

# Application And Research Progress of T Cells in Immunotherapy in Cancer Treatment

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**Abstract.** Chimeric Antigen Receptor T-cell therapy (CAR-T) has emerged as a revolutionary approach in cancer treatment, offering hope to patients with previously untreatable malignancies. This review paper provides a comprehensive overview of CART therapy, covering its structure, working mechanism, clinical applications, and strategies to mitigate adverse effects. Firstly, we delve into the structural intricacies of CAR-T cells, exploring how they are genetically engineered to express chimeric antigen receptors that enable them to target specific tumor-associated antigens. The paper elucidates the mechanisms by which CAR-T cells recognize and eliminate cancer cells, including the activation of immune responses and the production of cytokines. Next, we present an in-depth analysis of CAR-T cell therapy in clinical practice, highlighting its remarkable successes in treating various hematological malignancies and solid tumors. The review discusses the challenges associated with manufacturing, patient selection, and long-term persistence of CAR-T cells in vivo. Finally, we address the adverse effects associated with CAR-T therapy, such as cytokine release syndrome and neurotoxicity, and propose strategies to mitigate these side effects. These include the use of pharmacological interventions, patient monitoring, and the development of novel CAR-T cell constructs with improved safety profiles. In summary, this review paper provides a comprehensive and up-to-date perspective on CAR-T cell therapy, emphasizing its potential to transform the landscape of cancer treatment while addressing the challenges and risks that accompany this groundbreaking approach.

**Keywords:** Chimeric Antigen Receptor T-cell therapy; cancer treatment; immunotherapy.

## 1. Introduction

As individuals experience enhanced standards of living and societal and economic advancements, and in the context of the general increase in life expectancy of people in various countries, the incidence of cancer has gradually increased. With the increasing life pressure and poor living habits of young people, the incidence of various cancers is gradually getting younger. Therefore, the demand for cancer treatment is increasing day by day, and traditional treatment methods include radiotherapy and chemotherapy. Although they have achieved good results in some respects and surgery has the possibility of recovery, these methods are still very limited, including large amounts of radiotherapy, chemotherapy. The numerous side effects and irreversible damage to organs caused by surgery have prompted the need to find better treatments. Immunotherapy methods have attracted people's attention, among which cart therapy, as its representative therapy, has a good therapeutic effect. Compared to traditional treatment methods, the advantages of cart therapy include better efficacy with fewer side effects. This article introduces the basic principles, clinical applications, side effects and risks of cart therapy.

## 2. The structure and working mechanism of CART

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#### 2.1.1 single-chain variable fragment (scFv)

The antigen recognition domain is indispensable for the specific binding of the chimeric antigen receptor (CAR) to cancer antigens. The fundamental architecture of this entity is referred to as the single-chain variable fragment, often abbreviated as scFv [1]. The composition of this structure

consists of the light chain (VL) and the heavy chain (VH) components of a monoclonal antibody, interconnected by flexible joint. This configuration maintains the antibody's antigen specificity and affinity, forming the basis for CAR-T cell targeting [2]. T cells detect and attach to specific antigens owing to the single-chain variable segment which has the capability. In comparison to unmodified native TCR-T cells, CAR-T cells that have been engineered to express single-chain variable fragments (scFv) have a significantly enhanced binding affinity towards target antigens. When using single-chain variable fragments (scFv), it is important to consider that the recognition and binding of chimeric antigen receptor T (CAR-T) cells to their target antigens do not need antigen presentation by major histocompatibility complex (MHC) molecules. The presence of this dual feature serves the purpose of not only impeding the ability of cancer cells to evade immune recognition via the control of major histocompatibility complex (MHC), but also facilitating the capacity of chimeric antigen receptor T (CAR-T) cells to identify non-peptide antigens [3]. Presently, the majority of CAR-T research concentrates on targeting tumor-associated antigens (TAA) like CD19, CD20, BCMA, among others, which has contributed to the creation of antibodies targeting these markers. Nevertheless, a prevalent issue in CAR-T clinical research is the predominant use of scFv derived from mice. There is a potential for an elevated probability of rejection of CAR-T cells by the immune system of the host, leading to cytotoxicity or decreased persistence of CAR-T cells inside the organism [4]. Therefore, a challenge in CAR-T research is to develop humanized or fully human antibody-derived scFv to minimize the immunogenicity of CAR-T cells.

