

Development and Clinical Application of CAR-T Therapy

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Abstract. The problem of cancer is becoming more and more serious. As of 2021, the global of cancer patients has reached 14 million. Now how to treat cancer has become one of the key research topics. To treat cancer, people have found many cure methods, like salvage chemotherapy, radiotherapy, cytotoxic chemotherapy, and so on. But these therapies can only delay the patient's life. They cannot cure cancer. People want to find a therapy to completely empty the cancer cells. Until 1989, scientists have found a way to engineer T-cell called Chimeric antigen receptor T cell (CAR-T) to attack cancer cells, CAR-T therapy now has four generations. Good results have been achieved in the treatment of B-cell malignant lymphoma. However, CAR-T treatment in the area of solid tumors now still has many challenges. Therefore, the topic of this article is based on this structure of CAR-T cells, The development of CAR-T cell therapies and clinical application of CAR-T to reveal advantages and disadvantages of CAR-T treatment in cancer.

Keywords: CAR-T, Tumor, Clinical Therapy.

1. Introduction

Chimeric antigen receptor T-cell (CAR-T) treatment is a way that T-cells are created by editing genes that bind to antigens on the surface of cancer cells. To use the technology, T-cells can bind to cancer cells more precisely. The history of CAR-T therapy now has gone Through four generations. Each generation of improvements aims to reduce antigen escape, increased CAR-T cell movement, enhanced invasion of tumor cells, and adaptation to immunosuppressive microenvironment, and reduce the damage of CAR-T cells to human body [1]. Today CAR-T therapy against B cell malignancies resulted has become an unprecedented success. In comparison with traditional cancer therapy like Chemotherapy and radiation therapy CAR-T therapy is better at removing cancer and more precise targeting of cancer cells to increase the patient's life span even cure cancer. However, because of the invasiveness of solid tumors, the complexity of solid tumor microenvironment and heterogeneity of solid tumors. CAR-T targeting solid tumors still present significant challenges. This article probe into the structure of CAR-T cells, the development of CAR-T cell therapies, and the clinical application of CAR-T to Understand the advantages and challenges of CAR-T treatment in cancer therapy.

2. Structure of CAR-T cells

CAR-T cells are functionally divided into three functional regions (Figure 1) [2]. One of these, the extracellular structural domain, consists of a single variable chain fragment (scFv) responsible for recognizing and binding monoclonal antibodies to antigens and a hinge region that plays a linking role. The intracellular structural domain consists of a costimulatory domain and a signaling domain. The transmembrane structural domain links the intracellular structural domain to the extracellular structural domain.

