

Overview and future development of CAR-T therapy

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Abstract. Cancer, as one of the highest mortality rate diseases, and the research on its treatment is endless. Traditional therapies like chemotherapy or radiotherapy all have serious side effects and unstable therapeutic effects. The treatment process of patients is excruciating. Therefore, CAR-T cell therapy as a bright star in immunotherapy gradually emerged in the world. CAR-T therapy needs to alter a patient's own T cells to kill cancer cells, so it has high specificity for each individual and can design the most suitable CAR-T cells for different patients based on their immune systems and tumor cells status. Its strong proliferation and survival ability let patients have a long-term anti-tumor effect and prevent the recurrence of cancer very well. Although it still has side effects, unstable durability and the threat of immune escape mechanisms, it still shows good results in many clinical trials which undoubtedly proves that it is a way to treat cancer with great potential. CAR-T will gradually improve its safety and durability to address the demand of more patients as technology and instruments improve. This review discusses the basic principles of CAR-T, applications in clinical trials, difficulties and impending future of CAR-T therapy, gives a thorough overview of the present situation and potential development of CAR-T therapy in cancer treatment.

Keywords: cancer, CAR-T therapy, mechanism, clinical application.

1. Introduction

World Health Organization (WHO) data shows that cancer is the cause of 10 million deaths or one in six of all deaths worldwide in 2022. In the face of this most important health problem in the world, traditional therapies like radiotherapy, surgery and chemotherapy are the most commonly used cancer treatments. However, they have serious side effects. For instance, hair loss and loss of taste may be experienced after radiotherapy, and anemia and diarrhea may happen after chemotherapy, the treatment process is extremely painful and suffering. In addition, due to the drug resistance of cancer cells and other difficulties in cancer treatment, traditional therapies can no longer meet patients' needs, it is urgent to find new cancer treatment therapies.

In recent years, Immunotherapy is gradually becoming more noticeable due to its unique mechanism: killing tumor cells by utilizing a patient's own immunity, and excellent therapeutic effect [1]. Among them, CAR-T therapy was proposed and developed by Carl H. June and his team in 2010, which has attracted wide attention. CAR-T therapy has gone through the process from basic research to clinical trials and made major breakthroughs, it is a personalized immunotherapy with great potential. CAR-T therapy uses gene technology, patient's own T cells were engineered to have the specific ability to identify and contest cancer cells [2]. Hematologic neoplasms can be treated by CAR-T therapy extremely effective, particularly acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma

(NHL) during clinical trials. CAR-T therapy breaks the traditional therapeutic limitations, the treatment of cancer has greatly improved.

Meanwhile, CAR-T therapy is confronted with many challenges. For example, tumor cell may develop resistance to specific antigen on single antigen targeting CAR-T cells, or normal tissue cells may have the same antigen expressed on tumor cells that are targeted [3]. Hence, finding out suitable development direction is meaningful for it. This article will discuss the mechanism, clinical manifestations, limitations and future development of CAR-T cell therapy, then provide guidance for future development of CAR-T therapy.

2. CAR-T basic principles

2.1. CAR-T Structure

CAR-T stands for Chimeric Antigen Receptor T-cell, CAR is a special receptor on T cell that artificially add on it, figure1 illustrates a general structure of all CARs.. Three main parts are present on CAR: ectodomain, transmembrane domain and endodomain[4].

