasting immune memory, while remaining well tolerated with no evidence of autoimmunity [360]. Collectively, these findings indicate that while therapeutic cancer vaccines alone may be insufficient in unselected CRC populations, they represent a safe and biologically active platform that can be leveraged within multimodal treatment strategies to enhance antitumor immunity and improve long-term disease control.

7. Challenges in the Development of CRC Vaccines

As previously discussed, the development of therapeutic vaccines for CRC is hindered by the profoundly immunosuppressive TME and the sophisticated immune evasion strategies that characterize CRC immunopathology. Immunosuppression within the TME remains a major limiting factor affecting the clinical efficacy of vaccines in improving patient survival [492–495]. According to the cancer immunoediting paradigm, interactions between tumor cells and the immune system evolve through three stages: elimination, equilibrium, and escape. During the escape phase, tumors establish a highly suppressive microenvironment that is difficult to revert, thereby severely impairing vaccine-induced antitumor immunity [496]. Upregulation of immune checkpoint proteins such as PD-1 and PD-L1 further exacerbates immune escape, directly restraining the cytotoxic activity of vaccine-primed effector T cells [492–495]. Beyond these broadly recognized immunological barriers, CRC exhibits several unique biological, anatomical, and clinical features that impose additional and distinct challenges for the development of effective therapeutic vaccines.

As explained previously, MSS and pMMR tumors account for approximately 85% of CRC cases. These tumors are characterized by low TMB, limited neoantigen diversity, poor T-cell infiltration, and weak baseline interferon signaling, collectively resulting in an immunologically “cold” TME that is intrinsically resistant to both immune checkpoint inhibition and vaccine-induced immune priming [61, 497–499]. Consequently, vaccine strategies that rely on pre-existing antitumor immunity or robust neoantigen presentation face fundamental limitations in the majority of CRC patients. Metastatic patterns further compound these challenges. The liver, the most common site of CRC metastasis, represents a uniquely tolerogenic immune organ enriched with immunosuppressive Kupffer cells, liver sinusoidal endothelial cells, Tregs, and myeloid-derived suppressor cells. This hepatic immune milieu actively promotes T-cell dysfunction, deletion, and exhaustion, thereby attenuating systemic and immunotherapy-induced antitumor responses [500–502]. As a result, immunotherapy that demonstrate immunogenicity in primary cancer lesions may fail to generate meaningful clinical benefit in patients with liver-dominant metastatic disease [503].

In addition, CRC is uniquely influenced by the gut microbiome, which plays a critical role in shaping mucosal immunity, antigen presentation, and systemic immune responsiveness. Dysbiosis, microbial-derived metabolites, and bacterial translocation can modulate DC function, T-cell polarization, and vaccine adjuvant efficacy. Emerging evidence indicates that specific microbial taxa can either enhance or suppress responses to cancer immunotherapies, including vaccines, highlighting the microbiome as both a confounding variable and a potential therapeutic target in CRC vaccine development [504–507]. This layer of complexity is particularly relevant to CRC and less pronounced in non-gastrointestinal malignancies. Beyond microbiome-mediated immunomodulation, anatomical and physiological features of the colorectum also impose constraints on immune surveillance and vaccine delivery. The intestinal immune system is inherently biased toward tolerance due to constant exposure to dietary antigens and commensal microbes. This tolerogenic baseline can dampen vaccine-induced immune activation and necessitates strategies capable of overcoming mucosal immune suppression without triggering excessive inflammation or autoimmunity [508, 509]. Furthermore, regional lymphatic drainage and local immune compartmentalization may limit effective trafficking of vaccine-primed effector cells to tumor sites.

Finally, CRC vaccine development must contend with intense competition from well-established standard-of-care therapies, including cytotoxic regimens such as FOLFOX and FOLFIRI, molecularly targeted agents (anti-EGFR, anti-VEGF), and selected immunotherapies for MSI-H/dMMR tumors. These treatments set a high benchmark for clinical efficacy, particularly in terms of progression-free and overall survival, thereby raising the evidentiary threshold for vaccine-based interventions to demonstrate additive or synergistic benefit [510, 511]. In this therapeutic landscape, cancer vaccines must not only be safe and immunogenic but also provide clear clinical

advantages over, or in rational combination with, existing modalities.

In addition to these CRC-specific biological and anatomical constraints, therapeutic vaccine development also faces challenges involving the balance between personalization and scalability. Neoantigen-based vaccines are inherently patient-specific, yet their production requires extensive processing from tumor tissue acquisition and genomic characterization to individualized vaccine formulation, resulting in substantial cost and time burdens. These limitations are particularly consequential in mCRC, where rapid disease progression demands timely therapeutic intervention [512]. Tumor heterogeneity presents an additional barrier, enabling subclones to evade immune detection and limiting the durability and consistency of vaccine responses across patients [178].

