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REVIEW ARTICLE

Colorectal Cancer; Novel Approaches in Chimeric Antigen Receptors (CAR) -T cell

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ABSTRACT

Colorectal cancer (CRC) is a popular type of cancer, characterized by high mortality and a notable impression on the well-being of individuals. The success of adoptive chimeric antigen receptor T (CAR-T) cell therapy in treating hematological malignancies has been remarkable in recent years; however, its application in solid tumors like CRC has many challenges.

These obstacles encompass the immunosuppressive microenvironment of the tumor, the insufficient targeting of CAR-T cells, the limited lifespan of CAR-T cells within the body, and the constrained capacity for proliferation. Additionally, CAR-T cells face hurdles in effectively infiltrating the tumor site, which further complicates treatment outcomes. Diverse innovative strategies have been suggested to surmount these barriers in the context of CRC.

This comprehensive review endeavors to meticulously elucidate an exhaustive and detailed evaluation of the prevailing and contemporary landscape concerning CAR-T cell therapy as it pertains to the intricate management of CRC, while simultaneously offering a thorough indication of the various risk factors and the associated prevalence that are intricately linked with the manifestation and progression of CRC.

Keywords: Colorectal cancer, CAR-T cell, Treatment approaches

Introduction

Colorectal cancer (CRC), which includes cancers of the colon and rectum, is the second most lethal and the third most frequently diagnosed cancer globally, posing a significant public health challenge. Statistics from 2020 reveal that colorectal cancer was responsible for 9.4% of all cancer-related fatalities. However, a surge in incidences among the elderly is anticipated, suggesting that the global prevalence of CRC will more than double by 2035, with the most significant increase expected in less developed regions (1, 2). The progression of CRC is a gradual process that generally spans about 10 years for a polyp to transition into a malignant tumor. Consequently, timely identification and removal of polyps through routine screening are crucial for CRC prevention. Existing diagnostic techniques such as fecal immunochemical test and multitarget DNA test, colonoscopy, flexible sigmoidoscopy, CT colonography, and colon capsule, and tests based on blood identify only 40% of CRC cases in the early stages, leading to a risk of recurrence after surgery and treatment (3).

Chemotherapy targets cancer cells but also damages healthy cells, often leading to resistance in many CRC patients and reducing the efficacy of anticancer drugs, eventually resulting in chemotherapy failure. Therefore, an in-depth discussion on the epidemiology, risk factors, and preventive measures for CRC based on the latest evidence-based information is crucial to address future challenges associated with this disease (3, 4) Pharmacological approaches include non-specific drugs such as fluoropyrimidines (5-fluorouracil, usually combined with leucovorin, or capecitabine, a 5-fluorouracil prodrug), irinotecan and oxaliplatin, as well as targeted drugs such as angiogenesis inhibitors, EGFR inhibitors, and multikinase inhibitors. Currently, the landscape of cancer treatment has been greatly revolutionized by the utilization of immunotherapy strategies employing immune checkpoint inhibitors and adoptive cell therapy (ACT) in addition to chemotherapy.

Neoadjuvant therapy entails the delivery of radiotherapy, chemotherapy, and a blend of various treatment modalities before surgery. The main objective of this strategy is to reduce the extent of the tumor, thereby diminishing the chances of local recurrence and enhancing the general prognosis (5). This evaluation focuses on novel therapeutic agents created for cancer-specific interventions. It explores methods of screening and their application in tailored treatments targeting distinct molecular pathways. Lastly, it provides a forward-looking assessment of upcoming trends in therapeutic agents and screening within the domain of targeted therapies.

Literature Search and Selection of Articles

An extensive review of the current literature on recent advancements in therapeutic agents for CRC was conducted. The inclusion criteria encompassed articles written in English, available in full-text, comprehensive, and directly pertinent to the subject under investigation. A comprehensive search was carried out in the PubMed and Scopus databases in December 2023, utilizing keywords related to drugs, therapeutic agents, colorectal cancer/CRC, CAR T-cell, and novel therapeutic methods.

Initially, 159 articles were identified based on their titles, abstracts, and publication dates. After eliminating duplicate entries, 84 distinct articles were retained. These articles were thoroughly analyzed, and 5 articles relevant to the research question were selected. In March 2024, a supplementary search using Google Scholar, PubMed, and Scopus identified and included nine additional articles relevant to the topic. To enhance the clarity and coherence of our arguments, nine additional references were integrated throughout the writing process (Figure 1).

CRC prevalence and risk factors

In 2023, approximately 153,020 individuals will be diagnosed with CRC and 52,550 will die from the disease, including 19,550 cases and 3750 deaths in individuals younger than 50 years(2). In the year 2020, approximately 19.3 million new cases of cancer were documented on a global scale, resulting in approximately 10 million fatalities linked to cancer. CRC represented an estimated 1.93 million novel cases, which accounts for around 10% of the total, and around 0.94 million deaths, constituting 9.4% of the total (2). The occurrence and fatality rates of CRC vary notably between countries and regions, often correlating with the economic status of countries. Regions with higher income levels tend to have a higher prevalence of new cases and fatalities, whereas lower-income areas tend to report fewer incidences and

deaths. In 2019, East Asia was the region most impacted by colorectal cancer, with 637,096 new cases, 275,604 deaths, and 6.7 million DALYs (6).

In 2019, China, the USA, and Japan had the highest number of new cases of the condition for both males and females combined. The highest number of deaths related to the condition occurred in China with 261,777 deaths, followed by India with 79,098 deaths, and the USA with 84,026 deaths. Greenland, Brunei, and Hungary had the highest rates, while Bangladesh, Somalia, and Nepal had the lowest rates among the 204 countries and territories analyzed in 2019 (7).

