Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/21/20230899

Limitations of CAR-T therapy and possible directions of improvement

Xingtong Lin

Beijing 101 Middle School, 11 Yiheyuan Road, Haidian District, Beijing, 100091, China

1811121113@mail.sit.edu.cn

Abstract. Chimeric antigen receptor T-cell (CAR-T) therapy is a new biological immunotherapy approach. This paper is an overview of the limitations of CAR-T therapy and possible directions of improvement. This paper starts with the mechanism of CAR-T, briefly describes the pathogenesis, and then provides a detailed description of the three types of CAR-T toxicity and their mechanisms, including cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and CAR-T-associated encephaly syndrome (CRES). In the end, the paper proposes several possible improvements to improve the current situation, summarizes the corresponding improvements, and looks forward to the future.

Keywords: CAR-T, toxicity, CRS, ICANS, CRES.

1. Introduction

The goal of CAR-T therapy is to isolate and remove T lymphocytes from the patient's body, then use genetic engineering technology to transform, process, and culture them. Then, T cells are activated and given the CAR gene, which allows T cells to specifically recognize tumor cells, and release a significant number of effector factors by immunological activity. Finally, the cells were injected back into body to alter proteins at the site of the tumor to eliminate or reduce the destructive power of cancer cells. It is mainly used for refractory malignant hematologic diseases, such as leukocyte, lymphoma, multiple myeloma and so on. CAR-T therapy can treat cancer accurately, quickly and efficiently. However, this method also has certain adverse reactions, patients may have high fever, chills, nausea, muscle pain, general edema, cardiac insufficiency, breathing difficulties, kidney function damage. Due to the incidence and resulting burden of CRS, CRES, and ICANS in therapy, the use of CAR-T therapy is now restricted to some extent [1].

In many studies now, researchers have suggested ways to deal with these side effects. This paper will summarize the mechanism of CAR-T therapy, three toxicities and possible ideas for improvement, hoping to provide a guide for the future development.

2. Mechanism

CAR-T therapy is the development of antigen-binding T cell receptors containing immunoglobulins through genetic cloning. Genetic material containing specific CAR domains and T cell activation signals

DOI: 10.54254/2753-8818/21/20230899

has been introduced into T cells. As a result, they bind directly to specific antigens on the surface of tumor cells and activate them [2].

After recognizing tumor antigens, CAR activates the immune pathway of the working cells, and the expression of related genes is up regulated. Granulocyte, perforin, and other substances are secreted to directly mediate tumor cell lysis. Meanwhile, inflammatory factors, such as GM-CSF, is also secreted.

Several key points are involved in the application of CAR-T therapy: antigen binding site, binding, transmembrane domain and signal transduction domain. These are also important areas of concern for improving CAR-T therapy.

3. Toxicity

3.1. CRS

The most frequent immediate side effect of CAR-T treatment is CRS. CAR-T cells' activation following tumor detection in vivo causes the production of cytokines, which in turn causes systemic inflammatory reactions, or CRS. The pathophysiology of CRS is influenced by inflammatory cytokines, which are released by bystander immune cells such macrophages after being activated by CAR-T cells. In more severe cases, CRS can result in numerous organ malfunction. It typically manifests as systemic symptoms such as fever, myalgia, chills, lethargy, and appetite loss. CRS are entirely reversible, though, if handled correctly [3].

In the five stages, "CRS" has the following pathological changes: In the first and second stages, many malignant cells spread to the tumor source, CAR-T cells recognize and proliferate, produce cytokines to trigger the occurrence of CRS, and kill the tumor cell. In phase 3, cytokinesis actively enters the peripheral blood, and systemic inflammation occurs concurrently. This is because the CAR-T cell population in the peripheral blood increases, which can cause complications such as hypoxia, hypotension, and organ damage. This is an imbalance of osmotic pressure caused by damage to multiple organs or tissues, as well as holes in blood vessels with leakage and endothelial damage. In the fourth stage, peripheral blood enters the cerebrospinal fluid (CSF) and central nervous system (CNS) by allowing cytokine diffusion, CAR-T cells, endogenous T cells, and peripheral activated monocytes, including the destruction of the blood-brain barrier (BBB) [4]. In turn, it leads to the remission of symptoms in the fifth stage: the decrease of cytokines, the weakening of systemic inflammation, the gradual end of CRS/ICANS symptoms, and the generation of long-standing memory T cells. It has been demonstrated that serum IL-6 levels are correlated with the severity of CRS, and that tolumab, an anti-IL-6 receptor antibody, blocks IL-6 to reverse CRS. It has also been demonstrated that other cytokines and chemokines, including IL-8, IL-10, IL-15, IFN-, and MCP-1, are related to severe CRS. Patients with CRS and mice models have both shown elevated levels of these key cytokines. Corticosteroids are employed as a result because they produce global immunosuppression, decrease the production of several cytokines and chemokines, and directly impact CAR-T cell proliferation and function, if blocking IL-6 alone is insufficient to manage CRS. For the clinical management of CRS, tocilizumab and other core cytokine blocking inhibitors have also been approved as treatment options. CRS can be diagnosed by tracing the genesis of its main cytokines.

