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Review

CAR-T cell therapy: Advances in digestive system malignant tumors

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Malignant tumors of the digestive system have had a notoriously dismal prognosis throughout history. Immunotherapy, radiotherapy, surgery, and chemotherapy are the primary therapeutic approaches for digestive system cancers. The rate of recurrence and metastasis, nevertheless, remains elevated. As one of the immunotherapies, chimeric antigen receptor T cell (CAR-T) therapy has demonstrated a promising antitumor effect in hematologic cancer. Despite undergoing numerous clinical trials, the ineffective antitumor effect and adverse effects of CAR-T cell therapy in the treatment of digestive system cancers continue to impede its clinical translation. It is necessary to surmount the restricted options for targeting proteins, the obstacles that impede CAR-T cell infiltration into solid tumors, and the limited survival time *in vivo*. We examined and summarized the developments, obstacles, and countermeasures associated with CAR-T therapy in digestive system cancers. Emphasis was placed on the regulatory functions of potential antigen targets, the tumor microenvironment, and immune evasion in CAR-T therapy. Thus, our analysis has furnished an all-encompassing comprehension of CAR-T cell therapy in digestive system cancers, which will generate tremendous enthusiasm for subsequent in-depth research into CAR-T-based therapies in digestive system cancers.

INTRODUCTION

Malignant tumors of the digestive system refer to a range of malignant tumors that occur in the digestive tracts and some associated organs.[1] The primary categories of digestive system cancer are esophageal cancer, gastric cancer (GC), colorectal cancer (CRC), liver cancer, pancreatic cancer, and gallbladder cancer.[2] Based on data from Global Cancer Observatory statistics (GLOBOCAN), CRC has the third-highest occurrence rate among all types of cancer (10.2%) in both males and females.[3–5] It also has the highest rate among all digestive system cancers, followed by GC (5.7%; ranked 6th among all cancers), liver cancer (4.7%; ranked 7th among all cancers), esophageal cancer (3.2%; ranked 8th among all cancers), and pancreatic cancer (2.5%; ranked 13th among all cancers).[3–5] CRC has the second-highest fatality rate among all types of cancer (9.2%), and the highest fatality rate among all digestive system cancers, followed by liver cancer (8.2%), stomach cancer (8.2%), esophageal cancer (5.3%; ranking 6th among all cancers), and pancreatic cancer (4.5%; ranking 7th among all cancers).[3–5] The primary modalities utilized for digestive system cancer treatment are surgical intervention, chemotherapeutic agents, and immunotherapeutic approaches.[3–6] However, it continues to have a significant mortality rate. For instance, in the case of CRC, 20% of individuals with CRC are identified at stage V, which has a 5-year relative survival rate of 12.5%.[7] For individuals with metastatic CRC, treatment options other than for liver and lung metastasis primarily consist of palliative measures such as systemic chemotherapy and supportive therapy. Their lifespan typically does not exceed 12 months.[8] Tumors affecting the digestive system, such as those in the bile ducts, pancreas, and stomach, are highly malignant and have limited success with conventional treatments.

The chimeric antigen receptor (CAR) is a modular fusion protein that typically consists of a co-stimulatory molecule derived from an antibody single-chain variable fragment (scFv), an extracellular target-binding domain, and a spacer domain.[9,10] The receptor 4-1BB is associated with the intracellular signaling domain of CD3z, similar to CD28.[11] CARs facilitate the identification of tumor-associated antigens (TAAs) by T cells in a manner that is not dependent on the major histocompatibility complex (MHC).[11,12] CAR-T cell immunotherapy has demonstrated significant progress in the treatment of hematological malignancies since 2012.[10] Specifically, CD19-targeting CAR-T therapy has been effectively implemented to treat diffuse large B cell lymphoma and B cell acute lymphoblastic leukemia (B-ALL).[11–13] Following the remarkable progress made in the treatment of solid malignancies, CAR-T is advancing in a novel, more generalized direction.[11]

Presently, CAR-Ts that target numerous novel targets have emerged in solid tumors of the digestive tract, providing an additional layer of protection for these tumors in situations where immunotherapy

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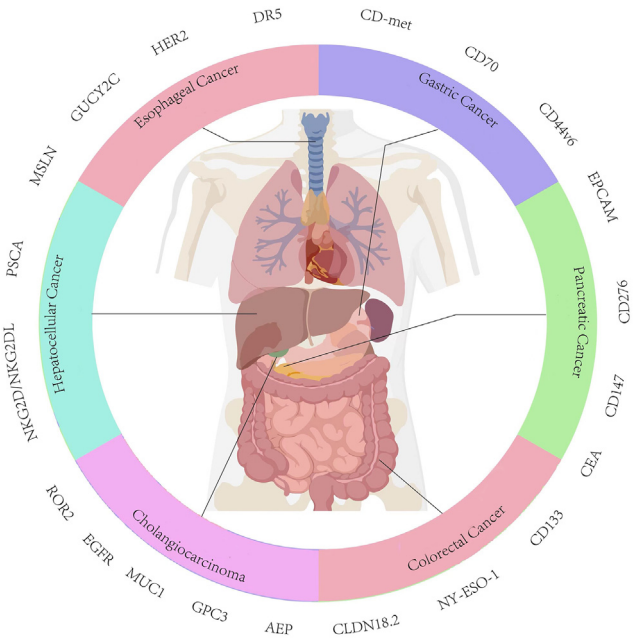

Figure 1. Different digestive tract malignancies and their corresponding targets

fails.[14] However, CAR-T therapy encounters several challenges due to the intricate nature of solid tumors of the digestive tract and their location within the human body.[15,16] These obstacles include an inhibitory microenvironment within the tumor, the elution of antigens, off-target toxicity, undesired antigen specificity, and a delivery mechanism that is not highly efficient.[16–19] Thankfully, novel approaches are being suggested to address a variety of challenges associated with the use of CAR-T in the treatment of solid tumors. These approaches include modifying the structure of CAR, preventing extratumor toxicity of CAR-T, and developing new delivery systems. Utilizing CAR-T cells to treat solid malignancies of the digestive tract are anticipated to advance to a new level of development in the near future, as these strategies offer fresh perspectives[18,20–24] (Figure 1).