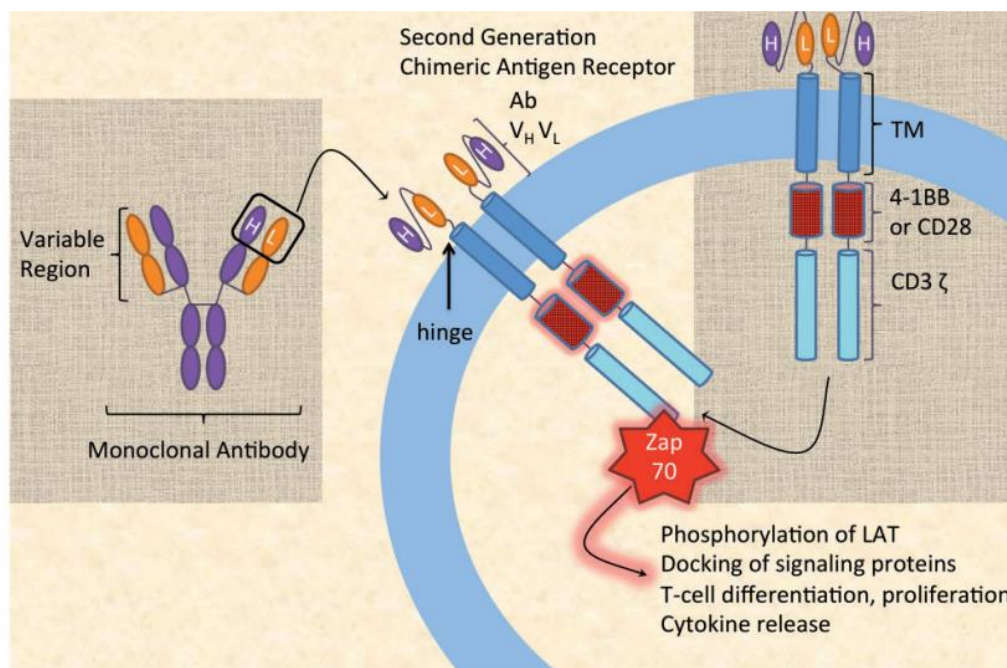


Figure 1. Structure of second-generation CAR-T cells. The monoclonal antibody's terminal variable region's heavy and light chains are reorganized into a single chain that connects the transmembrane structural domain (TM).

The hinge region is extracellular, linking the extracellular region to the transmembrane structural domain, and is used to increase the conformational range of the antigen-binding domain. The T-cell-specific co-stimulatory molecule 4-1BB or CD28 is found in an activation domain. The T-cell receptor CD3 ζ also possesses an intracellular signaling domain [2].

The single chain variable fragment (scFv), the primary element of the antigen recognition domain, is the mechanism through which tumor-associated antigens specifically attach to the CAR (TAAs) [3]. The monoclonal antibody's heavy and light chains are connected by a polypeptide. SCFV allows CAR-T cells to deliver MHC antigens to identify and integrate target antigens. SCFV prevents cancer cell escape by regulating MHC molecules and allows CAR-T cells to recognize non-peptide antigens.

The hinge region connects scFv to the transmembrane structural domain. A large portion of the hinge region in car cells is generated from the hinge CD8 or CD28 extracellular region of IgG. The target cell antigen epitope's position and the level of exposure both influence how long the hinge region is. By adjusting the length of the hinge region, CAR- t cells can be kept at the most suitable distance from the target cells. The distance between CAR-T cells and the target tumor cells affects how well CAR cells signal.

The transmembrane structural domain (TM structural domain) links the extracellular structural domain of CAR to the intracellular signaling structural domain. Also, it plays a role in anchoring the receptor to the T cell membrane. The CD3 ζ transmembrane structural domain can be used to enhance the activity of CAR-T cells. [1]. However, it is gradually being abandoned as it does not require binding to endogenous TCRs to activate T cells highly. In addition, structural domains derived from t-cell molecules are also included in the commonly used transmembrane structural domains [4]. Most clinical studies presently use CD28 or CD8, which can improve the expression of CARs on cell surfaces.

Typically, CD27, CD28, OX40, 4-1BB, inducible co-stimulatory molecules with ICOS and CD278, and glucocorticoid-induced tumor necrosis factor receptor-related protein are the sources of co-stimulatory structural domains (GITR) [5]. The most widely adopted are CD28, OX40, and 4-1BB molecules. The costimulatory domain enables dual activation of costimulatory molecules and intracellular signaling, resulting in sustained T cell proliferation and release of cytokines to enhance the anti-tumor capacity of T cells. Typically, CD28 provides early secondary signaling and promotes the secretion of high levels of IL-2 [4].

The signaling domain is a TCR CD3 ζ chain containing immune receptor tyrosine activation motifs (ITAMs) or an immunoglobulin Fc receptor Fc ϵ RI γ chain [3]. The signal transduction structural domain plays a crucial role in performing T cell signaling functions, triggering antigen binding by regulating the downstream signaling cascade T cells activate [6].

