Evaluation of Safety and Therapeutic Efficacy of CAR-T Therapy

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Abstract. Chimeric antigen receptor (CAR)–T therapy has been developed as revolutionary treatment in cancer treatment. It has been clinically proven to be effective and durable towards certain subtypes of blood cancer especially in B-cell leukemia or lymphoma. CARs are synthetic receptor which are designed to redirect T lymphocytes to eliminate cancer cells with a specific antigen on the surface. However, limited therapeutic efficacy and safety concern of CAR-T therapy present as obstacles to its application in both solid and hematological cancer. Hence more research is being conducted to study the mechanism背后 therapeutic limitation and how to improve its therapeutic effectiveness. Furthermore, innovative strategies should be investigated to limit its toxicities. This review firstly explains the process of CAR-T therapy and its current milestone. Then the review discusses its current challenges and characterize some reasons for its limited therapeutic applications and strategies to improve its efficacy and safety in clinical settings.

Keywords: CAR-T cell therapy; leukemia, blood cancer, lymphomas.

1. Introduction

Leukemia is most prevalent oncologist disease that commonly affects pediatric patients and also continuously claim high mortality rate for young adult with leukemia, individuals who diagnosed with leukemia often experience poor quality of life with huge financial burden to both patients and their family member. Adults who are diagnosed with leukemia often suffer suboptimal survival rate of around 40 to 50 percent despite the progress on current treatment. Young children and young adults with relapsed disease continue to have poor outcomes regarding their quality of life.

Immunotherapy aims to boost patients’ own immune systems to detect and attack abnormal cells [1]. The concept of using a patient’s own immune cells to fight cancer was explored in the 1980s and 1990 and then rapidly developed through advanced understanding of genetic and cellular modification techniques. For instance, the immune checkpoint inhibitors now have been widely used to treat different types of cancer lymphoma [1,2].

Another immunotherapy, named CAR-T cell has appeared to be revolutionized the treatment landscape for cancer treatment. In the early 2000s, researchers engineered chimeric antigen receptors (CARs) which can be added to T lymphocytes and enable T cell to better recognize a specific cancer-associated antigen and strengthen their capacities to eradicate tumor. Followed by that discovery, innovative CAR T cells were designed to target CD19 which is highly expressed in B cell malignancies. Moreover, new secondary generation CAR-T cell encodes co-stimulatory domains which help to further stimulate T cells to destroy abnormal B cell cancer. In clinical settings, CAR T cell was surprisingly successfully to treat B cell acute lymphoblastic leukemia (ALL) with outstanding complete remission up to 90% [3]. Most patients with relapse leukemia can achieve complete remission after receiving CD-19-specific CAR T therapy. Despite its success in leukemia, particularly in B leukemia, patients receiving treatment can still experience relapse (over 60% of patients within the first 12 months) and drug resistance due to antigen loss or decreased antigen expression post-infusion [4]. it is also associated with immune stimulation and inflammation which lead to severe side effects and toxicity [4].

This article is going to review the current status of therapeutic CART therapy with main focus on leukemia. This article will give a brief introduction on the mechanism of CAR T therapy and then its development. This article is also going to address the current challenges faced by CAR therapy in
other tumors treatment and propose some practical strategies to overcome current therapeutic limitation such as antigen escape, tumor infiltration, immunosuppressive microenvironment, and CAR-T cell associated toxicities.

2. Mechanism of Action

As shown in figure1, the CAR-T therapy starts by extracting a patient's T cells through leukapheresis. This involves the separation of blood components to obtain a concentrated amount of T cells. Next step involves genetic modification of extracted T cells in a laboratory. A viral vector (often a deactivated virus) commonly gets employed for introduction of a desired gene into the T cells [1]. In this case, the gene introduced to T cells encodes for a CAR, which is a synthetic receptor that enables the T cell to recognize a specific antigen on the cancer cells for eradication. The third phase is expansion and activation phases where the modified T cells are cultured to amplify to larger population of CAR T cells. During multiplication process, they are also activated to enhance their ability to target and destroy cancer cells. Once a sufficient number of engineered CAR T cells are generated, they are infused back into the patient's bloodstream to circulate in the patient's body and they job is to recognize the specific antigen expressed on the surface of cancer cells. Followed by injection, the fifth phase involves CAR T cells attack cancer cells. Upon binding to specific antigens on the targeted cancer cells, the CAR T cells then become fully activated, and the CAR T cells initiate a series of immune responses which eventually lead to the destruction of the cancer cells. This destruction can occur through the release of cytotoxic substances or by signaling other immune cells to attack the cancer cells. In the final phase, some of the CAR-T cells continue to persist in the body after the initial blood infusion as memory T cells, those memory T cell can provide ongoing surveillance and potential long-term protection. Once they encounter the same cancer cells in the future, it can be reactivated to fight against tumor cells and prevent cancer recurrence.

