

# Manufacturing Process of CAR-T Therapy and Assessment of the Optimization Potential

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**Abstract.** One ground-breaking novel treatment that is being utilized extensively in the field of cancer treatment is chimeric antigen receptor (CAR)-T cell therapy. Three axes are used by CAR-T cells to promote tumor killing: Targeting the antigen-positive fraction is the Perforin and Granzyme axis; Targeting the antigen-negative fraction is the Fas and FasL axis; and Cytokine release is focused on the Stromal cell. As a result of this technology's widespread use, its benefits and applications have increased; yet, drawbacks and toxicities have also been discovered. The wide range of uses for this cutting-edge technology has resulted in an increase in its advantages and uses. Alongside these benefits, studies have also discovered negative effects and toxicities related to its usage. This work analyzes the complexities of the specialized immunotherapy mechanism, the difficulties associated with this technology, toxicity issues, and future directions in the treatment of CAR-T cells. Its potential to completely transform the way cancer is treated is becoming more and more evident as the medical community works to understand the intricacies of this innovative therapy.

**Keywords:** CAR-T; manufacturing process; gene editing.

## 1. Introduction

Along with the development of cancer research, more immunotherapies targeting cancers are developed, including monoclonal antibodies, cancer vaccines, cytokine, checkpoint inhibitors, and CAR-T therapy. Adoptive T cell therapy (ATC) is a class of therapy that kills cancer cells through the use of transferred T cells, and CAR-T cell is one of the main ATC approaches. The primary job of T cells, which are white blood cells derived from the bone marrow, is to attach to a particular antigen on antigen-presenting cells and create an MHC complex. Different types of immunotherapies have different fields they are better than other therapies, based on their mechanism and limitations. T cells include cytotoxic T cells that kill infected cells and helper T cells that send signals to other immune cells. T cells focus on more specific immune responses, they target specific antigens and destroy cells with that antigen. CAR-T therapy uses genetically modified T cells to increase the efficiency of targeting and destroying cancer cells. CAR molecules are the part that is added by genetic modification. A hinge region, a transmembrane domain, an external target antigen-binding domain, and one or more intracellular signaling domains are the four fundamental components of CARs. The current CAR-T cell is after 4 times of iterations, each generation has its improvement and limitations. However, the current CAR molecule still has the space to optimize, especially in its manufacturing and preparation process.

CAR-T therapy is a promising new therapeutic option for cancer, but its broad application is limited by its high cost and danger. Further research, resources, and time will be needed to investigate this technique fully. To assure the safety and cost-effectiveness of this therapy and enable more individuals to get it, these issues must be resolved via further research and development. Efficacy is needed to increase its usefulness for both the general public and more cancer sufferers. This paper will focus on the working mechanism and the preparation process that most CAR-T therapy currently uses, and will discuss their advantages and disadvantages based on current procedures in use. Based on this, this article will further evaluate the possibility of customization and mass production of this therapy.



## 2. General Information of CAR-T Therapy

Several factors have contributed to the development of CAR-T therapy during the last few decades. For starters, T cells may target different malignancies by recognizing tumor-associated antigens that are shared by different types of tumors and individuals. TCRs, which are receptors that can cause T cells to become reactive to malignancies, have also boosted the likelihood of T cell treatment for cancers. T cell treatment has also benefited from the colne technology, which can replicate tumor-reactive T cells, sequence them, and genetically edit them. All of this information and technological advancement led to the creation of CAR-T therapy. AT is a class of therapy that kills cancer cells through the use of transferred T cells, such as engineered TCR treatment, CAR-T therapy, natural killer (NK) cell therapy, and tumor-infiltrating lymphocyte (TIL) therapy. One of the primary ACT methods is the use of CAR-T cells. The primary job of T cells, which are white blood cells derived from the bone marrow, is to attach to a particular antigen on antigen-presenting cells and create an MHC complex. [1].

Artificially modified T cells are called CAR T cells. The T cell was modified to include CAR. CAR T cells can exert their anti-tumoral activity by sensitizing the tumor stroma through the production of cytokines, the Fas and Fas ligand axis, and the perforin and granzyme axis, all through the formation of a non-classical immunological synapse. CAR--T cells destroy MHC-unrestricted tumor cells by allowing T cells to connect with target cell surface antigens via a single-chain variable fragment (scFv) recognition domain. After PBMCs are virally transduced, CAR-T cells may be grown and greatly enlarged before to being given to a patient, enabling the creation of this therapy autologously. [2].

