Advances in Developing CAR-T Cell Therapy for Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is a common malignant tumor of digestive system. Current traditional treatment options, including surgery, radiotherapy and chemotherapy, are frequently ineffective to fight against CRC due to late diagnosis and high recurrence. Therefore, there is a vital need to develop novel and effective therapeutic agents. In recent years, immunotherapies have become more widely used in cancer therapy. Among these treatments, chimeric antigen receptor T (CAR-T) cell immunotherapy constitutes the most promising approach. CAR-T cells can target and kill tumor cells efficiently so that malignant tumors can be effectively treated. At present, CAR-T therapy has achieved favorable results in hematological cancers, but in solid tumor it is still in its infancy and the research about this area is limited. Nowadays, some achievements have been made in the treatment of CRC with CAR-T cells, but there are also many challenges. This article mainly reviews the research progress of different therapeutic targets of CAR-T in CRC (existing clinical trials / ongoing clinical trials) and challenges.

Keywords

Immunotherapy; CAR-T cell therapy; Colorectal cancer; Clinical trials.

1. INTRODUCTION

CRC is a common malignant tumor of digestive tract. The deaths of CRC in 2018 was 880792 (9.2%), ranking second in the global proportion of cancer deaths. Many patients of CRC are found to be in advanced stage and one-third of patients are under metastasis[Miller, et al., 1]. As a result, the prognosis remains poor. The traditional therapeutic schedule for CRC includes surgery, chemotherapy, and radiotherapy. These methods play an important role in the treatment of CRC, but all of them have limitations. In order to break through the limitations of traditional treatments, it is imperative to look for a treatment plan that may be even more optimal.

In recent years, immunotherapy has become more widely used in cancer therapy. Among them, chimeric antigen receptor T (CAR-T) cell has emerged as a novel arm to target tumor antigens and effectively kill tumors, which brings new hope to patients and doctors. CAR is the core component of CAR-T cell therapy. It lead T cells to recognize tumor cells without recourse to the classical major histocompatibility complex (MHC) presentation[Eshhar, et al., 2], and can identify a wider range of targets than natural T cell antigen receptor (TCR). Modified CAR- T cells can distinguish normal cells and tumor cells that express the same antigen in vivo, even if they are low expression[Caruso, et al., 3]. Furthermore, CAR-T cells also have the potential that they exist in vivo for a long time because T cells can produce memory T cells. The Food and Drug Administration approved Tisagenlecleucel for the treatment of relapsed or refractory B cell precursor ALL of patients younger than or equal to 25 years old. The success of phased CAR-T

therapy has stimulated researchers to continue to explore CAR-T therapy. Its advantages will also be incomparable to traditional cancer therapy methods.

At present, CAR-T therapy has brought considerable benefits to hematological tumors, but there are many difficulties in solid tumors. Noticeable, many researchers are still trying to fight against CRC by CAR-T cells and some progress has been made in these years[Sur, et al., 4]. In this article, we summarized the results of recent clinic trials and then analyses the current problems of CAR-T cell therapy against CRC.

2. CRC-RELATED TARGETS IN CLINICAL TRIALS

2.1. CEA specific CAR-T cells

Carcinoembryonic antigen (CEA) is a sensitive biomarker of gastrointestinal tumors, which is widely expressed in colorectal cancer tissues and sera[Martin, et al., 5]. In addition to its low level expression in gastrointestinal tract and endoderm cells, CEA cannot be detected in most normal adult tissues[Leusch, et al., 6]. Therefore, CEA is presumed to be a powerful target for colorectal cancer by CAR-T cell therapy.

In 2017, Chengcheng et al.[C. Zhang, et al., 7] conducted CEA-specific CAR-T cell phase I clinical trials for relapsed and refractory CEA+ CRC liver-lung metastasis patients. The clinical study included 10 patients, 8 patients had liver metastasis and 5 patients had lung metastasis. During the experiment, 5 incremental dose levels (1×105 to 1×108/CAR+/kg cells) were used for intravenous drip of CAR-T cells respectively. Two of the patients who received two different doses of infusion (DL4 for the first time and DL5 for the second time) were accompanied by liver, lung and lymph metastasis. After the second infusion, the serum CEA levels of both patients were significantly reduced, especially after long-term monitoring, and remained at a relatively low level. It is worth mentioning that no patient had serious adverse events associated with CAR-T therapy and all of them showed good tolerance. One patient found obvious reduction of liver lesions through MRI examination before and four weeks after the first infusion, and another patient found obvious reduction of lung tumor activity through PET/CT examination after the second infusion, which confirmed the effectiveness of CAR-T cells in treating solid tumors and promoted the development of CAR-T therapy in treating solid tumors.