3. Development of CAR-T therapies

3.1. First generation

The first generation of CAR-T cells invented by Gross's group in 1989 [7]. Its main structural feature was a CD3 ζ - chain (Figure 2). This Single chain antibody was important to transmit the pathways from endogenous T -cell receptor (TCR). Never less, a substance secreted by a tumor can reduce the response of CAR-T to tumors. This modified T cells could not persist in patients, and the clinical benefit was limited. In the treatment of cancer, people needed to inject IL-2 to activate T-cells to kill the cancer [1, 8].

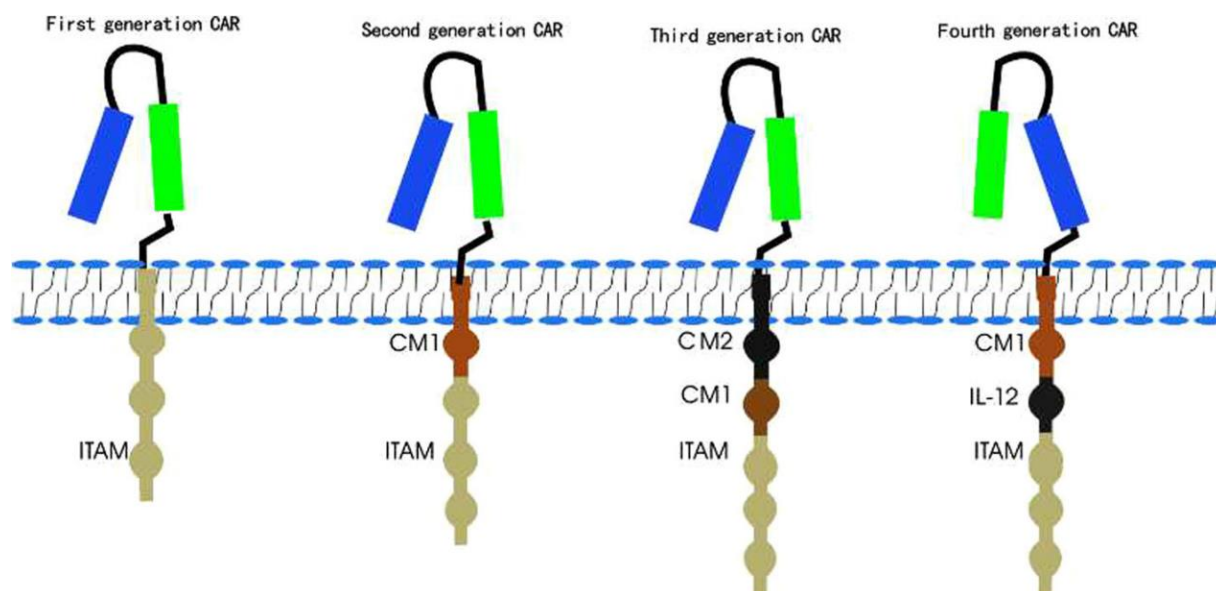


Figure 2. Evolutionary engineering of CAR.

First generation CAR is a combination of single chain antibody and transmembrane domain. The second-generation CAR adds co-stimulation domain to the first generation. Third generation CAR adds multiple co-stimulation domains in hopes of enhancing the effect. Fourth generation CAR adds IL-2 to activate T lymphocytes [1].

3.2. Second generation

Because the first generation could not persist in tumor microenvironment. The second-generation CAR-T appeared. The main feature of this generation was a dual signal. On the basis of the first generation inserted a segment of co-stimulatory signal like CD28 was to promoting IL-2 secretion enhances T cell activation and reduces apoptosis [1]. According to the clinical experiment confirmed that after adding a co-stimulatory signal, CAR-T cells in treatment B cell lymphoma were much better than the last [9]. However, some co-stimulatory signals can cause early failure and may not have the desired effect on the tumor [10, 11].