DIFFERENT TUMOR ANTIGENS FOR THE DIGESTIVE SYSTEM

In the progress of cancer, tumor tissues express different antigens with specificity. Due to these antigens are only presented at a low level outside the tumor, differences in tumor antigen expression between tumor tissues and other tissues make them the possible targets for CAR-T cell therapy of human solid tumors (Table 1).[18]

Glypican-3

Glypicans are a group of cell surface glycoproteins among which heparan sulfate glycosaminoglycan chains are covalently linked to the protein core.[25] Glypican-3 (GPC3) is a member of the heparan sulfate glycoproteins family, and it is attached to the cell membrane through a glycophosphatidylinositol (GPI) anchor. In digestive tract tumors, the abnormal expression of GPC3 regulates tumor proliferation and progression by modulating Wnt signaling pathways.[26] GPC3 is highly expressed in GC, especially hepatocellular carcinoma (HCC). Recent evidence from preclinical experiments indicated that CAR-T cells targeting GPC3 could inhibit the growth of HCC cells. In preclinical animal experiments, GPC3 CAR-T cells also demonstrated significant tumor elimination effects.[27,28] Clinical trials of GPC3-CAR in the treatment of $GPC3^{+}$ gastrointestinal solid tumors have started in recent years. In 2020, the results of the phase 1 clinical study of the world's first second-generation CAR-T cell therapy targeting GPC3, CAR-GPC3 T cells, in the treatment of advanced HCC were released.[29] The results showed that 2 of the 13 subjects achieved partial remission (PR); the 3-year, 1-year, and 6-month survival rates were 10.5%, 42.0%, and 50.3%, respectively; and the median survival time (OS [overall survival]) was 278 days (95% confidence interval [CI]: 48,615 days).[29] In addition, a large number of clinical studies are in progress for GPC3-CAR-T.

Epidermal growth factor receptor

In numerous forms of cancer, epidermal growth factor receptor (EGFR) and EGFR variant III (EGFRvIII) are highly expressed.[30] The amplification of EGFRvIII has been observed in around 30% of patients with glioblastoma (GBM) and has been investigated in clinical trials as a potential target for therapy of GBM tumors.[31] Nonetheless, this trial did not have the most severe and prevalent toxic reactions, such as cytokine release syndrome and neurological toxicity; furthermore, no patients exhibited statistically significant regression of MRI lesions.[31] Patients who tested positive for EGFR and had recurrent or metastatic cholangiocarcinoma were administered one to three cycles of EGFR CAR-T cell infusion. Among these patients, 10 achieved disease stability and 1 achieved complete remission; however, 3 patients developed acute fever or chills of grade 3 or higher. Additionally, some patients developed level 1/2 target-mediated toxicity associated with pre-treatment therapy, which included mucosal/skin toxicity and acute pulmonary edema, in addition to $\geq$ level 3 lymphocyte and thrombocytopenia.[32] The therapeutic efficacy and safety profile of CAR-T cells that specifically target EGFRvIII and EGFR in the context of solid malignancies remain to be established.

Mucin-1

Mucin-1 (MUC-1; epithelial membrane antigen) is a transmembrane glycoprotein that is expressed on the apical membrane of epithelial cells.[33] MUC-1 belongs to the mucin family with extensive O-linked glycosylation in the extracellular domain and is physiologically expressed on the apical surface of epithelial cells in stomach, intestine, and several other organs.[34] Overexpression of transmembrane mucins contributes to oncogenesis by promoting receptor tyrosine kinase signaling, loss of epithelial cell polarity, and constitutive activation of growth and survival pathways (example.g., the Wnt-β-catenin and nuclear factor-κB pathways).[35] MUC-1 is widely overexpressed in GC, liver cancer, and pancreatic cancer. It is interesting to note that MUC-1 linked to cancer is hypoglycosylated as opposed to the highly glycosylated variant present in normal cells. This means that

Table 1. Different targets for CAR-T cell therapy

Target molecule	Protein function	Cellular localization	Applicable cancer	Representative clinical trials
GPC-3	cell surface proteoglycan that bears heparan sulfate	cell surface	liver cancer	NCT05123209
EGFR	plays an important role in multiple aspects of cell growth, differentiation, migration, invasion, survival, and apoptosis	cell surface	esophageal cancer, hepatoma, pancreatic cancer	NCT03941626
MUC-1	plays an important role in epithelial renewal and differentiation, maintaining epithelial integrity, and the occurrence and metastasis of cancer	type I transmembrane protein	intrahepatic cholangiocarcinoma	NCT03633773
AFP	binds copper, nickel, and fatty acids as well as, and bilirubin less well than, serum albumin; only a small percentage (<2%) of the human AFP shows estrogen-binding properties	protein located outside the cell membranes	liver cancer, liver neoplasms, metastatic liver cancer	NCT03349255
CEA	closely related to various functions of endothelial cells, including cell adhesion, proliferation, and migration *in vivo* and *in vitro*	cell surface	CRC, liver metastasis, esophageal cancer, stomach cancer, pancreatic cancer, metastatic tumor, recurrent cancer	NCT04513431
EpCAM	involved in processes such as tumor cell proliferation, formation, invasion, migration, diagnosis, drug resistance, and antitumor therapy	cell surface	colon cancer, esophageal carcinoma, pancreatic cancer, GC, hepatic carcinoma	NCT05028933
CD133	highly expressed in EPCs	cell surface	liver cancer, pancreatic cancer, CRC	NCT02541370
NKG2D	a multifunctional receptor that can bind directly to various ligand molecules expressed on the surface of target cells, without the need for antigen presentation, leading to activation or co-stimulation of immune effector factors	cell surface	HCC, colon cancer, liver metastasis	NCT05248048
HER2	a receptor tyrosine kinase that binds to the surface of cell membranes and is involved in signaling pathways leading to cell growth and differentiation	cell surface	esophageal cancer, CRC, pancreatic adenocarcinoma	NCT02713984
MSLN	abnormal expression plays a positive role in the malignant transformation and invasiveness of tumors by promoting cancer cell proliferation, local migration/invasion, and metastasis	cell surface	CRC, pancreatic cancer	NCT05089266
CD147	a highly glycosylated transmembrane immunoglobulin	cell surface	advanced HCC	NCT03993743
CLDN18.2	plays a major role in tight junction-specific obliteration of the intercellular space, through calcium-independent cell-adhesion activity	cell surface	gastric adenocarcinoma, pancreatic cancer, gastroesophageal junction adenocarcinoma	NCT05539430