At present, clinical tests are being conducted to verify the safety and effectiveness of CEA+CAR-T cells in tumor patients. At the same time, it is expected to explore the safe dose range and optimal dose for CAR-T cells infusion, and to provide an appropriate infusion plan so as to lay a foundation for subsequent clinical tests or clinical applications (NCT02349724, NCT04348643, NCT03682744, NCT02416466)

2.2. TAG-72 specific CAR-T cells

Tumor associated antigen 72 (TAG-72) is overexpressed in most human adenocarcinoma epithelial cells, and its expression is mainly in tumor cells[Thor, et al., 8].The expression of TAG-72 antigen is usually not detected in most normal tissues and organs of adults[Povoski, et al., 9]. Previous clinical trials have verified the anti-CRC effect of humanized anti-TAG-72 single-chain variable fragment for CAR-T cell therapy and the outcomes provide hope for TAG-72 targeted CAR-T cells to treat patients with CRC.

Kristen M et al[Hege, et al., 10]. investigated the effectiveness and safety of a first generation of TAG-72 specific CAR-T cell for metastatic CRC patients in two open phase I clinical trials. Expression of TAG-72 was detected in 5% tumor cells by immunohistochemistry. All the patients involved in the C-9701 test had received surgical treatment and chemotherapy, and 3 of them received radiotherapy. In the C-9701 test, 10 patients were administered by intravenous infusion, while in the C-9702 test, 6 patients were administered by direct hepatic artery infusion. In test C-9701, 5 patients in part A (108, 109, 1010, 1010 total T cells) and 2 patients in part B

(3×1010 total T cells) completed all planned doses once every two weeks. In test C-9702, 4 patients received all planned doses (109, 1010, 1010, 1010 total T cells). Persistence of CAR-T cells can be observed in blood, but most of them are short-term (≤14 weeks). One patient could still detect CAR-T cells 48 weeks after treatment. In one patient's tumor biopsy, the metastasis of CAR-T cells to tumor tissue was observed, but it was only concentrated around the large tumor mass and was not detected inside the tumor. There are also some patients whose liver metastasis can be detected. The progress of the disease was observed according to radiological evidence. Thus, an anti-CAR immune response was considered.

The persistence of CAR-T cells indicates that the costimulatory domains of CD3 and CD28 play a certain role in the integration of CAR design. In the strategy of CAR-T cell therapy for advanced solid tumors, injection of targets to enhance the homing and tumor penetration of CAR-T cells may make the therapeutic effect more significant.

2.3. CD133 Specific CAR-T Cells

CD133 is a ubiquitous protein expressed on tumor stem cell (CSC). It has been confirmed to be present in CSC markers of CRC[Boman and Wicha, 11, Ferrandina, et al., 12].The cancer cell population with high expression of CD133 not only has the ability of self-renewal, proliferation, and differentiation into different cells [Hemmati, et al., 13, Singh, et al., 14, Singh, et al., 15], but also has a high degree of metastasis, and it will be resistant to treatment by chemical drugs, radiation, etc [Liou, 16].Tumor metastasis and drug resistance are the main causes of death in CRC patients, and CD133 is crucial in these two aspects, so targeting CD133 is an effective method for treating patients with CRC and metastasis.

In a recently reported phase I clinical trial of CAR-T cell therapy targeting CD133(CART-133) [Y. Wang, et al., 17], there were 2 patients with CRC. Through the dose increasing scheme of CART-133 cells, the acceptable dose of CART-133 cells was determined to be 0.5-2 GB 106/kg. Two patients with CRC were treated to observe the efficacy, and the results showed that both CRC patients had stable disease (SD) and no neoplastic cells were detected. In patients with CRC, autoimmune reaction occurred in the normal tissues expressing target antigen during CART-133 cell therapy, and hematopoietic system toxicity was found to recover within 1 week. The results demonstrate the feasibility and effectiveness of CART-133 in the treatment of refractory / recurrent advanced CRC.

At the same time, there are other clinical trials (NCT02541370) on the treatment of relapsed and/or chemotherapy-refractory advanced malignant tumors through CART-133, to determine the safety and feasibility of CART-133 cell immunotherapy, and then promote the clinical application of CART-133 cell immunotherapy in the treatment of CRC.