3.3. Third generation

The third generation of CAR-T cells differs from the second generation because the third generation added multiple signaling fields like CD3 ζ -CD28-OX40 / CD3 ζ -CD28-41BB [1]. The aim is to increase the potency of the drug by increasing the production and killing of cytokines [12]. But actually, according to the clinical experiment confirmed is that the treatment in lymphoma and colon

cancer, the third generation of CAR-T therapy is not much better than Previous generation CAR-T cells [12]. The main reason may be the small number of experimental samples. As a result, in order to maximize the effects of cytokine production and killing ability enhancement, it is also necessary to investigate the effectiveness and safety of these treatments [1].

3.4. Fourth generation

The fourth generation is called CAR-T redirecting for Cytokine activated killing (TRUCKs). The most special framework of this fourth generation is that the fourth generation was supplemented with IL-12 on this basis of the second generation structure. IL-12 might activate T lymphocytes to destroy cancer cells that lack an antigen in certain lesions. It is a valuable way in the research shaping the tumor environment. What is more, TRUCK T cells can also treat diseases caused by viruses, autoimmune diseases [13].

4. Application

4.1. Leukemia

Blood cancer, also known as leukemia, is one of the significant diseases targeted by CAR-T therapy. In treating leukemia, CD19 target is the target choice of CAR-T treatment, which is widely used in the clinic. This is because there are many molecular markers of B cells, and CD19 is one of the most important ones. Compared with other B cell surface molecules, CD19 is very common in B cells and malignant B cells [14]. So far, of five CAR-T methods for clinical use in treating cancer issued by the U.S. Food and Drug Administration (FDA), four types target to antigen CD19 [15]. In recent years, researchers worldwide have continued to explore CD20, CD22, CD70, and other targets, resulting in different therapeutic effects.

In terms of CAR-T treatment of acute lymphoblastic leukemia (ALL), Tisagenlecleucel became a second-generation product modified on the basis of the first generation. Its cell surface is activated (CD3 ζ) and provoked signal (4-1BB) [16]. It is approved to aiming to CD19, and is a treating way aimed to cure children and youths who have acute lymphoblastic leukemia (ALL) which is relapsed or refractory [17]. For this treatment, the University of Pennsylvania and the Philadelphia Children's Hospital researched a single-center Phase 1-2a clinical study investigating 60 children and young adults who have relapsed or refractory B-cell ALL. The results showed 93% of customers are completely relieved. At the same time, the long-term disease control of Tisagenlecleucel lasted for four years. On this basis, the researchers started the second phase key multi-site study of Tisagenlecleucel. The results showed that among 75 patients who received Tisagenlecleucel infusion, event-free survival and overall survival at 6 months were 73% (60 to 82) and 90% (81 to 95), respectively. At 12 months, they were 50% (35 to 64) and 76% (63 to 86), respectively. All data confidence intervals [CI] are taken as 95% [18].

At the same time, researchers are also beginning to explore whether CAR-T cell treatment has a significant effect in relapsed or high-risk chronic lymphocytic leukemia (CLL), mainly CD19 CAR-T cell treatment. Some clinical investigation shows that although the expansion and proliferation reaction ability of cells in patients is not ideal, they still have a good effect on the immune deficiency of patients who suffer from chronic lymphoblastic leukemia [14]. At one shot, experimenters transfused engineered T cells into patients who have refractory chronic lymphoblastic leukemia (CLL), and a series of adverse reactions occurred. Data collection on patients on day fourteen after the end of the first injection, this patient presented with tremors and low-grade fever, which were associated with grade 2 fatigue. In the following about five days, shivering worsened, and this patient's temperature to 39.2 °C, accompanied by chills, sweating, anorexia, nausea and diarrhea, and the patient was found having tumor lysis syndrome 22 days after receiving treatment [19].

4.2. Lymphatic cancer

In addition to leukemia, lymphatic cancer is also one of the leading clinical applications of CAR-T treating way. Human lymphoma includes Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL). Besides, NHL has lymphoid malignant tumor cells in different types, which are caused by cloning and amplifying B, T, and natural killer (NK) cells of varying degrees of differentiation. Hematopoietic markers are abnormally expressed in HL malignant Reed Sternberg (RS) cells [20].

