http://pmjournal.ir Review Article

Winter 2024, Volume 9, Issue 32 (1-7)

New Developments in Cancer Treatment Using CAR T cell Therapy, a Kind of Gene Therapy

Azin Sohrabi[1], Mahnaz Saremi[2]

[1]Department of Biology, Faculty of Basic Sciences, East Tehran Branch, Islamic Azad university, Tehran, Iran.
[2]Reference Health Laboratory, Ministry of Health and Medical Education.

**Corresponding author: Azin Sohrabi, Department of Biology, Faculty of Basic Sciences, East Tehran Branch, Islamic Azad university, Tehran, Iran. Email: azin.sihrabi1998@gmail.com.*

DOI: 10.22034/pmj.2024.2024374.1034

Submitted: 2024-03-06
Accepted: 2024-03-20

Keywords:
Chimeric antigen receptor
Solid tumors
Immunotherapy
Cancer

How to Cite this Article:

A. Sohrabi, M. Saremi. "New Developments in Cancer Treatment Using CAR T cell Therapy, a Kind of Gene Therap " Personalized Medicine Journal, Vol. 9, no. 32, pp. 1- 7.

Abstract:

Recent research has pinpointed cancer as the primary cause of death on a global scale. Various traditional medications and cytotoxic immunotherapies have been established and are now accessible on the market. Given the intricate nature of tumor activity and the multitude of genetic and cellular elements implicated in the development and spread of cancers, it is imperative to create a highly effective immunotherapy that can specifically target tumors at both the cellular and genetic levels. In the clinical context, cancer immunotherapy is growing more and more significant, particularly for tumors that are resistant to traditional chemotherapy and targeted treatments. Chimeric antigen receptor (CAR) T cell therapy is a new method of modifying T cells taken from a patient's blood in a laboratory setting. These modified T cells are created to express artificial receptors that specifically target a particular tumor antigen. These specifically recognize the tumor antigen without the participation of the major histocompatibility complex. The use of CAR therapy has the promise of providing a prompt and more secure treatment regimen for both non-solid and solid malignancies. This study provides a comprehensive analysis of the benefits and progress made in CAR immunotherapy.

INTRODUCTION

Immunological checkpoints are natural mechanisms that impede the immune system's ability to recognize and eliminate normal cells inside the organism. Cancerous cells use this defense route to avoid the detrimental effects of the immune system's activities. If tumor cells cannot use these immunological barriers for defense, the immune system can target and remove them (1). Immune checkpoint blockers are a specific kind of medication created specifically to treat solid tumor malignancies. These medications hinder the function of immunological checkpoints, hence enhancing immune reactions to cancerous growths, namely T-cell reactions (2). Multiple trials have shown the efficacy of immune checkpoint blockade treatment in eliminating tumours. Nevertheless, this treatment has limits since it has been seen to not produce a sufficient amount of tumor-reactive T cells. Furthermore, the responses to tumors that are often activated have limited efficacy, and the generation of memory T cells is inadequate(3).

Adoptive cellular therapy (ACT) is a kind of cancer immunotherapy where cells are taken from an individual or someone else, altered in a lab, and then given back to the recipient (4). ACT has shown greater effectiveness when compared to immune checkpoint blockade therapy, namely in terms of increasing the number of T-cells that fight against tumours and triggering specific immune responses. The progress of modern CAR-T treatment relies on the knowledge and understanding acquired from first ACT methods (5). Research indicates that the ability of T cells to fight tumors is greatly reduced by the suppressive microenvironment of tumors. Subsequently, the concept of isolating, cultivating, and transferring these infiltrating cells was suggested as a potential medicinal therapy. This method has shown to be successful in eliminating several forms of cancer in both experimental and clinical trials (6). CD8+ T cells possess the potential to produce proinflammatory cytokines when they are activated. Proinflammatory cytokines has the capacity to inflict enduring damage on malignant cells. CD4+ T lymphocytes are essential for stimulating B cells to produce antibodies, particularly when they differentiate into plasma cells that generate antibodies. Furthermore, these cells have an essential

part in initiating CD8+ T cell immune responses (7, 8).

