

# The Development of CAR-T Immunotherapy

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**Abstract:** With the advancement of tumor biology, molecular biology and cell biology, the treatment of cancer has been greatly promoted. So many scientific researches have given messages that new tumor immunotherapy methods such as CAR-T cell immunotherapy have become another new type of treatment can replace targeted therapy, cancer surgery, radiotherapy, and chemotherapy. This paper focuses on the development prospect, research progress and research problems of CAR-T cell immunotherapy. Meantime, the advantages, martyrs and differences of various immunotherapies were compared, which provided reference for the research and preparation of tumor immunotherapy drugs. This review will review contemporary and recent major research on CAR T cells, with a focus on overcoming the adverse effects of CAR-T therapy and exploring new treatments

**Keywords:** CAR-T Cell Therapy; Tumor; Immunotherapy; Drug Development.

## 1. Introduction

In recent years, cancer is becoming one of the world's leading causes of death. With the increase of the number of patients, the demand for cancer treatment technology is increasing day by day [1]. The number of new cases of breast cancer in 2020 was 2.26 million, surpassing lung cancer for the first time and becoming the "first cancer" in the world.

At present, tumor treatment methods mainly include radiotherapy, surgery, chemotherapy, surgical treatment and targeted drug therapy, but all the methods have some limitations. In conventional surgery, it is difficult to remove tumor cells and it is easy to recur after surgery. [2]. Although radiation therapy and chemotherapy can inhibit the proliferation and metastasis of tumor cells to some extent, they can also inhibit normal functional cells, especially those that divide rapidly [3]. Compared with surgical resection and chemotherapy, targeted drug therapy can reduce adverse drug reactions, however, drug resistance to malignant tumors develops, leading to the recurrence of malignant cells. With the major research results of molecular biology, cell biology and tumor biology, as well as the continuous maturity of medical equipment and theory, cell-targeted immunotherapy has become a new means of tumor treatment. Unlike the above treatment options, tumor immunotherapy mainly targets immune cells. It activates the body's immune system by inhibiting negative immunomodulators, improving the ability of immune cells to recognize tumor cell antigens. In this way, tumor cells can be removed, which has the advantages of significant effect, small side effects and low recurrence rate[4].

Contemporary research into the mechanism of tumor immune evasion has deepened, and many new and different immunotherapies have been discovered. For example, the tumor immunotherapy drug IFN- $\alpha$ , which is produced by white blood cells, is involved in innate immunity from viral infections. Investigations have shown that improving IFN- $\alpha$  expression in invasive macrophages leads to more efficient activation of dendritic cells and cytotoxicity of immune effectors [5].

## 2. CAR-T immunotherapy

### 2.1. CAR-T Cell Definition and Structure

CAR-T cell therapy, it is an active drug that clears tumors and can be unaffected by MHC, that is, a certain precondition antigen presenting cell is required to interact with T cells, and T cells can be activated only when the two MHC molecules are the same, that is, T cells can only recognize antigen peptides presented by their own MHC molecules, but not antigen peptides presented by non-self MHC molecules. Malignancies resistant to other therapies [6]. CAR is a membrane protein and recombinant receptor composed of different protein functional domains in tandem, which has the characteristics of flexibility and specificity in recognizing antigen. Taking the currently commercialized CARs as an example, the functional domain consists of five parts: single-stranded antibody region, hinge region, transmembrane domain, co-stimulation domain and T cell activation domain [7]. TAA is a kind of antigen molecule which exists on tumor cells or normal cells and is often used in clinical diagnosis of tumor. It is not specific to tumor cells, Normal cells are microsynthesized and highly expressed during tumor cell proliferation. Single-chain variable fragment (scFv) is composed of the heavy chain region (VH) and light chain region (VL) through a flexible short peptide composed of 10-25 amino acids. It is the smallest recombinant antibody form (about 27 kDa).

