Proceedings of the 3rd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/98/2025.21496

A Review on Immunotherapy with Cancer Treatment and CAR-T Cell Processing

Jingyuan Chen[1,a,*]

[1]*Haide college, Ocean University of China, Shandong province, 266100, China*
a. 23220001001@stu.ouc.edu.cn
**corresponding author*

Abstract: Chimeric Antigen Receptor T-Cell Immunotherapy is known as CAR-T, The clinical therapeutic benefits in cancer and tumor cells are significant. Cancer and tumor cells have long been among the most serious threats to human life safety. The advent of CAR - T has made it possible to use immunotherapy to specifically target and eliminate tumor cells. However, there is still a need for a systematic review to summarize the recent advancements in the genetic research of CAR-T cells, thereby assisting researchers in the development of this significant technology. In this review, first of all, expound on the principles of CAR - T therapy for the treatment of cancerous tumor cells. Subsequently, reflect on the gene-editing cases of the technology cells over the past five years since 2018, summarizing the efficacy and significance of gene editing for CAR-T. Lastly, review the application of the technology in cancer therapy and outline the future research directions and development prospects for CAR-T cells. Over the past five years, the introduction of a plethora of innovative CAR-T cell therapies in the field of gene editing has indicated that gene editing has addressed some of the inherent issues in CAR-T cells to varying degrees, significantly enhancing the feasibility and controllability of the technology in clinical applications. I believe that this review can provide researchers studying gene editing of CAR-T cells with a clear summary of research findings. This review article will discuss function of the technology cells and the benefits of gene editing with CAR-T cell, which might help the researchers know the current research direction.

Keywords: Cancer treatment, CAR-T, gene editing, tumor cell

1. Introduction

Chimeric Antigen Receptor T-cells (CAR-T) are a novel and precise targeted therapy for treating tumors . However, this groundbreaking treatment technology was first proposed by Prof. Zelig Eshhar in 1988 (Italy)[1]. The therapy of CAR-T cells, once proposed, has garnered global attention and become a hot topic in the field of biotherapeutics. Consequently, CAR-T technology has advanced rapidly, evolving from the first generation of CAR-Ts to the recent fourth generation[3]. The structure of CAR-Ts has been more thoroughly explored, and the genetic modification techniques within them have become increasingly sophisticated. The development of successive generations of CAR-Ts has been trending towards universality, high efficiency, safety, and tolerability, progressing step by step in these directions . CAR-T cells have shown significant clinical benefits in the treatment of cancer and tumor cells , but also some problems. For instance, [2] CAR-T have achieved complete remission

© 2025 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/98/2025.21496

rates of up to 50-90% in the treatment of certain B-cell malignancies. However, [3] CAR-T are associated with toxicity issues, consisting of cytokine release syndrome and neurotoxicity[4]. CAR - T has demonstrated efficacy in treating specific types of B - cell leukemia or lymphoma. Nevertheless, [5] CAR - T cells may experience functional impairment, and two main states of dysfunction are exhaustion and senescence[6]. The therapeutic efficacy of CAR-T cells is relatively low in solid tumors compared to hematological malignancies. [7] For patients who have H3K27M-mutated diffuse intrinsic pontine glioma (DIPG) or spinal cord diffuse midline glioma (DMG), GD2-CAR-T cell therapy has a bright future[8]. CT041 may become an significant treatment option for the patients with advanced gastric cancer. [9] The combination of CRISPR and CAR-T technology addresses issues related to the use of viral vectors and random integration[10]. CAR-T cells which are in vivo generation have great potential as a therapeutic platform for various diseases.

2. The Origin and Principle of Car-T

T-cells, a kind of T lymphocyte, originate from bone marrow stem cells and are a subset of white blood cells in the human body. They possess receptors on their cell surface that can specifically recognize certain foreign cells and induce apoptosis. The CAR-T technology essentially involves the use of genetic engineering to activate lymphocytes T-cells and equip them with chimeric antigen receptors that are specific to tumor cells. This allows the T-cells to specifically recognize these particular tumor cells and release immune factors through an immune response, efficiently and specifically killing tumor cells, ultimately achieving the goal of treating malignant tumors.This groundbreaking treatment technology was first proposed by Prof. Zelig Eshhar in 1989 (Italy).