CAR-T cells can selectively target it without having an off-tumor effect while remaining on target.[36] MUC-1 participates in intracellular signaling, acting as an adhesion ligand for stromal cells and endothelial cells and affecting cell mobility, which makes it an excellent candidate for immunotherapy target.[37] Preclinical experiments have shown that MUC-1 is a promising therapeutic target to clear tumor cells even for solid tumors like pancreatic ductal adenocarcinoma(PDAC) and cholangiocellular carcinoma.[38,39] MUC-1 specific CAR-T cells efficiently destroy tumor cells, but require more research to further confirm the efficiency and safety in clinical applications. Clinical trials of CAR-T therapy for liver cancer, GC, pancreatic cancer, and colon cancer MUC1 have been widely carried out. There are currently two phase 1/2 clinical trials in progress for patients with advanced refractory solid tumors, including HCC. These trials involve the use of anti-MUC-1 CAR-T and CAR-natural killer (NK) cell therapies. These trials are registered at ClinicalTrials.gov (NCT02587689 and NCT02839954).

α-etoprotein

α-Fetoprotein (AFP) is a 70-kDa glycoprotein that can be detected in the serum of early mammalian embryos.[40] It is synthesized at the site of embryonal hematopoiesis, specifically the yolk sac. Following birth, the levels decrease rapidly, and by the second year, only minuscule amounts can be detected in the bloodstream. Adult levels usually fall within the range of 1–40 ng/mL.[41] Several conditions can lead to the reappearance or elevated serum levels of certain substances. These conditions include pregnancy, hepatic disorders, and various malignancies such as HCCs, germ cell tumors (particularly those with yolk sac tumor components), breast, esophagus, cervical, pancreatic, endometrial, GC, lung, and rectal cancers.[41,42] Elevated

expression of AFP in tumor and serum is found in liver cancer and GC, and is commonly associated with poor prognosis.[43] Although high specificity makes AFP an ideal target for HCC CAR-T cell therapy, it is expressed and secreted intracellularly as a soluble protein, which is incapable of binding to traditional antibody-based antibodies, making it impossible to be used by traditional antibody-based CAR-Targeting.[44] To overcome this obstacle, a novel CAR-Targeting the peptide-MHC complex (AFP-MHC) was generated and demonstrated robust antitumor activity *in vitro* and *in vivo* in a preclinical setting.[43] Three of the six patients in a phase 1 trial assessing the safety and effectiveness of ET1402L1-CAR-T cells (anti-HLA-A02/AFP complex) saw a decrease in tumor size without experiencing drug-related neurotoxicity or cytokine-release syndrome (CRS). This represents a novel approach to intracellular antigen CAR-T therapy (this trial is registered at ClinicalTrials.gov [NCT03349255]).[43]

Carcinoembryonic antigen

Carcinoembryonic antigen (CEA) is a structural acid glycoprotein of the cell membrane originally discovered in colon cancer and fetal intestinal tissue.[45] CEA has been shown to be widespread in endodermal-derived digestive system tumors, such as GC, liver, pancreatic, and CRC.[46,47] A preclinical study showed that CEA-specific CAR-T cells could contribute to the delay of tumor growth and an extension in the survival of mice with GC.[48] The first clinical trial of CEA CAR-T cells was carried out in the United Kingdom to treat CEA^+ cancers and collected 14 patients with liver metastasis before premature closure due to acute respiratory toxicity.[49] Later, intrahepatic artery transfusion of CEA CAR-T cells has been proved safe and potentially efficient for unresectable CEA^+ liver metastasis in a clinical trial.[50] The trial also revealed that neutrophil:lymphocyte ratios are correlated with prognosis of intrahepatic artery transfusion of CEA CAR-T cells. High neutrophil:lymphocyte ratios are associated with poor responses following CAR-T therapy.[51] In 2017, another trial included 10 CEA^+ metastatic CRC patients. Among these patients, 7 reached a stable state after CAR-T cell therapy and the tumors of 2 patients have shrunk. No severe complications occurred during this process of treatment, which showed great efficiency and safety of CAR-T cell therapy in CEA^+ colon cancer.[52] So far, CEA CAR-T cell therapy seems to benefit more patients with colon cancer and metastatic liver cancer, but further investigation is still needed to improve clinical outcomes.

Epithelial cell adhesion molecule

Epithelial cell adhesion molecule (EpCAM) is a type I transmembrane glycoprotein expressed on the basolateral cell surface in most human simple epithelia.[53] EpCAM is highly expressed in solid tumors, including CRC, gallbladder cancer, pancreatic cancer, esophageal cancer, and GC, but a lower level of expression was identified in HCC.[54] As such, EpCAM has the potential to be a prognostic and therapeutic marker in either cancer progression or metastasis formation. In contrast to normal epithelia, in which EpCAM is expressed mostly on the basal or basolateral cell membrane, EpCAM distribution varies in carcinoma, depending on the type of carcinoma.[55] Because it is uniformly expressed over the entire surface of tumor cells, EpCAM is considered an excellent target for various therapeutic approaches, including immunotherapy.[56] A preclinical study demonstrated that EpCAM CAR-T, while having potent antitumor efficacy, may also lead to lethal toxicity in an immunodeficient mouse model due to basal EpCAM expression in normal lung.[53] Meanwhile, another preclinical study showed that EpCAM CAR-T therapy significantly inhibited the formation and growth of colon tumors in mice without systemic toxicity to the mice.[57] At present, clinical trials of EpCAM CAR-T for solid tumors of the digestive system are being carried out in China to test the efficacy and safety of EpCAM CAR-T.

