Mechanism and Future Application of CAR-T Therapy in DLBCL

Yunqi Hou *

Culver Academies, 1300 Academy Rd, Culver, Indiana, 46511, United States

* Corresponding author: yunqi.hou@culver.org

Abstract. The idea of CAR-T cell therapy emerged in the early 1990s, as scientists discovered the application of CD3ζ in its effectiveness of activating T cells. Through generations of improvements by implementing costimulatory domains, the therapy demonstrated an excellent prognosis in refractory DLBCL patients. This paper is going to address the mechanism and basic structures of CAR-T therapy. In addition, this paper will analyze the result of several clinical trials and compare the efficacy of the therapy to the current second-line treatments while pointing out its limitations and efficacy in DLBCL. It is the hope that this paper can give an overview of the therapy while suggesting a superior treatment in DLBCL patients and address some current applications of CAR-T therapy in DLBCL patients while suggesting some future improvements and applications in CAR-T cell therapy. Although Car-T cell therapy showed prominent results over stem cell transplantation in elder patients, its efficacy still required further examinations in order to replace auto-HCT as the second-line treatment of DLBCL.

Keywords: CAR-T Therapy, Diffused Large B Cell Lymphoma (DLBCL), Mechanism.

1. Introduction

Diffused large B cell lymphoma, one common NHL in the United States. For 2/3 of the patients, first line immunochemotherapy combinations that contain rituximab. Using domain of CD20 as a classic antibody, it showed promising results in the treatment of diffused large B cell diffusion lymphoma. However, about 10 to 15% of patients demonstrated primary refractory disease three months after the initial treatment, and another 20 to 35% of patients have a relapse. For relapsed or refractory DLBCL patients, only 40 to 60% of them are responding to current second line therapy. Among these patients, 50% received autologous hematopoietic stem-cell transplantation, while only 26% of the patients respond to the therapy. According to statistics, for patients that were unable to receive high-dose chemotherapy and hematopoietic stem-cell transplantation, a poor result is shown, and median survival rates were only 23% and 16% respectively. On the other hand, the CAR-T cell therapy demonstrated high levels of efficacy with no significant side effects. In one phase 2 study of tisagenlecleucel, the overall response rate was found to be 52%, with patterns of complete responses shown in 40% of the patients. Among patients that died, none of them were attributed to the widely regarded side effects of CAR-T therapy, including CRS and cerebral edema that is triggered by the rapid release of cytokines. In another phase 2 study of CAR-T therapy where axicabtagene ciloleucel was used as the codomain, the objective response rate was 82% for 101 administered patients, and over half of the administered patients remained to survive after 18 months of the initial therapy. As a result, the therapy is commonly found in primary refractory DCBCL patients for its prominent efficacy and side effects and it has a great chance of taking the place of Auto-SCT as the second treatment of DLBCL.

In this paper, mechanisms, and effects of the Axl-cel and tisagel CAR-T therapy, acting on two different codomains, on the refractory patients will be discussed in detail, and more of the data comparing the efficacy of auto-SCT to CAR-T cells therapy will be analyzed. In order to compare the differences between the two therapies and the uses of CAR-T cell therapy in DLBCL patients, the limitations of the therapy will also be presented.

2. Overview of the Mechanism of CAR-T therapy

Three parts constitute the CAR-T cells. An extracellular antibody-like surface domain, a transmembrane domain, and an intracellular signaling domain activate the T-cells [1]. Within those three general parts, the Ectodomain, the extracellular region of the CAR-T cells, contains ScFv and a spacer.

On the extracellular region, a short peptide that presents on the N-terminus of the new protein helps to guide it into the ER. The newly synthesized proteins are formed by the ribosome before it folds into its acting shape. Within the ectodomain, heavy and light chains of immunoglobulins fused together with a short linker peptide form a single chain variable fragment. ScFv facilitates phage display, and CAR-T cells often use it to bind its antigen, because it allows rare clones to be screened and isolated by using desirable antigens. Besides, it also helps the receptor to recognize tumor antigens [2]. In adopting the CAR-T cells, one traditional way of adopting the scFv antibodies is by isolating the mRNA first from lymphoma cells, and then reverse transcribing it into cDNA. The cDNA will then allow the antibody gene to be amplified through Polymerase Chain Reaction. Using this method, great diversities of antibodies can then be created. To successfully isolate and display the scFv, one common approach is to use E. coli as the host of the fragments [3]. Following by the productor of the antigen binding domain, a spacer connects scFv to the transmembrane domain. The transmembrane domain serves a crucial role in connecting the extracellular and intercellular parts together. The transmembrane is crucial in ensuring the functions of the receptor and it consists of a hydrophobic structure that crosses the membrane. The CD3-zeta transmembrane ensures the transmission between scFv and the intracellular domain. Shifting to the cross-membrane domain of CAR-T cells, a combination of CD3, CD8, and CD28 mediates the dimerization of chimeric antigen receptors through cellular activation and functional interaction with the endogenous TCR. At the end of the receptor, the CD3 ζ is the most common component that includes in three ITAMs, and costimulatory domains also assist in the growth, effects, and persistence of the enhanced T cells. Aggregation of the receptor often is attributed to the interactions between TCR and cognate antigens, phosphorylation of CD3 proteins, and intracellular signaling down the pathway. These interactions rely on the crucial activation of antigen-specific T lymphocytes. Besides, costimulatory receptors are important in determining the T cell response quality, proliferation, the distribution of cytokines, and the memory in remaining cells. Costimulatory receptors activate PI3K kinase, which leads to activation of protein kinase B, whereas the TNF receptor is used to activate NF-kB.