3. OTHER ONGOING CLINICAL TRIALS OF CRC

3.1. NKG2D Specific CAR-T Cells

NKG2D, which plays an important role in immune response, activates effector cells by binding to ligands and produces corresponding immune response. There are many types of ligands that bind to NKG2D. The expression of NKG2D ligand is strictly regulated to prevent autoimmune tissue damage[Dhar and Wu, 18]. Because MIC is the most expressed NKG2D ligand in human tumors, there are many studies on MIC. Some studies [Wu, et al., 19, Liu, et al., 20, Groh, et al., 21]have shown that targeting pathways related to sMIC release or immunosuppression may be a feasible way to treat cancer.

The safety and efficacy of NKG2D CAR-T cell targeted therapy for NKG2D ligand-positive tumors are currently being explored and evaluated. Different studies have demonstrated that NKG2D CAR-T effectively controls the growth of multiple tumor types in mouse models,

including multiple myeloma[Barber, et al., 22], ovarian cancer[Barber, et al., 23], and osteosarcoma[Fernández, et al., 24].

There are two ongoing phase I studies of a similar design for NKG2D CAR-T cells to evaluate the safety, efficacy, and tolerance of metastatic CRC treated with standard chemotherapy combined with CYAD-01/CYAD-101 (both NKG2D CAR-T cell therapy). The preliminary data of Allo-SHRINK study did not reveal evidence of graft-versus-host disease (GvHD) and the host response to allogeneic CYAD-101 cells was controllable; both autologous CYAD-01 and allogeneic CYAD-101 combined with FOLFOX showed good safety; the preliminary data showed that both of them had dose-dependent effects on cell dynamics. Compared with the same dose of autologous CYAD-01 products, allogeneic CYAD-101 products provide a higher level of relative transplantation. To sum up, NKG2D CAR-T cells have effective killing effect on CRC tumor cells. Similarly, HER-2 is also a developing target for CRC therapy which is expressed in normal epithelial cells and overexpressed in colorectal tumors, and T cells targeting HER-2 cause fatal damage to the normal lungs of patients.

3.2. EpCAM Specific CAR-T Cells

In 1979, epithelial cell adhesion molecule (EpCAM) was first found to be a tumor antigen associated with colon cancer[Herlyn, et al., 25], and subsequently found to be overexpressed to varying degrees in most human tumors[Went, et al., 26].Previous studies have shown that EpCAM is related to cell movement, proliferation, carcinogenesis, metastasis and poor prognosis [Baeuerle and Gires, 27, Liao, et al., 28, Patriarca, et al., 29]. EpCAM has been identified as a biomarker of circulating tumor cells (CTC) and CSC. [Schulze, et al., 30, Gires, et al., 31, O'Brien, et al., 32], CTCs are potential precursor cells for tumor metastasis [Joosse, et al., 33], CSCs initiate and maintain tumor growth ,Therefore, specific targeting EpCAM makes it possible to isolate CTCs and tumor cells with CSC characteristics, which also makes EpCAM a potential target for tumor immunotherapy[B. L. Zhang, et al., 34]. Through in vivo and in vitro studies on human colon cancer mouse model[B. L. Zhang, Li, 34], it was found that EpCAM specific CAR-T cells have specific and efficient targeting to EpCAM positive tumor cells. In addition, EpCAM specific CAR-T cell therapy did not cause significant systemic toxicity in mice, indicating the safety of EpCAMCAR-T cells in xenogeneic mice. The results have not been clinically verified. The clinical study(NCT03013712) of CAR-T cells targeting EpCAM positive tumors is still under way.
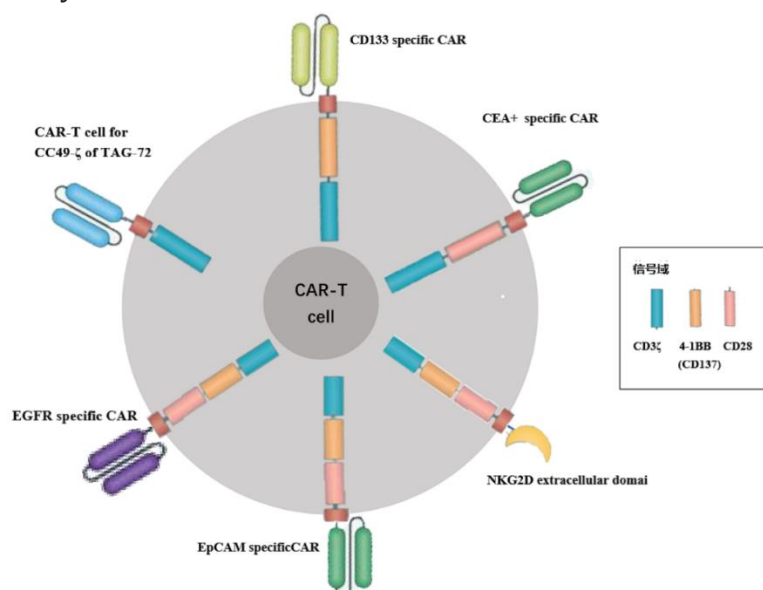