CD133

CD133 (prominin-1), a pentaspan membrane glycoprotein a plasma membrane cholesterol-binding pentaspan glycoprotein (lipid microdomain).[58] CD133 is expressed in normal organ and tissues like kidney, prostate, bone marrow, liver, pancreas, and skin, and also in solid tumors, including liver cancer, pancreatic cancer, and colon cancer.[59] Higher-stage tumors are correlated with the increased expression of CD133, particularly in HCC cells, which generally indicates a poor prognosis for patients.[59] Furthermore, it has been established that endothelial progenitor cells (EPCs) and cancer stem cells are involved in the spread and recurrence of tumors. CD133 is a marker for these cells. Patients with advanced $CD133^+$ malignancies may find CD133 a suitable target for immunotherapy because of these features.[60] A preclinical study in 2021 revealed that CD133 CAR-T cells could kill GC cells in a xenograft model efficiently.[61] Results of a phase 1 clinical trial aimed at advanced metastatic cancers of the digestive system completed in 2018 showed that of 23 patients enrolled (14 with HCC, 7 with pancreatic cancer, and 2 with CRC), 3 achieved partial responses and 14 achieved stable disease. The 3-month disease control rate (DCR) was 65.2%, and the median progression-free survival was 5 months.[62] Results from a phase 2 clinical trial completed in 2020 demonstrated a median OS of 12 months (95% CI: 9.3–15.3 months) and a median PFS of 6.8 months (95% CI: 9.3–15.3 months) among 21 evaluable patients enrolled (95% CI: 4.3–8.4 months). Of the 21 evaluable patients recruited, 1 had a partial response, 14 had stable disease for 2–16.3 months, and 6 progressed after T cell infusion. The clinical trial results showed that CAR-T-133 showed promising antitumor activity, manageable safety, and potent activity in treating solid tumors of the digestive system.[63]

NKG2D

NKG2D is composed of two disulfide-linked copies of a type II transmembrane glycoprotein.[64] NKG2D is expressed on most human NK cells, γδ T cells, $CD8^+$ T cells, and a small subset of $CD4^+$ T cells.[65] However, the expression of NKG2DL on most cell surfaces is commonly restricted under physiological circumstances, but it might be upregulated in tumor cell lines or primary tumors while experiencing infection, overproliferation, or malignancy.[66] NKG2DLs are commonly expressed among solid tumors of the digestive system, and they could be an extraordinary target for CAR-T therapy.[67] Recently, more than four preclinical studies have revealed the potential of NKG2D CAR-T cells killing tumor cells *in vitro* and their antitumor activity in xenograft models, including liver cancer, GC, CRC,

and pancreatic cancer.[68–70] NKG2D provides a good target for CAR-T treating solid tumors of the digestive system, but some preclinical studies also cast doubt on its toxicities, especially if delivered subsequent to lymphodepletion regimens, like cyclophosphamide.[71] NKG2D-based CARs have the potential to induce significant toxicities *in vivo*, especially if delivered subsequent to lymphodepletion regimens, like cyclophosphamide. Many clinical studies are in progress to evaluate the efficiency and safety of NKG2D CAR-T.

HER2

Human EGFR 2 (HER2) is a transmembrane glycoprotein.[72] HER2 consists of three parts: a domain containing a ligand-binding site, a transmembrane domain, and a domain with tyrosine kinase activity.[73] Imbalanced HER2 signal transduction could destroy the polarity and adhesion of cells, disturb cell cycles, promote invasiveness, and have dramatic effects on the initiation of carcinogenesis and further tumor growth.[74] HER2 is highly expressed in GC, pancreatic cancer, esophageal cancer, and CRC, while only weak expression is detected in gallbladder cancer and extrahepatic cholangiocarcinoma.[75] Therefore, HER2 might serve as a promising target for applying CAR-T therapy in solid tumors of the digestive system.[76,77] A preclinical study demonstrated the diversity and efficiency of HER2 CAR-T therapy in a human-derived pancreatic cancer xenograft model.[78] Another study focused on a human-derived GC xenograft model showed that HER2 CAR-T constantly inhibited HER2-overexpressed tumors.[75] Moreover, in a CRC patient-derived xenograft (PDX) mouse model, HER2 CAR-T implantation was detected to reduce even clear tumor tissues and also protected mice from recurrent CRC, which further improved survival.[79] Based on a clinical trial published in 2017, 11 advanced cholangiocarcinoma and pancreatic cancer patients with positive HER2 expression underwent CAR-T therapy, and among these, 1 obtained a 4.5-month-long partial response and 5 achieved stable disease. The median progression-free survival was 4.8 months (range, 1.5–8.3 months).[80] These studies support the potential application of HER CAR-T therapy in $HER2^{+}$ solid tumors, and many studies are under way.

MesothelinMesothelin (MSLN) is a GPI-anchored protein bound to the cell surface and a differentiation antigen.[81] It is expressed at a low level in mesothelial cells of the pleura, pericardium, and inner peritoneum. MLSN is expressed in 30% of cancers, including esophageal cancer, pancreatic cancer, cholangiocarcinoma, and GC.[82] The involvement of MLSN in tumorigenesis may be related to the promotion of cell proliferation and migration by activating PI3K, ERK, and MAPK signaling pathways, and the promotion of tumor cell invasion and metastasis.[83] MLSN is also considered to be a promising target for CAR-T therapy. In 2019, multiple models in a preclinical CAR-T study on GC with high MSLN expression demonstrated the effectiveness of MSLN as a new target for CAR-T treatment of GC.[82] A preclinical study in 2021 showed that MSLN CAR-T had a good antitumor effect in a PDX mouse model of GC and CRC and a cell line-derived xenograft mouse model of CRC.[84] A preclinical study in 2022 noted the antitumor effect of MSLN CAR-T cell therapy on pancreatic cancer in orthotopic human pancreatic cancer animal models, indicating its therapeutic potential.[85] In 2018, researchers reported the clinical treatment results of stage I PDAC targeting MSLN. A total of six patients were treated without cytokine-release syndrome (CRS) and dose-related toxicity. The stable disease(SD) reactions of two patients were 3.8 and 5.4 months, respectively. In one patient, the active metabolic signal of tumor tissue decreased by 69.2%, and all liver metastases disappeared. After using MSLN CAR-T cells in pancreatic cancer, positive results were observed, leading to anticancer reactions in patients with metastasis.[86] In 2019, a phase 1 clinical trial confirmed the feasibility and safety of MSLN CAR-T, but the clinical effects were limited.[87] Clinical trials of MSLN CAR-T against solid tumors of the digestive system, especially pancreatic cancer, are being carried out extensively, and the future is promising.