Viruses are the most often used carrier in both fundamental science and clinical settings because of their speed in transferring to the T cells, quick time reaching the required amount of grown T cells, and accessibility of several viruses with various expression properties. The majority of viral systems may accept genes from beneficial and intriguing cells as well as offer the enzymes and proteins necessary for the production of modified carriers that include adenovirus, adeno-associated viruses, and retroviruses. Among these, genetically modified retroviruses are the most widely used agents in the delivery of the cells. The viral vectors, however, might be dangerous. The carrier capacity is potentially constrained, with insufficient titer if the insertion mutation employed elicit the immune response that cause tumorigenesis and toxicity. By contrast, non-viral gene become an alternate approach because of its superior effectiveness, specificity, unrestricted carrier capacity, regulated composition, and abundant production. Non-viral gene therapy becomes an effective method for treating lymphoma cancer. The innovative non-viral minicircle DNA vectors, produced from a cellular plasmid. It can continuously express genetic material at high levels, and it is devoid of plasmid bacterial DNA sequences.

To produce CAR-T cells, leukapheresis is used to first remove any remaining leukocytes from the patients. In the body of patients, T cells are required to detach from the leukocytes. So, the cells will then be washed and enriched. Third, distinct antibody will link to a specific tag to differentiate the types of T cells based on CD4/CD8 makeup. Through the process of naturing, the T cells can then be activated. Cells that contain autologous antigen from donors must be purified for this procedure, as well as monoclonal anti-CD3/anti-CD28 antibodies, independently or in conjunction with feeder cells.

The most widely utilized agent, IL-2, stimulates a faster expansion of T cells. The culture conditions are further optimized to alter phenotype of the T cells. Bioreactor system like WAVE is utilized to cultivate CAR-T cells. A single device—the CliniMACS Prodicy system—can efficiently activate, transduce, and grow the cells. They are then gathered and given to the patients after they have reached the quantity needed for therapeutic applications.

3. Generations of CAR-T cell therapy

For the initial generation of CARs, a single structure from the CD3ζ -chain or Fcε RI from the intracellular domain is the usual characteristic. It administers exogenous interlukin-2, because simply the CAR-T cells were unable to destroy the tumor cells along. As a result, the simultaneous injection of cytokines had an important positive effect on the initial generation of CAR-T cell treatment that utilized single-chain receptors. Recent research shows that the phosphorylation of ITAM A and C is involved in the apoptotic signal presents in CD3, which is beneficial to the transgene's ability to continuous expression. However, in clinical settings, CAR-T cells containing CD3-chain instead of the Fc-RI-chain were used in more investigations. The fact that the CD3-chain comprised three ITAMs while the Fc-RI-chain only had one might be the cause. On the other hand, while having lower expression levels in vitro, the cells containing the CD3-chain showed batter successful rate in triggering the function of T cells and eliminating cancer cells. A combination of dimer of CD3, CD8, and CD28 makes up the transmembrane domain of CAR-T cells, which activate cells through the dimerization of CARs and the contact with the endogenous TCR through this receptor. Due to many limiting factors of the first CAR-T cells therapy, expected result didn’t appeared on patients as the CAR-T cells didn’t reach adequate proliferation as well as sufficient secreted cytokines due to the lack of costimulatory domains.