Furthermore, it has been shown that the application of interleukin (IL)-2, whether administered systematically or used to grow TILs outside the body, might augment the effectiveness of ex vivo-expanded TILs in fighting against tumors (9). Subsequently, it was shown that the combination of lymphodepletion chemotherapy with ACT may augment the in vivo proliferation and long-term presence of T-cells in individuals with solid tumors or blood cancers (10, 11). CAR-T cells are T cells that have been manipulated genetically to express chimeric antigen receptors (CARs). CARs are synthetic receptors made up of an antigen-binding region derived from the single-chain variable fragment (scFv) of a monoclonal antibody (mAb), linked to an intracellular T-cell activation signaling domain and one or two co-stimulatory domains. Chimeric antigen receptors (CARs) may guide genetically modified T lymphocytes to cancerous cells expressing a specific specific epitope (12, 13). Hence, this article focuses on the introduction of CAR-T cell-based treatment and examines the most recent strategies to overcome the significant challenges that hinder the effectiveness of CAR-T therapy in different tumours.

Overview of CAR-T treatment

Immunotherapy with CAR has been shown to be an effective treatment for several types of malignancy. This therapeutic method is a novel kind of gene therapy that directs T lymphocytes to eradicate cancer cells (14). The initial part of this therapy comprises leukapheresis, which is the procedure of separating an individual's peripheral blood. Apheresis is a frequently used method for removing blood from humans and separating it into its component components, which are then genetically changed before being returned into the patient's system. Blood banks have used apheresis to collect platelets and other blood components for the treatment of many conditions, including hematologic and renal diseases. Therefore, it is seen as a wise strategy for both those in excellent physical condition and those who are unwell (14). The CAR is composed of four elements: a single-chain variable fragment (scFv) serving as the extracellular domain for binding to the target, a spacer domain, a intermembrane domain, and an intracellular area responsible for signalling and activation. When comparing CAR T cells to T cell receptor (TCR) modified cells, CAR T cells have the capability to recognize tumour antigens located on the cell surface independently of HLA molecules. This leads to the stimulation, proliferation, and production of cytokines by antigen-specific T cells, enabling them to combat tumours. CARs has the capacity to recognize different antigens without relying on major histocompatibility complex (MHC) molecules. This characteristic expands their potential use in therapeutic contexts (14).

CAR Architecture

CARs are artificial receptors with a modular structure including four primary elements: (1) an extracellular domain that binds to target antigens, (2) a flexible area that allows movement, (3) a domain that spans the cell membrane, and (4) several domains responsible for intracellular signaling. In this discussion, we will examine the existing ideas that form the foundation of CAR architecture (15).

Antigen binding domain

The antigen binding domain is the specific region of the CAR that provides specificity for the target antigen of interest. Conventionally, the antigen-binding portions are derived from the variable heavy (VH) and light (VL) chains of monoclonal antibodies. Subsequently, these areas are interconnected using a pliable linker to form a unified single-chain variable fragment (scFv) (16). Typically, the single-chain variable fragments (scFvs) present in CARs are engineered to specifically identify cancer antigens located on the external membrane of cells. This recognition triggers the activation of T lymphocytes, bypassing the need for major histocompatibility complex (MHC) molecules. Nevertheless, there have been documented instances of CARs that possess the ability to identify tumor-associated antigens inside cells, using a method like to that of TCRs and necessitating MHC molecules. Several characteristics of the single-chain variable fragment (scFv) have a significant impact on the function of CARs beyond their basic ability to recognize and bind the target epitope. The affinity of CARs plays a vital role in defining their activity since it directly impacts the ability of the antigen-binding domain to bind. For effective detection of antigens on tumor cells, activation of CAR signaling, and T cell activation, CARs must possess an elevated specificity for binding to antigens. Nevertheless, it is important to note that an excessively strong affinity should be avoided, as it might result in the demise of CAR-expressing T cells owing to activation-induced processes and give rise to toxicity (17, 18).

Hinge region

The hinged or spacers area is the extracellular architectural region that links the bound domains to the transmembrane domain. The hinge functions give flexible for overcoming steric hindrance and aid in extending the antigen-binding domain to reach the specific epitope. The chosen hinge significantly affects the operation of CARs due to variations in the size and composition of the hinge area (19). These alterations may impact adaptability, CARs formation, signaling, epitope recognition, activation intensity,

and epitope recognition. Additionally, it has been suggested that the length of the gap is crucial for maintaining a sufficient distance between cells, which is necessary for the development of an immunological connection. The ideal separator dimensions depends on the precise location of the targeted epitope and the level of obstruction on the target cell. Increased spacer length provides enhanced adaptability and improved reachability for epitopes in proximity to the cellular membrane or exhibiting intricate glycosylation patterns. Conversely, shorter spacers demonstrate greater efficacy in binding epitopes that are located at a greater distance from the cell membrane (20).