### 2.1.2 Hinge area

The hinge region assumes a pivotal function for CAR T-cell treatment as it serves to link the single-chain variable segment with the transmembrane domain responsible for antigen recognition. Both the extracellular domain of CD8/CD28 which situated at the cellular membrane and the hinge region of immunoglobulin G are possible sites for the initiation of this specific hinge region.

Several studies examining CAR-T cells have yielded findings indicating a positive association between the level of CAR-T cell activation and the dimensions of the cell's hinge region. The adjustment of the length of the hinge area allows for the attainment of the desired spatial separation between the CAR-T cells and the target cells. Due to the potential interference of large phosphatases with the antigen-antibody binding process, resulting in a decrease in the CAR signal. In certain instances, the accessibility of the antigenic epitope may present difficulties, necessitating the presence of a longer hinge region [5]. The optimal length of the hinge region may vary based on the individual antigenic epitope being targeted. Furthermore, it may be necessary to modify the length of the hinge area when targeting neoantigens, in order to match the unique properties shown by these targets.

### 2.2. Transmembrane domain

In addition to possessing an extracellular domain, the chimeric antigen receptor (CAR) also encompasses an intracellular signal transduction region. The interconnection between these two segments of the receptor is facilitated by the transmembrane domain of the molecule, enabling them to synergistically cooperate. Most of the transmembrane domains are composed of CD4, CD8a, CD28 and other proteins, which are non-synthetic natural proteins [6]. There is a potential for enhancing the development of chimeric antigen receptors (CARs) and the functionality of CAR-T cells via the formation of homodimers or heterodimers between the transmembrane domain of CD3 and the conventional T cell receptor (TCR). This approach has the potential to decelerate the progressive elimination of hyperactive T cells due to its ability to reduce the need for interaction between the native T-cell receptor and the foreign T-cell receptor (TCR). The use of the transmembrane domains of CD8 and CD28 has been extensively employed in many clinical investigations owing to their capacity to augment the surface display of chimeric antigen receptors (CARs) [7].

## 2.3. Intracellular domain

### 2.3.1 Costimulatory Domain

Researchers often associate with other members of the CD28 receptor family when attempting to determine the precise placement of the costimulatory domain. T lymphocytes demonstrate the capacity to sustain their proliferation and cytokine production. This phenomenon is attributed to the pivotal function of the costimulatory domain in facilitating the activation of costimulatory molecules and intracellular signaling pathways. Meanwhile, the occurrence of this event leads to a significant enhancement in the efficacy of T cells as agents against malignancy. The inclusion of the CD28 costimulatory region in these cells facilitates the reliance of chimeric antigen receptor T cells (CAR-T cells) on glycolytic metabolism. Thus, the ability of these cells to differentiate into effector T cells increases.

Extant research has shown that the 4-1BB costimulatory domain assumes a significant function in promoting mitochondrial biogenesis. This process enhances the rate at which cells may engage in respiration, thus facilitating the oxidation of fatty acids. Chimeric antigen receptor T cells (CAR-T cells) have been seen to have a predilection for differentiating into central memory T cells subsequent to their initial activation by antigens and subsequent exposure to the same antigens. This growth preference becomes evident subsequent to the activation of CAR-T cells by antigens, followed by further exposure to those same antigens. However, it remains a matter of inquiry as to whether costimulatory domain exhibits superior performance. In a study published in the journal *Science Signaling* in 2018, a comparison was made between the two structures, CD28 and 4-1BB. The findings revealed that CD28 exhibited more rapid and robust signaling activity, whilst 4-1BB displayed comparatively slower and milder signaling activity. Nevertheless, the use of 4-1BB has the inherent capability to prolong the longevity of T cells while simultaneously preserving their anti-cancer properties.

### 2.3.2 Signaling Domain

A signaling domain refers to a distinct area inside a protein or biomolecule that has a particular functionality, enabling the transmission of signals within the cellular environment. This transmission subsequently triggers a specific biological response or initiates a metabolic process. Signaling domains often include a series of amino acid residues that are crucial for the protein's interaction with other biomolecules. The aforementioned areas are also of utmost importance in the context of cellular signaling, intercellular communication, and regulatory mechanisms inside living organisms.