Fig. 1 CAR-T cell transformation process [5].
As shown in Figure 1, although CAR-T cells have been targeted to specific antigens through biotechnology during the production process, these modified T cells have essentially similar functions, which also makes them specific. Sexuality faces huge challenges. CAR-T cells are mainly composed of four parts, namely scFv, hinge region, transmembrane domain and intracellular signaling domain composed of CD3z [6]. The antigen binding domain confers specificity for the targeted antigen. The fundamental principle is the affinity of CAR for its target because high binding affinity is required to induce full activation of T cell function. However, if the CARs antigen binding affinity is too high, it can result in the death of T cells. In terms of hinge region, the antigen binding site is located outside cell membrane of the cells, aims to provide flexibility for the antigen-binding domains to interact with target epitope. Transmembrane domain help to anchor the CAR to the T cell membrane [2]. Intracellular domains are internal part which composed of co-stimulatory domain for transmitting signals to T cells after the receptor has recognized a specific cancer associated antigen.

Regarding the selection of an appropriate antigen for Car-T cell to direct at, CD19 has attracted researchers’ attention. There are several features favor CD19 as a suitable target, firstly it is displayed with high frequency in B cell malignancies and required for B cell growth in cell cycle. And most importantly CD19 is not expressed outside of the B cell lineage hence make the therapy highly specific for B cell malignancies without affecting other normal cells lines [7].

3. Development of CAR-T Therapy

In the mid-20th century, researchers began to identify the role of T cells in the immune system. It is recognized that T cells play a crucial role in recognizing and attacking foreign or abnormal cells present in the body. Advancements in monoclonal antibody technology in the 1980s allowed scientists to create synthetic antibodies that could specifically target cellular antigens [5]. The first-generation CAR T cells were created based on the ideals of combination of synthetic antibodies and T cell receptor, first-generation CARs. However, the initial designs lack some features of later-generation CARs including the costimulatory domain, hence the preclinical results did not demonstrate promising outcome in in laboratory settings [7,8]. Early in vivo models of B-cell malignancies highlight significance of co-stimulatory domain in anti-CD-19-specific CAR-T cell. It was found that T cell proliferation was significantly enhanced by co-stimulation through a co-stimulatory molecule, hence such findings then raised the concept of second-generation CAR- T therapy.

From the mid-2000s, clinical trials began to evaluate CAR-T therapy in patients with tumors, with main focus on hematological malignancies like certain types of leukemia and lymphoma. For example, in 2017, the FDA approved the first CAR-T cell therapy, tisagenlecleucel, a second-generation CAR T cell immunotherapy, for pediatric and young adult patients up up 25 years of age who are diagnosed with refractory B- ALL as well as in second later relapse phase [9]. This approval is based on two studies, ELIANA and JULIET. These studies respectively investigated the efficacy and safety of CD-19-targeted CAR-T therapy in children and adolescents with relapsed/refractory B-ALL [4,9]. Since the approval of this therapy, more research focus has been placed on the development of CAR-T therapy, the more typical of which is research on various blood cancers, and multiple clinical trials are ongoing [10]. Clinical trial results from multiple institutions have also made significant progress in recent years. CAR-T therapies targeting CD19, CD22 and CD30 have been approved by the FDA for specific tumors. These therapies target tumors including B- ALL, B-NHL, CLL and HL etc. Among them, Yescarta is suitable for some HLs.

4. Challenges and Limitations

Although CAR-T therapy has been considered as a revolutionary cancer treatment tool, high rates of toxicities with some fatalities and development of tumor resistance has restricted the role of CAR-T therapy in first-line treatment.
4.1. Toxicity of CAR-T

Major challenges observed in CAR-T cells immunotherapy are managing adverse events like cytokine release syndrome (CRS) and neurotoxicity. Close follow-up care is often required to monitor potential side effects and assess the therapy's long-term effectiveness. Currently there are plenty of ongoing trials continue to assess the safety and efficacy of CAR-T therapy in both pediatric and adult populations with various hematological malignancies.