## 3. General Information on CAR Molecules

An external target antigen-binding domain, a hinge region, a transmembrane domain, and one or more intracellular signaling domains are the four core components of CARs. Modular synthetic receptors are known as CARs.

### 3.1. Antigen-binding Domain

Derived from the variable heavy (VH) and light (VL) chains of monoclonal antibodies, the antigen-binding domain may target individual antigens. A flexible linker connects the two chains to generate a scFv. The binding affinity of the antigen-binding domain must be high enough to detect antigens on tumor cells and initiate CAR signaling, but not so high as to result in toxicities or activated T cell death in CAR-expressing cells. [3].

### 3.2. Hinge Region

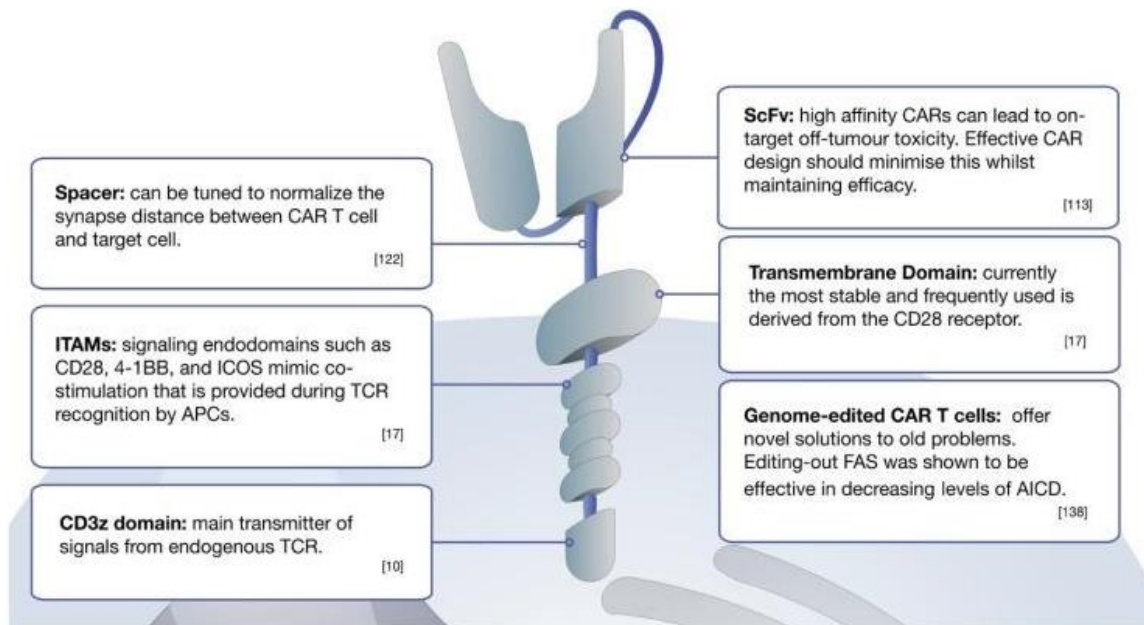
The extracellular structural area that extends the binding units from the transmembrane domain is called the hinge region. It can induce length, make the molecule flexible, get past steric hindrance, and improve the antigen-binding domain's efficiency in reaching the target. The position and degree of the epitopes' hindrance on the target determine the length. Long hinges will work better against complex glycosylated antigens or membrane-proximal epitopes; shorter hinges work better against membrane-distal epitopes. Hinges sections are most frequently used and are taken from amino acid sequences found in CD8, CD28, IgG1, or IgG4.

### 3.3. Transmembrane Domain

The process of targeting CAR to the cell membrane of T cells involves the delivery of a transmembrane domain. As shown in Figure 1, relevant studies have found that CAR can maintain its cell activity and participate in protrusion formation and signal transduction during the delivery process. This can also be reflected in its interaction with inner signaling molecules and promotion of dimer formation. Most transmembrane domains are derived from naturally occurring proteins, such as CD3z, CD4, CD8 $\alpha$ , etc. Research on these transmembrane domains and CAR-T cells has found that the CD8 or CD28 structure can enhance the binding probability and stability of CAR [4]. This

article further speculates that the best way to activate CAR-T cells is to activate proximal cells. The inner domain is connected to the right transmembrane domain.

Different functional and metabolic profiles are displayed by the co-stimulatory domains: CARs with CD28 domains differentiate effector memory T cells, which mainly rely on aerobic glycolysis; CARs with the 4-1BB domain, on the other hand, differentiate central memory T cells, which are distinguished by increased mitochondrial biogenesis and oxidative metabolism.



