



Emerging Frontiers in Colorectal Cancer Therapy: From Targeted Molecules to Immunomodulatory Breakthroughs and Cell-Based Approaches

Nayeralsadat Fatemi¹ · Seyedeh Nasim Mirbahari^{1,2,3} · Sascha Tierling⁴ · Fatemeh Sanjabi⁵ · Shabnam Shahrivari⁵ · Mandana AmeliMojarad⁶ · Melika Amelimojarad⁶ · Meygol Mirzaei Rezaei⁷ · Parsa Nobaveh⁷ · Mehdi Totonchi^{1,3} · Ehsan Nazemalhosseini Mojarad^{8,9}

Received: 26 May 2024 / Accepted: 20 November 2024 / Published online: 27 January 2025
© The Author(s) 2025

Abstract

Colorectal cancer (CRC) is ranked as the second leading cause of cancer-related deaths globally, necessitating urgent advancements in therapeutic approaches. The emergence of groundbreaking therapies, including chimeric antigen receptor-T (CAR-T) cell therapies, oncolytic viruses, and immune checkpoint inhibitors, marks a transformative era in oncology. These innovative modalities, tailored to individual genetic and molecular profiles, hold the promise of significantly enhancing patient outcomes. This comprehensive review explores the latest clinical trials and advancements, encompassing targeted molecular therapies, immunomodulatory agents, and cell-based therapies. By evaluating the strengths, limitations, and potential synergies of these approaches, this research aims to reshape the treatment landscape and improve clinical outcomes for CRC patients, offering new found hope for those who have exhausted conventional options. The culmination of this work is anticipated to pave the way for transformative clinical trials, ushering in a new era of personalized and effective CRC therapy.

Keywords Colorectal cancer · Targeted molecular therapies · CAR-T-cell therapies · Oncolytic viruses · Immune checkpoint inhibitors

Introduction

Colorectal cancer (CRC) is the second most common cause of cancer-related deaths globally [1]. The incidence is slightly higher in men and it peaks around the age of 50. Genetics, environmental, and lifestyle risk factors, all have

a role in the development and progression of CRC [2]. The progression of tumors from benign early adenomas and polyps to malignant late-stage tumors is a complex and multifaceted process governed by intricate molecular signaling pathways. Two critical factors influencing this progression are microsatellite instability and chromosomal instability

✉ Ehsan Nazemalhosseini Mojarad
Nazemalhosseini@sbmu.ac.ir; Ehsanmojarad@gmail.com

¹ Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

² Department of Developmental Biology, School of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran

³ Department of Genetics, Reproductive Biomedicine Research Center, ACECR, Royan Institute for Reproductive Biomedicine, Tehran, Iran

⁴ Department of Genetics/Epigenetics, Faculty NT, Life Sciences, Saarland University, Saarbrücken, Germany

⁵ Department of Medical Biotechnology, School of Allied Medicine, Iran University of Medical, Tehran, Iran

⁶ Department of Biology, Faculty of Basic Science, Kharrazi University, Tehran, Iran

⁷ School of Advanced Sciences and Technology, Islamic Azad University, Tehran Medical Branch, Tehran, Iran

⁸ Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Yeman St, Chamran Expressway, P.O. Box 19857-17413, Tehran, Iran

⁹ Department of Surgery, Leiden University Medical Center, Leiden, Netherlands

(CIN). Each of these factors plays a distinct role in tumor development [3]. Additionally, various gene mutations contribute to the transformation of normal cells into cancerous ones. Some of the most crucial pathways implicated in tumor progression include the MAPK/ERK, PI3K/AKT/mTOR, and Wnt/ β -catenin pathways [4]. Mutations in genes involved in these pathways, such as *KRAS*, *BRAF*, and *APC*, can trigger aberrant signaling, driving the transition from early adenomas to malignant tumors (Fig. 1) [5].

In recent years, the discourse surrounding novel treatments for CRC has garnered unprecedented attention and significance within the medical community. This surge in interest is propelled by a confluence of factors. Firstly, the escalating incidence of CRC worldwide has underscored the urgency to develop and optimize therapies that can effectively combat this prevalent malignancy. Furthermore, the emergence of groundbreaking techniques like natural killer (NK) cell therapy, chimeric antigen Receptor-T (CAR-T) cell therapy, oncolytic virus therapy, and immune checkpoint inhibitors represents a paradigm shift in oncology, presenting promising avenues for targeted, less toxic, and more efficacious interventions (Fig. 2). As these innovative treatments progress through rigorous clinical trials, their potential to significantly enhance patient outcomes and quality of life cannot be overstated.

Moreover, the individualized nature of these therapies holds the promise of tailoring treatments to specific genetic and molecular profiles, offering a level of precision medicine previously unimaginable. Therefore, delving into these approaches is paramount, as it signifies not only a quantum leap in the fight against CRC, but also a beacon of hope for patients and clinicians alike in the pursuit of more effective and personalized treatment modalities. With an in-depth exploration of the latest clinical trials and advancements in cell-based therapies, immunomodulatory agents, and innovative approaches, this article aims to provide a comprehensive overview of the strategies revolutionizing CRC treatment, paving the way for more effective and personalized therapeutic interventions. By examining the strengths, limitations, and potential synergistic effects of these various methodologies, it is anticipated that this research will offer valuable insights that can revolutionize the treatment perspective and improve clinical outcomes for CRC patients. Ultimately, the final aim is to bring about a paradigm shift in CRC therapy, offering new hope and avenues for patients who have exhausted conventional treatment options.

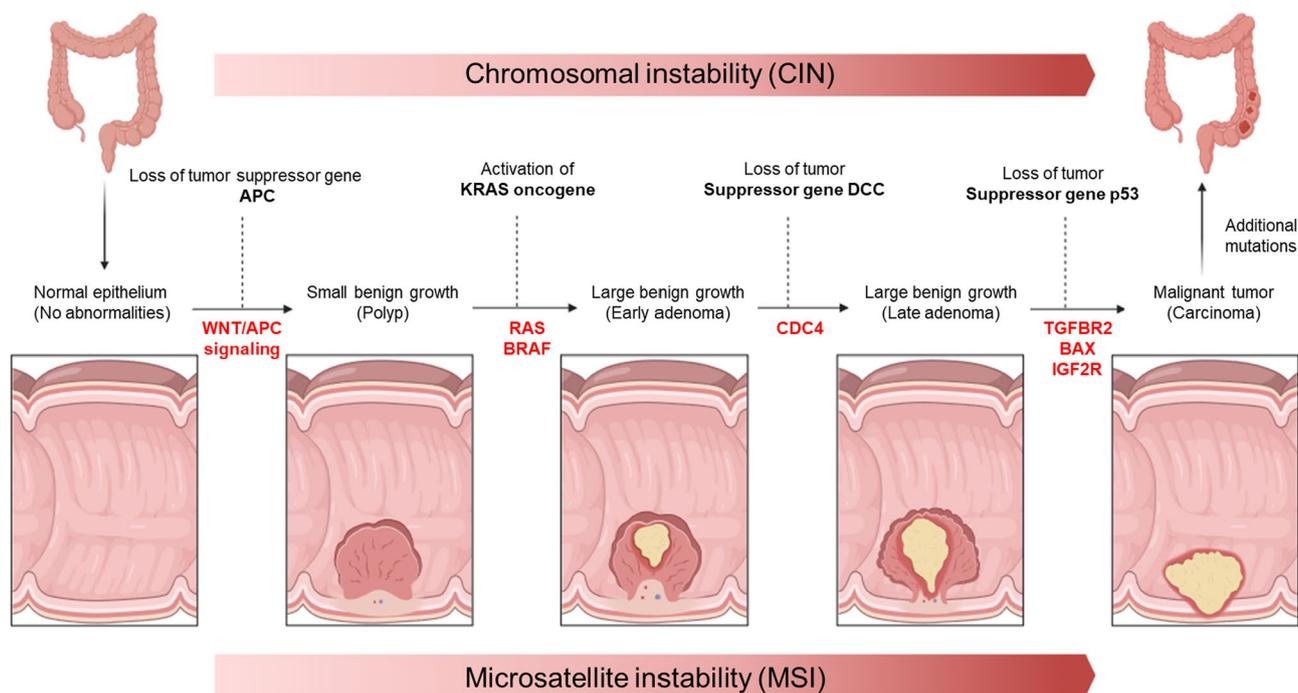


Fig. 1 Visualizing the Tumorigenesis Cascade. Beginning with normal epithelium, the Wnt/APC signaling pathway is disrupted, leading to the formation of polyps due to the loss of tumor suppressor genes. Subsequently, activation of the oncogene *KRAS* in the RAS/BRAF pathway transforms these small polyps into large benign adenomas.

As the journey continues, defects in the P53 gene, coupled with additional mutations and escalating microsatellite instability and chromosomal instability, culminate in the transition from large benign polyps to malignant tumors, providing a vivid portrayal of the intricate molecular evolution underlying tumorigenesis

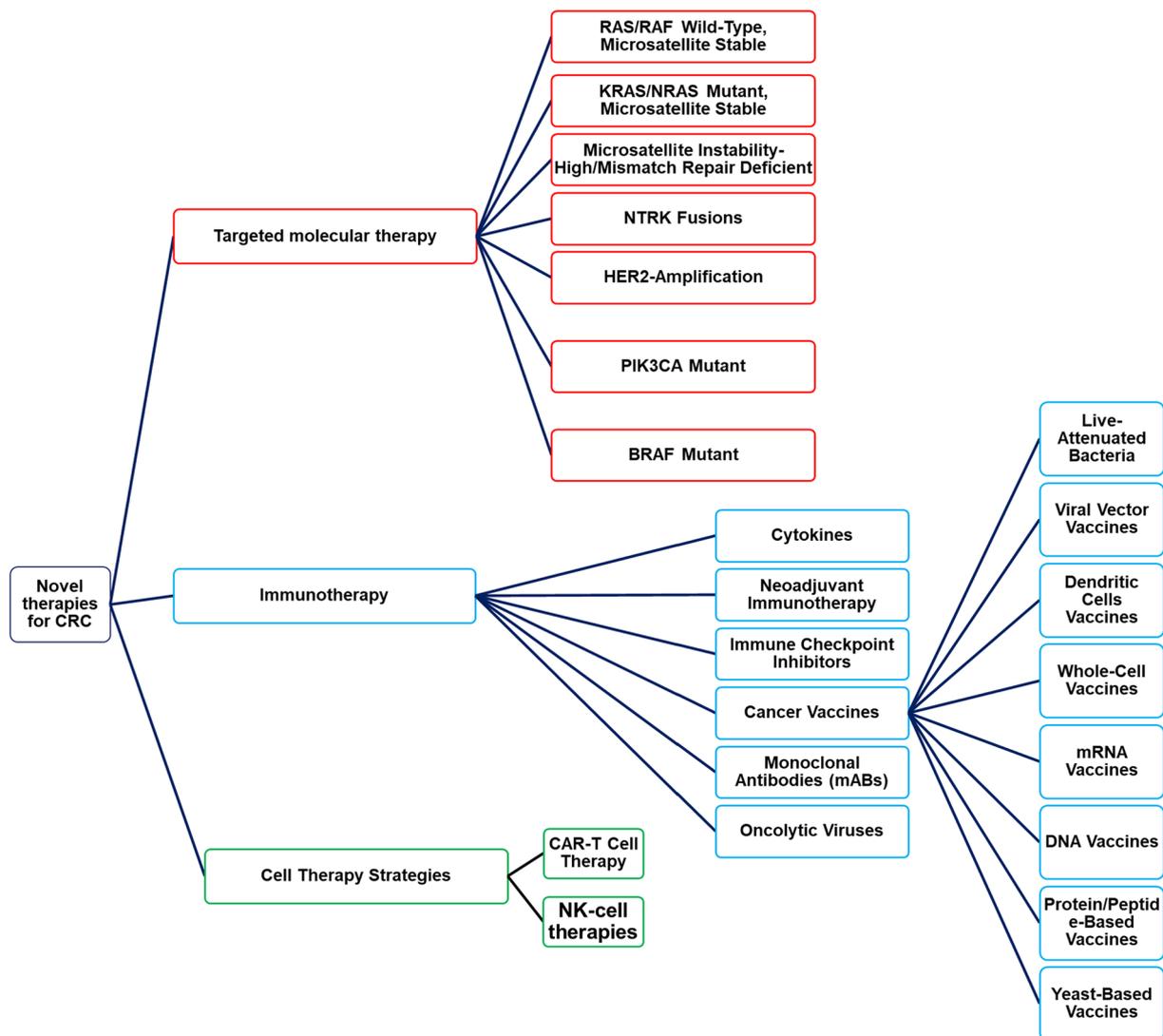


Fig. 2 Novel therapies for CRC. This diagram shows the various types of cancer vaccines and targeted therapies that are being investigated for the treatment of CRC. The vaccines target different molecular targets and signaling pathways, including RAS, RAF, HER2,

PIK3CA, BRAF, NTRK, and mismatch repair. The targeted therapies include monoclonal antibodies (MABs), cytokines, and viral vector vaccines

Molecular Targets and Signaling Pathways

The primary signaling pathways involved in regulating key cellular processes such as proliferation, differentiation, apoptosis, and survival in CRC include Sonic Hedgehog (SHH), Wnt/ β -catenin, and transforming growth factor- β (TGF- β)/SMAD. These pathways provided promising targets during past decades for precision therapy in CRC [6]. The Food and Drug Administration (FDA) approved cetuximab (ErbixTM) as the first targeted drug for CRC in February 2004 [7]. Several targeted drugs for colorectal cancer (CRC), such as the EGFR-specific antibody cetuximab, have been approved by the FDA and introduced to the market. Table 1 summarizes the molecular subtypes of colorectal cancer

(CRC) along with associated therapeutic approaches and key clinical findings. However, challenges in completely inhibiting specific biologic interactions and managing complex downstream signaling have limited the scope of existing data.

