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REVIEW

Updated Clinical Perspectives and Challenges of Chimeric Antigen Receptor-T Cell Therapy in Colorectal Cancer and Invasive Breast Cancer

Yu Cao[1] · Sergey K. Efetov[1] · Mingze He[1] · Yu Fu[1] · Narasimha M. Beeraka[1,2] · Jin Zhang[1] · Xinliang Zhang[1] · Namitha Bannimath[4] · Kuo Chen[3]

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Abstract

In recent years, the incidence of colorectal cancer (CRC) and breast cancer (BC) has increased worldwide and caused a higher mortality rate due to the lack of selective anti-tumor therapies. Current chemotherapies and surgical interventions are significantly preferred modalities to treat CRC or BC in advanced stages but the prognosis for patients with advanced CRC and BC remains dismal. The immunotherapy technique of chimeric antigen receptor (CAR)-T cells has resulted in significant clinical outcomes when treating hematologic malignancies. The novel CAR-T therapy target antigens include GUCY2C, CLEC14A, CD26, TEM8/ANTXR1, PDPN, PTK7, PODXL, CD44, CD19, CD20, CD22, BCMA, GD2, Mesothelin, TAG-72, CEA, EGFR, B7H3, HER2, IL13Ra2, MUC1, EpCAM, PSMA, PSCA, NKG2D. The significant aim of this review is to explore the recently updated information pertinent to several novel targets of CAR-T for CRC, and BC. We vividly described the challenges of CAR-T therapies when treating CRC or BC. The immunosuppressive microenvironment of solid tumors, the shortage of tumor-specific antigens, and post-treatment side effects are the major hindrances to promoting the development of CAR-T cells. Several clinical trials related to CAR-T immunotherapy against CRC or BC have already been in progress. This review benefits academicians, clinicians, and clinical oncologists to explore more about the novel CAR-T targets and overcome the challenges during this therapy.

Keywords CAR-T · Colorectal cancer · Breast cancer · Solid tumor · Immunotherapy · Novel CAR-T targets · Challenges

✉ Kuo Chen
chenkchenk2008@outlook.com

Yu Cao
drcaoyu0124@gmail.com

Sergey K. Efetov
efetov@mail.ru

Mingze He
hemingze1997@gmail.com

Yu Fu
a840534855@gmail.com

Narasimha M. Beeraka
bnmurthy24@gmail.com

Jin Zhang
alexander600229@gmail.com

Xinliang Zhang
zhangxinliang980620@gmail.com

Namitha Bannimath
namithabannimath@gmail.com

[1] I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), Moscow 119991, Russia

[2] Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Chiyyedu, Anantapuramu, Andhra Pradesh 515721, India

[3] Department of Breast Surgery, The First Affiliated Hospital of Zhengzhou University, #1 Jianshedong Str., Zhengzhou 450052, People's Republic of China

[4] Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education and Research (JSS AHER), Mysuru, Karnataka, India

Introduction

Colorectal cancer (CRC) and breast cancer (BC) are responsible for a significant number of fatalities every year worldwide. According to GLOBOCAN 2020, CRC accounts for approximately 10% of the more than 19.3 million newly diagnosed cancers worldwide. Breast cancer is another devastating cancer considered the most commonly diagnosed cancer, accounting for approximately 11.7% of these cases. The number of deaths due to cancer is ten million worldwide, with which CRC and BC account for about 9.4% and 6.9%, ranking second and fifth, respectively (Sung et al. 2021). In developed countries, early screening strategies have improved the 5-year survival rate of CRC and BC patients, but 25% of patients are still in stage IV of the disease, with a median overall survival of 30 months (Lech et al. 2016; Peterse et al. 2018; Siegel Rebecca and Miller Kimberly 2019; Van Cutsem et al. 2016). Currently, the main modalities for the treatment of CRC and BC are surgery, radiotherapy, and chemotherapy. Current pharmacological agents such as fluoropyrimidine, irinotecan, and platinum derivatives to target solid tumors are constrained by nonspecificity, and adverse toxicity (Lech et al. 2016). Other reported anticancer drugs such as angiogenesis inhibitors (bevacizumab), epidermal growth factor receptor inhibitors (cetuximab), multikinase inhibitors (regorafenib), and anti-HER2-targeted therapy (trastuzumab) can be effective against breast cancer but their clinical usage yet to clear clinical validations (Martínez-Lago et al. 2022; Sertesen et al. 2022; Xie et al. 2023; Yu and Cheung 2018). Although the combination of regimens and targeted therapy has improved the survival conditions of patients to a certain extent, morbidity and mortality rates associated with CRC or BC are significantly higher.

Thus, with the technological advances in immunotherapy and oncology, chimeric antigen receptor (CAR)-T cell therapy has also become a hot topic of research. Previous studies described the pharmacological efficacy of CAR-T as immunotherapy against solid hematological cancers (June et al. 2018). US FDA approved the application of CAR-T cell-based therapy to target hematological tumors (Fournier et al. 2017). Furthermore, although a few basic and clinical studies pertinent to CAR-T implications against CRC and BC has been published current significant progress in CAR-T therapy has not been published (D'Aloia et al. 2018; Zhang et al. 2017b). CAR is composed of the components such as the extracellular region, transmembrane region, intracellular signal component, and hinge region (Lipowska-Bhalla et al. 2012). A scFv in monoclonal antibodies exhibits significant splicing ability and modulates antigen specificity (Zhang et al. 2014). CD4, CD8, CD28, or IgG4 are the significant proteins existing in the hinge domain in CAR and this domain connects the extracellular domain to the transmembrane domain (Hudecek et al. 2015). CD8α, CD4, CD3ζ, CD28, or inducible T cell co-stimulator (ICOS is a immunoglobulin super family, that can modulate CAR-T efficacy) are confined in the transmembrane domain which connects the extracellular domain to the intracellular domain and anchors the cell membrane (Bridgeman et al. 2010; Guedan et al. 2018). The intracellular domain could induce signal transmission into the cell during CAR activity (Zhang et al. 2017a).

In the case of hematologic tumors, the usage of the CAR-T cell in the treatment is a major advancement. Adults and adolescents with relapsed or refractory acute lymphoblastic leukemia, and chronic lymphocytic leukemia have experienced complete remission rates of 70–90% when treated with CAR-T cell therapy accompanying the activity of CD19 (Pehlivan et al. 2018; Wang et al. 2017). Due to the lack of ideal targets in solid tumors like CD19 and CD20, which are common in hematologic tumors, CAR-T is employed less frequently in solid tumors than in the hematologic system because of the difficulty in identifying tumor-specific antigens (TSAs) which are amenable in case of CAR-T cell treatment and low tumor remission rate (Newick et al. 2017; Wagner et al. 2020). A significant breakthrough in the implications of CAR-T to target solid tumors has been reported mainly in patients with extensive intracranial metastases of glioblastoma achieving complete remission (Majzner et al. 2022; Mount et al. 2018). Very limited reports described related to the CAR-T efficacy against CRC and BC. In this review, we have described the current clinical perspectives of CAR-T for modulating cancer cell proliferation across tumor microenvironment by targeting novel anticancer signaling, and oncogenic signaling proteins involved in tumor progression; we discussed recently updated information pertinent to several novel targets of CAR-T for CRC, and BC. We vividly described the challenges of CAR-T therapies when treating CRC or BC subsequently, subsequently discussed the updated clinical information related to the implications of CAR-T in the BC and CRC.