Transmembrane domain

The transmembrane domain is the most enigmatic component among all the sections of CARs. The fundamental function of the membrane domain is to attach the CAR to the outer surface of the T cell. Nevertheless, there is evidence suggesting that this transmembrane domain may have a role in the functioning of CAR-T cells. Research suggests that the transmembrane domains of CAR have a direct influence on the level and durability of CAR expression. Moreover, they may also participate in signalling or synapse formation and possess the ability to form dimers with endogenous signalling molecules. Most membrane domains are derived from naturally occurring proteins like CD3ζ, CD4, CD8α, or CD28. The study on the influence of different transmembrane domains on CAR function is still inadequate, since the transmembrane domain is often modified to accommodate the requirements of the extracellular spacer region or the intracellular signalling areas. The transmembrane domain of CD3ζ has a crucial function in promoting the activation of T cells via CAR signalling. This is because it plays a crucial role in the process of CAR dimerization and its incorporation into the natural TCRs (21, 22).

Intracellular signaling domain

The first version of CARs, which emerged in the final years of the 1990s, had a signalling domain consisting of either CD3ζ or FcRγ. The stimulation of CAR-T cells in the majority of CARs primarily relies on immunoreceptor tyrosine-based activation motifs generated from CD3ζ. However, relying just on these patterns for signalling is inadequate to stimulate effective T cell responses. The lifetime and durability of these first generation CARs are restricted in vitro (23). The clinical research have substantiated these findings, revealing a low or negligible level of efficacy. The importance of co-stimulation in preserving the durability of CD-19-targeted CAR-T cells was shown via the use of first in in vivo studies of B-cell carcinoma. Introducing a co-stimulatory domain improved the production and multiplication of IL-2 when the antigen was encountered many times (24). Due to the acknowledgment of the importance of co-stimulation in providing durable CAR-T cell therapy, a new version of CARs, referred to as second generation CARs, were created. These CARs are composed of a single co-stimulatory domain linked in a sequential manner with the CD3ζ intracellular signalling domain. Both CD28 and 4-1BB (CD137), which are the two most often approved co-stimulatory motifs by the FDA, have a significant association with heightened levels of patient response. The co-stimulatory domains have unique structural and metabolic features (25, 26).

Antigens used in clinical trials for CAR T-cell therapy targeting solid tumours

EGFRvIII

The rise of CARs has attracted much attention and examination as a result of the favourable results seen from using CD19 CARs in tumour therapy. An in-depth examination is conducted on many antigens linked to tumors to improve the chances of achieving maximum effectiveness. Invasive glioblastoma (GB) is caused by the excessive synthesis of epidermal growth factor receptor (EGFR) and EGFR variant III (EGFRvIII) in different kinds of tumors (27, 28). EGFRvIII presence in a cell often results in cell survival, aggression, formation of new blood vessels, and resistance to both radiation and chemotherapy. EGFRvIII-specific CAR T-cells, which have demonstrated substantial anti-tumor efficacy in preclinical investigations, are now being assessed for inclusion in clinical trials (29). EGFR can maintain a binding site for cetuximab after modifications, however it may lose its domains I and II, as well as its cytoplasmic tail. Consequently, cetuximab has the ability to detect the shortened version of EGFR (huEGFRt), enabling the detection, monitoring, and removal of CAR T-cells that exhibit the truncated EGFR after cetuximab treatment. EGFRvIII is detected in around between 25 and 30% of newly identified GB tumours and is now being examined in clinical trials as a possible therapeutic target for the treatment of GB tumours. Despite the potential for manufacturing cells for therapy and administering them intravenously without complications, two clinical experiments conducted on patients with GB who received treatment with EGFRvIII-targeting CAR T-cells, either co-stimulated solely with 4-1BB or in combination with CD28, did not demonstrate any beneficial effects in radiographic imaging (30, 31).

IL13Rα2

Decreased longevity in people has been connected with a glioma-specific protein called Interleukin 13 receptor α2 (IL13Rα2). A study shown that the application of CAR T-cell treatment led to tumour

decrease. IL13Rα2-specific CARs have the capacity to also identify IL13Rα1 (32). The IL13Rα1-specific scFv is classified as an antigen binding domain, resulting in improved specificity. PET imaging studies show that IL13Rα2-specific CAR T-cells may efficiently migrate to the brain parenchyma, particularly in areas impacted by tumors. Studies enhanced these findings by using an updated design with 4-1-BB co-stimulation (33, 34). The findings demonstrated favourable outcomes for the treatment of GB and a satisfactory degree of tolerance. A different study discovered that although brain and spinal tumors showed a significant decrease in size by therapeutic and PET scans, this improvement was short-lived, lasting around seven months, before the tumors reappeared in other areas (35).