Immunotherapy

Every person’s immune system is the first defense line to attack cancer cells, thus potentially representing an effective assistance in cancer treatment and diagnosis. About a century ago, the idea of activating the host’s immune system to eradicate cancer was proposed [8]. The limited efficacy of traditional treatments like chemotherapy and radiotherapy

Table 1 Advances in colorectal cancer: molecular subtypes, targeted therapies, and immunotherapy strategies

Molecular subtype	Key characteristics	Therapeutic approach	Outcome/challenges	References
RAS/RAF Wild-Type, MSS	Found in ~30–40% of CRC cases. Loss of APC leads to dependence on EGFR signaling	Cetuximab and panitumumab combined with chemotherapy; VEGF-targeting bevacizumab tested in CALGB/SWOG 80405 trial	Median OS: 31.2 months (CALGB/SWOG 80405). No significant OS difference between bevacizumab and cetuximab. Up to 60% of patients develop resistance to EGFR inhibitors	[8–15]
KRAS/NRAS Mutant, MSS	KRAS mutations in >40% of CRC cases, NRAS in 2–5%; activates RAS/MAPK pathway, causing resistance to EGFR inhibitors	Cobimetinib (MEK inhibitor) + atezolizumab (anti-PDL1) tested in phase I–III trials; regorafenib as comparator	Phase III trial showed no OS improvement. Efficacy for KRAS mutant-MSS still under investigation	[16–19]
MSI-High/dMMR	~4–5% of mCRC cases; characterized by mismatch repair deficiency or high microsatellite instability	Anti-PD1 therapies (pembrolizumab, nivolumab). CheckMate 142 trial tested nivolumab + ipilimumab	Pembrolizumab: 78% PFS (20 weeks), Nivolumab: 69% PFS (> 12 weeks). Combination therapy: 60% ORR, 83% OS (12 months). FDA-approved pembrolizumab and nivolumab as second-line treatments	[20–25]
BRAF Mutant	~10% of CRC cases, 80% are V600E mutations; associated with poor prognosis (median OS: 11 months)	BEACON trial: Encorafenib (RAF inhibitor) + cetuximab, ± binimetinib (MEK inhibitor). FDA-approved encorafenib-cetuximab	Triplet vs. doublet therapy showed no significant PFS/OS difference but better toxicity profile. Combined therapies with ICIs showed promise. Molecular heterogeneity and resistance remain challenges	[26–33]
PIK3CA Mutant	~18% of CRC cases. Mutations in PI3K/mTOR pathway lead to potential drug resistance	PI3K inhibitors (GDC-0941, GDC-0980, MEN1611) evaluated in phase I trials; mTORC1/2 inhibitors tested in preclinical models	Moderate anti-tumor activity observed; resistance noted in patient-derived spheroid cultures. MEN1611 in combination with cetuximab under evaluation (expected completion July 2023)	[34–37]
HER2-Amplification	Found in 3–5% of CRC cases; activates RAS/MAPK and PI3K/mTOR pathways, causing resistance to anti-EGFR therapies	HERACLES trial: Trastuzumab + lapatinib. MyPathway trial: Pertuzumab + trastuzumab. FDA-approved tucatinib + trastuzumab for HER2-positive mCRC	HERACLES: 30% ORR. MyPathway: 38% ORR. Tucatinib approved for cases resistant to prior chemotherapy	[38, 39]
NTRK Fusions	Fusion of NTRK genes leads to overexpression or activation of Trk kinases. Rare in CRC	FDA-approved larotrectinib and entrectinib for CRC with NTRK fusions	Resistance to NTRK fusion blockers common; need for new molecules to overcome resistance	[40–42]
Immunotherapy	Focuses on enhancing immune system's ability to target tumor-specific antigens. Complementary to traditional therapies	Immune checkpoint inhibitors, combination therapies with chemotherapy or biologic treatments	Promising clinical trial results, but resistance remains an issue in some patients. Ongoing research into combination therapies to improve response rates	[43]

in colorectal cancer (CRC) has highlighted the need for immune-based approaches as complementary or alternative therapies. Tumor-specific antigens, resulting from mutations and structural changes during tumor formation, are recognized by the immune system as foreign, triggering immune responses. Recent advances in CRC immunotherapy have demonstrated its potential to enhance the body's natural defense against malignant cells, as supported by clinical trials.

Monoclonal Antibodies (mABs)

Monoclonal antibodies are synthetic proteins that mimic the role of human antibodies in the immune system. They are considered a form of immunotherapy as they enhance the immune response, helping the body to identify and combat cancer cells with greater efficiency [9]. CRC expresses several receptors that can be targeted by monoclonal antibodies [10]. Table 2 shows the target of common mABs. Bevacizumab, cetuximab, panitumumab, and ramucirumab are among the most commonly used monoclonal antibodies for treating colorectal tumors. Of these, bevacizumab specifically targets VEGF [11] and

cetuximab and panitumumab act on EGFR [12]. Clinical studies have shown that the combination of bevacizumab and chemotherapy, i.e., shown by Hurwitz et al., significantly increases PFS in CRC patients. Hurwitz et al. combined bevacizumab with irinotecan leading to the approval of bevacizumab for use in combination with chemotherapy as a first-line treatment for mCRCs [13]. There is evidence to support the use of cetuximab as a preferred drug in the initial treatment of mCRCs that have wild-type *RAS* and *BRAF* genes and proficient mismatch repair [14, 15]. The SWOG 1406 trial demonstrated that the combination of vemurafenib, irinotecan, and cetuximab improved PFS. This suggests that when the activity of either BRAF or EGFR inhibition alone is minimal, the combination of BRAF and EGFR inhibition can provide clinical benefit [16]. Furthermore, in a randomized clinical trial (NCT02394795) involving 802 patients with CRC, it was found that adding panitumumab to first-line chemotherapy improved OS compared to bevacizumab. This improvement was observed in patients with left-sided tumors and in the overall population [17]. Among mABs, only cetuximab, bevacizumab, and panitumumab have been approved by the FDA and broadly used in the USA. Figure 3 indicates FDA-approved mABs.

Table 2 Monoclonal antibodies and their receptors for CRC targeting

Monoclonal antibody	Trade name	Target	Function	References
Pembrolizumab	Keytruda®	PD1	Inhibition of PD1	[133]
Cetuximab	Erbix™	EGFR	Treatment of K-Ras mutated CRC	[134]
Bevacizumab	Avastin®	VEGF	Antiangiogenic agents	[135]
Conatumumab	–	DR5	Agonist against (tumor necrosis factor-related apoptosis-inducing ligand)	[136]
Trastuzumab	Herceptin	HER2/neu	Treatment of HER-2-overexpressing metastatic cancer	[137]
Adecatumumab	–	EpCAM	Agonist against the tumor-associated antigen	[138]
Ensituximab	–	MUC5AC	A novel chimeric mAb targeting a glycosylated variant of MUC5AC	[139]

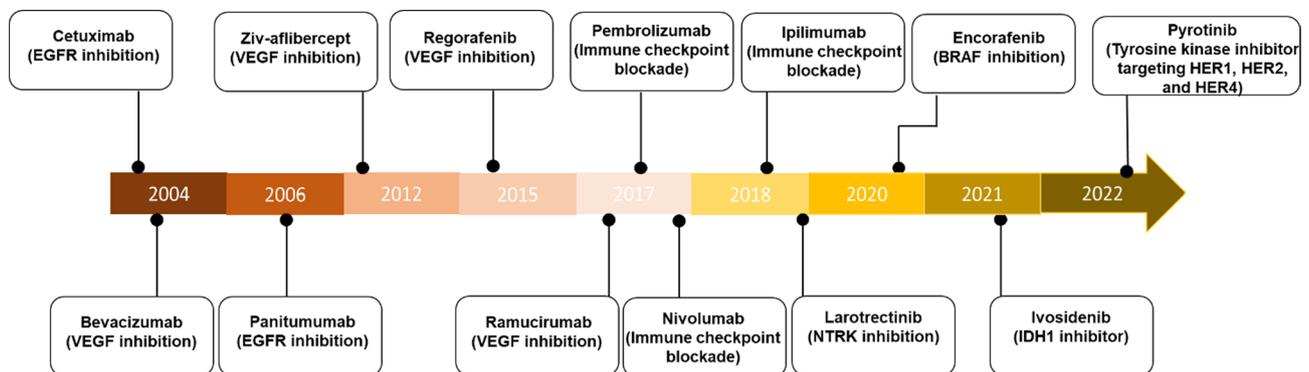


Fig. 3 Timeline of FDA-approved targeted therapy for colorectal cancer. *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor, *NTRK* neurotrophic tropomyosin receptor kinase, *IDH1* isocitrate dehydrogenase 1

Immune Checkpoint Inhibitors (ICIs)

ICIs prevent checkpoint proteins from binding to receptor proteins, enabling T cells to attack cancer cells effectively. CTLA4, PD1, and PDL1 are the molecules that are currently approved checkpoint inhibitor targets [18]. Figure 4 indicates the ligands and receptors which can be inhibited by ICIs.

ICIs enhance the activity of T cells by preventing the negative regulators of T-cell function from binding. However, this can also result in uncontrolled immune responses leading to autoimmune effects on normal organs known as immune-related side effects [10]. CTLA4 inhibitors have a higher incidence of immune-related adverse events (irAEs) than programmed cell death 1 (PD1) and its ligand 1 (PDL1) inhibitors. Combining CTLA4 and PD1 inhibitors further increases the risk of irAEs [19]. While ICIs have shown efficacy in treating various cancers, mCRC has been less responsive. Nonetheless, the FDA has approved certain PD1 inhibitors as first-line therapy for unresectable or metastatic MSI-H or dMMR CRC [20].

Neoadjuvant Immunotherapy

Adjuvants refer to a variety of substances, such as organic or inorganic molecules, colloids, and polymers, that are employed alone or in combination with other agents to stimulate the immune system. Their purpose is to enhance the immune response [21]. They can activate antigen-presenting cells, which help to present epitopes on the major histocompatibility complex class I (MHC-I), thereby promoting the activity of cytotoxic T lymphocytes. This process can lead to the destruction of cancer cells [22]. Ipilimumab is a neoadjuvant that stimulates the

immune response by targeting cCTLA-4, a protein receptor responsible for suppressing the immune system. By blocking this receptor, it eliminates the inhibitory mechanism displayed by cytotoxic T cells, enabling them to combat cancer cells better. In other words, it enhances the body's immune response to fight cancer cells [23]. In a study published in 2020, neoadjuvant immunotherapy was suggested for the treatment of patients with early-stage CRC. In this research, patients with dMMR or pMMR tumors were given a single dose of ipilimumab and two doses of nivolumab prior to surgery. The treatment was well tolerated among all patients, and they exhibited a favorable response to the treatment [24]. Overall, it appears that neoadjuvant immunotherapy could be the standard treatment for specific groups of CRC patients. However, this assertion requires further confirmation through more extensive studies. Dostarlimab is a checkpoint inhibitor that has been shown to be effective in the treatment of a variety of cancers, including rectal cancer. In a recent phase 2 clinical trial, Cercek et al. reported that dostarlimab was well tolerated and led to the absence of residual disease, as proven by rectal magnetic resonance imaging [25], 26. In all 12 patients with locally advanced rectal cancer [27] who were ineligible for surgery. The results of this trial are very promising, as they suggest that dostarlimab may be a new and effective treatment option for patients with locally advanced rectal cancer [27]. This is the first time that a complete clinical response has been reported in a phase 2 clinical trial of a cancer immunotherapy. The long-term follow-up of these patients is still ongoing, and it is important to see if the complete clinical responses are durable or long-lasting. However, the results of this trial suggest that dostarlimab may be a promising new treatment option for patients with LARC.

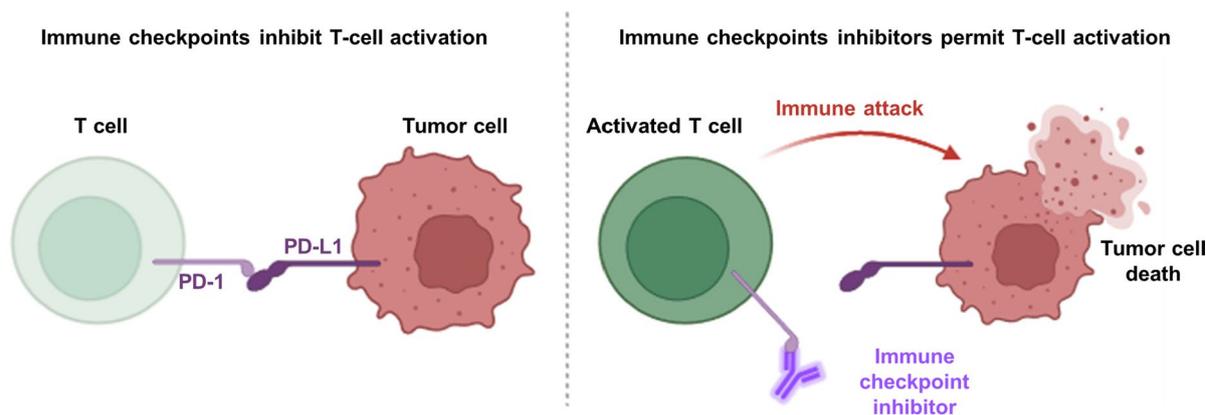


Fig. 4 Unlocking Immune Potential: in the first frame, PD1 engages with PDL1, stifling T-cell activation through immune checkpoint inhibition. The second frame depicts the pivotal role of immune checkpoint inhibitors, releasing this suppressive interaction and rein-

vigorating T-cell activation, showcasing the promise of immunotherapy in unleashing the body's innate defenses against cancer

Oncolytic Viruses

Oncolytic viruses are viruses that have been genetically engineered to infect and kill cancer cells. They work by infecting specifically cancer cells, replicating inside, leading to lysis and subsequent death of the cells. The use of viruses in the treatment of cancer dates back to about a century ago, but with the presence of genetic engineering methods and the ability to remove pathogenic genes from viruses, oncolytic viruses entered the field as a new and promising line of treatment. Oncolytic viruses have been shown to be effective in the treatment of a variety of tumors, including melanoma, head and neck cancer, and bladder cancer. No oncolytic virus has yet been approved for the treatment of CRC. However, promising oncolytic viruses that are currently being investigated in clinical trials for the treatment of CRC include talimogene laherparepvec, coxsackievirus, and adenovirus. T-VEC is a modified herpes simplex virus that has been engineered to infect and kill melanoma cells. T-VEC has been shown to be effective in shrinking tumors and improving survival in patients with melanoma. Coxsackievirus is a virus that is naturally found in humans and has been modified to infect and kill cancer cells. Coxsackievirus is currently being investigated in combination with chemotherapy for the treatment of pancreatic cancer. Similarly, naturally found human adenovirus has been modified to infect and kill cancer cells and is currently being investigated in combination with immunotherapy for the treatment of CRC. There are several reports from oncolytic viruses which indicates their promising role in treatment of KRAS-mutated CRC [28]. Pelareorep is an oncolytic virus containing non-enveloped dsRNA that can destroy KRAS-mutated colorectal tumor cells. It induces autophagic mechanisms and up-regulates autophagic proteins, leading to increased apoptosis and cell death in CRC cells [29]. In addition, pelareorep enhances immune efficacy by increasing the expression of MHC-I molecules and activating CD4 and CD8 T-cell populations in CRC patients with KRAS mutations [30]. The results of clinical trials that are investigating oncolytic viruses in combination therapy for CRC are still pending. However, the early results are promising, and oncolytic viruses may one day become a standard treatment for CRC. In addition, there are several different

types of oncolytic viruses that are being investigated in combination therapy for CRC (Table 3).