Literature Search

Databases such as Pubmed, Medline, Scopus, and relemed were searched for the published reports pertaining to the implications of CAR-T cell therapy in colorectal cancer, and breast cancer. We have used keywords such as colon cancer, breast cancer, colorectal and breast cancer clinical trials, cytokine release syndrome, guanylate cyclase 2C, tumor-associated glycoprotein-72, carcinoembryonic antigen (CEA), human epidermal growth factor receptor-2

(HER-2), epidermal growth factor receptor (EGFR), mesothelin (MSLN), and epithelial cell adhesion molecules.

Overview of CAR-T Cell Therapy

"CAR-modified T-cell therapy" involves genetically engineering the sequence of an antibody variable gene that recognizes an antigen molecule and splicing it with the sequence of the intracellular region of the lymphocyte immune receptor, transfecting it into T lymphocytes through a vector. The retrovirus is a specific vector that can be used in this design and the fusion protein in this retroviral vector is confined in the vicinity of T cell membranes to kill tumor cells through non-major tissue. The major histocompatibility complex (MHC) recognizes specific antigens to kill tumor cells in a restricted manner (Fesnak et al. 2016; Guedan et al. 2019; Liu et al. 2019), thereby increasing cytotoxic T lymphocyte-dominated anti-tumor cell immunity. Human T cells when combined with CAR can inhibit the subsets of tumor cells' growth in MHC- and Fas-independent fashion. Hence, the CAR-T cells could identify the target tumor cells irrespective of the patient's MHC type. Specifically, the cytolytic degranulation of granzymes is the major pathway involved for targeted cell killing by CAR-T mediated therapy (Sheykhhasan et al. 2022) (Fig. 1).

CAR is the central component of CAR-T, allowing T cells to identify tumor antigens in a human leukocyte antigen-independent manner and recognize a broader spectrum of target antigens than the native T cell surface receptor (Lu and Jiang 2022; Sadelain et al. 2003). The mechanism of CAR-T therapy depends on the components of a CAR, which include the scFv of the extracellular antigen recognition domain; this domain fuses the heavy and light chains of the antigen recognition region of an antibody specific for a tumor antigen; this binding determines the specificity of the CAR and binds to the target molecule through a mechanism of antigen–antibody interaction (Rahbarizadeh et al. 2019). The CAR is activated by a specific antigen to release a large number of cytokines that can destroy tumor cells when recognition is performed by the antigen. The role of intracellular transduction structural domains has been continuously explored and improved over four generations of CAR-T cells. The first-generation CAR-T contains only one intracellular transduction domain CD3ζ to provide signals (Subklewe et al. 2019); thus CAR-T is poorly active, short-lived in vivo, and unable to initiate resting T cells and continuously direct T cell responses and cytokine release (Aoe et al. 1994). Thus, first-generation CAR is composed of signaling domains such as scFv and CD3ζ (Hwu et al. 1993, 1995; Stancovski et al. 1993) and exhibited limited antitumor efficacy because of the absence of co-stimulation and interleukin (IL)-mediated signaling (Thistlethwaite et al. 2017). Second-generation CAR is composed of the costimulatory domain, which is associated with 4-1BB or CD28 to induce costimulatory signals at the time of activation (Subklewe et al. 2019). CAR-T pertinent to the second generation incorporates an intracellular co-stimulatory molecule like CD28 or 4-1BB (Patel et al. 2014), to its base, enabling T cells to prolong their value-added time and subsequently induce substantial tumor cell-killing efficacy. Two costimulatory domains are located in the CATR pertinent to the third generation which is potentially involved in fostering the functional aspects of CAR (Huang et al. 2020a, b). The third-generation CAR-T adds another co-stimulatory molecule to the second generation. For instance, OX40, DAP-10, and ICOS are protein domains (Webb et al. 2016; Wikenheiser and Stumhofer 2016), which allow CAR-T to

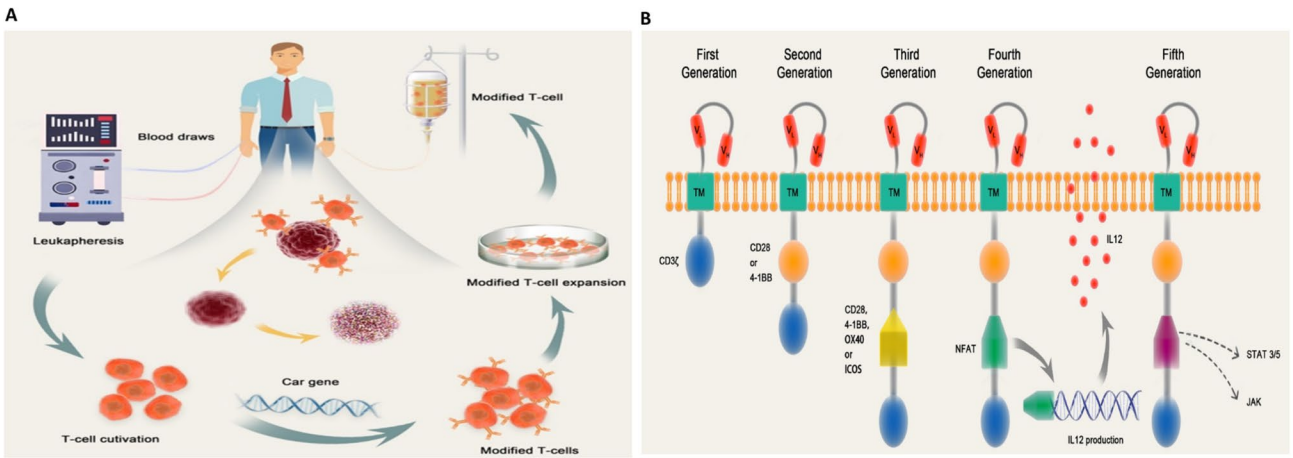

Fig. 1 Preparation of CAR-T cells and reinfusion through several step-wise strategies such as T cell cultivation subsequently to generate modified T cells; the four generations of CAR-T cells and their subsequent targets and generation of IL-12. **a** Preparation of CAR-T cells; **b** Scheme of different generations of CAR-Ts

proliferate continuously in vivo and secrete cytokines to increase the effective killing of cancer cells. The fourth generation of CAR is relying upon the generation of cytokines, IL-2, and IL-12 (Tokarew et al. 2019; Yeku and Brentjens 2016). Cells expressing CAR-T in a combinatorial regimen with focused ultrasound were designed and these cells subsequently express heat-inducible genes (Wu et al. 2021). It has been demonstrated that the ultrasound-exposed tumor cells are killed by CAR-T which concludes the efficacy of focused ultrasound enhancing CAR-T targeting efficacy (Qin et al. 2022). The fourth generation of CAR-T cells are T cell redirected for universal cytokine killing cells which can incorporate cytokines or co-stimulatory ligands that can secrete specific cytokines in the tumor, thereby altering the tumor microenvironment and recruit and activate other immune cells to produce an immune response (Chmielewski and Abken 2015). Fifth-generation of CAR-T is vividly described by recent research studies. This kind of CAR-T therapy development was based on the second generation of CARs and they composed of truncated cytoplasmic IL-2 receptor β-chain. This domain is composed of binding region for signal transducer and activator of transcription (STAT)3 transcription factor (Corti et al. 2022; Tokarew et al. 2019). Antigen-specific activation of this cytoplasmic IL-2 receptor concomitantly induce activation of T cell receptor via the domains such as CD3ζ, CD28, and Janus kinase (JAK)-STAT3/5 signalling (Kagoya et al. 2018), and these signals could collectively promote the T cell activation and proliferation (Tokarew et al. 2019; Yang et al. 2022) (Fig. 1).