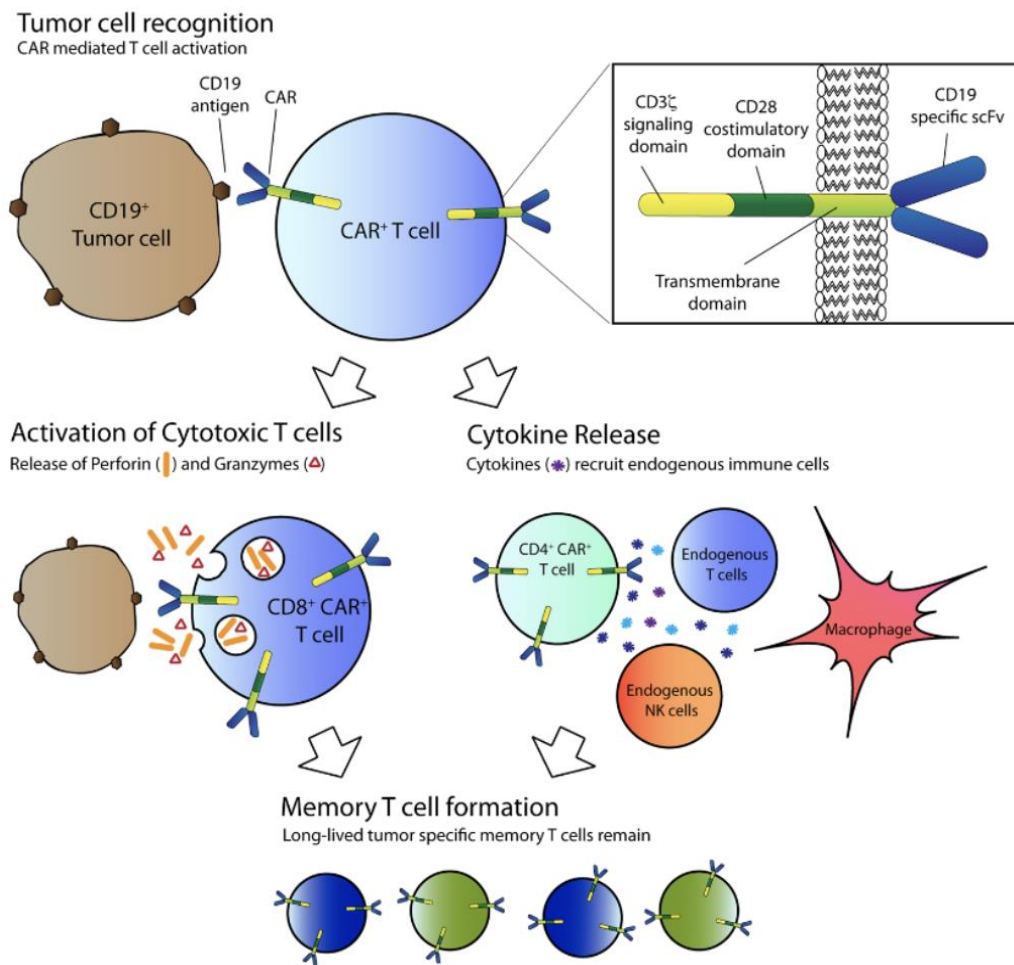
**Figure 1.** The composition of CARs [5].

#### 4. Working Mechanism of CAR-T Cells

As a brand new technology, CAR T cell therapy combined some important roles of conventional T cells. It combines the specificity of a monoclonal antibody and memory functions, as well as cytotoxic functions [6]. As mentioned in the above sections, the specificity of CAR comes from the antigen-binding domain, which is a site of a monoclonal antibody. As shown in figure2, there are three axes of tumor killing mediated by CAR T cells. The first one is the perforin and granzyme axis: When the antigen-binding domain binds to its antigen on the cancer cell, it initiates the process of CAR T cell immune response. Once the transduction pathway is activated, the intracytoplasmic signaling domains are going to directly kill the cancer cell by CAR-T cell-mediated release of granzyme and perforin. After the CAR is initiated, the granules in Cytotoxic T cells will transfer and fuse into the plasma membrane of cSMAC. The cytolytic vesicles are released into the synaptic cleft, where pro-apoptotic granzymes can enter because perforin induces core formation on the cancer cell membrane. Because it can eliminate the target cells without triggering other chain reactions, the cancer cell-killing method carried out by CAR-T cells is effective in reducing response times. Tumor death receptor molecules are not necessary for this mode of action; instead, it depends simply on the synthesis of antigens associated with the tumor.

