

# CAR-T Therapy: Defective Currently but Promising in the Future

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**Abstract.** Since CAR-T therapy was approved by the FDA in 2017, this therapy has become one of the most important cancer treatments for patients with some certain hematological malignancies. However, severe adverse effects, such as cytokine release syndrome (CRS) and neurotoxicity, cause low survival. The existed and approved drugs against these side effects would possibly become the trigger of other side effects or reduce the effect of therapy. Besides, CAR-T therapy that has been successfully applied in hematological malignancies shows weak efficacy in solid tumors. In this article, we introduce main limits of this promising therapy, integrate existed solutions and explore possible strategies to overcome these challenges. With more profound studies about CAR-T cell and associated side effects and limitations, we believe that there would be more novel strategies to optimize this revolutionary therapy will appear and be realized in the foreseeable future.

**Keywords:** chimeric antigen receptor, cancer immunotherapy, cytokine release syndrome, Immune effector cell-Associated Neurotoxicity Syndrome

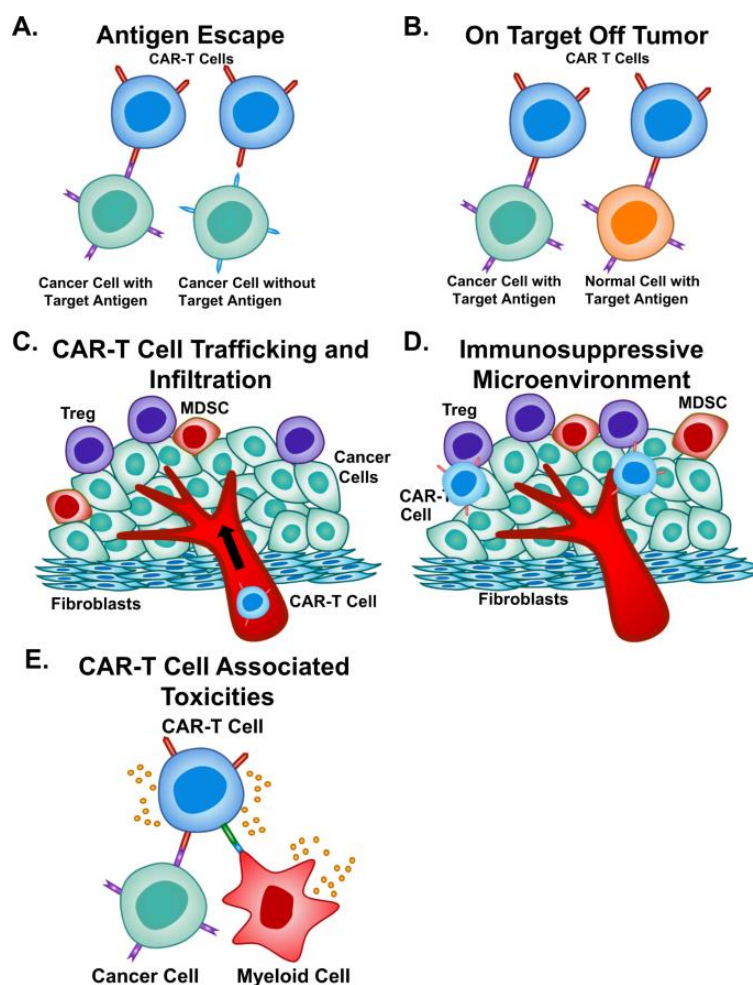
## 1. Introduction

Chimeric antigen receptor T cell therapy, or CAR-T therapy, is a novel therapy against cancer. This therapy isolates T cells from blood of the patient or a donor, then the T cells are activated and genetically engineered to express the CAR. The costimulatory domains (CD28 and 4-1BB for instance) and single chain variable fragment (scFv) are fused to the signaling domain as well. After the engineered T cells are delivered to the cancer cells, the CAR constructs detect and ligase with some specific membrane proteins of tumor cells. Consequently, the tumor cells are induced to death.

Compared with other cancer therapies, CAR-T therapy shows better specificity to tumor cells, which means that CAR - cell can better prevent killing normal cells.

This technique was approved by the FDA in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) treatment in 2017 [1].

However, this therapy has some side effects, and some are rather severe, just like other cancer therapies (Fig. 1E). Among them, CRS and neurotoxicity are the most common side effects. CRS is one of the side effects shared by different types of immunotherapies, and the result of CRS can be fatal [2]. And the clinical data shows that CAR-T therapy would cause a series of neural symptoms, including delirium, headache and language disturbance [3].