Targets of CAR-T for Colorectal Cancer and Breast Cancer

Colorectal Cancer: CEA and CAR-T

CEA is an acidic glycoprotein with human embryonic antigen properties. It is a classical tumor marker, especially in CRC where it is more than 80% positively expressed (Fakih and Padmanabhan 2006; Ghazi et al. 2022; Rizeq et al. 2018). CEA expression is localized to the luminal side of epithelial cell membranes in mature tissues (Wagener et al. 1983). CEA loses its apical polarity of expression in the luminal epithelium with tumor transformation (Hauck and Stanners 1991). As a result, CEA enters the capillaries and is considered a significant marker for identifying CEA^+ carcinoma. Therefore, targeting CEA with CAR-T therapy for solid tumors is well-established (Holzinger and Abken 2017). Zhang et al. (2017b) examined CAR-T therapy for the patients with CEA-positive CRC metastases in ten patients with five incremental doses of CAR-T cells. However, there were no severe adverse events associated with CAR-T, and two out of ten patients were in stable condition for more than 30 weeks. CEA is a good target for CAR-T cell therapy because it is overexpressed in certain types of tumors, including colorectal cancer, and it has been shown to be effective in preclinical and clinical studies as a target for CAR-T cell therapy. The shrinkage of tumor size was evident which was exemplified by the decline in serum CEA in two other patients. Most of the patient patients showed evidence of CAR-T cell proliferation, and tolerability even at higher dosages in the CEA-positive patients. These results have been indicating the safety and efficacy of CAR-T for CEA-positive CRC. Another study by Cha et al. (2021) used the injection of second-generation anti-CEA CAR or mock-transduced T cells formulated from CEA-specific antibodies into the CEA-transfected mice with colon cancer (MC38) and mice with breast cancer (E0771). Results of this study reported that CEA^+ tumor growth was much slower in mice treated with anti-CEA CAR-T cells than the mice treated with mock-transduced T cells. There is no systemic toxicity, diarrhea, or loss of motility during the CAR-T treatment in these experimental models of mice. CAR-T treatment alone is not sufficient to mitigate tumor cell proliferation. Cyclophosphamide (CY) is a preferred drug that can be used along with anti-CEA CAR-T to make anti-CEA CAR-T cell monotherapy more effective. CY selectively depletes immunosuppressive cells to enhance the antitumor activity (Owen et al. 2022). Injecting CY 24 h before CAR-T cell treatment into CEA-positive tumor-bearing mice induced a reduction in the tumor size and subsequently promoted median survival without systemic toxicity. To further enhance the anti-tumor efficacy of anti-CEA CAR-T cells, the addition of "immunocytokine (ICK)" to "CY" combined with "anti-CEA CAR-T cell therapy" has enhanced the anti-tumor efficacy against MC38/CEA and E0771/CEA tumors and also promoted pro-inflammatory responses by recruiting interferon (IFN)-γ^+/PD1-$CD8^+$ T cells into the tumor microenvironment. Thus, ICK treatment after the regimen of CY in combination with anti-CEA CAR-T cell therapy was not only sufficient to eradicate tumors but also to establish antitumor immunity.

Colorectal Cancer: HER-2 and CAR-T

The expression of HER-2 can be observed in several cancers including breast, ovarian and colorectal cancer (Carney et al. 2007; Nathanson et al. 2003; Slamon et al. 1989; Vasaikar et al. 2018). HER-2 is an important member of the human epidermal growth factor receptor family and plays a significant role in the regulation of cell growth, differentiation, and apoptosis. HER-2 is composed of three major components, including the extracellular domain, a transmembrane domain, and intracellular structural domains. Among these two ligand-binding domains and two cysteine-rich regions constitute the extracellular domain, and the transmembrane contains 23 amino acids which anchor the periplasmic

membrane (Budi et al. 2022; Moasser 2007). HER-2 is normally in a non-activated state, and when stimulated by oncogenic factors, it regulates vascular endothelial growth factor expression, generates tumor neo-vessels (angiogenesis), and enhances tumor cell invasion and metastasis (Drago et al. 2022). The expression of HER-2 in CRC specimens was detected by immunohistochemistry through the experimental models of CRC xenografts, and it was evident that the adoptive transfer of HER-2-specific CAR-T cells led to the regression or even clearance of CRC xenografts and protected the relapsed colon cancer tissues which suggest it might be a promising approach for mitigating the progression of tumors (Teng et al. 2019).

Szöőr et al. (2020) described that "T cells engineered with a trastuzumab-derived HER-2-specific CAR-T" to overcome the tumor extracellular matrix barrier and can effectively eliminate tumors that have developed resistance to trastuzumab. Tóth et al. (2020) also observed that HER-2-positive trastuzumab-resistant breast tumors killed second-generation CAR-T cells which possess the "CD28 costimulatory domain". In this study, even a small number of CAR-T cells could concentrate the activity of xenogeneic T cells to cause strong anti-tumor responses. CAR-T cell therapy exerts significant potential for improving the prognosis of patients with HER2-positive tumors. Globerson-Levin et al. (2014) also reported that tumor relapse is significantly evident with a single injection of CAR-T cells. Furthermore, multiple administrations of CAR-T cells can be used to regulate recurrent cancer without damaging normal tissues. However, Morgan et al. (2010) concluded that the cytokine release syndrome was triggered when "CAR-T cell therapy targeting HER-2" was administered in a colon cancer patient. This subsequently caused respiratory failure in the patient, which led to death. Therefore, both safe and optimal strategies of CAR-T immunotherapy require further preclinical and clinical exploration.

Colorectal Cancer: Tumor-associated Glycoprotein-72 and CAR-T

Tumor-associated glycoprotein-72 (TAG-72) is a high-molecular-weight mucinous protein which mainly expressed in human ovarian, colon, gastric, breast, and lung cancers (Cho et al. 2019; Minnix et al. 2020; Ouyang et al. 2010; Zhang et al. 2012). Hege et al. (2017) described the application of autologous CAR-gene-modified T cells targeting TAG-72 in the treatment of metastatic colorectal cancer. In the C-9701 and C-9702 experiments, treatment with retrovirally transduced first-generation CAR-T cells was examined in two patients diagnosed with metastatic CRC infused back into the body via intravenous and transrectal hepatic artery infusion,**** respectively; these patients were administered concomitantly with IFN-α to upregulate TAG-72 expression. In this study, a progressive enhancement in the dose administration induced typically a low degree of "cytokine release syndrome". During this study, CAR-T cells can circulate continuously and be detectable in one patient at 48 weeks. These CAR-T cells can reach the tumors to impair the growth of tumor cells. The human CAR constructions can reduce immunogenicity and it is crucial to design CAR with the inclusion of co-stimulatory structural domains. Hombach et al. (2019) developed an anti-CD30/TAG-72 CAR for targeting TAG-72^{+} tumor cells and compared it with a monospecific anti-TAG-72 CAR. The anti-CD30/TAG-72 CAR-T cells exhibited a higher elimination rate of tumor cells with TAG-72^{+}. Hence TAG-72 can be considered as the potential target and require significant research studies to act against tumor cells.

Colorectal Cancer: Guanylate Cyclase 2C and CAR-T

Guanylate cyclase C (GUCY2C) is an intestinal tissue-specific peptide and a member of the receptor guanylate cyclase family. This protein is specifically expressed in intestinal epithelial cells (Pattison et al. 2016). GUCY2C is also expressed in CRC cell lines, primary and metastatic CRC. This protein could regulate intestinal epithelial cell transformation and inhibits CRC cell proliferation and DNA synthesis. GUCY2C antibodies may regulate small intestinal mucosal epithelial cell proliferation and differentiation through a cyclic guanine monophosphate-dependent mechanism (Mishra et al. 2021). Overexpression of GUCY2C is mainly confined to the colorectum, and its expression has been reported in esophageal and gastric adenocarcinomas (He and Wang 2020). GUCY2C-specific antibody fragment that detects GUCY2C can direct CAR-T cells that are then measured by cytokines production (Magee et al. 2016). CAR-T cells increased the survival of "mice with metastatic CRC" expressing GUCY2C and subsequently decreased the number and incidence of tumors. As a result, CAR affinity and surface expression were successfully mirrored by GUCY2C-directed T cells.

