

Limitations in the CAR-T therapy for diffuse large B-cell lymphoma

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Abstract. Diffuse large B-cell lymphoma (DLBCL) therapy has been the subject of much investigation for many years. CAR-T cell therapy (CAR-T therapy) is now a new treatment for DLBCL. Human T cells are genetically altered to create new CAR-T cells, which are then injected into patients' bodies to kill cancer cells via an immune reaction. This process is known as CAR-T therapy. When treating refractory/relapsing (r/r) DLBCL, CAR-T therapy has showed some fairly thrilling clinical responses in comparison to conventional therapy and has a high cure rate. A few CAR-T treatments for DLBCL have also been authorized and put on the market. CAR-T cell therapy, however, has several drawbacks and difficulties when used to treat DLBCL. Some individuals are resistant to the therapy, leading to unsuccessful treatment, for the immunosuppressive microenvironment (TME) of DLBCL tumors and the phenomena of antigen escape. Additionally, CAR-T cell treatment may have major adverse effects including CRS and ICANS. If these adverse effects are not taken into consideration, people might die. Additionally, CAR-T treatment is quite pricey and common people might not be able to afford it, which will lead to a number of ethical issues. Naturally, scientists are working to provide answers to these problems and have made some success. The fundamentals of CAR-T therapy and its drawbacks for treating DLBCL will be covered in this review (including drug resistance, side effects, ethical and financial issues). Of course, the corresponding solutions, such as developing multiple targeted CARs and inhibiting the activity of certain cytokines, will also be described in this review.

Keywords: CAR-T therapy, lymphoma.

1. Introduction

The most popular non-lymphoma Hodgkin's (NHL) is DLBCL. It's a B-lymphoid tumor made up of medium-sized to big cells. DLBCL can be seen in different places of the human body, however it is more frequently found in areas with a lot of lymph nodes, such the armpit and neck. The yearly incidence of DLBCL in the United States and the United Kingdom is between 0.07 and 0.08 percent. In China, accounting for 45-50% of newly found NHL patients, DLBCL is still the NHL type with the highest incidence rate. The pathogenesis of this disease is unknown so far, which may be related to human infection with the virus. DLBCL can occur in people of any age, but it most often occurs in the elderly (the median age is 65-70 years) [1], and among male DLBCL patients are slightly more than females. Some severe DLBCL symptoms might finally result in mortality. In short, this is a relatively serious disease, but under developed medical conditions, it can be well controlled and even cured.

The prognosis of DLBCL varies according to the patient's age, treatment method and disease stage, and the treatment mode plays a decisive role. Approximately 30% of NHL patients can be cured with the conventional CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone) therapy. This treatment has been utilized for more than 40 years. The birth of more advanced R-CHOP therapy (adding rituximab on the basis of CHOP therapy) has brought good news to DLBCL patients. This treatment scheme has greatly improved the cure rate of DLBCL patients. In fact, more than 60% of patients can be cured by R-CHOP [1]. However, it must be pointed out that R-CHOP therapy also has its limitations. More than 20% of patients relapse after receiving R-CHOP treatment, and once R-CHOP treatment failed, the prognosis of patients will be not optimistic. R-CHOP is also difficult to achieve good results for relapsed and refractory DLBCL.

Faced with this dilemma, some new therapies for DLBCL were invented. CAR-T therapy is one of those that has received a lot of interest. This therapy has revealed encouraging outcomes even for resistant or recurrent DLBCL, which surely offers patients in despair a new treatment alternative. Patients with refractory DLBCL had a considerably higher survival rate thanks to CAR-T treatment. After clinical studies, 50% to 70% of patients continued to get therapy for 12 months [2]. Until today, at least two kinds of CAR-T cell products have obtained medical approval in the United States and Europe, and have been marketed locally. Axicabtagene ciloreucel injection, a CAR-T cell treatment medication, was introduced to the Chinese market in 2021, offering a solid medical assurance for the recovery of Chinese DLBCL patients. Currently, deeper researches are ongoing. Nevertheless, the treatment has several significant drawbacks.

This review will go over what CAR-T cells are, how they work, and the currently approved CAR-T treatment for DLBCL. The limits of the present CAR-T therapy for DLBCL, such as drug resistance, side effects, as well as ethical and practical issues, will also be discussed in this review.

2. CAR-T therapy

CAR-T cell is a type of genetically modified T cell. Its noticeable property is that it synergizes with T cells and CAR protein. As a recombinant receptor, CAR can target native cells antigens on their surface. When it is engineered with T cells, these cells will be endowed with the ability of CAR to target specific tumor-associated antigens without relying on MHCs. Ordinary T cells may not recognize cancer cells because of their camouflage, let alone eliminate them. But the CAR protein can enable them to recognize specific antigens on the tumor surface (such as CD19), thus stimulating these T cells to acquire a powerful anti-tumor reaction [3]. Therefore, CAR-T cell can specifically kill tumors.