The second axis is cytokine secretion. Cytokine production by activated CAR T cells enhanced the anti-tumoral abilities of the modified cell, it is also important for tumor lysis. The tumor stroma may express the interferon-gamma (IFN- $\gamma$ ) receptor in response to cytokines produced by CAR T cells that are specific to HER-2 [7]. IFN- $\gamma$  is a crucial macrophage activator that stimulates the synthesis of class II molecules of the major histocompatibility complex. The rededucation of immune cells also occurs at the same time, for example, the polarization of macrophages to the anti-tumoral phenotypes [8].

The third axis is the Fas and Fas Ligand (FasL) axis. Fas and FasL pathway often functions in non-pathogenic situations. Calcium-independent Fas and FasL work with calcium-dependent granule exocytosis to form one major axis of T Cells lysis of cancer cells [9]. The Fas ligand must trimerize the Fas receptor, activate caspase 8 and pro-caspase 8, and create a death-inducing signaling complex (DISC) in order to activate the Fas and FasL pathway. After that, caspase 8 converts pro-caspase 3 into mature caspase 3, which may induce apoptosis and separate 500 or more cellular substrates. [10]. Under the condition that has an antigen-negative fraction in an antigen-independent cell interaction, CD30 and CD19 targeting CAR T Cells can mediate cancer cell lysis (Only when both antigen positive and negative cells occur). Then FasL in CAR T cells is upregulated, mediating the tumor destruction process [10].



**Figure 2.** Working Mechanism of CAR T cell [11].

## 5. Application of biotechnology in CAR-T therapy optimization

At present, there are still many problems that need to be optimized regarding the side effects of CAR. This optimization is in addition to genetic engineering of the CAR protein itself. The purpose is to improve the effectiveness and safety of treatment by regulating the activity of T cells. The mechanisms by which CAR can cause toxicity come from various aspects. Previous studies have shown that compared with the transient expression of virally integrated CAR, the gene expression of CAR using mRNA electroporation technology will be more targeted, thereby reducing the toxicity of cross-recognition to healthy tissues. In addition, abnormal expression of EGFR can be detected in most tumors. Another measure to reduce toxicity is to use short EGFR transgenic technology to modify CAR and introduce suicide genes to promote related suicide mechanisms.

After modification, the CAR gene will enter the transcription and translation stages. At this stage, it can also be optimized accordingly, such as using transcriptional regulation to improve the safety of

CAR-T cells. For example, the small molecule drug DOX, in which the mechanism of action of the drug is to regulate the transcriptional ability of CAR by regulating tetracycline, respectively drives the "switch" of the system. This switch system can reversely inhibit the damage of CAR to T cells during the signaling process.

In addition, new gene editing technologies such as CRISPR also have great application prospects in optimizing CAR-T therapy. Although the application of this technology in this field is still in the laboratory research stage, relevant results have shown that, On the one hand, cell function can be enhanced by genetically knocking out factors related to toxic side effects. On the other hand, this technology can be used to modify the TME, such as inhibiting immune evasion [12]. In summary, the emergence of new technologies has made considerable progress in the application of CAR-T therapy for tumor treatment in recent years, but it is still in its infancy.

## 6. Conclusion

CAR-T therapy, as a cell therapy that has attracted much attention recently in the field of tumor treatment, has been widely and extensively used in acute lymphoblastic leukemia and non-Hodgkin lymphoma. Despite this, its targeting and cytotoxicity in clinical practice are still issues that need to be resolved. In addition, this therapy is currently limited to the treatment of blood tumors, and solid tumors are in their infancy, and this issue needs further exploration.

The currently used CAR-T includes the collection of T cells, the addition and modification of CAR, the expansion of CAR-T cells, the suppression of the patient's immune system through chemotherapy and other methods, and the final injection. However, after the injection is successful, the patient should continue to pay attention to whether there will be adverse reactions. Reducing adverse reactions has become a hot topic in recent research. This article reviews the structure and transformation process of CAR-T cells and recent related progress. In response to recent research findings, the core of CAR-T therapy lies in the targeted modification of T cells through biotechnology to enhance their targeting and reduce cytotoxicity. In the future research process, new biotechnologies such as CRISPR can be used to help achieve this goal.

The analysis results of this article can provide reference ideas for future research, but this article does not further analyze the potential adverse effects of CAR-T, so future research can pay more attention to improving its side effects.

## Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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