Cytokines

Cytokines are messenger molecules that facilitate communication between cells and coordinate immune system interactions. They are produced by both immune and non-immune cells in response to stress, such as infection, inflammation, and tumorigenesis [31]. Cytokines play a vital role in enabling the efficient and rapid propagation of immune signals, which elicit a coordinated and potent immune response to target antigens [32]. The latest findings indicate that it is possible to inhibit the development of CRC or enhance the effectiveness of chemotherapy or checkpoint inhibitors in treating CRC tumors by targeting particular cytokine pathways. A common form of cytokine therapy involves the use of high doses of IL2 to induce cancer regression in patients with metastatic cancer. Along with IL2, interferon-alpha (IFN- α) is also utilized as a therapeutic cytokine in cancer treatment [33]. To increase the efficacy of cytokine therapy in CRC, one should either inhibit several cytokines together or combine this method with other treatment methods. Indeed, blocking a single cytokine is not likely to produce significant outcomes as cytokine signals frequently overlap with each other [34], thus, this strategy is not likely to be effective in CRC treatment. Therefore, it is likely to be more effective targeting multiple cytokines or combining cytokine therapy with other treatment methods.

Cancer Vaccines

Cancer vaccines are one of the novel immunotherapy strategies to create a stronger immune response and to prevent cancer occurrence and micro-metastasis [35, 36]. Recently, effective cancer vaccines have been developed with the ability to strengthen the immune system and harbor low toxicity. There are various adjuvants, such as cytokines, pathogen-associated molecular pattern molecules (PAMPs), and Toll-like receptors (TLRs), that can be combined with cancer vaccines to enhance their ability to fight cancer synergistically [37, 38]. In the following,

Table 3 Available oncolytic viruses for colorectal cancer treatment

Virus	Strain	Results	References
Reovirus	Pelareorep	Lysis of KRAS-mutated CRC	[140]
HSV	oHSV-2	Recruitment of immune cells	[141]
Adenovirus	SPDD-UG	Apoptosis in CRC cells	[142]
Measles virus	MeVac FmIL-12	Apoptosis in CRC cells	[143]
Vaccinia virus	VG9-IL-24	Apoptosis in CRC cells	[144]

we focus on new vaccines for cancer treatment and experimental strategies in CRC immunotherapy.

Protein/Peptide-Based Vaccines Protein/peptide-based vaccines are a type of vaccine that uses specific proteins or peptides derived from pathogens, such as viruses or bacteria, to stimulate an immune response in the body. Unlike traditional vaccines, which may use weakened or inactivated forms of the entire pathogen, protein/peptide-based vaccines contain only selected protein fragments or peptides that are known to trigger a strong immune response [39]. These vaccines work by presenting these protein fragments or peptides to the immune system, which then recognizes them as foreign and mounts an immune response. This response involves the production of antibodies, specialized immune cells, and memory cells that “remember” the pathogen. This way, if the individual is later exposed to the actual pathogen, their immune system can quickly recognize and combat it, providing protection against infection. One of the advantages of protein/peptide-based vaccines is their precision [25]. By targeting specific proteins or peptides, scientists can design vaccines to focus on the most immunogenic parts of the pathogen, reducing the risk of unwanted side effects. Additionally, they can be produced using biotechnological methods, which can lead to more efficient and scalable vaccine production. These types of vaccines have been explored for various diseases, including certain types of cancer and infectious diseases. They are an important area of research in the development of new and innovative vaccines [40]. Immunization with proteins primarily leads to the activation of humoral immune response. However, for cancer treatment, it is essential to stimulate both humoral and cellular immune responses. Protein-based antibodies used for immunization must contain immunogenic sites that can be recognized by MHC-I/II molecules [41]. This process involves identifying and combining epitopes to stimulate anti-tumor or immune responses against tumor-associated antigens. Peptide antibodies for CRC patients are generally safe and well tolerated, but there are limitations to their application in clinical trials, such as frequent reactions at the injection site and restrictions based on patient's HLA type.

A phase II trial with 96 patients with advanced CRC demonstrated the safety of a vaccination mixture containing five HLA-A*2404-restricted peptides (RNF43, KOC1, TOMM34, VEGFR1, VEGFR2). The vaccine was administered concurrently with oxaliplatin-based chemotherapy [42]. In a phase I trial, a combination of KOC1, TTK, URLC10, DEPDC1, and MPHOSPH1 as an HLA-A*2404-restricted vaccine was found to be safe. The vaccine also demonstrated an OS of 9.4 months in comparison to untreated patients [43].

DNA Vaccines Naked nucleic acid vaccines have the potential to be effective in treating cancer patients [44]. DNA vaccines are being studied as therapeutic gene vaccines for CRC due to their potential to enhance the growth of CD8 T cells. The efficacy of DNA vaccines in CRC has not been extensively investigated in clinical trials, despite the wealth of information derived from *in vitro* and *in vivo* studies [45].

For example, Staff et al. administered a modified plasmid DNA vaccine encoding the CEA antigen (CEA66 DNA) along with T helper cell-related epitopes to patients with CRC. The study demonstrated that the vaccine was well tolerated and did not cause any autoimmune response [46]. In addition, pVAX1-HER2-CTLA-4 is a DNA vaccine that is currently under investigation in a clinical trial [47].

mRNA Vaccines Studies have shown that the administration of naked or vehicle-loaded mRNA vaccines that carry tumor antigens can activate antigen-presenting (APC) cells, leading to the stimulation of innate/adaptive immune responses [48]. Compared to other cancer vaccines, mRNA vaccines have several distinctive features: mRNA is a non-infectious, non-integrating platform, easier to modify for different purposes, and has a higher translation efficiency compared to DNA vaccines, leading to higher protein yields [49].

mRNA has the capability to encode multiple antigens concurrently or a complete protein containing epitopes that bind to both, MHC-I and MHC-II, enabling the facilitation of both humoral and cellular adaptive immune responses. mRNA vaccines are devoid of viral and cellular components, posing no risk of infection [48]. Clinical trials have shown that these vaccines are typically safe, with few reports of adverse reactions at the injection site [50]. Nevertheless, there are significant concerns regarding mRNA instability, innate immunogenicity, and the limited efficacy of *in vivo* delivery. Currently, a phase I trial (NCT03948763) is underway to investigate the efficacy of mRNA 5671, a vaccine targeting KRAS-positive cancers, in combination with Pembrolizumab for non-MSI-H patients. Another phase I trial (NCT03313778) is evaluating the clinical effectiveness of mRNA-4157, a cancer vaccine based on mRNA, in combination with Pembrolizumab in non-MSI-H patients [23].

Whole-Cell Vaccines Whole-cell vaccines offer the advantage of being applicable to multiple individuals, as they are not personalized for each patient. This characteristic makes them easier to manufacture, saving time and money, while also reducing the risk of cancer cells evading the treatment [51]. However, developing a universally applicable vaccine for all patients is challenging. Whole-cell vaccines often exhibit low immunogenicity and generate non-specific reactions. This is primarily due to the fact that the majority of antigens in these vaccines are shared with normal cells,

while only a small fraction of the proteins is specific to tumors [52]. Therefore, several combinational strategies are developed to enhance autologous vaccines efficiency. One of these strategies, involves injecting modified autologous tumor cells that have been infected with the nonlytic Newcastle disease virus (NDV) to express immune-stimulating antigens and alter the tumor cells [53]. OncoVax is a CRC cancer vaccine strategy that involves combining autologous cancer cells with the Bacillus Calmette–Guérin (BCG) vaccine. It is considered one of the extensively investigated CRC cancer vaccines and was the subject of early-phase clinical trials in the 1980s [54]. Subsequently, the combination of OncoVax with 5-fluorouracil (5FU) and leucovorin demonstrated a safe approach to minimize clinical effects in patients with stage III CRC [55]. Another vaccine called GVAX, which is an allogenic whole-cell vaccine engineered to secrete granulocyte–macrophage colony-stimulating factor (GM-CSF), exhibited an anti-tumor response in a phase II trial targeting advanced CRC patients with proficient mismatch repair. Currently, clinical trials (NCT01966289) are focused on enhancing the immunologic activity of GVAX [22]. In order to further improve GVAX, epigenetic therapy has been tried to enhance immunologic activity in both pre-clinical and clinical trials (NCT01966289) [56, 57]. A feasibility study of combined epigenetic and vaccine therapy in advanced CRC with pharmacodynamic endpoint [56]. Neo-antigen-based EpiGVAX vaccine initiates anti-tumor immunity in CRC [57]. Unlike whole-cell vaccines, autologous vaccines do not employ the entire tumor cell in their formulation [58]. Autologous vaccines are isolated and altered to be injected back into the same patient's body [59]. Utilizing the individual's own cells in autologous vaccines reduces the likelihood of rejection, and these cells specifically contain the antigens that are highly efficient in triggering an immune response [58].

Dendritic Cells Vaccines Dendritic cells (DCs) are powerful APCs utilized in cancer vaccines due to their capacity to initiate immune responses against tumors [60]. DC vaccines involve the manipulation of DCs outside the patient's body by exposing them to cancer cells or antigens. This process enhances the ability of the DCs to recognize and target cancer cells with the same antigens, leading to more effective attacks against cancer cells [61]. There are ongoing clinical studies to explore the effectiveness of DC vaccines for CRC patients. For instance, in one randomized clinical trial phase II study, autologous tumor lysate dendritic cell vaccine (ADC) combination with best supportive care (BSC) indicated that ADC induces a tumor-specific immune response but has no effect on PFS or OS [62]. DC vaccine based on Wilms' tumor (WT1) class I/II peptide confirmed the immunogenicity and safety of DC vaccine in advanced CRC patients through evaluation of WT1 expression in tis-

sue using the Enzyme-Linked Immunosorbent Spot (ELISPOT) assays [63].

Rodriguez et al. [64] conducted a Randomized Control Trial (RCT) involving 19 patients with surgically amenable liver metastasis of CRC. The patients were divided into two groups, with one group receiving DC vaccinations and the other group undergoing observation after surgery and chemotherapy. The median PFS was significantly longer in the vaccine arm compared to the observation arm [64].

MelCancerVac is a DCs vaccine being investigated in phase II clinical trials [65]. This vaccine is produced by pulsing allogeneic melanoma cell lysate from DDM-1.13, known for its high expression of MAGE-A3, which is a tumor-associated antigen [66] that is also overexpressed in CRC. The overall results of the trial did not demonstrate a significant improvement in OS for the patients. However, five patients experienced prolonged PFS of more than six months, with two patients remaining progression-free for over 27 and 37 months. A phase III trial of MelCancerVac for CRC patients is currently underway, and the results are expected to be available in future.

To enhance the effectiveness of the DC vaccine, further research is required to compare various subsets, including monocyte-derived DCs (moDCs), conventional DCs (cDCs), and plasmacytoid DCs (pDCs) [67]. Additionally, induced pluripotent stem cells (iPSCs) could potentially be a viable option for improving the DC vaccine [68].

Viral Vector Vaccines Cancer vaccines based on viral vectors are effective carriers for delivering tumor antigens [69]. Vaccinia virus, poxvirus, and adenovirus are naturally immunogenic and can directly infect and activate the APC cells (specifically DCs). Therefore, they are deemed as suitable vectors to transfect tumor antigens based on their ability to stimulate the cellular immune response against CRC antigens. Clinical trials have shown that these vaccines are effective in activating cellular immune response against antigens, such as CEA, EpCAM/KSA, p53, and 5T4, which are associated with CRC [70].

Morse et al. [71] conducted a phase I/II trial using adenovirus subtype 5 (Ad5) [E1- E2b-]-CEA (6D) to enhance CEA-specific T cell-mediated immune response in 32 mCRC patients. The treatment was proven to be safe and effective, with a 48% overall survival rate at 12 months. Another trial using E1/E3-deleted Ad5 with GUCY2C and PADRE sequences has also shown safety in a phase I trial [72] and a phase IIa trial is currently being explored (NCT04111172).

One promising approach involves utilizing a DC vaccine along with pox vectors encoding CEA and MUC1 (PANVAC) in patients with resected mCRC. This combination has shown promising results, with vaccinated patients exhibiting longer survival compared to an

unvaccinated group. These findings indicate the potential of the vaccine to stimulate immune responses against cancers that overexpress CEA and MUC1 [71].

Several other vaccines with intrinsic outcomes are currently in phase I/II clinical trials. These include TroVax (modified vaccinia Ankara encoding 5T4 antigen) [73, 74]. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma [75, 76].