CD147

CD147 is a transmembrane glycoprotein, which plays an important role in T cell activation, proliferation, migration, adhesion, and invasion.[88] The expression levels of CD147 vary in different types of normal cells—low in normal epithelial and fetal tissues, while significantly upregulated in invasive solid tumors, including HCC and CRC.[89] The upregulation of CD147 promotes tumor invasion, progression, and metastasis. CD147 seems to be a potential target of CAR-T, but its application is limited because of its expression in most normal tissues.[90] CD147 CAR-T therapy could improve its efficacy by using the Tet-On system to control its expression, which may be utilized in treating liver cancer.[91] These preclinical studies provide guidance for the subsequent clinical translation of CD147 and the safety improvement of clinical trials.

B7-H3

The B7-H3 molecule is a member of the B7 immunological co-stimulatory and co-inhibitory globulin family and is a type I transmembrane glycoprotein that was recently identified.[92] Numerous investigations have demonstrated that whereas B7-H3 is either not expressed at all or is very limited in normal tissues, it is overexpressed in leukemia, GC, breast cancer, non-small cell lung cancer, osteosarcoma, pancreatic cancer, and other human cancers.[93] It has also been documented that key elements of the tumor microenvironment (TME), including tumor stromal cells, tumor stem cells, and tumor neovascularization, express B7-H3 molecules.[94] According to clinical research, the overexpression of B7-H3 molecules usually results in a poor prognosis for a variety of malignancies, speeds up the progression of the disease, and decreases the infiltration of tumor-associated lymphocytes within tumors. Owing to these features, the B7-H3 molecule has gained popularity as a target for tumor immunotherapy.[95] The infusion of 1×10^{7} B7-H3 CAR-T into the tail vein of NSG mice produced total tumor regression and eradication as well as a considerable extension of mouse survival in *in situ* xenograft models of osteosarcoma ($n = 5$) and Ewing sarcoma ($n = 5$).[96] Effectively eliminating esophageal squamous cell carcinoma cells and extending the survival period of mice have been demonstrated by B7-H3-CAR-T cells in animal models; B7-H3-CD70-CAR-T cell therapy has also demonstrated therapeutic effects against esophageal cancer. Currently under construction is the third-generation CAR-T

(CD28-4-1BB-CD3ζ) that targets B7-H3.[97] B7-H3 CAR-T exhibited strong antitumor characteristics in the PDAC cell line *in situ* xenograft model, metastasis model, and PDAC-PDX model. Among these, B7-H3 CAR-T can successfully remove tumors and maintain tumor growth control for up to 80 days in the PDAC-PDX model.[98] The outcomes of employing B7-H3 CAR-T in a patient with recurrent anaplastic meningioma were initially published by domestic scholars. Using local intracavitary injection, the researchers treated B7-H3 CAR-T for three consecutive cycles. The patients tolerated the treatment well, with no adverse reactions of grade 3 or above. While B7-H3 CAR-T is unable to infiltrate distant tumor tissue, it can infiltrate tumor tissue close to the infusion device and successfully limit tumor growth there. After three treatment cycles, the expression level of B7-H3 in tumor tissue at the infiltrating site dropped dramatically, according to immunohistochemical examination, although B7-H3 was still highly expressed at distant, non-infiltrating sites.[99] Several B7-H3 CAR-T clinical trials are being carried out. To assess the safety and viability of B7-H3 CAR-T locally within the tumor or intraventricular region in a variety of pediatric recurrent or refractory CNS cancers, including diffuse gliomas, one of the phase 1 clinical trial plans is currently underway (this trial is registered at ClinicalTrials.gov [NCT04185038]). A domestic institution carried out a phase 1/2 trial (NCT04077866) to examine the safety and effectiveness of the local administration of B7-H3 CAR-T in combination with temozolomide against temozolomide monotherapy in patients with refractory or recurrent GBM.

Claudin18.2

Claudin18.2 (CLDN18.2) is an integrin membrane protein, located on the surface of the cell membrane.[100] It usually expresses at a low level in differentiated epithelial cells of the gastric mucosa, but it is significantly upregulated in 70% of primary GC and it metastasis, and 60% of pancreatic tumors. In addition, CLDN 18.2 activation is seen in esophageal cancer, making it a promising target for the potential CAR-T treatment of solid tumors of the digestive system.[101,102] A preclinical study of CLDN18.2 CAR-T in CDX or PDX models revealed the inhibition to tumors, and partially or even completely clear the tumor tissues.[103] In 2019, Jiang and colleagues reported updated data of CLDN12.8 CAR-T therapy in 12 metastatic adenocarcinomas (7 GC and 5 pancreatic cancer) and detected no serious adverse events, treatment related death, or severe neurotoxicity.[103] Among 11 evaluable patients, 1 was in complete remission, 3 were in PR, 5 were stable, and 2 were in progression; the overall objective remission rate was 33.3%. In 2022, Lin's team reported research on the efficacy and safety of CLDN18.2 CAR-T in digestive system tumors, which is the first systemic cellular immunotherapy targeting CLDN18.2. The interim results of a phase 1 clinical study showed that the objective response rate and DCR of all patients (37 patients with advanced gastrointestinal tumors) were 48.6% (95% CI: 31.9–65.6) and 73.0% (95% CI: 55.9–86.2), respectively; the 6-month response rate was 44.8%.[104] The above studies show that in the treatment of solid tumors in the digestive tract, the safety of CLDN18.2 CAR-T cell therapy is generally controllable, and the side effects are well tolerated. Follow-up CAR-T cell therapy has high reference value.