In addition, there are clinical studies pertinent to CD133 (Vora et al. 2020), MUC1 (Nalawade et al. 2021), ICAM1 (Yang et al. 2021b), and other targets are yet to require substantial studies to explore specific targets for CAR-T cells in CRC treatment, which could lead to breakthroughs in CAR-T immunotherapy against CRC treatment. Specific CAR-T cell target antigens are summarized in Tables 1 and 2.

Colorectal Cancer: Placental Alkaline Phosphatase and CAR-T

Placental alkaline phosphatase (PLAP) is the human metalloenzyme reported in the placental tissue and testis and it is highly expressed in the cervical cancer and colorectal

Table 1 Several specific CAR-T cell target antigens are summarized in colorectal cancers

Author	Year	Type of tumor cell	Preclinical or clinical	CAR antigen
Zhang et al.	2017a, b	Colorectal cancer cell	Clinical	CEA
Cha et al.	2021	Colon and breast carcinomas cell	Preclinical	CEA
Zhang et al.	2019	Colorectal cancer cell	Preclinical	EpCAM
Teng et al.	2019	Colorectal cancer cell	Preclinical	HER-2
Morgan et al.	2010	Colorectal cancer cell	Clinical	HER-2
Liu et al.	2021a, b	Colon cancer cell	Preclinical	MSLN
Liu et al.	2021a, b	Colon cancer cell	Preclinical	MSLN
Masoumi et al.	2020	Mesothelin-expressing cell	Preclinical	MSLN
Hege et al.	2017	Colorectal cancer cell	Clinical	TAG-72
Hombach et al.	2019	Colorectal cancer cell	Preclinical	TAG-72
Magee et al.	2016	Colorectal cancer cell	Preclinical	GUCY2C

Table 2 Several specific CAR-T cell target antigens are summarized in breast cancers

Author	Year	Type of tumor cell	Preclinical or clinical	CAR antigen
Sahm et al.	2012	Breast cancer cell	Preclinical	EpCAM
Szöőr et al.	2020	Breast cancer cell	Preclinical	HER-2
Tóth et al.	2020	Breast cancer cell	Preclinical	HER-2
Globerson-Levin et al.	2014	Breast cancer cell	Preclinical	HER-2
Xia et al.	2020	TNBC cell	Preclinical	EGFR
Xia et al.	2021	TNBC cell	Preclinical	EGFR
Zhou et al.	2022	TNBC cell	Preclinical	EGFR
Li et al.	2020b	Breast cancer cell	Preclinical	MSLN
Yang et al.	2021a	TNBC cell	Preclinical	MSLN

TNBC triple-negative breast cancer

adenocarcinomas, and seminomas (Golubovskaya et al. 2020; Li et al. 2020a). PLAP-redirected CAR-T exemplified by the humanized PLAP-specific scFVs domains and they can target the PALP-positive colon cancer cells with a higher selectivity (Golubovskaya et al. 2020; Li et al. 2020a). For instance, the humanized PLAP-redirected CAR-T could impaired the tumor progression in the PLAP-positive colon cancer cells in xenograft models (Golubovskaya et al. 2020; Li et al. 2020a). However, the combinatorial regimen of checkpoint inhibitors such as PD-1, PD-L1 inhibitors could foster the overall therapeutic efficacy of humanized PLAP-CAR-T therapies against colorectal cancers (Kozani et al. 2021).

Colorectal Cancer: Protein Tyrosine Kinase 7 and CAR-T

Protein tyrosine kinase 7 (PTK7) is involved in the modulating the cell proliferation, migration and programmed cell death through the Wnt signaling (Levitsky et al. 2020). Upregulated expression of PTK7 is reported in the solid tumors of colon and it is also referred to as the colon cancer carcinoma kinase 4. Allogeneic PTK-7 redirected CAR-Ts were evaluated against several cancers including colon cancer xenograft mouse models and produced effective outcome (Sachdev et al. 2016).

Breast Cancer: EGFR and CAR-T

The EGFR is a complex arginine kinase receptor that is widely expressed in epithelial cells, glial cells, and fibroblasts in mammals (Jones and Rappoport 2014; Li et al. 2020b; Voldborg et al. 1997). The binding of ligand and EGFR could induce a conformational change and form a dimer in the extracellular domain region. As the dimer of EGFR is formed, it will allow phosphorylation of multiple intracellular complexin residues to initiate EGFR downstream signal transduction pathways, such as JAK/STAT, phospholipase C to regulate cell proliferation, division, differentiation, and apoptosis (Cruz-Duarte et al. 2022; Freed et al. 2017; Verhoeven et al. 2020). As a result, EGFR overexpression leads to uncontrolled cell growth, division, and differentiation which results in the formation of tumors. Xia et al. (2020) developed a third-generation CAR that targets EGFR most effectively. In this study, IL-12 is considered as the signal peptide because it could increase EGFR scFv secretion and improve antigen recognition. Thus. the signaling peptide of IL-12 was used in the CAR construct so that

the EGFR scFv derived CAR can express on T cell surface and target the antigen (Xia et al. 2020). T cells infected with EGFR CAR lentivirus have inhibited triple-negative breast cancer (TNBC) cell growth and metastasis both in vivo and in vitro; a high dose of CAR-T cell infusion has exhibited significant efficacy to induce substantial cytokine secretion and causes cytokine release syndrome, which leads to the reduced anti-tumor efficiency (Schepisi et al. 2023). However, the expression of EGFR is also confined to most of the normal cell surfaces, therefore, the toxicity of EGFR CAR is one of the significant limitations during CAR-T therapy. Therefore, a safety strategy is required to optimize CAR-T by regulating the dosage and integrating suicide genes using CAR-T cells, pointing the way toward future CAR-T safety in the treatment of solid tumors. Xia et al. (2021) also observed that CAR-T cells that can target EGFR subsequently impair the proliferation of TNBCs and drug-resistant breast cancers. EGFR CAR-T produced a large number of immunosuppressive factors inside the TNBCs, which were primarily induced by IFN-γ regulation. THZ1 is a CDK7 inhibitor and has proven effective in attenuating the expression of immunosuppressive genes expressed by the EGFR CAR-T cells. Therefore, transcriptional regulation by epigenetic inhibitors could overcome the immune resistance induced by CAR-T cell therapy, providing evidence for effective clinical treatment modalities against TNBCs. Zhou et al. (2022) demonstrated a drastic elevation of CAR-T cells by combining "radiotherapy with EGFR-CAR-T immunotherapy", and radiation-activated NF-κB signaling which results in the pronounced enhancement of ICAM1 expression on TNBCs, thereby promoting CAR-T cell infiltration and killing of tumor cells. These results suggest that CAR-T cell therapy combined with radiotherapy is also a promising therapeutic strategy against TNBCs.

