Advanced Pharmaceutical Bulletin

Accepted Manuscript (unedited)

The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form.

Review Article

How to cite this article:
Abuhassan Q, Sayed U, N. Patel P, Jyothi S.R, Singh G, Nayak PP, Arora V, Shakhmurova G. Targeting the Immunosuppressive Microenvironment in Colorectal Cancer: Pharmacological and Nanomedicine Strategies. Advanced Pharmaceutical Bulletin, doi: 10.34172/apb.47456

Targeting the Immunosuppressive Microenvironment in Colorectal Cancer: Pharmacological and Nanomedicine Strategies

Qamar Abuhassan[1], Usamah Sayed[2], Pareshkumar N. Patel[3], S. Renuka Jyothi[4], Gunjan Singh[5], Priya Priyadarshini Nayak[6*], Vimal Arora[7], Gulnora Shakhmurova[8]

[1]Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, University of Jordan, Amman, 11942, Jordan
[2]Faculty of Allied Medical Sciences, Hourani Center for Applied Scientific Research, Al-Ahliyya Amman University, Amman, Jordan
[3]Department of Pharmacy, Faculty of Pharmacy, Gokul Global University, Sidhpur, Gujarat, India
[4]Department of Biotechnology and Genetics, School of Sciences, JAIN (Deemed to be University), Bangalore, Karnataka, India
[5]Department of Pharmacy, Sharda School of Pharmacy, Sharda University, Greater Noida, India
[6]Department of Medical Oncology, IMS and SUM Hospital, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha-751003, India
[7]University Institute of Pharma Sciences, Chandigarh University, Mohali, Punjab, India
[8]Department of Biological Sciences, National Pedagogical University of Uzbekistan named after Nizami, Tashkent, Uzbekistan

ARTICLE INFO

Keywords:
Colorectal cancer,
Tumor microenvironment,
Antitumor immunity,
Nanomedicine,
Immunomodulation

Article History:
Submitted: May 27, 2026
Revised: August 05, 2026
Accepted: August 14, 2026
ePublished: September 05, 2026

ABSTRACT

The immunosuppressive stroma in colorectal cancer (CRC) plays a central role in therapeutic resistance. Modulating key targets within this stromal compartment has been proposed as a promising strategy for restoring antitumor immunity and overcoming resistance mechanisms. Several immunomodulatory agents, including next-generation immunotherapies, small-molecule inhibitors targeting specific receptors or signaling pathways, and natural compounds, have shown potential for remodeling the tumor immune microenvironment (TIME). In addition, nanoparticle-based delivery systems may further enhance the therapeutic efficacy of these agents. Evidence from both preclinical and clinical studies suggests that targeting stromal and immunosuppressive cells can effectively remodel the antitumor immune response in CRC. Furthermore, specific inhibitors, natural products, and their nanoformulations may enhance antitumor immunity by suppressing the recruitment and activity of immunosuppressive stromal and immune cells. This review summarizes the current knowledge of emerging pharmacological agents, including immunomodulators, therapeutic drugs, and their nanoformulations, for restoring antitumor immunity in CRC. It first discusses the fundamental immunosuppressive interactions within the TME and subsequently examines how these agents enhance immune responses by reprogramming stromal and immune cells, as well as their secreted mediators within the TIME.

***Corresponding Author**
Priya Priyadarshini Nayak: Email: drpriyanayak.sjh@gmail.com. ORCID: 0009-0003-9422-7758

Introduction

Colorectal cancer (CRC) remains a major challenge in cancer research and clinical practice. It is the third most frequently diagnosed malignancy worldwide and the second leading cause of cancer-related mortality.[1] Standard treatment strategies for CRC include surgery,[2] radiotherapy (particularly for rectal cancer), cytotoxic chemotherapy (e.g., fluoropyrimidine- and oxaliplatin- or irinotecan-based regimens),[3] and targeted therapies such as anti-vascular endothelial growth factor (VEGF) and anti-epidermal growth factor receptor (EGFR) agents in biomarker-selected patients.[4,5] More recently, novel therapeutic agents, including antibodies targeting pro-survival and pro-angiogenic factors, have also been introduced for the treatment of metastatic CRC.[6] Despite these advances, several limitations continue to compromise treatment efficacy. Both primary and acquired resistance to chemotherapy and targeted therapies frequently arise through molecular adaptations.[7,8] Moreover, tumor recurrence remains a major clinical challenge following treatment.[9] These challenges indicate a need for new approaches.

A critical determinant of treatment response in CRC is the tumor immune microenvironment (TIME), which consists of malignant epithelial cells, stromal components—particularly cancer-associated fibroblasts (CAFs) and the extracellular matrix (ECM)—vascular cells, and diverse immune cell populations.[10] In many tumors, especially mismatch-repair–proficient/microsatellite-stable (pMMR/MSS) CRC, the TIME is characterized by the exclusion of $CD8^+$ T cells, accumulation of myeloid-derived suppressor cells (MDSCs), metabolic reprogramming, and the secretion of pro-tumorigenic cytokines and chemokines, including transforming growth factor (TGF)-β, IL-6, and C-X-C motif chemokine ligand 12 (CXCL12). Together, these factors impair the cytotoxic functions of cytotoxic $CD8^+$ T lymphocytes (CTLs) and natural killer (NK) cells.[11] By contrast, mismatch-repair-deficient/microsatellite instability-high (dMMR/MSI-H) CRC generally exhibits greater immunogenicity and responds more favorably to immune checkpoint blockade.[12] This distinction underscores the importance of understanding the unique cellular and molecular interactions within the TIME when designing immunotherapeutic and other targeted treatment strategies.[13]

This review focuses on druggable immunosuppressive mechanisms in CRC and evaluates pharmacological agents, as well as formulation and delivery strategies, that can reprogram the TIME. Particular emphasis is placed on actionable therapeutic barriers, including stromal (CAF- and ECM-mediated) immune exclusion, immunosuppressive immune cell populations, and metabolic and hypoxia-associated pathways that contribute to the suppression of antitumor immunity. We discuss representative therapeutic approaches, including small-molecule inhibitors, monoclonal antibodies, selected immunomodulatory natural products, vaccine- and immune-priming strategies, and nanomedicine-based delivery systems. The evidence reviewed encompasses in vitro studies, animal models, early-phase clinical trials, randomized clinical studies, and approved clinical applications. Finally, we discuss key translational challenges relevant to pharmaceutical development, including pharmacokinetics, toxicity, manufacturability, and regulatory considerations.

Literature Identification

A targeted narrative literature search was conducted using PubMed/MEDLINE, Scopus, and Web of Science to identify studies investigating pharmacological and formulation-based approaches for modulating the CRC TIME. The primary search period covered January 2015 through December 2025. Earlier landmark publications were

also included when necessary to provide background on established biological mechanisms or first-in-class therapeutic interventions. The search strategy combined disease-specific terms with keywords related to the TIME and therapeutic interventions, including colorectal cancer, colon cancer, rectal cancer, tumor microenvironment, immune microenvironment, CAF, cancer-associated fibroblast, macrophage, TAM, MDSC, Treg, immune checkpoint, PD‑1, PD‑L1, CTLA‑4, STING, TLR, TGF‑beta, chemokine, adenosine, hypoxia, HIF, lactate, metabolism, drug, inhibitor, antibody, small molecule, vaccine, natural product, polyphenol, nanoparticle, and nanomedicine. In addition, the reference lists of relevant review articles and eligible primary studies were manually screened to identify additional pertinent publications. Only peer-reviewed English-language original research articles (including both in vitro and in vivo studies), clinical trials, and translational studies focusing on CRC were included in this review. For critical evaluation, priority was given to clinical trial reports that provided molecular stratification (e.g., dMMR/MSI-H vs. pMMR/MSS, tumor mutational burden, and POLE/POLD1 mutation status), trial phase, clinical endpoints, and safety outcomes. Evidence from non-CRC malignancies was considered only when CRC-specific data were unavailable and was used primarily to explain conserved mechanisms and interactions within the TIME.

TIME in CRC

TIME in CRC consists of a diverse population of stromal and immune cells that interact through complex signaling networks and soluble mediators. Among these, Cancer-associated fibroblasts (CAFs) are major stromal cells in CRC, which may arise from several cellular sources, including resident fibroblasts, mesenchymal stem cells (MSCs), pericytes, and, in some cases, cells undergoing epithelial-to-mesenchymal transition (EMT).[14] These cells are commonly identified by the expression of markers such as α-smooth muscle actin (α-SMA), fibroblast activation protein (FAP), platelet-derived growth factor receptors (PDGFRα/β), and S100A4. Importantly, CAFs constitute a highly heterogeneous cell population within the tumor microenvironment.[15] They secrete a broad spectrum of cytokines and chemokines, promote glycolytic metabolism and lactate production,[16] and produce major extracellular matrix (ECM) components, including collagen types I, III, and IV, fibronectin, laminins, and several additional matrix proteins.[17]

Tumor-associated macrophages (TAMs) represent another major population of immunosuppressive cells within the CRC microenvironment. These cells originate from both infiltrating monocytes and tissue-resident macrophages. Rather than existing as discrete subsets, TAMs occupy a spectrum of transcriptional and functional states that are shaped by their spatial localization and local microenvironmental cues.[18] M1-like TAMs predominantly produce IL-12, tumor necrosis factor (TNF)-α, and reactive oxygen species (ROS). They also participate in antigen presentation and can carry tumor antigens and stimulate cytotoxic responses by CTLs. In contrast, alternatively activated (M2-like) TAMs secrete IL-10, TGF-β, and arginase-1, thereby suppressing CTL activity and promoting tumor growth.[19] M2-like macrophage programs are frequently enriched within immunosuppressive regions of CRC. Although the terms M1-like and M2-like are commonly used to describe predominantly inflammatory and immunosuppressive macrophage phenotypes, macrophages in the CRC microenvironment often display intermediate or mixed activation states that dynamically shift in response to local factors, including cytokines, hypoxia, and metabolites.[19] Cytokines and mediators such as colony-stimulating factor-1 (CSF-1), IL-34, IL-4, IL-13, lactic acid, and prostaglandins are among the major factors that promote macrophage polarization toward an M2 phenotype.[20]

Regulatory T cells (Tregs) constitute another important immunosuppressive population enriched in CRC. These $CD4^+$ T-cell subsets are typically characterized by the expression of CD4, high levels of CD25, and the transcription factor FOXP3. Tregs suppress immune responses through several mechanisms. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expressed by Tregs interacts with CD80/CD86 on antigen-presenting cells (APCs), while the secretion of immunosuppressive cytokines, including IL-10, TGF-β, and IL-35, directly inhibits the activity of both APCs and CTLs. In addition, Tregs suppress the functions of NK cells and $CD4^+$ helper T cells. High expression of CD39 and CD73 enables Tregs to convert extracellular ATP into adenosine, a potent inhibitor of CTL-mediated cytotoxicity.[21]

MDSCs are another key immunosuppressive component of the CRC stroma. These cells are recruited into tumors in response to tumor-derived chemokines.[22] MDSCs suppress adaptive immune responses through the production of arginase-1, ROS, and inducible nitric oxide synthase (iNOS). Arginase-1 depletes arginine, an amino acid required for optimal CTL function, whereas nitric oxide and ROS impair T-cell receptor (TCR) signaling and may induce apoptosis in CTLs. In addition, MDSCs express programmed death ligand 1 (PD-L1) and secrete immunosuppressive cytokines, including IL-10 and TGF-β, further contributing to immune evasion.[23]

CTLs and NK cells are the principal effector cells responsible for antitumor immunity. CTLs are commonly identified by the expression of CD8, TCR, granzyme B, and perforin. Tumor-infiltrating CTLs exhibit considerable phenotypic heterogeneity and may exist in naïve, effector, memory, or exhausted states.[24] Exhausted CTLs are characterized by the expression of inhibitory immune checkpoint receptors, including programmed death 1 (PD-1), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte activation gene 3 protein (LAG-3), T cell immunoreceptor with Ig and ITIM (TIGIT), and CTLA-4, together with diminished cytokine production, and impaired cytotoxic function.[25,26] Effective CTL-mediated antitumor immunity depends on efficient antigen presentation by dendritic cells (DCs) and M1-like TAMs, as well as supportive cytokines such as IL-2 and IL-12. However, these immune responses are frequently attenuated by multiple immunosuppressive factors limit CTL cytotoxicity.[27]

NK cells also play a critical role in antitumor immunity. These cells are commonly identified by markers such as natural killer cell protein 46 (NKp46) and natural killer group 2, member D (NKG2D) and eliminate tumor cells through the recognition of stress-induced ligands, including histocompatibility complex class I (MHC-I) chain-related proteins A and B (MICA/B) and ULBPs, as well as the absence of MHC-I expression. Activated NK cells exert their cytotoxic activity through the release of granzymes, perforin, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α).[28]

Tumor vessels, ECM stiffness, and hypoxia are other distinct features of the tumor stroma that can affect antitumor immunity within the tumor. Tumor angiogenesis is driven by VEGF family ligands, angiopoietins, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) released by cancer, endothelial, and stromal cells.[29] Abnormal vessel architecture results in heterogeneous perfusion and hypoxia. These vascular changes impair T-cell trafficking and create hypoxic niches that promote polarization toward immunosuppressive immune cell subsets and facilitate adenosine accumulation.[30]

Hypoxia-inducible factor (HIF)-1α and HIF-2α are key mediators activated under hypoxic conditions. These transcription factors reprogram cellular metabolism and angiogenic signaling. HIF signaling upregulates glycolytic enzymes and carbonic anhydrases, while HIF-1α can induce VEGF expression. Elevated VEGF

promotes the formation of chaotic, leaky blood vessels and contributes to poor tissue perfusion.[31] Hypoxia also induces the HIF-1α/PD-L1 axis on in both cancer and myeloid cells.[32] In addition, adenosine accumulates through the activity of CD39 and CD73 in the hypoxic microenvironment, suppressing the functions of CTLs and NK cells.[33] Furthermore, hypoxia promotes the recruitment of MDSCs and monocytes and facilitates their differentiation toward M2-like TAMs.[34] Lactate accumulation is another characteristic feature of the tumor microenvironment that is further enhanced by hypoxia. The resulting acidification of the tumor stroma inhibits the proliferation and effector functions of CTLs.[35] (Figure 1)

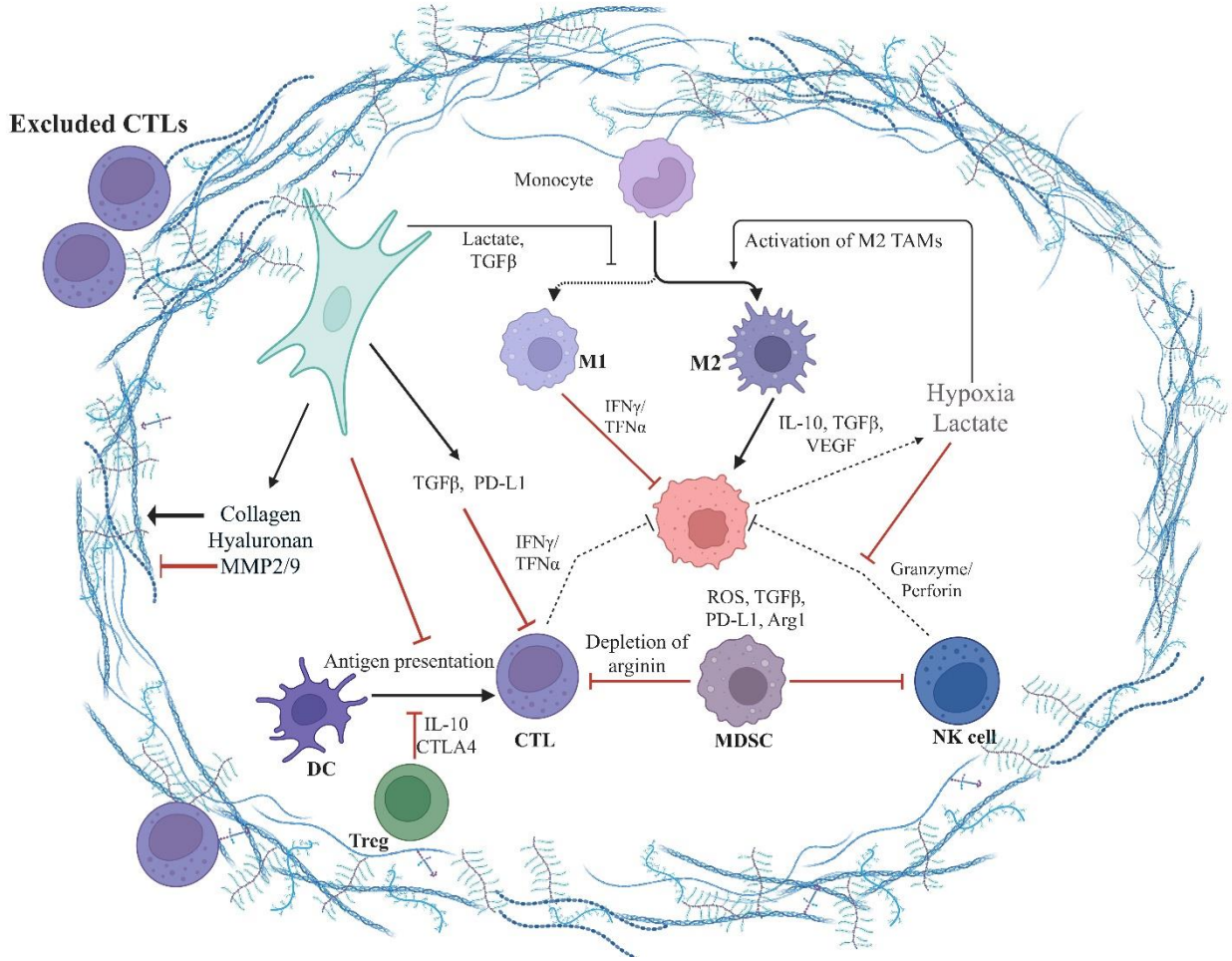

Figure 1. The tumor stroma in CRC and the immunosuppressive secretions and interactions mediated by stromal and immune cells. (This figure is original and was not adapted or reproduced from previously published sources)

