

# The Brief Introduction Of CAR-T Cell Therapy in Cancer Tumors

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**Abstract.** CAR-T cell therapy shows revolutionary promises in the therapeutic treatment of cancer, offering the possibility of long-lasting remissions and potential cures for certain cancers. The principle of this immunotherapy is to improve patient's own T cells. It shows clinically significant efficacy in difficult-to-treat cancers. However, when overcoming solid tumors, this therapy encounters limitations of tumor infiltration, the multiple limitations of the tumor microenvironment, and the toxicity produced by CAR-T cells themselves. All these factors make the efficacy of CAR-T cell immunotherapy significantly reduced. The investigation of the causes leading to these issues is a central focus of research. The focus of this paper is on the accomplishments made in CAR-T cell therapy and the drugs that are currently available on the market. It will also cover current shortcomings of this immunotherapy and difficulties that need to be overcome. The paper will also cover a vision and prediction of future CAR-T cell therapy technology.

**Keywords:** CAR-T cell therapy; TME; Limitations of CAR-T.

## 1. Introduction

Immunotherapy is a method of harnessing the body's immune mechanisms to reinforce the patient's immune function in order to eliminate tumor cells [1]. Immunotherapy has a critical role to perform in the treatment of cancer. Nowadays, many new types of immunotherapies are continuously being emerged. Adoptive T cell Therapy (ACT), Cancer vaccines, Anti-tumor antibodies, Stimulatory factors (cytokines) and Immune checkpoint inhibitors as several kinds of therapies for cancer immunotherapy are constantly at its forefront.

Chimeric antigen receptor (CAR) T-cell therapy, a prominent treatment in therapeutic immunotherapy, is an adoptive T-cell therapy. This is attributed to its precise targeting, personalization of treatment, relatively long-lasting effects, and immune memory. This high degree of specificity facilitates the minimization of damage to healthy cells and mitigates potential side effects compared to traditional chemotherapy and radiation therapy [1]. The principle of this therapy, in a nutshell, enables the patients' T-cells to be genetically programmed to create a synthetic receptor called a "CAR". The CAR displays a fragment of a specific antibody fused to an intracellular T-cell signaling domain that binds to antigen. The patient is then reinfused with the amplified CAR-T cells.

In this review, we are going to discuss some of the latest therapeutic approaches and outcomes of CAR-T cell therapy for haematological malignancies as well as in solid tumors. We will also focus on the potential dilemmas and therapeutic approaches to TME in solid tumors.

## 2. The current application of CAR-T cell therapy

### 2.1. Achievements of CAR-T cell therapy in treating hematological tumors

CAR-T cell therapy represents a significant milestone in the area of hematologic malignancy treatment with demonstrated efficacy in Acute Granulocytic Leukemia (AGL) and Multiple Myeloma (MM). In recent trials and product applications of this therapy, third-generation CAR-engineered T cells were demonstrated to have favourable clinical effectiveness and significantly lower treatment-related toxicity. A separate academically motivated study with third-generation retroviral vectors of good production specification was conducted by a research team consisting of Schubert et al. The study treated adult r/r ALL patients with increasing doses of CAR-T. This trial validated the Third-

generation CAR-T product as very safe and promising after a 90-day observation and response evaluation of 13 patients. It also demonstrated that there is a strong possibility that the product can completely cure ALL patients [2]. In solid tumors, this therapy also made some breakthroughs in recent years. Progress was made in enhancing tumor penetration and targeting multiple antigens. The effects of combination therapies, in which CAR-T cells are combined with radiotherapy, targeted therapies, or other immunotherapies to produce synergistic effects and improve tumor response, are also under exploration.

In recent years, the Food and Drug Administration (FDA) and the National Medical Products Administration (NMPA) have authorised eight products for CAR-T cell therapies (Table 1).

**Table 1.** NMPA and FDA authorised CAR-T cell therapies.

Commercial Name	Target	R&d Pharmaceutical Company	Approval Time	Approval	Indication [1]
Kymriah	CD19	novartis	2017.8	FDA	B-ALL DLBCL
Yescarta	CD19	Gilead Sciences	2017.1	FDA	DLBCL FL HGBCL
Tecartus	CD19	Gilead Sciences	2020.7	FDA	MCL B-ALL
Brevanzi	CD19	Bristol Myers Squibb	2021.3	FDA	LBCL FL3B HGBCL
Abecma	BCMA	Bristol Myers Squibb	2021.6	FDA	MM
Yescarta	CD19	FOSUNKite and Gilead Sciences	2021.6	NMPA	DLBCL PMBCL NOS
Carteyva	CD19	JW Therapeutics	2021.9	NMPA	r/r LBCL
Carvykti	BCMA	Legendbiotech	2022.2	FDA	MM
Fucaso	BCMA	IASO Bio	2023.6	NMPA	MM