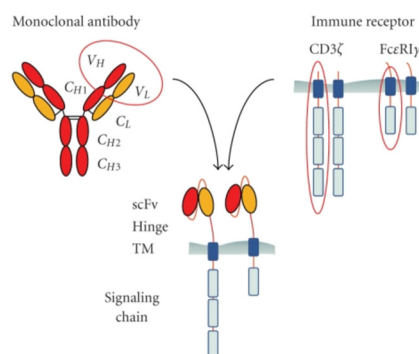


Figure 1. Structure of CAR-T cells (Source: CN-Healthcare)

## 2.2. Definition and Mechanism of Action of scFv

Essentially, scFv is a protein that preserves the specificity of the original immunoglobulin for antigens. The small molecular size of scFv brings the advantages of strong tumor penetration, rapid degradation in blood and small negative feedback in human body, which also lays the foundation for clinical application of scFv. The specificity and low immunogenicity of scFv make them an alternative to previous outdated treatments, improving the precision of targeting specific molecules and preventing some undesirable effects [8]. The intracellular signaling domain after scFv binds to TAA can promote the release of T cell activity, and co-stimulatory molecules can allow stable proliferation of T cells .

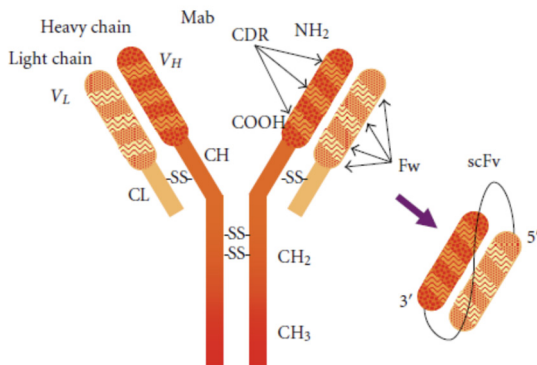


Figure 2. Single-chain fragment variable (Source: Clinical and developmental immunology)

## 3. Clinical Risk CRS

### 3.1. Cytokine Release Syndrome Needs to be Solved Urgently

CAR-T therapy is currently significant in the treatment of tumors, but there are also some risks in the process of clinical use. Among them, CRS and ICANS are the most common side effects of CAR-T cell immunotherapy, and clinically they often present with fever, hypotension, hypoxia, cognitive impairment and other symptoms. Therefore, how to accurately test and deal with the toxicity generated during CAR-T therapy and avoid adverse events related to CAR-T therapy as much as possible is the challenge and difficulty of current CAR-T cell therapy.

### 3.2. CRS Production and Treatment

CRS and ICANS are two major causes of complications after CAR-T cell therapy. Therefore, understanding the pathogenesis of CRS and ICANS is of great help in the treatment of complications. CRS is an inflammatory response that spreads throughout the body, including infections, medications, and viral infections[9] . Between the first day and the fourteenth day after CAR-T cells are infused into the body, CRS usually occurs, manifesting as fever, myalgia, hypotension, arrhythmia and other symptoms. [10] When CAR-T cells target the binding site, it causes a large release of inflammatory factors and chemokines, and excessive secretion of these factors can lead to CRS [11]. Whereas, in addition to the cytokines produced by CAR-T cells, activated peripheral immune cells also secreted a large number of cytokines, while non-peripheral immune cells also secreted them, and found that IL-6 was mainly secreted by monocytes, macrophages and dendritic cells. Since IL-6 is so important

in CRS, IL-6 plays a huge advantage in treating inflammation in the brain[12]. Autoimmune encephalitis is caused by autoantibodies against brain proteins that activate immune cells and induce the release of inflammatory cytokines such as IL-6, IL-17 and IL-1 $\beta$  produced by non-immune cells such as neurons and astrocytes, and is also caused by immune cells such as T cells and macrophages .The excessive release of GM-CSF signals is associated with several human diseases, such as rheumatoid arthritis and chronic myelocytic unicytic leukemia, and inhibition of GM-CSF signaling pathway is also an effective way to alleviate CRS symptoms. [13,14] Thus, the spatial structure of CAR-T cells is designed to stimulate CAR-T cell activity and maintain CAR-T cells while attenuating the activation of monocytes and macrophages. To study and produce a new generation of CAR-T cells, S. Balagopal et al. propose the following options to improve CRS: 1) based on the adapter strategy, 2) 3) orthogonal regulation of cytokine receptors of macrophage cytokine activity, 4) autonomic neutralization of major cytokines, 5) off, 6) reversible CAR inhibition method. Based on the above strategy, CAR-T cell therapy in the future will better inhibit the onset of CRS and reduce pain in patients.