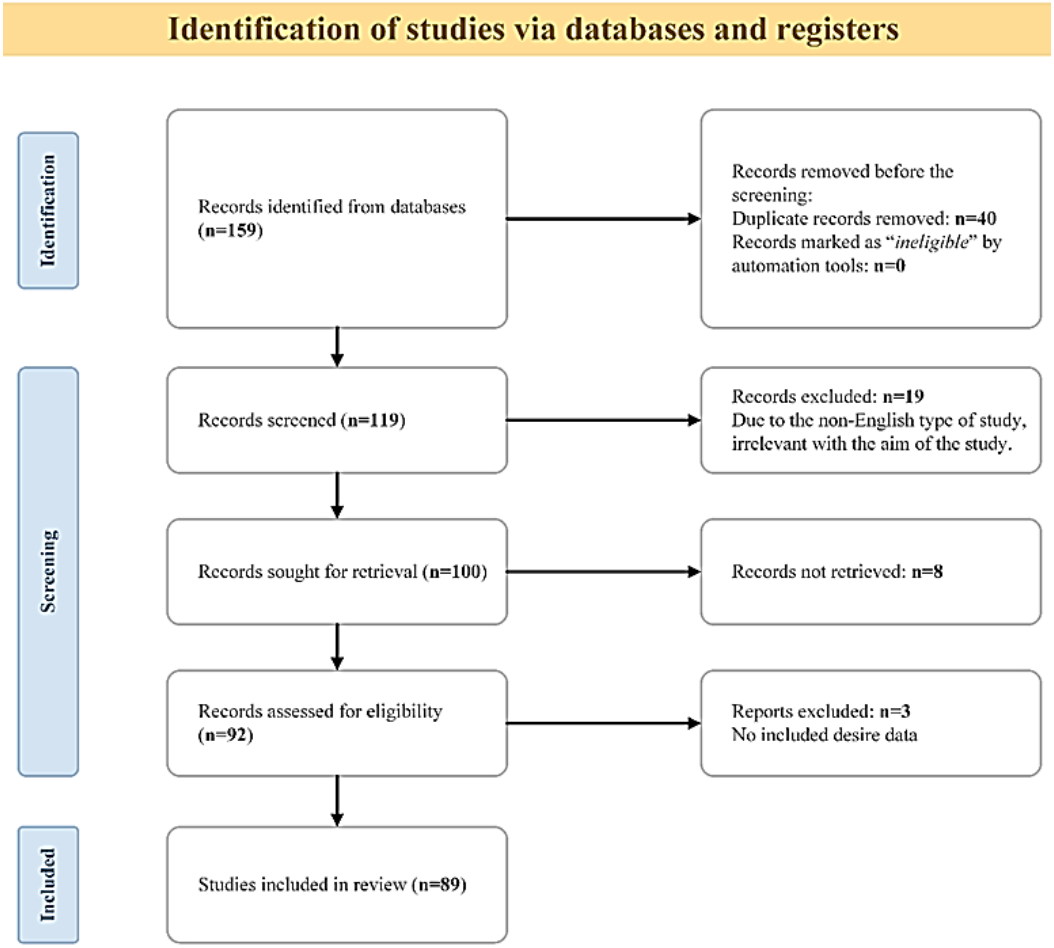

Figure 1. Flow diagram of the steps for including studies in the review study

Looking ahead to 2040, it is projected that the global burden of colorectal cancer will significantly escalate, reaching an estimated 3.2 million new cases annually, reflecting a substantial 63% increase. Similarly, the sum of deaths recognized by CRC is predicted to rise to 1.6 million per year, marking a considerable 73% surge compared to current statistics (2).

This anticipated surge in both incidence and mortality rates underscores the urgent need for enhanced preventive measures, early detection strategies, and innovative treatment approaches to effectively address the growing impact of colorectal cancer on global public health. The CDC reports that CRC occurrence and mortality rates vary by sex, race, and ethnicity. Black individuals have the highest rates, followed by white individuals, Asian or Pacific Islanders, and American Indians or Alaska Natives (8). In the realm of epidemiological studies, it has been consistently observed that the male population exhibits a significantly elevated propensity for the development of colorectal cancer when contrasted with their female counterparts, a disparity that is further compounded by the increased incidence of inflammatory bowel disease among males. Early-onset CRC patients are 1.44 times more likely to be male compared to those with late-onset CRC (50 years or older), as well as having higher odds of being black or Asian and having IBD. The prevalence of colorectal cancer, along with the

corresponding rates of mortality associated with this disease, is currently experiencing an upward trajectory in nations characterized as developing, although it is noteworthy that these rates continue to exhibit a significantly elevated level in countries classified as high-income, thereby highlighting a stark contrast between different economic contexts (9, 10).

In 2020, CRC is more prevalent in men than in women and is over four times more common in high-income countries compared to low-income nations (10, 11). Mortality rates for CRC have dropped in many regions, but some countries still face challenges in obtaining necessary treatment and additional therapies. CRC represents a critical non-modifiable risk factor, with a preponderance of cases manifesting in individuals exceeding the age of 50 years. In the United States, approximately 153,020 individuals will receive a colorectal cancer diagnosis in 2023, with approximately 52,550 cases and 3750 deaths among those under 50. The occurrence of CRC has decreased from 3% to 4% in the 2000s to 1% per year from 2011 to 2019(12). An observable shift towards left-sided tumors is also noted, with the percentage of rectal cancer cases escalating from 27% in 1995 to 31% in 2019. The age groups with the highest relative survival rates (RSRs) for colon cancer in most Asian and North American nations and regions are 45-54 years old and under 44 years old (13). A familial history of CRC represents a considerable risk factor, as individuals with such a background are nearly twice as likely to develop the condition. A comprehensive meta-analysis encompassing 8,091 cases of CRC from 16 different studies revealed that individuals aged 18 to 49 with a family history of CRC face a markedly higher risk of the disease compared to their counterparts without such a familial link (14, 15).