HER2

Studies have shown that human epidermal growth factor receptor 2 (HER2) is excessively produced in different tumors. This overexpression is associated with the development of cancer, indicating that HER2 might be used as a useful indicator for predicting outcomes and as an option for therapy in cancer patients (36). Despite the substantial research conducted in clinical studies on CAR T-cells targeting HER2-expressing tumors, safety concerns have arisen due to the unfortunate death of a colorectal cancer patient who was administered 1×10^{10} third-generation HER2-CAR T-cells (37). Conversely, the phase 1 trial involved administering increasing doses of second-generation HER2-CAR cytomegalovirus (CMV) pp65-bispecific cytotoxic T lymphocytes (CTLs) to 17 patients who had late-stage HER2-positive GB. The trial found that the patients tolerated one or more infusions of 1×10^{8} CTLs well, without experiencing severe toxicities associated to the therapy. Out of the 16 patients who were able to be evaluated, one had a partial response for a duration of over nine months, while seven patients exhibited stable illness that lasted between eight weeks and 29 months. Unfortunately, the status of eight patients worsened after receiving medication. However, the quantities of CAR T-cells in the blood gradually decreased over time, and after 12 months, only two patients still tested positive (38). However, after 18 months, neither patient showed any signs of CAR T-cells, indicating that the HER2-CAR T-cells did not multiply after being administered but managed to survive for almost a year (38, 39).

Difficulties presented by CAR-T cell therapy for solid tumors

Multiple obstacles hinder the use of CAR-T cell therapy in treating solid tumors. Initially, there is a restricted variety of antigens that may be precisely aimed at. Moreover, solid tumors often show varied expression of these antigens. Furthermore, the configuration of the CAR itself presents difficulties. Additionally, there are difficulties linked to the manufacturing of CAR-T cells (40). Furthermore, the capacity of CAR-T cells to efficiently detect and penetrate tumour tissue is inadequate. Furthermore, the presence of an immunosuppressive milieu inside the tumour creates an obstacle. Therefore, there is a pressing need for innovative therapeutic approaches that use lower dosages of CAR-T cells, including highly effective CARs and combination therapies. This section outlines the difficulties linked to CAR-T cell treatment for solid tumors, mostly caused by internal and external variables affecting T cells (40).

Native T-Cell factors

T cell exhaustion

Prolonged activation of effector T cells by receptor-mediated stimulation may induce their differentiation into an exhausted state, leading to decreased longevity of T cells and heightened expression of inhibitory receptors (41). Modifying the CD28 co-stimulatory domain to reduce its activity has been shown to improve the effectiveness of CAR-T cells in organisms. Evidence indicates that CD28 signaling boosts the glycolytic process and promotes the effector memory characteristics, whereas 41BB signaling may improve the oxidative phosphorylation process and sustain the primary memory state (42). The injection of the third-generation CAR, which combines CD28 and 41BB, resulted in a more vigorous proliferation compared to the second-generation CAR that simply utilizes CD28. The density of chimeric antigen receptors on T cells, or on a particular subset of T cells, might influence both the efficacy and possible adverse consequences. Studies indicate that the immunological contact between CAR-T cells and target cells is crucial for the functionality of these cells. Remarkably, the strong attraction of CAR-T cells to certain antigens allows them to identify very little amounts of these antigens. However, this characteristic also raises the possibility of harmful effects on healthy cells around the tumor (43, 44).

CAR-T cell toxicity

The intricacies of CAR-T cell treatment may be categorized under the following groups: Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) affect the targeted cells and unintended organs due to CAR-T cell therapy, leading to cardiotoxicity and hypersensitivity responses (45). Severe CRS is caused by multiple organ failure, coagulation problems, and respiratory system impairment, presenting a severe danger. ICANS, or immune effector cell-associated neurotoxicity condition, may occur in individuals undergoing CAR-T cell treatment, with a prevalence

between 0% and 50% (46). The symptoms of ICANS include hallucinations, encephalopathy, seizures, and cerebral edema. To reduce these negative occurrences, monoclonal antibodies aimed at the IL-6 receptor, IL-1 receptor antagonist, or granulocyte-macrophage colony-stimulating factor (GM-CSF) might be used. Tocilizumab is licensed for treating CRS, although anakinra and lenzilumab have demonstrated effectiveness in treating both CRS and neurotoxicity. Evaluating and forecasting possible hazards before treatment is still difficult to determine (47).

