

# Toxicities and related improvements of CAR-T Cell in the treatment of cancer

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**Abstract.** Chimeric antigen receptor (CAR)-T cell therapy is a revolutionary new therapy. Since its first discovery, CAR-T therapy has become the fastest growing and most widely used branch of anti-cancer immunotherapy. The field of hematological malignancies has undergone a rapid transformation due to the advent of this technology, which is responsible for over 50% of all cell therapies that are being developed or sold nowadays. With the extensive use of CAR-T cell technologies, more and more advantages and uses have emerged. However, as CAR-T was approved for marketing, shortcomings and toxicities such as CRS, CRES, and cytopenias were also discovered in clinical practice. A number of limitations, including high prices, limited accessibility, and unregulated quality also restrict CAR-T cell therapy's further promotion and application. This review focuses on CAR-T cell therapy's mechanisms, toxicities, related improvements, and future developments. Also, this article makes an analysis and summary of CAR-T therapy, hoping to provide a feasible reference for future research.

**Keywords:** Cancer treatment; CAR-T cell; Toxicities.

## 1. Introduction

Adoptive T cell therapy (ATC), which includes Tumor-Infiltrating Lymphocyte (TIL) therapy, Engineered T Cell Receptor (TCR) therapy, Natural Killer (NK) Cell therapy, and CAR-T Cell therapy, uses transplanted T cells to kill cancer cells. Among these, a primary one is the CAR-T cells [1]. CAR is a modular synthetic receptor and has four major components: an external target antigen-binding domain, a hinge region, a transmembrane domain, and one or more intracellular signaling domains. T cells are the white blood cells which are created in the bone marrow. Their primary job is to attach themselves to a particular antigen on antigen-presenting cells and create an MHC complex. T cells have the ability to target various malignancies through their recognition of tumor-associated antigens, which are common to the tumor types and individuals. As a cutting-edge technology for cancer treatment, CAR-T cell therapy has great potential. However, because of its toxicities and expensive price, which limit its wide application to a large extent, more money, time, and manpower are needed to explore this technology. Future research and development work are needed to solve these problems and more efforts are needed in this area to ensure the safety and cost-effectiveness of this therapy.

## 2. The mechanism of the CAR-T cell therapy

CAR-T cells are artificially modified T cells. Specifically, CAR was added to the T cell by modification. To mediate their anti-tumoral activities, CAR-T Cells can form a non-classical immunological synapse and utilize various mechanisms such as the granzyme and perforin axis, the Fas and Fas ligand axis, and cytokine production to sensitize the tumor stroma [2]. CAR-T cells can be grown and greatly enlarged when Peripheral blood mononuclear cells (PBMCs) are virally transduced, enabling the autologous manufacturing of this therapy prior to patient administration. CAR-T cell treatment as a novel technology, integrated several crucial functions of traditional T cells. It combines cytotoxic properties with memory and the specificity of a monoclonal antibody. Usually, the patient's T-cells are taken out of their body and sent to the lab for alteration. In this instance, the lab uses genetic engineering to introduce a protein into the CAR somatic cells. When reinjected into

the patient's body, these CAR-T cells seek for the cancer cells and precisely clear them in order to accomplish therapeutic objectives. These CARs function as the receptors that enable the CAR-T cells to recognize cancer cells more quickly. CAR-T cells enhance the immune system's capacity to react against leukemia and lymphoma cells, hence improving cell detection and eradication.

### 3. The toxicities

There are several obstacles which impede the growth and adoption of CAR-T cell therapy. Firstly, a variety of factors, including the extrinsic (tumor suppressive microenvironment and tumor cells with target deletions or mutations) and intrinsic (inadequate CAR-T cell proliferation or short persistence) ones may both affect the failure of CAR-T cell therapy. Secondly, the safety issues still require scientists' special attention. In addition to driving the clearance of tumors, CAR-T cells can have potentially fatal side effects, such as neurotoxicity and cytokine release syndrome (CRS), which are caused by the "on-target/off-tumor effect" that comes from low antigen expression specificity, excessive cytokine release, and CAR-T cell overactivation. Moreover, the highly proliferative tumors continue to progress during the approximately two-week duration of the current production cycle. Furthermore, following multiple chemotherapies, cancer patients often experience lymphocytopenia or congenital immunodeficiency, which results in poor T cells that are insufficient for CAR-T cell manufacturing. Thirdly, the labor-intensive and expensive production process of CAR-T cells continues to be a barrier to the widespread use of CAR-T cell therapy. The cost of a single infusion of Kymriah is \$475,000, and the entire cost of treatment with Kymriah or Yescarta comes to around \$1 million for every single patient [3]. Last but not the least, the resistance after CAR T-cell therapy is also a serious difficulty.