Body mass index (BMI) plays a crucial role in the expansion of cancer. Adipose tissue, an endocrine organ, regulates energy intake and the inflammatory response. Abnormal or excessive fat accumulation can lead to changes in hormone secretion by adipose tissue, leading to increased levels of factors such as leptin, TNF-α, IL-1, IL-6, IL-7, and IL-8. A comprehensive meta-analysis examining the association between CRC and BMI across over 66,000 CRC patients in 23 studies identified a substantial link between BMI and CRC risk. The risk of developing CRC was found to increase by 10% for every 8 kg/m2 increase in BMI (16). Several investigations have explored the correlation between different measures of obesity and the risk of early-onset colorectal cancer (EOCRC). After adjustment for BMI, waist circumference was independently linked to a twofold increase in the likelihood of colon cancer, with a notably strong association observed among inactive individuals. Additional study is desirable to precisely elucidate the specific impact of excess weight and abdominal obesity on EOCRC risk in both genders (17).

Higher levels of exercise have been demonstrated to improve the survival rate, however, physical inactivity has also been related to an augmented risk of the disease (17, 18). The prevalence and impact of colorectal cancer on a global, regional, and national scale from 1990 to 2019 revealed that certain factors predominantly influenced certain factors at a global level. These factors included a low milk intake (15.6%), smoking (13.3%), insufficient calcium in the diet (12.9%), and alcohol use (9.9%). Consistent alcohol intake has been notably linked to a higher risk of colorectal cancer, with moderate drinkers experiencing a 21% increased risk and heavy drinkers facing a 52% increased risk (19). In the community population aged 50-74, research findings suggest a positive association between alcohol consumption and an increased risk of CRC (20).

The act of smoking is identified as a contributing risk factor for colorectal cancer, with evidence suggesting that the risk of developing this type of cancer escalates following the volume of cigarettes consumed. Various research findings indicate that the correlation between ongoing smoking and rectal cancer is notably more pronounced in comparison to proximal or distal cancers, particularly when contrasted with individuals with no history of smoking. Moreover, male smokers are confronted with a 39% elevated risk of distal cancer, whereas women who have ever smoked exhibit a 20% higher susceptibility to proximal cancer than females who have never engaged in smoking. These observations highlight the intricate relationship between smoking habits and the differential risks of various types of CRC among different genders (21, 22).

Red meat consumption, such as beef, pork, and lamb, has been shown to elevate the likelihood of developing CRC by 20-30%, as supported by various research studies. A study conducted in the United Kingdom revealed that persons who have an average daily intake of 76 grams of red meat are faced with a

20% higher risk of CRC compared to those who consume only 21 grams per day. To mitigate this risk, individuals should limit their consumption of red meat to a maximum of 500 grams per week or 70 grams per day, with a particular emphasis on moderating the intake of processed meat due to its detrimental effects. Scientific evidence indicates that dietary patterns abundant in high-fiber foods, including fruits, vegetables, whole grains, and cereals, provide a protective benefit against colorectal cancer. Furthermore, research has shown that there is a correlation between vitamin D insufficiency and colorectal cancer, with lower levels of vitamin D being associated with a higher incidence of this type of cancer. Therefore, it is suggested that individuals consider supplementing their diet with additional calcium and vitamin D to potentially lower the risk of developing colorectal cancer (23).

Therapeutic approaches

Local therapeutic approaches in the treatment of rectal cancer often involve neoadjuvant therapy, which includes radiotherapy and chemotherapy, either separately or in combination. The main focus of neoadjuvant therapy is on locally advanced rectal cancer and select cases of resectable metastatic colorectal cancer. Research has shown that this treatment can successfully reduce tumor size in intermediate and advanced-stage cancer patients (24).

The goals of radiation therapy in rectal cancer treatment are to improve overall survival and diminish the risk of local reappearance. Neoadjuvant radiation therapy can potentially enhance the effects of anti-PD-1/PD-L1 therapy by boosting various aspects of the immune response. Additionally, neoadjuvant chemotherapy may increase the effectiveness of immune checkpoint inhibitor treatments. Two studies have highlighted the benefits of neoadjuvant immunotherapy before surgery in colorectal cancer patients, showing significant improvements in tumor response and survival rates (24, 25). Advanced radiation therapy methods like intensity-modulated radiation therapy (IMRT) have become increasingly common in clinical practice for rectal cancer treatment. Based on previous studies, IMRT has shown potential benefits by reducing toxicity through lower radiation dosages. It allows for accurate delivery of radiation to the tumor while diminishing exposure to nearby healthy tissues. Studies comparing IMRT with traditional 3D conformal radiation therapy (3DCRT) have demonstrated lower toxicity levels with IMRT, particularly in the treatment of gynecologic malignancies and anal cancer(26, 27). Research by Georgios Kouklidis indicated that IMRT can reduce acute bowel side effects in patients with locally advanced rectal cancer undergoing neoadjuvant radiotherapy. Individuals in the UK suffering from stage II-III rectal cancer have undergone treatment with either 3D conformal radiation therapy or intensity-modulated radiation therapy, and there has been a growing inclination towards the use of IMRT in recent years. The utilization of IMRT varies among different institutions and sociodemographic groups, but it seems to be more consistently embraced in specific clinical settings (28).

Moving on to the systemic therapeutic approaches for colorectal cancer, chemotherapy shows an important role in the treatment of the disease. Chemotherapeutic drugs like 5-FU (fluorouracil), capecitabine, cisplatin, oxaliplatin, and irinotecan target cancer cells through various mechanisms. For instance, 5-FU inhibits DNA synthesis in cancer cells, while capecitabine is converted into active 5-FU within the body. Cisplatin forms DNA adducts that disrupt DNA replication, and oxaliplatin interferes with DNA replication and transcription processes. Irinotecan inhibits an enzyme necessary for DNA transcription, leading to DNA damage and cell death. Combination regimens containing these chemotherapeutic drugs have become standard in clinical practice, showing superior efficacy in advanced colorectal cancer cases compared to single-agent treatments (29, 30).