CAR T cell therapy in conjunction with vaccinations

Therapeutic cancer vaccines are a significant breakthrough in cancer therapy since they specifically activate T cells to identify and eliminate cancerous growths. Several studies have shown that dual-specific T cells, which possess tumor-specific chimeric antigen receptors (CARs) as well as their own T cell receptors (TCRs), display strong expansion and dramatic anti-cancer effects when stimulated with a vaccination containing highly effective immunogens, such as influenza viruses [48]. Preliminary experiments have shown tremendous potential in using virus-specific cytotoxic T lymphocytes (CTLs) for the development of CAR T cells. Notably, in patients with neuroblastoma, cytotoxic T lymphocytes (CTLs) that exclusively target Epstein-Barr virus (EBV) and express GD2 CARs showed a longer period of being active compared to non-virus-specific T cells that were activated (48).

Vaccination offers an alternate approach to enhance the proliferation, activation, and effectiveness of CAR T cells in targeting particular cells inside the body. A commonly used kind of immunization is a viral-based vaccine, such a cytomegalovirus (CMV)-based vaccine, which, when combined with T cell infusion, significantly augments tumour eradication. The administration of a viral vaccine containing gp100 led to enhanced proliferation of T cells and decreased tumour growth in several mouse models (49). A recent study conducted on individuals with B cell acute lymphoblastic leukemia (B-ALL) has shown that viral vaccinations may be used to stimulate natural TCRs, even in the absence of lymphodepletion. This activation enables the efficient proliferation and sustained preservation of CD19-modified virus-specific T lymphocytes. However, the reactivation of viruses and the presence of viruses in the circulation pose possible risks, which necessitates a more comprehensive evaluation of the safety of viral vaccines (50).

Studies conducted in both preclinical and clinical settings have shown that cancer vaccines containing soluble tumor-associated antigens (TAAs) and dendritic cell (DC) adjuvants may successfully activate TAA-specific effector cells and stimulate the production of antibodies. Dendritic cells (DCs) are highly specialized immune cells that have a vital function in both innate and adaptive immunity. They are crucial for the efficacy of immunotherapy. A study showcased the enhanced stimulation, reproduction, and anti-tumor effect of ACT by the implementation of a DC vaccine in live organisms (51). Participants in a melanoma clinical trial received injections of dendritic cells with tumour antigens, followed by the infusion of T lymphocytes that had infiltrated the tumour. Consequently, one man had complete remission while two others maintained a stable state of sickness (52).

THE WAY FORWARD AND CONCLUSION

The realm of synthetic biology and cell engineering offers boundless potential, serving as a basis for the development of groundbreaking pharmaceuticals. Ongoing attempts are being made to create creative strategies to enhance the effectiveness of CAR T-cell treatment. Potential engineering strategies may boost CAR T-cell biological sciences, expanding the method's use in cancer therapy. Enhancing the intricacy of CAR architectures and altering the genes on T-cells might heighten the possible hazards linked to CAR T-cell treatment. Both gene editing and viral transmission technologies include the danger of unintentionally modifying genes that are not the intended target (53).

A targeted insertion of the CAR gene occurred at the TET2 locus, leading to the proliferation of T-cells in a clonal manner. Following that, the clonal population of T-cells saw a natural decrease, emphasizing the possible hazards linked to the use of genetically-modified cells for patient therapy (54, 55). A pilot clinical study has started to evaluate a genetically modified T-cell product that has a transgenic TCR engineered to target NY-ESO-1. This product has been genetically modified utilizing the CRISPR-Cas9 technology. Ongoing monitoring of obstacles linked to gene-editing in trials of CAR T-cell products will aid in detecting potential long-term dangers connected with the emerging field of gene-editing in medicine and assist in devising viable solutions for these issues. The high manufacturing cost of CAR T-cells may rise further because of the use of innovative technological methods. Techniques include using non-viral vectors may reduce manufacturing costs and perhaps enhance affordability. Due to the shorter duration of clinical trials for CAR T-cells in comparison to other medications, it is likely that many CARs may get approval for different illnesses over the next 5-10 years.

REFERENCES

1.Safarzadeh Kozani, P., Safarzadeh Kozani, P., Ahmadi Najafabadi, M., Yousefi, F., Mirarefin, S. M. J., & Rahbarizadeh, F. (2022). Recent advances in solid tumor CAR-T cell therapy: driving tumor cells from hero to zero?. *Frontiers in Immunology*, *13*, 795164.