### 3.3. CAR-T Study Progress

In 2012, CD19-targeting CAR-T cells were used in the treatment of acute lymphoid leukemia in patients Emily, enabling tumor-free survival to this day. Patients with diffuse large B-cell lymphoma were collected for up to 5 years of clinical efficacy, and the data showed that the therapy significantly extended the survival of patients . More than 60% of adult LBCL patients treated with CD19 CAR-T do not achieve a sustained treatment response[15]. Treatment failure in some of these recurrent patients is associated with CD19 recurrence, the remainder is associated with CAR-T proliferation disorders and in vivo T cell depletion. The data showed that PD-L1 was elevated in the gene expression profile of LBCL tissue biopsy in patients with recurrent episodes after CD19 CAR-T treatment, and PD-L1 was significantly active in 62% of patients with disease progression. Ways to overcome the above problems and improve LBCL CAR-T therapy mainly include targeting double antigens and using PD-1 or PD-L1. There have been more than 500 trials based on CAR-T cell therapy in the world, especially in 2017, FDA approved the marketing of two CD19CAR-T therapies, so that CAR-T therapy has a good development space in clinical application[16]. Until June 2021, there are 5 FDA-certified CAR-T products, focusing on the dual-target CAR-T cell therapy currently being tried for hematological tumors. The dual-target CAR-T cell therapy currently being tried can avoid the problem of CAR-T off-target and may also have better drug resistance. Dual-target CAR can independently recognize the target antigen, and recognition of TAA can fully activate T cells. CD19/CD22 is the first dual-target CAR-T, and the results of the treatment with monoclonal antibody have shown surprising anti-tumor activity Universal CAR-T therapy is also the focus of current research. The researchers designed universal CAR-T cell products such as CTA101 based on CD19 and CD22, which provide a new way to cure patients with relapsed and refractory acute lymphoblastic leukemia.

However, CAR-T therapy is not omnipotent and harmless. CAR-T treatment process will produce relevant toxicity and CAR-T treatment-related adverse events. Due to the large amount of cytokines produced during treatment, CRS is the

most popular SAE in the immune system after CAR-T cell therapy. In addition, CRS can also cause systemic SAEs in a variety of systems, and therefore, CRS is the initiating factor for various SAEs. Philipp Karschia et al. investigated the clinical manifestations of adverse reactions in 25 adult patients after CAR-T cell injection, of which 52% showed grade 3-4 neurotoxicity [17]. Blood oxygen deficiency is a respiratory system prone to SAE, and the disease can progress to respiratory failure, which is life-threatening. The study found that respiratory complications are the main morbidity after CAR-T cell therapy. Based on these studies, neurological toxicity is common in CAR-T cell therapy and preventive treatment should be carried out. The researchers used FAERS to test 996 patients who reported adverse events, including arrhythmia in 77.6%, heart failure in 14.3%, and myocardial infarction in 0.5%. In addition, it was found that cardiovascular adverse events are also closely related to death caused by CAR-T cell therapy.

#### 4. Conclusion

Therefore, the progress in the field of CAR-T therapy is surprising and exciting, playing a key role in the treatment of lymphoma and hematologic tumors, but the field of CAR-T still faces some big challenges. One challenge is how to avoid CRS, which is to understand the pathogenesis of ICANS and effectively treat it, another major challenge is the re-emergence of the disease after CAR T cell injection, and future efforts should also focus on finding suitable CAR T cell therapy antigens while preserving normal tissues to maintain homeostatic function. Equally important, standardizing and optimizing protocols to produce clinical-grade and sufficient quantities to deliver treatment to a larger patient population is another key consideration in this area.

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