Regarding to the causes of CRS, a large number of cytokines are released due to poor targeting of CAR-T cells in patients. The clinical manifestations of CRS are also divided into mild and severe. Mild symptoms include fever, fatigue, etc., while severe symptoms include hypotension, renal failure, multiple organ failure and other symptoms. Further development of CRS may lead to the death of the patient, so the seriousness of CRS cannot be ignored. Further research found that CRS is mainly mediated by IL-6, so tocilizumab and IL-6 inhibitors can be used to relieve symptoms during treatment. However, in order to prevent the occurrence of CRS from the root cause, future research should also focus more on the targeting of CAR-T modification to reduce toxicity to other cells [2,4].

4.2. Relapse

Another challenge faced by clinicians when use CAR-T therapy in practice is duration of response and potential relapse. Clinical trials have also found that duration of responses of CAR-T can vary among individuals with some patients achieve 100% disease remission while others experience relapse after receiving the treatment. Antigen loss can be a potential cause of relapse. Even though, single antigen targeting CAR-T cells therapy can achieve high response rates at the start of the treatment, the malignant cells can escape the destruction of CAR-T cells by either partially display tumor-associated antigen or commence complete loss of antigen. According to recent follow-up data, a study examining patient relapse following CAR-T cure revealed that while 70–90% of ALL patients can demonstrate sustained responses targeting CD19 after treatment in the later recovery stage, widespread resistance to this therapy has developed over time. According to this data, CD19 antigen is either downregulated or lost in 30–70% of relapsed patients. Tumor escape is primarily caused by CAR-T cells targeting and eliminating CD19 antigen. This phenomenon may be primarily caused by the high frequency (approximately 28%) of CD19 negative mutations [7].

More efforts should be put on understanding the mechanisms of relapse including antigen loss and developing strategies to address relapses. Currently, more trials are exploring CAR-T therapies directing at different antigens beyond CD19 or CD20 to manage antigen loss. Recently some studies are investigating combination therapy combining CAR-T therapy with other treatments such as checkpoint inhibitors in order to enhance efficacy and overcome the shortages with CAR-T therapy [2].

4.3. Application in T Cell Malignancy

Although CAR-T immunotherapy has achieved remarkable success in the treatment of B-cell leukemia, its application in T-cell malignancies faces many challenges. One of the major issues is the existence of shared antigens between T-cell leukemia and CAR-T cells, which may inhibit the proliferation of CAR-T cells. This proliferation increases the probability of healthy T cells being attacked, leading to the generation of CRS. In addition, product contamination by leukemia cells is another major reason that hinders the development of this therapy, because CAR molecules may mask the antigens on T-cell lymphoma and prevent immune cells from recognizing them, which may lead to the generation of people who are immune to CAR-T therapy. Powerful modified tumor cells. These challenges highlight the complexity of applying CAR-T cell immunotherapy to T-cell malignancies, and further research is needed to overcome these obstacles [3,4].

4.4. Application in Solid Tumor

When attempting to treat solid tumors, one of the challenges is that tumor antigens are present at varying levels on healthy normal tissues. Therefore, it is crucial to select appropriate antigens in CAR
design, not only to ensure binding specificity but also to limit "off-tumor" toxicity. Additionally, compared to hematologic malignancies, CAR-T cell therapy for solid tumors is restricted by the immunosuppressive tumor microenvironment and physical tumor barriers that limit the transport of CAR-T cells to solid tumors and their ability to penetrate them. For instance, systemic delivery of CAR-T cell immunotherapy to diseased organs increases the risk of drug encountering normal tissues compared to local administration, leading to treatment-related toxicity [2].

5. Conclusion

The development of CAR-T cell represents a remarkable achievement in cancer treatment, offering hope for patients with incurable blood cancers and paving the way for further advancements in immunotherapy. However, there remain several difficulties to the broad application of CAR-T therapy in clinic. Limitation can include on-target/off tumor toxicities associated with the therapy resulting from the CAR-T cells' recognition of normal healthy cells as a target for attack, its restricted efficacy against solid tumor mainly due to hostile tumor microenvironment, and therapeutic resistance in treatment of multiple malignancies due to antigen loss.

This article mainly reviews the mechanism of CAR-T therapy, its progress and application in recent years, and its side effects in practical application. It is found that although CAR-T therapy has made great progress, it is still limited to ALL. The treatment of blood cancers and the treatment of solid cancers still face huge challenges. Therefore, scientists continue to refine CAR-T cell therapies, exploring ways to improve their efficacy, reduce side effects, and expand their use to treat other types of cancers beyond hematological malignancies. Continued research should be conducted to improve CAR design, including the incorporation of co-stimulatory domains to enhanced CAR T cell functionality.

References