Live-Attenuated Bacteria Bacterial vaccine vectors employed as live vaccine vectors over the last 30 years [77]. They possess many advantages based on their easy and inexpensive manufacture. Their susceptibility to antibiotics and well-defined mutations for reducing virulence make it straightforward to manage any adverse reactions caused by viral vector-based vaccines [77]. Moreover, these vaccines can be administered via multiple routes, including oral mucosal delivery [78]. The use of enteric bacterial vectors presents a distinct advantage in developing mucosal vaccines because they have a propensity to target lymphoid antigen-presenting cells (such as dendritic cells and macrophages) in the intestinal mucosal tract [79]. A phase I trial (NCT03189030) is currently underway to assess the safety of a personalized live-attenuated, double-deleted (pLADD) *Listeria monocytogenes* vaccine in patients with CRC. Another ongoing phase I trial (NCT03265080) is investigating the combination of the *L. monocytogenes* platform with neoantigens in patients with metastatic solid tumors, known as ADX-NEO [22]. The phase I trial of pLADD *Listeria monocytogenes* vaccine in patients with CRC (NCT03189030) has completed enrollment and is ongoing. The phase I trial of ADX-NEO in patients with metastatic solid tumors (NCT03265080) is also ongoing. The primary endpoint of NCT03189030 and NCT03265080 is to assess the safety of the combination of the *L. monocytogenes* platform with neoantigens. The secondary endpoints of the trials are to assess the immunogenicity of the combination therapy and its efficacy in treating metastatic solid tumors. In addition to these two trials, there are several other trials that are investigating the use of *L. monocytogenes* in cancer treatment. For example, a phase 2 trial (NCT04225205) is currently underway to assess the efficacy of *L. monocytogenes* in combination with Pembrolizumab in patients with mCRC. The results of these trials are still pending, but the early results are promising. These trials suggest that *L. monocytogenes* has the potential to be a promising new candidate for the treatment of CRC.

Yeast-Based Vaccines *Saccharomyces cerevisiae*, also known as baker's yeast, is a non-harmful yeast strain widely used as a vector in therapeutic vaccines [80]. Research has

shown that utilizing whole recombinant *S. cerevisiae* can activate dendritic cells, leading to the stimulation of specific cytotoxic T lymphocyte (CTL) responses. Moreover, it has demonstrated the ability to induce cell-mediated immunity that provides protection against tumor challenges in mice [80, 81]. There is some vaccine using Heat-killed *Saccharomyces cerevisiae* as a vector, encoded with tumor-associated antigens or carcinoembryonic antigen (CEA) to form GI-6207 [82], (GI-4000) [83], and in multiple phase I trials, (GI-6301), which encodes brachyury [84], was deemed safe with no indication of serious adverse effects or autoimmunity. Yeast-derived β -glucan particles loaded with short DNA sequences that contain unmethylated CpG motifs and MC38 lysates (lysed cells from the MC38 tumor cell line) showed a strong antibody response in murine models [85]. Different trials are investigating the effects of most vaccine trials that either use one or more antigenic elements in the form of whole tumor cell vaccines which contain irradiated tumor cells or tumor cell lysate, dendritic cell-based cancer (DC-linked peptides) vaccines or viral vector-based cancer vaccines [70]. Currently, clinical trials are underway to explore the use of different tumor antigens, such as CEA, PDL1, MUC1, and SART3, which are known to be highly expressed in the digestive system, for vaccination of CRC patients [86]. These trials are also evaluating various types of vaccines, including DC-based vaccines, DNA vaccines, RNA vaccines, and viral vaccine vectors. The clinical trials are primarily in phase I and II, aiming to assess the safety and efficacy of these vaccine approaches [87]. A summary of clinical trials of immunotherapy in CRC is presented in Table 4.

Hurdles of Cancer Vaccines

Therapeutic cancer vaccines have been demonstrated to improve the clinical outcomes of patients and increased tumor burden, despite the great efforts to discover possible target antigens for CRC vaccines and several clinical trials over the past two decades [88]. These therapeutic vaccines do not have a significant effect as monotherapy in CRC, especially in the advanced stages based on the existence of Co-inhibitory and inhibitory receptors that suppress T-cell function in the TME and make them dysfunctional. Also, most clinical trials showed no considerable survival benefit when using a single-peptide vaccine [89]. A promising new approach could be genetically modified cancer vaccines that harness both innate and adaptive immune responses to elicit long-lasting anti-tumor effects and prevent tumor recurrence. Unlike whole-cell vaccines, autologous vaccines do not employ the entire tumor cell in their formulation [58]. Autologous vaccines are isolated and altered to be injected back into the same patient's body [59]. Utilizing the individual's own cells in autologous vaccines reduces the likelihood

Table 4 A summary of vaccines used in clinical trials of CRC treatment

Name	Vaccine Type	Outcome	Stage	References
Fowlpox and vaccinia viruses encoding the CEA antigen and TRICOM (B7.1, ICAM-1, and LFA-3)	Viral vector	Induction of CEA-specific CTL	Metastatic	[145]
Cholera	Viral vector	Decreased the mortality rate of CRC,	I–IV	[106]
Nonreplicating canarypox virus (ALVAC-CEA/B7.1)	Viral vector	Increases in CEA-specific T cells were detected in patients treated with chemotherapy and booster vaccination	Metastatic	[75]
pVAX1-HER2, coding HER2 antigen	DNA-based vaccines	Increases the susceptibility of cancer cells to lysis by CTL	Metastatic	[146]
pcDNA-hNIS, expressing human sodium/iodide symporter (hNIS)	DNA-based vaccines	Promote IFN- γ production and reduced the tumor growth	–	[147]
OX40L OX86	DNA-based vaccines	Murine OX40L promote T-cell proliferation	–	[148]
MYB	DNA-based vaccines	Enhanced tumor target lysis and memory of T cells	–	[149]
CpVR-MS and CpDV-IL2-MS, encoding a fusion gene of human surviving S8 and human 33 MUC1, plus IL2)	DNA-based vaccines	Increase in anti-tumor effects and extended the rate of survival about 2.5-fold	Metastatic	[56]
NDV-infected irradiated autologous tumor cells	Whole tumor cell	Did not significantly improve overall survival		[150]
Autologous tumor cells combined with BCG	Whole tumor cell	Benefits in terms of disease-free survival ($P=0.078$) and overall survival ($P=0.12$)	stage II—III	[151]
DC pulsed with CEA	DC-based vaccines	The majority of CRC patients demonstrated induction of CEA-specific T-cell responses	IV	[152]
DCs with CEA- altered peptides combined with the Flt3 ligand	DC-based vaccines	Expansion of CD8 + T cells	IV	[153]
Autologous tumor antigen-loaded DC	DC-based vaccines	Increased IL-12 production for immunization against neoantigens	Metastatic	[154]
13-mer mutant ras	Peptide	The anti-tumor immune response was significantly associated with prolonged overall survival	–	[155]
β -hCG	Peptide	Prolongation of survival and induction of serum antipeptide antibody	Metastatic	[156]
SART3	Peptide	Increased CTL activity and induction of serum antipeptide	Metastatic	[157]
Survivin-2B	Peptide	Increase of Survivin-2B-specific CTL frequency	Metastatic	[158]
Set of 10 overlapping p53 synthetic long peptides	Peptide	Induction of p53-specific CD4 + and CD8 + T-cell responses and p53-specific CTL reactivity	Metastatic	[159]
mRNA-4157	RNA-based vaccine	Induce strong neoantigen-specific T=cell responses	Metastatic	[42]
NCI 4650 (mRNA 4650)	RNA-based vaccine	A portion of results showed that this vaccine was safe and significantly induced neoantigen-specific CD8 and CD4 T cells responses against CRC neoepitopes	Metastatic	[160]
GI-6207	Yeast	Decreased serum CEA and calcitonin, and improve CD8 + T cells and CD4 + T responses,	Metastatic	[82]
GI-6301	Yeast	Decreased tumor density and level of serum CEA of in CRC-treated patients therapeutic	Metastatic	[84]
GI-4000	Yeast	Demonstrated a favorable safety profile and immunogenicity in the majority of subjects	Metastatic	[83]

of rejection, and these cells specifically contain the antigens that are highly efficient in triggering an immune response [58]. Future research on antigens, adjuvants, and methods of delivery are crucial to develop new cancer vaccines with low toxicity and high benefits in many patients.

Cell Therapy Strategies

CAR-T-Cell Therapy

CAR-T-cell immunotherapy is a new method of treatment that genetically modifies T cells to fight against cancer [90].

Several techniques are currently under development to improve CAR-T-cell therapy. These include methods to enable them to counteract the immunosuppressive microenvironment of cancer by targeting and destroying PD1 and CTLA-4, as well as CARs that can target two different cancer antigens, among other approaches, to enhance specificity and safety [91]. Besides, there are ongoing efforts to develop genetic modification techniques that would make CAR-T cells from healthy donors suitable for allogeneic use as a treatment option [92–94].

To address the challenges of reducing complications associated with CAR-T-cell therapy in CRC, new approaches are being proposed. These include techniques such as engineering T cells with immune-activating molecules, administering T cells regionally, using bispecific T-cell engagers, and employing combinatorial target antigen recognition [95]. Patient-derived xenograft (PDX) mouse models of CRC are created by engrafting tumor tissue from patients. These models accurately represent the molecular heterogeneity of the patients and replicate the tumor's immunosuppressive microenvironment. PDX models have been established as a trustworthy model, and humanized models have been developed for two preclinical studies of anti-CRC CAR-T cells [96]. In xenograft models, CAR-T cells are either injected together with cancer cells or separately a few days after the cancer cell administration, once the tumor has formed. A few examinations utilizing CAR-T cells focusing on CEA or EpCAM showed that co-injection of CAR-T and growth cells repressed or deferred cancer development [97]. In 2017, Ang et al. conducted preclinical tests on CAR-T cells targeting CRC, focusing on the cytotoxic effects of EpCAM-directed CAR-T cells. Their study found that multiple infusions of EpCAM CAR-T cells, created through mRNA electroporation, delayed the progression of cancer in mice with CRC xenografts and showed promising results [98].

One study demonstrated significant anti-tumor effectiveness of third-generation MSLN-CAR-T cells, which remained active for at least 10 days after their administration. Another study showed that HER2-CAR-T cells could lead to tumor relapse or elimination in a PDX mouse model of

CRC and protect the treated animals from cancer recurrence [99]. Due to the positive results obtained in preclinical studies, numerous CAR-T-cell therapies developed against CRC are currently undergoing evaluation in clinical trials to determine their effectiveness and safety in combating tumors.

Furthermore, second-generation CAR-T cells that target CEA have shown excellent anti-tumor effects both in laboratory tests and animal studies, and their effectiveness can be increased by combining them with interleukins, such as IL-12 [100]. Tandem CAR-T cells that target both CEA and CD30 have demonstrated increased cytotoxicity, persistence, and the release of Perforin and granzyme B compared to CAR-T cells that target only CEA. Similarly, CD30/TAG72-CAR-T cells have shown increased cytotoxicity compared to TAG72-CAR-T cells [101]. By contrast, CAR-T cells that target both CEA and CD25 showed increased cell persistence but similar cytotoxicity compared to anti-CEA CAR-T cells in CRC models. In *in vivo* studies using mouse models of CRC, the CAR-T-cell doses typically range from 2×10^6 to 2×10^7 [102]. Moreover, when CEA-CAR-T cells were co-administered with mesenchymal stem cells expressing IL-7 and IL-12, tumor inhibition was significantly enhanced, leading to prolonged survival. Typically, in treatments where CAR-T cells are administered after tumor formation, multiple doses are usually given [95]. One of these studies, conducted by Huang et al., demonstrated cancer eradication using EGFRvIII-CAR-T cells in combination with miR-153, which suppresses indoleamine 2,3-dioxygenase 1 (IDO1), a protein that is negatively associated with patient survival [103]. In addition, combining CEA-CAR-T cells with recombinant human IL-12 has shown to have a superior anti-tumor effect compared to using CAR-T cells alone. These findings indicate that the use of cytokines or other therapeutic approaches in conjunction with CAR-T cells can improve their ability to fight tumors, as seen in animal models of CRC. However, preclinical studies have also shown that CAR-T-cell therapy directed toward CRC antigens such as CEA, EpCAM, GUCY2C, NKG2DL, and PLAP can be effective in inhibiting tumor growth [104]. In addition to intravenous administration, the effectiveness and safety of CAR-T cells targeting EpCAM and GUCY2C have also been evaluated through local administration via intraperitoneal implantation [105].

There has been much progress in developing CAR-T cells to treat hematologic tumors in clinical trials, but fewer trials are conducted on solid tumors, such as CRC. This is partly because of the lack of appropriate tumor-specific antigens that can be targeted by CAR-T cells, potentially causing on/off-tumor toxicity due to the accidental killing of surrounding non-malignant cells that express the target antigen [106, 107].

In Table 5, we provide an overview of all of the clinical trials involving CAR-T cells against CRC that are currently registered at clinicaltrials.gov. Various studies were found on the effect of CAR-T cells on the removal of colorectal tumor cells. There are 11 different antigens on CRC that have been targeted by CAR-T-cell studies registered at clinicaltrials.gov, including HER2 [108], MUC1, guanylate cyclase-C [56], NKG2D ligands (7), CD133 [108], EPCAM [108], CEA (7), B7-H3, mesothelin [56], C-met [108], and EGFR [56].

For example, B7-H3 is a co-stimulatory molecule for T cells that make T cells proliferate and differentiate into cytotoxic T cells. Healthy tissues only express a very limited level of B7-H3. Nonetheless, B7-H3 is overexpressed in CRC, causing NK cells to be unable to recognize and kill tumor cells [109].

Some CRC cells have MUC1 overexpression, an immunogenic molecule related to TCR and BCR epitopes, which mediates the disease's metastasis, chemical resistance, and worse prognosis [110].

Most studies do not specify what method was used to introduce chimeric antigen receptors to T cells. Only two studies have used lentiviral methods for this purpose. CAR Formats are not explained in most studies; only in a few cases that are listed in Table 5.