**Fig. 1** Main limitations of CAR-T therapy [3].

CAR-T therapy is more used in non-solid tumor and its efficacy against solid tumor is limited [1]. There are two major factors, properties of tumor microenvironment (TME) that differs between solid tumors and hematological malignancies, and the loss of detectable targets for immune system [3]. Multiple immune cells are compressed within immunosuppressive TME, such as regulatory T cells and CD4<sup>+</sup> T helper 2 (Th2) cells. Immunosuppressive TME helps tumor cells to escape from the detection of immune system and would also suppress and inhibit the activity and infiltration of CAR-T cells [3] (Fig. 1A-D). Beyond, though CAR-T therapies have been successful in hematology, the major clinical properties of hematological malignancies are not much similar as those of solid tumor [3].

Aiming at the two aspects of limitations, this article will show some possible and promising solutions towards these problems.

## 2. Side Effects

### 2.1. CRS

CRS, is one of the most frequently reported side effects in different kinds of immunotherapies and can be observed after some surgeries or some viral infections. It is the excess-inflammatory response due to over-reaction of immune system. There are several factors that could trigger CRS, such as infections and immune-modulating drugs, especially treatment with T cell engaged [4]. Increase of cytokines level (IL-6, TNF- $\alpha$ , INF- $\gamma$ , for instance) is observed in patients with CRS, and it seems to be a critical element in CRS mechanism.

In CAR-T therapy, CRS is the most common acute toxicity and the occurrence rate ranges between 37% to 93% in a series of reports [4]. Most symptoms of CRS occur in a few hours to 2 weeks after the CAR-T cell injection [5]. It is likely to be detected that IL-6 level increases and this increase can be considered to be correlated with CART-cell-associated CRS [4]. The major symptom is fever ( $\geq 38^{\circ}\text{C}$ ). The other nonspecific symptoms include myalgia, malaise, fatigue, gastrointestinal complaints, and rash that is sometimes observed in clinical reports [6]. In some clinical cases, CRS is self-limiting, which can be relieved and resolved after proper care and sufficient rest. However, CRS sometimes might be fatal, which triggers pulmonary edema caused by capillary leak, hypotension, multiorgan failure, and even circulatory collapse [4, 7].

## **2.2. Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)**

ICAN is another group of frequent adverse effects during and after CAR-T therapy. Though the detailed mechanism remains unknown, researches show that ICAN is possibly related to destruction of blood-brain barrier triggered by cytokine accumulation in cerebrospinal fluid (CSF) [8]. The early symptoms, including expressive aphasia, tremor etc., could develop to more severe symptoms like global aphasia and seizures [8].

## **2.3. Treatments of CRS and ICANS**

Currently, corticosteroid therapy and tocilizumab (TCZ) treatment are the only two therapies approved by many clinical guidelines to treat CRS relative to CAR-T therapy [9].

Corticosteroids (dexamethasone, for instance) have potent anti-inflammatory effects and this family of drug is widely used in surgery and CRS treatment. Though a few researches show that corticosteroids, even in large dose, would not negatively influence the effect of CAR-T therapy as what some early studies doubted, its ability to inhibit immune system is still an issue which would increase the risk of infection [9-11].

TCZ, one of the most broadly used therapy against CRS during CAR-T therapy, is an IL-6 receptor inhibitor antagonist. It can inhibit IL-6 binding to its corresponding receptor, which blocks IL-6-mediated signal transduction. Clinical reports show that patients treated with TCZ show higher possibility and worsened degree to be affected by neurotoxicity [9]. Main IL-6 elimination is achieved through IL-6 receptor-regulated exhaustion. Markable rise of IL-6 in serum is frequently reported after TCZ treatment [12]. This rise can promote the penetration of IL-6 through the blood-brain barrier into CSF, however TCZ possesses poor ability of penetration compared to IL-6 [13]. It is believed in theory that cytokine-mediated neurotoxicity would be aggravated by TCZ which contributes to IL-6 increase in CSF [9]. And as an inhibitor of the immune factor, IL-6, long-term or large-amount utilization of TCZ would lead to higher risk of infection as well.

These treatments both have disadvantages and side effects and the treatment against CRS is still insufficient. To reduce the effect of CRS, improvement of CAR-T cell itself would be a resolution.

## **2.4. Possible Strategies to Reduce the Side Effects**

One strategy is to alter the CAR structure. One route is to reduce the affinity of binding domain. To achieve the anti-tumor activity, the affinity should be higher than a certain threshold; however, if the affinity exceeds another higher certain threshold, the cytokine and inflammatory response would reach a hazardous level. However, this modification requires higher level of expression of tumor cells to maintain the efficacy. Therefore, to reduce the toxicity, CAR affinity should be controlled between the two thresholds. In another possible way, altering the CAR structure and transmembrane regions can modulate the cytokine secretion when CAR-T cell is activated. The costimulatory domain could be another possible domain to modify. According to researches, the less toxic 4-1BB costimulatory domains are suggested to be preferentially included in high body burden and in tumor with high antigen density [14, 15]. Anti-CD19 CAR molecule (CD19-BBz (86)) is a type of engineered CAR

molecule, which is designed with the CD19-BBz as the prototype and is combined with less toxic co-stimulatory domains (for example, 4-1BB and CD3 $\zeta$ ) [15]. Compared to its prototype, this CAR molecule produces less cytokine and it is less likely to cause CRS [15].