2.1. Treatment of DLBCL with CAR-T cells

There have been some CAR-T cell therapies developed up to this point for treating DLBCL. In general, the current CAR-T cells therapies used to treat DLBCL target CD19, because DLBCL expresses CD19. Therapists usually draw the patient's blood first, and then filter the naive T cells from the blood by using leukocyte isolation. These T cells will then receive a genetic instruction that will enable them to make CAR that can detect CD19 and develop into anti CD19 CAR-T cells. These cells will be cultured and proliferated in the laboratory until therapists gain enough cells. If the patient's physical condition does not allow CAR-T therapy temporarily, These CAR-T cells will be kept by freezing [4]. After chemotherapy makes immune system no longer reject CAR-T cells, the therapists will inject these cells into the patient. Once these CAR-T cells enter the human body, they will proliferate and use their receptors to lock DLBCL cancer cells. Eventually, cancer cells will be destroyed by the CAR-T cells by cytolysis. Besides, these cells can stay in blood lastingly to keep an eye on and destroy any cells that express the CD19 antigen in order to stop the recurrence of DLBCL. Of course, the corresponding medical monitoring is also necessary to reduce the damage of side effects to patients [4], this process will last about 7-10 days.

To maximize the effectiveness of this therapy, these specific CAR-T cells will release some cytokines to promote the proliferation of T cells after binding with the CD19 antigen. Taking the axicabtagene ciloreucel therapy as an example, these T cells will proliferate to the maximum in 1-2 weeks after being injected into the human body. At the same time, the number of cytokines will also reach a peak within two weeks. It is worth noting that these cytokines can also cause serious side effects while promoting CAR-T cell proliferation.

2.2. Approved CAR-T cell treatments to treat DLBCL

There have been six CAR-T cell treatments authorized thus far. The first CAR-T therapy among them, axicabtagene ciloreucel (axi-cel), was authorized by FDA to treat refractory/relapsing(r/r) DLBCL. The treatment has demonstrated good effectiveness for r/r DLBCL with inadequate response

to conventional therapy (like R-CHOP). The FDA has also approved the use of the treatments lisocabtagene maraleucel and tisagenlecleucel. The three treatments mentioned above are crucial in the treatment of DLBCL (particularly r/r DLBCL). However, these treatments are quite expensive, which is often not affordable for ordinary families. Even insurers also expressed financial concerns about DLBCL's CAR-T coverage.

3. Limitation of CAR-T therapy in treating DLBCL

Although CAR-T therapy is a developing medical technology, it still has significant limitations in treating DLBCL despite having numerous advantages over conventional treatments. These numerous limitations will have an impact on CAR-T therapy treatment and may have catastrophic outcomes.

3.1. Drug resistance

Clinical study has discovered that many individuals who have undergone CAR-T cell treatment may experience both primary and secondary medication resistance. The term "primary drug resistance" refers to the issue with CAR-T cells themselves, which is typically connected to the NHL-specific T cell life span shortening and the proliferation of CAR on CAR-T cells. Drug resistance in DLBCL patients is caused by secondary drug resistance, which is a mutation in the body or tumor that develops accompanied with CAR-T cell treatment. However, both primary and secondary drug resistance are related to the molecules produced by tumor, human body or CAR-T cells themselves. According to different mechanisms, drug resistance can be divided into the following categories.

3.1.1 Immunosuppressive tumor microenvironment (TME)

Numerous immunosuppressive cells that can release different chemokines and cytokines to inhibit the immunological effect of CAR-T cells can be found in the tumor microenvironment. Patients can become resistant to CAR-T cell treatment as a result of some immune cells and their corresponding proinflammatory factors. The fight for nutrients in the TME will also cause CAR-T cells to malfunction. In addition, if the burden of tumors is too high, it may lead to abnormal immune microenvironment, which will eventually lead to exhaustion of T cells. To combat this microenvironment, researchers can engineer CAR-T cells and then provide immune stimulation signals in the form of cytokines. This improves CAR-T cell survival and growth and motivates these T cells to attack tumors. Tumor's microenvironment is appropriately balanced. In reality, several research have created these "armored-CARs" and created novel varieties of CAR-T cells using cytokines.