Current Treatment Modalities for CRC

A combination of treatment modalities, including surgery, radiotherapy, chemotherapy, targeted molecular therapy, and immunotherapy, can be suggested for patients with CRC. Surgery followed by adjuvant chemotherapy may be recommended for the early stages of CRC. For stage I–III colon cancer and most rectal tumors, complete mesocolic or mesorectal excision can provide long-term disease control. Nevertheless, microscopic residual cancer cells can reduce the efficacy of this approach.[36] Locally advanced tumors may receive neoadjuvant chemoradiotherapy or total neoadjuvant therapy. Patients with metastatic CRC require systemic treatment and may also undergo metastasectomy or ablative therapy.[37] Adjuvant and neoadjuvant chemotherapy are well-established treatment modalities for CRC, particularly for stage III and high-risk stage II cancers.[38] In

rectal cancer, neoadjuvant chemoradiotherapy or chemotherapy alone can be prescribed to downstage tumors and facilitate sphincter preservation.[39] Systemic chemotherapy for metastatic CRC can improve survival.[40]

The response of CRC to treatment is strongly influenced by the TME. Conversely, various drugs and radiotherapy can reshape this ecosystem by remodeling immunosuppressive mechanisms, reducing pro-tumor and anti-apoptotic signaling, and restoring the function of CTLs.[41] Chemotherapy drugs may induce immunogenic cell death (ICD). This form of cell death releases tumor antigens and danger-associated signals that activate APCs, such as M1 TAMs and DCs, which in turn prime naïve $CD8^+$ T cells and promote CTL expansion.[42] However, chronic exposure to tumor antigens and the sustained release of inflammatory mediators by CTLs and NK cells can induce the expression of immune checkpoint molecules and increase the infiltration of Tregs, MDSCs, and M2-like TAMs. Under these conditions, treatment with immune checkpoint inhibitors (ICIs) or other immunomodulators that reduce checkpoint molecule expression may improve the therapeutic response.[43] Dense stroma and hypovascular regions also limit drug penetration, promoting spatially restricted tumor survival.[44] In addition, dose-limiting toxicity, neuropathy, and cumulative myelosuppression can reduce treatment efficacy.[45]

Radiotherapy is widely used in rectal cancer and selectively in oligometastatic CRC, particularly for liver and lung lesions.[46,47] Ionizing radiation (IR) can increase MHC expression and enhance antigen presentation. Type I interferon signaling can also be activated by IR. Together, these changes can convert irradiated tumors into in situ vaccines.[48] However, the response is context-dependent. High-dose or fractionated regimens may also damage endothelial cells, promote fibrosis, and recruit immunosuppressive MDSCs and macrophages.[49] In addition, hypoxic tumor regions are relatively radioresistant. Irregular vasculature and poor perfusion further exacerbate this problem.[50] Combining radiotherapy with immunomodulators may enhance antitumor immune responses while attenuating radiation-induced immunosuppression in CRC.[49]

Targeted molecular therapy is another treatment option for advanced CRC. Bevacizumab and other anti-VEGF antibodies can be used to inhibit tumor neovascularization. This class of drugs improves the efficacy of chemotherapy by normalizing abnormal tumor vasculature. Improved perfusion within the tumor stroma enhances both drug delivery and CTL trafficking. However, tumors may activate alternative pro-angiogenic pathways and develop hypoxia in response to this treatment.[51] Antibodies and drugs that inhibit EGFR and its downstream signaling pathways, such as cetuximab and panitumumab, are other therapeutic options for patients with RAS- and BRAF-wild-type metastatic CRC.[52] These agents can also modulate the TME. For example, EGFR inhibition can alter cytokine secretion.[53]

ICIs are effective for CRC with dMMR or MSI-H. Pembrolizumab and nivolumab have demonstrated good efficacy and durable tumor control in patients with dMMR/MSI-H gastrointestinal cancers.[54] These tumors harbor a high neoantigen burden and abundant infiltrating CTLs. As a result, ICIs can produce favorable therapeutic responses in this type of tumor stroma. However, most CRCs are microsatellite stable (MSS) and derive minimal benefit from ICI monotherapy. The MSS TME is typically characterized as cold or immune-excluded. These tumors contain few neoantigens, abundant CAFs, and immunosuppressive TAMs and MDSCs. In addition, tumors can lose antigen-presentation machinery, upregulate alternative immune checkpoint molecules, or recruit Tregs following immunotherapy.[55]Lower tumor immunogenicity and impaired antigen presentation in these tumors limit the pool of tumor-reactive T cells available for reinvigoration.[56,57] Many MSS tumors exhibit immune exclusion even after CTL activation. CAF-driven ECM remodeling, high concentrations of TGF-β, and abnormal

vasculature contribute to this phenotype by restricting immune cell trafficking and sustaining immunosuppressive cytokine programs.[58,59] Myeloid cell dominance, hypoxia, and altered metabolism are additional mechanisms that contribute to ICI resistance in these tumors.[60,61] A smaller subset of CRC harbors POLE/POLD1 proofreading alterations that generate an ultramutated phenotype with a high tumor mutational burden (TMB). These alterations can enhance sensitivity to ICIs even in MSS tumors.[62] However, although TMB may enrich for treatment response, its predictive value in CRC depends on the sequencing platform, the cutoff used, and whether the hypermutated phenotype results from defective DNA repair rather than other mechanisms.[63]

Disease site also influences immune responses and clinical outcomes. The location of the primary tumor (right- vs. left-sided colon, or rectum vs. colon) and the site of metastasis can shape the immune composition of the TME and influence therapeutic responsiveness. For instance, right-sided CRC may exhibit higher cytotoxic immune activity. In addition, increased expression of VEGF and TAM-associated signatures can markedly influence immune responses in right-sided tumors but not in left-sided tumors.[64] In particular, liver metastases are frequently associated with stronger myeloid-mediated immunosuppression and attenuated systemic antitumor immunity. These characteristics may reduce the efficacy of ICIs despite adequate systemic drug exposure.[65] Cancer vaccines and adoptive cellular therapy (ACT) are additional strategies with growing relevance in CRC.[66,67] However, these modalities have not yet been approved for the treatment of solid tumors such as CRC in humans. Furthermore, they face several resistance mechanisms, including the presence of fibrotic, immunosuppressive, and hypoxic niches within the CRC microenvironment.[67] (Figure 2)

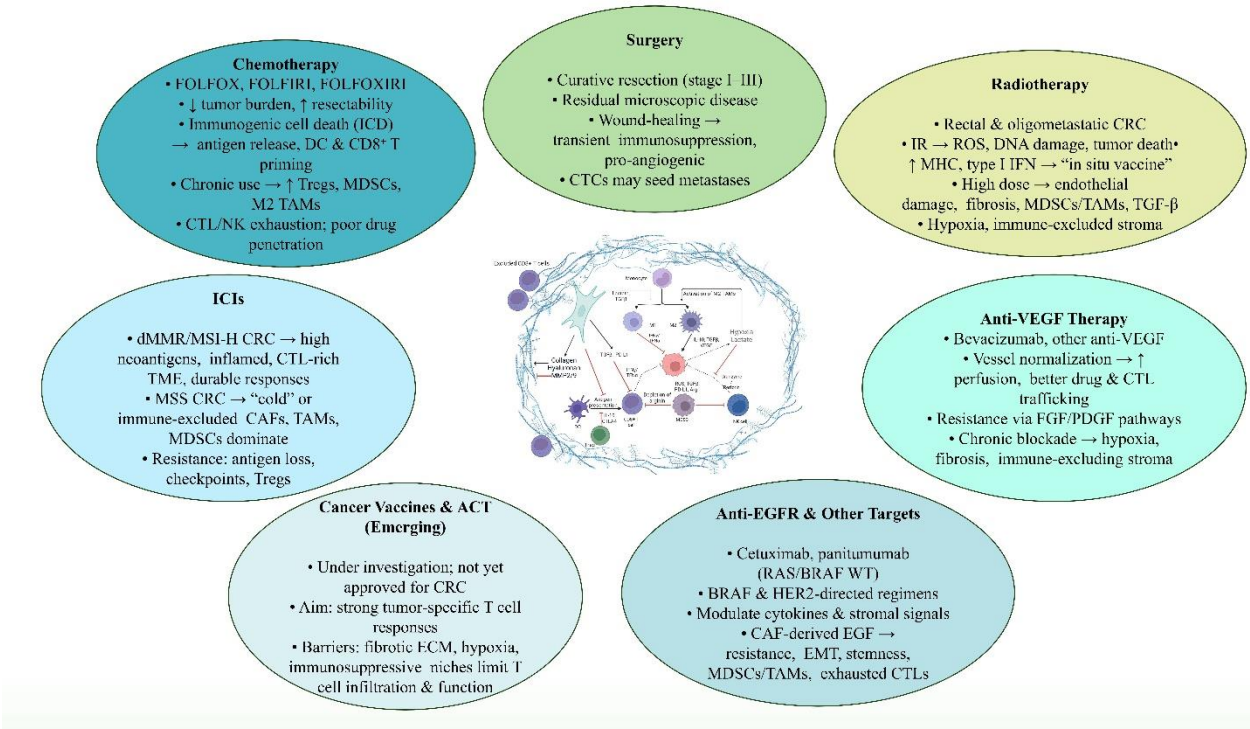

Figure 2. Various treatment modalities for CRC and their impact on the tumor stroma and antitumor immunity. (This figure is original and was not adapted or reproduced from previously published sources)

Targeting CAFs and ECM to Induce Antitumor Immunity

Several factors can activate CAFs, whereas others are secreted by CAFs and promote stemness in CRC cells or further expand the CAF population within the tumor. IL-1, TGF-β, and IL-6 are well-known factors that not only

promote CAF expansion but are also secreted by CAFs and contribute to therapeutic resistance in CRC.[68] A study showed that CAF-specific IL1R1 knockout and treatment with the IL-1 receptor antagonist anakinra remarkably reduced tumor growth in preclinical CRC models. Blockade of this pathway also resulted in reduced PD-L1 expression in CRC.[69] TGF-β and its receptor, TGFBR1, are other important therapeutic targets in CAFs. The TGF-β/TGFBR1 axis in CAFs is closely associated with IL-1β signaling. Both IL-1β and TGFBR1 have been shown to promote CAF recruitment and the polarization of normal fibroblasts into CAFs, leading to increased secretion of immunosuppressive cytokines and reduced production of inflammatory cytokines. IL-1β and TGF-β activate fibroblasts through the janus kinase (JAK)/signal transducer and activator of transcription (STAT)3 and nuclear factor of κappa B (NF-κB) signaling pathways. Blockade of either IL-1β or TGFBR1 (using galunisertib) reduced CAF accumulation, thereby decreasing the resistance of CRC xenografts to oxaliplatin. In addition, inhibition of these pathways significantly reduced the levels of C-C motif chemokine ligand (CCL)5, IL-6, hepatocyte growth factor (HGF), and granulocyte-macrophage colony-stimulating factor (GM-CSF).[70]

NADPH oxidase 4 (NOX4) is a pro-oxidant factor in CAFs that can induce apoptosis in CTLs. It is activated by TGF-β, while NOX4 activation can also stimulate TGF-β release and further promote CAF expansion. A study reported that NOX4 inhibition reversed TGF-β1-mediated CAF activation, resulting in reduced ECM deposition, increased CTL infiltration into tumors, and an enhanced response to anti-PD-1 therapy.[71] CAFs in CRC also suppress T-cell proliferation through PD-L1 expression. This molecule can be regulated by several CAF-derived secreted factors. CXCL5 is one of the key mediators. Secreted CXCL5 activates CXCR2 and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling in CRC cells, thereby enhancing PD-L1 expression on cancer cells and promoting immunosuppressive responses within the TME.[72] CAFs can also regulate PD-L1 expression in CRC through the release of circEIF3K-containing exosomes. This factor suppresses miR-214 in cancer cells, resulting in PD-L1 overexpression. Since miR-214 normally acts as a negative regulator of PD-L1 in CRC cells, its suppression by CAF-derived circEIF3K enhances tumor-mediated immunosuppression.[73]

In addition, CAF-derived factors can induce PD-L1 upregulation in CRC cells through activation of extracellular signal-regulated kinase (ERK)5 phosphorylation.[74] CAFs can also promote PD-L1 expression in TAMs. In response to overexpression of Yes-associated protein (YAP) in CRC cells, CAFs upregulate hyaluronan synthase 2 (HAS2). This process is associated with increased secretion of connective tissue growth factor (CTGF) by cancer cells, which stimulates HAS2 production. HAS2 binds to CD44 on M2-like TAMs and promotes PD-L1 upregulation. This interaction further contributes to CTL exhaustion in CRC. Under these conditions, inhibition of HAS2 synthesis in CAFs using specific inhibitors can restore CTL activity by reducing PD-L1 expression, thereby improving the response of CRC to anti-PD-1 therapy.[75]

Some other agents have also shown the potential to remodel the ECM. The ECM-modifying enzyme lysyl oxidase (LOX) may promote CRC progression, particularly in advanced-stage tumors. This enzyme can enhance tumor invasion and metastasis by increasing collagen cross-linking, thereby promoting tumor stiffness. A preclinical study demonstrated that LOX induces focal adhesion kinase (FAK) and steroid receptor coactivator (Src) phosphorylation in CRC cells, which subsequently enhances cancer cell migration and invasion. Inhibition of LOX attenuated these effects. The authors suggested that targeting LOX activity may represent a novel and effective therapeutic strategy for patients with metastatic CRC.[76] Discoidin domain receptor 1 (DDR1) is another factor involved in collagen remodeling that promotes immune exclusion in CRC. A study reported that DDR1 silencing disrupts collagen barriers and enhances CTL infiltration, revealing a novel role of the DDR1-mediated

collagen-rich matrix in tumor resistance.[77] Although ECM remodeling has shown promising results in preclinical studies, it may also facilitate metastasis. Therefore, the clinical translation of this strategy faces greater challenges than approaches targeting CAFs or other immunosuppressive cells and components.[78] (Figure 3)