FOLFOX, leucovorin calcium (folinic acid, FOL), fluorouracil (5-FU, F), and oxaliplatin (OX), and FOLFIRI, leucovorin calcium (folinic acid), fluorouracil, and irinotecan hydrochloride, regimens, which combine different chemotherapy drugs, have demonstrated significant therapeutic potential in metastatic colorectal cancer. The implementation of these combination therapies has led to enhanced progression-free survival and overall survival rates among patients diagnosed with stage II and III colorectal cancer. Clinical studies have also shown that regimens like XELOX, capecitabine (Xeloda), and oxaliplatin, have comparable efficacy and toxicity profiles to traditional 5-FU/LV treatments. Adjuvant therapy with XELOX has been associated with upgraded disease-free survival and complete survival

rates compared to 5-FU/LV therapy. However, each regimen may have specific side effects, with 5-FU/LV being linked to stomatitis and neutropenia, while XELOX may cause thrombocytopenia and hand-foot syndrome (29, 31).

In a prospective study on metastatic colorectal cancer, the efficacy and the toxicity of three different chemotherapy regimens - FOLFOX-4 vs. FOLFIRI vs. IROX were compared. The objective response rates were recorded at 63.6% for the FOLFOX group, 44% for the FOLFIRI arm, and 53% for the IROX arm. The stable disease rates were 27.3% in the FOLFOX arm, 44.4% in the FOLFIRI arm, and 35.3% in the IROX arm. The disease control rates were 91% for FOLFOX, 88.8% for FOLFIRI, and 88.2% for IROX. The primary grade 3–4 toxicities included neutropenia at rates of 22.7%, 16.66%, and 29.4%; febrile neutropenia at 4.5%, 5.5%, and 11.76%; diarrhea at 9%, 22.2%, and 23.5%; nausea/vomiting at 4.5%, 5.5%, and 11.76%; and neuropathy at 18.18%, 0%, and 17.6%, respectively. All three treatment regimens were well tolerated, yielding comparable outcomes, with the FOLFOX arm demonstrating a slightly superior disease control rate and the IROX group showing a trend towards a higher complete response rate. The FOLFOX regimen exhibited lower incidences of nausea, vomiting, and diarrhea, while sensitive neuropathy and neutropenia were more prevalent in regimens that included oxaliplatin.

The IROX regimen was associated with a higher frequency of adverse effects (32). The treatment of colorectal cancer should prioritize surgical resection of the tumor, either as a standalone option or in conjunction with chemotherapy and radiotherapy, as this approach has proven to be the most effective in enhancing patient survival rates. Nevertheless, surgical intervention is not beneficial for colorectal cancer diagnosed at advanced stages, which accounts for roughly 25% of cases. Pharmacological treatments encompass non-specific agents such as fluoropyrimidines, irinotecan, and oxaliplatin, alongside targeted therapies like angiogenesis inhibitors (bevacizumab), EGFR inhibitors (cetuximab), and multikinase inhibitors (regorafenib).

The integration of combination therapies and targeted agents has significantly advanced the treatment of colorectal cancer in recent years, particularly for patients with metastatic disease. However, the estimated 5-year overall survival rate for colorectal cancer patients in the United States stands at 64% across all stages, dropping to a mere 12% for those with metastatic colorectal cancer, underscoring the critical need for the development of new therapeutic strategies for this condition. Overall, neoadjuvant and systemic therapeutic approaches, along with advanced delivery methods like IMRT, have shown promising results in the treatment of colorectal cancer, highlighting the importance of personalized and comprehensive treatment strategies for better patient outcomes.

Adoptive cell transfer therapy

Chimeric antigen receptor T-cell therapy, frequently mentioned to as CAR-T therapy, is classified as a form of adoptive T-cell immunotherapy (ACT). This groundbreaking method involves utilizing the patient's immune cells, specifically T cells, to enhance their ability to recognize and destroy cancerous cells. By modifying T cells to express chimeric antigen receptors, CAR-T therapy emerges as a promising approach for addressing multiple cancer types by capitalizing on the body's inherent immune response (33). The modified T cells are reintroduced into the patient to enhance their ability to identify and destroy cancer cells. There are three primary forms of adoptive cell therapy.

The first is tumor-infiltrating lymphocytes, which involves isolating T cells that are specific to the tumor from the patient, expanding them in a controlled lab setting, and then reinfusing them to strengthen the immune response against the tumor. The second method involves the introduction of a genetically engineered T-cell receptor designed to recognize a particular cancer antigen. Lastly, chimeric antigen receptor T-cell therapy involves genetically modifying the patient's T cells to express a synthetic chimeric antigen receptor on their surface, enhancing their ability to detect and eliminate cancer cells (33, 34). To this point in time, the predominant technique utilized for producing CAR T-cells involves the employment of viral vectors. Nonetheless, the utilization of vectors derived from viruses may present contests in the developed process of CAR T-cells due to stringent regulations and high-cost requirements. Alternative methods could assist in the advancement of this technology and its implementation in clinical settings. Transposon-derived vectors, particularly those based on the sleeping beauty transposon, have emerged as the

most encouraging alternatives to virus-derived vectors. These vectors exhibit considerable coding potential and guarantee a secure integration profile, while simultaneously maintaining production expenses at a relatively low level. CAR T-cell production involves isolating T cells from a patient's blood, activating them, genetically modifying them to express a chimeric antigen receptor, and expanding them before reinfusion into the patient. The process typically involves several steps: leukapheresis to collect blood, isolation of T cells, activation, genetic modification, expansion, and quality control (Figures 2 and 3) (35). While CARs are not exclusively associated with T cells, they are often connected to them. These genetically engineered artificial receptors, termed chimeric due to their composition of various antibody elements, can be integrated into immune effector cells to enhance their functionality. CARs effectively take over the role of the T-cell receptor, utilizing the binding domain of a monoclonal antibody in a single-chain variable fragment (scFv) format, which is linked to intracellular T-cell activation domains such as CD28 and CD3ζ(36, 37). As a result, CARs allow for the direct recognition of surface antigens without requiring the presence of MHC molecules to facilitate antigen recognition. One of the current TCR-independent approaches for T cell-based therapies is CAR-T. It has taken more than 50 years of study to create and apply CAR-T therapy, and there are still projects underway to improve this cancer treatment strategy. The following lists the significant turning points in the development of CAR-T cells (38). T cells altered with chimeric antigen receptors have presented encouraging findings in the combat contradiction of B cell leukemia and lymphoma, showcasing their potential as a therapeutic method against cancer (39, 40).