CD19, as a critical target, is often used in CAR-T therapy for lymphatic cancer. Recently, a researcher performed CAR-T immunotherapy on a patient who suffered from advanced follicular lymphoma (FL, a kind of NHL). They first gave a preparatory chemotherapy scheme and then used autologous T cells genetically engineered to express specific receptors. This receptor recognizes the CD19 antigen on the surface of B cells. These results showed that the patient's lymphoma experienced significant regression. After infusing CD19 T cells, the researchers observed the patient's bone marrow and removed qualified B cell precursors from it. The patient suffered an acute toxicity which was cytopenia (due to chemotherapy) and 2-day fever with a maximum temperature of 38.5 °C. The patient was discharged 11 days after the second anti-CD19-CAR T cell infusion and returned to full-time work. In addition, after treatment, CT scan showed that the patient's lymphoma had a certain amount of partial remission, lasting for 32 weeks [21].

In addition to CD19, CD20 is also one of the essential targets for NHL treatment. In one clinical trial, patients who have relapsed or refractory inert B-cell lymphoma or mantle cell lymphoma are used autologous T cell treating, and the T cells were genetically changed by CD20 special chimeric T. Seven patients were given T cell transfusions. The first three patients in this study received a limited dilution method to generate T cells, and these reformed T cells were stored at patient's body for 1-3 weeks. However, the following four patients receiving cultured T cells lasted for 5 to 9 weeks, and then 14 days after low-dose subcutaneous interleukin-2 (IL-2) injection. Seven patients in this study were investigated. Two patients maintained the perfect remission, one patient showed certain relief, besides four patients were in steady condition [22].

CD30 is another common target, also belonging to the TNFR superfamily. Many researchers use it as a target in treating Hodgkin's lymphoma. A clinical trial allows patients who have recurrent or refractory Hodgkin's lymphoma to receive pretreatment chemotherapy and then infuse CD30 CAR-T cells. The researchers studied 18 patients. Among these individuals, a significant number of patients have a history of severe treatment or multiple neoplastic lesions. They received injections of an average of 1.56×10^7 CAR-positive T cells per kilogram. The results showed that in 18 patients, 7 patients were under control and 6 patients had stable disease. In the results, it was observed that the curative effect of treatment on different conditions was inconsistent: the treatment effect of lymph nodes was better than that of extra lymph node lesions, while the treatment effect of lung disease seems to be relatively unsatisfactory [23]. Another researcher conducted a clinical trial where 9 individuals received CD30 CAR-T treatment, including 7 individuals who have HL and 2 individuals who have anaplastic large cell lymphoma (ALCL, one of NHL). Six weeks after treating, one patient was complete remission (CR), one patient showed partial remission (PR), four patients were under stable control, and there was no change from 1.5 to 8 months. Three patients continued to develop [24]. Brentuximab vedotin (BV), a CD30-targeting drug, is clinically indicated for treating to adults classic Hodgkin lymphoma (HL). This investigation was aiming to check whether BV is safe and efficacious in treating refractory/relapsed HL patients under 18 years of age [25].

4.3. Glioblastoma

Glioblastoma is a common tumor, which deteriorates in the central nervous system (CNS). The patients who have this dangerous cancer do not fare well in the later stages of their illness, and only 9.8% of patients were alive after five years and show systemic central nervous system symptoms, which will worsen over time, including headache and epilepsy [26]. Although CAR-T treating way in application in solid tumors remains an initial study, experiments show that CAR-T has anti-tumor activity in solid tumors (such as neuroblastoma) [27].