Moreover, determining the cause of suboptimal vaccine immunogenicity in cancer patients can be challenging. Reduced immune responses may result from inappropriate antigen selection (e.g., TAAs that largely resemble host molecules and are therefore subject to self-tolerance), suboptimal vaccine design (such as weak adjuvants), profound immunosuppression within the TME, the deleterious effects of prior therapy, advanced patient age, or a combination of these factors [360]. Despite considerable advances, identifying optimal tumor antigens for vaccine development remains difficult in most malignancies, including CRC, underscoring the need for refined antigen discovery approaches and rigorous antigen selection criteria [442].

Beyond biological barriers, financial constraints also significantly influence the development, accessibility, and commercial viability of cancer vaccines. The economic sustainability of these products depends on an appropriate balance between manufacturing costs, pricing strategies, and demonstrable clinical benefit. High-cost vaccines must provide substantial improvements in survival outcomes to justify premium pricing. For example, Sipuleucel-T, the first FDA-approved therapeutic cancer vaccine, continues to face adoption challenges due to its high-cost relative to its modest clinical benefit [513]. Its complex manufacturing workflow, involving leukapheresis, ex vivo antigen loading of autologous dendritic cells, and reinfusion, contributes to treatment costs exceeding $90,000 per patient [514, 515]. By contrast, ICIs such as nivolumab and pembrolizumab remain widely used despite their high prices, largely due to their proven durable clinical responses and compatibility with combination therapy regimens [516, 517]. These market dynamics demonstrate that pricing strategies in oncology are strongly shaped by perceived therapeutic benefit and competitive alternatives within the treatment landscape [518].

8. Utilization of Bioinformatics in Cancer Vaccine Development

Bioinformatics is an interdisciplinary field that integrates computer science, information technology, mathematics, and statistics to analyze, interpret, and model biological data, including predictions of gene regulatory networks [519, 520]. By enabling high-resolution interpretation of complex biological information, bioinformatics provides powerful computational frameworks for solving scientific challenges across diverse biomedical domains [521, 522]. In the healthcare sector, bioinformatics has become indispensable for overcoming the limitations of traditional drug discovery pipelines, which are often hindered by high development costs, prolonged timelines, and discrepancies between preclinical predictions and clinical outcomes. These capabilities are further strengthened by the rapid expansion of biological databases and the development of increasingly sophisticated, internationally validated computational platforms that support accurate prediction of pharmacological, toxicological, and molecular characteristics of therapeutic candidates [523].

A notable example of bioinformatics-driven therapeutic innovation is the development of imatinib mesylate (Gleevec), a targeted therapy for chronic myeloid leukemia designed to inhibit abnormal protein production resulting from the BCR-ABL fusion gene. Bioinformatics tools were instrumental in identifying and validating the relevant genetic drivers, enabling the rational design of this highly effective drug. Additionally, in time-sensitive global health emergencies, such as the COVID-19 pandemic, bioinformatics played a central role in rapidly characterizing the SARS-CoV-2 genome, predicting viral protein structures, identifying therapeutic targets, and accelerating vaccine development, thereby establishing foundational biological insights and underscoring its critical contribution to modern biomedical research [523].

Immunoinformatics, a specialized subfield of bioinformatics, integrates computational tools with immunological principles to analyze and model immune system behavior, predict disease mechanisms, and identify potential antigenic determinants [520, 524–526]. This approach supports the prediction of immunogenicity, allergenicity, and antigenicity of candidate antigens to ensure the generation of effective humoral and cellular immune responses [527–529]. Immunoinformatics significantly reduces the time and

resources required for vaccine target identification, often shortening the process to approximately 1–2 years compared with conventional vaccine development strategies [524, 530–532]. Immunoinformatics-guided vaccine research has demonstrated success across preclinical, clinical, and post-vaccination stages, highlighting its transformative impact on rational vaccine design and precision immunotherapy [519, 520, 526].

In the CRC context, these computational strategies directly address several key themes discussed in earlier sections of this review. For tumor antigens, bioinformatics enables systematic evaluation of gene and protein expression across CRC cohorts, in silico epitope mapping, and population-level HLA coverage analysis, thereby refining antigen selection and minimizing off-target toxicity [519, 533]. Moreover, bioinformatics provides the computational foundation for the neoantigen concept introduced throughout this manuscript, offering a rational solution to the antigen selection challenges highlighted in Section 7 [534–536]. Importantly, the personalized vaccine approaches discussed in Section 5, including mRNA-based platforms such as mRNA-4157 and mRNA-5671, are fundamentally dependent on bioinformatics pipelines for patient-specific mutation calling, epitope prioritization, and vaccine construct design, underscoring the integrative role of bioinformatics across CRC vaccine development [537, 538].