To start with, one of the most frequent toxicities of CAR-T cell therapy is CRS. Numerous variables, such as illness features, tumor load, CAR structure, and CAR-T cell dosages, all influence the occurrence of CRS. CRS can present with a wide range of clinical symptoms. Fever, myalgia, exhaustion, hypoxia, poor appetite, hypotension, and even organ malfunction are common ones. Furthermore, if left untreated, it may quickly develop into potentially fatal diseases such as numerous organ dysfunction and hemodynamic instability. The results of a new trial, however, showed that CRS patients with > grade 2 had higher rates of remission and longer progression-free survival (PFS) than patients with < grade 2, suggesting that Adequate CRS could enhance CAR-T treatment efficacy. Since IL-6 is the primary mediator of CRS, tocilizumab, an IL-6 receptor antagonist, is largely advised to treat the clinical symptoms of CRS. Different treatment plans are chosen based on the CRS grading. For grade 1 CRS, both symptomatic and supportive therapy are recommended. For grade 2 CRS with severe symptoms, as well as grade 3 and 4 CRS, tocilizumab plus corticosteroids are advised [4]. CRS has diverse manifestations and is partially similar to infections. Furthermore, it is proved that severe CRS may be correlated with the higher risk of coagulopathy and CAR-T-cell-related encephalopathy syndrome (CRES). Therefore, to facilitate the prevention and management of these adverse events, it is better to clarify their underlying mechanisms [3].

As mentioned above, CRES is a CAR-T cell-related neurotoxicity which typically happens after CRS or concurrently with it. Some of the signs and symptoms of CRES are cerebral edema, headache, dizziness, delirium, and seizures. Because there aren't enough appropriate animal models, it is unclear what the underlying pathogenic mechanisms of CRES are. However, high tumor burden, excessive CAR-T cell growth, and severe CRS may all be connected to an elevated risk of CRES. At the moment, immune-mediated endothelial activation is a recognized mechanism that contributes to CRES. Furthermore, the rupture of the blood-brain barrier is proved to have the ability to allow macrophages and T cells, which includes CAR-T cells, to penetrate the central nervous system. The activation of the brain-resident macrophages called the microglia may be brought on by these infiltrating immune cells and cytokines, which would then intensify regional inflammatory reactions and ultimately cause neurotoxicity. Consequently, immune-mediated endothelium damage serves as a CRES trigger. For instance, Tocilizumab has a low effectiveness in managing CRES due to the fact that it is unable cross the blood-brain barrier. Corticosteroids are advised for the treatment of CRES

due to their increased CNS penetration; they do not influence the proliferation or antitumor actions of CAR-T cells [3]. Moreover, by preventing myeloid cell and T cell infiltration locally, GM-CSF deficit or suppression can both improve CAR-T cell anti-tumor activities and mitigate CRS and CRES.

During CAR-T cell therapy, cytopenia is also a commonly observed common symptom. It usually lasts from a couple of days to months. In clinical studies, cytopenia can occur 30% to 100% of the time., and it includes three common types, anemia, thrombocytopenia, and leukopenia. It has been established that severe CRS and cytopenia are related. Cytopenia may also result from the reduced hematopoietic capacity caused by previous chemotherapy and HSCT. Moreover, since healthy hematopoietic stem or progenitor cells co-express certain target antigens, CAR-T cells may directly drive the death of hematopoietic cells. In a clinical setting, cytopenia can be treated with platelet and red blood cell transfusions, granulocyte colony-stimulating factor (G-CSF), hematopoietic growth factors such as thrombopoietin (TPO), TPO receptor agonists, and sirolimus [3]. The incidence of coagulopathy may be decreased by the prompt and efficient management of CRS. Some individuals with coagulopathy may progress to disseminated intravascular coagulation (DIC), which has a dismal prognosis if prompt and efficient management is not provided [3].

In order to provide an effective therapeutic response, CAR-T cell activation and cytokine production need to be stimulated by reaching a minimum threshold level of the CAR-T cell antigen-binding domain and binding to its target epitope. Nevertheless, there is also a highest threshold level of activation which will result in hazardously high cytokine levels and immune system activation if it's crossed. That is, a CAR-T cell needs to stay within its “therapeutic window” to keep clinically useful. Otherwise, going outside of it will result in a variety of toxicities [5].