Breast Cancer: MSLN and CAR-T

Mesothelin, a glycosylphosphatidylinositol-anchored cell surface glycoprotein, is expressed at minor levels in the normal tissues such as the pleura, peritoneum, and pericardium, but is highly expressed in various malignancies such as ovarian, pancreatic, breast, and colorectal cancers (Hagemann et al. 2019; Hou et al. 2022; Hsu et al. 2021; Wickstroem et al. 2019). MSLN plays an essential role in tumor invasion and metastasis. Differential expression of mesothelin is considered the diagnostic marker for diagnosing tumors (Faust et al. 2022). MSLN expression was higher on cell lines of ovarian cancer than colon cancer. Liu et al. (2021a, b) formulated MSLN-targeted CAR-T cells that expressed a PD-1-targeted shRNA to impair PD-1 mRNA in T cells. PD-1 expression level on the MSLN-targeted CAR-T cells could be downregulated by the PD-1-targeted shRNA, which in turn enhanced the cytotoxicity and cytokine secretion induced through CAR-T cells against ovarian and colon cancer cell lines. The pharmacological responses of MSLN-targeted CAR-T cells against cancer cells with differential expression levels of MSLN. In this study, shRNA-mediated PD-1 disruption enhanced the antitumor activity of CAR-T cells, and thus PD-1 silencing of MSLN CAR-T cells with a stable PD-1-targeted shRNA might improve the clinical outcome of CAR-T cells. It has also been shown (Huang et al. 2020a, b; Liu et al. 2021a, b; Masoumi et al. 2020), that the enhanced antitumor efficacy resulted from "shRNA-mediated adenosine 2A receptors" (A2AR) disruption in the anti-MSLN CAR-T cells. This approach is demonstrating that attenuating A2AR signaling by genetically targeting the receptor may be a promising approach to improve CAR-T cell function and has the potential to improve therapeutic outcomes in the clinical setting.

In another study (Li et al. 2020b), targeting the "TGF-β of oncolytic adenovirus (rAd.st) in combination with MSLN-CAR-T immunotherapy" has been reported to show therapeutic efficacy in xenografts model of breast cancer. Both rAd.st and MSLN CAR-T in a combinatorial regimen have induced impairment of tumor growth, but the efficacy is substantial when both treatments were administered together against these xenograft models. Moreover, this combination therapy also enhanced the production of IL-6, and IL-12, and is considered a promising therapeutic approach. In addition, Yang et al. (2021a) isolated "exosomes from MSLN-targeted CAR-T cells", and these CAR-bearing exosomes could significantly inhibit the growth of TNBCs' without side effects.

Breast Cancer: Epithelial Cell Adhesion Molecules and CAR-T

Epithelial cell adhesion molecule (EpCAM, CD326) is a transmembrane glycoprotein that mediates cell adhesion between the epithelial cells. It consists of an extracellular structural domain with epidermal growth factor and thyroglobulin-like structural domains, a transmembrane structural domain, and an intracellular structural domain consisting of 26 amino acids. This is located in most epithelial cells in adults and is involved in cell conduction, migration, proliferation, and differentiation (Brown et al. 2021; Moon et al. 2018; Patriarca et al. 2012). Biomarkers for circulating tumor cells (CTCs) and cancer stem cells have recently been found to be EpCAM. CTCs are potential precursors for tumor metastasis, which actively invade or shed from the primary tumor into the circulation. So, it is possible to use EpCAM-based gated capture of CTCs for most types of cancers, especially breast cancer (Ahn et al. 2021; Li et al. 2018; Weissenstein et al. 2012).

Previous reports demonstrated the correlated implications pertinent to the expression of epithelial adhesion

factors and overall survival in tumor patients (Fong et al. 2014; Seeber et al. 2016; Warneke et al. 2013). EpCAM is now used as a monoclonal antibody for the treatment of various cancers (Liao et al. 2015). One of these trifunctional monoclonal antibodies has been approved by the European Medicines Agency for the treatment of $EpCAM^+$ malignant ascites cancer (Knödler et al. 2018). According to Zhang et al. (2019), the lentiviruses were created by using the calcium phosphate method and splicing "anti-EpCAM single-stranded variable fragment sequences". In addition, the sequences constructed from the CD8 hinge region, the CD8 transmembrane structural domain, the intracellular region, and CD3 to form third-generation EpCAM-specific CARs using the overlapping polymerase chain reaction, and finally inserting the EpCAM-specific CARs into the lentivirus. The authors co-cultured CAR-T cells with $EpCAM^-$ and $EpCAM^+$ tumor cell lines and discovered that CAR-T cells recognized $EpCAM^+$ tumor cell lines and produced significant levels of IFN-γ and IFN-α. In a xenograft tumor model of two colon tumor cell lines with high EpCAM expression, EpCAM-specific CAR-modified T lymphocytes significantly mitigated the formation and tumor growth. During this study, the mice have not exhibited adverse events. So, this clinical trial involving EpCAM CAR-T cells in cancer patients showed that CAR-T cells could target EpCAM in EpCAM-positive solid tumors. Sahm et al. (2012) reported that natural killer (NK-92) cells could induce antitumor efficacy if they were administered through a lentiviral vector encoding IL-5 and CAR-targeted epithelial adhesion molecule (EpCAM). These cells could induce a high and selective cytocidal activity against EpCAM-expressing breast cancer cells.

Breast Cancer: HER Expression and CAR-T

HER is widely expression antigen and it can be targeted by the CAR-Ts in solid tumors. The overexpression of HER2 is associated with breast cancers (Kumar et al. 2017). In case of TNBCs, the TEM8.CAR-Ts were resulted in tumor rejection in preclinical animal models (Baybutt et al. 2020). However, the PTK7.CAR-Ts resulted in the good clinical outcomes in the xenograft models of breast (Sachdev et al. 2016).

TNBC cells are highly proliferative cancer devoid of estrogen receptor, progesterone receptor, and HER-2 receptors. These cells exhibit stage-specific embryonic antigen-4 and the efficacy of CAR-T has been proven to target these antigens in vitro and in vivo (Pfeifer 2018). Hence, it is crucial to explore several clinical studies for studying the efficacy of CAR-T cells against these TNBCs (Nasiri et al. 2022).

Mucin 1: A Common Target for CAR T Cell Against BC and CRC

Mucin 1 expression is confined to 90% of breast cancers (Keshavarz et al. 2022) as well as its expression also reported in colon cancers (Niv 2008). For instance, the expression of Mucin 1 is observed in 95% of TNBCs. TAB004-derived MUC28Z CAR-T cells have shown significant pharmacological efficacy in vitro and in vivo (Zhou et al. 2019). The MUC1 expression mainly evident in the epithelium-derived solid tumors of breast cancer subtypes. The MUC28Z CAR-T cells have shown prominent efficacy for targeting solid tumors (Keshavarz et al. 2022; Zhou et al. 2019).

Challenges of CAR-T for CRC and BC

Absence of Tumor-Specific Antigens

Tumor-specific antigens (TSAs) is the key to effective CAR-T immunotherapy. TSAs in the tumor cells of solid tumors are rarely candidates for CAR-T cell treatment. However, the absence of ideal targets such as CD19, and CD20 in solid tumors is highly evident subsequently paving a path to implicate CAR-T therapy (Qu et al. 2022).