NK-Cell Therapy

NK cells are a type of immune cell that play a critical role in the body's defense against infectious agents and cancer cells. The mechanism of action involves their ability to recognize and eliminate abnormal cells without prior sensitization or the need for the immune system to be activated [111]. NK cells have a range of receptors on their surface that can detect the presence of stress-related molecules or proteins on the surfaces of abnormal cells, such as infected cells or tumor cells [112]. These receptors include killer cell immunoglobulin-like receptors (KIRs), natural cytotoxicity receptors (NCRs), and NKG2D receptors. When NK cells recognize abnormal cells, they release cytotoxic granules containing Perforin and Granzymes. Perforin creates pores in the target cell's membrane, allowing the entry of Granzymes. Granzymes then trigger a cascade of events within the target cell, leading to cell death through apoptosis or programmed cell death [113]. Additionally, these cells can also induce cell death by directly engaging death receptors on the target cell surface, such as Fas receptor and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor. NK cells can also produce cytokines, such as interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α), which help in stimulating other immune cells, enhancing the immune response, and aiding in the clearance of infected or abnormal cells [114]. NK-cell therapy involves usage of

these specialized immune cells as a treatment for various types of cancer. The therapy focuses on enhancing the activity and efficacy of NK cells to target and destroy cancer cells more effectively. NK-cell therapy can be used for various types of cancer, including CRC, as NK cells can recognize and target a broad range of cancer cells [115]. These cells can selectively attack cancer cells while sparing healthy cells, reducing the likelihood of side effects compared to traditional treatments like chemotherapy or radiation. Unlike other forms of cell-based immunotherapy, such as T-cell therapy, NK cells do not carry the risk of causing GVHD, a potentially severe immune reaction [115]. Despite all of these advantages, NK cells have a relatively short lifespan compared to other immune cells, which may limit the long-term efficacy of the therapy. Obtaining a sufficient number of high-quality NK cells for therapy can be challenging and may require advanced laboratory techniques, making it less accessible than other treatments [116]. Fortunately, in the context of CRC, NK-cell therapy has shown promising results in preclinical and early-phase clinical trials. Studies have demonstrated that NK-cell therapy can enhance the anti-tumor immune response and improve patient outcomes. However, further research is needed to optimize NK-cell therapy approaches and explore combination treatments to maximize efficacy against CRC [117]. Several clinical trials have explored the intratumoral infusion of NK cells in CRC patients. Intratumoral administration allows direct delivery of NK cells to the tumor microenvironment, enhancing their cytotoxicity and potential anti-tumoral effect. A phase I/II clinical trial by Hsu et al. (2015) demonstrated the feasibility and safety of intratumoral NK-cell infusion in mCRC patients and reported objective tumor responses. Adoptive NK-cell transfer involves expanding and activating autologous or allogeneic NK cells *ex vivo* and subsequently infusing them back into the patient to enhance anti-tumor activity [118]. The results of a phase II trial evaluating the efficacy of adoptive transfer of activated NK cells in patients with advanced CRC. The trial reported significant improvements in progression-free survival and overall survival in the NK-cell therapy cohort compared to the control group [119]. Several ongoing clinical trials are exploring the potential of combining NK cell-based therapies with other treatment modalities. For example, a phase I/II trial by Hu, Hung, and Huang (2020) is investigating the safety and efficacy of combining NK-cell therapy with immune checkpoint inhibitors in advanced CRC patients. Preliminary results showed enhanced anti-tumor responses and improved overall survival of patients receiving the combination therapy. The genetic engineering of NK cells has emerged as a promising strategy to enhance their anti-tumor activity. Clinical trials exploring the use of NK cells in CRC have shown promising results, both as monotherapy and in combination with other treatment approaches. Intratumoral NK-cell infusion,

Table 5 CAR-T cells used in clinical trials of CRC patients

Number	Identifier	phase	Type of cancer	Status	Antigen	CAR Formats	participant	References
1	NCT02349724	1	Colorectal, lung, gastric, breast, and pancreas cancer	Unknown	CEA	scFv-CD28/CD3 ζ	75	https://clinicaltrials.gov/ct2/show/NCT02349724
2	NCT03682744	1	Colorectal, peritoneal, gastric, breast, and pancreas cancer, peritoneal metastases	Withdrawn	CEA	N/A	0	https://clinicaltrials.gov/ct2/show/NCT03682744
3	NCT03542799	1	Metastatic CRC	Unknown	EGFR/IL-12	TRUCKs	20	https://clinicaltrials.gov/ct2/show/NCT03542799
4	NCT03152435	½	EGFR-positive Colorectal Cancer	Unknown	EGFR	4-1BB-CD28:CD3	20	https://clinicaltrials.gov/ct2/show/NCT03152435
5	NCT02617134	½	Malignant Glioma of Brain, Colorectal Carcinoma, Gastric Carcinoma	Unknown	MUC1	N/A	20	https://clinicaltrials.gov/ct2/show/NCT02617134
6	NCT03310008	1	Colon Cancer Liver Metastasis	Unknown	NKG2D ligands	NKG2D link to CD3 ζ	36	https://clinicaltrials.gov/ct2/show/NCT03310008
7	NCT03370198	1	Colon Cancer Liver Metastasis	Unknown	NKG2D ligands	N/A	1	https://clinicaltrials.gov/ct2/show/NCT03370198
8	NCT03692429	1	Unresectable Metastatic Colorectal Cancer	Recruiting	NKG2D ligands	NKG2D link to CD3 ζ	49	https://clinicaltrials.gov/ct2/show/NCT03692429
9	NCT02959151	½	Hepatocellular, Pancreatic Cancer, Metastatic Colorectal Cancer	Unknown	CEA	N/A	20	https://clinicaltrials.gov/ct2/show/NCT02959151
10	NCT04503980	1	Colorectal Cancer, Ovarian Cancer	Recruiting	mesothelin	N/A	10	https://clinicaltrials.gov/ct2/show/NCT04503980
11	NCT05089266	1	Colorectal Cancer	Not yet recruiting	mesothelin	N/A	30	https://clinicaltrials.gov/ct2/show/NCT05089266
12	NCT05240950	1	Colorectal Cancer, Metastatic Liver Cancer	Recruiting	CEA	N/A	18	https://clinicaltrials.gov/ct2/show/NCT05240950
13	NCT05248048	1	Refractory Metastatic Colorectal Cancer	Recruiting	NKG2D ligands	N/A	9	https://clinicaltrials.gov/ct2/show/NCT05248048
14	NCT04513431	1	Stage III Colorectal Cancer, Colorectal Cancer Liver Metastasis	Not yet recruiting	CEA	N/A	18	https://clinicaltrials.gov/ct2/show/NCT04513431
15	NCT05190185	1	Malignant Melanoma, Lung Cancer, or Colorectal Cancer	Recruiting	B7-H3	N/A	18	https://clinicaltrials.gov/ct2/show/NCT05190185
16	NCT04550663	1	Solid Tumor, Hepatocellular Carcinoma, Colorectal Cancer, Glioma	Not yet recruiting	NKG2D ligands	N/A	10	https://clinicaltrials.gov/ct2/show/NCT04550663
17	NCT04348643	½	Solid Tumor, Lung Cancer, Colorectal Cancer, Liver Cancer, Pancreatic Cancer, Gastric Cancer, Breast Cancer	Recruiting	CEA	N/A	40	https://clinicaltrials.gov/ct2/show/NCT04348643

Table 5 (continued)

Number	Identifier	phase	Type of cancer	Status	Antigen	CAR Formats	participant	References
18	NCT05415475	1	Colorectal Cancer, Esophageal Cancer, Stomach Cancer, Pancreatic Cancer, Metastatic Tumor Recurrent Cancer	Recruiting	CEA	N/A	36	https://clinicaltrials.gov/ct2/show/NCT05415475
19	NCT05396300	1	Colorectal Cancer, Esophageal Cancer, Stomach Cancer, Pancreatic Cancer, Metastatic Tumor Recurrent Cancer	Recruiting	CEA	N/A	60	https://clinicaltrials.gov/ct2/show/NCT05396300
20	NCT02713984	½	Breast Cancer, Ovarian Cancer, Lung Cancer, Gastric Cancer, Colorectal Cancer, Glioma, Pancreatic Cancer	Withdrawn	HER2	N/A	0	https://clinicaltrials.gov/ct2/show/NCT02713984
21	NCT03638206	½	B-cell Acute Lymphoblastic Leukemia, Lymphoma, Myeloid Leukemia, Multiple Myeloma, Hepatoma, Gastric Cancer, Pancreatic Cancer, Mesothelioma, Colorectal Cancer, Esophagus Cancer, Lung Cancer, Glioma, Melanoma, Synovial Sarcoma, Ovarian Cancer, Renal Carcinoma	Recruiting	C-met	N/A	73	https://clinicaltrials.gov/ct2/show/NCT03638206
22	NCT05028933	1	Advanced Hepatocellular Carcinoma, Advanced Colorectal Cancer, Advanced Gastric Cancer, Advanced Pancreatic Cancer	Recruiting	EPCAM	N/A	48	https://clinicaltrials.gov/ct2/show/NCT05028933
23	NCT05239143	1	Breast Cancer, Ovarian Cancer, Non-Small Cell Lung Cancer, Colorectal Cancer, Pancreatic Cancer, Renal Cell Carcinoma, Nasopharyngeal Cancer, Head and Neck Squamous Cell Carcinoma, Gastric Cancer	Recruiting	MUC1	N/A	100	https://clinicaltrials.gov/ct2/show/NCT05239143

Table 5 (continued)

Number	Identifier	phase	Type of cancer	Status	Antigen	CAR Formats	participant	References
24	NCT03740256	1	Bladder Cancer, Head and Neck Squamous Cell Carcinoma, Cancer of the Salivary Gland, Lung Cancer, Breast Cancer, Gastric Cancer, Esophageal Cancer, Colorectal Cancer, Pancreatic Adenocarcinoma, Solid Tumor	Recruiting	HER2	N/A	45	https://clinicaltrials.gov/ct2/show/NCT03740256
25	NCT04991948	1	Unresectable Metastatic Colorectal Cancer	Recruiting	NKG2D ligands	N/A	34	https://clinicaltrials.gov/ct2/show/NCT04991948
26	NCT04107142	1	Colorectal Cancer, Triple-Negative Breast Cancer, Sarcoma, Nasopharyngeal Carcinoma, Prostate Cancer, Gastric Cancer	Unknown	NKG2D ligands	N/A	10	https://clinicaltrials.gov/ct2/show/NCT04107142
27	NCT02541370	½	Liver Cancer, Pancreatic Cancer, Brain Tumor, Breast Cancer, Ovarian Tumor Colorectal Cancer, Acute Myeloid and Lymphoid Leukemias	Completed	CD133	anti-CD133 scFv-CD137ζ	20	https://clinicaltrials.gov/ct2/show/NCT02541370
28	NCT05319314	1	Colorectal Cancer	Not yet Recruiting	guanylate cyclase-C	N/A	30	https://clinicaltrials.gov/ct2/show/NCT05319314
29	NCT05287165	1	Advanced Solid Tumors, Digestive System Neoplasms, Pancreatic Cancer, Resectable Colorectal (Colon or Rectal) Cancer	Recruiting	guanylate cyclase-C	N/A	19	https://clinicaltrials.gov/ct2/show/NCT05287165

adoptive NK-cell transfer, and combination therapies have demonstrated improved clinical outcomes in CRC patients. Further studies incorporating NK-cell engineering and optimized treatment protocols are necessary to fully harness the potential of NK cell-based therapies for CRC treatment.

CAR-NK-Cell Therapy

Chimeric antigen receptor (CAR) NK cells are a type of immunotherapy that involves engineering NK cells to express a CAR. A CAR is a synthetic receptor that can be designed to recognize specific tumor antigens. When CAR NK cells encounter tumor cells that express their target antigen, they can kill them directly or activate other immune cells to attack the tumor [120]. CAR NK cells have several advantages over other types of immunotherapy, such as CAR-T cells. First, NK cells are less likely to cause cytokine release syndrome (CRS), a severe side effect that can occur with CAR-T-cell therapy. Second, NK cells can be obtained from allogeneic donors, which means that they can be used to treat patients who are not eligible for autologous CAR-T-cell therapy (i.e., therapy that uses the patient's own NK cells). Third, NK cells can be expanded and engineered more efficiently than T cells [121]. Several clinical trials are currently underway to evaluate the safety and efficacy of CAR NK cells for the treatment of colorectal cancer (CRC). For example, the results of a phase I trial of CAR NK cells targeting the EpCAM. The trial showed that CAR NK-cell therapy was safe and feasible, with no patients experiencing CRS or other serious side effects. In addition, 8 of the 11 patients who received CAR NK-cell therapy had a clinical response to treatment, including 2 patients who achieved complete remission [122]. Another clinical trial is evaluating the safety and efficacy of CAR NK cells targeting the NKG2D ligand MICA/B in patients with advanced CRC. The trial is still ongoing, but preliminary results have shown that CAR NK-cell therapy is safe and well tolerated. In addition, some patients have experienced tumor shrinkage and/or prolonged survival. Overall, the results of early clinical trials suggest that CAR NK-cell therapy is a promising new treatment approach for CRC. However, more research is needed to confirm the long-term safety and efficacy of this therapy.

Hurdles of Cell Therapy Strategies

Cell therapy strategies, specifically CAR-T-cell therapy, have been successful in treating blood cancers but face challenges when it comes to treating solid tumors. These challenges include finding an ideal target antigen, reaching the tumor, and survive in the tumor. Additionally, the toxicity of CAR-T cells to the human body and the potential for antigen escape are other concerns [123]. Solid tumors pose difficulties in

targeting antigens because they are expressed at different levels on normal tissues, increasing the risk of on-target off-tumor toxicity. Furthermore, the dense extracellular matrix [124] and lack of certain chemokines [125] in solid tumors hinder the migration and invasion of CAR-T cells. However, there are potential strategies to address these hurdles, such as targeting tumor-specific post-translational modifications and using anti-angiogenic therapy to normalize tumor vasculature [126]. The tumor microenvironment also plays a role in limiting the effectiveness of CAR-T-cell therapy, and efforts are being made to modify the metabolic profiles of CAR-T cells to enhance their function. For example, several chemotherapeutic drugs, including sunitinib, modulate tumor microenvironment components (such as Treg and MDSCs). Researchers reported that sunitinib combined with carbonic anhydrase IX (CAIX) targeting CAR-T cells increased infiltration and proliferation of CAIX-specific CAR-T cells by decreasing the presence of MDSCs and increasing the expression of the target antigen [5]. Toxicities associated with CAR-T-cell therapy include anaphylaxis, tumor lysis syndrome, infectious diseases, cytokine release syndrome (CRS), and neurologic toxicities [127–129]. Although most of these symptoms have not been reported in solid tumors, they should be considered when designing CAR-T cells for solid tumors, such as CRC. Various approaches, such as using complete human sequences in CAR construction to prevent allergic reactions and engineering CAR-T cells to secrete IL-1 receptor antagonists to control CRS, are being explored to address these toxicities [23, 130]. Another challenge is tumor resistance to antigen-targeting CAR-T cells, with loss of antigen expression in malignant cells being reported in most patients [131, 132].