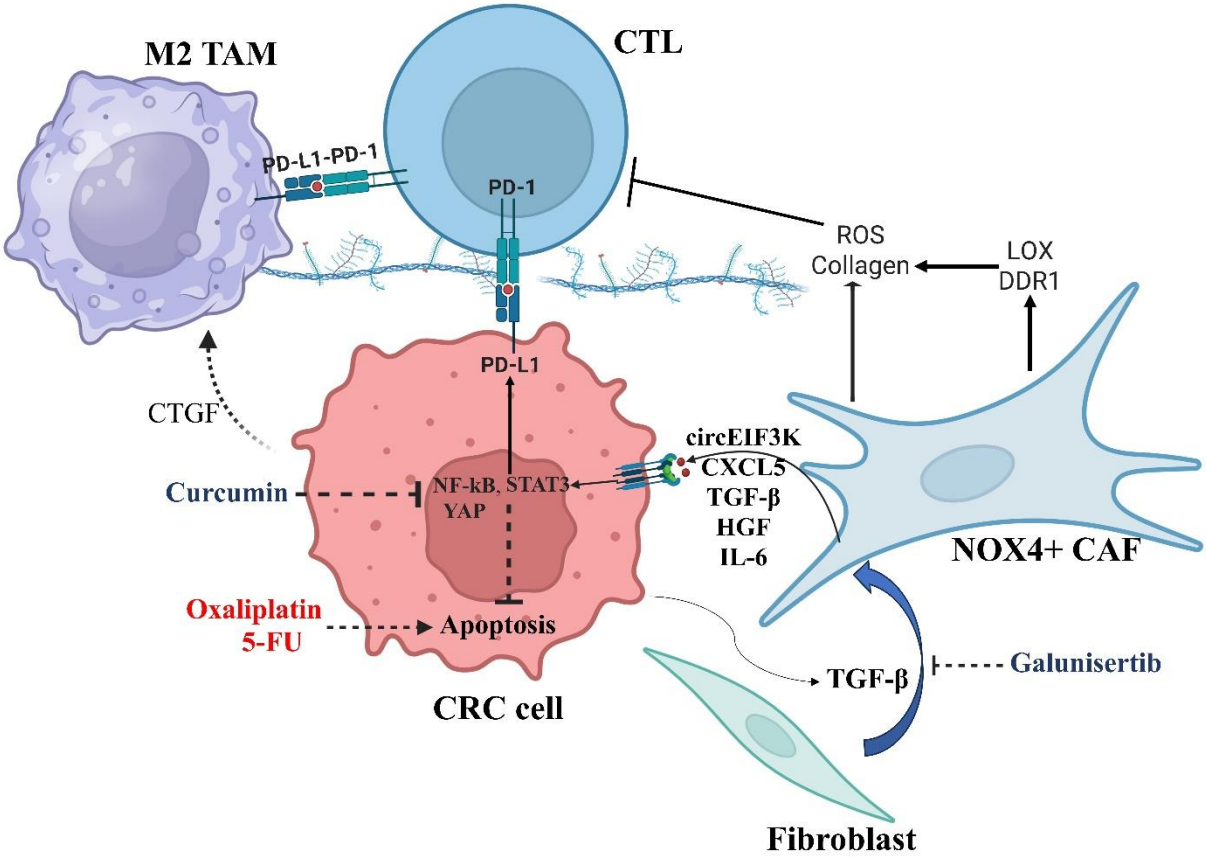

Figure 3. Mechanisms of CTL exhaustion and cancer cell resistance mediated by CAFs. As illustrated, CAFs release several factors that induce drug resistance and anti-apoptotic mediators in CRC cells. CAFs also produce collagen, which limits CTL infiltration into the tumor. In addition, CAFs stimulate CTGF release by CRC cells, thereby promoting the recruitment of M2-like TAMs and the upregulation of PD-L1 expression in these cells, ultimately leading to CTL exhaustion. Treatment with curcumin can reduce the activity of drug resistance mediators, such as NF-κB, in CRC cells. Furthermore, blockade of TGF-β can attenuate CAF expansion and subsequently reduce resistance mechanisms in CRC. (This figure is original and was not adapted or reproduced from previously published sources)

Targeting TAMs to Induce Antitumor Immunity

Immunosuppressive TAMs express immune checkpoint molecules, engage in metabolic competition through indoleamine 2,3-dioxygenase (IDO) and arginase activity, secrete immunosuppressive cytokines and chemokines, and facilitate ECM remodeling.[79,80] These characteristics provide several potential therapeutic targets for reducing the protumor activity of these cells. CSF-1R and CCL2 are well-established targets for depleting and reprogramming M2-like TAMs. An in vivo study reported that the CSF-1R inhibitor PLX3397 effectively reduced M2 TAM populations in orthotopic CRC xenograft models. When combined with anti-PD-1 therapy, this treatment significantly suppressed tumor growth. The findings also demonstrated that CAFs promote the recruitment of M2 TAMs and subsequent CTL exhaustion through CSF-1R signaling. Blockade of this pathway effectively reversed these effects in CRC.[81]

Another study showed that CCL2 blockade suppresses macrophage recruitment and polarization toward the M2 phenotype, thereby sensitizing CRC to 5-FU. This combination therapy reduced tumor growth approximately fivefold compared with chemotherapy alone.[82] Interestingly, several studies have reported that MSI-H CRC tends to express high levels of PD-L1, which promotes the recruitment of M2 TAMs through the PD-L1/PD-1 axis. Moreover, sustained PD-1 overexpression on M2 TAMs reduces their phagocytic activity and contributes to CTL exhaustion. Consequently, PD-1 blockade decreases M2 TAM infiltration, promotes M1-like polarization, and enhances CTL activation in CRC.[83] Combining TAM-reprogramming strategies with immune checkpoint inhibitors therefore offers synergistic therapeutic potential for CRC treatment.

As discussed earlier, the CRC stroma contains a broad spectrum of TAM populations with diverse functional states. Human single-cell RNA sequencing and spatial profiling studies have further refined the macrophage landscape in CRC. These analyses have identified multiple TAM programs beyond the traditional M1/M2 classification, including antigen-presenting/inflammatory macrophages, monocyte-like macrophages, and immunoregulatory macrophages. These distinct macrophage states are associated with differences in phagocytic capacity, angiogenesis, and ECM remodeling.[84] Spatial mapping studies have also shown that immunosuppressive myeloid programs frequently colocalize with CAF-rich, TGF-β-associated stromal niches and are depleted in T cell-inflamed regions of digestive tumors.[85] These human CRC atlases support a therapeutic strategy that combines targeting TAM recruitment and polarization pathways with stromal modulation and immune checkpoint blockade.

Targeting MDSCs for Inducing Antitumor Immunity

Several therapeutic strategies have been proposed to target MDSCs and restore antitumor immunity. Experimental studies suggest that these approaches include promoting the differentiation of MDSCs into mature myeloid cells, blocking their recruitment, directly inhibiting their immunosuppressive functions, and targeting their metabolic pathways.[86] As explained, GM-CSF, VEGF, IL-6, IL-10, TGF-β, and prostaglandin E2 (PGE2) can expand MDSCs by inducing STAT3/6 phosphorylation in these cells. In addition, CSF-1, CCL2, and CCL5 can enhance the recruitment of MDSCs. These factors form an interactive communication network promoting MDSC accumulation. These factors can be suggested as promising targets for inhibiting MDSCs. Maraviroc, a C-C chemokine receptor (CCR)5 antagonist, combined with pembrolizumab, is being evaluated in metastatic CRC.[87] However, its effects on MDSCs in these patients need to be determined. Histamine dihydrochloride (a NOX2 inhibitor) has been shown to reduce MDSC accumulation in MC-38 cell-bearing mice. This effect resulted in improved tumor response to anti-PD-1/PD-L1 therapy.[88] Similarly, embelin, an inhibitor of X-linked inhibitor of apoptosis protein (XIAP), significantly decreased MDSC populations in both peripheral lymphoid organs and tumor tissues by reducing ROS production and arginase-1 expression. Furthermore, naringin suppressed colitis-associated CRC development by regulating the immunosuppressive activity of MDSCs through inhibition of the NF-κB/IL-6/STAT3 signaling pathway. TLR7/8 agonist resiquimod (R848) is another agent that can induce an inflammatory response in CRC. Treatment with this agent can sensitize CRC to oxaliplatin by reducing MDSCs within tumors and polarization of these toward M1 TAM.[89] (Figure 4)

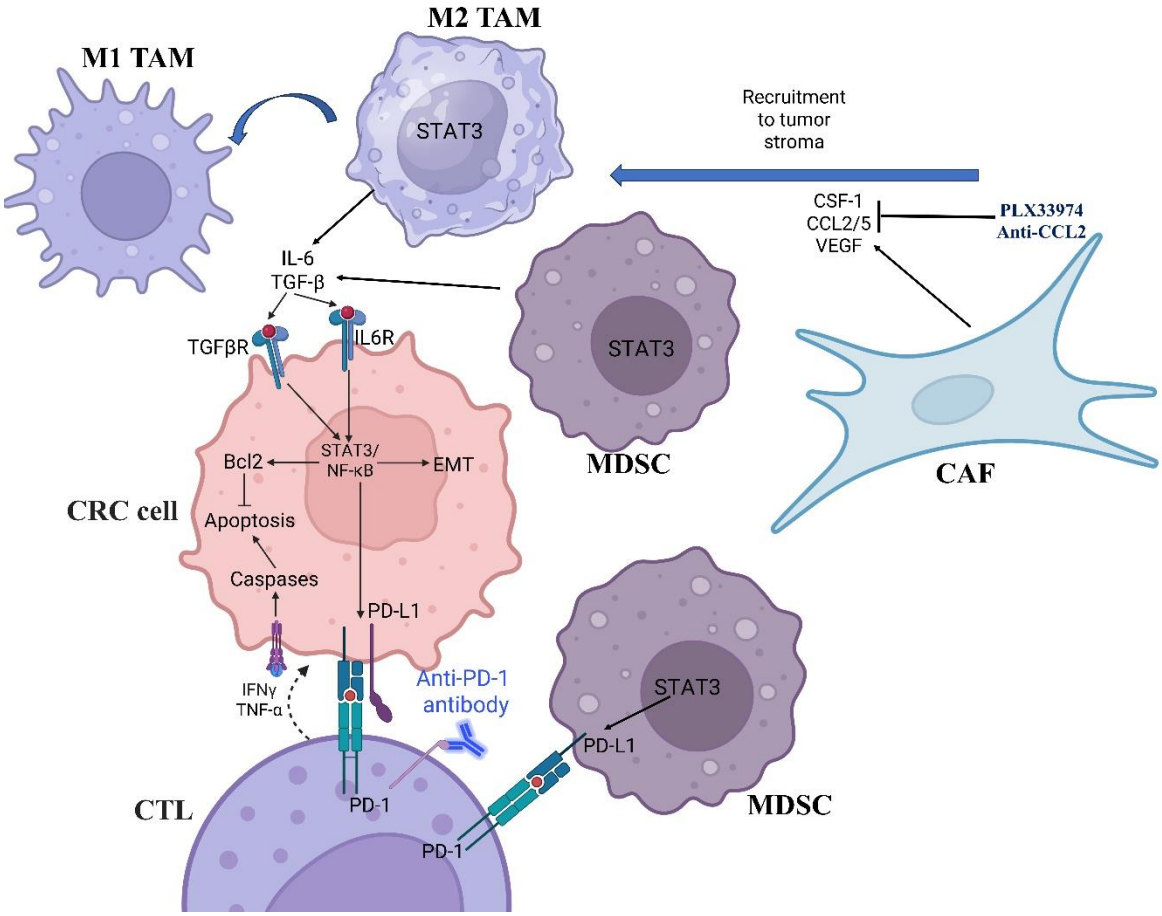

Figure 4: Targeting TAMs and MDSCs in CRC for boosting antitumor immunity. Chemokines such as CSF-1 and CCL2/5 can induce the recruitment of MDSCs and M2 TAMs. These cells induce CTL exhaustion by expressing PD-L1 and enhance drug resistance in CRC by releasing IL-6 and TGF-β. As illustrated, the blockade of CCL2/5 and CSF-1 can reduce the recruitment of these cells and subsequent resistance to therapy. (This figure is original and was not adapted or reproduced from previously published sources)

Targeting Tregs for inducing antitumor immunity

Tregs are potent immunosuppressive cells and represent a promising target for enhancing antitumor immunity. CCR8 has emerged as an attractive target for selective Treg depletion because its expression distinguishes tumor-infiltrating Tregs from those residing in lymphoid organs. CCR8-expressing Tregs migrate into tumors in response to the CCL1 ligand secreted by CAFs and M2-polarized TAMs. Selective CCR8 depletion has been reported to preserve peripheral immune tolerance while eliminating tumor-infiltrating Tregs.[90] Targeting CCR8 in CRC models has also been shown to enhance the expansion and activation of Th1 $CD4^+$ cells and CTLs.[91] This improvement in antitumor immunity has been associated with enhanced responses to anti-PD-1 therapy and vaccine-based strategies in murine CRC models.[92,93]

CCL5 is another important chemoattractant for Tregs in CRC. Increased CCL5 expression promotes the expansion of TGF-β^+ Tregs, thereby inducing CTL apoptosis.[94] Immunosuppressive mediators such as TGF-β are major drivers of Treg expansion. Therefore, reducing TGF-β levels within the tumor microenvironment may decrease Treg accumulation. TGF-β produced and secreted by TAMs has been shown to promote Treg expansion in CRC.[95] Moreover, TGF-β expression in CRC is positively correlated with Foxp3 expression and distant metastasis.[96] Similar to MDSCs, Treg populations can also be reduced following treatment with certain chemotherapeutic

agents. Low-dose cyclophosphamide has been reported to deplete Tregs in CRC.[97] Tregs are major suppressors of CTL activity in cancer and can also impair DC function. Colon cancers contain $CCR4^{+}CTLA4^{hi}$Foxp3 Tregs that may serve as therapeutic targets for restoring antitumor immunity.[98]

Several studies have suggested that targeting CTLA-4 with monoclonal antibodies is an effective strategy for depleting or reducing Treg populations.[99] However, other studies have reported limited efficacy or even increased Treg expansion following CTLA-4 blockade.[100,101] In preclinical models, treatment of CRC-bearing mice with an anti-CTLA-4 antibody significantly reduced tumor-infiltrating Tregs.[102] Clinical trials have also demonstrated improved antitumor immunity in patients with CRC following treatment with anti-CTLA-4 antibodies.[103,104] Nevertheless, experimental studies indicate that cells within the CRC stroma can express alternative immune checkpoint molecules that diminish the efficacy of anti-CTLA-4 therapy. Therefore, Treg depletion using anti-CTLA-4 antibodies in combination with other agents, such as anti-PD-1 or anti-PD-L1 antibodies, may further improve therapeutic efficacy.[105]

Targeting Hypoxia for Inducing Antitumor Immunity

Targeting hypoxia, HIF-1α signaling, metabolic pathways, and tumor oxygenation has emerged as a promising strategy for remodeling antitumor immunity in CRC. Treatment with PX-478, a HIF-1α inhibitor, has been shown to enhance oxaliplatin sensitivity by suppressing the HIF-1α/miR-338-5p/IL-6 feedback loop in CRC xenograft models. The findings demonstrated that IL-6 is a direct target of miR-338-5p and mediates STAT3/Bcl-2 activation under hypoxic conditions. Moreover, the HIF-1α/miR-338-5p/IL-6 signaling loop was found to be essential for the development of chemoresistance, and disruption of this pathway restored chemosensitivity.[106] Hypoxia also promotes the secretion of TGF-β2 by CAFs, thereby enhancing chemoresistance in CRC. Consequently, alleviating tumor hypoxia may attenuate the immunosuppressive effects of CAFs and improve therapeutic responses.[107] Collectively, these findings indicate that targeting hypoxia-related pathways can reverse immunosuppression and enhance the efficacy of anticancer therapies in CRC.

Targeting Tumor Acidity and Altered Metabolism for Inducing Antitumor Immunity

The Warburg effect promotes excessive lactate production, resulting in acidification of the tumor stroma. This acidic milieu suppresses antitumor immunity and contributes to resistance against multiple anticancer therapies. Therefore, targeting this acidic stroma with pH-modulating drugs can modulate antitumor efficacy against CRC. A study showed that cariporide treatment induced TNF-α production and improved antitumor immunity, although it was also associated with elevated IL-6 levels.[108] Inhibition of monocarboxylate transporters (MCTs) reduces lactate export and accumulation, thereby sensitizing CRC cells to chemotherapy.[109] Similarly, proton pump inhibitors have been reported to improve the survival of patients receiving FOLFOX.[110]

Additional metabolic targets have also been proposed for remodeling the immunosuppressive microenvironment. In human CRC, CAFs are known as the prominent $CD73^{hi}$ population. These cells can be expanded through an adenosine–A2B receptor-mediated feedforward loop. Inhibition of CD73 has therefore been shown to restore antitumor immunity.[111] TAMs has also emerged as an attractive therapeutic strategy. The STING agonist GB2 induces metabolic remodeling of TAMs by enhancing the glycolysis–ROS–HIF-1α axis while suppressing oxidative phosphorylation (OXPHOS), thereby promoting M1 polarization and increasing macrophage phagocytic activity. These changes restore CTL function and enhance the therapeutic efficacy of anti-PD-1

immunotherapy.[112] Metformin is another promising metabolic modulator that affects both TAMs and MDSCs. Metformin activates adenosine monophosphate-activated protein kinase (AMPK) while inhibiting mechanistic target of rapamycin (mTOR) signaling in these immunosuppressive cells. In addition, it suppresses the mevalonate pathway, which plays a critical role in tumor metabolism. Treatment with metformin has also been shown to reduce Treg frequencies in murine CRC models without significantly affecting peripheral lymphoid tissues.[113]

Targeting CTLs and NK Cells

In addition to strategies aimed at suppressing immunosuppressive mechanisms within the TME, direct activation of NK cells and CTLs has also attracted considerable attention. Therapeutic cancer vaccines represent a promising approach for enhancing antitumor immunity while minimizing systemic toxicity. Current CRC vaccine platforms include whole tumor cell-based vaccines, DC-based vaccines, peptide vaccines, nucleic acid vaccines, and viral vector-based vaccines.[114] Among these approaches, the ELI-002 2P vaccine has demonstrated a favorable safety profile in a Phase I clinical trial involving patients with KRAS-mutated CRC (NCT05726864). Recent findings showed that most patients developed robust antigen-specific immune responses characterized by activation of $CD4^+$ Th1 cells and CTLs.