Tumor-associated antigen (TAA), natural killer group 2 ligand (NKG2D), GUCY2C, and HER-2 are commonly implicated in CAR-T cell research, and CAR-T cells co-cultured with these targets have shown significant anti-tumor effects (Fan et al. 2021; Li et al. 2020c; Magee et al. 2018). However, the ideal CAR-T cell target should also have a higher expression only in the tumor cells but not in the healthy tissues with neither immune escape nor mutation. However, the current TAA targets are widely expressed across the normal tissues, and therefore are often associated with off-target effects during the treatment process (Cerrano et al. 2020). To this end, researchers have proposed various optimization strategies to improve the targeting efficacy of CAR-T cells, including dual-TAA targeting systems, and synthetic Notch (synNotch) receptor CAR-T cells (Marei et al. 2019; Moghimi et al. 2021; Srivastava et al. 2019). The CAR cells were edited to contain the first antigen-binding CAR structure providing the first signal. A second antigen-binding chimeric co-stimulatory receptor structure provides the second signal to enhance targeting efficacy when combined with dual TAA. It has been shown that a dual-receptor CAR (dCAR) model can effectively target CEA and MSLN, the MSLN-4/1BB and CEA-CD3ζ signaling domains, respectively (Zhang et al. 2018). The cytotoxicity of dCAR-engineered T cells is successfully controlled by homologous tumor cells (AsPC-1) expressing CEA and MSLN. CAR-T cell treatment can be used precisely due to minimal toxicity and higher safety. Hyrenius-Wittsten et al. (2021) described

alkaline phosphatase placenta-like 2 (ALPPL2) as a TSA in several solid tumors, which used a synNotch CAR. ALPPL2 can either be the individual target of CAR or combined with the melanoma adhesion molecules in synNotch CAR combination antigens. Thus, synNotch CAR-T could exhibit a significant control of tumor load and durable anti-tumor efficacy (Hyrenius-Wittsten et al. 2021).

Similarly, other studies (Hyrenius-Wittsten et al. 2021; Srivastava et al. 2019) selected EpCAM and receptor tyrosine kinase-like orphan receptor 1 (ROR1) as target antigens to achieve specific antitumor efficacy on $EpCAM^{+}ROR1^{+}$ tumor tissues. In these studies, synNotch receptors T cells, after CAR-T cells and synNotch are disengaged, T cell can migrate to adjacent tissues and exert toxic effects on $ROR1^{+}$ normal tissues. There is co-expression of EpCAM and ROR1 in normal gastric and colonic epithelium. This kind of CAR-T cell system enhances its anti-tumor specificity and tumor-killing efficacy but fails to induce the off-target effect due to its dual-TAA specific targeting, which can still be activated by single-TAA-positive tissues.

Cytokine Release Syndrome

In addition to the off-target effect caused by the lack of tumor-associated antigens that can damage normal tissues, cytokine release syndrome (CRS) could affect the usage of CAR-T immunotherapy. CAR-T cells are activated after recognition of tumor cells in vivo, subsequently promoting the release of higher levels of cytokines, and triggering a series of systemic inflammatory responses. The released cytokines further can activate immune cells to release large amounts of inflammatory factors contributing to the generation of CRS, which often manifests as fever and impaired function of multiple organs and systems (Kotch et al. 2019; Schubert et al. 2021). There are two main mechanisms for the occurrence of CRS: (1). The uncontrolled inflammatory response is associated with IL-1, IL-6, and the dynamic balance between pro-and anti-inflammation in the body. Activated CAR-T cells lead to the secretion of large amounts of pro-inflammatory factors by monocytes, which disrupt the balance between the proinflammation and anti-inflammation process. (2). Activated CAR-T cells, monocytes IL-6, IFN, and tumor necrosis factor activates endothelial cells, which subsequently produce higher levels of IL-6 and also initiate their own mediated pro-inflammatory pathways, further disrupting the pro-inflammatory and anti-inflammatory balance (Orning et al. 2019). Clinically, tocilizumab and chimeric anti-IL-6 antibody cetuximab are the drugs of choice for the treatment of moderate to severe CRS. In most patients, both drugs allow rapid reversal of CRS symptoms, but there is a long treatment interval between the two drugs; henceforth, there is a lag in acute CRS (Neelapu 2019; Oved et al. 2019). It has been reported that when CRS occurs, the activation of vascular endothelial and administration of tocilizumab can enhance the passive diffusion of IL-6 into the central nervous system, which leads to the disruption of the blood–brain barrier and increases the potential for neurotoxicity (Nishimoto et al. 2008). Therefore, CAR-T cell proliferation and differentiation need to be precisely regulated to mitigate the occurrence of CRS.

Neutralizing granulocyte–macrophage colony-stimulating factor (GM-CSF) was used to mitigate the toxicity associated with CART19 cells (Sterner et al. 2019); it has been described that lenzilumab, which neutralizes GM-CSF, and impair CART19 cell function in vivo or in vitro and fostered CART19 proliferation. GM-CSF neutralization prevented cytokine release syndrome and significantly reduced neuroinflammation. In addition, the inactivation of CAR-T cells is one of the key measures to prevent CRS, including the construction of CAR receptors containing suicide genes. For example, cells from CART containing ganciclovir (GCV) and herpes simplex virus thymidine kinase complexes were effectively cleared after exposure to GCV (Shao et al. 2014). Amatya et al (2021) constructed a CD28 CAR with a dimeric structure domain, anti-signaling lymphocytic activation molecule F7 (SLAMF7), and a caspase-9 structural domain. Cancer cells in vitro were selectively removed by T cells carrying anti-SLAMF7 CAR and suicide genes, and the constructs-T cells were afterward destroyed by the dimer AP1903. Although these measures minimize the occurrence of CRS, there is also a risk of premature and irreversible clearance of CAR-T cells by suicide genes that reduces the therapeutic effect (Fig. 2).

Unfavorable Tumor Microenvironment

Tumor microenvironment (TME) across the colorectal tumors and breast tumors are divergent when compared to the hematologic tumors as these colorectal or breast tumors grow with tighter intercellular connections. The high pressure inside the TME of solid tumor tissue makes it difficult for CAR-T cells to enter, subsequently limiting efficacy (Burga et al. 2015). The efficacy of the CAR-T cells is reported to be higher in small size tumors when compared to large tumors. Therefore, the administration of CAR-T cells could improve the therapeutic efficacy by increasing the levels of CAR-T cells inside the tumor. For example, the amount of intrahepatic administration of CAR-T cells should be sufficient enough to deliver across the tumor region in patients with CRC with concomitant liver metastases (Guizhen et al. 2022). The administration of CAR-T cells through the percutaneous hepatic arterial route was effective in CEA^{+} CRC liver metastases conditions in Phase I clinical trials (Katz et al. 2015). In the subsequent Phase 1b trial, serum CEA was stable or reduced in all subjects by local infusion, and no severe CRS or neurotoxicity was