The usual features of T cell activation were dual signals, and second-generation CAR-T therapies are among the most common one on the market. This process includes three separate receptor types: co-stimulatory receptors, cytokine receptors, and T-cell antigen receptors. The TCR activates the first signal, a unique signal that detects cells that presents antigenic MHC complex on its surface. Then, the second signal will encourage the production of IL-2. So, T cell activation will fully functions and reduce unnecessary apoptosis. Without the presence of costimulatory signal, naive T cells cannot carry out their typical function; this is true even if the antigen stimulates the T cells. So, CARs that contain the CD3 sequence will be unable to activate the cells without the signal of costimulatory domains. Trying to improve its efficacy, second generation CARs added one significant feature. It included various protein receptors like CD28 to the end of the cytoplasm. In vivo, such improvement has led to sustained response, longer duration, sustained response, and reduce its cytotoxicity. The control of lymphocyte proliferation and survival by CD28-mediated co-stimulation is crucial for the development of memory cells and effector cells. When the B7 molecules CD80 and CD86 bind on APCs, CD28 and CTLA4, a coinhibitory receptor is regarded as model T cell costimulatory receptors.CD28 is a costimulatory molecule that is presents on T cells. By contrast, other costimulatory molecules are exclusively produced after T cell activation. CTLA-4 deficient mice that demonstrate lymphoproliferative syndrome serve as examples of the effects of non-regulated CD28 signaling. Traditionally, it is believed that dual signals are necessary to activate the T-cells. However, it is disproved by the discovery that this CD28 agonist may activate T cells even in the absence of TCR ligation.

Both PI3K and Grb2 are bound and activated by the CD28 cytoplasmic motif YMNM. As a result, transcription and mRNA stability are improved, leading to an increase in amount of T cells and IL2 production. By uplifting the availability of IL2 and upregulating pro-survival pathways, CD28 signaling increases T cell resistance to AICD and encourages the production of Th1 cytokines. However, CD4+ T cell expansion is favored by synthetic microbeads or artificial APCs, and CD8+ T cell fatigue or anergy may result. One common approach in the therapy is the use of CD28 codomain, and it was recently authorized by the FDA in treating DLBCL. Verifying its significance

in multiple centers, the influential study draws the conclusion that patients who received axi-cel showed significant durable responses with a low risk of myelosuppression and cytokine release syndrome [4]. Another significant study analyzed the use of Tisa-gel, which adopts the codomain of 4-1BB in T cell activation. Similar to the use of axi-cel, tisagel revealed high rate of durable responses on relapsed/refractory DLBCL patients [5]. Both current CAR-T therapies demonstrate their potentials in the treatment of DLBCL.

Third-generation CARs promote better cell multiplication and persistence. According to one study in 2018, Ramos et al. concurrently gave second-generation CAR T cells (axicel) and new generation of CAR T cells, which is combined with both axi-cel and tisa-gel costimulatory domains, to patients with Refractory/Relapse Non-Hodgkin B cell lymphoma in a phase I trial [6]. Six of the 11 patients with active illness and three of them completely responded to therapy. Third-Version CAR-T cells expanded its cells more rapidly in 10 out of 11 patients with active illness than second-generation cells did. The infusion of CAR-T cells remained at satisfactory level after 160 days. In addition, because third generation CAR-T cells were given after remissions of autologous stem cell transplantation, it demonstrated that CD19 antigen has little effects on the therapy. Therefore, Ramos et al. come to the conclusion that the new advancement in the therapy may be effective in areas like curing residual disease and have better duration of responses [6].

4. Current Second Line Treatment

Traditionally, hematopoietic stem cell transplantation like allo-SCT and auto-SCT are widely used in relapse/refractory NHL patients. For auto-SCT, it is commonly used as the secondary treatment in DLBCL. When the experiment is conducted, the patient's own stem cells will be removed from their bone marrow or blood. Then the extracted stem cell will receive the procedure of purification, which the leukemia cells in the sample will be cleared, and the goal is to avoid any leftover tumor cells. Autologous transplantation is generally safer than the allogenic cell transplantation as the patients is using their own cells to build up new immune system. This treatment usually complied with high dose chemotherapy that might lead to significant side effects. Compared to allogenic stem cell transplantation, autologous cell transplantation has various advantages like quick recovery time, lesser side effects, and a cheap price compared to traditional autografts. However, for allogenic stem cell treatment, it is usually adopted for multiple refractory/relapse patients. Though, it is not as safe as the ASCT, it contains no tumor cells, and it builds a completely new immune system against the diffused large B cell lymphoma.