The previous study has already shown the detail of the function and natural structure of the T cell, especially the Recognition between T cell receptor and MHC, which is the basic of Car-T [2]. It is this fundamental principle that serves as an essential cornerstone for the genetic engineering of T cells to enable their specific cancer therapy.Besides,the other one study [3] also introduces the treatment mechanism of the technology. It mentions CAR, which stands for Chimeric Antigen Receptor, is a transmembrane molecule on the cell membrane composed of several functional elements. These transmembrane molecules require the fusion of the scFv (corresponding to the antigen recognition sequence of the antibody) with a hinge/spacer module and a transmembrane domain, which is then connected to the intracellular domain through this system. This crucial step for signal transduction eventually leads to the immune response.However, according to previous research[4], CARs have not only these advantages, but also a modular design, which allows their antigen-binding domains and other structural components to be altered, enabling CARs to work more effectively in specific environments.

These various advantages have made this new technology shine successfully in clinical trials.

3. CAR-T with Gene Editing Engineering the Application of CAR-T in Cancer Therapy.

The development of editing engineering makes the CAR-T come true.Thanks to the researchers efforts the 4th CAR-T have already been published, which is exactly based on the editing engineering [3]. Researchers have innovatively designed the technology with the aim of making it safer and more efficient, enabling CAR - T therapy to be more widely applied to the treatment of various complex diseases [4].. This includes research on modifying the stimulation region of CAR-T cells through gene editing, altering the hinge and transmembrane domains, and the particularly typical research on UCART (Universal CAR-T cells), which utilizes gene editing technology to knock out a portion of the TCR and address issues such as the autoimmunity and indiscriminate attack on host cells caused by the transplanted cells.In CAR-T cell therapy, issues such as rapid aging and exhaustion of CAR-T cells have been observed. To mitigate these problems, review articles [5] have suggested methods

DOI: 10.54254/2753-8818/98/2025.21496

like gene editing to knock out genes that regulate immune checkpoint molecules like PD-1. This can help to alleviate such issues, allowing the therapy to be more durable and effective.

Four parts: antigen-binding domain, hinge region, transmembrane domain, and one or more intracellular signal domains constitute the basic structure of CAR-T cells. Research [6] has also proposed that gene editing engineering can precisely modify each of these regions. Of course, emerging the technology are also being proposed[9]. By utilizing the CRISPR-Cas9 gene editing technology, specific genes can be accurately inserted into specific locations of the T cell genome without the need for viral vectors. This "two-in-one" approach cleverly solves both the problem of viral vector use and the issues brought about by random integration at the same time. Previous investigators first optimized the gene-specific integration of T cells without viral vectors, using dsDNA as a homology-directed repair (HDR) template, pioneering the reality of high homologous recombination efficiency and cell survival rate. By inserting the anti-CD19 CAR gene fragment into the AAVS1 safe harbor site, a new type of anti-CD19 CAR-T cell was developed, which can better eliminate tumor cells in xenograft models. About 2 months later,another research team [10] has developed a method for producing CAR-T cells in vivo by delivering modified mRNA via lipid nanoparticles (LNPs). This technology can generate transiently effective CAR-T cells. With the promotion of CAR-T therapy, an increasing amount of clinical data has demonstrated its feasibility to the world. In 2018 CAR-T cells targeting CD19 have achieved complete remission rates of up to 50-90% in the treatment of certain B-cell malignancies[2]. Testing of various types of CAR-T cells is also being emphasized by major laboratories, such as evaluating the feasibility, safety, and tolerability of GD2-CAR T cells, and determining the maximum tolerated dose or the recommended phase II dose[7]. Researchers are also evaluating the clinical activity of these cells. Given the randomness of cancer occurrence sites, technologies for different parts of the body have also been developed. For instance,CLDN18.2 is a protein that is highly expressed in multiple cancers and can become a potential target for anti-tumor treatment in digestive system cancers[8]. CAR T cells (CT041) can specifically recognize this protein, which can achieve good therapeutic effects. The security, high efficiency, pharmacokinetics, and immunogenicity of CT041 in patients with digestive system cancers have also been evaluated, making it safer and more reliable for the treatment of related digestive system cancers.