Furthermore, patients with stronger CTL responses experienced prolonged relapse-free periods. The vaccine also generated durable antitumor immunity, suggesting its potential to prevent or delay cancer recurrence in high-risk patients.[115] Some other vaccine platforms, including Listeria monocytogenes-based immunotherapy and ADX-NEO, which combines a L. monocytogenes platform with neoantigens, have demonstrated acceptable safety profiles in Phase I clinical trials (NCT03189030 and NCT03265080). These approaches are currently being evaluated as monotherapies or in combination with other anticancer agents for the treatment of solid cancers.[116] Gemogenovatucel-T (Vigil) is another promising vaccine that has demonstrated the ability to induce durable adaptive immune responses when combined with FOLFOX-6 chemotherapy. The available evidence suggests that this vaccine is safe and exhibits potential antitumor activity in patients with advanced CRC.[117] Messenger RNA (mRNA)-based vaccine technology has recently emerged as a promising therapeutic strategy, offering greater specificity, enhanced efficacy, and fewer adverse effects than traditional approaches.[118] Results of clinical trials investigating novel ICIs, vaccines, and combination treatment strategies is presented in Table 1. (Table 1)

Table 1: Summary of Clinical Trials Evaluating Immunomodulatory Strategies in CRC

Population	Design	Key Efficacy	Safety	
mKRAS PDAC/CRC; MRD+ (AMPLIFY-201)	Phase 1 (N=25; 5 CRC); ELI-002 2P vaccine	• 84% biomarker reduction; 24% clearance • mRFS: 16.3 mo • T cell response: 84% • mOS: 16.3 mo	• No grade ≥3 AEs • No DLTs/CRS • Fatigue (24%)	[119]
pMMR/MSS mCRC	Phase 2 (N=114) serplulimab plus	• PFS: 17.2 vs. 10.7 • OS wasn't reached to the aim of study	Grade ≥3 TRAEs: 65.5% vs 56%1	[120]

	HLX04 and serplulimab vs placebo			
pMMR/MSS mCRC	Phase 2 (N=201) FOLFOXIRI plus bevacizumab alone or in combination with atezolizumab	No improvement in PFS compared to conventional therapy	No additional TRAEs was reported for immunotherapy arm compared to FOLFOXIRI plus bevacizumab	[121]
dMMR/MSI-H mCRC; ≥1 prior line (CheckMate 142)	Phase 2 (N=74); Nivolumab	• ORR: 31.1% • 12-mo PFS: 50%; 12-mo OS: 73% • Responses in all subgroups	• Grade 3-4: 20% • No treatment-related deaths	[103]
MSI-H/dMMR mCRC; first-line (KEYNOTE-177)	Phase 3 (N=307); Pembrolizumab vs Chemotherapy	• PFS: 16.5 vs 8.2 mo • ORR: 45% vs 33%	• Grade ≥3 TRAEs: 22% vs 66% • No pembrolizumab-related deaths	[122]
MSI-H/dMMR mCRC; first-line (CheckMate 8HW)	Phase 3 (N=303); Nivo+Ipi vs Chemotherapy	• 24-mo PFS: 72% vs 14% • Benefit across all subgroups	• Grade 3-4: 23% for Nivo+Ipi vs 48% for chemotherapy • 2 treatment-related deaths for Nivo+Ipi	[123]
Refractory CRC; all standard therapies (CO.26 studies)	Phase 2 (N=180); Durva+Treme+BSC vs BSC	• OS: 6.6 mo for Durva+Treme+BSC vs 4.1 mo for BSC only	• Grade ≥3: 64% for combination therapy vs 20% for BSC only	[124]
Refractory mCRC (BACCI study)	Phase 2 (N=128); Cape+Bev+Atezo vs Cape+Bev+Placebo	• PFS: 4.4 for Cape+Bev+Atezo vs 3.6 mo for Cape+Bev+Placebo	• Similar AE rates • 1 treatment-related death	[125]
Advanced solid tumors, including MSS-CRC	Phase 1 (N=192; 42 CRC); Oleclumab alone or with Durvalumab	• CRC: ORR 2.4% (1 CR) • mPFS: 5.4 mo	• TRAEs: 60% • CRC: grade 3-4 19%	[126]

MSS-CRC; recurrence after chemo	Phase 1 (N=6); Personalized vaccine (Neoantigen Vaccine)	• Immune response: 67% • PFS: 19 vs 11 mo (responders vs non)	• Grade 1 injection reactions, rash, headache	[127]
Advanced CRC; ≥2 prior lines	Phase 1 (N=6); NKG2D CAR-NK ± anti-PD-1	• Best: SD (1 patient) • 2 patients OS >700 days	• TRAEs: 100% • Hematologic toxicities (lymphodepletion)	[128]

Abbreviations: AE, adverse event; AESI, adverse event of special interest; Atezo, atezolizumab; Bev, bevacizumab; BICR, blinded independent central review; BSC, best supportive care; Cape, capecitabine; CI, confidence interval; CR, complete response; CRC, colorectal cancer; CRS, cytokine release syndrome; ctDNA, circulating tumor DNA; DLT, dose-limiting toxicity; dMMR, deficient mismatch repair; DOR, duration of response; Durva, durvalumab; EGFR, epidermal growth factor receptor; FOLFIRI, folinic acid + fluorouracil + irinotecan; FOLFOX, folinic acid + fluorouracil + oxaliplatin; FOLFOXIRI, folinic acid + fluorouracil + oxaliplatin + irinotecan; HLX04, bevacizumab biosimilar; HR, hazard ratio; Ipi, ipilimumab; IRRC, independent radiology review committee; ITT, intention-to-treat; mCRC, metastatic colorectal cancer; mKRAS, mutant KRAS; MRD, minimal residual disease; MSI-H, microsatellite instability-high; MSS, microsatellite stable; MTD, maximum tolerated dose; Nivo, nivolumab; NR, not reached; NSCLC, non-small cell lung cancer; OR, odds ratio; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; pMMR, proficient mismatch repair; PR, partial response; Q2W, every 2 weeks; Q3W, every 3 weeks; Q4W, every 4 weeks; QOL, quality of life; RFS, relapse-free survival; RMST, restricted mean survival time; RP2D, recommended phase 2 dose; SAE, serious adverse event; SD, stable disease; TEAE, treatment-emergent adverse event; TMB, tumor mutational burden; TRAE, treatment-related adverse event; Treme, tremelimumab; WT, wild-type; XELOX, capecitabine + oxaliplatin.

The findings from clinical trials reveal a clear dichotomy based on the molecular status of the tumor. In total, these findings suggest that ICIs are the standard of care for MSI-H/dMMR CRC. These trials have shown that nivolumab plus ipilimumab provides the most durable disease control. No immunotherapy strategy has demonstrated a transformative benefit in patients with MSS/pMMR CRC. The modest signals from combinations and emerging data on vaccines suggest that converting MSS tumors to an immune-responsive state requires more sophisticated approaches, including better patient selection using biomarkers such as TMB and ctDNA dynamics.

Natural products for boosting antitumor immunity in CRC

Natural products, particularly polyphenol-rich compounds, have been investigated as immunomodulatory candidates for CRC, mainly in preclinical models. Agents such as curcumin, resveratrol, quercetin, and epigallocatechin gallate (EGCG) have been reported to influence inflammatory signaling (e.g., NF‑κB and STAT3), cytokine profiles, and immunosuppressive pathways within the TIME. These modulatory effects are associated with changes in CTL activity and reduced MDSCs or Treg programs in experimental systems.[129] However, the strength of evidence varies substantially across compounds and model systems, and findings from in vitro assays should not be extrapolated to clinical efficacy without exposure and pharmacokinetic support.

Curcumin is the most frequently studied compound for CRC immunomodulation. Preclinical studies suggest that curcumin can attenuate immunosuppressive signaling in the TIME (including TGF-β- and NF-κB signaling pathways) and may reduce immunosuppressive TAMs and Treg-associated features in CRC models.[130-132] Curcumin has been shown to attenuate M2 TAMs by lowering IL-10 secretion and arginase-1 expression. These effects reversed the M2 polarization of TAMs and attenuated CRC cell invasion. [133] Curcumin treatment has been shown to polarize MDSCs toward an M1-like phenotype by inhibiting STAT3 and NF-κB and subsequently

attenuating IL-6 release by MDSCs.[134] Some phytochemicals like curcumin, Cucurbitacin B, and Astragaloside IV suppress M2 polarization of TAMs by inhibiting NF-κB/STAT3. These agents can also block VEGF/HIF-1α-induced angiogenesis and restore antitumor immunity by downregulating PD-L1.[135-137]

In a limited clinical investigation, curcumin was associated with immunological changes consistent with reduced Treg activity and enhanced Th1-type responses. However, clinical efficacy outcomes remain insufficiently defined.[138] Resveratrol and EGCG have also shown immunomodulatory effects in CRC-related models. These products have shown the ability to suppress tumor-promoting stromal/metabolic interactions and attenuate signaling pathways involved in angiogenesis and hypoxia responses.[139-141] Additional natural compounds, including luteolin, piceatannol, glycyrrhizin, tanshinone IIA, and acriflavine, have been reported to affect TAM/MDSC/Treg-associated pathways or HIF-related signaling in preclinical CRC models.[142-146] All-trans retinoic acid (ATRA) promotes MDSC differentiation into mature cells, thereby attenuating their immunosuppressive effects.[147] However, most studies mentioned have limited validation in immune-competent CRC models and lack human evidence. (Figure 5)

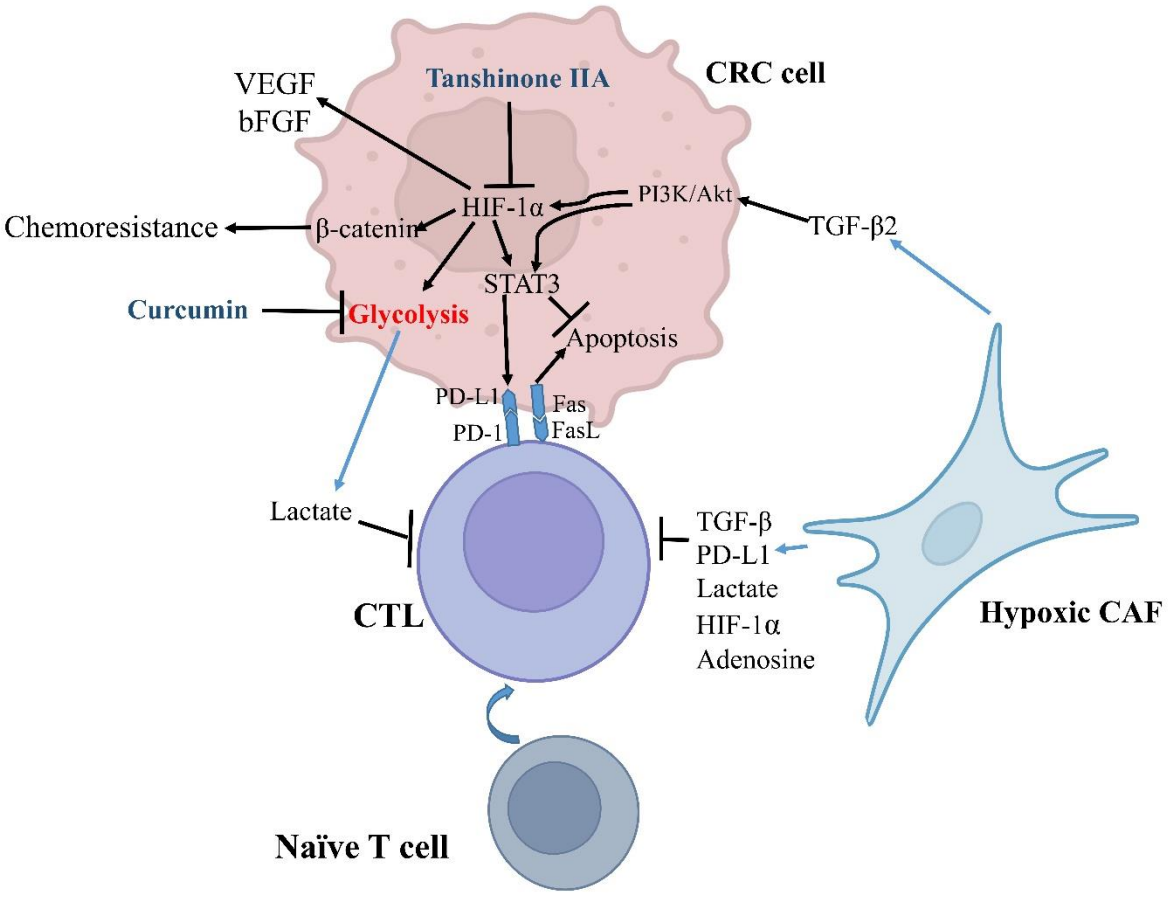

Figure 5: Natural products can remodel the TIME in CRC by modulating hypoxia and metabolism. Hypoxia can potentiate both glycolysis and lactate production. Hypoxia can induce the release of TGF-β2, lactate, and adenosine, which not only suppress CTLs but also induce chemoresistance in CRC cells. The modulation of HIF-1 or glycolysis by natural products can mitigate these effects. (This figure is original and was not adapted or reproduced from previously published sources)

From a pharmaceutical-development perspective, the translation of natural products faces serious challenges, including poor aqueous solubility, extensive first-pass metabolism, short systemic half-life, and limited and

variable oral bioavailability.[148,149] These limitations can prevent achieving intratumoral exposures comparable to those used in mechanistic in vitro studies. In addition, variability in formulation and product standardization (extracts vs. purified compounds) causes significant obstacles. Other limitations include potential drug–drug interactions, enhanced normal tissue toxicity by anticancer drugs, and the absence of consistent biomarker-driven clinical studies.[150-152] Overall, natural products are best viewed as adjunct immunomodulatory candidates or lead structures rather than an alone anticancer approach in CRC. Future studies should prioritize standardized formulations, exposure–response relationships, immune-competent CRC models with defined endpoints, and biomarkers. Appropriate endpoints, such as CTL infiltration/function, can predict the direct immunomodulatory effects of different natural products. This can also facilitate the comparison of the potential of various products in different forms. Biomarker-stratified clinical investigations are also needed to determine whether these immunological effects translate into clinically meaningful benefit.[153,154]

Nanomedicine for boosting antitumor immunity in CRC

Nanomedicine platforms can improve the intratumoral delivery of immunomodulatory agents, including cytotoxic drugs that induce immunogenic cell death, targeted inhibitors, natural products, and ICIs. Nanoparticles can accumulate in tumors through the enhanced permeability and retention (EPR) effect. This effect results from tumor vasculature.[155] However, the magnitude of the EPR is heterogeneous and frequently less pronounced in patients than in murine models. Accordingly, active targeting (e.g., ligand- or antibody-mediated binding to tumor or stromal markers) and stimulus-responsive release (pH-, redox-, or enzyme-triggered systems) are commonly explored to enhance tumor retention. Specific polymers in nanoparticles can provide controlled release within the acidic or enzyme-rich tumor stroma. This can amplify local killing and prevent severe side effects in normal tissues.[156] Nanocarriers also enable the co-delivery of combination payloads (e.g., antigen plus immune stimulant or chemotherapy plus immunomodulator). These formulations can carry ECM-degrading enzymes or stromal-targeting moieties that reduce tumor rigidity and improve drug penetration into the stroma of the tumor.[157,158] These properties provide opportunities for remodeling the TIME in different malignancies, including CRC.