## 2.2. Recent clinical trials

Although the FDA and NMPA have now approved some products on CAR-T cell therapy. This therapy for hematologic malignancies has had somewhat of an achievement, but there are still a large number of dilemmas that need to be overcome. Based on recent studies, some patients with hematological malignancies experience recurrence after CAR-T cell injection due to this cell over-expansion. CRISPR/Cas9 technology has demonstrated efficacy as a means of enhancing the multiplication as well as durability of this cell in vivo. Armed with this strategy, it is possible to decrease attrition, generate memory phenotypes and find out new targets to increase antitumor effect. It suggests a new line of research for some of the current refractory hematologic malignancies [3]. Recently, in a study by Guo, it was shown that the immune checkpoint B7-H3 can be useful in the immunotherapy of refractory hematologic and solid malignancies. B7-H3 has immunomodulatory properties and is a B7 family member. B7-H3 was shown to inhibit the proliferation and persistence of both CD4+ and CD8+ effector T cell propagation and activity. By virtue of this ability this leads to immunosuppression and tumor progression. Accordingly, B7-H3 serves as an aid to tumor growth in the tumor microenvironment. And this immune checkpoint could provide new insights for the treatment of some cellular tumors with high recurrence. Therefore, B7-H3 can be a target for this therapy, especially in the diagnosis and therapy of brain tumours [4].

### **2.3. The principle of CAR-T cell therapy**

Since concept of CAR-T cell therapy is to ameliorate patients' own T-cells. Thus, this therapy can be tailor-made for the patient, offering a more efficient and customized therapeutic effect. This therapy is an immunotherapy that uses CAR gene technology to give T-cells the ability to kill tumors in a tumor-specific manner. The therapy represents a novel approach in the fight against cancer, with the potential to revolutionise current treatments. The first step in utilizing CAR-T cells to deal with cancer is the collection of T cells. Then genetic engineering is utilized to modify the T cells to introduce genes for the CAR structure. Cultivation and expansion is performed in the laboratory. The infused cells are subsequently reintroduced into the patients' body. Circulating CAR-T cells can then identify cancer cells through the CAR's scFv structural domain, which recognizes specific antigens [5]. Furthermore, this therapy overcomes setbacks linked with traditional cancer treatments by utilizing immune system's potency to pinpoint and eliminate cancerous cells. Potentially, it can lead to complete remission or even cure in patients who have failed standard treatments or who suffer from relapsed/refractory disease.

## **3. The brief introduction and shortages of CAR-T cell therapy**

### **3.1. The structure of CAR-T cells**

The construction of CAR-T cells is well-designed to merge specificity of antibody with cytotoxicity capability. The structure of this cell consists of extracellular, transmembrane and intracellular domains [1]. The structure of CAR-T cell is intended to equip T cells with a specific receptor that empowers them to selectively eliminate cancer cells. In this therapy, selecting and designing the receptor involves various factors to consider. It involves a balance between affinity, specificity and signal strength to both ensure effective targeting and killing of cancer cells while minimizing off-target effects.

Significant advancements have been made in the field of hematological malignancies through current state of CAR-T cell therapy technology. A major issue that continues to cause headaches for researchers is the difficulties that this therapy needs to be overcome in solid tumors. Difficulties faced in solid tumors include restricted tumour transport and infiltration at the affected site, hypoxia, immunosuppression in the TME, heterogeneity, and antigen escape, in addition to CAR-T cell exhaustion which can prove fatal. Daei's research team summarized EGFRvIII-specific CAR-T cells for the field of malignant glioblastoma (GB) clinical trial. These trials employed EGFRvIII as a therapeutic target and displayed potent anti-tumor properties in preclinical trials. The clinical trial evaluation, with some modifications, still possesses a monoclonal antibody binding site [6]. However, the sophisticated environment with a diverse range of properties in the TME results in a plethora of challenges that persist in this therapy.

### **3.2. Shortage of CAR-T cell therapy**

For the past several years, CAR-T cell therapy has attained a noteworthy degree of achievement in the management of leukemia and lymphoma. Similarly, there is a moderate success in the field of solid carcinomas. It has also received increasing attention as a potential treatment for a variety of cancers. However, this cell therapy is characterized by Cytokine Release Syndrome (CRS), limited applicability to solid tumors, complexity and cost of manufacturing, and risk of treatment-related toxicity and off-target adverse events [1]. CAR-T immunotherapy remains to encounter many shortcomings due to both the limitations of this immunotherapy itself and the complexity of the malignancies it targets.