2.Pardoll, D. M. (2012). The blockade of immune checkpoints

in cancer immunotherapy. *Nature reviews cancer*, *12*(4), 252-264.
3.Jenkins, R. W., Barbie, D. A., & Flaherty, K. T. (2018). Mechanisms of resistance to immune checkpoint inhibitors. *British journal of cancer*, *118*(1), 9-16.
4.Rohaan, M. W., Wilgenhof, S., & Haanen, J. B. (2019). Adoptive cellular therapies: the current landscape. *Virchows Archiv*, *474*, 449-461.
5.Rosenberg, S. A., Spiess, P., & Lafreniere, R. (1986). A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science*, *233*(4770), 1318-1321.
6.Borst, J., Ahrends, T., Bąbała, N., Melief, C. J., & Kastenmüller, W. (2018). CD4+ T cell help in cancer immunology and immunotherapy. *Nature Reviews Immunology*, *18*(10), 635-647.
7.Whiteside, T. L. (2008). The tumor microenvironment and its role in promoting tumor growth. *Oncogene*, *27*(45), 5904-5912.
8.Baxevanis, C. N., Voutsas, I. F., Tsitsilonis, O. E., Gritzapis, A. D., Sotiriadou, R., & Papamichail, M. (2000). Tumor-specific CD4+ T lymphocytes from cancer patients are required for optimal induction of cytotoxic T cells against the autologous tumor. *The Journal of Immunology*, *164*(7), 3902-3912.
9.Umeshappa, C. S., Nanjundappa, R. H., Xie, Y., Freywald, A., Xu, Q., & Xiang, J. (2013). Differential requirements of CD 4+ T-cell signals for effector cytotoxic T-lymphocyte (CTL) priming and functional memory CTL development at higher CD 8+ T-cell precursor frequency. *Immunology*, *138*(4), 298-306.
10.Eberlein, T. J., Rosenstein, M., & Rosenberg, S. A. (1982). Regression of a disseminated syngeneic solid tumor by systemic transfer of lymphoid cells expanded in interleukin 2. *The Journal of experimental medicine*, *156*(2), 385-397.
11.Rosenberg, S. A., Packard, B. S., Aebersold, P. M., Solomon, D., Topalian, S. L., Toy, S. T., ... & White, D. E. (1988). Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. *New England Journal of Medicine*, *319*(25), 1676-1680.
12.June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., & Milone, M. C. (2018). CAR T cell immunotherapy for human cancer. Science, 359(6382), 1361-1365.
13.Park, J. H., Rivière, I., Gonen, M., Wang, X., Sénéchal, B., Curran, K. J., ... & Sadelain, M. (2018). Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *New England Journal of Medicine*, *378*(5), 449-459.
14.Mohanty, R., Chowdhury, C. R., Arega, S., Sen, P., Ganguly, P., & Ganguly, N. (2019). CAR T cell therapy: A new era for cancer treatment. *Oncology reports*, *42*(6), 2183-2195.
15.Sterner, R. C., & Sterner, R. M. (2021). CAR-T cell therapy: current limitations and potential strategies. *Blood cancer journal*, *11*(4), 69.
16.Chailyan, A., Marcatili, P., & Tramontano, A. (2011). The association of heavy and light chain variable domains in antibodies: implications for antigen specificity. *The FEBS journal*, *278*(16), 2858-2866.
17.Liu, X., Jiang, S., Fang, C., Yang, S., Olalere, D., Pequignot, E. C., ... & Zhao, Y. (2015). Affinity-tuned ErbB2 or EGFR chimeric antigen receptor T cells exhibit an increased therapeutic index against tumors in mice. *Cancer research*, *75*(17), 3596-3607.