Nanoformulations have been investigated to improve the pharmacological performance of poorly soluble natural products and support immune activation by various immunomodulators. For example, delivery systems such as liposomes, micelles, gold nanoparticles, and polymeric or chitosan nanoparticles have been used to increase the efficacy of curcumin against CRC.[159] In an SW620 xenograft model, gold nanoparticles reduced α-SMA$^+$ CAF features and ECM-associated mediators, including collagen I and CTGF. Nanoparticles also inhibited TGF-β1 and VEGF, which was associated with improved vascular function and reduced hypoxia.[160] Several nanomedicine systems have also been designed to reprogram suppressive myeloid programs in immune-competent CRC models. A study by Mao et al. developed lentinan-ursolic acid polymeric nanomedicine to target CRC in mice. This nanocomplex promoted M2 to M1 polarization by inducing ICD in the tumor. In addition, mannose-modified erythrocyte membrane-wrapped nanoparticles loaded with artesunate and chloroquine repolarized M2 TAMs to M1 TAMs, thereby inhibiting the immunosuppressive effects of TAMs.[161] Aptamer-conjugated-2c-loaded nanoparticles (Apt-2cNP) and retinoic acid-loaded PLGA nanoparticles are other nanocomplexes that have been utilized to modulate TAM polarization within colorectal TME by inhibiting NF-κB/STAT3 signaling pathway associated with M2 polarization.[162,163]

Despite these opportunities, the translation of immuno-nanomedicine faces substantial barriers. The major challenges include variable tumor delivery (due to tumor heterogeneity), uptake by the reticuloendothelial system, and potential immunotoxicity. Furthermore, challenges in scalable manufacturing, reproducibility, and regulatory characterization add another layer of barriers to the clinical translation of nanoparticles. Therefore, comparative reporting of carrier composition, physicochemical properties, pharmacokinetics, toxicity, and model quality is essential for each type of nanomedicine. The results of important factors and modulatory effects of different nanoparticles for remodeling antitumor immunity in CRC are summarized in Table 2. (Table 2)

Table 2. Comparison of Nanoparticle-Based Immunomodulatory Strategies in CRC Preclinical Models

Formulation & Targeting	Mechanism of Action	In Vivo Model & Efficacy	PK Profile	Safety & Manufacturing	Ref.
M1EVs + OXA, RA, LF (Intrinsic EV homing)	M2→M1 repolarization; STAT3/NF-κB/AKT↓; ↑$CD8^+$ T cells; ↓IL-10, PD-L1; ↓MDR1/survivin	CT-26 syngeneic (flank + peritoneal); ↓ primary tumor & metastases; ↑ apoptosis	Not reported	Safety: Basic H&E only. Manufacturing: Ultracentrifugation	[164]
Nanocurcumin + resveratrol (320 nm) + mEHT	ICD via HSP70/CRT; ↑$CD3^+$ T cells & $F4/80^+$ macrophages; apoptosis activation	CT-26 syngeneic (flank); ↓ tumor volume & weight; ↑ T-cell infiltration	Mean concentration: CUR nanoparticle: 7.85 vs 0.7 ng/mL for free at 1 h; and 646 for resveratrol nanoparticlevs 76 ng/mL for free at 15 min	Safety: Not reported Manufacturing: Physical grinding	[165]
PLGA + Betulinic acid analogue (2c) (EpCAM DNA aptamer)	↑M1 macrophages; ↑$CD8^+$ T cells; ↑Bax/caspase-3; ↓Bcl-2; ↓NF-κB	DMH-induced carcinogen model (immunocompetent); ↓ tumor burden (67.5%); ↑ tumor accumulation	$T_1/_2$: 36 h (vs 6 h free); AUC: increased by 2.58 fold	Safety: Hemolysis <5%; liver/kidney histology improved in H&E study. Manufacturing: PLGA (FDA-approved); aptamer costly. Regulatory: Clear path for PLGA.	[166]
AuNPs (15 nm) + Cisplatin	Vessel decompression: ↓CAFs, ↓collagen I, ↓TGF-β/VEGF;	SW620 xenograft (BALB/c nude) – immunodeficient (major limitation); ↓	AuNP PK not reported; enhanced cisplatin	Safety: No weight loss; no organ injury in H&E staining. Manufacturing: Simple 15 nm synthesis; scalable. Regulatory:	[167]

	↑ perfusion; ↓hypoxia; enhances cisplatin delivery	tumor growth; perfusion ↑23→43%	accumulation in the tumor	AuNPs have FDA precedent.	
PLGA-lecithin-PEG + Curcumin (EpCAM RNA aptamer)	Targeted endocytosis → apoptosis; ↑ intracellular curcumin	In vitro only (HT29/HEK293T); no in vivo efficacy data (major gap)	$T_1/_2$: ~5.93 h (vs 1.07 h free); AUC enhanced by 2.2 fold; sustained release 24 h	Safety: Not reported (PK only in healthy rats). Manufacturing: Nanoprecipitation; RNA aptamer costly but scalable. Regulatory: No in vivo data limits regulatory path.	[168]
LNT-UA (carrier-free; LNT as carrier + immunoadjuvant)	UA → ICD; LNT → DC maturation & M2→M1 repolarization; ↑$CD8^+/CD4^+$; ↑IFN-γ/TNF-α; ↓IL-10	CT-26 syngeneic (flank + bilateral); AOM/DSS spontaneous CRC (best model); ↓ primary & distant tumors; 2.2× survival extension	Not quantified; oral bioavailability assumed improved	Safety: No weight loss; normal ALT/AST/BUN/creatinine; H&E staining. Manufacturing: Simple nanoprecipitation. Regulatory: LNT/UA are GRAS; path as botanical supplement simpler.	[161]
Cholesterol-coated PLGA + RA (Cholesterol membrane targeting)	IL-10R/STAT3/NF-κB↓; M2-TAM repolarization; ↓EMT; ↓PD-L1; synergy with αPD-L1	CT-26 syngeneic (flank) + mice model; ↓ tumor growth & metastasis	Not reported	Safety: Assumed standard. Manufacturing: PLGA (FDA-approved); cholesterol/RA approved; simple formulation. Regulatory: Highly feasible; all components approved.	[169]

Abbreviations: AUC, area under the curve; CAFs, cancer-associated fibroblasts; CUR, curcumin; DC, dendritic cell; DMH, dimethylhydrazine; EPR, enhanced permeability and retention; GMP, Good Manufacturing Practice; GRAS, generally recognized as safe; ICD, immunogenic cell death; LNT, lentinan; mEHT, modulated electro-hyperthermia; M1EVs, M1 macrophage-derived extracellular vesicles; OXA, oxaliplatin; PLGA, poly(lactic-co-glycolic acid); RA, retinoic acid; SEC, size-exclusion chromatography; TMA, tissue microarray; UA, ursolic acid.

Preclinical evidence for nanoparticle-based immunomodulation demonstrates considerable promise. However, significant translational gaps remain in this field. Among the formulations mentioned in Table 2, M1 macrophage-derived extracellular vesicles, lentinan-ursolic acid, and PLGA-betulinic acid exhibited the most robust immunological activity. Pharmacokinetic profiling remains a critical limitation in this field. Future studies should focus on pharmacodynamic and safety profile factors.

Limitations and future perspectives

Many findings on CRC microenvironment targeting are based on cell lines or short-term murine experiments. However, clinical validation of these models is limited. Preclinical models cannot replicate the complexity of human stroma. This issue is a serious limitation in translating these findings into clinical practice. The reported effects are also sensitive to model choice, dosing schedule, and tumor implantation site. Consequently, the apparent consistency across studies may reflect methodological similarity rather than biological robustness.[170] Publication bias is an additional concern. Negative or neutral findings are rarely reported in detail.[171] This distorts the perceived efficacy of the strategies discussed above. Therefore, the strength of evidence differs markedly between intervention classes. For instance, ICIs in dMMR/MSI-H tumors have been adopted clinically and show durable activity in prospective CRC trials.[172] However, most stromal, myeloid, and metabolic modulation strategies remain preclinical or early phase. Furthermore, natural products and nanoformulations are predominantly at the discovery stage.[173] Therefore, we should weigh these differences by comparing different approaches. Uniform reporting of model type, exposure, comparator, and immune endpoints would improve their comparability. Without such standardization, translational prioritization remains subjective.

Patient selection and biomarker gaps are other important issues. Molecular stratification is essential for interpreting the immunotherapeutic potential in CRC. Mismatch repair status remains the dominant clinical biomarker in CRC. POLE/POLD1 alterations may extend the benefit to selected MSS tumors. Tumor mutational burden adds information.[174] However, considering assay thresholds are inconsistent at this moment. Anatomical variables also influence the response to therapy. These differences may suggest diverse treatment options for tumors at different locations. Liver metastases, prior chemotherapy, and radiotherapy alter immune responses.[175] These variables are unevenly reported in published studies. However, all of these factors can significantly affect the response of CRC to anticancer modalities. Emerging spatial and single-cell analyses of human CRC have revealed significant heterogeneity across the CRC stroma. These findings demonstrate that CAFs, TAMs, and MDSCs occupy specific tumor neighborhoods. On the other hand, CTLs are often confined to peripheral regions.[176,177] This unique organization of stromal and immune cells may explain resistance despite adequate systemic drug exposure. However, spatial assays have not yet been standardized for routine clinical use. Cost, throughput, and interpretation remain other important barriers. Simplified multiplex panels may prove more feasible. Prospective validation is required before spatial metrics can guide therapy selection or trial enrollment in CRC.[178]

Stromal and ECM-directed strategies face significant challenges. Targeting CAFs and ECM remains conceptually attractive. However, the clinical translation of these strategies is complex. Fibroblast populations in the tumor stroma are heterogeneous and have diverse functions. Some CAF subsets prevent tumor progression. These subsets promote antigen presentation and CTL activation. Therefore, broad depletion of CAFs may be harmful.[179] Marker-based targeting of these cells is also imperfect because FAP and α-SMA are expressed in normal tissues. ECM modification carries additional risks. Reducing tumor stiffness can improve perfusion and drug delivery. However, it may simultaneously facilitate invasion or dissemination.[180] The timing and degree of remodeling are likely critical. Similar concerns exist regarding pathway inhibition. For instance, TGF-β signaling regulates immunity, epithelial homeostasis, and cardiovascular function. Therefore, sustained systemic blockade can cause serious side effects.[181] Localized or tumor-restricted delivery of TGF-β may mitigate these side effects. However, evidence for such approaches in CRC is limited. Furthermore, most stromal interventions have been evaluated as

monotherapies in preclinical studies. The efficacy of these interventions within multimodal regimens is poorly defined. Sequencing relative to chemotherapy, radiotherapy, or ICIs requires systematic studies rather than empirical combinations.

Suppressive myeloid cells are also compelling targets in CRC. However, the translation has been disappointing. CSF-1R inhibition efficiently reduces TAMs. However, compensatory recruitment of TAMs or MDSCs through alternative chemokine axes reduces the efficacy of this strategy. Myeloid plasticity further complicates the interpretation.[182] Treg targeting raises different concerns. Systemic depletion may precipitate autoimmune toxicity. CTLA-4–directed antibodies illustrate this trade-off, particularly in combination regimens.[183] Selective approaches, such as CCR8-directed depletion, appear more attractive mechanistically. However, clinical findings on this strategy are limited.[184] Pharmacodynamic monitoring is another unresolved issue. Peripheral measurements do not necessarily capture the multi-compartment tumor–stroma–immune interactions that determine intratumoral function and repeated on-treatment tumor biopsies are difficult to scale in routine practice.[185]

Nanomedicine-based approaches have been proposed to overcome stromal barriers. However, the clinical translation of these approaches is constrained by variable tumor accumulation, reticuloendothelial clearance, and potential immunotoxicity. Manufacturing complexity is an additional obstacle. In addition, regulatory characterization requirements are correspondingly demanding.[186] Similar considerations apply to natural products. However, poor solubility, extensive metabolism, and inconsistent standardization limit their reproducibility. The interaction potential with anticancer agents is insufficiently characterized.[173] Consequently, both approaches require rigorous pharmacokinetic and toxicological evaluations before efficacy claims can be substantiated in patients with CRC.

The progress of current approaches will depend on disciplined prioritization rather than broader exploration. Near-term opportunities are relatively evident. Checkpoint blockade has shown promising results and should continue to be optimized for dMMR/MSI-H CRC. Combination strategies for MSS tumors should be considered with regard to specific barriers. For instance, specific strategies should be considered for myeloid-rich or stromal-rich tumors.[187] In addition, particular combinations may be needed for vascular normalization.[188] These hypotheses should be tested prospectively. The trial design must evolve according to these challenges and perspectives. Biomarker-defined cohorts can overcome challenges by reducing heterogeneity. Furthermore, biomarker-based strategies can facilitate adaptive therapies to refine treatment doses and sequences.[189] Preclinical standards also require improvements. Immunocompetent, orthotopic, and metastatic models should be prioritized. Patient-derived systems incorporating immune and stromal components deserve wider adoption in the future. The reporting of exposure, toxicity, and comparator selection should be routine in these studies.[170,190] Finally, translational success will require realistic framing of the results. Microenvironment modulation is unlikely to produce durable responses alone. Integrating pharmacology, formulation science, and immunological biomarkers can provide the most credible route to meaningful clinical benefits in CRC.

Conclusion

CRC remains a therapeutically challenging disease, particularly in tumors with an immunosuppressive microenvironment. In such settings, CAF/ECM remodeling, suppressive myeloid programs, Tregs, hypoxia, and metabolic remodeling converge to limit CTLs and NK cell function. These features also reduce drug penetration

and blunt responses to chemotherapy, targeted therapy, and ICIs. The strength of evidence supporting TIME-modulating strategies in CRC is uneven and should guide prioritization. The most mature and clinically validated approach is immune checkpoint blockade in dMMR/MSI-H CRC. Clinical trials and combination regimens (e.g., PD-1 ± CTLA-4 blockade) have confirmed that this strategy can provide durable responses in patients. In contrast, most interventions aimed at stromal remodeling (CAF/ECM), myeloid reprogramming (TAM/MDSC), adenosine/metabolic pathways, and hypoxia remain supported mainly by preclinical studies or early-phase trials. Accordingly, these strategies should be interpreted as hypothesis-generating unless corroborated by biomarker-stratified clinical data. Some strategies can also be suggested as near-term clinical options. These options either have regulatory approval in defined CRC subsets or rely on agents with established clinical availability that can be rationally combined. According to the reviewed studies, biomarker-guided ICI use in dMMR/MSI-H tumors can be suggested as a promising option for CRC patients. In addition, combination strategies for pMMR/MSS CRC that aim to convert cold tumors into hot tumors can be classified into this category. Chemotherapy or radiotherapy to trigger inflammatory responses, anti-angiogenic therapy for vascular normalization, and carefully selected ICIs in combination with stroma/myeloid targeting are promising approaches. Immune-priming approaches with early human safety signals, including selected therapeutic vaccine platforms in molecularly defined settings, can be suggested as another option. Importantly, success in MSS CRC will likely require matching combinations to the dominant resistance mechanisms.

Several approaches have shown limited clinical translation, despite a strong mechanistic rationale. Monotherapy with immune checkpoint blockade is largely ineffective in pMMR/MSS CRC. In addition, depletion or broad inhibition of MDSCs or TAMs (e.g., CSF-1/CSF-1R-axis strategies) has not consistently produced durable benefits. This highlights pathway redundancy, compensatory recruitment, and diverse TAM functions. Similarly, although aggressive ECM-modifying strategies may improve drug perfusion, they may trigger invasion, side effects, or unpredictable stromal remodeling. These findings emphasize that TIME targets should be prioritized not only by biological plausibility but also by human feasibility, safety margins, and biomarker-defined vulnerability.

Other strategies remain in the early stage or preclinical phases. Selective stromal reprogramming, spatially informed strategies that disrupt CAF–myeloid suppressive neighborhoods, and targeting of adenosine, lactate transports, and hypoxia-associated pathways can be classified into this category. Furthermore, nanomedicine-based approaches can be suggested as another example that has been designed to improve intratumoral exposure and boost immune priming by ICIs. These approaches remain promising but require rigorous validation in immunocompetent and clinically relevant CRC models. These strategies face remarkable challenges, such as the need for standardized immune endpoints and careful toxicology and manufacturability assessments prior to clinical translation. Natural products and nutraceutical candidates have shown immunomodulatory activity in experimental CRC models. However, their clinical relevance is limited by bioavailability, variability in formulation, and limited controlled trial data.

In summary, advancing CRC immunotherapy will require biomarker‐stratified and mechanism‐matched combinations. Integration of human single‐cell/spatial evidence to identify dominant suppressive niches is essential for targeting the TIME. In addition, development programs that explicitly address pharmacokinetics, toxicity, and translational feasibility are crucial for future preclinical studies and clinical trials. Such a

prioritization framework can separate clinically actionable strategies from early exploratory concepts and may accelerate durable immune control in CRC.

Competing Interests

The authors declare no conflict of interests.

Ethical Approval

Not applicable.

Funding

No funds, grants, or other support was received

Data availability statements

No new data has been created in this study

Authors contributions

Conceptualization: Qamar Abuhassan, Usamah Sayed, Priya Priyadarshini Nayak
Supervision: Priya Priyadarshini Nayak, Usamah Sayed
Writing–original draft: Qamar Abuhassan, Usamah Sayed, Pareshkumar N. Patel, S. Renuka Jyothi, Gunjan Singh, Priya Priyadarshini Nayak, Vimal Arora, and Gulnora Shakhmurova.
Writing–review & editing: Priya Priyadarshini Nayak and Usamah Sayed. All authors approved final manuscript before submission.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-263. doi: 10.3322/caac.21834.
2. Rentsch M, Schiergens T, Khandoga A, Werner J. Surgery for Colorectal Cancer - Trends, Developments, and Future Perspectives. Visc Med. 2016;32(3):184-91. doi: 10.1159/000446490.
3. Neugut AI, Lin A, Raab GT, Hillyer GC, Keller D, O'Neil DS, Accordino MK, Kiran RP, Wright J, Hershman DL. FOLFOX and FOLFIRI Use in Stage IV Colon Cancer: Analysis of SEER-Medicare Data. Clin Colorectal Cancer. 2019;18(2):133-140. doi: 10.1016/j.clcc.2019.01.005.
4. Allegra CJ, Yothers G, O'Connell MJ, Beart RW, Wozniak TF, Pitot HC, et al. Neoadjuvant 5-FU or Capecitabine Plus Radiation With or Without Oxaliplatin in Rectal Cancer Patients: A Phase III Randomized Clinical Trial. J Natl Cancer Inst. 2015;107(11):djv248. doi: 10.1093/jnci/djv248.
5. Liu X, Ou K, Ma X, Gao L, Wang Q, Zhang H, Yang L. Safety and efficacy of irinotecan, oxaliplatin, and capecitabine (XELOXIRI) regimen with or without targeted drugs in patients with metastatic colorectal cancer: a retrospective cohort study. BMC Cancer. 2022;22(1):807. doi: 10.1186/s12885-022-09889-3.
6. Ding C, Li L, Yang T, Fan X, Wu G. Combined application of anti-VEGF and anti-EGFR attenuates the growth and angiogenesis of colorectal cancer mainly through suppressing AKT and ERK signaling in mice model. BMC Cancer. 2016;16(1):791. doi: 10.1186/s12885-016-2834-8.
7. Zhou J, Ji Q, Li Q. Resistance to anti-EGFR therapies in metastatic colorectal cancer: underlying mechanisms and reversal strategies. J Exp Clin Cancer Res. 2021;40(1):328. doi: 10.1186/s13046-021-02130-2.