#### **3.2.1 Infiltration**

One of the crucial elements of CAR-T cell therapy for solid carcinomas is achieving CAR-T cell infiltration into the tumor. Infiltration denotes the capability of this cell to migrate into TME, thus efficiently targeting tumor cells. In the hematologic system, CAR-T cells are presented with

increasing number opportunities to interact with hematologic tumor cells. In contrast, CAR-T cell faces greater obstacles to penetrate solid tumors through the bloodstream. In addition, the lack of chemokine expression during T-cell infiltration of tumour tissue. Also, compact fibrotic mesenchyme is present in solid cancers. Thus, the ability of this cell to migrate and infiltrate tumour cells is reduced. To address this issue, researchers have proposed that one approach is to modify these cells to enhance their ability to infiltrate the TME. The migration of CAR-T cell toward tumor cell can be improved by engineering this cell to express chemokine receptors that are highly expressed in the TME. Molecules that enable them to degrade the extracellular matrix could facilitate their infiltration into tumors. To enhance its infiltration and reprogramme the TME, the researchers engineered lysosomal adenoviruses (oAds) to express the chemokine CXCL11 [7].

### 3.2.2 Balance between CAR-T cell efficacy and toxicity

Another great challenge is how to balance CAR-T cell's efficacy and toxicity. This cell can cause on-target/off-tumor toxicity. It can target and destroy not only cancerous cells, but also healthy tissues that express the specific antigens. For example, CD19 is a common target antigen in this therapy for B-cell lymphoma. Nevertheless, healthy B cells also express CD19 [7]. It can cause serious side effects and border of the use of this immunotherapy, which can be potentially harmful to patients, as well as unknown side effects. This cell is designed to recognize and target specific antigens on tumor cells. Identifying targets that are exclusive to tumor cells and contribute significantly in their growth is crucial, while minimizing their expression in normal tissues. Therefore, we need to select an appropriate target. But the range of targets that can be selected by CAR is small. Moreover, there are not so many targets on CAR-T cells. The reason for this is that CAR is unresponsive to MHC. Detection techniques can be used to determine if surface antigens are present on the plasma membrane. In addition, the antigen sensitivity of CAR is also significantly lower. CAR requires a considerable number of antigens, over 1000, to be activated. Thus, it largely suggests that low antigen density is disadvantageous for CAR-T cell therapy. Anti-CD19 CAR-T cell therapy then eliminates both cancerous and healthy B cells, resulting in lower serum immunoglobulin levels. In the case of solid tumors, the search for highly specific targets becomes even more difficult due to the considerable heterogeneity of these tumors. Despite ongoing investigations, there are varying degrees of side effects with this immunotherapy. To address this issue, next generation of this cell is being developed with logic-gate control. Additionally, researchers are exploring the potential of combining oncolytic viruses (OVs) with CAR-T cell therapy. These strategies aim to improve target specificity, overcome antigenic heterogeneity, and enhance the effectiveness of this therapy. Overall, target selection is a key aspect of CAR-T cell therapy, and finding right target to minimize non-tumor toxicity and maximize therapeutic efficacy is critical to the success of this therapeutic approach [8].

### 3.3. Mechanisms of TME limiting immunotherapy

The TME is characterized by abnormalities in the vascular system, dense extracellular matrix, interstitial fusion pressure, hypoxia, and the presence of immunosuppressive cells. There are various Influences within TME that significantly contribute to the progression of tumors. In the initial stages of tumor development, the tumor microenvironment facilitates the development of premature cancer cells, which enables the tumor to subsist, infiltrate, and proliferate. It is also known to promote angiogenesis to overcome the hypoxic and acidic environment.

In contrast to blood tumor, solid tumor is located deep within the body, which makes it difficult for cells to approach them. Although this therapy has had remarkable success in treating malignant hematologic cancers, complex interactions within the TME may limit its effectiveness. Immunosuppressive environment, heterogeneity, antigen escape, stromal cells and ECM, hypoxia, metabolic competition and cytokine depletion are some of the key factors that contribute to TME resistance to CAR-T cell therapy.

These features can be key factors preventing CAR-T cell penetration. It is precisely because TME is a sophisticated and evolving of an environment that its characteristics continue to promote tumor progression. Therefore, it renders CAR-T cell unable to function under the background conditions of

TME. Immune checkpoints are a major research target in TME. Immune checkpoints are utilized by tumor cells to evade immune surveillance and suppress CAR-T cell activity. Immune checkpoints are regulatory mechanisms that are responsible for maintaining immune homeostasis and preventing excessive immune responses. Tumor cells can hijack these checkpoints to suppress the immune response, making it difficult for immune system, including CAR-T cell, to effectively target and eliminate tumors [9]. One of these checkpoints is Lymphocyte Activation Gene 3 (LAG-3), a checkpoint receptor. When it interacts with the ligand MHC class II molecules, it can inhibit T cell responses. Tumor cells can express MHC class II and bind to LAG-3 on T cells, which causes a decrease in CAR-T cell activity.