18.Huang, R., Li, X., He, Y., Zhu, W., Gao, L., Liu, Y., ... & Zhang, X. (2020). Recent advances in CAR-T cell engineering. *Journal of Hematology & Oncology*, *13*(1), 1-19.
19.Hirobe, S., Imaeda, K., Tachibana, M., & Okada, N. (2022). The effects of chimeric antigen receptor (CAR) hinge domain post-translational modifications on CAR-T cell activity. *International Journal of Molecular Sciences*, *23*(7), 4056.
20.Xu, H., Hamburger, A. E., Mock, J. Y., Wang, X., Martin, A. D., Tokatlian, T., ... & Kamb, A. (2020). Structure-function relationships of chimeric antigen receptors in acute T cell responses to antigen. *Molecular Immunology*, *126*, 56-64.
21.Bridgeman, J. S., Hawkins, R. E., Bagley, S., Blaylock, M., Holland, M., & Gilham, D. E. (2010). The optimal antigen response of chimeric antigen receptors harboring the CD3ζ transmembrane domain is dependent upon incorporation of the receptor into the endogenous TCR/CD3 complex. *The Journal of Immunology*, *184*(12), 6938-6949.
22.Guedan, S., Posey Jr, A. D., Shaw, C., Wing, A., Da, T., Patel, P. R., ... & June, C. H. (2018). Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. *JCI insight*, *3*(1).
23.Wu, L., Wei, Q., Brzostek, J., & Gascoigne, N. R. (2020). Signaling from T cell receptors (TCRs) and chimeric antigen receptors (CARs) on T cells. *Cellular & Molecular Immunology*, *17*(6), 600-612.
24.Wu, W., Zhou, Q., Masubuchi, T., Shi, X., Li, H., Xu, X., ... & Xu, C. (2020). Multiple signaling roles of CD3ε and its application in CAR-T cell therapy. *Cell*, *182*(4), 855-871.
25.Maher, J., Brentjens, R. J., Gunset, G., Rivière, I., & Sadelain, M. (2002). Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor. *Nature biotechnology*, *20*(1), 70-75.
26.Kawalekar, O. U., O'Connor, R. S., Fraietta, J. A., Guo, L., McGettigan, S. E., Posey, A. D., ... & June, C. H. (2016). Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity*, *44*(2), 380-390.
27.Daei Sorkhabi, A., Mohamed Khosroshahi, L., Sarkesh, A., Mardi, A., Aghebati-Maleki, A., Aghebati-Maleki, L., & Baradaran, B. (2023). The current landscape of CAR T-cell therapy for solid tumors: Mechanisms, research progress, challenges, and counterstrategies. *Frontiers in immunology*, *14*, 1113882.
28.Tang, C. K., Gong, X. Q., Moscatello, D. K., Wong, A. J., & Lippman, M. E. (2000). Epidermal growth factor receptor vIII enhances tumorigenicity in human breast cancer. *Cancer research*, *60*(11), 3081-3087.
29.Johnson, L. A., Scholler, J., Ohkuri, T., Kosaka, A., Patel, P. R., McGettigan, S. E., ... & Maus, M. V. (2015). Rational development and characterization of humanized anti–EGFR variant III chimeric antigen receptor T cells for glioblastoma. *Science translational medicine*, *7*(275), 275ra22-275ra22.
30.O'Rourke, D. M., Nasrallah, M. P., Desai, A., Melenhorst, J. J., Mansfield, K., Morrissette, J. J., ... & Maus, M. V. (2017). A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Science*