Figure 1. CAR-T structure diagrams for the treatment of CRC

TABLE 1. Ongoing clinical trials on CAR-T cell therapy for colorectal cancer

Target	Pathology	Patient Number	Administration	Other features
EGFR-IL12	Metastatic CRC	20	Infusion	KPS scores over 80; Expected survival > 3 months; EGFR+ in patients with metastatic colorectal cancer
EGFR	EGFR+ CRC	20	Infusion	KPS scores over 80; Expected survival >3 months; Metastatic colorectal cancer patients with EGFR+
CEA	Metastasis of CRC	20	Vascular interventional therapy or intratumoral injection	Expected survival >6 months; Histological examination of CEA+ tumors;
MUC1	MUC1+CRC	20	Infusion	Patients who have no therapeutic treatment available and whose prognosis is limited and this treatment is available
NKR-2	Unresectable liver metastatic CRC	18	Hepatic artery administration	Adenocarcinoma of colon or rectum; Incurable liver metastasis was treated by surgical resection or local ablation
NKR-2	Potentially resectable liver Metastasis of CRC	36	Infusion	Receive first-line metastatic chemotherapy with FOLFOX as a neoadjuvant
NKG2D	CRC	36	Infusion	Histologically confirmed metastatic adenocarcinoma of the colon or rectum
CEA	CRC	75	Infusion	KPS scores over 60; Expected survival time>3 months
CEA	CRC	40	Intravenous administration	KPS score of not less than 60 Expected survival time>12weeks Received first-line standard treatment but failed
CEA	Peritoneal metastasis or malignant ascites metastasis of CRC	18	Intraperitoneal infusion	Expected survival time>12weeks and ECOG score ≤2; CEA + canceration or malignant ascites
C -met	CRC	73	Infusion	Estimated survival of ≥ 12 weeks, but ≤ 2 years ECOG score ≤2
HER2	CRC	39	Intratumoral injection	Not suitable for radical surgery, radiotherapy, systematic therapy or any combination of the above; Have at least one tumor site appropriate for intratumoral injection.
CD133	CRC	20	Infusion	ECOG performance status: 0-2; Chemotherapy refractory or relapsed CD133-positive cancer (include CRC)
NKG2DL	CRC	10	Intravenous infusion	ECOG performance status: 0/1/2
CEA	Liver Metastasis of various Gastrointestinal tumors	5	Hepatic artery infusion	Patients with histologically confirmed diagnosed CEA+ adenocarcinoma and liver metastasis; Life expectancy>4 months;

	including CRC in CEA+			Failed at least one line of standard systemic chemotherapy and has unresectable disease.
CEA	CEA+Adeno carcinoma with liver Metastasis of Gastrointest inal tumors including CRC	8	Arterial infusion	Patients with histologically confirmed diagnosed CEA+ adenocarcinoma and liver metastasis; Life expectancy> 4 months; Failed at least one line of standard systemic chemotherapy and has unresectable disease.

4. CHALLENGES

The success of CAR-T cell therapy in the treatment of hematological tumors has inspired people to extend this technique to the treatment of solid tumors. The above is a summary of the relevant targets and clinical trials about the use of CAR-T cell immunotherapy in CRC so far (TABLE , Figure 1). All the clinical trials are registered with the clinical trials website [http://www.clinicaltrials.gov/ct]. A few of them have been achieved, but others are also faced with great challenges.

4.1. The Influence of Tumor Microenvironment

Solid tumors such as CRC usually form solid masses in some organs in the early stage of carcinogenesis, which not only causes many obstacles for the recruitment of immune cells to the affected areas, but also leads to the aggregation of a variety of immunosuppressive cells and molecules[Kasakovski, et al., 35], forming a special tumor microenvironment (TME). Generally speaking, TME can be divided into non-immune microenvironment dominated by fibroblasts and extracellular matrix and immune microenvironment dominated by immune cells.