Neoantigen prediction pipelines have emerged as a critical application of bioinformatics in CRC vaccine development. Computational frameworks such as pVACtools, MuPeXI, and NeoPredPipe integrate whole-exome or whole-genome sequencing data with RNA sequencing to identify nonsynonymous somatic mutations, predict mutant peptide sequences, and rank candidate neoantigens based on their likelihood of being presented by patient-specific HLA molecules [534, 539–541]. These pipelines are particularly relevant for CRC, where MSI-H tumors exhibit elevated TMB and increased neoantigen load, making them more amenable to neoantigen-based vaccine strategies compared with MSS tumors [497, 499].

Accurate HLA typing and epitope–MHC binding prediction constitute another indispensable component of CRC vaccine bioinformatics. Tools such as NetMHCpan, MHCflurry, and MixMHCpred are widely used to predict peptide binding affinity and stability across diverse HLA class I and II alleles, enabling prioritization of epitopes capable of eliciting robust CTL and HTL responses [542–545]. These predictions are particularly important in CRC, given the extensive interpatient HLA diversity and the need to design vaccines with sufficient population coverage or individualized specificity.

Beyond in silico prediction, bioinformatics-driven immunopeptidomics approaches have gained increasing importance in validating CRC neoantigens. Mass spectrometry–based profiling of HLA-bound peptides directly isolated from CRC tissues enables empirical confirmation of naturally presented tumor antigens, thereby refining computational predictions and reducing false-positive epitope selection [546, 547]. Integration of immunopeptidomics data with sequencing-based pipelines has been shown to improve the translational relevance of candidate vaccine epitopes and enhance the likelihood of clinical efficacy.

Importantly, bioinformatics-guided CRC vaccine development has already progressed into early-phase clinical evaluation. Several personalized neoantigen vaccines for CRC, often developed using integrated pipelines combining mutation calling, HLA binding prediction, and epitope prioritization, have demonstrated feasibility, safety, and immunogenicity in phase I trials, particularly in MSI-H CRC and in combination with immune checkpoint inhibitors [537, 538, 548]. These studies highlight how bioinformatics not only accelerates antigen discovery but also enables precision stratification of CRC patients most likely to benefit from vaccine-based immunotherapy.

Collectively, these advances underscore the indispensable role of bioinformatics and immunoinformatics in overcoming CRC-specific barriers to vaccine development. By enabling systematic neoantigen discovery, epitope validation, and patient stratification, computational approaches provide the conceptual and technical framework required for the next generation of effective CRC vaccines.

9. Conclusions

CRC represents an immunologically complex malignancy in which tumor progression, metastatic dissemination, and therapeutic resistance are strongly shaped by the TME. Accumulating evidence indicates that immunosuppressive cellular and stromal networks, including TAMs, Tregs, impaired DCs, CAFs, TANs, endothelial abnormalities, and ECM remodeling, collectively attenuate anti-tumor immunity and restrict the efficacy of current immunotherapeutic approaches. These features provide an important biological context for understanding the limited clinical efficacy observed with current vaccine-based strategies in CRC.

Therapeutic cancer vaccines are supported by a strong biological rationale, particularly their capacity to induce

antigen-specific T-cell responses and modulate tumor immunogenicity. This review has summarized key classes of CRC-associated antigens, including selected TAAs and neoantigen-based approaches, as well as major vaccine platforms evaluated to date. However, available clinical evidence remains largely confined to early-phase studies, with immunogenicity often exceeding demonstrated clinical benefit, underscoring the persistent translational gap in CRC vaccine development.

Synthesis of the current literature suggests that this gap reflects CRC-specific immunopathological constraints rather than insufficient antigen immunogenicity alone. The predominance of immunologically cold MSS/pMMR tumors, early immune exclusion, and tolerogenic pressures within metastatic niches, particularly the liver, distinguish CRC from more immunotherapy-responsive malignancies and limit the impact of vaccine monotherapy. Within this framework, vaccines appear to function primarily as immune primers, necessitating additional interventions to enable effective T-cell infiltration, persistence, and effector function within the TME.

Consistent with this concept, emerging data indicate that rational combination strategies, especially those integrating cancer vaccines with immune checkpoint inhibition, may partially restore T-cell functionality, although robust and durable clinical benefit has yet to be conclusively demonstrated. In parallel, computational and bioinformatics-based approaches have increasingly been applied to support antigen prioritization, neoantigen identification, and patient stratification, offering conceptual solutions to several challenges discussed in this review, while their clinical utility in CRC requires further prospective validation.

In summary, while meaningful progress has been made in elucidating CRC immunobiology and exploring vaccine-based immunotherapy, substantial challenges remain. Future advances will likely depend on improved and CRC-tailored antigen selection strategies, deeper integration of immunopathological insights into vaccine design, rational combination regimens that directly address dominant immunosuppressive mechanisms, and rigorous clinical evaluation incorporating robust immune monitoring. A mechanistically informed and critically assessed translational framework will be essential to define the ultimate clinical role of therapeutic cancer vaccines in CRC.

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