Brexucabtagene autoleucel and lisocabtagene maraleucel are further medications that have been accepted for the treatment of ALL and mantle lymphoma, as well as DBCL, follicular lymphoma, and high-grade lymphoma, in that order (41, 42).

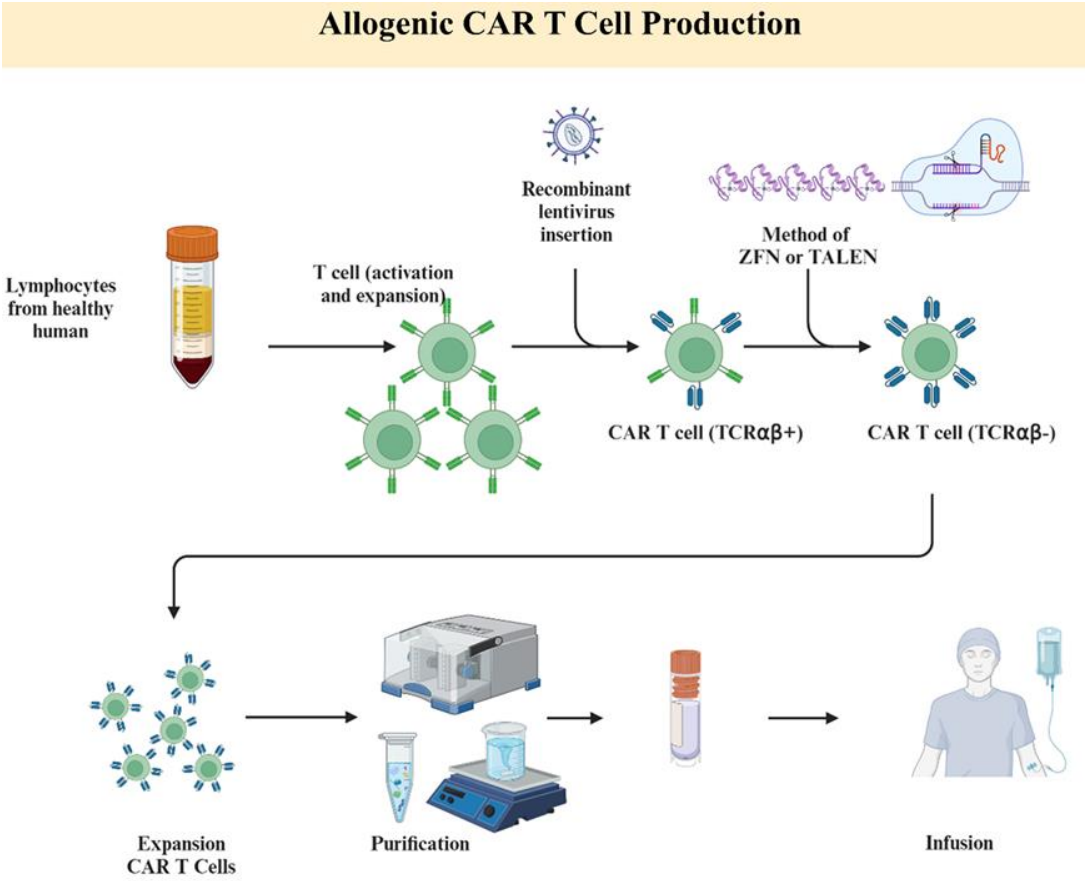

Figure 2. The process of CAR T cell production

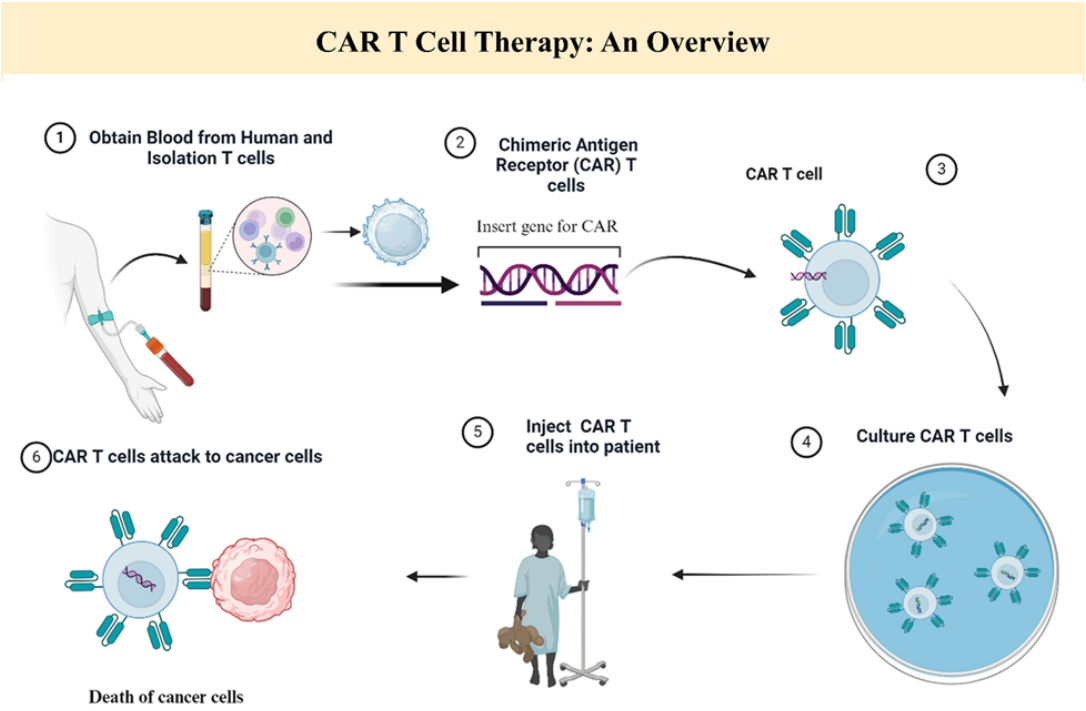

Figure 3. CAR T cell overview

The target antigen CD19 on B-cells, which independently of MHC elicits a T cell-mediated immune response against the malignant B cells, is responsible for the treatment's efficacy. Targeted antigens include CD38 and BCMA, which are present in myeloma cells. Consequently, by obtaining high response rates, low recurrence rates, and controllable cytokine release syndrome, the application of BCMA-CD38-CAR-T cell treatment has demonstrated promise in managing relapsed and refractory multiple myeloma (43, 44). It is important to highlight that BCMA-CAR-T therapies, including Ciltacabtagene-autoleucel and Idecabtagene-vicleucel, are currently available for the treatment of multiple myeloma patients. These significant developments in the management of blood cancers support the potential use of CAR-T cell therapy in the treatment of solid tumors (45, 46).

Recent years have witnessed a rise in CAR-T cell clinical trials targeting solid tumors, with this section reporting on the encouraging clinical outcomes based on the greatest corporate target antigens, as evidenced by data from ClinicalTrials.gov and related literature. Currently, over 27 CAR-T cell therapies have received approval for initial clinical trials, with relevant clinical data for CAR-T cell therapy in colorectal cancer (CRC) compiled CAR -T cell for Colorectal Cancer /Haki M, et al in Table 1 on ClinicalTrials.gov.

Chimeric antigen receptors have been used to genetically modify peripheral T cells, and this has shown to have a main effect on the treatment of hematologic malignancies. Chimeric antigen receptors have been used to genetically modify peripheral T cells, and this has shown to have a main effect on the treatment of hematologic malignancies. Still, there are a lot of challenges in the way of using CAR-T cell therapy for solid tumors. Carcino-embryonic antigen (CEA), mesothelin (MSLN), guanylyl cyclase C (GUCY2C), epithelial cell adhesion molecule (EpCAM), human epidermal growth factor receptor-2 (HER2), and doublecortin-like kinase 1 (DCLK1) are among the targets for CAR-T cell treatment in colorectal cancer (CRC)(47, 48).