The efficacy of CAR-T treatment over autologous treatment after first round salvage chemotherapy treatment was recently analyzed by the Fred Hutchinson cancer research center using the data from the Blood Website1. In the experiment, they included DLBCL patients that received an auto-HCT treatment from 2013-2019, and patients received CAR-T therapy (axi-cel) from 2018-2019 [7]. Using Kruskal-wallis test and pearson test, scientists tried to make comparison of the fundamental characteristics between auto-HCT and CAR-T cohorts. Multivariable linear regression model was utilized, and the error bar was set to be at the significant level of 0.05. After analyzing 411 patients with DLBCL in PR by computed tomography, the study then concluded that the progression free survival rate was 10% higher in auto-HCT than the CAR-T group in the two-year period, and the relapse rate over two years were 40% and 52% percent respectively. Consolidated by both subgroup analysis and multivariable regression analysis, the study presented the result that the CAR-T therapy still has a long way to go before it replaced auto-HCT as the second line treatment of DLBCL despite its grand potentials.

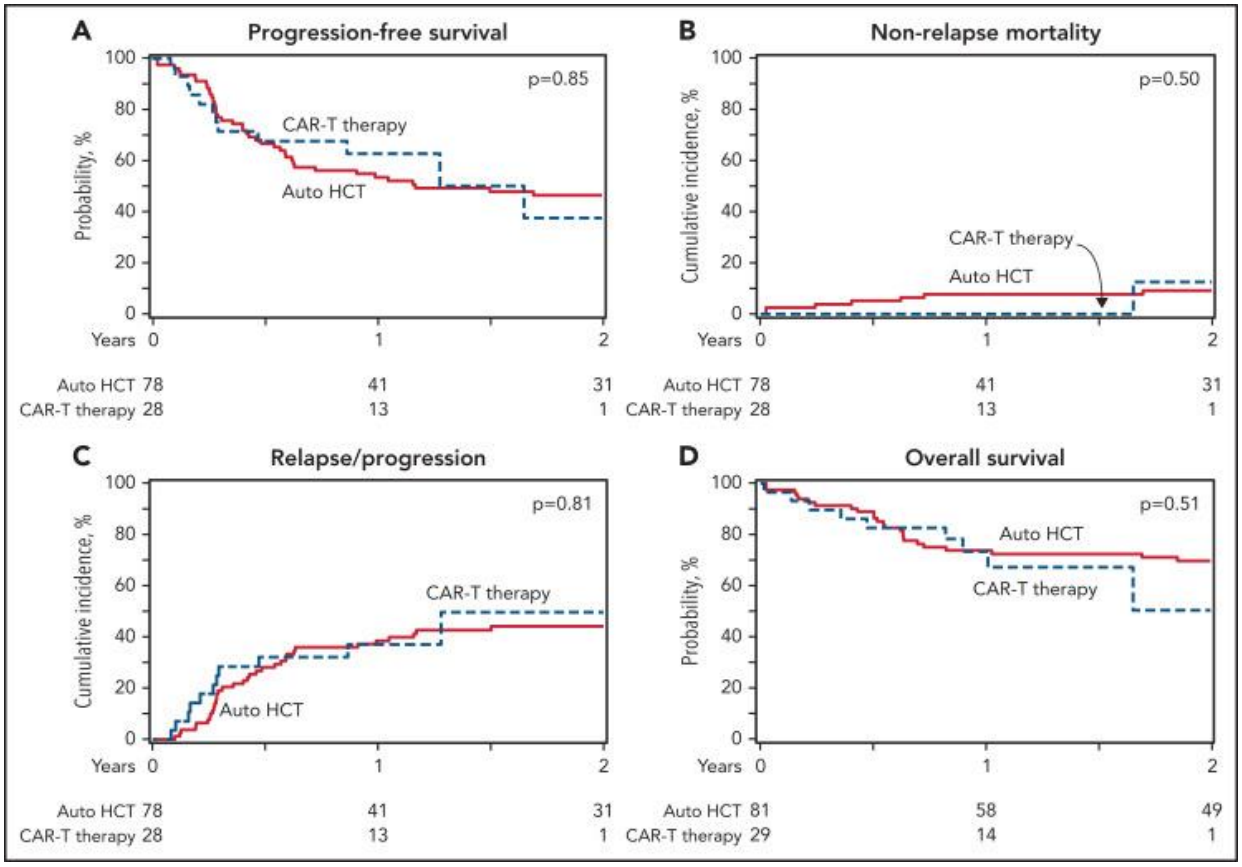

Figure 1. Comparison between Auto-HCT to CAR-T therapy [7].

However, in other studies, the use of the therapy was proven to be not inferior than the uses of allogenic stem cell transplantation patients, and a higher response rate was shown in CAR-T cell cohort [8]. In another research co-authored by Hamadani and published in the British Journal of Haematology, study shows that the probability of disease progression or relapse for CAR-T therapy at 3 years was superior to the allo-SCT. This result was drawn to the poor prognosis of high chemoresistance patients, which was often correlated with the age of the patients. As a result, the CAR-T cell therapy demonstrated its capability to replace allogenic stem cell transplantation therapy among elder patients [9].

