

Side Effects and Solutions of CAR-T Treatment

Zhijie Yao *

Shenzhen High School of Science, No. 26, Shenhui Road, Longgang District, Shenzhen City,
Guangdong Province, 518129

* Corresponding author: jroman77832@student.napavalley.edu

Abstract. Cancer poses a significant threat to human health. It is a kind of complex and severe diseases characterized by the expanding of abnormal cells in the whole body due to uncontrolled growth. If left untreated, cancer with metastasis can invade nearby tissues, spread to other parts of the body, and impair the normal functioning of organs and systems. There are some treatment methods for it, such as surgery, chemical therapy and radiation. The field of immunology has made tremendous strides in understanding how the immune system works and how it can be harnessed to fight disease. Immunotherapy has become a rising solution in recent years. CAR-T therapy belongs to immunotherapy using genetically modified T cells with receptors of tumor antigens to specifically eliminate cancer cells, which is a promising approach for cancer patients. However, there are still some issues that remain to be resolved to make it wider using in treatment of cancer patients. For example, CAR-T therapy occurs severe side effects sometimes, even induced patients die, although it has been approved application in clinic since 2017, however, limited studies investigated its adverse reactions. Therefore, in the present, the author provides a comprehensive overview of the side effects of CAR-T therapy from reduction of therapeutic effects of antigen escape, to induction patients die of severe cytokine storms, then discusses corresponding treatment measures, which purpose is to help guide treatment for patients, make clinical staff focus on its side effects and provide some ideas for research and development in the future.

Keywords: CAR-T; side effects; treatment; CRS.

1. Introduction

Cancer poses a significant threat to human health. It is a kind of complex and severe diseases characterized by the expanding of abnormal cells in the whole body due to uncontrolled growth. If left untreated, cancer with metastasis can invade nearby tissues, spread to other parts of the body, and impair the normal functioning of organs and systems. There are some treatment methods for it, such as surgery, chemical therapy and radiation. The field of immunology has made tremendous strides in understanding how the immune system works and how it can be harnessed to fight disease.

Immunotherapy has become a rising solution in recent years. Chimeric antigen receptor (CAR)-T therapy belongs to immunotherapy using genetically modified T cells with receptors of tumor antigens to specifically eliminate cancer cells, which is a promising approach for cancer patients, continuing revolutionary its structure to gain the optimal functions since CAR-T cell production [1]. It is a synthetic receptor expressed on immune cells through genetic engineering methods. The most common modified cell is killer T cells, which purpose is to specifically kill tumor cells through recognizing antigen molecules of tumor cells. This kind of recognizing and binding through modified receptor did not dependent on MHC molecular on APCs, thus causing stronger activation of T cells to kill tumor cells [2]. FDA approved CAR-T cells using in clinic because of its unprecedented success in treatment of B cell malignancies with anti-CD19 CAR-T cell in 2017. Therefore, CAR-T cell therapy expanded from the initial application for treating hematological malignancies to solid tumors at present, and some significant achievements have been made. However, there are still some CAR-T cell-associated toxicities, even induced patients die such as cytokines storm, that remain to be resolved to make it wider using in treatment of cancer patients including developing new CAR molecules, modify existing molecules, and combination treatment with other immune agents, targeted

agents, conventional anti-cancer drugs and/or methods to improve anti-tumor efficacy, expand clinical application, as well as limit its toxicities [3].

In the present, the author provides a comprehensive overview of the side effects of CAR-T therapy from reduction of therapeutic effects of antigen escape, to induction patients die of severe cytokine release syndrome (CRS), then discusses corresponding treatment measures to overcome current limitations, which purpose is to help guide treatment for patients, make clinical staff focus on its side effects, provide some ideas for research and development in the future. With ongoing improvements and advancements, the use of this kind of therapeutic strategy has the potential to become even more effective for cancer patients while minimizing negative impacts on patients.

2. Side Effects and Measures of CAR-T Treatment

In this review, the author focuses on four kinds of side effects of CAR-T therapy from reduction of therapeutic effects of antigen escape and on-target off-tumor effects, to induction patients die of CRS and immune effector cell-associated neurotoxicity syndrome (ICANS) (Table 1).

Table 1. Side Effects and Measures of CAR-T Treatment

Side effects	Mechanisms	Symptoms	Solutions
Antigen escape	Reduction of target antigen expression	Resistance and relapse	Modified CAR or designed dual/triple CAR
On-target off-tumor effects	Overreaction immunity and not specific antigen	Fever, chills, vomiting, diarrhea, organ dysfunction	Post-translationally modify targeted tumor antigens
CRS	Extensive activation of CAR-T cells	High fever, low blood pressure, respiratory failure, organ dysfunction	Tocilizumab, glucocorticoids, high-flow oxygen
ICANS	Massive cytokines releasing, eg. TGF- β	Increasing of CD14+ cells, hypotension, hypoxia	Tocilizumab, glucocorticoids, low-flow oxygen