8. Yuan S, Tao F, Zhang X, Zhang Y, Sun X, Wu D. Role of Wnt/β-Catenin Signaling in the Chemoresistance Modulation of Colorectal Cancer. Biomed Res Int. 2020 18;2020:9390878. doi: 10.1155/2020/9390878.

9. Wang R, Su Q, Yan ZP. Reconsideration of recurrence and metastasis in colorectal cancer. World J Clin Cases. 2021;9(24):6964-6968. doi: 10.12998/wjcc.v9.i24.6964.

10. Chen Y, Zheng X, Wu C. The Role of the Tumor Microenvironment and Treatment Strategies in Colorectal Cancer. Front Immunol. 2021 2;12:792691. doi: 10.3389/fimmu.2021.792691.

11. Liu JL, Yang M, Bai JG, Liu Z, Wang XS. "Cold" colorectal cancer faces a bottleneck in immunotherapy. World J Gastrointest Oncol. 2023;15(2):240-250. doi: 10.4251/wjgo.v15.i2.240.

12. Ros J, Balconi F, Baraibar I, Saoudi Gonzalez N, Salva F, Tabernero J, Elez E. Advances in immune checkpoint inhibitor combination strategies for microsatellite stable colorectal cancer. Front Oncol. 2023;13:1112276. doi: 10.3389/fonc.2023.1112276.

13. Wang L, Geng H, Liu Y, et al. Hot and cold tumors: Immunological features and the therapeutic strategies. MedComm 2023;4(5):e343. doi: 10.1002/mco2.343.

14. Kotsiliti E. Origin of CAFs in colorectal cancer. Nat Rev Gastroenterol Hepatol. 2022;19(2):79. doi: 10.1038/s41575-021-00573-8.

15. Sandberg TP, Stuart MP, Oosting J, Tollenaar RA, Sier CF, Mesker WE. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. BMC cancer 2019;19(1):284. doi: 10.1186/s12885-019-5462-2.

16. Wang X, Qu Y, Ji J, et al. Colorectal cancer cells establish metabolic reprogramming with cancer-associated fibroblasts (CAFs) through lactate shuttle to enhance invasion, migration, and angiogenesis. Int Immunopharmacol. 2024;143(Pt 2):113470. doi: 10.1016/j.intimp.2024.113470.

17. Nakai N, Hara M, Takahashi H, et al. Cancer cell-induced tissue inhibitor of metalloproteinase-1 secretion by cancer-associated fibroblasts promotes cancer cell migration. Oncol Rep. 2022;47(6):112. doi: 10.3892/or.2022.8323.

18. Li J, Li L, Li Y, et al. Tumor-associated macrophage infiltration and prognosis in colorectal cancer: systematic review and meta-analysis. Int J Colorectal Dis. 2020;35(7):1203-1210. doi: 10.1007/s00384-020-03593-z.

19. Wang H, Tian T, Zhang J. Tumor-associated macrophages (TAMs) in colorectal cancer (CRC): from mechanism to therapy and prognosis. Int J Mol Sci. 2021;22(16):8470. doi: 10.3390/ijms22168470.

20. Ge Z, Ding S. The crosstalk between tumor-associated macrophages (TAMs) and tumor cells and the corresponding targeted therapy. Front Oncol. 2020:10:590941. doi: 10.3389/fonc.2020.590941

21. Olguin JE, Medina-Andrade I, Rodriguez T, Rodriguez-Sosa M, Terrazas LI. Relevance of regulatory T cells during colorectal cancer development. Cancers 2020;12(7):1888. doi: 10.3390/cancers12071888.

22. Zeng W, Liu H, Mao Y, et al. Myeloid‐derived suppressor cells: Key immunosuppressive regulators and therapeutic targets in colorectal cancer. Int J Oncol. 2024;65(3):85. doi: 10.3892/ijo.2024.5673.

23. He J, Chai X, Zhang Q, et al. The lactate receptor HCAR1 drives the recruitment of immunosuppressive PMN-MDSCs in colorectal cancer. Nat Immunol. 2025;26(3):391-403. doi: 10.1038/s41590-024-02068-5

24. Glaire MA, Domingo E, Sveen A, et al. Tumour-infiltrating CD8+ lymphocytes and colorectal cancer recurrence by tumour and nodal stage. Br J Cancer. 2019;121(6):474-482. doi: 10.1038/s41416-019-0540-4.

25. Saleh R, Taha RZ, Toor SM, et al. Expression of immune checkpoints and T cell exhaustion markers in early and advanced stages of colorectal cancer. Cancer Immunol Immunother. 2020;69(10):1989-1999. doi: 10.1007/s00262-020-02593-w.

26. Liang R, Zhu X, Lan T, et al. TIGIT promotes CD8+ T cells exhaustion and predicts poor prognosis of colorectal cancer. Cancer Immunol Immunother. 2021;70(10):2781-2793. doi: 10.1007/s00262-021-02886-8.

27. Ding J-T, Yang K-P, Zhou H-N, Huang Y-F, Li H, Zong Z. Landscapes and mechanisms of CD8+ T cell exhaustion in gastrointestinal cancer. Front Immunol. 2023 25:14:1149622. doi: 10.3389/fimmu.2023.1149622.

28. Krijgsman D, de Vries NL, Skovbo A, et al. Characterization of circulating T-, NK-, and NKT cell subsets in patients with colorectal cancer: the peripheral blood immune cell profile. Cancer Immunol Immunother. 2019;68(6):1011-1024. doi: 10.1007/s00262-019-02343-7.

29. Vimalraj S. A concise review of VEGF, PDGF, FGF, Notch, angiopoietin, and HGF signalling in tumor angiogenesis with a focus on alternative approaches and future directions. Int J Biol Macromol. 2022:221:1428-1438. doi: 10.1016/j.ijbiomac.2022.09.129.

30. Najafi M, Mortezaee K, Majidpoor J. Stromal reprogramming: A target for tumor therapy. Life Sci. 2019;239:117049. doi: 10.1016/j.lfs.2019.117049.

31. Tuomisto A, Garcia-Solano J, Sirniö P, et al. HIF-1α expression and high microvessel density are characteristic features in serrated colorectal cancer. Virchows Archiv. 2016;469(4):395-404. doi: 10.1007/s00428-016-1988-8.

32. Sun J, Zhao Z, Lu J, et al. The tumor microenvironment mediates the HIF-1α/PD-L1 pathway to promote immune escape in colorectal Cancer. Int J Mol Sci. 2024;25(7):3735. doi: 10.3390/ijms25073735.

33. Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. J Immunother Cancer. 2018;6(1):57. doi: 10.1186/s40425-018-0360-8.

34. Gharzeddine K, Prieto CG, Malier M, et al. Metabolic reprogramming of hypoxic tumor-associated macrophages through CSF-1R targeting favors treatment efficiency in colorectal cancers. J Immunother Cancer. 2024;12(9):e009602. doi: 10.1136/jitc-2024-009602.

35. Graboń W, Otto-Ślusarczyk D, Chrzanowska A, et al. Lactate formation in primary and metastatic colon cancer cells at hypoxia and normoxia. Cell Biochem Funct. 2016;34(7):483-490. doi: 10.1002/cbf.3211.

36. Bertelsen CA, Neuenschwander AU, Jansen JE, et al. 5-year outcome after complete mesocolic excision for right-sided colon cancer: a population-based cohort study. Lancet Oncol. 2019;20(11):1556-1565. doi: 10.1016/S1470-2045(19)30485-1.

37. Adebayo AS, Agbaje K, Adesina SK, Olajubutu O. Colorectal cancer: disease process, current treatment options, and future perspectives. Pharmaceutics. 2023;15(11):2620. doi: 10.3390/pharmaceutics15112620.

38. Baratelli C, Zichi C, Di Maio M, et al. A systematic review of the safety profile of the different combinations of fluoropyrimidines and oxaliplatin in the treatment of colorectal cancer patients.
Crit Rev Oncol Hematol. 2018:122:21-29. doi: 10.1016/j.critrevonc.2017.12.010.

39. Baik SH, Kim NK, Lee KY, Sohn SK, Cho CH. Analysis of anal sphincter preservation rate according to tumor level and neoadjuvant chemoradiotherapy in rectal cancer patients. J Gastrointest Surg. 2008;12(1):176-82. doi: 10.1007/s11605-007-0254-4.

40. Khali KA, Musalm HS, Hassan MA, Mahmoud IA. Triplet (FOLFOXIRI) versus doublet (FOLFOX or FOLFIRI) regimen as first line treatment in metastatic colorectal carcinoma, a prospective phase II, randomized controlled trial. Asian Pac J Cancer Prev. 2022;23(10):3421-3429. doi: 10.31557/APJCP.2022.23.10.3421.

41. Plundrich D, Chikhladze S, Fichtner-Feigl S, Feuerstein R, Briquez PS. Molecular mechanisms of tumor immunomodulation in the microenvironment of colorectal cancer. Int J Mol Sci. 2022;23(5):2782. doi: 10.3390/ijms23052782.

42. Bains SJ, Abrahamsson H, Flatmark K, et al. Immunogenic cell death by neoadjuvant oxaliplatin and radiation protects against metastatic failure in high-risk rectal cancer. Cancer Immunol Immunother. 2020;69(3):355-364. doi: 10.1007/s00262-019-02458-x.

43. Limagne E, Thibaudin M, Nuttin L, et al. Trifluridine/tipiracil plus oxaliplatin improves PD-1 blockade in colorectal cancer by inducing immunogenic cell death and depleting macrophages. Cancer Immunol Res. 2019;7(12):1958-1969. doi: 10.1158/2326-6066.CIR-19-0228.

44. Mezheyeuski A, Hrynchyk I, Herrera M, et al. Stroma-normalised vessel density predicts benefit from adjuvant fluorouracil-based chemotherapy in patients with stage II/III colon cancer. Br J Cancer. 2019;121(4):303-311. doi: 10.1038/s41416-019-0519-1.

45. Liu Y-X, Le K-J, Zhang C, et al. A fatal myelosuppression, diarrhea and neurotoxicity induced by combination of irinotecan and tegafur-gimeracil-oteracil potassium in the treatment of colon cancer: A case report. Transl Cancer Res. 2020;9(1):382-387. doi: 10.21037/tcr.2019.11.39.

46. Jingu K, Matsushita H, Yamamoto T, et al. Stereotactic radiotherapy for pulmonary oligometastases from colorectal cancer: a systematic review and meta-analysis. Technol Cancer Res Treat. 2018:17:1533033818794936. doi: 10.1177/1533033818794936.

47. Kobiela J, Spychalski P, Marvaso G, et al. Ablative stereotactic radiotherapy for oligometastatic colorectal cancer: systematic review. Crit Rev Oncol Hematol. 2018:129:91-101. doi: 10.1016/j.critrevonc.2018.06.005.

48. Seyedin SN, Hasibuzzaman M, Pham V, et al. Combination therapy with radiation and PARP inhibition enhances responsiveness to anti-PD-1 therapy in colorectal tumor models. Int J Radiat Oncol Biol Phys. 2020;108(1):81-92. doi: 10.1016/j.ijrobp.2020.01.030.

49. Wu C, Shao Y, Gu W. Immunotherapy combined with radiotherapy to reverse immunosuppression in microsatellite stable colorectal cancer. Clin Transl Oncol. 2023;25(7):1916-1928. doi: 10.1007/s12094-023-03091-y.

50. Fok M, Hill R, Fowler H, et al. Enhancing radiotherapy outcomes in rectal cancer: A systematic review of targeting hypoxia-induced radioresistance. Clin Transl Radiat Oncol. 2023:44:100695. doi: 10.1016/j.ctro.2023.100695.

51. Pavlidis ET, Pavlidis TE. Role of bevacizumab in colorectal cancer growth and its adverse effects: a review. World J Gastroenterol. 2013;19(31):5051-60. doi: 10.3748/wjg.v19.i31.5051.

52. Da Silva WC, De Araujo VE, Lima EMeA, et al. Comparative effectiveness and safety of monoclonal antibodies (bevacizumab, cetuximab, and panitumumab) in combination with chemotherapy for metastatic colorectal cancer: a systematic review and meta-analysis. BioDrugs. 2018;32(6):585-606. doi: 10.1007/s40259-018-0322-1.

53. Suh KJ, Sung JH, Kim JW, et al. EGFR or HER2 inhibition modulates the tumor microenvironment by suppression of PD-L1 and cytokines release. Oncotarget. 2017;8(38):63901-63910. doi: 10.18632/oncotarget.19194.

54. Wang Z, Cheng S, Yao Y, et al. Long-term survivals of immune checkpoint inhibitors as neoadjuvant and adjuvant therapy in dMMR/MSI-H colorectal and gastric cancers. Cancer Immunol Immunother. 2024;73(9):182. doi: 10.1007/s00262-024-03764-9.

55. Shan J, Han D, Shen C, Lei Q, Zhang Y. Mechanism and strategies of immunotherapy resistance in colorectal cancer. Front Immunol. 2022:13:1016646. doi: 10.3389/fimmu.2022.1016646.

56. Anderson P, Aptsiauri N, Ruiz-Cabello F, Garrido F. HLA class I loss in colorectal cancer: implications for immune escape and immunotherapy. Cell Mol Immunol 2021;18(3):556-565. doi:10.1038/s41423-021-00634-7

57. Grasso CS, Giannakis M, Wells DK, et al. Genetic Mechanisms of Immune Evasion in Colorectal Cancer. Cancer Discov 2018;8(6):730-749. doi:10.1158/2159-8290.Cd-17-1327

58. Tauriello DVF, Palomo-Ponce S, Stork D, et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature 2018;554(7693):538-543. doi:10.1038/nature25492

59. Calon A, Espinet E, Palomo-Ponce S, et al. Dependency of colorectal cancer on a TGF-β-driven program in stromal cells for metastasis initiation. Cancer Cell 2012;22(5):571-84. doi:10.1016/j.ccr.2012.08.013

60. Nie SC, Jing YH, Lu L, Ren SS, Ji G, Xu HC. Mechanisms of myeloid-derived suppressor cell-mediated immunosuppression in colorectal cancer and related therapies. World J Gastrointest Oncol 2024;16(5):1690-1704. doi:10.4251/wjgo.v16.i5.1690

61. Yu M, Guo G, Huang L, et al. CD73 on cancer-associated fibroblasts enhanced by the A(2B)-mediated feedforward circuit enforces an immune checkpoint. Nat Commun 2020;11(1):515. doi:10.1038/s41467-019-14060-x

62. Ambrosini M, Rousseau B, Manca P, et al. Immune checkpoint inhibitors for POLE or POLD1 proofreading-deficient metastatic colorectal cancer. Ann Oncol 2024;35(7):643-655. doi:10.1016/j.annonc.2024.03.009

63. Marques A, Cavaco P, Torre C, Sepodes B, Rocha J. Tumor mutational burden in colorectal cancer: Implications for treatment. Crit Rev Oncol Hematol 2024;197:104342. doi:10.1016/j.critrevonc.2024.104342

64. Zhang L, Zhao Y, Dai Y, et al. Immune Landscape of Colorectal Cancer Tumor Microenvironment from Different Primary Tumor Location. Front Immunol 2018;9:1578. doi:10.3389/fimmu.2018.01578

65. Yu J, Green MD, Li S, et al. Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. Nat Med 2021;27(1):152-164. doi:10.1038/s41591-020-1131-x

66. Liu C, Liu N, Zhang T, Tu Y. Adoptive immune cell therapy for colorectal cancer. Front Immunol. 2024:15:1433315. doi: 10.3389/fimmu.2024.1433315.