The signaling domain functions as a medium via which signals may be sent between different components within the cellular environment. The organism has the capacity to react to molecular signals or external stimuli, so initiating the activation of internal biochemical processes or pathways, eventually leading to distinct cellular responses.

The ability of signaling domains to effectively facilitate signal transmission is often closely correlated with their capability to interact with a diverse array of proteins and chemicals. Instances of biological activity that may occur as a direct result of interactions encompass the phosphorylation of proteins, the formation of protein complexes, and changes in the structural composition of proteins. The development of cellular connections has significant importance as it is essential for ensuring the unhindered transmission of information between cells and for the initiation of diverse biological processes.

Signaling domains play a crucial role in regulating intracellular signaling pathways. The chemical in question has the capacity to function as either the initial point or an intermediary along a signaling pathway. The entity has the ability to transmit or modulate signals and has the potential to impact the functioning of molecules situated farther along in the route. The regulatory mechanism described herein facilitates the maintenance of homeostasis within intracellular signaling cascades, so enabling the organism to effectively respond to and adjust to changes in its environment.

There are notable variations in both the structural and functional characteristics across different signaling domains. Common signaling domains include several types such as tyrosine kinase domains,

tyrosine phosphatase domains, Src homology domains, and PDZ domains, among several others. Every domain has its own unique signaling system and collection of biological activities.

Aberrations such as cancer, inflammation, and neurological disorders have been associated with atypical functioning or genetic alterations within signaling domains. In the pursuit of developing therapeutic therapies that effectively target signaling pathways for the management of various diseases, researchers often prioritise signaling domains as crucial areas for potential pharmacological advancements.

Signaling zones play a crucial role not just in intracellular signaling but also in several biological processes. Cell signalling molecules are of significant importance in facilitating intracellular communication and are essential for the proper functioning of several physiological systems in animals. Engaging in thorough investigation of signaling domains facilitates a comprehensive comprehension of cellular signaling processes, as well as the aetiology and therapeutic approaches for various diseases [1].

### **3. CAR-T cell therapy in clinical practice**

#### **3.1. Long-term efficacy data for CD19-targeted chimeric antigen receptor (CAR) T cells in B-cell lymphoma and/or CLL/SLL patients has grown in recent years.**

To begin with, analyzing the comparison of treating the same disease with different drugs, we can draw the following conclusions [8]:

In the treatment of DLBCL, Chong et al. (2021) [9] used Tisagenlecleucel, which had an ORR of 66% and a CRR of 55%, while Schuster et al. (2021) also used Tisagenlecleucel, but the ORR was 53% and the CRR was 39%. In addition, Jacobson et al. (2021) used Axicabtagene ciloleucel to treat DLBCL, with an ORR of 74% and a CRR of 54%, which performed well in patients with DLBCL. From the data, Axicabtagene ciloleucel is relatively more effective in terms of ORR and CRR.

Regarding the treatment of FL (inguinal lymphoma), Chong et al. (2021) [9] used Tisagenlecleucel, which had an ORR of 66% and a CRR of 55%, while Hirayama et al. (2019) used Lisocabtagene maraleucel, which had an ORR of 46% and a CRR of 55%. 88%. IN the research by Chong et [9], the PFS of DLBCL and FL were 31% and 43%, respectively. Therefore, Lisocabtagene maraleucel showed better effects in terms of ORR and CRR, but the PFS of Tisagenlecleucel was slightly higher in the study by Chong et al [9].

In terms of MCL treatment, Wang et al. (2023) used Brexucabtagene autoleucel, with an ORR of 91% and a CRR of 68%, showing good efficacy.

In summary, the response of various cancer types to CAR-T cell therapy can vary across different studies and clinical trials. Certain therapies have shown higher ORR and CRR in treating DLBCL but relatively lower in CLL patients. In addition, some therapies may have shorter duration of response despite having higher ORR and CRR, while other therapies may provide longer duration of response. For example, Brexucabtagene autoleucel demonstrated higher CRR and sustained response time in MCL patients. Finally, age may also have an impact on treatment efficacy; for example, younger patients may have better survival rates with the therapy, as was observed in research of tisagenlecleucel in B-ALL.

#### **3.2. Long-term outcomes in patients with B-cell acute lymphoblastic leukemia receiving CAR T-cell therapy with CD19.**

Shah et (2021) examined the experiment on individuals and kids between the ages of 4 and 30 [8]. The results show a median Event-Free Survival (EFS) of 93 days and a complete remission (CR) rate of 62%. It is important to highlight that all patients who reached MRD-negative CR eventually had relapse, even if EFS may be extended among those who attained this condition.