While TME affects the infiltration, activation and function of T cells, TME consists of a variety of cells in an altered fibrous ECM. ECM stromal cells and the ECM constitute a mechanical shield that hampers the entry of T cells. Treg cells (regulatory T cells) and CAR-T cells share a connection, which is so complicated that it has both positive and negative effects. On the one hand, Treg cells can impede CAR-T cells' activity and restrain their anti-tumor function. Treg cells play a role in immunomodulation and tolerance. They can inhibit the activity of effector T cells, including CAR-T cells, through various mechanisms, such as secretion of immunosuppressive cytokines such as IL-10 and TGF- $\beta$ . Such inhibition by Treg cells weakens effectiveness of the therapy and diminishes tumor deaths. On the flip side, Treg cells can also offer advantageous effects in this immunotherapy. They are able to contribute to controlling excessive immune responses and preventing immune-related toxicity. Treg cells work to help regulate these immune responses and limit the toxicity linked with this therapy.

Overall, the relationship between Treg cells and CAR-T cells is complex and context-dependent. Treg cells not only inhibit CAR-T cell activity, but also act as immunomodulators to prevent excessive immune responses. More research is needed to better control the delicate relationship between the two to balance the pros and cons [3].

### 3.3.1 Hypoxia

In addition, relatively low pH, hypoxia and high levels of immunosuppressive metabolites characterise the TME. This environment can suppress effector lymphocytes, including CAR-T cells, with the deprivation of nutrients some nutrients essential for cell growth such as glucose. One specific metabolic pathway that can be upregulated by tumour cells is the expression of indoleamine 2,3 dioxygenase (IDO). It catalyses changes of intracellular amino acids into an immunosuppressive metabolite. Insufficient oxygen supply in the TME can cause hypoxia, which impairs anti-tumour immune responses through a range of mechanisms. Hypoxia-inducible factor proteins, such as HIF-1 $\alpha$ , play a role in upregulating immune checkpoint molecules on tumor cells as well as myeloid-derived suppressor cells (MDSC) [7]. In this way, it can suppress T cell function.

### 3.3.2 IFP and Stiffness

Moreover, the high interstitial fluid pressure (IFP), mechanical stress, and stiffness linked with the secretion of cytokines and chemokines by tumor-associated cells is augmented, which results in inhibition of T cell activity. Specifically, CD8+ T cell function decreases while Tregs activity increases. TGF- $\beta$  is an immunosuppressive cytokine that suppresses the immune response by late stage. TGF- $\beta$  also promotes the conversion of CD4+ T cells into suppressor Tregs and triggers epithelial mesenchymal transition and cancer-associated fibroblast proliferation. In addition, high IFP and mechanical stress within the TME prevents T cells from effectively resulting in tumor deaths. IFP and fluid flow within the TME creates a challenging barrier to T cell infiltration and promotes the production of immunosuppressive factors. Fluid and macromolecules in the TME increase osmotic pressure as they accumulate. Inflammation and cancer-associated fibroblasts (CAF) were triggered, whereupon ECM was produced. Elevated IFP and mechanical stress imposed on the tumor margins due to high IFP and fluid flow lead to furthermore complications [10].

## 4. Summary

Several related drugs have been introduced in hematologic malignancies, driving CAR-T cell therapy to be further explored. Among problems encountered by this immunotherapy in the treatment of solid carcinomas, how the CAR-T cells fully infiltrate tumor and how to balance efficacy and toxicity of CAR-T cells determine upper limit of this therapy. In solid tumors, however, TME is characterized by immune checkpoints, hypoxia, limitations of infiltration, IFP, and stiffness. All of these factors affect the efficacy and effectiveness of the therapy. Clinical trials continue to explore the efficacy and impact of strategies made in the face of these dilemmas, and a growing number of technologies and several generations of CAR-T cell therapy products demonstrate importance of understanding the mechanisms of action in tumor cancers. Further research is needed in the future to explore ways to address the balance of efficacy and toxicity of this therapies, as well as to not limit CAR-T cell infiltration into tumors. There could be a focus on exploring the use of combination therapies to improve synergistic effects. Multiple targets could also be explored for effective and customized multiple combination targeted therapies.

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