CAR-T therapy is inextricably linked to gene editing technology. CAR-T therapy serves as a vital gateway through which the practical applications of gene editing technology make a real impact on our daily lives. As CAR-T therapy evolves, gene editing technology also advances, with the two complementing and progressing together, promising a broad and bright future.

4. The Future Research Directions and Development Prospects for CAR-T .

Though CAR - T cell therapy is known for its significant benefits in treating cancer and tumor cells, there are also numerous problems to be solved, which are precisely the future research directions and development prospects for CAR - T cells. Cytokine release syndrome (CRS) and neurotoxicity are closely related to the severity and probability of the onset of CAR - T - associated encephalopathy syndrome. Study demonstrated this and provided data describing the linear relationship between them[3]. They also discussed the research progress of universal CAR-T cells (UCART) and their toxicity characteristics. However, a clear solution was not directly provided, which implies that enhancing safety and efficacy could be a way to develop CAR-T. There's another research mentioned that even if the success of CAR-T cells in treating hematological cancers, there are fewer successes in treating solid tumors[4]. It means the applications of CAR-T are still problems that are limited to some areas, and there are more areas to explore, such as solid tumor cells. The researchers also believe that synthetic biology and gene - editing techniques should be more widely applied to CAR - T therapy. From a certain perspective, regarding the two critical states of CAR - T dysfunction [5], how

DOI: 10.54254/2753-8818/98/2025.21496

to restore dysfunctional CAR - T cells by directly modulating intrinsic pathways and/or utilizing exogenous signals can also be a significant research direction.In many related research processes [3, 4, 6, 8, 10, 11], a common problem has also been found and raised,the security of CAR-T.As a genetically engineered T-cell, we can imagine how dangerous it would be if it would attack our healthy cells, like autoimmune diseases. Regardless of how the technology develops, safety should come first.

Some of the researchers clearly announced that the future of the new technology development is very positive, and many researchers also put forward constructive suggestions for the development of CAR-T [4, 6, 11], and also believe that the development of CAR-T will add a strong color to the development of modern medical development [2, 3, 5, 7, 8, 9, 10] .

5. Conclusion

CAR - T, as a highly specific and efficient method for treating cancer and tumors, holds great research value in an era when cancer mortality accounts for 18.7% of all deaths. Clinically, successful cases of CAR - T therapy undoubtedly encourage researchers to explore this field further. However, CAR-T cell therapy still has several issues: The emergence of functional impairments such as exhaustion and senescence; Severe potential lethal toxicities-- CRS and Neurotoxicity. By meticulously analyzing these issues, we can gain insights into the current state of CAR-T therapy development, identify the shortcomings of this technology, and provide assistance in setting more defined goals for the future development of CAR-T.

The relentless efforts of researchers have led to a comprehensive understanding of CAR-T cell therapy's structural principles, providing a robust foundation for future advancements, while the field is also witnessing promising developments in gene editing engineering that have resulted in the creation of four generations of the technology, each featuring genetic constructs that enhance safety and efficiency. Furthermore, the pursuit of universal CAR - T cells, which can bring the advantages of convenience and lower therapy costs, is emerging as a dominant trend in this field. If the challenge of maintaining specificity while enhancing universality can be successfully overcome, it has the potential to further enhance CAR - T therapy.