Conclusion and Future Perspectives

In conclusion, the treatment landscape for CRC has witnessed significant evolution with the advent of targeted therapies. Chemotherapy backbones like FOLFOX/CAPOX and FOLFIRI remain central for CRC treatment, while newer combinations with oxaliplatin may pose neurotoxicity concerns. Targeted therapies such as Cetuximab and Panitumumab benefit *RAS/RAF* wild-type, microsatellite stable CRC, while immunotherapies like Pembrolizumab and Nivolumab offer promise for high microsatellite instability/mismatch repair-deficient cases. Combination therapies with immune checkpoint inhibitors are being explored to tackle resistance. Specific molecular subtypes, like *BRAF* mutant CRC, *HER2*-amplified CRC, and *NTRK* fusions, have seen breakthroughs with targeted therapies, expanding treatment options. Recent advancements in immunotherapy, encompassing monoclonal antibodies, checkpoint inhibitors, and oncolytic viruses, provide hope for novel CRC treatments

Table 6 Comparison of different immunotherapies

Treatment Type	CAGR	Advantages	Disadvantages	Commercial products	Number of clinical trials
NK-Cell Therapy	45–50% from 2021 to 2028	Enhanced anticancer response	Limited persistence	Yescarta (axicabtagene ciloleucel) by Kite Pharma/Gilead Sciences	Over 200 registered trials
Oncolytic Virus	30–35% from 2021 to 2028	Direct tumor cell killing	Potential off-target effects	Imlygic (talimogene laherparepvec) by Amgen	Over 600 registered trials
Cytokines	5–10% from 2021 to 2028	Stimulate immune response	Systemic toxicity	Proleukin (aldesleukin) by Prometheus Laboratories	Over 100 registered trials
Immune Checkpoint Inhibitor Therapy	20–25% from 2021 to 2028	Enhanced immune response	Autoimmune side effects	Keytruda (Pembrolizumab) by Merck, Opdivo (Nivolumab) by Bristol Myers Squibb, and others	Over 2,500 registered trials
Cell-Based Therapy	30–40% from 2021 to 2028	Target-specific cancer cells	Difficult manufacturing	Provenge (sipuleucel-T) by Dendreon Pharmaceuticals, Kymriah (tisagenlecleucel) by Novartis, and Yescarta (axicabtagene ciloleucel) by Kite Pharma/Gilead Sciences	Over 1000 registered trials
Monoclonal Antibodies	10–15% from 2021 to 2028	High specificity	Risk of immunogenicity	Herceptin (Trastuzumab) by Genentech/Roche, Rituxan (Rituximab) by Roche, and numerous others	Over 1000 registered trials
Cancer Cell Vaccines	5–10% from 2021 to 2028	Induce immune response	Variable effectiveness	Provenge (sipuleucel-T) by Dendreon Pharmaceuticals	Over 100 registered trials
CAR-T-Cell Therapy	40–45% from 2021 to 2028	Highly effective in some cases	Costly and complex	Commercial products: Kymriah (tisagenlecleucel) by Novartis, Yescarta (axicabtagene ciloleucel) by Kite Pharma/Gilead Sciences, Tecartus (brexucabtagene autoleucel) by Kite Pharma/Gilead Sciences	Over 100 registered trials

All of these methods have a variety of advantages and disadvantages

The compound annual growth rate (CAGR) is a useful index for comparison

(Table 6). Although these approaches are in early stages, they hold potential for improving outcomes. Continued research and clinical trials are essential for refining these therapies, overcoming resistance, and advancing CRC treatment in future.

Author's contribution NF, SNM, ST, FS, SS, MA, MA, MMR, and PN wrote the manuscript and prepared the tables and figures; NF and SNM collected the references and carried out the primary literature search; and ENM and MT designed and revised the article. All the authors read and approved the final manuscript.

Funding The authors have not disclosed any funding.

Data availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc/4.0/>.

References

- Alluri P, Newman LA. Basal-like and triple-negative breast cancers: searching for positives among many negatives. *Surg Oncol Clin N Am* 2014;23:567–577. <https://doi.org/10.1016/j.soc.2014.03.003>.
- Collaborators GBDCC. Global, regional, and national burden of colorectal cancer and its risk factors, 1990–2019: a systematic analysis for the global burden of disease study 2019. *Lancet Gastroenterol Hepatol* 2022;7:627–647. [https://doi.org/10.1016/S2468-1253\(22\)00044-9](https://doi.org/10.1016/S2468-1253(22)00044-9).
- De Palma FDE, D'Argenio V, Pol J, Kroemer G, Maiuri MC, Salvatore F. The molecular hallmarks of the serrated pathway in colorectal cancer. *Cancers (Basel)*. 2019. <https://doi.org/10.3390/cancers11071017>.
- Stefani C, Miricescu D, Stanescu S II et al. Growth factors, PI3K/AKT/mTOR and MAPK signaling pathways in colorectal cancer pathogenesis: where are we now? *Int J Mol Sci*. 2021. <https://doi.org/10.3390/ijms221910260>.
- Fennell LJ, Kane A, Liu C et al. APC mutation marks an aggressive subtype of BRAF mutant colorectal cancers. *Cancers (Basel)*. 2020. <https://doi.org/10.3390/cancers12051171>.
- Tiwari A, Saraf S, Verma A, Panda PK, Jain SK. Novel targeting approaches and signaling pathways of colorectal cancer: an insight. *World J Gastroenterol* 2018;24:4428–4435. <https://doi.org/10.3748/wjg.v24.i39.4428>.
- Hainsworth JD, Meric-Bernstam F, Swanton C, Hurwitz H, Spigel DR, Sweeney C et al. Targeted therapy for advanced solid tumors on the basis of molecular profiles: results from mypathway, an open-label, phase iia multiple basket study. *J Clin Oncol* 2018;36:536–542. <https://doi.org/10.1200/JCO.2017.75.3780>.
- Jin JY, Kim DW, Lee JW, Han CW, Min WS, Park CW et al. Immune suppression therapy in aplastic anemia: influencing factors on response and survival. *Korean J Intern Med* 1995;10:25–31. <https://doi.org/10.3904/kjim.1995.10.1.25>.
- Kumar M, Thangavel C, Becker RC, Sadayappan S. Monoclonal antibody-based immunotherapy and its role in the development of cardiac toxicity. *Cancers (Basel)*. 2020. <https://doi.org/10.3390/cancers13010086>.
- Zhan L, Feng HF, Liu HQ et al. Immune checkpoint inhibitors-related thyroid dysfunction: epidemiology, clinical presentation, possible pathogenesis, and management. *Front Endocrinol (Lausanne)* 2021;12:649863. <https://doi.org/10.3389/fendo.2021.649863>.
- Ranieri G, Patruno R, Ruggieri E, Montemurro S, Valerio P, Ribatti D. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. *Curr Med Chem* 2006;13:1845–1857. <https://doi.org/10.2174/092986706777585059>.
- 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;40:328. <https://doi.org/10.1186/s13046-021-02130-2>.
- Hurwitz H, Fehrenbacher L, Novotny W et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335–2342. <https://doi.org/10.1056/NEJMoa032691>.
- Rouyer M, Francois E, Sa Cunha A et al. Effectiveness of first-line cetuximab in wild-type RAS metastatic colorectal cancer according to tumour BRAF mutation status from the EREBUS cohort. *Br J Clin Pharmacol* 2021;87:1120–1128. <https://doi.org/10.1111/bcp.14472>.
- Wei L, Lin Z, Xie S et al. Complete response with cetuximab-based treatment of metastatic colorectal cancers: two case reports and literature review. *Front Oncol* 2022;12:798515. <https://doi.org/10.3389/fonc.2022.798515>.
- Kopetz S, Guthrie KA, Morris VK, Lenz HJ, Magliocco AM, Maru D et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG S1406). *J Clin Oncol* 2021;39:285–294. <https://doi.org/10.1200/JCO.20.01994>.
- Watanabe J, Muro K, Shitara K, Yamazaki K, Shiozawa M, Otori H et al. Panitumumab vs bevacizumab added to standard first-line chemotherapy and overall survival among patients with RAS wild-type, left-sided metastatic colorectal cancer: a randomized clinical trial. *JAMA* 2023;329:1271–1282. <https://doi.org/10.1001/jama.2023.4428>.
- Shiravand Y, Khodadadi F, Kashani SMA, Hosseini-Fard SR, Hosseini S, Sadeghirad H et al. Immune checkpoint inhibitors in cancer therapy. *Curr Oncol* 2022;29:3044–3060. <https://doi.org/10.3390/curroncol29050247>.
- He R, Zhao X, Liu J, Zhou Y, Zhang X, Cheng F. PD-1 and CTLA-4 inhibitors in combination vs alone for the treatment of advanced melanoma: a systematic review and meta-analysis.

- Medicine (Baltimore)* 2022;101:e30561. <https://doi.org/10.1097/md.00000000000030561>.
20. Borelli B, Antoniotti C, Carullo M, Germani MM, Conca V, Masi G. Immune-checkpoint inhibitors (ICIs) in metastatic colorectal cancer (mCRC) patients beyond microsatellite instability. *Cancers (Basel)*. 2022. <https://doi.org/10.3390/cancers14204974>.
 21. Awate S, Babiuk LA, Mutwiri G. Mechanisms of action of adjuvants. *Front Immunol* 2013;4:114. <https://doi.org/10.3389/fimmu.2013.00114>.
 22. Jia W, Zhang T, Huang H et al. Colorectal cancer vaccines: the current scenario and future prospects. *Front Immunol* 2022;13:942235. <https://doi.org/10.3389/fimmu.2022.942235>.
 23. Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. *Mol Cancer* 2021;20:41. <https://doi.org/10.1186/s12943-021-01335-5>.
 24. Chalabi M, Fanchi LF, Dijkstra KK et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med* 2020;26:566–576. <https://doi.org/10.1038/s41591-020-0805-8>.
 25. Lekshmy M, Dhanya CR, Smrithi JS et al. Peptide vaccines as therapeutic and prophylactic agents for female-specific cancers: the current landscape. *Pharmaceuticals* 2023;16:1054.
 26. Cercek A, Lumish M, Sinopoli J et al. PD-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. *N Engl J Med* 2022;386:2363–2376. <https://doi.org/10.1056/NEJMoa2201445>.
 27. Szklarczyk D, Gable AL, Lyon D et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019;47:D607–d613. <https://doi.org/10.1093/nar/gky1131>.
 28. Zolaly MA, Mahallawi W, Khawaji ZY, Alahmadi MA. The clinical advances of oncolytic viruses in cancer immunotherapy. *Cureus* 2023;15:e40742. <https://doi.org/10.7759/cureus.40742>.
 29. Jiffry J, Thavornwatanayong T, Rao D et al. Oncolytic reovirus (pelareorep) induces autophagy in kras-mutated colorectal cancer. *Clin Cancer Res* 2021;27:865–876. <https://doi.org/10.1158/1078-0432.CCR-20-2385>.
 30. Parakrama R, Fogel E, Chandy C et al. Immune characterization of metastatic colorectal cancer patients post reovirus administration. *BMC Cancer* 2020;20:569. <https://doi.org/10.1186/s12885-020-07038-2>.
 31. Liu C, Chu D, Kalantar-Zadeh K, George J, Young HA, Liu G. Cytokines: from clinical significance to quantification. *Advanced Science* 2021;8:2004433.
 32. Waldmann TA. Cytokines in cancer immunotherapy. *Cold Spring Harbor perspectives in biology* 2018;10:a028472.
 33. Lee S, Margolin K. Cytokines in cancer immunotherapy. *Cancers (Basel)* 2011;3:3856–3893. <https://doi.org/10.3390/cancers3043856>.
 34. Bhat AA, Nisar S, Singh M et al. Cytokine- and chemokine-induced inflammatory colorectal tumor microenvironment: emerging avenue for targeted therapy. *Cancer Commun (Lond)* 2022;42:689–715. <https://doi.org/10.1002/cac2.12295>.
 35. Dubensky TW Jr, Reed SG. Adjuvants for cancer vaccines. *Semin Immunol* 2010;22:155–161. <https://doi.org/10.1016/j.smim.2010.04.007>.
 36. Ni G, Wang T, Walton S et al. Manipulating IL-10 signalling blockade for better immunotherapy. *Cell Immunol* 2015;293:126–129. <https://doi.org/10.1016/j.cellimm.2014.12.012>.
 37. Perrie Y, Kirby D, Bramwell VW, Mohammed AR. Recent developments in particulate-based vaccines. *Recent Pat Drug Deliv Formul* 2007;1:117–129. <https://doi.org/10.2174/187221107780831897>.
 38. Goodwin TJ, Huang L. Investigation of phosphorylated adjuvants co-encapsulated with a model cancer peptide antigen for the treatment of colorectal cancer and liver metastasis. *Vaccine* 2017;35:2550–2557. <https://doi.org/10.1016/j.vaccine.2017.03.067>.
 39. Skwarczynski M, Toth I. Peptide-based synthetic vaccines. *Chem Sci* 2016;7:842–854. <https://doi.org/10.1039/c5sc03892h>.
 40. O'Neill CL, Shrimali PC, Clapacs ZE, Files MA, Rudra JS. Peptide-based supramolecular vaccine systems. *Acta Biomaterialia* 2021;133:153–167. <https://doi.org/10.1016/j.actbio.2021.05.003>.
 41. Klebanoff CA, Acquavella N, Yu Z, Restifo NP. Therapeutic cancer vaccines: are we there yet? *Immunol Rev* 2011;239:27–44. <https://doi.org/10.1111/j.1600-065X.2010.00979.x>.
 42. Hazama S, Nakamura Y, Tanaka H et al. A phase I trial study of five peptides combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study). *J Transl Med* 2014;12:108. <https://doi.org/10.1186/1479-5876-12-108>.
 43. Murahashi M, Hijikata Y, Yamada K et al. Phase I clinical trial of a five-peptide cancer vaccine combined with cyclophosphamide in advanced solid tumors. *Clin Immunol* 2016;166:48–58. <https://doi.org/10.1016/j.clim.2016.03.015>.
 44. Manickan E, Karem KL, Rouse BT. DNA vaccines—a modern gimmick or a boon to vaccinology? *Crit Rev Immunol* 2017;37:483–498. <https://doi.org/10.1615/CritRevImmunol.v37.i2-6.140>.
 45. Azadi A, Golchini A, Delazar S et al. Recent advances on immune targeted therapy of colorectal cancer using bi-specific antibodies and therapeutic vaccines. *Biol Proced Online* 2021;23:13. <https://doi.org/10.1186/s12575-021-00147-7>.
 46. Staff C, Mozaffari F, Haller BK, Wahren B, Liljefors M. A phase I safety study of plasmid DNA immunization targeting carcinoembryonic antigen in colorectal cancer patients. *Vaccine* 2011;29:6817–6822. <https://doi.org/10.1016/j.vaccine.2010.12.063>.
 47. Huang S, Yu X. Antitumor immunity of DNA vaccine based on CTLA-4 fused with HER2 against colon carcinoma. *European Journal of Inflammation* 2018;16:2058739218768144. <https://doi.org/10.1177/2058739218768144>.
 48. Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA vaccines for infectious diseases. *Front Immunol* 2019;10:594. <https://doi.org/10.3389/fimmu.2019.00594>.
 49. Liu T, Liang Y, Huang L. Development and delivery systems of mRNA vaccines. *Front Bioeng Biotechnol* 2021;9:718753. <https://doi.org/10.3389/fbioe.2021.718753>.
 50. Faghfuri E, Pourfarzi F, Faghfour AH, Abdoli Shadbad M, Hajiasgharzadeh K, Baradaran B. Recent developments of RNA-based vaccines in cancer immunotherapy. *Expert Opin Biol Ther* 2021;21:201–218. <https://doi.org/10.1080/14712598.2020.1815704>.
 51. Li C, Liang S, Zhang C et al. Allogenic dendritic cell and tumor cell fused vaccine for targeted imaging and enhanced immunotherapeutic efficacy of gastric cancer. *Biomaterials* 2015;54:177–187. <https://doi.org/10.1016/j.biomaterials.2015.03.024>.
 52. Mosolits S, Nilsson B, Mellstedt H. Towards therapeutic vaccines for colorectal carcinoma: a review of clinical trials. *Expert Rev Vaccines* 2005;4:329–350. <https://doi.org/10.1586/14760584.4.3.329>.
 53. Takamura-Ishii M, Nakaya T, Hagiwara K. Regulation of constitutive interferon-stimulated genes (ISGs) in tumor cells contributes to enhanced antitumor response of Newcastle disease virus-infected tumor vaccines. *Cancers (Basel)*. 2018. <https://doi.org/10.3390/cancers10060186>.
 54. Berry J, Vreeland T, Trappey A et al. Cancer vaccines in colon and rectal cancer over the last decade: lessons learned and future