CEA, a glycoprotein produced by cells in the colon, is a recognized sensitive marker for CRC. Ongoing research is focusing on CAR-T therapy for CEA, with promising results when CEA is targeted alongside other agents like CD30 antibody. The integration of CEA-CAR-T cells with recombinant human IL-12 has demonstrated a notable reduction in tumor growth (49, 50).

Table 1. Clinical trial of CAR-T cells in CRC (https://clinicaltrials.gov/)

Antigen	phase	Clinicaltrials. gov identifier	Recruitment Status
NKG2DL	Early Phase 1	NCT05248048	Recruiting
	Phase 1	NCT04550663	Not yet recruiting
	Phase 1	NCT03370198	Active, not recruiting
	Phase 1	NCT04107142	Unknown
	Phase 1	NCT03310008	Active, not recruiting
	Phase 1	NCT03692429	Recruiting
CEA	Phase 1	NCT02850536	Completed
	Phase 1	NCT02416466	Completed
	Early Phase 1	NCT04513431	Not yet recruiting
	Phase 1	NCT05240950	Recruiting
	Phase 1 Phase 2	NCT04348643	Recruiting
	Phase 1	NCT02349724	Completed
	Phase 1	NCT03682744	Active, not recruiting
	Phase 1 Phase 2	NCT02959151	Unknown
MSLN	Phase 1	NCT05089266	Not yet recruiting
	Early Phase 1	NCT04503980	Recruiting
EpCAM	Phase 1	NCT05028933	Recruiting
MUC1	Phase 1	NCT05239143	Recruiting
	Phase 1 Phase 2	NCT02617134	Unknown
	Phase 1 Phase 2	NCT02839954	Recruiting
HER2	Phase 1	NCT03740256	Recruiting
	Phase 1	NCT04660929	Recruiting
B7-H3	Phase 1	NCT05190185	Recruiting
EGFR	Phase 1	NCT03542799	Unknown
	Phase 1 Phase 2	NCT03152435	Unknown
	Phase 1	NCT01869166	Recruiting
CD133	Phase 1 Phase 2	NCT02541370	Completed
Guanylate Cyclase guanylate cyclase-C (GCC)	Phase 1	NCT05319314	Recruiting
c-Met	Phase 2	NCT03638206	Recruiting
***α*PD-1-MESO**	Phase 1 Phase 2	NCT03615313	Recruiting
HILA	Phase 1 Phase 2	NCT05736731	Recruiting

NA, not available; d(s), dose(s); DL, dose levels

Mesenchyme cells in the peritoneum, pleura, and pericardium spontaneously express MSLN, a glycoprotein present on cell surfaces. MSLN overexpression has been linked to colorectal cancer, construction it an important target for CAR-T cell treatment. Research examining the effectiveness of MSLN-CAR-T cells on colon cancer xenografts demonstrated higher peripheral blood T lymphocyte counts and greater granzyme B infiltrates in tumor tissue, suggesting a strong anti-tumor effect in contrast to control groups (51, 52). GUCY2C is essential for maintaining cellular stability by activating its ligand, which leads to the production of the second messenger cGMP. Abnormalities in GUCY2C signaling are associated with the development of colorectal cancer. This receptor is significantly expressed in both primary and metastatic CRC, making it an important tumor marker. CAR-T cells engineered to target GUCY2C have shown effectiveness in eliminating CRC cells that express this receptor while leaving normal intestinal epithelial cells unharmed.