On the first generation as a basis, the researchers carried out the second-generation transformation of CAR-T and clinical trials. Referring research, researchers contrasted two perfusion sites' effect in the efficacy of intracranial CAR-T cells in respondents - tumor cavity resection and ventricular system resection. In this patient, the two pathways were alike in low toxin characteristics, nevertheless they differ in their results to inhibit the expansion of tumors at the far end. Although intracavitary treating seems to avoid certain tumor recurring, researchers have observed the emergence of glioblastoma in remote areas, especially the emergence of new lesions. On the contrary, il 13 bb is injected into the cerebral ventricle ζ — after CAR-T cell treating way, patient's all central nervous system cancer cells were controlled, including spinal tumors, which showed regression. Although the patient had a significant clinical response, the division and copy in CSF's CAR-T cells became limited at the subsequent cycle and 7-day infusion cycle [28].

In general, CAR-T therapy has a particular therapeutic effect in the clinical application of glioblastoma patients, but the research is still in the initial stage. Due to limited data, its safety and effectiveness need further investigation.

4.4. Pancreatic cancer

Pancreatic cancer is a kind of refractory solid tumor of the digestive tract. Its refractory is mainly reflected in the fact that pancreatic cancer cells can establish an immune suppressive tumor microenvironment by mobilizing host immune cells and then escaping the host's immune surveillance. In this situation, CAR-T therapy has also achieved substantial advancements in treating pancreatic cancer, and tumor immunotherapy has become a hotspot in tumor therapy [29-31]. Researchers at the University of Pennsylvania generated T cells by the requirements of the Investigational New Drug (IND) authorized by the FDA for use in a Phase I study (NCT01355965) in patients with malignant pleural mesothelioma (MPM) at the Abramson Cancer Center, PA [32]. Eligible patients received bulk T-cell therapy. These T cells underwent the same processing as those that had been stimulated and bead-fixed anti-CD3 antibodies. These T cells were isolated, amplified and electroporated in vitro using an anti-mesodermal SS1 scFv CAR mRNA construct containing the 4-1BB and TCR ζ signaling modules [33]. In the trial, Beatty used a second-generation CAR-T infusion to treat recurrent and metastatic pancreas, and in one patient, the liver metastases were successfully resolved [34]. At this stage, the efficacy of CAR-T cells constructed from dermatitis and HER genes and pancreatic cancer overexpressing autoantigens has been confirmed by researchers through experiments in a mouse model of pancreatic cancer. The majority of clinical trials testing late-stage CAR-T therapies have, however, produced insufficient evidence.

5. Conclusion

Fourth-generation CAR-T cell treatments are already available and are widely regarded as having tremendous research promise for treating malignant tumors. The findings of clinical trials using CAR-T cells to treat hematopoietic tumors, particularly CD19 CAR-T cells, have been positive. Nevertheless, CAR-T cell therapies still face an urgent clinical need in solid tumor-oriented treatments. Maintaining effective CAR-T cell proliferation in vivo and enhancing CAR-T cell efficiency remain challenging tasks. Here is where CAR-T's design and the investigation of various tumor types and immune microenvironments must be created. Additionally, there are still severe and apparent side effects associated with CAR-T cell therapy in clinical settings, which poses considerable therapeutic risks. Therefore, for the clinical treatment of CAR-T therapy, avoiding toxic side effects and improving safety are issues that need to be addressed. Researchers are proposing a more appropriate approach to tumor therapy: combining CAR-T cell therapy targeting specific antigenic epitopes with non-specific needle tract therapy targeting fine tumor cells. This can not only comprehensively kill tumor cells but also reduce the possibility of immune escape. CAR-T treatment is critical for lowering the dose and toxicity of non-targeted medicines like chemotherapy. Moreover, distinct hematological tumor cells display the antigenic epitopes CD22, CD20, and CD34, which can

be exploited as therapeutic targets for other resistant hematological illnesses. This approach opens up new possibilities for the disease's therapy. CAR-T therapy has achieved satisfactory therapeutic effects, bringing hope to patients with refractory hematologic malignancies. However, further studies and more experiments are needed to accumulate clinical experience. The experience of novel targeted therapies at multiple levels and angles may bring more effective clinical effects to CAR-T treatment. It is promising for hematologic tumors, solid tumors, and even more benign or chronic diseases.

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