At now, it makes sense for researchers to concentrate on IL-6 production to understand the mechanism of CRS [4]. It has been demonstrated that IL-6 is a pleiotropic cytokine released under a variety of circumstances, including stress, infection, and tissue damage. In addition to immune cells, fibroblasts, mesenchymal cells, and vascular endothelial cells also release IL-6.

Uncertainty surrounds the pathophysiological mechanism of CRS. The primary source of core cytokines in CRS may be macrophages. The combination of modified T cells with their target cells can cause on-target effects, which can then attract and activate bystander immune or non-immune cells. Myeloid macrophages have been implicated in the pathophysiology of CRS in an increasing number of studies.

Macrophages were the primary source of Nitric oxide synthase (iNOS), a kind of inflammatory cytokine. Vasodilation and hypotension, which are serious life-threatening clinical symptoms during

DOI: 10.54254/2753-8818/21/20230899

CRS brought on by the infusion of CAR-T, are produced by abnormal NO generation. Treatment with the iNOS inhibitor L-NIL or 1400 W in Sadelain's trial both increased survival and decreased toxicity in the case of severe CRS. Yet, there is also evidence that iNOS are engaged in additional CRS processes. In plasma cells, George et al. discovered that IL-6 promoted Nos 2 transcription. Furthermore, IL-1 can promote iNOS expression and synthesis. IL-6 and IL-1 strongly induce CRS, and iNOS is thought to be involved in this process [5].

3.2. ICANS

ICANS can happen while having CRS or, more frequently, after it has passed. In more severe cases, it can develop to lower levels of awareness, coma, seizures, motor weakness, and cerebral edema. It often manifests as toxic encephalopathy with difficulties speaking, aphasia, and bewilderment. Similar to CRS, the degree of neurotoxicity was linked with cytokine, chemokine, and CAR-T cell amplification [6].

The neurotoxicity may result from endothelial cell activation and BBB disruption. 10 Other findings point to the CNS's involvement with myeloid cell activation. The relevance of IL-1 in the pathophysiology of the neurotoxicity has recently been shown in two mice models, and both toxicities may be reversed by inhibiting IL-1 using the IL-1 receptor antagonist analbinin. ICANS handles low-grade toxicity mostly with supportive treatment because to a poor knowledge of pathogenesis, although corticosteroids are frequently utilized to treat more severe toxicity. ICANS has a self-limiting course and is totally reversible in the majority of patients, just like CRS.

3.3. CRES

Some patients with acute B-cell leukemia develop symptoms of severe neurotoxicity after treatment with CRES CAR-T cells often manifest as toxic dementia. The first symptoms are problems concentrating, speaking and writing. Other signs and symptoms include confusion, disorientation, emotional disturbances. Aphasia, drowsiness and tremors, cerebral edema is the most severe neurotoxic complications. CRES can happen simultaneously with CRS or separately, and typically occurs 4-5 days following CAR-T cell injection [5].

CRES is often accompanied by CRS, but the pathogenesis of CRES is different from that of CRS, and the mechanism of CRES is still unclear. One mechanism for the occurrence of CRES may be the activation of independent monocytes triggered by IL-1, which then produces the expression of multiple cytokines such as IL-6, which in turn activates T cells and macrophages, increasing systemic inflammation [5]. Another possible mechanism is BBB. The destruction of BBB leads to T cell migration in the brain parenchyma and leads to elevated levels of cytokines and proteins in the cerebrospinal fluid, leading to central nervous system inflammation and toxicity. And endothelial activation can aggravate systemic inflammation and BBB destruction [7]. In addition, CAR-T cells can directly damage the CNS. The detection CSF indicates that CAR-T cells can enter CNS and that patients with neurotoxicity have a considerably larger number of CAR-T cells than patients without neurotoxicity.

4. Possible improvements

4.1. Cytokine-expressing

Upregulation of IL-12 secretion has been demonstrated in research using immunocompetent and immunocompromised mice models, however toxicity has been noted in human investigations using in vitro growth of tumor-infiltrating lymphocytes. In a different strategy, Sachdeva et al. demonstrated that in response to IL-12 induction, an IL-12 inducer arms IL-12, suggesting a potential loss of NFAT promoter activity. To connect cytokine secretion with active CAR phage activity, the IL-12 gene has really been inserted under the IL-2 Ra regulator or PDCD 1. enhances plasticity and effector activity. IL-15 preserved the naive nature of central memory T cells when it was examined in vitro and in immunoreactive mice models. decreased expression of PD-1 and elevated levels of the anti-apoptotic protein Bcl-2 [8]. When IL-15 attached to the CAR-T cell membrane, similar outcomes were seen. In a solid tumor mouse model, the release of IL-7 and CCL19 by CAR-T cells improved antitumor responses

DOI: 10.54254/2753-8818/21/20230899

and raised overall survival. In mice treated with CAR-T, both these antigen-positive and antigen-negative tumors were eliminated. T cells that release CCL 19 and IL-7.