References

[1] *Gross, G., Waks, T., & Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proceedings of the National Academy of Sciences of the United States of America, 86(24), 10024-10028. https://doi.org/10.1073/pnas.86.24.10024*

[2] *Labanieh, L., Majzner, R. G., & Mackall, C. L. (2018). Programming CAR-T cells to kill cancer. Nature Biomedical Engineering, 2, 377–391. https://doi.org/10.1038/s41551-018-0235-9*

[3] *Titov, A., Petukhov, A., Staliarova, A., Motorin, D., Bulatov, E., Shuvalov, O., Soond, S. M., Piacentini, M., Melino, G., Zaritskey, A., & Barlev, N. A. (2018). The biological basis and clinical symptoms of CAR-T therapy-associated toxicities. Cell Death & Disease, 9, 897. https://doi.org/10.1038/s41419-018-0918-x*

[4] *Rafiq, S., Hackett, C. S., & Brentjens, R. J. (2020). Driving CAR T-cells forward. Nature Reviews Clinical Oncology, 17, 147-167. https://doi.org/10.1038/s41571-019-0297-y*

[5] *Poorebrahim, M., Melief, J., Pico de Coaña, Y., Wickström, S. L., Cid-Arregui, A., & Kiessling, R. (2021). Counteracting CAR T cell dysfunction. Oncogene, 40, 421–435. https://doi.org/10.1038/s41388-020-01501-x*

[6] *Sterner, R. C., & Sterner, R. M. (2021). CAR-T cell therapy: current limitations and potential strategies. Blood Cancer Journal, 11(69), 1-11. https://doi.org/10.1038/s41408-021-00459-7*

[7] *Majzner, R. G., Ramakrishna, S., Yeom, K. W., Patel, S., Chinnasamy, H., Schultz, L. M., Richards, R. M., Jiang, L., Barsan, V., Mancusi, R., Geraghty, A. C., Good, Z., Mochizuki, A. Y., Gillespie, S. M., Shaw Toland, A. M., Mahdi, J., Reschke, A., Nie, E. H., Chau, I. J., Rotiroti, M. C., Mount, C. W., Baggott, C., Mavroukakis, S., Egeler, E., Moon, J., Erickson, C., Green, S., Kunicki, M., Fujimoto, M., Ehlinger, Z., Reynolds, W., Kurra, S., Warren, K. E., Prabhu, S., Vogel, H., Rasmussen, L., Cornell, T. T., Partap, S., Fisher, P. G., Campen, C. J., Filbin, M. G., Grant, G., Sahaf, B., Davis, K. L., Feldman, S. A., Mackall, C. L., & Monje, M. (2022). GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. Nature, 603, 934–940. https://doi.org/10.1038/s41586-022-04489-4*

DOI: 10.54254/2753-8818/98/2025.21496

[8] *Qi, C., Gong, J., Li, J., Liu, D., Qin, Y., Ge, S., Zhang, M., Peng, Z., Zhou, J., Cao, Y., Zhang, X., Lu, Z., Lu, M., Yuan, J., Wang, Z., Wang, Y., Peng, X., Gao, H., Liu, Z., Wang, H., Yuan, D., Xiao, J., Ma, H., Wang, W., Li, Z., & Shen, L. (2022). Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. Nature Medicine, 28, 1189–1198. https://doi.org/10.1038/s41591-022-01800-8*

[9] *Zhang, J., Hu, Y., Yang, J., Li, W., Zhang, M., Wang, Q., ... & Huang, H. (2022). Non-viral, specifically targeted CAR-T cells achieve high safety and efficacy in B-NHL. Nature, 609, 369-374. https://doi.org/10.1038/s41586-022-05140-y*

[10] *Rurik, J. G., Tombácz, I., Yadegari, A., Méndez Fernández, P. O., Shewale, S. V., Li, L., ... Epstein, J. A. (2022). CAR T cells produced in vivo to treat cardiac injury. Science, 375(6585), 91–96. https://doi.org/10.1126/science.abm0594*

[11] *Cappell, K. M., & Kochenderfer, J. N. (2023). Long-term outcomes following CAR T cell therapy: what we know so far. Nature Reviews Clinical Oncology, 20, 359–371. https://doi.org/10.1038/s41571-023-00754-1*