Additionally, a combined therapy with checkpoint blocking can somewhat lessen the immunosuppressive effects of TME. To lessen the harm that tumor-killing T cells to cancer cells in TME, tumor cells produce cytokines and enlist the help of certain immunosuppressive cells, namely CD4⁺T cells. Furthermore, to control certain CAR-T cells utilized to treat DLBCL, PD-L1 can bind to PD-1 (the receptor of PD-L1) [5]. Combining these two factors will result in a reduction in T cells (such CD8⁺T cells, which are utilized to fight malignancies) activities, as well as even apoptosis. Therefore, inhibiting the PD-1/PD-L1 checkpoint is required to increase the effectiveness of CAR-T cell tumor treatment. Scientists have suggested that employing corresponding antibodies can help them do this. When researching ways to enhance the effectiveness of CAR-T therapy used to treat DLBCL, it is essential to discover and control biological targets associated to PD-1/PD-L1 in DLBCL.

3.1.2 Antigen escape

Some DLBCL patients' cancer cells may not fully or partially express the target antigen after undergoing CAR-T cell treatment. This phenomenon is called antigen escape. Antigen escape is an important reason for drug resistance and tumor recurrence in DLBCL patients. In an analysis experiment not long ago, the researchers investigated the expression of CD19 antigen in B cells and concluded two different types of B cell tumor recurrence. They are CD19 antigen loss (CD19⁻) and CD19 positive (CD19⁺) [6]. At present, there are fewer cases of CD19-positive lymphoma recurrence,

and the solution is not yet clear. It is worth noticing that antigen loss is responsible for 20% to 30% of recurrences following CAR-T cell treatment targeting CD19 antigen [6]. And the CD19 target is crucial for identifying and destroying DLBCL cancer cells by CAR-T cells. Therefore, once the CD19 antigen is lost, the traditional CAR-T cell therapy for DLBCL will hardly work.

Nowadays, many improved strategies prefer to produce CAR-T cells targeting multiple antigens. Generally, double CAR constructs or tandem CARs are used to make these cells recognize and combine multiple tumor antigens [7]. This method theoretically reduces the negative impact of CD19 antigen escape on the treatment of DLBCL. It is proved that CAR-T treatment employing dual targeted CAR-T cells had the anticipated positive impact on DLBCL patients. Double-antigen-targeted CAR-T cells often exhibit superior therapeutic results as compared to previous kinds. In addition, selecting more appropriate antigen targets is also an important means to avoid the impact of antigen escape, and some scientists are also working in this direction.

3.2. Side effects of DLBCL treatment with CAR-T therapy

Using CAR-T treatment might have negative side effects, and they often lead to the deterioration of the patient's condition and even death. Many CAR-T cell therapies targeting different cancers (including DLBCL) have the same or similar side effects. Next, this review will describe two representative side effects of CAR-T therapies in DLBCL at length.

3.2.1 Cytokine release syndrome (CRS)

CAR-T cell treatment has a major cytotoxicity known as CRS. Its mechanism is that CAR-T cells are activated after being injected into the human body and release a large quantity of cytokines, thus affecting the function of the human body. Symptoms of CRS vary in severity. Mild CRS can cause patients have fever, fatigue, joint pain and other clinical phenomena. Severe CRS can cause patients to have systemic symptoms, such as coagulation disorder, respiratory failure and systemic inflammatory reaction [8], which are often deadly for CAR-T cell therapy users. What's more, patients receiving CAR-T therapy have a huge probability of developing CRS. About 37% - 93% of lymphoma patients developed CRS when using CAR-T therapy [7]. And there are 13% - 18% of DLBCL patients who have used the therapies of axiobtagene ciloleucel and tisagenlecleucel had grade 3 or more severe CRS symptoms. Because of such serious consequences, therapists must closely monitor the patient's physical condition after using CAR-T therapy. If the patient exhibits CRS symptoms, corresponding measures must be taken immediately.

Since blood IL-6 levels are connected with CRS severity, some scientists believe that the cytokine IL-6 catalyzes the development of CRS, so therapists need to use chemicals to block the IL-6 receptor. Such substances can be tocilizumab or some kinds of corticosteroids. However, even with approved IL-6 receptor inhibitors such as tocilizumab, patients still often suffer from severe CRS and death. As for corticosteroids (such as dexamethasone), in order to prevent this substance from interfering with the expansion of CAR-T cells, it can inhibit CRS only after tocilizumab fails [9]. In addition, cytokines include IL-8 and IL-12 also promote CRS. Thanks to the complex mechanism of CRS, up to now, the specific drug to inhibit CRS has not been invented.