T Laetsch et (2023) employed a CAR design that included the 4-1BB co-stimulatory domains, CD8 $\alpha$  hinge and transmembrane (H/T), and scFv. Patients who were paediatric and adolescent, ranging in age from 3 to 24, had this course of treatment. An exceptional 82% complete remission

(CR) rate and a noticeably longer Event-Free Survival (EFS) of 24 months were found in the experimental data. The longer EFS could be a symptom of the beneficial influence of the CAR design, which increases the CAR-T cells' persistence and leads to better results. Jacoby et al. (2022): This study encompassed both pediatric and adult patients aged between 1 and 36 years old. The findings revealed a notable rate of complete remission (CR) at 86%, along with a median Event-Free Survival (EFS) of 1.4 years. In the event that conservative transplantation therapy is not available, a subset of patients will have a relapse of their ailment. Although the inclusion of the CD28 costimulatory domain in CAR T cells has the capacity to enhance their activation kinetics, it does not guarantee the prevention of relapse.

Wayne and others (2022) Mice scFv, CD28, and CD28 H/T co-stimulatory domains are used. A median RFS of 158 days and a CR rate of 67% were discovered in the paediatric and adolescent patients, ages 3 to 20, who were included in the experiment. While most RFS patients recovered successfully, one patient passed away as the illness worsened.

The study done by Hay et al. (2019) included a sample of individuals within the adult age range of 20 to 76 years. The study had a complete response (CR) rate of 85% and an average event-free survival (EFS) duration of 7.6 months. The idea suggests that patients who achieve MRD-negative complete remission (CR) may have a longer event-free survival (EFS). However, no specific data have been provided to substantiate this claim.

Park et al.'s (2018) investigation included adult patients with an average EFS of 6.1 months and a CR rate of 83%. Although the EFS of the majority of patients was favorable, 17/44 patients experienced relapse or mortality.

His investigation included adult patients with an average EFS of 6.1 months and a CR rate of 83%. Although the EFS of the majority of patients was favorable, 17/44 patients experienced relapse or mortality.

Shah et al. (2021) use brexucabtagene autoleuce whose domain is Mouse scFv, CD28 H/T, and CD28 co-stimulatory domains. In adults, the CR rate was 69% and the RFS was approximately 7 months. A few patients received transplants, but the majority of patients with CR did not receive transplants. This may suggest that a subset of CR patients can maintain remission without transplantation.

Seventeen out of twenty adult patients demonstrated MRD-negative CR. Nonetheless, the EFS at 24 months according to MRD relapse criteria was 44%. This may suggest that despite the fact that some patients achieve CR, the durability of the therapy remains problematic.

Using hCART19s, Wang et al. (2002) administered Humanized scFv,4-1BB, CD8 H/T, co-stimulatory domains to paediatric patients, and 20/24 patients achieved CR [10]. In terms of 3-year EFS, the EFS is 37%. However, a subset of patients still experience relapse.

Frey et al. (2020) used Mouse scFv, 4-1BB, CD8 $\alpha$  H/T co-stimulatory domains, and tisagenlecleucel in adult patients, and 24/35 patients achieved CR. In terms of EFS, the median is 5.6 months. However, some patients do not achieve durable complete remission.

An et al. (2020), Sino19 used CAR's Mouse scFv, 4-1BB, IgG4 hinge, and CD28 TM co-stimulatory domains. This study included both paediatric and adult patients, with 38/47 achieving CR. The median RFS duration is 10.5 months. A subset of patients, however, experience relapse.

These investigations investigated the effects of various CAR T-cell therapies on various patients and conditions. Overall, they demonstrate that the CAR design, patient population, and disease classification impact treatment outcomes, such as complete response rate and survival. Several treatments have yielded relatively positive outcomes, but relapse remains a challenge.