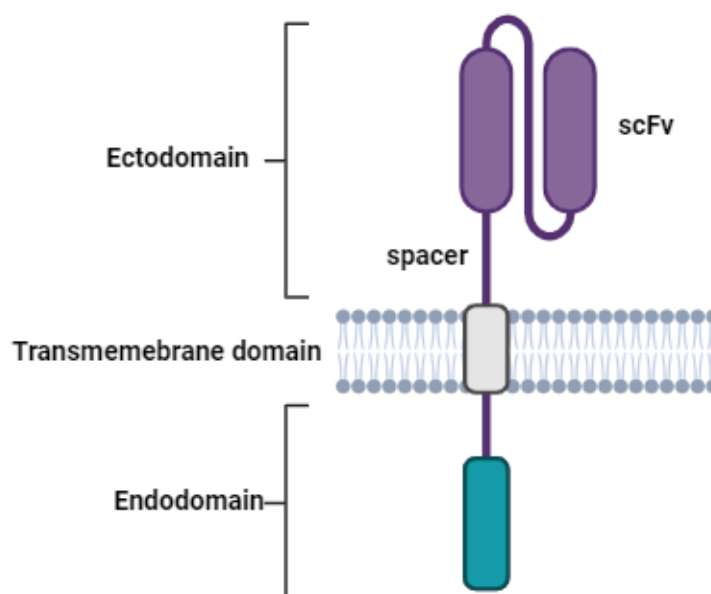


Figure 1. General structure of CARs

2.1.1. Ectodomain

This part is located at the outside of T cell membrane, composed of antigen recognition domain and linker region. Antigen recognition domain is also be called scFv (single chain variable fragment), a peptide linker links a fragment of an antibody's variable heavy and light chains to form a single-stranded structure. It has the ability of specify antigen recognition and binds tumor cells' particular antigens on its surface. Once bound to the antigen of cancer cells, T cells will be triggered and kill off cancer cells. Therefore, the design and selection of antigen recognition domain are crucial for CAR-T cell therapy, which determine the specificity and selectivity of CAR-T cells, so that they can distinguish cancer cells from normal cells. According to its function, in the design of scFv specificity of antigen should be considered. Ensure that CAR-T cells only bind to the antigen on the targeted cancer cell, not to the normal cell, when designing scFv by choosing an appropriate antigen as the target. The affinity is also an important factor that should be considered when designing. Relatively high affinity can augment the aptitude of CAR-T cells recognition and binding of antigen thus improving therapeutic effect. However,

high affinity may cause non-specific binding or side effect caused by excessive binding, so it is important to control the affinity in an appropriate range.

Linker region can also be called “spacer”, they all refer to the part that connect antigen recognition domain and transmembrane domain. They mainly play a role in connection and fixation as well as providing flexibility in structure. They are responsible for CAR location and connection as well as regulation of the distance between two domains. Based on their functions, the length of polypeptide sequence, hydrophilicity and hydrophobicity should be considered in the design of CAR to achieve appropriate connection and stable CAR structure [4].

2.1.2. Transmembrane domain

Transmembrane domain is usually hydrophobic amino acid, commonly including α helices, which link the antigen recognition domain and intracellular signaling domain of CAR. Therefore, cell signaling ability and stability should be considered when designing, so it is necessary to choose appropriate length and sequence of amino acids to ensure the functional integrity of CAR structure [5].

2.1.3. Endodomain (Intracellular signaling domain)

Intracellular signaling domain is composed of one or more cell signaling modules, so there are many choices of its combination and order. Common cell signaling modules used in CAR-T cell therapy are CD3 ζ , CD28 and 4-1BB (CD137). CD3 ζ is the most common component that activates the cell-killing mechanisms by interacting with other signaling molecules, while the instigation and expansion effect of T cells can be enhanced by CD28, a co-stimulatory molecule. Another co-stimulatory molecule, 4-1BB, can improve T cells' viability and persistence, to resist tumor for a relatively long time. When designing CAR, different combination of signal transduction modules are used to make highly individualized CAR-T cells for patients and cancer types [6,7].

2.2. CAR-T cell mechanism

After introducing the structure of CAR-T, we will now explore its basic mechanism and understand how CAR-T therapy plays its role in killing tumor cells.

First of all, the antigen recognition domain is in charge of identifying and attaching itself to particular antigens present on the outside of cancerous cells. After binding cell signaling modules will be activated and then a series of reaction in the intracellular signal pathway will be triggered and release including pore-forming toxin and some products that cause cell lysis, then directly kill cancer cells. In addition, activated CAR-T cells can also proliferate, increasing their impact and leaving memory cells that can allow patients to have long-term anti-cancereffects [8].

2.3. CAR-T manufacturing process

Collecting T cells from the patient's peripheral blood sample is the initial step in producing CAR-T, and use density gradient centrifugation or magnetic bead separation to separate various types of cells in the blood, the T cell population can be selected and increased by using magnetic beads or cell surface markers [9]. The number and viability of cells are then determined by cell counter and cell staining reagents. T cells are activated by exposure to an activation stimulator, usually an antibody that is similar to the signal used to activate T cells in real human body. Activated T cells then proliferate and grow under specific media and culture conditions. Then, a suitable virus vector, such as retrovirus or adenovirus, is selected, and the CAR gene is loaded into the appropriate site in the vector using restriction enzyme and DNA recombination technology. The constructed vector DNA was transfected with appropriate host cells to generate virus particles with CAR gene, which were purified and concentrated after extraction from the cells. Triggered T cells are infected with the constructed virus and the infected T cells will accept the CAR gene into their genome and then begin to express the CAR. Following the cultivation and development of modified T cells, a quality inspection is required after obtaining a sufficient number of CAR-T cells, and the CAR-T cells that pass the quality inspection will be reinjected into the patient.