The second strategy is to decrease CAR immunogenicity. In some cases, cytokine toxicities are caused by that CAR constructs are not recognized as “magic bullet” but heterogenous antigens by the immune system of the patient. Therefore, including or fusing purely human-sourced or humanized antibody fragments instead of those widely used antigen fragments extracted from murine could be promising to reduce the CRS. Additionally, humanized antibody fragments possess the ability to reduce the potency of cytokine-triggered toxicity and increase the duration of CAR activity [16]. However, this strategy would probably increase the cost of CAR-T therapy that has been too expensive compared to other therapies.

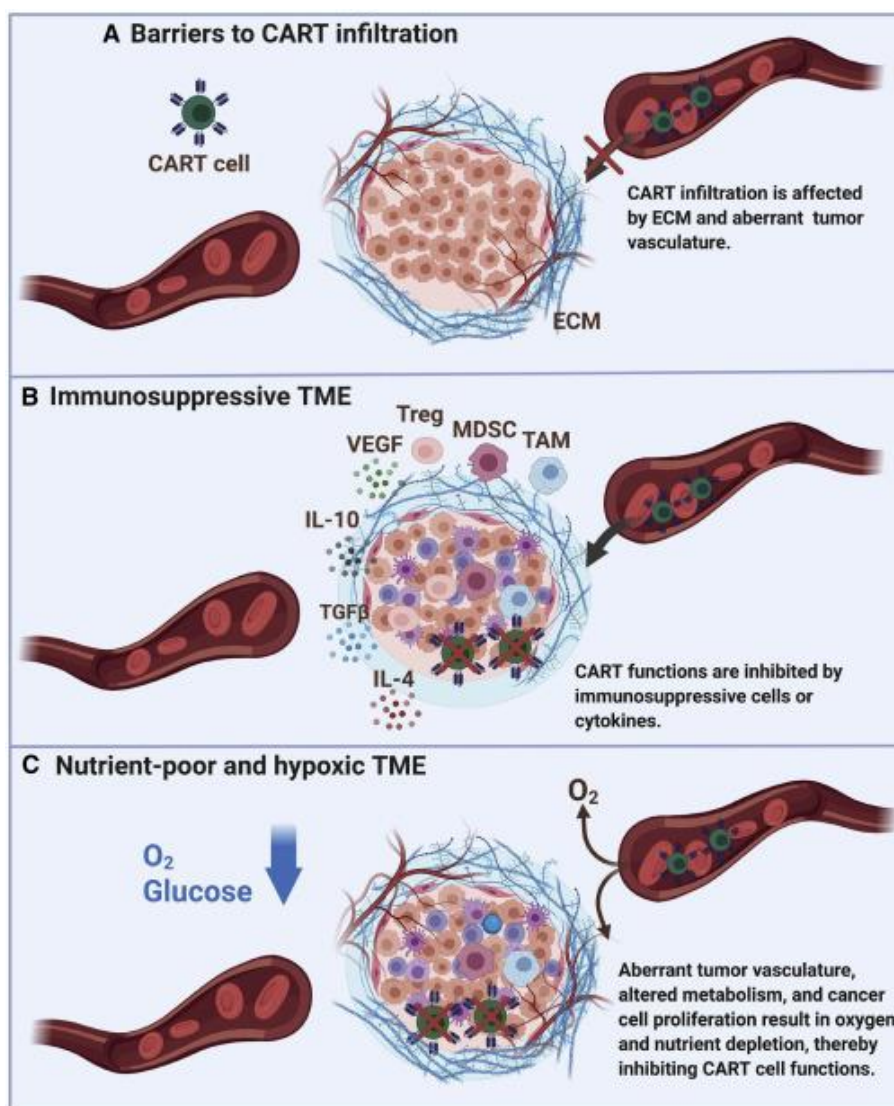
Recently, a novel inspiration has been researched: CAR “off-switches” or suicide gene strategies. This strategy can selectively reduce the amount of CAR-T cells when adverse events, such as CRS, occur [2, 15]. This strategy could be realized through a secondary inducing agent. For instance, the clearance of CAR-T cells after rituximab injection is facilitated by the expression of CAR constructs modified to express full length CD20 or CD20 mimotopes. Nonetheless, CAR “off-switch” method that shows effect of antibody-mediated in a relative slow rate would be restricted by the limited efficacy in patients who are suffered by severe, acute CRS and require immediate treatment to reverse such situation [2, 15]. Such strategy and other similar approaches share the biggest limitation: though they can ensure the safety of patients in adverse events, the trigger of suicide would stop the whole CAR-T therapy abruptly [2]. Therefore, the existence of CAR “off-switch” approaches would encourage doctors to make more safety-ensuring strategy and regard CAR “off-switch” as the last countermeasure.