DIFFICULTIES ENCOUNTERED WITH CAR-T THERAPY FOR GASTROINTESTINAL CANCERS

Compared with various hematological malignancies, CAR-T cell immunotherapy of solid tumors, especially solid tumors of the digestive system, still faces many obstacles. In the use of CAR-T cell immunotherapy for solid tumors of the digestive system, we need to consider the following factors: (1) lack of appropriate antigenic targets and antigenic heterogeneity, (2) insufficient CAR-T cell transport and tumor infiltration efficiency, (3) CAR-T cell-mediated toxicity, and (4) immunosuppressive TMEs.

Lack of appropriate antigenic targets

Tumor antigen refers to a collection of proteins and peptide molecules that are recently synthesized and highly expressed during the malignant transformation of cells.[9] On the basis of the association between tumor antigens and malignancies, tumor antigens are categorized primarily as tumor-specific antigen (TSA) or TAAs.[105] TAAs are antigens that are not exclusive to tumor cells and exhibit minimal expression on normal tissue cells.[106,107] TSA, which is alternatively referred to as neoantigen, denotes an antigen that exclusively manifests on tumor cells subsequent to protein sequence alterations precipitated by gene fusion, non-synonymous point mutations, codon insertion/deletion, frameshift mutations, or frameshift mutations.[106] TAA is less specific in comparison. The optimal CAR-Target ought to exhibit remarkable homogeneity of expression across the entire tumor, across multiple patients, while displaying negligible or absent expression in critical normal tissues. Solid tumors are, unfortunately, highly heterogeneous, which complicates the acquisition of optimal TSA for CAR-T cell therapy.[24,108]

Conversely, diminished expression levels of the CAR-T target antigen are a significant determinant in the inefficacy of CAR-T therapy and the recurrence of solid malignancies. In their research, Anurathapan et al. discovered that the level of tumor cell eradication facilitated by CAR-T cells is positively correlated with the quantity of tumor cells expressing target antigens.[109] Furthermore, the efficacy of CAR-T is dependent on the intensity of antigen expression; tumor cells lacking antigen expression are capable of eluding CAR-mediated eradication, which ultimately results in tumor recurrence.[110] Furthermore, the administration of targeted CAR-T cells via infusion for the treatment of solid tumors may result in the suppression of target antigen expression, thereby impeding the ability of CAR-T cells to identify tumor cells and ultimately causing treatment inefficacy.[111] According to the findings of Fry et al., the median remission period for patients with B-ALL who have received CAR-T cell therapy that targets CD22 is 6 months; recurrence is correlated with a reduction in CD22 site density.[112] The efficacy of CAR-T cells against solid tumors is significantly restricted due to the impact that both the density of tumor cell antigen expression and the design of tumor-specific antibodies have on the antitumor response.[18]

Insufficient CAR-T cell transport and tumor infiltration efficiency

CAR-T cell therapy for malignant solid tumors operates on the fundamental principle that to bind with antigens on the surface of tumor

cells, the cell must be transported from the circulation to the site of the tumor.[106] To be more precise, CAR-T cells traverse the vascular system and migrate toward tumor tissue via the following mechanisms: (1) CAR-T cell edge aggregation and wall attachment are facilitated by selectin ligand interactions; (2) chemokines present in the target tissue stimulate chemokine receptors on CAR-T cells, which in turn induce the expression of integrin, and this adheres firmly to vascular endothelial cells[113]; and (3) CAR-T cell migration across endothelium is facilitated by the synergistic effects of intercellular cell adhesion molecules, vascular cell adhesion molecules, and chemokines.[114]

CAR-T cells that are designed to migrate, transport, and infiltrate solid tumors must surmount the aforementioned physical obstacles to locate cancer cells for interaction; this demonstrates the challenging nature of their transportation mechanism.[115] Concurrently, cancer cells will develop resistance to CAR-T cells to impede their migration, infiltration, and exudation. The attachment and migration of CAR-T cells can be hindered by an aberrant vascular system that is characterized by aberrant expression of adhesion molecules.[113,114] Additionally, the expression of several chemokines, including CCL5, CXCL9, and CXCL10, which rely on the migration, infiltration, and transportation of CAR-T cells to tumor tissue, is either downregulated or absent.[116–118] Physically, the dense extracellular matrix of the tumor prevents CAR-T cells from penetrating the site. T cell function is directly inhibited by ligands that inhibit the expression of immune checkpoints in tumor cells, such as programmed death-ligand 1/ligand 2 (PD-L1/L2).[119–122] Hence, alongside the dearth of TSAs, the optimal method of delivering CAR-T cells to tumor tissue constitutes an additional formidable obstacle.[120]

CAR-T cell-mediated toxicity

Although CAR-T has become a very promising method for the treatment of solid tumors of the digestive tract, it cannot become a first-line treatment due to certain toxic and side effects.[123] Antigen receptors on the surface of CAR-T cell target tumor cells by recognizing specific antigens on the surface of tumor cells. However, most of the antigens that highly express on tumor cells also express on other non-tumor cells, which makes it a dilemma to kill tumor cells while avoiding harm to normal tissue cells.[123,124] CAR-T cells may damage normal tissues and organs, and this is the so-called off-target effect. Some adverse effects have been reported with current CAR-T in clinical trials for the treatment of solid tumors of the digestive system.[125] For example, anti-ERBB2 CAR-T therapy was reported to cause respiratory distress in a colon cancer patient after 15 min and eventual death after 5 days. At the same time, the off-target effect of CAR-T cell may lead to normal organ dysfunction, and some could even be life-threatening.[126] As CAR-T cell therapy works *in vivo*, it also causes the release of cytokines and activation of the immune system. Toxic levels of systemic cytokine release and severe immune cell cross-activation may lead to CRS, which is typically manifested by fever, chills, muscle pain, generalized weakness, and ultimately, systemic organ failure.[126–129] Activated CAR-T cells are the major cause of CRS and may lead to a marked increase in the secretion of pro-inflammatory factors by immune cells. CAR-T cell therapy can also lead to immune effector cell-associated neurotoxicity syndrome (ICANS), which is characterized by elevated levels of cerebrospinal fluid cytokines and disruption of the blood-brain barrier.[130–133] Neurotoxicity is characterized by a variety of neurological symptoms, including headache, aphasia, delirium, and even cerebral hemorrhage, seizures, and death. If the incidence and severity of CRS and/or ICANS could be reduced, then CAR-T cell therapy will be more safe and practical clinically.[130]