- directions. *Expert Rev Clin Immunol* 2017;13:235–245. <https://doi.org/10.1080/1744666X.2016.1226132>.
55. Baars A, Claessen AM, Wagstaff J et al. A phase II study of active specific immunotherapy and 5-FU/Leucovorin as adjuvant therapy for stage III colon carcinoma. *Br J Cancer* 2002;86:1230–1234. <https://doi.org/10.1038/sj.bjc.6600254>.
 56. Bever KM, Thomas DL 2nd, Zhang J et al. A feasibility study of combined epigenetic and vaccine therapy in advanced colorectal cancer with pharmacodynamic endpoint. *Clin Epigenetics* 2021;13:25. <https://doi.org/10.1186/s13148-021-01014-8>.
 57. Kim VM, Pan X, Soares KC et al. Neoantigen-based EpiGVAX vaccine initiates antitumor immunity in colorectal cancer. *JCI Insight*. 2020. <https://doi.org/10.1172/jci.insight.136368>.
 58. Theiler J, Korber B. Graph-based optimization of epitope coverage for vaccine antigen design. *Stat Med* 2018;37:181–194. <https://doi.org/10.1002/sim.7203>.
 59. Chung Y, Qin H, Kang CY, Kim S, Kwak LW, Dong C. An NKT-mediated autologous vaccine generates CD4 T-cell dependent potent antilymphoma immunity. *Blood* 2007;110:2013–2019. <https://doi.org/10.1182/blood-2006-12-061309>.
 60. Turnis ME, Rooney CM. Enhancement of dendritic cells as vaccines for cancer. *Immunotherapy* 2010;2:847–862. <https://doi.org/10.2217/imt.10.56>.
 61. Sugiyama H. Cancer immunotherapy targeting Wilms' tumor gene WT1 product. *Expert Rev Vaccines* 2005;4:503–512. <https://doi.org/10.1586/14760584.4.4.503>.
 62. Maurel J, Caballero-Baños M, Mila J et al. Phase II randomized trial of autologous tumor lysate dendritic cell vaccine (ADC) plus best supportive care (BSC) compared with BSC, in pre-treated advanced colorectal cancer patients. *Journal of Clinical Oncology*. 2015. https://doi.org/10.1200/jco.2015.33.15_suppl.3048.
 63. Higuchi Y, Koya T, Yuzawa M et al. Enzyme-linked immunosorbent spot assay for the detection of wilms' tumor 1-specific t cells induced by dendritic cell vaccination. *Biomedicines* 2015;3:304–315. <https://doi.org/10.3390/biomedicines3040304>.
 64. Rodriguez J, Castanon E, Perez-Gracia JL et al. A randomized phase II clinical trial of dendritic cell vaccination following complete resection of colon cancer liver metastasis. *J Immunother Cancer* 2018;6:96. <https://doi.org/10.1186/s40425-018-0405-z>.
 65. Toh HC, Wang WW, Chia WK et al. Clinical benefit of allogeneic melanoma cell lysate-pulsed autologous dendritic cell vaccine in MAGE-positive colorectal cancer patients. *Clin Cancer Res* 2009;15:7726–7736. <https://doi.org/10.1158/1078-0432.CCR-09-1537>.
 66. van den Bent MJ, Tesileanu CMS, Wick W et al. Adjuvant and concurrent temozolomide for 1p/19q non-co-deleted anaplastic glioma (CATNON; EORTC study 26053–22054): second interim analysis of a randomised, open-label, phase 3 study. *Lancet Oncol* 2021;22:813–823. [https://doi.org/10.1016/s1470-2045\(21\)00090-5](https://doi.org/10.1016/s1470-2045(21)00090-5).
 67. Wooster AL, Girgis LH, Brazeale H, Anderson TS, Wood LM, Lowe DB. Dendritic cell vaccine therapy for colorectal cancer. *Pharmacol Res* 2021;164:105374. <https://doi.org/10.1016/j.phrs.2020.105374>.
 68. Maruoka S, Ojima T, Iwamoto H, Kitadani J, Tabata H, Tomi-naga S et al. Tumor RNA transfected DCs derived from iPS cells elicit cytotoxicity against cancer cells induced from colorectal cancer patients in vitro. *Sci Rep* 2022;12:3295. <https://doi.org/10.1038/s41598-022-07305-1>.
 69. Panagioti E, Klenerman P, Lee LN, van der Burg SH, Arens R. Features of effective T cell-inducing vaccines against chronic viral infections. *Front Immunol* 2018;9:276. <https://doi.org/10.3389/fimmu.2018.00276>.
 70. Jiang S, Good D, Wei MQ. Vaccinations for colorectal cancer: progress, strategies, and novel adjuvants. *Int J Mol Sci*. 2019. <https://doi.org/10.3390/ijms20143403>.
 71. Morse MA, Chaudhry A, Gabitzsch ES, Hobeika AC, Osada T, Clay TM et al. Novel adenoviral vector induces T-cell responses despite anti-adenoviral neutralizing antibodies in colorectal cancer patients. *Cancer Immunol Immunother* 2013;62:1293–1301. <https://doi.org/10.1007/s00262-013-1400-3>.
 72. Snook AE, Baybutt TR, Xiang B et al. Split tolerance permits safe Ad5-GUCY2C-PADRE vaccine-induced T-cell responses in colon cancer patients. *J Immunother Cancer* 2019;7:104. <https://doi.org/10.1186/s40425-019-0576-2>.
 73. Harrop R, Connolly N, Redchenko I et al. Vaccination of colorectal cancer patients with modified vaccinia ankara delivering the tumor antigen 5T4 (TroVax) induces immune responses which correlate with disease control: a phase I/II trial. *Clin Cancer Res* 2006;12:3416–3424. <https://doi.org/10.1158/1078-0432.CCR-05-2732>.
 74. Elkord E, Dangoor A, Drury NL et al. An MVA-based vaccine targeting the oncofetal antigen 5T4 in patients undergoing surgical resection of colorectal cancer liver metastases. *J Immunother* 2008;31:820–829. <https://doi.org/10.1097/CJI.0b013e3181876ab3>.
 75. Kaufman HL, Lenz HJ, Marshall J et al. Combination chemotherapy and ALVAC-CEA/B7.1 vaccine in patients with metastatic colorectal cancer. *Clin Cancer Res* 2008;14:4843–4849. <https://doi.org/10.1158/1078-0432.ccr-08-0276>.
 76. Erika JC, Amy CH, Donna N et al. Long-term survival of patients with stage III colon cancer treated with VRP-CEA(6D), an alphavirus vector that increases the CD8+ effector memory T cell to Treg ratio. *Journal for Immunotherapy of Cancer* 2020;8:e001662. <https://doi.org/10.1136/jitc-2020-001662>.
 77. Shata MT, Stevceva L, Agwale S, Lewis GK, Hone DM. Recent advances with recombinant bacterial vaccine vectors. *Mol Med Today* 2000;6:66–71. [https://doi.org/10.1016/s1357-4310\(99\)01633-0](https://doi.org/10.1016/s1357-4310(99)01633-0).
 78. da Silva AJ, Zangirolami TC, Novo-Mansur MT, Giordano Rde C, Martins EA. Live bacterial vaccine vectors: an overview. *Braz J Microbiol* 2014;45:1117–1129. <https://doi.org/10.1590/s1517-83822014000400001>.
 79. Leclerc C. New technologies for vaccine development. *Med Sci (Paris)* 2007;23:386–390. <https://doi.org/10.1051/medsci/2007234386>.
 80. Stubbs AC, Martin KS, Coeshott C et al. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. *Nat Med* 2001;7:625–629. <https://doi.org/10.1038/87974>.
 81. Lu Y, Bellgrau D, Dwyer-Nield LD et al. Mutation-selective tumor remission with ras-targeted, whole yeast-based immunotherapy. *Cancer Res* 2004;64:5084–5088. <https://doi.org/10.1158/0008-5472.CAN-04-1487>.
 82. Bilusic M, Heery CR, Arlen PM et al. Phase I trial of a recombinant yeast-CEA vaccine (GI-6207) in adults with metastatic CEA-expressing carcinoma. *Cancer Immunol Immunother* 2014;63:225–234. <https://doi.org/10.1007/s00262-013-1505-8>.
 83. Cohn A, Morse MA, O'Neil B et al. Whole recombinant saccharomyces cerevisiae yeast expressing ras mutations as treatment for patients with solid tumors bearing ras mutations: results from a phase 1 trial. *J Immunother* 2018;41:141–150. <https://doi.org/10.1097/CJI.0000000000000219>.
 84. Heery CR, Singh BH, Rauckhorst M et al. Phase I trial of a yeast-based therapeutic cancer vaccine (GI-6301) targeting the transcription factor brachyury. *Cancer Immunol Res* 2015;3:1248–1256. <https://doi.org/10.1158/2326-6066.cir-15-0119>.
 85. Hou Y, Liu R, Hong X et al. Engineering a sustained release vaccine with a pathogen-mimicking manner for robust and durable