Shah et al. (2021) successfully implemented the administration of chimeric antigen receptor (CAR) T cells in adult subjects, using a treatment strategy that used the CD28 and CD28 H/T co-stimulatory domains [11], and the CR rate was 69% in this study. Frey et al. (2020) used CAR T cells with the 4-1BB and CD28 H/T co-stimulatory domains to treat adult patients; the study's CR rate was 69%. While both researches included CD28 H/T in their CAR designs, the function and effectiveness of CAR T cells may change depending on which co-stimulatory domain is used. This suggests that the

therapeutic efficiency of CAR T cells may be influenced by the precise design of the CAR, and that analysing the 4-1BB component may result in extended event-free survival (EFS) and prolonged activity of CAR T cells. These factors have the potential to improve the overall success of the therapy.

Brexucabtagene Autoleucel was used to treat adult patients by Shah et al. (2021) [11], and the CR rate was 69%. In Laetsch et al. (2023), the same therapeutic drug was used to treat children and adolescent patients, and the CR rate was 82%. Diverse experimental results may indicate a more advantageous reaction to CAR T cell treatment in paediatric and adolescent populations, maybe linked to their immune system condition and cancer characteristics.

Several studies have produced a range of therapeutic outcomes by using various designs of chimeric antigen receptor (CAR) and CAR T cell products. The choice of the costimulatory domain has the potential to impact the long-term effectiveness and event-free survival (EFS) of chimeric antigen receptor (CAR) T cell therapy. While a significant number of patients are able to attain complete remission (CR), there exists a subset of individuals who continue to face recurrence, so underscoring the persistent challenge associated with CAR T-cell therapy: the ability to maintain long-term full remission.

All in all, the therapy is a complex treatment whose efficacy is influenced by lots of numbers of factors, including CAR design, patient characteristics, drug selection, and post-treatment maintenance strategies. Improve the effectiveness of the treatment and enhancing patient survival may be facilitated by individualized treatment strategies and expanded research.

#### **4. Principal adverse effects and mitigating strategies of CAR-T therapy**

Due to immune activation, patients often experience multiple toxicities when receiving CAR-T therapy. Here introduce the three most important acute toxicities.

##### **4.1. Cytokine release syndrome**

Since numerous cart cells are imported into the body, the cells release a large number of cytokines, which will cause an inflammatory response in the body's cells, causing symptoms such as fever, chills, hypotension, shortness of breath, and pulmonary symptoms.

In order to alleviate cytokine syndrome, cytokine interleukins are clinically used to symptomatically treat grade 2-4 cytokine syndrome. Clinical experimental studies have shown that interleukin antagonists can effectively relieve symptoms and improve the overall survival rate of patients. The combined use of interleukins is more effective than either alone [12].

##### **4.2. Neurotoxicity**

The pathophysiological mechanism remains obscure. Current studies suggest that the CD19 protein may be related to neurotoxicity in CAR-T therapy. Neurotoxicity usually manifests as disturbances in attention and consciousness, which can progress to depression, coma, seizures, and fatal cerebral edema. For isolated neurotoxicity, steroids are the main treatment; for patients with cerebral edema, symptomatic treatments such as mannitol or hypertonic saline and hyperventilation can also be given; due to the poor blood barrier permeability of tocilizumab, here It has limited efficacy and is therefore only used when neurotoxicity is accompanied by cytokine release syndrome.

##### **4.3. Reduce blood cells**

CAR-T therapy that targets CD19 depletes the normal B cells in the patient's body, leading to cytopenias (B cell aplasia and hypoglotia). Proteinemia) has become a more common adverse reaction after treatment. It manifests as anemia, easy bleeding caused by low platelets, and infection symptoms caused by low white blood cells. Immunoglobulin replacement therapy is often used as an alternative treatment for cytopenias. Prsistent cytopenias after the therapy have been resolved through autologous blood transfusion or allogeneic stem cell support.

In addition to the three major adverse reactions listed above, cart has additional severe adverse reactions for which solutions must be found.

## 5. Conclusion

In general, CART cells, in a broad sense, have a favorable influence on some forms of hematoma; nonetheless, their efficacy is not notably optimal when used in the context of solid tumors. Furthermore, the manufacturing and preparation process of chimeric antigen receptor T-cell therapy (CAR-T) poses considerable challenges, necessitating a substantial investment of time and financial resources. Consequently, the achievement of large-scale production and commercial viability of this product becomes arduous. Furthermore, it is essential to identify a resolution for the potentially life-threatening adverse reactions, such as cytokine storm syndrome, in order to ensure the widespread adoption of the intervention. However, this development represents a positive progression.

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