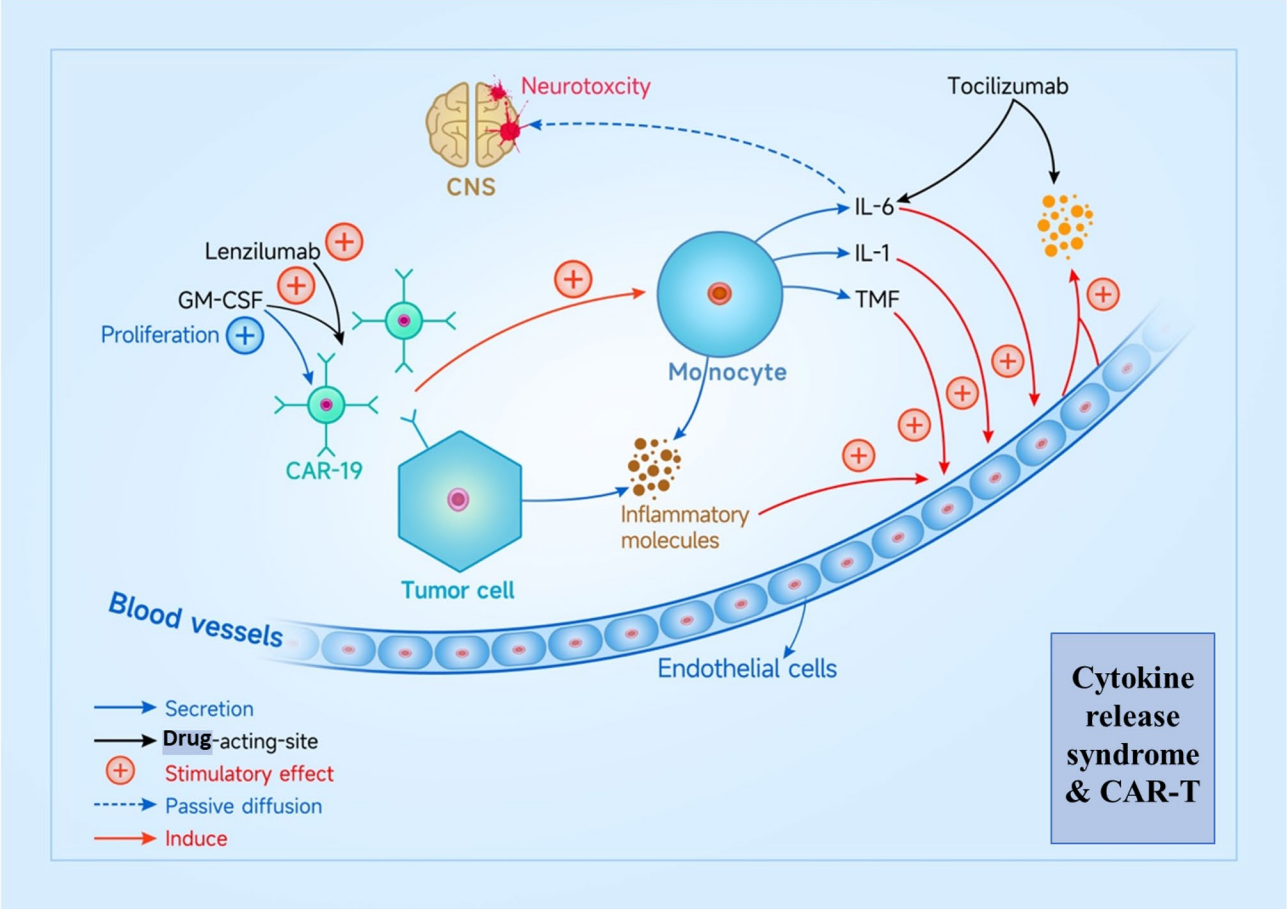

Fig. 2 Numerous systemic inflammatory and immunological responses contributed to the development of cytokine release syndrome (CRS). CAR-T cells initially recognize tumor cells and then activate monocytes after their interactions. Several inflammatory molecules and cytokines (IL-6, IL-1, TMF) are then secreted by tumor cells and monocytes. Surrounding molecules initiate activation of endothelial cells, which subsequently produce substantial levels of IL-6 and also initiate pro-inflammatory pathways, further disrupting the pro-inflammatory and anti-inflammatory balance. Tocilizumab and Lenzilumab are the drugs of choice used to correct CRS. Tocilizumab temporarily promotes the passive diffusion of IL-6 into the central nervous system by disrupting the blood–brain barrier thereby increasing the potential for neurotoxicity while lenzilumab, which neutralizes granulocyte–macrophage colony-stimulating factor (GM-CSF) with promoted CART19 proliferation but preserved CART19 cell function in vivo or in vitro

observed which is indicating the effective delivery without systemic toxicity (Katz et al. 2020). The condition where T cells cannot infiltrate and exhibit activity in solid tumors can be improved by local infusion of CAR-T cells due to intra-tumor hypertension. In addition, CAR-T cells effectively should cross extracellular matrixes to overcome the surrounding immunosuppressive TME. It has been reported that CXCR1/CXCR2-modified CARs significantly improve T cell tumor migration (Kremer et al. 2017; Liu et al. 2020). The accumulation of metabolic waste released from the tumor cells in solid tumors negatively impacts not only proliferation but also the expression of CAR-T cells. Therefore, several strategies targeting the tumor microenvironment have also been proposed, and include IL-12 (Singh et al. 2011), IL-18 (Huang et al. 2020a, b), IL-23 (Ma et al. 2020), TRANSF14 (Boice et al. 2016), which can improve the immunosuppressive microenvironment and enhance the killing of tumor cells to a certain extent.

Different Mechanisms for Overcoming the Difficulties Related to the Use of CAR-T in Solid Tumors

Immunocheckpoint inhibition is a significant process that can enhance the immune system efficacy to target tumor cells. As we discussed above, anti-PD-1 and PD-1 ligands, and anti-CTLA4 are the prominent examples for the targeted modality that can be used with CAR-T therapies to target tumor cells (Gay et al. 2017; Nasiri et al. 2022; Schmidts and Maus 2018). Although the CAR-T therapy has proven efficacy for targeting haematological malignancies, the

treatment of solid tumors with CAR-T could be a significant challenge due to the minimal persistence, and trafficking and T cell inhibitory le inside the tumor microenvironment (Lim and June 2017; Martinez and Moon 2019; Newick et al. 2016; Schepisi et al. 2023; Ye et al. 2018; Yeku et al. 2017). Hence, the combinatorial regimen of immunotherapy and CAR-T along with immunecheckpoint inhibitors could bestow significant pharmacological efficacy to target solid tumors (Chen et al. 2017; Hu et al. 2019; John et al. 2013; Rupp et al. 2017; Yoon et al. 2018). Gene-editing in CAR-T cells is a significant approach used to damage the immune checkpoints, and this approach could be a prominent way to increase the anti-tumor pharmacological efficacy of CAR-T cells (Lloyd et al. 2013; Yin et al. 2019). Thus, the anti-tumor pharmacological efficacy of CAR-T cells could be improved by the PD-1 disruption (McGowan et al. 2020).

CAR-T Trials on Colorectal and Breast Cancer Clinical Trials

In recent years, prospective on-target clinical trials were extensively conducted worldwide to validate the clinical feasibility and safety of this new type of therapy. In a Phase I trial (NCT02349724), CEA-targeted CAR-T cells were infused into 10 patients, and this treatment demonstrated acceptable efficacy and tolerability even at higher doses, with seven progressive patients having stable disease, two patients out of them in remission for more than 30 weeks. Two patients having tumor shrinkage who did not develop complications associated with CAR-T therapy (Zhang et al. 2017b). In another clinical trial with CAR-T cell-based therapy focused on CD133 (NCT02541370), a total of 23 patients were involved in this study, including two CRC patients who showed no signs of having the disease (Wang et al. 2018). In a Phase, I clinical trial on c-met (NCT01837602), comparatively low levels of c-met-CAR-T cells were detectable among 33% of patients with breast cancer after injecting "mRNA-c-met-CAR-T cells" intratumorally. Clinical trials and updated NCT numbers for the remaining targets:

NKG2D: NCT05248048, NCT04550663

CEA: NCT04513431, NCT05240950, NCT04348643, NCT05396300, NCT03682744

MSLN: NCT05089266

MUC1: NCT02617134, NCT05239143, NCT04020575, NCT02587689

EpCAM: NCT05028933, NCT02915445

HER-2: NCT02713984, NCT02547961, NCT04430595, NCT04650451

IM96: NCT05287165

EGFR: NCT05341492

Reports from these trials were not found specific clinical trials are summarized in Table 3.