- immune responses. *J Control Release* 2021;333:162–175. <https://doi.org/10.1016/j.jconrel.2021.03.037>.
86. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. *J Exp Med* 1965;121:439–462. <https://doi.org/10.1084/jem.121.3.439>.
 87. Lopes A, Vandermeulen G, Preat V. Cancer DNA vaccines: current preclinical and clinical developments and future perspectives. *J Exp Clin Cancer Res* 2019;38:146. <https://doi.org/10.1186/s13046-019-1154-7>.
 88. Thomas S, Prendergast GC. Cancer vaccines: a brief overview. *Methods Mol Biol* 2016;1403:755–761. https://doi.org/10.1007/978-1-4939-3387-7_43.
 89. Gordon B, Gadi VK. The role of the tumor microenvironment in developing successful therapeutic and secondary prophylactic breast cancer vaccines. *Vaccines (Basel)*. 2020. <https://doi.org/10.3390/vaccines8030529>.
 90. Zhao Z, Xiao X, Saw PE et al. Chimeric antigen receptor T cells in solid tumors: a war against the tumor microenvironment. *Science China Life Sciences* 2020;63:180–205.
 91. Rodriguez-Garcia A, Palazon A, Noguera-Ortega E, Powell DJ Jr, Guedan S. CAR-T cells hit the tumor microenvironment: strategies to overcome tumor escape. *Front Immunol* 2020;11:1109.
 92. Hayakawa T, Harris I, Joung J et al. Report of the international regulatory forum on human cell therapy and gene therapy products. *Biologicals* 2016;44:467–479.
 93. Graham C, Jozwik A, Pepper A, Benjamin R. Allogeneic CAR-T cells: more than ease of access? *Cells*. 2018. <https://doi.org/10.3390/cells7100155>.
 94. Martinez Bedoya D, Dutoit V, Migliorini D. Allogeneic CAR T cells: an alternative to overcome challenges of CAR T cell therapy in glioblastoma. *Front Immunol* 2021;12:640082. <https://doi.org/10.3389/fimmu.2021.640082>.
 95. Li H, Yang C, Cheng H, Huang S, Zheng Y. CAR-T cells for colorectal cancer: target-selection and strategies for improved activity and safety. *Journal of Cancer* 2021;12:1804.
 96. Rizzo G, Bertotti A, Leto SM, Vetrano S. Patient-derived tumor models: a more suitable tool for pre-clinical studies in colorectal cancer. *J Exp Clin Cancer Res* 2021;40:178. <https://doi.org/10.1186/s13046-021-01970-2>.
 97. Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front Immunol* 2019;10:128. <https://doi.org/10.3389/fimmu.2019.00128>.
 98. Ang WX, Li Z, Chi Z, Du S-H, Chen C, Tay JC et al. Intra-peritoneal immunotherapy with T cells stably and transiently expressing anti-EpCAM CAR in xenograft models of peritoneal carcinomatosis. *Oncotarget* 2017;8:13545.
 99. Li H, Yang C, Cheng H, Huang S, Zheng Y. CAR-T cells for colorectal cancer: target-selection and strategies for improved activity and safety. *J Cancer* 2021;12:1804–1814. <https://doi.org/10.7150/jca.50509>.
 100. Jin K-T, Chen B, Liu Y-Y, Lan H, Yan J-P. Monoclonal antibodies and chimeric antigen receptor (CAR) T cells in the treatment of colorectal cancer. *Cancer Cell International* 2021;21:1–15.
 101. Alnefaie A, Albogami S, Asiri Y et al. Chimeric antigen receptor T-cells: an overview of concepts, applications, limitations, and proposed solutions. *Front Bioeng Biotechnol* 2022;10:976.
 102. Cha SE, YazakiKujawski MJP, Brown C, Shively JE. Tumor regression and immunity in combination therapy with anti-CEA chimeric antigen receptor T cells and anti-CEA-IL2 immunocytokine. *Oncimmunology* 2021;10:1899469.
 103. Akbari B, Ghahri-Saremi N, Soltantoyeh T, Hadjati J, Ghassemi S, Mirzaei HR. Epigenetic strategies to boost CAR T cell therapy. *Molecular Therapy* 2021;29:2640–2659.
 104. Chen N, Xu Y, Mou J et al. Targeting of IL-10R on acute myeloid leukemia blasts with chimeric antigen receptor-expressing T cells. *Blood cancer journal* 2021;11:1–11.
 105. Xu J, Meng Q, Sun H et al. HER2-specific chimeric antigen receptor-T cells for targeted therapy of metastatic colorectal cancer. *Cell Death & Disease* 2021;12:1–11.
 106. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010;18:843–851. <https://doi.org/10.1038/mt.2010.24>.
 107. Lamers CH, Sleijfer S, van Steenbergen S et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. *Mol Ther* 2013;21:904–912. <https://doi.org/10.1038/mt.2013.17>.
 108. Sur D, Havasi A, Cainap C et al. Chimeric antigen receptor T-cell therapy for colorectal cancer. *J Clin Med*. 2020. <https://doi.org/10.3390/jcm9010182>.
 109. Ingebrigtsen VA, Boye K, Tekle C, Nesland JM, Flatmark K, Fodstad O. B7–H3 expression in colorectal cancer: nuclear localization strongly predicts poor outcome in colon cancer. *Int J Cancer* 2012;131:2528–2536. <https://doi.org/10.1002/ijc.27566>.
 110. Guo M, You C, Dou J. Role of transmembrane glycoprotein mucin 1 (MUC1) in various types of colorectal cancer and therapies: current research status and updates. *Biomed Pharmacother* 2018;107:1318–1325. <https://doi.org/10.1016/j.biopha.2018.08.109>.
 111. Guillerey C. NK cells in the tumor microenvironment. *Adv Exp Med Biol* 2020;1273:69–90. https://doi.org/10.1007/978-3-030-49270-0_4.
 112. Becker PSA, Suck G, Nowakowska P et al. Selection and expansion of natural killer cells for NK cell-based immunotherapy. *Cancer Immunology. Immunotherapy* 2016;65:477–484. <https://doi.org/10.1007/s00262-016-1792-y>.
 113. Liu M, Liang S, Zhang C. NK cells in autoimmune diseases: protective or pathogenic? *Front Immunol* 2021;12:624687. <https://doi.org/10.3389/fimmu.2021.624687>.
 114. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: development, maturation, and clinical utilization. *Front Immunol* 2018;9:1869. <https://doi.org/10.3389/fimmu.2018.01869>.
 115. Minetto P, Guolo F, Pesce S et al. Harnessing NK cells for cancer treatment. *Front Immunol* 2019;10:2836. <https://doi.org/10.3389/fimmu.2019.02836>.
 116. Streltsova MA, Erokhina SA, Kanevskiy LM et al. Recurrent stimulation of natural killer cell clones with K562 expressing membrane-bound interleukin-21 affects their phenotype, interferon- γ production, and lifespan. *Int J Mol Sci* 2019;20:443.
 117. Zhang Q, Zhang H, Ding J et al. Combination therapy with EpCAM-CAR-NK-92 cells and regorafenib against human colorectal cancer models. *Journal of Immunology Research* 2018;2018:4263520.
 118. Lee S-C, Shimasaki N, Lim JS et al. Phase I trial of expanded, activated autologous NK-cell infusions with trastuzumab in patients with HER2-positive cancers. *Clinical Cancer Research* 2020;26:4494–4502.
 119. Ishikawa T, Okayama T, Sakamoto N et al. Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with IgG1 antibody in patients with gastric or colorectal cancer. *Int J Cancer* 2018;142:2599–2609. <https://doi.org/10.1002/ijc.31285>.
 120. Della Chiesa M, Setti C, Giordano C et al. NK cell-based immunotherapy in colorectal cancer. *Vaccines (Basel)*. 2022. <https://doi.org/10.3390/vaccines10071033>.

121. Zhang L, Meng Y, Feng X, Han Z. CAR-NK cells for cancer immunotherapy: from bench to bedside. *Biomarker Research* 2022;10:12. <https://doi.org/10.1186/s40364-022-00364-6>.
122. Xiao L, Cen D, Gan H, Sun Y, Huang N, Xiong H et al. Adoptive transfer of NKG2D CAR mRNA-engineered natural killer cells in colorectal cancer patients. *Molecular Therapy* 2019;27:1114–1125.
123. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. *Mol Ther Oncolytics* 2016;3:16011. <https://doi.org/10.1038/mt.2016.11>.
124. Henke E, Nandigama R, Ergun S. Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. *Front Mol Biosci* 2019;6:160. <https://doi.org/10.3389/fm.2019.00160>.
125. Poznansky MC, Olszak IT, Evans RH et al. Thymocyte emigration is mediated by active movement away from stroma-derived factors. *J Clin Invest* 2002;109:1101–1110. <https://doi.org/10.1172/JCI13853>.
126. Daenen LG, Shaked Y, Man S et al. Low-dose metronomic cyclophosphamide combined with vascular disrupting therapy induces potent antitumor activity in preclinical human tumor xenograft models. *Mol Cancer Ther* 2009;8:2872–2881. <https://doi.org/10.1158/1535-7163.MCT-09-0583>.
127. Kochenderfer JN, Dudley ME, Kassim SH et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol* 2015;33:540–549. <https://doi.org/10.1200/JCO.2014.56.2025>.
128. Porter DL, Hwang WT, Frey NV et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015. <https://doi.org/10.1126/scitranslmed.aac5415>.
129. Turtle CJ, Hanafi LA, Berger C et al. CD19 CAR-T cells of defined CD4+CD8+ composition in adult B cell ALL patients. *J Clin Invest* 2016;126:2123–2138. <https://doi.org/10.1172/JCI85309>.
130. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med* 2018;24:731–738. <https://doi.org/10.1038/s41591-018-0041-7>.
131. Maude SL, Teachey DT, Porter DL, Grupp SA. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood* 2015;125:4017–4023. <https://doi.org/10.1182/blood-2014-12-580068>.
132. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer Journal* 2021;11:69. <https://doi.org/10.1038/s41408-021-00459-7>.
133. 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;23:659–670. [https://doi.org/10.1016/s1470-2045\(22\)00197-8](https://doi.org/10.1016/s1470-2045(22)00197-8).
134. Jonker DJ, O'Callaghan CJ, Karapetis CS et al. Cetuximab for the treatment of colorectal cancer. *New England Journal of Medicine* 2007;357:2040–2048. <https://doi.org/10.1056/NEJMoa071834>.
135. Rosen LS, Jacobs IA, Burkes RL. Bevacizumab in colorectal cancer: current role in treatment and the potential of biosimilars. *Target Oncol* 2017;12:599–610. <https://doi.org/10.1007/s11523-017-0518-1>.
136. Liu Y, Zhang H, Cui H et al. Combined and targeted drugs delivery system for colorectal cancer treatment: conatumumab decorated, reactive oxygen species sensitive irinotecan prodrug and quercetin co-loaded nanostructured lipid carriers. *Drug Deliv* 2022;29:342–350. <https://doi.org/10.1080/10717544.2022.2027573>.
137. Sartore-Bianchi A, Trusolino L, Martino C et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016;17:738–746. [https://doi.org/10.1016/s1470-2045\(16\)00150-9](https://doi.org/10.1016/s1470-2045(16)00150-9).
138. Li G, Suzuki H, Ohishi T, Asano T, Tanaka T, Yanaka M et al. Antitumor activities of a defucosylated anti-EpCAM monoclonal antibody in colorectal carcinoma xenograft models. *Int J Mol Med*. 2023. <https://doi.org/10.3892/ijmm.2023.5221>.
139. Kim RD, Azad NS, Morse MA et al. Phase II study of ensituximab, a novel chimeric monoclonal antibody, in adults with unresectable. *Metastatic Colorectal Cancer. Clin Cancer Res* 2020;26:3557–3564. <https://doi.org/10.1158/1078-0432.ccr-20-0426>.
140. Jiffry J, Thavornwatanayong T, Rao D et al. Oncolytic reovirus (pelareorep) induces autophagy in KRAS-mutated colorectal carcerautophagy augments pelareorep activity in KRAS-mutated CRC. *Clinical Cancer Research* 2021;27:865–876.
141. Yang H, Peng T, Li J et al. Treatment of colon cancer with oncolytic herpes simplex virus in preclinical models. *Gene Ther* 2016;23:450–459. <https://doi.org/10.1038/gt.2016.15>.
142. Yuan S, Wu Y, Wang Y, Chen J, Chu L. An oncolytic adenovirus expressing SNORD44 and GAS5 exhibits antitumor effect in colorectal cancer cells. *Hum Gene Ther* 2017;28:690–700. <https://doi.org/10.1089/hum.2017.041>.
143. Wang J, Liu T, Chen J. Oncolytic measles virus encoding interleukin-12 mediated antitumor activity and immunologic control of colon cancer in vivo and ex vivo. *Cancer Biother Radiopharm* 2021;36:774–782. <https://doi.org/10.1089/cbr.2019.3084>.
144. Deng L, Yang X, Fan J et al. IL-24-armed oncolytic vaccinia virus exerts potent antitumor effects via multiple pathways in colorectal cancer. *Oncol Res* 2021;28:579–590. <https://doi.org/10.3727/096504020x15942028641011>.
145. Högri H, Lee DS, Konkright W et al. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol Immunother*. 2000. <https://doi.org/10.1007/s002620000146>.
146. Danishmalik SN, Sin JI. Therapeutic tumor control of HER2 DNA vaccines is achieved by an alteration of tumor cells and tumor microenvironment by gemcitabine and anti-Gr-1 Ab treatment in a HER2-expressing tumor model. *DNA Cell Biol* 2017;36:801–811. <https://doi.org/10.1089/dna.2017.3810>.
147. Son HY, Apostolopoulos V, Chung JK, Kim CW, Park JU. Protective efficacy of a plasmid DNA vaccine against transgene-specific tumors by Th1 cellular immune responses after intradermal injection. *Cell Immunol* 2018;329:17–26. <https://doi.org/10.1016/j.cellimm.2018.04.003>.
148. Ali SA, Ahmad M, Lynam J et al. Anti-tumour therapeutic efficacy of OX40L in murine tumour model. *Vaccine* 2004;22:3585–3594. <https://doi.org/10.1016/j.vaccine.2004.03.041>.
149. Cross RS, Malaterre J, Davenport AJ et al. Therapeutic DNA vaccination against colorectal cancer by targeting the MYB oncoprotein. *Clin Transl Immunology* 2015;4:e30. <https://doi.org/10.1038/cti.2014.29>.
150. Ockert D, Schirmacher V, Beck N et al. Newcastle disease virus-infected intact autologous tumor cell vaccine for adjuvant active specific immunotherapy of resected colorectal carcinoma. *Clin Cancer Res* 1996;2:21–28.
151. Harris JE, Ryan L, Hoover HC Jr et al. Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: eastern cooperative oncology group study E5283. *J Clin Oncol* 2000;18:148–157. <https://doi.org/10.1200/JCO.2000.18.1.148>.

152. Nair SK, Boczkowski D, Morse M, Cumming RI, Lysterly HK, Gilboa E. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. *Nat Biotechnol* 1998;16:364–369. <https://doi.org/10.1038/nbt0498-364>.
153. Fong L, Hou Y, Rivas A et al. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc Natl Acad Sci U S A* 2001;98:8809–8814. <https://doi.org/10.1073/pnas.141226398>.
154. Dillman RO, Nistor GI, Keirstead HS. Autologous dendritic cells loaded with antigens from self-renewing autologous tumor cells as patient-specific therapeutic cancer vaccines. *Hum Vaccin Immunother* 2023;19:2198467. <https://doi.org/10.1080/21645515.2023.2198467>.
155. Toubaji A, Achar M, Provenzano M et al. Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers. *Cancer Immunol Immunother* 2008;57:1413–1420. <https://doi.org/10.1007/s00262-008-0477-6>.
156. Moulton HM, Yoshihara PH, Mason DH, Iversen PL, Triozzi PL. Active specific immunotherapy with a beta-human chorionic gonadotropin peptide vaccine in patients with metastatic colorectal cancer: antibody response is associated with improved survival. *Clin Cancer Res* 2002;8:2044–2051.
157. Miyagi Y, Imai N, Sasatomi T et al. Induction of cellular immune responses to tumor cells and peptides in colorectal cancer patients by vaccination with SART3 peptides. *Clin Cancer Res* 2001;7:3950–3962.
158. Tsuruma T, Hata F, Torigoe T et al. Phase I clinical study of anti-apoptosis protein, survivin-derived peptide vaccine therapy for patients with advanced or recurrent colorectal cancer. *J Transl Med* 2004;2:19. <https://doi.org/10.1186/1479-5876-2-19>.
159. Speetjens FM, Kuppen PJ, Welters MJ et al. Induction of p53-specific immunity by a p53 synthetic long peptide vaccine in patients treated for metastatic colorectal cancer. *Clin Cancer Res* 2009;15:1086–1095. <https://doi.org/10.1158/1078-0432.CCR-08-2227>.
160. Chehelgerdi M, Chehelgerdi M. The use of RNA-based treatments in the field of cancer immunotherapy. *Molecular Cancer* 2023;22:106. <https://doi.org/10.1186/s12943-023-01807-w>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.