These specialized CAR-T cells improve antitumor activity through antigen-specific T-cell activation and the release of cytokines (53, 54). EpCAM, a prominent tumor-associated antigen on the surface of CRC cells, is involved in promoting migration, proliferation, and tumor growth. Experimental treatment utilizing EpCAM-CAR-T cells in CRC has shown superior lethality and specificity against cancer cells expressing EpCAM, highlighting its potential as a therapeutic strategy (55, 56). HER2, a protein overexpressed in colorectal cancer (CRC), serves as a crucial target for CAR-T cell therapy. The application of HER2-CAR-T cells has displayed potent and selective cytotoxic effects on colon cancer cells. Studies on murine models reveal that treatment with HER2-CAR-T cells results in remarkable tumor regulation, meaningfully enhanced overall survival rates, and suppression of distant CRC metastasis to the organs (57). The use of DCLK1-targeted CAR-T cell therapy has effectively subdued xenograft tumor growth without causing any observable toxicity (58, 59).

Cbl-b, functioning as an E3 ubiquitin ligase involved in ubiquitination, plays a role in mediating the removal of Cbl-b from CAR-T cells, consequently boosting the antitumor activity of these cells. In comparison to the control group, cells have been found to significantly increase the cytotoxic capability of CAR-T cells against CRC cells, as evidenced by elevated secretion levels of IFN-γ, TNF-α, and granzyme B (59).

The FDA has granted orphan drug designation to A2B530, a novel cell therapy aimed at treating colorectal cancer patients with germline heterozygous HLA-A*02-positive disease that expresses carcinoembryonic antigen and has lost HLA-A*02 expression (60). The orphan drug status provides A2 Biotherapeutics with various benefits, such as tax credits for clinical trials, user fee exemptions, and potential market exclusivity for up to 7 years (61). A2B530 is currently under investigation in the phase 1/2 EVEREST-1 study (NCT05736731) (60, 62). T cell amplification therapy, such as adoptive cell therapy, encompasses chimeric antigen receptor T cell therapy, T cell receptor modification, and enhancing T cell activity through tumor-infiltrating lymphocytes to counteract cancer cell growth. In the realm of medical advancements, adoptive cell transfer therapy stands out as a remarkable treatment method that bolsters the immune system. This groundbreaking therapy employs cells from the patient or altruistic donors to optimize immune function (63).

In several cancer forms, including non-small-cell lung carcinoma, breast, gastric, and colorectal cancers, the epidermal growth factor receptor (EGFR) is acknowledged as a major target for therapy and plays a critical role in the development and progression of lumps. The application of CAR-T cells targeting EGFR in the treatment of EGFR-positive solid tumors has been investigated in a number of clinical trials. For example, promising results were seen in a phase-I clinical trial (NCT01869166) that included EGFR CAR-T cell therapy in 11 patients with EGFR+ refractory/relapsed non-small cell lung cancer (64, 65).

Also, in another phase-I clinical trial, 10 patients with recurrent EGFRvIII+ glioblastoma (GBM) were cured with EGFRvIII engineered CAR-T cells (NCT02209376) showing a significant anti-tumor effect with a median OS of around 8 months for all patients (66-68). Other antigens like ephrin type-A receptor 2 (EphA2) (NCT02575261) (69, 70) and mucin 1 (MUC1) (NCT02839954 (71), NCT02617134 (72)) are also being targeted by CAR-T cells for GBM therapy. Moreover, nine patients with non-small cell lung cancer were included in the phase I clinical trial (NCT03182816) to inspect the use of non-viral

piggyBac transposon system-engineered EGFR-CAR T-cell therapy (73). Neoantigens originating from mutations, such as the KRAS (G12V) and KRAS (G12D) mutants, have a close association with pancreatic and colorectal cancers, making them highly promising targets for therapeutic interventions. The present emphasis of CAR-T cell therapies targeting solid tumors is centered on mesothelin, glypican-3, GD2, HER2, B7-H3, and claudin18.2 (74, 75). Despite the potential of these targets, ongoing clinical trials have revealed that CAR-T therapies for solid tumors are still in the early stages, mainly encompassing phase 1 or phase 2 trials (74, 76).

The HER2-targeted CAR-macrophage (CAR-M) therapy known as CT-0508 exhibited an acceptable safety profile and demonstrated promising antitumor effects in a diverse group of patients with solid tumors, as outlined in the results of a phase 1 trial (NCT04660929) (77). CYAD-01 is designed based on the natural killer group 2D (NKG2D) receptor, which interacts with eight ligands that are commonly overexpressed in various hematological malignancies but are typically absent in non-neoplastic cells (NCT03018405) (73). In contrast, CYAD-101 represents an innovative non-gene edited allogeneic CAR T-cell therapy, combining the broad tumor-targeting capabilities of the NKG2D-based CAR with a peptide-driven strategy to manage graft versus host disease (GvHD) (78).

NKG2D, which fixes eight ligands frequently overexpressed in multiple tumor types, is a key component of the CYAD-101 therapy. Moreover, the co-expressed T-cell receptor (TCR) inhibitory (TIM) peptide plays a crucial role in disrupting signaling pathways associated with the endogenous TCR (79). A variety of CYAD-101 cells sourced from a single donor were extensively assessed in the AlloSHRINK phase 1 trial (NCT03692429), which included patients facing challenges with advanced respectable metastatic colorectal cancer(79). Additionally, a noteworthy trial focusing on Allogeneic NKG2DL-targeting Chimeric Antigen Receptor-grafted γδ T Cells for Relapsed or Refractory Solid Tumors is currently ongoing (NCT04107142) (80). NKG2D-based CAR T-cells Immunotherapy for patients with r/r NKG2DL+ solid tumors clinical trials carried out in phase 1 (NCT05131763 and NCT04270461) (81, 82).