translational medicine, *9*(399), eaaa0984.
31.Goff, S. L., Morgan, R. A., Yang, J. C., Sherry, R. M., Robbins, P. F., Restifo, N. P., ... & Rosenberg, S. A. (2019). Pilot trial of adoptive transfer of chimeric antigen receptor transduced T cells targeting EGFRvIII in patients with glioblastoma. *Journal of immunotherapy (Hagerstown, Md.: 1997)*, *42*(4), 126.
32.Brown, C. E., Alizadeh, D., Starr, R., Weng, L., Wagner, J. R., Naranjo, A., ... & Badie, B. (2016). Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *New England Journal of Medicine*, *375*(26), 2561-2569.
33.Krenciute, G., Krebs, S., Torres, D., Wu, M. F., Liu, H., Dotti, G., ... & Gottschalk, S. (2016). Characterization and functional analysis of scFv-based chimeric antigen receptors to redirect T cells to IL13Rα2-positive glioma. *Molecular Therapy*, *24*(2), 354-363.
34.Keu, K. V., Witney, T. H., Yaghoubi, S., Rosenberg, J., Kurien, A., Magnusson, R., ... & Gambhir, S. S. (2017). Reporter gene imaging of targeted T cell immunotherapy in recurrent glioma. *Science translational medicine*, *9*(373), eaag2196.
35.Brown, C. E., Badie, B., Barish, M. E., Weng, L., Ostberg, J. R., Chang, W. C., ... & Jensen, M. C. (2015). Bioactivity and safety of IL13Rα2-redirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. *Clinical cancer research*, *21*(18), 4062-4072.
36.Vranić, S., Bešlija, S., & Gatalica, Z. (2021). Targeting HER2 expression in cancer: New drugs and new indications. *Bosnian journal of basic medical sciences*, *21*(1), 1.
37.Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., & Rosenberg, S. A. (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Molecular therapy*, *18*(4), 843-851.
38.Daei Sorkhabi, A., Sarkesh, A., Saeedi, H., Marofi, F., Ghaebi, M., Silvestris, N., ... & Brunetti, O. (2022). The basis and advances in clinical application of cytomegalovirus-specific cytotoxic T cell immunotherapy for glioblastoma multiforme. *Frontiers in Oncology*, *12*, 818447.
39.Vitanza, N. A., Johnson, A. J., Wilson, A. L., Brown, C., Yokoyama, J. K., Künkele, A., ... & Park, J. R. (2021). Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis. *Nature medicine*, *27*(9), 1544-1552.
40.Chung, H., Jung, H., & Noh, J. Y. (2021). Emerging approaches for solid tumor treatment using CAR-T cell therapy. *International journal of molecular sciences*, *22*(22), 12126.
41.Ghajari, G., Heydari, A., & Ghorbani, M. (2023). Mesenchymal stem cell-based therapy and female infertility: limitations and advances. *Current Stem Cell Research & Therapy*, *18*(3), 322-338.
42.Piri-Gharaghie, T., Ghajari, G., Hassanpoor, M., Jegargoshe-Shirin, N., Soosanirad, M., Khayati, S., ... & Mirzaei, A. (2023). Investigation of antibacterial and anticancer effects of novel niosomal formulated Persian Gulf Sea cucumber extracts. *Heliyon*, *9*(3).
43.Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., & Rosenberg, S. A. (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Molecular therapy*, *18*(4), 843-851.
44.Ramos, C. A., Rouce, R., Robertson, C. S., Reyna, A., Narala, N., Vyas, G., ... & Dotti, G. (2018). In vivo fate and activity of second-versus third-generation CD19-specific CAR-T cells in B cell non-Hodgkin's lymphomas. *Molecular Therapy*, *26*(12), 2727-2737.
45.Sun, S., Hao, H., Yang, G., Zhang, Y., & Fu, Y. (2018). Immunotherapy with CAR-modified T cells: toxicities and overcoming strategies. *Journal of immunology research*, *2018*.
46.Norelli, M., Camisa, B., Barbiera, G., Falcone, L., Purevdorj, A., Genua, M., ... & Bondanza, A. (2018). Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nature medicine*, *24*(6), 739-748.
47.Sterner, R. M., Sakemura, R., Cox, M. J., Yang, N., Khadka, R. H., Forsman, C. L., ... & Kenderian, S. S. (2019). GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR-T cell function in xenografts. *Blood, The Journal of the American Society of Hematology*, *133*(7), 697-709.
48.Huang, M., Deng, J., Gao, L., & Zhou, J. (2020). Innovative strategies to advance CAR T cell therapy for solid tumors. *American journal of cancer research*, *10*(7), 1979–1992.
49.Grenier, J. M., Yeung, S. T., Qiu, Z., Jellison, E. R., & Khanna, K. M. (2018). Combining adoptive cell therapy with cytomegalovirus-based vaccine is protective against solid skin tumors. *Frontiers in Immunology*, *8*, 1993.
50.Lapteva, N., Gilbert, M., Diaconu, I., Rollins, L. A., Al-Sabbagh, M., Naik, S., ... & Rooney, C. M. (2019). T-cell receptor stimulation enhances the expansion and function of CD19 chimeric antigen receptor–expressing T cells. *Clinical Cancer Research*, *25*(24), 7340-7350.
51.Carreno, B. M., Magrini, V., Becker-Hapak, M., Kaabinejadian, S., Hundal, J., Petti, A. A., ... & Linette, G. P. (2015). A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science*, *348*(6236), 803-808.
52.Poschke, I., Lövgren, T., Adamson, L., Nyström, M., Andersson, E., Hansson, J., ... & Kiessling, R. (2014). A phase I clinical trial combining dendritic cell vaccination with adoptive T cell transfer in patients with stage IV melanoma. *Cancer immunology, immunotherapy*, *63*, 1061-1071.
53.Poirot, L., Philip, B., Schiffer-Mannioui, C., Le Clerre, D., Chion-Sotinel, I., Derniame, S., ... & Smith, J. (2015). Multiplex genome-edited T-cell manufacturing platform for "off-the-shelf" adoptive T-cell immunotherapies. *Cancer research*, *75*(18), 3853-3864.
54.Rafiq, S., Hackett, C. S., & Brentjens, R. J. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nature reviews Clinical oncology*, *17*(3), 147-167.
55.Dimitri, A., Herbst, F., & Fraietta, J. A. (2022). Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. Molecular Cancer, 21(1), 78.