Immunosuppressive TME

Regretfully, a number of studies have verified that the TME may significantly impede T cell metabolism.[134] Through glycolytic metabolism, tumor cells produce a microenvironment that is marked by hypoxia, elevated lactate, and high concentrations of immunosuppressive metabolites.[134] This greatly reduces the activity and functionality of antitumor immune cells. In addition, the TME contains a large number of stromal cells linked to cancer, such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and cancer-associated fibroblasts (CAFs).[24] Through a variety of processes, such as the release of growth factors, cytokines, and chemokines that stimulate tumor growth, metastasis, and angiogenesis, tumor-associated neutrophils (TANs) and regulatory T cells (Tregs) contribute to the development of tumors.[135] The primary constituents of the TAM are CAFs, which have the ability to release extracellular matrix proteins, trigger neovascularization and inflammation, create an immunosuppressive TME, modify the extracellular matrix, and impede immune cell activity to advance tumor development.[136] A subpopulation of T cells (Tregs) are essential in suppressing immune responses against tumors and are responsible for controlling a number of immune cell functions.[134] Most people agree that TAMs in the TME are primarily of the M2 type, which release tumor necrosis factor α. Interleukin-10 (IL-10) also increases the expression of PD-L1, which inhibits antitumor T cell activity and mediates immunological escape. Based on their activation, cytokine status, and effect on tumor cell development, TANs are categorized as either N1 (antitumor) or N2 (protumor).[137] TANs can be stimulated by transforming growth factor β (TGF-β) to develop into N2-TAN, which then secretes CCL17 to attract Tregs. Conversely, chemokines CCL2, CCL4, CXCL1, and CXCL12, as well as vascular endothelial growth factor, IL-1, and interferon-γ, can promote the amplification of MDSCs. These cells can be drawn to the tumor site by PEG-2 and produce inducible nitric oxide synthase, arginase, IL-10, and TGF-β. NK cells, CD4 T cells, and CD8 T cells are inactivated through the promotion of immunosuppressive checkpoint molecules, recruitment of Tregs, and inhibition of lymphocyte activity.[137,138]

POSSIBLE APPROACHES IN CAR-T TO COMBAT DIGESTIVE SOLID TUMORS

Various approaches have been developed to improve the safety and efficacy of CAR-T cell in the treatment of solid tumors, which are described below.

Choose appropriate patients

Priority number one when considering CAR-T therapy for solid malignancies should be patient selection. At this time, the most rapid

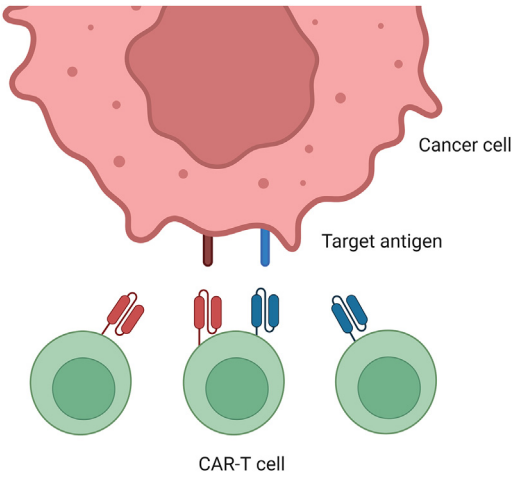

Figure 2. Dual-target CAR-T cell therapy

development of CAR-T for solid tumors in China is phase 2 research, and no commercially viable products have been approved. Data on the products' overall safety and efficacy are scarce, and the duration of use remains brief. In the meantime, certain CAR-T-related adverse effects are hazardous and potentially fatal.[139] Hence, rigorous criteria are applied when selecting patients for clinical research, taking into consideration factors such as disease stage, organ functionality, and physical condition. Patients who are ineligible for standard treatment are the initial criteria.[140] However, in cases where the tumor burden is excessively high or when there are an excessive number of treatment lines, the overall condition is frequently compromised, making CAR-T treatment difficult to tolerate. At this time, enrollment in clinical trials is typically contingent on a physical condition score ranging from 0 to 1 point, with a maximum of 1 point. Organ functions, such as coagulation function, white blood cells, hemoglobin, platelets, liver and kidney function, cardiopulmonary function, and coagulation function, must be restricted to a specific range to enhance the safety profile of CAR-T therapy.[141]

Design multitarget CAR-T cells

The next development in CAR-T cell therapy may begin with targeting more than one antigen due to the heterogeneity and antigen escape of tumors.[142] This will increase the possibility of simultaneously eliminating multiple subclonal populations by targeting multiple TAAs or other factors in the TME. Multitarget CAR-T cells have demonstrated potent antitumor activity, decreased immune escape capability, and enhanced T cell viability in numerous preclinical investigations.[143] In particular, Hegde et al. employed IL-13R and targeted HER2 at the same time for GBM.[76] In animal models, a subset of CAR-T cells demonstrated greater tumor-killing efficacy and less antigen escape.[144] EphA2- and fibroblast activation protein (FAP)-targeting CAR-T cells were employed by Kakarla and Gottschalk to treat lung cancer in the well-established A549 animal model.[145] FAP-targeted CAR-T cell infiltration dramatically slowed the growth of tumors and FAP^+ stromal cells.[146,147] Dual-target CAR-T cell therapy greatly increases antitumor effectiveness and extends animal lifetime as compared to treating either alone[148,149] (Figure 2).