4.1.1 The Influence of Tumor Microenvironment

The physical barrier formed by tumor blood vessels and extracellular matrix prevents CAR-T cells from effectively infiltrating into the tumor area[Tahmasebi, et al., 36]. The formation and growth of tumor blood vessels require the interaction of vascular endothelial growth factor receptor 2 (VEGFR2) and vascular endothelial growth factor (VEGF2)[Chinnasamy, et al., 37]. Fibroblast activating protein (FAP) is the surface marker of tumor-associated stromal cell (CASCs) [L. C. Wang, et al., 38]. CAR-T cells targeting VEGFR2/ FAP can be selected to break down the physical barrier of CRC, improving the penetration and anti-tumor activity intravenously.

4.1.2 The Effect of Immunosuppressive Tumor Microenvironment

Tumor cells and supporting stromal cells of colorectal cancer inhibit the anti-tumor activity of CAR-T cells by displaying and secreting a series of immunosuppressive signals, such as cytokines such as IL-6, IL-10, TGF-β, regulatory T cells (Tregs) and myelogenic suppressor cells (MDSCs)[Zhao and Cao, 39]. Neutralization/blocking antibodies against immunosuppressive signals can be used to weaken or even reverse immunosuppressive microenvironment and enhance the immune effect of CAR-T cells on tumor[Scott, et al., 40].

Colorectal cancer cells activate immune checkpoint receptors (PD1, CTLA4) [Eisenberg, et al., 41, Labanieh, et al., 42], on CAR-T cells through the tumor microenvironment to transmit immunosuppressive signals, which make CAR-T cells enter a state of failure, tolerance or dysfunction[Speiser, et al., 43, Davoodzadeh Gholami, et al., 44]. Non-specific PD-1 and CTLA-4 antibodies are used to bind to CAR-T cell receptors to prevent tumor cells from activating T cell immune checkpoint receptors, thus improving the anti-tumor activity of CAR-T cells[Scott, Duffy, 40, Kosti, et al., 45],as shown in Figure 2.

Chemokines (such as CXCL12) secreted by CRC cannot recognize the chemokine receptors on the surface of normal T cells, preventing T cells from migrating to tumor[Feig, et al., 46] and inhibiting the killing effect of T cells on tumor cells. It can be considered to modify the structure of CAR-T cells according to the chemokine characteristics of CRC to express receptors that can match the characteristics of tumor chemokine, so as to increase the proportion of CAR-T cells homing to CRC and enhance the anti-tumor efficacy of CAR-T cells[Yong, et al., 47].
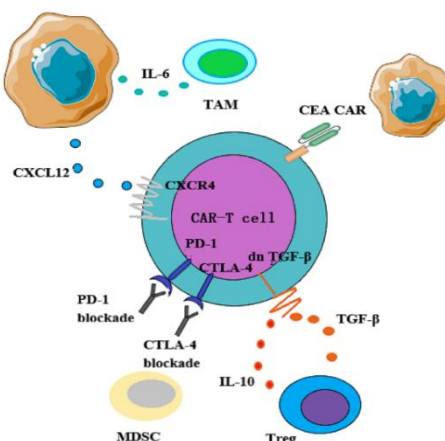

Figure 2. The effect of immunosuppressive tumor microenvironment on CAR-T cells

4.2. Antigen Targeting

In the treatment of CAR-T cells, the ideal target antigen is tumor-specific. However, the target antigens found in CRC are not specific, and there are still varying degrees of expression in normal tissues and cells[Z. Wang and Han, 48]. CAR-T cell therapy in patients with CRC may cause immune response in normal tissue cells, resulting in "on-target, out-of-tumor" effect, which is the most common side effect in clinical application of CAR-T cells. In order to improve the recognition rate of CAR-T cells, reducing toxic reactions and avoiding immune escape, a multi-target strategy was proposed. Some studies have shown[Kloss, et al., 49] that T cells transduced by CAR co-activated by two antigens only destroy the tumors that express both antigens, but have no effect on the cells that express only one of the antigens. This method can avoid some side effects of CAR-T cell therapy, and promoting the application of treatment. When serious side effects occur, remedial measures can be taken to selectively activate suicide or elimination genes added to CAR-T cells to consume CAR-T cells[Barrett, et al., 50], but the corresponding effect of CAR-T cells is also lost. In addition, the recognition ability of CAR T cells can also be improved by increasing antigen density. For example, IL-22 secreted by CAR-MUC1-IL22T cells enhances the recognition and cytotoxicity of head and neck squamous cell carcinoma[Mei, et al., 51]. The result provides another experimental way to improve the recognition of tumor associated antigens by CAR-T cells of CRC.

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