The effectiveness of CAR-T cell therapy may be hampered potentially by the cells' limited activity. It has been shown that several immune-stimulatory molecules, including certain cytokines or co-stimulatory molecules, such as CD40L, IL-7, IL-12, IL-15, IL-18, and IL-21, are crucial in controlling the growth and activity of T cells. They might encourage the CAR-T cells to proliferate vigorously, produce more memory-phenotype CAR-T cells, and finally result in the enhancement of their durability. In addition to the injection of these exogenous cytokines, the function of CAR-T cells could also be enhanced by genetic alterations which enables constitutive expression of these immunostimulatory molecules or their receptors.

#### **4. The related improvements**

One of the difficulties in targeting the CAR-T cells is that solid tumor antigens are often expressed to varying degrees in normal tissues. Thus, antigen selection plays an important role in CAR design to prevent "on-target off-tumor" toxicity while also maintaining therapeutic effectiveness. Targeting tumor-restricted post-translational modifications, including shortened O-glycans like sialyl-Tn and Tn overexpressed in solid tumors, may provide a way route from the targeting of antigens on solid tumors that are also found in normal tissues. To increase the clinical usage of CAR-T cell treatments in hematological malignancy, etc., more creative techniques to minimize antigen escape and select antigens capable of generating sufficient antitumor efficacy will need to be developed while minimizing toxicity issues [4].

Numerous combination strategies involving CAR-T cell therapy are currently under investigation and appear to be effective immunotherapies. All products of the commercial CAR-T cell are now made with autologous T cells; however, their continued use has been limited by considerably long manufacturing cycles, expensive manufacturing prices, and decreased lymphocyte quantity and function following multiline chemotherapies. Therefore, as CAR-NK cells and universal CAR-T (UCAR-T) are readily available off the shelf and have minimal manufacturing costs, they have significant promise in the treatment of cancer. Firstly, CAR-NK cells are recently being investigated because of the superior natural killing capabilities of NK cells and their plentiful sources, including peripheral blood, cord blood, NK92 cell line, and induced pluripotent stem cells, in addition to the

fact that graft versus host disease (GVHD) is not induced. Secondly, because the UCAR-T cells are derived from healthy donors, they are considered “off-the-shelf” products. This makes the manufacturing costs decrease while their accessibility increases. Unfortunately, there is a chance that these allogeneic UCAR-T cells will cause GVHD. Moreover, these UCAR-T cells produced from donors can be rejected by the host immune system, which reduces their durability [3].

It looks promising to combine CAR-T cell with small-molecule medicines, and it may have synergistic effects to make the CAR-T cell therapy more efficient. An effective Bruton's tyrosine kinase inhibitor, imatinib, has been licensed for the management of chronic lymphocytic leukemia (CLL) and Mantle cell lymphoma (MCL). Actually, in addition to enhancing the anti-tumor activity of CAR T cells in preclinical and the following clinical trials, ibrutinib can also decrease the incidence of severe CRS. Moreover, it has been shown that the demethylating drugs decitabine and azacitidine may further increase the cytotoxic effect of CAR-T cells. Additionally, the resistance brought on by anti-apoptotic proteins may be reversed by CAR-T cell therapy in conjunction with inhibitors of these proteins. Although this technology is still in its early stages, conjuncting with small-molecule medications is now being investigated through a variety of combinatorial approaches [3].

In 2011, CAR-T cells were employed to treat patients with lymphoblastic leukemia and got impressive outcomes. Kymriah, CTL-019 from Novartis, was the first CAR-T cell therapy treatment approved by FDA in 2017 for patients with relapsed/refractory B-cell acute lymphoblastic leukemia (r/r B-ALL) [6]. The approved indications were supposed to be able to treat the patients younger than 25 years old with refractory or second or subsequent relapse of ALL and the relapsed or refractory diffuse large B-cell lymphoma. This was based on several clinical trials that produced impressive outcomes [7]. Since then, to assess the effectiveness and safety of CAR-T treatment for various solid and hematologic malignancies, scientists all over the world have conducted extensive research activities on these diseases and several more drugs of similar nature have also been approved. Patients who receive the CD19 CAR-T typically have a complete remission (CR) rate of 30–70%; in certain trials, this percentage even exceeded 90%. The fact that these products were produced utilizing a variety of technological approaches indicates that CAR-T cells are remarkably effective in treating lymphoblastic leukemia [6].