67. Jia W, Zhang T, Huang H, et al. Colorectal cancer vaccines: The current scenario and future prospects. Front Immunol. 2022:13:942235. doi: 10.3389/fimmu.2022.942235.

68. Cui J-Y, Ma J, Gao X-X, et al. Unraveling the role of cancer-associated fibroblasts in colorectal cancer. World J Gastrointest Oncol. 2024;16(12):4565-4578. doi: 10.4251/wjgo.v16.i12.4565.

69. Koncina E, Nurmik M, Pozdeev VI, et al. IL1R1+ cancer-associated fibroblasts drive tumor development and immunosuppression in colorectal cancer. Nat Commun. 2023;14(1):4251. doi:10.1038/s41467-023-39953-w

70. Guillén Díaz-Maroto N, Sanz-Pamplona R, Berdiel-Acer M, et al. Noncanonical TGFβ Pathway Relieves the Blockade of IL1β/TGFβ-Mediated Crosstalk between Tumor and Stroma: TGFBR1 and TAK1 Inhibition in Colorectal Cancer. Clin Cancer Res. 2019;25(14):4466-4479. doi:10.1158/1078-0432.CCR-18-3957

71. Ford K, Hanley CJ, Mellone M, et al. NOX4 Inhibition Potentiates Immunotherapy by Overcoming Cancer-Associated Fibroblast-Mediated CD8 T-cell Exclusion from Tumors. Cancer Res. 2020;80(9):1846-1860. doi:10.1158/0008-5472.CAN-19-3158

72. Li Z, Zhou J, Zhang J, Li S, Wang H, Du J. Cancer-associated fibroblasts promote PD-L1 expression in mice cancer cells via secreting CXCL5. Int J Cancer 2019;145(7):1946-1957. doi:https://doi.org/10.1002/ijc.32278

73. Yang K, Zhang J, Bao C. Exosomal circEIF3K from cancer-associated fibroblast promotes colorectal cancer (CRC) progression via miR-214/PD-L1 axis. BMC Cancer 2021;21(1):933. doi:10.1186/s12885-021-08669-9

74. Zhang M, Shi R, Guo Z, He J. Cancer-associated fibroblasts promote cell growth by activating ERK5/PD-L1 signaling axis in colorectal cancer. Pathol Res Pract. 2020;216(4):152884. doi:https://doi.org/10.1016/j.prp.2020.152884

75. Yang YM, Kim J, Wang Z, et al. Metastatic tumor growth in steatotic liver is promoted by HAS2-mediated fibrotic tumor microenvironment. J Clin Invest 2025;135(7)doi:10.1172/jci180802

76. Baker AM, Bird D, Lang G, Cox TR, Erler JT. Lysyl oxidase enzymatic function increases stiffness to drive colorectal cancer progression through FAK. Oncogene 2013;32(14):1863-1868. doi:10.1038/onc.2012.202

77. Cui G, Deng S, Zhang B, et al. Overcoming the Tumor Collagen Barriers: A Multistage Drug Delivery Strategy for DDR1-Mediated Resistant Colorectal Cancer Therapy. Adv Sci. 2024;11(33):2402107. doi:https://doi.org/10.1002/advs.202402107

78. Di Martino JS, Akhter T, Bravo-Cordero JJ. Remodeling the ECM: implications for metastasis and tumor dormancy. Cancers 2021;13(19):4916. doi: 10.3390/cancers13194916.

79. Jiang H, Ge H, Shi Y, Yuan F, Yue H. CAFs secrete CXCL12 to accelerate the progression and cisplatin resistance of colorectal cancer through promoting M2 polarization of macrophages. Med Oncol. 2023;40(3):90. doi: 10.1007/s12032-023-01953-7.

80. Feng Y, Qiao S, Chen J, et al. M2-type macrophages and cancer-associated fibroblasts combine to promote colorectal cancer liver metastases. Onco Targets Ther. 2024:17:243-260. doi: 10.2147/OTT.S447502.

81. Shimizu D, Yuge R, Kitadai Y, et al. Pexidartinib and Immune Checkpoint Inhibitors Combine to Activate Tumor Immunity in a Murine Colorectal Cancer Model by Depleting M2 Macrophages Differentiated by Cancer-Associated Fibroblasts. Int J Mol Sci 2024;25(13)doi:10.3390/ijms25137001

82. Bess SN, Greening GJ, Rajaram N, Muldoon TJ. Macrophage-targeted anti-CCL2 immunotherapy enhances tumor sensitivity to 5-fluorouracil in a Balb/c-CT26 murine colon carcinoma model measured using diffuse reflectance spectroscopy. BMC Immunol. 2022;23(1):20. doi:10.1186/s12865-022-00493-5

83. Gordon SR, Maute RL, Dulken BW, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545(7655):495-499. doi:10.1038/nature22396

84. Zhao T, Luo Y, Sun Y, Wei Z. Characterizing macrophage diversity in colorectal malignancies through single-cell genomics. Review. Front Immunol. 2025; 21:16:1526668. doi:10.3389/fimmu.2025.1526668

85. Zhang J, Huang Y, Zhang M, et al. Spatial-Niche Perspective on the Heterogeneity and Functional Reprogramming of Tumor-Associated Macrophages in Digestive System Tumors. Cells 2026;15(13):1198. doi: 10.3390/cells15131198.

86. Li K, Shi H, Zhang B, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct Target Ther. 2021;6(1):362. doi: 10.1038/s41392-021-00670-9.

87. Haag GM, Springfeld C, Grün B, et al. Pembrolizumab and maraviroc in refractory mismatch repair proficient/microsatellite-stable metastatic colorectal cancer - The PICCASSO phase I trial. Eur J Cancer 2022;167:112-122. doi:10.1016/j.ejca.2022.03.017

88. Grauers Wiktorin H, Nilsson MS, Kiffin R, et al. Histamine targets myeloid-derived suppressor cells and improves the anti-tumor efficacy of PD-1/PD-L1 checkpoint blockade. Cancer Immunol Immunother 2019;68(2):163-174. doi:10.1007/s00262-018-2253-6

89. Liu Z, Xie Y, Xiong Y, et al. TLR 7/8 agonist reverses oxaliplatin resistance in colorectal cancer via directing the myeloid-derived suppressor cells to tumoricidal M1-macrophages. Cancer Lett 2020;469:173-185. doi:10.1016/j.canlet.2019.10.020

90. Campbell JR, McDonald BR, Mesko PB, et al. Fc-Optimized Anti-CCR8 Antibody Depletes Regulatory T Cells in Human Tumor Models. Cancer Res 2021;81(11):2983-2994. doi:10.1158/0008-5472.Can-20-3585

91. Chen Q, Shen M, Yan M, et al. Targeting tumor-infiltrating CCR8+ regulatory T cells induces antitumor immunity through functional restoration of CD4+ Tconvs and CD8+ T cells in colorectal cancer.
J Transl Med. 2024;22(1):709. doi: 10.1186/s12967-024-05518-8.

92. Villarreal DO, L'Huillier A, Armington S, et al. Targeting CCR8 Induces Protective Antitumor Immunity and Enhances Vaccine-Induced Responses in Colon Cancer. Cancer Res 2018;78(18):5340-5348. doi:10.1158/0008-5472.CAN-18-1119

93. Van Damme H, Dombrecht B, Kiss M, et al. Therapeutic depletion of CCR8(+) tumor-infiltrating regulatory T cells elicits antitumor immunity and synergizes with anti-PD-1 therapy. J Immunother Cancer 2021;9(2)doi:10.1136/jitc-2020-001749

94. Chang L-Y, Lin Y-C, Mahalingam J, et al. Tumor-Derived Chemokine CCL5 Enhances TGF-β–Mediated Killing of CD8+ T Cells in Colon Cancer by T-Regulatory Cells. Cancer Res 2012;72(5):1092-1102. doi:10.1158/0008-5472.CAN-11-2493

95. Ma X, Gao Y, Chen Y, et al. M2-Type Macrophages Induce Tregs Generation by Activating the TGF-β/Smad Signalling Pathway to Promote Colorectal Cancer Development. Onco Targets Ther. 2021;14(null):5391-5402. doi:10.2147/OTT.S336548

96. Miteva LD, Stanilov NS, Cirovski GM, Stanilova SA. Upregulation of Treg-Related Genes in Addition with IL6 Showed the Significant Role for the Distant Metastasis in Colorectal Cancer. Cancer Microenviron. 2017;10(1):69-76. doi:10.1007/s12307-017-0198-5

97. Scurr M, Pembroke T, Bloom A, et al. Low-dose cyclophosphamide induces antitumor T-cell responses, which associate with survival in metastatic colorectal cancer. Clin Cancer Res. 2017;23(22):6771-6780. doi: 10.1158/1078-0432.CCR-17-0895

98. Svensson H, Olofsson V, Lundin S, et al. Accumulation of CCR4+ CTLA-4hi FOXP3+CD25hi Regulatory T Cells in Colon Adenocarcinomas Correlate to Reduced Activation of Conventional T Cells. PLOS ONE 2012;7(2):e30695. doi:10.1371/journal.pone.0030695

99. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 2006;116(7):1935-45. doi:10.1172/jci27745

100. Marangoni F, Zhakyp A, Corsini M, et al. Expansion of tumor-associated Treg cells upon disruption of a CTLA-4-dependent feedback loop. Cell 2021;184(15):3998-4015. e19. doi: 10.1016/j.cell.2021.05.027.

101. Ha D, Tanaka A, Kibayashi T, et al. Differential control of human Treg and effector T cells in tumor immunity by Fc-engineered anti–CTLA-4 antibody. Proc Natl Acad Sci U S A. 2019;116(2):609-618.
doi: 10.1073/pnas.1812186116.

102. Selby MJ, Engelhardt JJ, Quigley M, et al. Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. Cancer Immunol Res. 2013;1(1):32-42. doi:10.1158/2326-6066.Cir-13-0013

103. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18(9):1182-1191. doi:10.1016/s1470-2045(17)30422-9

104. Chen EX, Jonker DJ, Loree JM, et al. Effect of combined immune checkpoint inhibition vs best supportive care alone in patients with advanced colorectal cancer: the Canadian Cancer Trials Group CO. 26 Study. JAMA Oncol. 2020;6(6):831-838. doi: 10.1001/jamaoncol.2020.0910.

105. Overman MJ, Lonardi S, Wong KYM, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol. 2018;36(8):773-779. doi:10.1200/jco.2017.76.9901

106. Xu K, Zhan Y, Yuan Z, et al. Hypoxia Induces Drug Resistance in Colorectal Cancer through the HIF-1α/miR-338-5p/IL-6 Feedback Loop. Mol Ther. 2019;27(10):1810-1824. doi:10.1016/j.ymthe.2019.05.017

107. Tang YA, Chen YF, Bao Y, et al. Hypoxic tumor microenvironment activates GLI2 via HIF-1α and TGF-β2 to promote chemoresistance in colorectal cancer. Proc Natl Acad Sci U S A. 2018;115(26):E5990-e5999. doi:10.1073/pnas.1801348115

108. Huntington KE, Louie A, Zhou L, Seyhan AA, Maxwell AW, El-Deiry WS. Colorectal cancer extracellular acidosis decreases immune cell killing and is partially ameliorated by pH-modulating agents that modify tumor cell cytokine profiles. Am J Cancer Res. 2022;12(1):138-151.

109. Andreucci E, Ruzzolini J, Peppicelli S, et al. The carbonic anhydrase IX inhibitor SLC-0111 sensitises cancer cells to conventional chemotherapy. J Enzyme Inhib Med Chem. 2019;34(1):117-123. doi:10.1080/14756366.2018.1532419

110. Wang X, Liu C, Wang J, Fan Y, Wang Z, Wang Y. Proton pump inhibitors increase the chemosensitivity of patients with advanced colorectal cancer. Oncotarget. 2017;8(35):58801-58808. doi:10.18632/oncotarget.18522

111. Yu M, Guo G, Huang L, et al. CD73 on cancer-associated fibroblasts enhanced by the A2B-mediated feedforward circuit enforces an immune checkpoint. Nat Commun. 2020;11(1):515.
doi: 10.1038/s41467-019-14060-x.

112. Deng A, Fan R, Hai Y, et al. A STING agonist prodrug reprograms tumor-associated macrophage to boost colorectal cancer immunotherapy. Theranostics. 2025;15(1):277-299. doi:10.7150/thno.101001

113. Kang J, Lee D, Lee KJ, et al. Tumor-Suppressive Effect of Metformin via the Regulation of M2 Macrophages and Myeloid-Derived Suppressor Cells in the Tumor Microenvironment of Colorectal Cancer. Cancers. 2022;14(12)doi:10.3390/cancers14122881

114. Gallio C, Esposito L, Passardi A. Therapeutic Cancer Vaccines in Colorectal Cancer: Platforms, Mechanisms, and Combinations. Cancers 2025;17(15):2582. doi: 10.3390/cancers17152582.

115. Pant S, Wainberg ZA, Weekes CD, et al. Lymph-node-targeted, mKRAS-specific amphiphile vaccine in pancreatic and colorectal cancer: the phase 1 AMPLIFY-201 trial. Nature Med. 2024;30(2):531-542. doi:10.1038/s41591-023-02760-3

116. Hecht JR, Goldman JW, Hayes S, et al. Abstract CT007: Safety and immunogenicity of a personalized neoantigen-Listeria vaccine in cancer patients. Cancer Res. 2019;79(13_Supplement):CT007-CT007. doi:10.1158/1538-7445.AM2019-CT007

117. Barve V, Adams N, Stanbery L, et al. Case Report: Marked Survival Advantage of Two Colorectal Cancer Patients with Liver Metastases Treated with Vigil and FOLFOX-6. Vaccines. 2021;9(10)doi:10.3390/vaccines9101201

118. Liu C, Papukashvili D, Dong Y, et al. Identification of Tumor Antigens and Design of mRNA Vaccine for Colorectal Cancer Based on the Immune Subtype. Original Research. Front Cell Dev Biol. 2022;Volume 9 - 2021doi:10.3389/fcell.2021.783527

119. Pant S, Wainberg ZA, Weekes CD, et al. Lymph-node-targeted, mKRAS-specific amphiphile vaccine in pancreatic and colorectal cancer: the phase 1 AMPLIFY-201 trial. Nat Med. 2024;30(2):531-542. doi:10.1038/s41591-023-02760-3

120. Wang Z-X, Peng J, Liang X, et al. First-line serplulimab in metastatic colorectal cancer: Phase 2 results of a randomized, double-blind, phase 2/3 trial. Med. 2024;5(9):1150-1163. e3. doi: 10.1016/j.medj.2024.05.009.

121. Antoniotti C, Borelli B, Rossini D, et al. AtezoTRIBE: a randomised phase II study of FOLFOXIRI plus bevacizumab alone or in combination with atezolizumab as initial therapy for patients with unresectable metastatic colorectal cancer. BMC Cancer. 2020;20(1):683. doi:10.1186/s12885-020-07169-6

122. Diaz LA, Jr., Shiu KK, Kim TW, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. Lancet Oncol. 2022;23(5):659-670. doi:10.1016/s1470-2045(22)00197-8

123. Andre T, Elez E, Van Cutsem E, et al. Nivolumab plus Ipilimumab in Microsatellite-Instability-High Metastatic Colorectal Cancer. N Engl J Med. 2024;391(21):2014-2026. doi:10.1056/NEJMoa2402141

124. Chen EX, Jonker DJ, Loree JM, et al. Effect of Combined Immune Checkpoint Inhibition vs Best Supportive Care Alone in Patients With Advanced Colorectal Cancer: The Canadian Cancer Trials Group CO.26 Study. JAMA Oncol. 2020;6(6):831-838. doi:10.1001/jamaoncol.2020.0910

125. Mettu NB, Ou FS, Zemla TJ, et al. Assessment of Capecitabine and Bevacizumab With or Without Atezolizumab for the Treatment of Refractory Metastatic Colorectal Cancer: A Randomized Clinical Trial. JAMA Netw Open. 2022;5(2):e2149040. doi:10.1001/jamanetworkopen.2021.49040

126. Bendell J, LoRusso P, Overman M, et al. First-in-human study of oleclumab, a potent, selective anti-CD73 monoclonal antibody, alone or in combination with durvalumab in patients with advanced solid tumors. Cancer Immunol Immunother. 2023;72(7):2443-2458. doi: 10.1007/s00262-023-03430-6.

127. Yu Y-J, Shan N, Li L-Y, et al. Preliminary clinical study of personalized neoantigen vaccine therapy for microsatellite stability (MSS)-advanced colorectal cancer. Cancer Immunol Immunother. 2023;72(7):2045-2056. doi: 10.1007/s00262-023-03386-7.

128. Wang D, Li B, Shen G, et al. NKG2D CAR-NK adoptive cellular immunotherapy combined with or without PD-1 blockade in the treatment of patients with metastatic colorectal cancer: an exploratory study. Cancer Immunol Immunother. 2025;74(11):1-11. doi: 10.1007/s00262-025-04196-9.