### **3. Limitations in Solid Tumors**

#### **3.1. TME**

Solid tumors possess some properties that differ from properties of non-solid tumors. TME is able to obstacle T cell trafficking to the target site, affect the function of CAR-T cell, and lead to CAR-T cell exhaustion. TME possesses more functions in solid tumors, and it additionally forms a physical barrier that prevents infiltration of CAR-T cell or other immune cells. One major barrier is that the cancer associated fibroblasts (CAFs) and other stromal cells form dense fibrogenic barrier [17].

Transforming growth factor  $\beta$  (TGF- $\beta$ ), which controls the activation and triggers tumoral secretion of extracellular matrix (ECM) proteins, is what causes this T-cell-excluding barrier [18] (Fig. 2A). Another major barrier, aberrant vasculature of solid tumors, would reduce the amount of tissue oxygen and nutrients, blocking T cell infiltration of TME through a series of pathways [17] (Fig. 2C).



**Fig. 2** Three pathways that limit effect of CAR-T therapy in solid tumor [17].

TME can create the immunosuppressive microenvironment that attracts immunosuppressive Treg cells, which release immunosuppressive cytokines, such as competitive consumption of interleukin-2 (IL-2), to prevent the activation of T cell. (Fig.2B) Some metabolites are proved to be suppressive on effector T cells [17, 19]. Moreover, some TMEs show the ability to induce CAR-T cells into dysfunctional exhaustion [20].

### 3.2. Strategies Against Barrier of TME

TME limits CAR-T cell infiltration mainly in a few methods, including formation of physical barrier, escape of the tumor antigen and abnormal tumor vasculature.

One direct strategy is localization of CAR-T cell delivery or injection. Intravenous administration is the most frequently employed CAR-T cell delivery approach. Nonetheless, more accurate and localized injection may help to better infiltrate solid tumor cells via bypassing barriers of TME to some extent. In this strategy, the dose requires about 30 less fold to ensure its safety and durable remission [21].

Another strategy is to further engineer CAR-T cell. Chemokines functions in building the TME-interior immune response. Only when chemokines ligase with their corresponding receptors will immune cells recognize the “invader”. Therefore, tumor evades from immune response through mismatches between chemokines and receptors or insufficient expression of chemokine receptors or even both. Some researchers have designed CAR-T cell with overexpressed chemokine receptors to improve the ability of engineered T cell to penetrate TME barrier [22]. Some groups attempt to

engineer CAR-T cell to recovery tumor vasculature to its normal phase. Abnormal tumor vasculature contributes to an environment against CAR-T cell infiltration. Structurally irregular blood vessels that squeeze the space, impaired blood flow that disturbs CAR-T cell affinity, partially hypoxic that impedes CAR-T cell respiration and higher interstitial fluid pressure, etc., which are all negative factors for CAR-T therapy [17]. Vascular endothelial growth factor receptor (VEGF-R) is found to be helpful to promote angiogenesis [17]. A few studies show that normalization of tumor vasculature is achievable under the utilization of VEGF-R targeting CAR-T cell, which therefore facilitates the infiltration [23]. Engineered CAR-T cell is also employed in studies of ECM density reduction. Researchers attempt to target fibroblast activation protein (FAP), the product of CAFs expression, to reduce the density of tumor ECM. Another idea is to degrade over-dense tumor ECM in the way of designing special CAR-T cells that express certain enzymes.

#### 4. Conclusion

Though CAR-T cell researches have got significant accomplishments in tumor suppression and treatment, more endeavors are demanded to guarantee better clinical efficacy and higher survivor rate. The two major factors that limit CAR-T therapy are severe side effects (and even higher mortality rate) and relatively poor efficacy against solid tumors. Recent studies show several strategies to overcome these defects, including CAR-T cell modification and engineer, CAR “off-switch” strategy, and localized delivery. These strategies, which employ novel gene editing technology and newly found mechanisms, not only enhance the CAR-T cell curative effect in different types of tumors, but also reduce the side effects and risk of death. More methods to optimize CAR-T therapy and more studies in undeclared CAR-T-associated mechanisms are still the key fields to study. Ultimately, the tasks to overcome the current defects and optimize CAR-T therapy is a multidimensional and complicated issue that demands approaches from different fields involved in the future.

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