In their initial endeavors to apply CAR T-cell therapy to colorectal cancer, numerous research groups have carried out clinical trials to evaluate the effectiveness of CAR T-cells targeting HER2, tumor-associated glycoprotein 72 (TAG-72), CEA, or CEACAM5 (50). The initial clinical trial evaluating CAR T-cell therapies for colorectal cancer focused on HER2 in a patient whose colon cancer had metastasized to the lungs and liver and had not responded to standard treatments. CAR T-cells targeting HER2 were developed utilizing the well-known humanized monoclonal antibody trastuzumab. Unfortunately, the patient experienced respiratory complications from a cytokine storm and ultimately succumbed to toxicity associated with the CAR T-cell therapy (83). No notable therapeutic benefits were observed with CAR T-cells aimed at the other three markers (TAG-72, CEA, or CEACAM5). In a separate study, Hege et al. reported the results of one of the pioneering CAR T-cell trials for metastatic colorectal cancer, which concentrated on CART72 cells targeting TAG-72. Two phase 1 trials were carried out using systemic (C9701) and regional hepatic artery infusion (C9702) as delivery methods for CART72. Although CART72 was considered safe, it exhibited limited presence in the bloodstream due to an immune response against the CAR and insufficient infiltration into the tumor masses (83, 84).

A clinical trial was conducted involving colorectal cancer patients with metastases that tested positive for the carcinoembryonic antigen to evaluate the protection and effectiveness of CAR T-cells targeting CEA. The results demonstrated a high degree of tolerability for CEA CAR T-cells, even at increased dosages, along with a sustained presence in the bloodstream and efficacy in most participants. Conversely, a phase I trial investigating CAR T-cell therapy aimed at carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) did not produce significant positive clinical outcomes. This lack of effectiveness resulted in the early termination of the study due to respiratory issues and the absence of measurable clinical benefits.

An ongoing phase I clinical trial is focused on assessing the safety and effectiveness of CAR T-cells that target the epidermal growth factor receptor (EGFR) in metastatic colorectal cancer. To improve the cytotoxicity and longevity of EGFR CAR T-cells and to tackle challenges posed by the tumor microenvironment, researchers have created fourth-generation EGFR-IL12 CAR T-cells, also known as T-

cells redirected for universal cytokines (TRUCKs), which are engineered to secrete interleukin-12 (85, 86). The evaluation of the highest acceptable dosages and the safety profile of EGFR-IL12 CAR T-cells is presently undergoing examination in a cohort of patients diagnosed with metastatic colorectal cancer. This assessment aims to determine the optimal dosage levels and potential adverse effects associated with the administration of this novel CAR T-cell therapy in individuals with progressive CRC (85). To overcome the challenges associated with conventional CAR T-cell therapies for solid tumors, GCC19CART has emerged as the leading clinical candidate from the Coupled CAR solid tumor platform. This innovative therapy combines CAR T-cells specifically targeting solid tumors with those directed against CD19, thereby improving the proliferation and activation of the CAR T-cell population aimed at solid tumors. The GCC19CART design strategically targets guanylate cyclase-C (GCC), a protein present in metastatic lesions in the intestinal tract of 70%–80% of colorectal cancer patients.

By utilizing this novel strategy, GCC19CART seeks to enhance the effectiveness and accuracy of CAR T-cell therapy for solid tumors, presenting a promising avenue for better treatment outcomes in colorectal cancer patients and potentially other types of cancer(87). αPD-1-MESO CAR T-cells, a fourth-generation CAR T-cell therapy that can generate and secrete anti-PD-1 nanobodies upon demand, is also being studied in colorectal cancer. The effectiveness of αPD-1-MESO CAR T-cell treatment in progressive solid tumors with MSLN positivity, such as ovarian and colorectal cancer, is being investigated in a Chinese trial. Premedication with cyclophosphamide is given to patients undergoing CAR T-cell generation to diminish their lymphocytes (88).

Discussion

There are multiple approaches to CRC adoptive cell therapy, with CAR-T cells being one of the most thoroughly researched and promising alternatives. Many studies have demonstrated CAR-T cells' safety and effectiveness in treating CRC. However, this therapy faces several obstacles that limit its practical application. Moreover, CAR-T cell therapy can result in various side effects, the most common being cytokine release syndrome (CRS), which occurs as a consequence of cytokine secretion following CAR-T cell infusion. CRS presents with a range of non-specific symptoms, such as fever, nausea, decreased heart function, and low blood pressure. It can also result in further systemic toxicities like shortness of breath, respiratory failure, irregular heartbeats, elevated cardiac markers, heart failure, liver impairment, gastrointestinal complications, clotting issues, muscle injury, neurotoxic effects, and more. Major obstacles in applying CAR-T cell therapy for solid tumors include pinpointing specific tumor antigens for effective targeting, overcoming tumor antigen evasion, and enhancing CAR-T cells' migration, infiltration, and persistence within the tumor environment. Additionally, boosting their functionality in a challenging tumor microenvironment is a pivotal concern.

Limitations and future directions

CAR-T cell immunotherapy has emerged as a highly promising approach in the realm of anticancer strategies, particularly for the treatment of B-cell malignancies. Nonetheless, when addressing solid tumors such as colorectal cancer, a variety of challenges and limitations necessitate careful consideration. A critical factor is the requirement for CAR-T cells to penetrate the tumor mass, given that the tumor core typically exhibits poor vascularization. To address these challenges, CAR-T cells could be engineered to express CXCR3 and CCR5 on their membrane and promote the release of heparanase, thereby emulating the behavior of tumor-infiltrating lymphocytes (TILs) and enhancing tumor infiltration.

Additionally, another approach involves designing CAR-T cells to target molecules that are overexpressed in the newly formed vasculature resulting from tumor angiogenesis, including αvβ6 integrin and VEGF-2, consequently limiting nutrient supply and reducing the metastatic potential of colorectal cancer. The immunosuppressive nature of the tumor microenvironment that typifies solid tumors represents a significant barrier to the successful application of CAR-T cell therapy. The elevated levels of hypoxia, inadequate nutrient availability, and excessive release of acidic byproducts contribute to the establishment of a detrimental environment that hinders the cytotoxic efficacy of CAR-T cells against the tumor. To counteract these hurdles, T cells that express a dominant negative TGF-β receptor have been

developed, and innovative chimeric receptors that convert immunosuppressive signals into stimulatory signals have been proposed.

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