## **5. The future of CAR-T cell therapy**

Although CAR-T cell treatment has shown considerable promise in the treatment of hematological malignancies, side effects including CRS, CRES, cytopenia, B cell aplasia, and CRS-related coagulopathy continue to pose a significant obstacle. These issues could potentially be fatal if proactive measures aren't taken. It is important to investigate the potential processes of these difficulties and identify them as early as possible for effective management [3]. Furthermore, a significant percentage of patients experience relapse following CAR-T cell therapy, even though the treatment is widely used for R/R B-cell malignancies. A variety of causes can lead to the relapse of the following CAR-T cell therapy. For instance, the limited durability of CAR-T cells, antigen escape, and the immunosuppressive tumor microenvironment are all possible causes. One of the most common mechanisms of antigen loss is the antigen mutations brought on by CAR-T cell therapy's therapeutic pressure, including lineage switching, splice variants, and biallelic mutations. Together with antigen alterations, tumor immune evasion may also be aided by the decreased antigen density on the surface brought on by CAR-T cell endocytosis. An effective approach to overcome relapse caused by antigens is through targeting distinct antigens. Along with targeting different antigens simultaneously, increasing the immunogenicity of target cells may also be a workable tactic [3]. There are several therapeutic approaches to conquer the CAR-T cell treatment resistance. For example, utilizing armored or bispecific CAR-T cells, enhancing the CAR architecture, and integrating CAR-T cell therapy with other techniques such as oncolytic viruses, small-molecule medications, and targeted radiation. However, a significant proportion of patients still experience treatment failure. One of the most common reasons for this is CAR antigen loss on cancer cells. Furthermore, the unfavorable immunosuppressive tumor microenvironment and the decreased in vivo persistence of

CAR-T cells may both contribute to immune evasion. Even though individuals with particular B cell-driven hematological malignancies have shown significant effectiveness with these treatments, more research is needed to increase the application of CAR-T cell therapy for other hematological cancers [8].

In previous years, scientists have paid great attention to the development of genetic engineering techniques, hoping to increase the potency of CAR-T cells. However, little research has been done up to this point on optimizing ex vivo cell expansion conditions. The therapeutic T cells' quality can be significantly impacted by the chemicals used for ex vivo CAR-T cell growth, which highlights the necessity for more thorough research. The majority of research has generally discovered that during ex vivo expansion, culture techniques that preserve less fatigued, less differentiated, and/or less glycolytic CAR T cells produced T cell products with increased antitumor effectiveness in vivo [9].

To handle the material and schedule the patients better during the therapeutic process, there must be effective coordination between the sites of collection, manufacture, and treatment. Therefore, in order to determine the essential quality attributes and desired product profile, it is crucial to build a standardized manufacturing method for CAR-T cells. Additionally, because the viability, phenotype, and positivity of CARs varied throughout products, it is critical to get additional knowledge with these methods. Because leukapheresis generates a variety of starting materials, it is challenging to compare products [10].

## 6. Conclusion

In conclusion, CAR-T cell therapy is a technology that is based on T cells taken from the bloodstream. After that, these T lymphocytes undergo transformation to produce CARs, which enable the altered T cells to identify and react to cancer cells without the need for a major histocompatibility complex interaction. These cells proliferate in vitro and are then reinfused into the patient to boost the body's defenses against the tumor. These CAR-T cells selectively identify target antigens and rapidly proliferate to produce anti-tumor actions in vivo. The field of antineoplastics has undergone significant advancement due to the rapid growth of cellular immunotherapy and the success of recent clinical trials. Ever since it was first discovered, CAR-T therapy has been developing at a rapid speed and applied to a wide range in anticancer cellular immunotherapy. The field of hematological malignancies has seen rapid transformation due to the advent of this technology, which is now responsible for over half of all cell treatments in development or available for purchase. This review mainly focuses on the toxicities and future developments of CAR-T cell therapy. Several examples and possible developing directions are stated in the review, however, the causes of the toxicities and the possible results of the developments aren't mentioned detailly. As CAR-T cell therapy has shown such promise in treating hematological malignancies, cellular immunotherapy has emerged as a new cornerstone of anticancer therapy. Nevertheless, a series of limitations, including expensive prices, low accessibility, and uncontrolled quality, have restricted its further application and dissemination. Thus, the primary goal of developing this therapy without a doubt should be developing new methods to reduce its toxicities and costs.

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