2.1. Antigen Escape

The most common reason for treatment failure is resistance to CAR-T of targeting a single antigen since the expression of the target antigen significantly decreases or even disappears with the progress of treatment, resulting in the inability to identify tumor cells, which also be defined immune escape. For example, more and more recent reported data showed that about half of the refractory patients who existed the downregulation or missing expression of CD19 in B lymphocyte tumors, although up to 70% of relapsed/refractory patients of ALL are still effective to CD19 targeted CAR-T cell therapy. Consistent with the research data from relapsed ALL patients, study showed that refractory multiple myeloma patients also occurred a similar phenomenon, who were resistant to CAR-T treatment of BCM due to the downregulation BCMA or loss of its expression. And then, similar to patients with hematological tumors, these phenomena have been observed during the treatment of patients with solid tumors. To solve this problem, dual-targeting CAR-T cells were developed with two kinds of receptors targeting tumor antigen in therapeutic T cells. Compared with single-targeted therapy, dual-targeting CAR-T has better anti-tumor effects. For example, in a study data from glioblastoma, researchers developed a dual-targeting CAR targeting HER2 and IL13Ra, and the experimental results suggested that the anti-tumor activity was improved and the risk of tumor recurrence was decreased in comparison with signal CAR treatment [2]. Another example is to use due-CAR targeted CD19 and CD22 in the treatment of B-ALL with an enhancement of effects, demonstrating the dual-targeting CAR is an alternative method to decrease relapse because of antigen escape, as well as increase therapeutic effects through optimal antigen screening [4].

2.2. On-target Off-tumor Effects

Another challenge in treatment of solid tumor using CAR-T technology is that the specificity of solid tumor antigens is relatively low, and some of them are also expressed in normal tissue cells at varying levels. Specific symptoms of these side effects include fever, chills, vomiting, diarrhea, low blood pressure, abnormal liver function, diffuse lung damage, headache, disturbance of consciousness and hallucination. Therefore, the optimization of antigen selection is crucial in designation of CAR receptor when applying CAR to treat solid tumors. An ideal antigen target must satisfy two conditions, not only to ensure the therapeutic effect, but also to limit the toxicity of off-target or off-tumor. A potential way to overcome the toxicity caused by killing normal tissues designing CAR for treating solid tumors is to post-translationally modify targeted tumor antigens, such as glycosylation, adding serine, etc [4-5]. In order to using CAR treatment of solid tumors, it is a optimistic strategies to develop dual CAR and optimize the screening of highly specific CAR molecular receptors dealing with tumor escape, improvement efficacy and mitigation toxic and side effects in the future.

2.3. CRS

The CRS has been identified a most serious and high incidence adverse reaction (more than 90%) of CAR therapy since it produced, although it is an effective treatment method, especially for hematological tumors and even some solid tumors. Moreover, the CRS can also induce other adverse effects, such as ICANS and cardiovascular, gastrointestinal, and solid organ toxic effects, which have seriously hindered CAR-T therapy in clinical application [6].

The possible mechanisms of CRS occurrence are the extensive activation of CAR-T cells due to drug administration, thereby releasing a large number of cytokines in short time, such as IFN- γ , TNF- α , GM-CSF, IL-2/8/10, which damaged nearby normal tissue cells at the same time killing tumor cells. For mild CRS patients, the clinical manifestations are high fever ($\geq 38^{\circ}\text{C}$), accompanied by fatigue, diarrhea, headache, rash, joint pain, myalgia and other symptoms, similar to common drug allergic reactions. For severe CRS reaction, patients occur systolic blood pressure less than 90 mmHg with cardiac and circulatory failure, oxygen saturation lower than 90% with respiratory failure, or other organs dysfunction, like renal failure, which induce death because of DIC and multi-organ dysfunction due to a number of inflammatory cytokines. The high-risk factors for severe CRS include the disease itself, patient conditions, and treatment strategies, such as B-ALL patients, elder patients, injection overload CAR-T cells, and combination with CD28 [6-7].

Tocilizumab is a mAb targeting IL-6 receptor that inhibits the IL-6 signaling pathway and reduces inflammation, which is the most common choice and has been widely used in clinical practice for managing CRS in CAR-T cell therapy. Besides that, glucocorticoids, such as dexamethasone and methylprednisolone have shown efficacy in treating CRS. They can suppress the function of various inflammatory cells and alleviate the inflammatory response using 10-20 mg dexamethasone or equidose of methylprednisolone. In addition, anakinra inhibit the action of IL-1 and are expected to reduce the severity of CRS since IL-1 is also a key factor in the inflammatory response associated with CRS. Anti-CD20 agents, like rituximab, can be used to eliminate B cells in B cell lymphoproliferative disorders induced by CAR-T cells, thus alleviating CRS symptoms. Furthermore, to prevent further deterioration of the condition, it's recommended to early administration of high-flow oxygen via nasal cannula or mask, or injection of vasopressors, except in patients with mild hypotension and hypoxia [6-7].