Regulate CAR-T cell apoptosis

The safety and effectiveness of CAR-T cells are correlated with their half-life *in vivo*. A currently active area of research is controlling CAR-T cell death: for instance, when dimeric small molecules like Rimed gel are introduced, the fusion of the apoptosis-promoting protein caspase-9 with the domain of FKBP12 (iCasp9) will promote the dimerization of the FKBP12 of iCasp9 and cause T cell apoptosis[20]; patients taking ganciclovir experienced CAR-T cell apoptosis when the herpes simplex virus thymidine kinase gene was introduced into CAR-T cells; and when the EGFR or CD20 gene was inserted into CAR-T cells, cetuximab or rituximab could, respectively, kill CAR-T cells expressing EGFR or CD20 molecules.[23] Thus, another area of research that merits consideration is the control of CAR-T cell apoptosis in the management of digestive system malignancies. However, using this approach will make it impossible for the body to continue growing CAR-T cells.[111] The body will not have any CAR-T cells with antitumor activities if the tumor relapses.

Combine CAR-T cell therapy with other immune or targeted therapies

With the advent of immune checkpoint inhibitors (ICIs) such as ipilimumab (anti-CTLA-4 antibody),[150] nivolumab, and pembrolizumab (both anti-PD-1 antibodies) in recent years,[119] the treatment landscape for patients with solid tumors has changed. ICIs may reverse CAR-T cell exhaustion and enhance their function. Therefore, a combinatorial immunotherapy strategy combining CAR-T cell therapy with ICIs is a promising treatment for solid tumors. At present, clinical trials of CAR-T cell combined with PD-1 blockade in the treatment of solid tumors are already under way, which have demonstrated significant antitumor effects.[119,120]

TGF-β is one of the key factors in TME that increase tumors immune surveillance. Galunisertib, a small-molecule inhibitor of TGF-β1, can reverse the inhibitory effect of TGF-β1 on the proliferation of $CD8^+$ T cells and the immunosuppressive effect of Tregs, and thus promote the infiltration of T cells into tumor tissues.[151] To boost the activity of CAR-T cells against solid tumors, combining them with a TGF-β1 inhibitor seems to be a good choice. Moreover, the combination of TGF-β1 inhibitors with CD133 CAR-T cells or HER2 CAR-T cells is reported to be effective against solid tumors in animal models.[152] MDSCs and Tregs in the TME have been reported to be inhibited by all-*trans* retinoic acid, vitamin D_3 derivatives, and anti-CD25, OX40, or CCR4 antibodies, respectively.[153] In the future, new strategies to combine the inhibition of MDSC or Treg cells with CAR-T cell therapy may further improve the curative effect.

Enhance CAR-T cells delivery or infiltration

Enhancing the infiltration of CAR-T cells into the TME is a priority to improve the therapeutic effect of digestive solid tumors, and one must choose the proper delivery method. Traditional intravenous administration makes CAR-T therapy less effective, mainly because cells are

difficult to find, infiltrate, and expand in the typical immunosuppressive TME.[154] To overcome the difficulties of such inefficient delivery and systemic toxicity, some researchers have explored the effect of direct regional delivery.[116] The findings suggest that local delivery of CAR-T cells into tumors can efficiently promote CAR-T cell trafficking and break through the physical barrier of TME, improving efficacy in the treatment of solid tumors.[110] A novel method has also been developed in regional delivery. By using injectable polymer-nanoparticle hydrogel storage technology to generate a local inflammatory niche, it could expand and maintain the storage of CAR-T cells and stimulatory cytokines, which has shown significant antitumor effect.[155] Moreover, it can break through the physical barrier caused by tumor-associated fibroblasts and abnormal tumor vascular fibrosis.[122] Studies show that CAR-T cells redirected by FAP can be used to remodel the TME and inhibit tumor growth by breaking the CAF barrier. At the same time, the abnormal vascular structure seriously affects the treatment of CAR-T cells. Targeting tumor blood vessels can promote CAR-T cells to infiltrate the tumor site and improve its therapeutic effect.[156]

In addition to physical barriers, mismatched chemokine/chemokine receptors can also make it difficult for CAR-T cells to enter solid tumors. Therefore, expressing chemokine receptors on CAR-T cells that match and respond to tumor-derived chemokines appears to significantly improve CAR-T cell trafficking and infiltration. Recent studies have shown that integrins αvβ6-CAR-T cells expressing CXCR2 or CAR-T cells overexpressing CXCR1 or CXCR2 can enhance infiltration and significantly improve antitumor efficacy.[157,158]

CONCLUSION

Cancers of the digestive system constitute a significant proportion of human cancers, where they account for almost 50% of all malignancies. Hence, it is imperative to discover efficacious treatment modalities for gastrointestinal neoplasms. Therapeutic application of CAR-T cells that specifically target GPC3, CEA, CD133, and other molecules holds promise for treating cancers in the digestive system.[14] Given that targets other than GPC3 often lack specificity, it is crucial to incorporate additional technologies, such as dual targets, to enhance the safety of CAR-T therapy. Furthermore, tumors affecting the digestive system frequently endure diverse inflammatory alterations, including the progression of liver cancer through stages of hepatitis, cirrhosis, and ultimately, liver cancer.[113] Hence, the tumor immune microenvironment is undeniably intricate. Investigating how to precisely enhance the antitumor effects of CAR-T cells inside the specific immunological milieu is an important area for future study and exploration. Given the challenges in replicating the human TME in animal models, it is imperative to conduct additional human research to identify genuinely efficacious CAR-T cells for antitumor therapy in the future. Overall, as CAR-T cells continue to advance, we have confidence that they will soon be effectively employed in the treatment of digestive system malignancies, leading to significant improvements in patient longevity.

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AUTHOR CONTRIBUTIONS

N.X. and Z.W. were the major contributors to reviewing the literature, writing the manuscript, and creating descriptive figures. J.P. was the major contributor to revising the manuscript and figures. Q.W. and X.X. designed the table and figures and supervised the completion of the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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