129. Focaccetti C, Izzi V, Benvenuto M, et al. Polyphenols as immunomodulatory compounds in the tumor microenvironment: friends or foes? Int J Mol Sci. 2019 Apr 6;20(7):1714. doi: 10.3390/ijms20071714.

130. Hou C, Hu Y, Zhang T. Research on curcumin mediating immunotherapy of colorectal cancer by regulating cancer associated fibroblasts. Anticancer Drugs. 2025;36(1):72-78. doi:10.1097/cad.0000000000001659

131. Hou C, Hu Y, Zhang T. Research on curcumin mediating immunotherapy of colorectal cancer by regulating cancer associated fibroblasts. Anti-Cancer Drugs 2025;36(1):72-78. doi: 10.1097/CAD.0000000000001659.

132. Buhrmann C, Kraehe P, Lueders C, Shayan P, Goel A, Shakibaei M. Curcumin suppresses crosstalk between colon cancer stem cells and stromal fibroblasts in the tumor microenvironment: potential role of EMT. PloS one 2014;9(9):e107514. doi: 10.1371/journal.pone.0107514.

133. Ge S, Sun X, Sang L, et al. Curcumin inhibits malignant behavior of colorectal cancer cells by regulating M2 polarization of tumor-associated macrophages and metastasis associated in colon cancer 1 (MACC1) expression. Chem Biol Drug Des 2023;102(5):1202-1212. doi:10.1111/cbdd.14330

134. Tu SP, Jin H, Shi JD, et al. Curcumin Induces the Differentiation of Myeloid-Derived Suppressor Cells and Inhibits Their Interaction with Cancer Cells and Related Tumor Growth. Cancer Prevent Res. 2012;5(2):205-215. doi:10.1158/1940-6207.CAPR-11-0247

135. Güllü N, Smith J, Herrmann P, Stein U. MACC1-Dependent Antitumor Effect of Curcumin in Colorectal Cancer. Nutrients. 2022;14(22)doi:10.3390/nu14224792

136. Yuan W, Zhang J, Chen H, et al. Natural compounds modulate the mechanism of action of tumour-associated macrophages against colorectal cancer: a review. J Cancer Res Clin Oncol. 2024;150(11):502. doi:10.1007/s00432-024-06022-8

137. Liu F, Ran F, He H, Chen L. Astragaloside IV Exerts Anti-tumor Effect on Murine Colorectal Cancer by Re-educating Tumor-Associated Macrophage. Arch Immunol Ther Exp. 2020;68(6):33. doi:10.1007/s00005-020-00598-y

138. Xu B, Yu L, Zhao LZ. Curcumin up regulates T helper 1 cells in patients with colon cancer. Am J Transl Res. 2017;9(4):1866-1875.

139. Chen S, Nishi M, Morine Y, et al. Epigallocatechin‐3‐gallate hinders metabolic coupling to suppress colorectal cancer malignancy through targeting aerobic glycolysis in cancer‐associated fibroblasts. Int J Oncol. 2022;60(2)doi:10.3892/ijo.2022.5309

140. Shimizu M, Shirakami Y, Sakai H, et al. (-)-Epigallocatechin gallate inhibits growth and activation of the VEGF/VEGFR axis in human colorectal cancer cells. Chem Biol Interact. 2010;185(3):247-52. doi:10.1016/j.cbi.2010.03.036

141. Buhrmann C, Shayan P, Brockmueller A, Shakibaei M. Resveratrol suppresses cross-talk between colorectal cancer cells and stromal cells in multicellular tumor microenvironment: A bridge between in vitro and in vivo tumor microenvironment study. Molecules. 2020;25(18):4292. doi: 10.3390/molecules25184292.

142. Jiang J, Zhu F, Zhang H, et al. Luteolin suppresses the growth of colon cancer cells by inhibiting the IL-6/STAT3 signaling pathway. J Gastrointest Oncol. 2022;13(4):1722-1732. doi:10.21037/jgo-22-507

143. Chiou YS, Lan YM, Lee PS, et al. Piceatannol Prevents Colon Cancer Progression via Dual-Targeting to M2-Polarized Tumor-Associated Macrophages and the TGF-β1 Positive Feedback Signaling Pathway. Mol Nutr Food Res. 2022;66(15):e2200248. doi:10.1002/mnfr.202200248

144. Wang G, Hiramoto K, Ma N, et al. Immunohistochemical analyses reveal FoxP3 expressions in spleen and colorectal cancer in mice treated with AOM/DSS, and their suppression by glycyrrhizin. PLoS One. 2024;19(8):e0307038. doi:10.1371/journal.pone.0307038

145. Sui H, Zhao J, Zhou L, et al. Tanshinone IIA inhibits β-catenin/VEGF-mediated angiogenesis by targeting TGF-β1 in normoxic and HIF-1α in hypoxic microenvironments in human colorectal cancer. Cancer Lett. 2017;403:86-97. doi:10.1016/j.canlet.2017.05.013

146. Shay JE, Imtiyaz HZ, Sivanand S, et al. Inhibition of hypoxia-inducible factors limits tumor progression in a mouse model of colorectal cancer. Carcinogenesis. 2014;35(5):1067-77. doi:10.1093/carcin/bgu004

147. Mirza N, Fishman M, Fricke I, et al. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. Cancer Res. 2006;66(18):9299-307. doi:10.1158/0008-5472.Can-06-1690

148. Lafta HA, AbdulHussein AH, Al-Shalah SAJ, et al. Tumor-associated Macrophages (TAMs) in Cancer Resistance; Modulation by Natural Products. Curr Top Med Chem. 2023;23(12):1104-1122. doi:10.2174/1568026623666230201145909

149. Aljabali AAA, Obeid MA, Bashatwah RM, et al. Phytochemicals in Cancer Therapy: A Structured Review of Mechanisms, Challenges, and Progress in Personalized Treatment. Chem Biodivers. 2025;22(8):e202402479. doi:10.1002/cbdv.202402479

150. Ashrafizadeh M, Taeb S, Haghi-Aminjan H, et al. Resveratrol as an Enhancer of Apoptosis in Cancer: A Mechanistic Review. Anticancer Agents Med Chem. 2021;21(17):2327-2336. doi:10.2174/1871520620666201020160348

151. Ayaz M, Nawaz A, Ahmad S, et al. Underlying Anticancer Mechanisms and Synergistic Combinations of Phytochemicals with Cancer Chemotherapeutics: Potential Benefits and Risks. J Food Qual. 2022;2022(1):1189034. doi:https://doi.org/10.1155/2022/1189034

152. Erdem M, Roth J, Knobloch J, et al. A mixture of plant polyphenols unexpectedly aggravates liver metastasis of colorectal cancer in mice. bioRxiv; 2026.

153. Tanjum FA, Rahman M, Hasan MM, et al. Mechanistic insights of clinically investigated natural products as anti-colorectal cancer agents. Precision Nut. 2026;5(2):e00137. doi:10.1097/PN9.0000000000000137

154. Khanehzar E, Shams F, Jafari A. The golden touch: a comprehensive network pharmacology-guided review of synergy between curcumin and PARP inhibitors. Med Oncol. 2025;43(1):20. doi:10.1007/s12032-025-03140-2

155. Jeon S, Jun E, Chang H, et al. Prediction the clinical EPR effect of nanoparticles in patient-derived xenograft models. J Cont Rel. 2022;351:37-49. doi: 10.1016/j.jconrel.2022.09.007.

156. Guo S, Qiao X, Ding L, et al. pH-responsive magnetic Fe3O4 modified chitosan nanoparticles loaded with β-acids to improve colorectal cancer treatment. Mat Today Bio. 2025:102151. doi: 10.1016/j.mtbio.2025.102151.

157. Shi L, Hu Y, Lin A, et al. Matrix metalloproteinase responsive nanoparticles for synergistic treatment of colorectal cancer via simultaneous anti-angiogenesis and chemotherapy. Bioconjug Chem. 2016;27(12):2943-2953. doi: 10.1021/acs.bioconjchem.6b00643.

158. Gong P, Wang F, Hua Y, Ying J, Chen J, Qiao Y. Collagenase-mediated extracellular matrix targeting for enhanced drug penetration and therapeutic efficacy in nanoscale delivery systems for cancer therapy. J Nanobiotechnology. 2025;23(1):733. doi: 10.1186/s12951-025-03815-y.

159. Idoudi S, Bedhiafi T, Hijji YM, Billa N. Curcumin and Derivatives in Nanoformulations with Therapeutic Potential on Colorectal Cancer. AAPS PharmSciTech. 2022;23(5):115. doi:10.1208/s12249-022-02268-y

160. Zhao X, Pan J, Li W, Yang W, Qin L, Pan Y. Gold nanoparticles enhance cisplatin delivery and potentiate chemotherapy by decompressing colorectal cancer vessels. Int J Nanomed. 2018;13(null):6207-6221. doi:10.2147/IJN.S176928

161. Mao Q, Min J, Zeng R, et al. Self-assembled traditional Chinese nanomedicine modulating tumor immunosuppressive microenvironment for colorectal cancer immunotherapy. Theranostics. 2022;12(14):6088-6105. doi:10.7150/thno.72509

162. Dutta D, Al Hoque A, Paul B, et al. EpCAM-targeted betulinic acid analogue nanotherapy improves therapeutic efficacy and induces anti-tumorigenic immune response in colorectal cancer tumor microenvironment. J Biomed Sci. 2024;31(1):81. doi:10.1186/s12929-024-01069-8

163. Júnior RFA, Lira GA, Schomann T, et al. Retinoic acid-loaded PLGA nanocarriers targeting cell cholesterol potentialize the antitumour effect of PD-L1 antibody by preventing epithelial-mesenchymal transition mediated by M2-TAM in colorectal cancer. Transl Oncol. 2023;31:101647. doi:10.1016/j.tranon.2023.101647

164. de Carvalho TG, Lara P, Jorquera-Cordero C, et al. Inhibition of murine colorectal cancer metastasis by targeting M2-TAM through STAT3/NF-kB/AKT signaling using macrophage 1-derived extracellular vesicles loaded with oxaliplatin, retinoic acid, and Libidibia ferrea. Biomed Pharmacother. 2023;168:115663. doi:10.1016/j.biopha.2023.115663

165. Kuo IM, Lee JJ, Wang YS, et al. Potential enhancement of host immunity and anti-tumor efficacy of nanoscale curcumin and resveratrol in colorectal cancers by modulated electro- hyperthermia. BMC Cancer. 2020;20(1):603. doi:10.1186/s12885-020-07072-0

166. Dutta D, Al Hoque A, Paul B, et al. EpCAM-targeted betulinic acid analogue nanotherapy improves therapeutic efficacy and induces anti-tumorigenic immune response in colorectal cancer tumor microenvironment. J Biomed Sci. 2024;31(1):81. doi:10.1186/s12929-024-01069-8

167. Zhao X, Pan J, Li W, Yang W, Qin L, Pan Y. Gold nanoparticles enhance cisplatin delivery and potentiate chemotherapy by decompressing colorectal cancer vessels. Int J Nanomedicine. 2018;13:6207-6221. doi:10.2147/ijn.S176928

168. Li L, Xiang D, Shigdar S, et al. Epithelial cell adhesion molecule aptamer functionalized PLGA-lecithin-curcumin-PEG nanoparticles for targeted drug delivery to human colorectal adenocarcinoma cells. Int J Nanomedicine. 2014;9:1083-96. doi:10.2147/ijn.S59779

169. Júnior RFdA, Lira GA, Schomann T, et al. Retinoic acid-loaded PLGA nanocarriers targeting cell cholesterol potentialize the antitumour effect of PD-L1 antibody by preventing epithelial-mesenchymal transition mediated by M2-TAM in colorectal cancer. Translat Oncol. 2023;31:101647. doi:https://doi.org/10.1016/j.tranon.2023.101647

170. Chen C, Schardey J, Schiergens TS, Werner J, Kühn F. Transplantable Orthotopic Mouse Models for Colorectal Cancer Research: Current Strategies and Future Directions. Visc Med. 2026;42(3):125-41. doi:10.1159/000551356

171. ter Riet G, Korevaar DA, Leenaars M, et al. Publication bias in laboratory animal research: a survey on magnitude, drivers, consequences and potential solutions. PLoS One. 2012;7(9):e43404. doi:10.1371/journal.pone.0043404

172. Lenz HJ, Van Cutsem E, Luisa Limon M, et al. First-Line Nivolumab Plus Low-Dose Ipilimumab for Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: The Phase II CheckMate 142 Study. J Clin Oncol. 2022;40(2):161-170. doi:10.1200/jco.21.01015

173. Rabbani SA, Sharma S, El-Tanani M, et al. Plant-Derived Polyphenols in Cancer Therapy: Bridging Molecular Mechanisms and Bioavailability Toward Clinical Translation. Pharmaceutics. 2026;18(6)doi:10.3390/pharmaceutics18060737

174. Stenzinger A, Allen JD, Maas J, et al. Tumor mutational burden standardization initiatives: Recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions. Genes Chromosomes Cancer. 2019;58(8):578-588. doi:10.1002/gcc.22733

175. Wang C, Sandhu J, Ouyang C, Ye J, Lee PP, Fakih M. Clinical Response to Immunotherapy Targeting Programmed Cell Death Receptor 1/Programmed Cell Death Ligand 1 in Patients With Treatment-Resistant Microsatellite Stable Colorectal Cancer With and Without Liver Metastases. JAMA Netw Open. 2021;4(8):e2118416. doi:10.1001/jamanetworkopen.2021.18416

176. Schürch CM, Bhate SS, Barlow GL, et al. Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front. Cell. 2020;182(5):1341-1359.e19. doi:10.1016/j.cell.2020.07.005

177. Pelka K, Hofree M, Chen JH, et al. Spatially organized multicellular immune hubs in human colorectal cancer. Cell. 2021;184(18):4734-4752.e20. doi:10.1016/j.cell.2021.08.003

178. Carranza FG, Diaz FC, Ninova M, Velazquez-Villarreal E. Current state and future prospects of spatial biology in colorectal cancer. Front Oncol. 2024;14:1513821. doi: 10.3389/fonc.2024.1513821.

179. Tommelein J, Verset L, Boterberg T, Demetter P, Bracke M, De Wever O. Cancer-associated fibroblasts connect metastasis-promoting communication in colorectal cancer. Front Oncol. 2015;5:63. doi: 10.3389/fonc.2015.00063.

180. Shah R, Johnson KA, Lippert AEL, et al. Cancer-Associated Fibroblast Proteins as Potential Targets against Colorectal Cancers. Cancers. 2024;16(18)doi:10.3390/cancers16183158

181. Huynh LK, Hipolito CJ, Ten Dijke P. A Perspective on the Development of TGF-β Inhibitors for Cancer Treatment. Biomolecules. 2019;9(11):743. doi: 10.3390/biom9110743.

182. Zeng W, Liu H, Mao Y, et al. Myeloid-derived suppressor cells: Key immunosuppressive regulators and therapeutic targets in colorectal cancer. Int J Oncol. 2024;65(3):85. doi: 10.3892/ijo.2024.5673.

183. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016;54:139-148. doi:10.1016/j.ejca.2015.11.016

184. Wang X, Kapoor VN, Chin DJ, et al. CHS-114: An Afucosylated Anti-CCR8 Monoclonal Antibody that Selectively Depletes Intratumoral Treg Cells and Induces Antitumor Immune Responses. Mol Cancer Ther. 2026;25(5):685-700. doi:10.1158/1535-7163.Mct-25-0367

185. Holder AM, Dedeilia A, Sierra-Davidson K, et al. Defining clinically useful biomarkers of immune checkpoint inhibitors in solid tumours. Nat Rev Cancer. 2024;24(7):498-512. doi:10.1038/s41568-024-00705-7

186. Cheng YH, He C, Riviere JE, Monteiro-Riviere NA, Lin Z. Meta-Analysis of Nanoparticle Delivery to Tumors Using a Physiologically Based Pharmacokinetic Modeling and Simulation Approach. ACS Nano. 2020;14(3):3075-3095. doi:10.1021/acsnano.9b08142

187. Becht E, de Reyniès A, Giraldo NA, et al. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. Clin Cancer Res. 2016;22(16):4057-66. doi:10.1158/1078-0432.Ccr-15-2879

188. Goel S, Duda DG, Xu L, et al. Normalization of the vasculature for treatment of cancer and other diseases. Physiol Rev. 2011;91(3):1071-121. doi:10.1152/physrev.00038.2010

189. Hu C, Dignam JJ. Biomarker-Driven Oncology Clinical Trials: Key Design Elements, Types, Features, and Practical Considerations. JCO Precis Oncol. 2019;3doi:10.1200/po.19.00086

190. Córdoba L, Cueto FJ, Cantero-Cid R, et al. Colorectal Air-Liquid Interface Organoids Preserve Tumour-Immune Architecture and Reveal Local Treg Expansion After PD-1 Blockade. Cancers. 2025;18(1)doi:10.3390/cancers18010132