2.4. ICANS

The ICANS is also a serious adverse reaction of CAR-T treatment. Studies have shown that it is related to the massive cytokines releasing because of CRS, especially cytokine of TGF- β , which incidence is about 30%, including severe cases accounting for a quarter in the total number of ICANS cases. In addition to releasing a lot of cytokines, myeloid-derived immune cells appear to be an important contributor to CAR-T cell therapy-induced neurotoxicity. A clinical trial reported that

CD14+ cells were significantly increased in patients with higher neurotoxicity after treatment using CAR-T cells. Unlike CRS, the treatment of hypotension should be injected intravenously hydration (20 ml/kg), while it's better to treat hypoxia with low-flow oxygen. Recently, a study data displayed that using modified CAR is benefit for preventing this kind of adverse reaction [8].

3. Challenges and Solutions

Although CAR-T therapy has achieved encouraging results in the clinical treatment of cancer patients, side effects are still problems that cannot be ignored. Some side effects have better countermeasures, but it has to be said that current solutions are relatively limited for some side effects, actually no solution. It's necessary to understand the mechanisms of side effects in CAR-T cell therapy, and conquer these difficulties are benefit to optimize treatment strategies, and improve patient outcomes without or low risk of toxicity. Several key factors that affect CAR T cell therapy include the designation of CAR molecular, the relapse ratio due to CAR resistance, dealing with toxic side effects [7, 9].

Toxic side effects limit the efficacy and application of CAR-T therapy because it is difficult to enhance the anti-tumor effect through increasing the number of CAR T cells or activation T cells. The immunotoxic side effects mainly associated with high tumor burden, elder patients, and intensive lymphocyte depletion pretreatment. With the increasing of treatment patients, more side effects have been gradually reported, such as macrophage activation syndrome, B-cell aplastic anemia and fatal infection because of immune damage, as well as fatal cerebral edema [9].

The CAR is consisted with scFvs, hinge regions, transmembrane and intracellular regions, and modified any domain can affect its therapeutic effects. For example, adding suicide genes inducing caspase-9 may be a means to reduce the toxic effects of CAR-T cells, but it may lead to reduction of therapeutic effects due to irreversible elimination of CAR-T cells. Mutating specific sites in the CD28 domain helps control stimulation intensity, reduce CAR-T toxic effects. In addition, modulating the strength of intracellular signal can also affect CAR T effects. For example, using CD3 ζ truncated short signaling domains with an ITAM, showed better effects in animal models [7-9]. Another promising direction is to focus on developing dual, triple or multi-targeted antigen CARs is common strategy to decrease antigen escape. Until now, the tandem or combined use of CD20, CD22 targets, CD19, and CAR-T cells has shown optimistic safety and ideal effects in clinical trials [8].

Besides, the optimization of CAR structure has broad development prospects. For example, CAR-T cells using CRISPR/Cas 9 technology to modify the PD-1 receptor gene have shown the feasibility of enhancing CAR-T efficacy through inhibiting PD-1. Alternatively, overexpression of the BATF protein and IRF-4 in CD8+ CAR-T cells promotes its survival and expansion, resulting in long-lived memory T cells to inhibit tumor recurrence [10].

4. Summary

The therapeutic CAR-T cells have achieved encouraging success in the treatment of hematological tumors. Therefore, more and more studies have been explored their application in patients of solid tumors including designation of new TCR-T cells. There is seven therapeutic CAR approved application in clinical to treated blood tumors until 2022. Although CAR-T therapy has gained great success, its severe adverse reactions have hindered CAR-T therapy. In the future, it is necessary to elucidate the mechanisms of risk factors, diagnostic criteria and measures, thereby providing valuable reference for the safety, effectiveness and certain population of patients for CAR-T therapy.

References

- [1] Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J*, 2021, 11 (4): 69.

- [2] Jogalekar MP, Rajendran RL, Khan F, et al. CAR T-Cell-Based gene therapy for cancers: new perspectives, challenges, and clinical developments. *Front Immunol*, 2022, 13: 925985.
- [3] Li Y, Ming Y, Fu R, et al. The pathogenesis, diagnosis, prevention, and treatment of CAR-T cell therapy-related adverse reactions. *Front Pharmacol*, 2022, 13:950923.
- [4] Singh N, Maus MV. Synthetic manipulation of the cancer-immunity cycle: CAR-T cell therapy. *Immunity*, 2023, 56 (10): 2296 - 2310.
- [5] Neelapu SS. Managing the toxicities of CAR T-cell therapy. *Hematol Oncol*, 2019, 37 Suppl 1: 48 - 52.
- [6] Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant*, 2019, 25(4):625-638.
- [7] Varadarajan I, Lee DW. Management of T-Cell Engaging Immunotherapy Complications. *Cancer J*, 2019, 25 (3): 223 - 230.
- [8] Xin T, Cheng L, Zhou C, et al. In-Vivo Induced CAR-T Cell for the Potential Breakthrough to Overcome the Barriers of Current CAR-T Cell Therapy. *Front Oncol*, 2022, 12: 809754.
- [9] Brudno JN, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for lymphoma. *Nat Rev Clin Oncol*, 2018, 15 (1): 31 - 46.
- [10] Seo H, González-Avalos E, Zhang W, et al. BATF and IRF4 cooperate to counter exhaustion in tumor-infiltrating CAR T cells. *Nat Immunol*, 2021, 22 (8): 983 - 995.