5. Future Application

One major challenge in identifying new targets is to avoid the “off target” effects. For instance, because the targeting antigen is not specifically existed in the immune system as it would also exists on normal cells, stimulated cells can cause damage and suppression to the unaffected cells. It might lead to long-term damage of the body, and potentially lethal side effects. Currently, scientists discovered that the malignancy of myeloid progenitor cells can be solved using alternate antigens like CD123 or CD33. However, how to utilize these two antigens are still unknown, because they present in the vital bone marrow system, and treatments in those two antigens can lead to long-term myelosuppression.

Overcoming the antigen-positive relapse brought on by the poor persistence and efficacy of CARS is another difficulty. One factor is the CAR costimulatory domain. Currently, CD28 and 4-1BB are the two main costimulatory domain that are used in axi-cel and tisa-gel. More costimulatory domains need to be found in order to improve the CD8/CD4 ratio and lessen patient tiredness, with tests that integrated both costimulatory domains or alternative domains in testing for third generation CAR-T treatment. Another factor is the source of single-chain variable fragment. Currently, scFv in CD19 CAR-T is mostly derived from the murine, and it leads to rapid exhaustion of the CAR-T cells for its high antigenicity. Limited T-cell might cause immunoregulatory response. Such limitation can be

solved by producing modified humanized scFv, so that a lower antigenicity will achieve in the therapy and the persistence of the CAR-T cells will be extended.

Another issue of the therapy was speed. Lentiviruses are used to create the majority of CAR-T treatments that are now on the market. This is a prolong process, as it requires many times to create the cell and expand it to sufficient amount that can be then injected into patients. Because of such limitation, many patients are unable to survive until that time, which is also a common phenomenon in the adoption of the treatment. As a result, we must shorten the time it takes to produce CAR-T cells so that they may be used on more patients and perform more functions before the patients' health conditions deteriorate. A recent innovation dubbed FasT CAR-T has significantly speed up the process of creating CAR-T cells. It undergoes phase I clinical testing at Xinqiao Hospital in China and cuts the production time to only a day [10].

6. Conclusion

CAR-T now serves as a prominent therapy in multiple r/r DLBCL patients, and it shows satisfying result in treating elder DLBCL patients. While CAR-T cell therapy hold huge potentials in future clinical applications of diffused large b cell lymphoma for its varied combination of codomains and characteristics, it also demonstrated some limitations that need to address before it replace hematopoietic stem cell transplantation as the second line therapy of DLBCL.

References

[1] Subklewe, Marion et al. “Chimeric Antigen Receptor T Cells: A Race to Revolutionize Cancer Therapy.” Transfusion medicine and hemotherapy: offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie vol. 46,1 (2019): 15 - 24.

[2] “Zhang, C., Liu, J., Zhong, J.F. et al. Engineering CAR-T cells. Biomark Res 5, 22 (2017).

[3] Ahmad, Zuhaida, and Wan Yong Ho."ScFv Antibody: Principles and Clinical. Application." Journal of Immunology Research, 15 Mar. 2012.

[4] Neelapu, Sattva S et al. “Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma.” The New England journal of medicine vol. 377, 26 (2017): 2531 - 2544.

[5] Schuster, Stephen J et al. “Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma.” The New England journal of medicine vol. 380,1 (2019): 45 - 56.

[6] Ramos CA, Rouce R, Robertson CS et al In vivo fate and activity of second- versus third-generation CD19-specific CAR-T cells in B cell non-Hodgkin's lymphomas. Mol Ther 2018.

[7] Shadman, Mazyar et al. “Autologous transplant vs chimeric antigen receptor T-cell therapy for relapsed DLBCL in partial remission.” Blood vol.139, 9 (2022): 1330 - 1339.

[8] Dreger, Peter et al. “CAR T cells or allogeneic transplantation as the standard of care for advanced large B-cell lymphoma: an intent-to-treat comparison.” Blood advances vol. 4,24 (2020): 6157 - 6168.

[9] Dhakal, Binod et al. “Promise and pitfalls of allogeneic chimeric antigen receptor therapy in plasma cell and lymphoid malignancies.” British journal of haematology vol. 197,1 (2022): 28 - 40.

[10] Caruso HG, Tanaka R, Liang J, Ling X, Sabbagh A, Henry VK, et al. Shortened ex vivo manufacturing time of EGFRvIII-specific chimeric antigen receptor (CAR) T cells reduces immune exhaustion and enhances antiglioma therapeutic function. J Neuro-Oncol. 2019; 145: 429 – 39.