Conclusion

CAR-T immunotherapy was primarily reported in 1989. The recurrent developments in CAR-T immunotherapy have significant pharmacological efficacy against hematologic cancers. Several clinical trials right now investigating the efficacy of CAR-T therapies to treat hematological malignancies significantly focusing on targeting antigens such as CD19, CD22, and BCMA (Kozani et al. 2021). Furthermore, the CS1, FLT3, CD7 and CD26 are considered as the non-solid tumor antigens implicated as the target antigens for CAR-T therapies in malignancies (Kozani et al. 2021). Investigation of novel antigens, which can be targeted by the antibody-based therapy or CAR-T therapy may confer to the promising antitumor pharmacological efficacy against hematological malignancies include B cell acute lymphoblastic leukemia, mantle cell lymphoma, diffuse large B cell lymphoma, R/R multiple myeloma (Mullard 2021; Munshi et al. 2021; Prasad 2018; Voelker 2020). However, CAR-T immunotherapy against CRC has not resulted in significant breakthroughs. The failure of significant breakthroughs pertinent to CAR-T immunotherapy against CRC are due to the tumor-associated complex vasculature across the tumor microenvironment and enriched stroma. The hostile tumor microenvironment could confer to the significant obstacles for the pharmacological efficacy of CAR-T therapy against CRC (Donnadieu et al. 2020; Kozani et al. 2021). Although several reports described higher CAR-T' cell efficacy and safety, these strategies have certain limitations. Tumor cells can undergo antigen-dependent changes to evade the CAR-T therapy which could cause raise in the therapeutic resistance of tumor cells. Antigen downregulation can induce mitigation in the antitumor pharmacological efficacy of CAR-T therapy against malignant cells (Shah and Fry 2019). Furthermore, the CAR-T therapy induced immune cells have to interact with substantial number of antigens to generate significant downstream signals to foster the cytolytic changes (Kozani et al. 2021). In addition, the development of colon cancer often experiences various environmental, dietary, and chronic inflammatory conditions. Therefore, CAR-T needs to be implicated in a combinatorial regimen with other anti-tumor therapeutic modalities such as traditional radiotherapy, targeted therapy, and immunotherapy. CAR-T cell immunotherapy exhibits a broad prospect in CRC and even against other solid tumors, which can bring new directions and hope for the treatment of breast tumors or CRC patients.

Table 3 Information pertinent to the several ongoing clinical trials to elucidate the efficacy of CAR-T therapy

Targeting antigen	Title of study	Enrollment estimates	Phase	Indication	Clinical trials ID	Status
NKG2D	Hepatic Artery transfusion of NKG2D CAR-T cells to treat the patients with previously treated liver metastatic colorectal cancer	9	Early Phase 1	Metastatic colorectal cancer	NCT05248048	Recruiting
CEA	A study evaluating the safety and preliminary efficacy of anti-CEA CAR-T cells for the prevention of postoperative recurrence and metastasis of stage III colorectal cancer or liver metastasis of colorectal cancer	18	Early Phase 1	Colorectal cancer liver metastasis	NCT04513431	Not yet recruiting
CEA		18	Phase 1	Colorectal cancer Metastatic liver cancer	NCT05240950	Recruiting
MSLN	Study of αPD1-MSLN-CAR T cells to evaluate the safety, tolerability, and effectiveness for patients with MSLN-positive advanced Solid Tumors	30	Phase 1	Colorectal cancer	NCT05089266	Not yet recruiting
MUC1	Immunotherapy with chimeric antigen receptor-modified T cells for MUC1 positive advanced refractory solid tumor	20	Phase 1 Phase 2	Colorectal carcinoma	NCT02617134	Unknown
NKG2DL	One-center, open-label, single-arm clinical study of the safety and efficacy of NKG2D CAR-T cells infusion in the treatment of relapsed/refractory NKG2DL + tumors	10	Phase 1	Colorectal cancer	NCT04550663	Not yet recruiting
EpCAM	Autologous T cells modified by chimeric antigen receptor targeting EpCAM for clinical research on advanced digestive system malignancies	48	Phase 1	Colorectal cancer	NCT05028933	Recruiting
CEA	Clinical research of chimeric antigen receptor (CAR) T cells targeting CEA-positive cancer	75	Phase 1	Colorectal cancer Breast cancer	NCT02349724	Unknown
CEA	Safety and efficacy of CEA-targeted CAR-T therapy for relapsed/refractory CEA + cancer	40	Phase 1 Phase 2	Colorectal cancer Breast cancer	NCT04348643	Recruiting
CEA	A clinical study of CEA-targeted CAR-T in the treatment of CEA-positive advanced malignant solid tumors	60	Phase 1	Colorectal cancer	NCT05396300	Recruiting
MUC1	A phase 1 dose escalation and expanded cohort study of P-MUC1C-ALLO1 in adult subjects with advanced or metastatic solid tumors	100	Phase 1	Colorectal cancer Breast cancer	NCT05239143	Recruiting
HER-2	A clinical research of CAR T cells targeting HER2 Positive cancer	0	Phase 1 Phase 2	Colorectal cancer Breast cancer	NCT02713984	Withdrawn
CD133	Clinical study of chimeric CD (cluster of differentiation)133 antigen receptor-modified T cells in relapsed and/or chemotherapy refractory malignancies	20	Phase 1 Phase 2	Colorectal cancer, Breast cancer	NCT02541370	Completed
IM96	Clinical Trial to evaluate the safety and efficacy of IM96 CAR-T cell-based therapy in patients with advanced digestive system neoplasms	19	Early Phase 1	Colorectal (colon or rectal) cancer	NCT05287165	Recruiting
HER-2	Chimeric antigen receptor-modified T cells for HER-2 positive recurrent and metastatic breast cancer	0	Phase 1 Phase 2	Breast cancer	NCT02547961	Withdrawn

Table 3 (continued)

Targeting antigen	Title of study	Enrollment estimates	Phase	Indication	Clinical trials ID	Status
EGFR	A single-arm, open, exploratory clinical study evaluating the safety and efficacy of EGFR/B7H3 CAR-T in patients with EGFR/B7H3-positive advanced solid tumors	30	Early Phase 1	Triple-negative breast cancer	NCT05341492	Recruiting
HER-2	Multiple 4SCAR-T cell therapy targeting breast cancer	100	Phase 1 Phase 2	Breast cancer	NCT04430595	Recruiting
MUC1	Adoptive immunotherapy for advanced MUC1* positive breast cancer with autologous T cells engineered to express a chimeric antigen receptor, huMNC2-CAR44 specific for a cleaved form of MUC1 (MUC1*)	69	Phase 1	Metastatic breast cancer	NCT04020575	Recruiting
EpCAM	T-cells associated with chimeric antigen receptor recognizing EpCAM for the patients with advanced solid tumors	30	Phase 1	Breast cancer recurrent	NCT02915445	Recruiting
MUC1	Phase I/II study of anti-MUC1 CAR t cells for patients with MUC1 + advanced refractory solid tumor	20	Phase 1 Phase 2	Triple-negative invasive breast carcinoma	NCT02587689	Unknown
HER-2	A phase 1/2, open-label, multicenter, non-randomized, safety and activity study of HER2-targeted dual switch CAR-T cells (BPX-603) in the subjects with previously treated advanced HER2-positive solid tumors	220	Phase 1	HER2-positive breast cancer	NCT04650451	Recruiting
CEA	Immunotherapy for peritoneal carcinomatosis (IPC)—a phase I study of the safety and efficacy of Anti-CEA CAR-T cell intraperitoneal infusions for the treatment of CEA-expressing adenocarcinoma peritoneal metastases or malignant ascites	0	Phase 1	Colorectal cancer Breast cancer	NCT03682744	Withdrawn
cMet	Clinical Trial of autologous cMet redirected T cells administered intratumorally into the patients with breast cancer	6	Phase 1	Metastatic breast cancer, TNBC	NCT01837602	Completed

NKG2D natural killer group 2 ligand

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Declarations

Conflict of interest Authors declare no conflict of interests.

Ethics approval This work has been approved by the institutional ethical committee of First affiliated hospital of Zhengzhou University, Henan. All the work related to this retrospective study was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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