4.2. Synchronous T cell delivery

Promoting local T-cell delivery can effectively reduce damage and avoid the risk of tumor overload. Such treatments have had some success and a degree of validation in treating specific cancers and diseases. When mice are exposed to MSC, the transgenic CSU neutralization is sufficient to prevent the spread of MSC and cell blocking, which limits immune molecules inhibition, such as pv l [8].

4.3. Improvement plans specifically for CRS

Clinical signs and severity of CRS can range greatly from non-life-threatening toxicity to severe symptoms. Accurate and prompt patient management and assessment can help prevent negative consequences [9]. After a CAR-T injection, CRS symptoms often go away in two weeks. IL-6 antagonists and/or corticosteroids may be needed for therapy, or the toxicity may be self-limiting and simply need symptomatic relief. The goal of CRS therapy is to maximize the anticancer benefits of cell therapy while minimizing hazardous side effects. There is disagreement on whether patients undergoing CAR-T treatment should be hospitalized (ZUMA-1 trial, JULIET trials, and ELIANA trial).

It is important to keep in mind that these treatment facilities have substantial expertise with CAR-T therapy and have well-established outpatient hematopoietic stem cell transplantation programs, even though this shows that the therapy may be administered in carefully supervised outpatient settings. Prior to the onset of severe symptoms, individuals who are at risk for developing severe early CRS still require rigorous monitoring and hospitalization, which requires further discovery of predictive biomarkers.

4.3.1. Supportive care. Throughout all stages of CRS, including the cell infusion, patients should receive supportive care. Complete blood counts with complete and different metabolites are often included in daily monitoring [10]. The risk of volume overload and pulmonary edema necessitates continuous monitoring of fluid balance, including daily body weight. Intravenous fluids are utilized to maintain hydration. Telemetry monitoring from CAR-T cell infusion until remission of CRS should be taken into consideration due to the danger of arrhythmia, especially in patients with concomitant cardiac risk factors.

4.3.2. Normal liver function patients. Acetaminophen can be administered to individuals with normal liver function to treat fever, and cooling blankets is another option. Alternative medications include non-steroidal anti-inflammatory medicines (NSAIDs); nevertheless, caution is needed while assessing thrombocytopenia. NSAIDs can also result in bleeding, gastritis, and renal failure. Numerous of these patients are neutropenic and have had lymphocyte clearance, therefore it is crucial to check for infections using chest x-rays, blood and urine cultures, and lymphocyte clearance. Furthermore, it is necessary to start taking broad-spectrum antibiotics.

5. Conclusion

Due to the incidence and resulting burden of CRS, CRES, and ICANS in therapy, the general use of CAR-T therapy is now restricted to some extent. The creation of efficient tailored medicines that lessen toxicity without sacrificing anticancer effectiveness will be made easier with a thorough understanding of the pathophysiology of these toxicities. New CAR architectures have been developed to enhance tumor antigen identification and efficient T-cell signaling while reducing the danger of causing CRS and ICANS. Finding a reliable and effective way to avoid these problems in the future will be one of the key components of CAR-T therapy's success.

DOI: 10.54254/2753-8818/21/20230899

References

[1] Vucinic, V., Quaiser, A., Lückemeier, P., Fricke, S., Platzbecker, U. and Koehl, U., "Production and Application of CAR T Cells: Current and Future Role of Europe," Frontiers in Medicine 8, 713401 (2021).

[2] Shah, N. N. and Fry, T. J., "Mechanisms of resistance to CAR T cell therapy," Nature Reviews Clinical Oncology 16(6), 372–385 (2019).

[3] Neelapu, S. S., "Managing the toxicities of CAR T-cell therapy," Hematological Oncology 37(S1), 48–52 (2019).

[4] Hao, Z., Li, R., Meng, L., Han, Z. and Hong, Z., "Macrophage, the potential key mediator in CAR-T related CRS," Experimental Hematology & Oncology 9(1), 15 (2020).

[5] Adachi, K., Kano, Y., Nagai, T., Okuyama, N., Sakoda, Y. and Tamada, K., "IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor," Nature Biotechnology 36(4), 346–351 (2018).

[6] Wang, M., Zhang, C. and Jiang, X., "CAR-T: a potential gene carrier targeting solid tumor immune microenvironment," Signal Transduction and Targeted Therapy 6(1) (2021).

[7] Li, Y., Huo, Y., Yu, L. and Wang, J., "Quality Control and Nonclinical Research on CAR-T Cell Products: General Principles and Key Issues," Engineering 5(1), 122–131 (2019).

[8] Li, H., Yang, C., Cheng, H., Huang, S. and Zheng, Y., "CAR-T cells for Colorectal Cancer: Target-selection and strategies for improved activity and safety," Journal of Cancer 12(6), 1804–1814 (2021).

[9] Glover, M., Avraamides, S. and Maher, J., "How Can We Engineer CAR T Cells to Overcome Resistance?" Biologics: Targets and Therapy 15, 175–198 (2021).

[10] Sermer, D. and Brentjens, R., "CAR T-cell therapy: Full speed ahead," Hematological Oncology 37(S1), 95–100 (2019).