3.2.2 Immune effector cell-associated neurotoxicity syndrome (ICANS)

ICANS is yet another substantial barrier to employing CAR-T cells to treat DLBCL. The second typical adverse reaction to CAR-T treatment is this one. Tremor, convulsions, disorientation, and cerebral edema are all signs of ICANS. The mechanism of this side effect has not been fully understood, so therapists have no particularly effective method to deal with ICANS. Laboratory research found that the blood-brain barrier of patients was destroyed by endodermal activation, which enabled CAR-T cells to enter the central nervous system. Subsequently, ICANS is caused by the cytokines like IL-1, IL-6, (particularly the brain) produced by CAR-T cells. Additionally common is the cellular neurotoxicity brought on by CAR-T treatment. According to statistics, 20% - 60% of patients have neurotoxicity, while 10% - 30% of patients have severe symptoms of neurotoxicity [9]. Fortunately, ICANS is mostly reversible and the mortality rate is low, so the risk is relatively low.

Under normal circumstances, ICANS occurs later than CRS, sometimes delaying several weeks before symptoms appear. Therefore, even if the patient using CAR-T cell therapy seems to have returned to normal, medical monitoring cannot be relaxed.

To relieve ICANS, since IL-6 inhibitors such as tocilizumab are generally ineffective for ICANS caused by CAR-T cell therapy, therapists will use corticosteroids (such as dexamethasone) to treat ICANS. If CRS and ICANS occur at the same time, therapists should use IL-6 inhibitors to treat CRS. Additionally, researchers also found that inhibition of certain cytokines can reduce the neurotoxicity of CAR-T cells. Lenzilumab has been shown in prior preclinical tests to be able to lessen neurotoxicity by inhibiting the activation of macrophages and monocytes to produce the cytokine GM-CSF. Moreover, the activity of CAR-T cells also increased. Hence, it is a good research direction to reduce the symptom intensity of ICANS by inhibiting the activity of GM-CSF.

3.3. Ethical and financial problems

Only patients with recurrent/refractory illnesses (such as r/r DLBCL) and a poor prognosis following conventional cancer treatment are currently given consideration for CAR T cell therapy. While there is the great likelihood of totally curing DLBCL when employing CAR-T cell treatment, it can potentially have catastrophic adverse effects. Such an environment with high uncertainty of prognosis is prone to ethical conflicts. Furthermore, using CAR-T cells to treat DLBCL is quite costly. CAR-T cell treatment is extremely expensive since it takes a lot of time and expensive medical resources to produce the cells. According to the statistical data, the total cost of each CAR-T treatment is 150000 to 400000 dollars [10]. An important DLBCL treatment drug, axicabtagene ciloleucel, can be sold at a very high price of 1.2 million RMB in China. Moreover, if the treatment of intensive care and complications is included, the total treatment price will be greatly increased, even greater than the cost of CAR-T cell medicine itself. This high price will block ordinary people who need to treat relapsed/refractory DLBCL but do not have enough money from the door of cure. Undoubtedly, this will result in moral dilemmas about the fair distribution of scarce financial and medical resources.

Unfortunately, as the production technology of CAR-T therapy is not developed, there is no good way to quickly solve this contradiction under current conditions. To solve these problems, there is no more effective way except to do more research on CAR-T cell therapy technology. These ethical and economic issues will no longer exist when relevant technologies advance and CAR-T therapy becomes safer and less expensive.

4. Conclusion

Although CAR-T therapy has become a symbol of high medical technology, it is still an immature DLBCL treatment method, so there are still many defects. Drug resistance still makes some patients unable to benefit from this advanced treatment. The dangerous side effects will lead to the death threat of patients, and the high price will make a large number of patients fall into the moral dilemma of "treat or not ". All of these have prevented CAR-T therapy to benefit the majority of DLBCL patients, setting up many obstacles for their recovery. However, medical workers have also explored many solutions by virtue of their hard work. Drug resistance has always been an important defect of CAR-T therapy. Blocking the PD-1/PD-L1 checkpoint can be used for the impact of DLBCL tumor's immunosuppressive microenvironment. For antigen escape, many current strategies prefer to target multiple antigens to reduce the negative impact of single antigen escape. Therefore, the transformation of CAR has become an ideal direction of improvement. Although there is no good way to reduce anaphylaxis, scientists have also found corresponding relief drugs and research directions. Now, therapists generally use tocilizumab to alleviate the dangerous CRS, while corticosteroid can be used to reduce the harm of ICANS. Inhibiting the activity of GM-CSF also provides an important idea for the treatment of ICANS. People are also increasing the investment in CAR-T therapy for DLBCL to make it cheaper and safer. Although CAR-T cell therapy still has many problems, researchers are also exploring new coping strategies. After all, CAR-T cell therapy is a

promising medical technique even if it is still in its infancy. It is strongly thought that future CAR-T therapies are expected to overcome these drawbacks and cure more DLBCL patients.

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