3. CAR-T cell therapy development

At the beginning, CAR-T cell therapy was not fully developed and many iterations have been made to achieve the current therapeutic effect. Right now, CAR-T therapy is available in four generations, which is showed in figure 2.

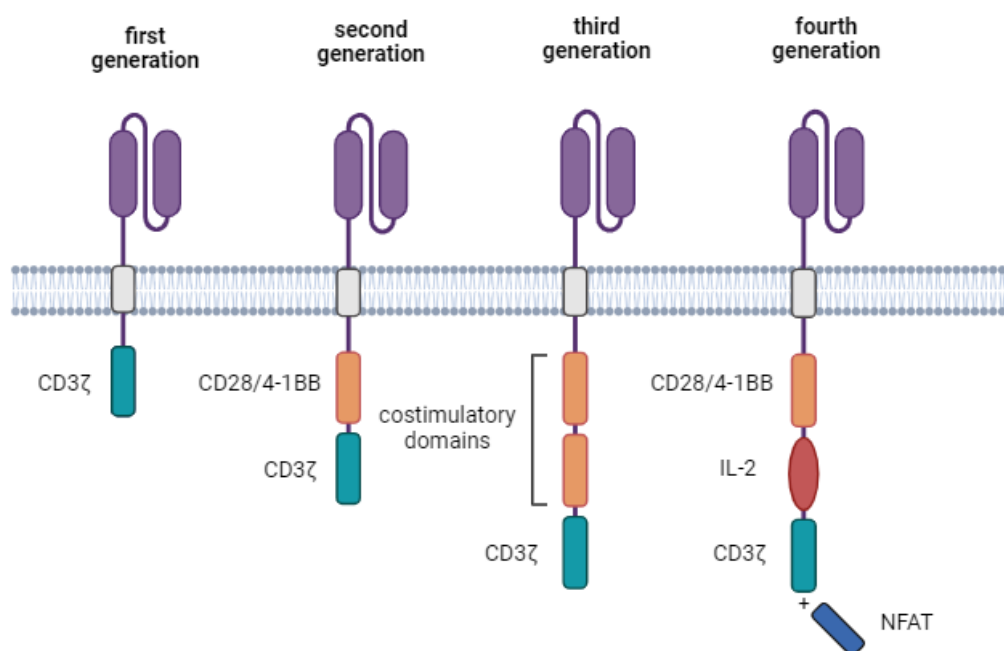


Figure 2. Four generations of CARs

The CAR-T of the first generation already consists of two parts: the external antigen-recognition domain and an internal cell signaling domain. The antigen-recognition domain consists of the variable region of the monoclonal antibody. Internal cell signaling domain typically uses CD3ζ activation domains, which can activate intracellular signaling pathways and promote T cells to be activated and grow. The design of the first generation is quite simple, introducing only a signaling module that can directly activate T cells and boost the antitumor of T cells. Due to the absence of more than one signaling domain, first-generation CAR-T therapies were restricted in antitumor efficacy. Only one activation signal cannot produce sufficient cell proliferation and anti-tumor effects. In addition, inhibitory pathways in T cells may also inhibit the activity of CAR-T cells. Moreover, safety concerns have been raised with first-generation CAR-T therapies. The potential for potent antitumor effects may be allied with shortcomings like CRS and neurotoxicity, which may manifest as fever, hypotension, dyspnea, and neurological symptoms. While the first cohort of CAR-T therapies has been efficacious in treating certain hematologic malignancies, their efficacy has been limited. First-generation CAR-T therapies laid the foundation for future improvements and innovations in CARs.

The second generation of CAR-T represents a great development. Compared with the first generation, in structure, it adds a costimulatory domain that promotes the initiation of CAR-T cells, strengthening antitumor effects. Expressed are costimulatory domains CD28 or 4-1BB; it belongs to the second generation. These kinds of costimulatory domains can further enhance the activation of CAR-T cells and their proliferation ability. Also, the introduction of costimulatory domains modifies survival times of CAR-T cells and increases effector cell production, improving adoptive transfer [5].

Third-generation CAR-T therapy is further refinement in the evolution of CAR-T therapy. Generally, such third-generation CAR-T therapies introduce other costimulatory domains into the structure of the CAR, including CD27, CD28, 41BB, ICOS, and OX-40. These domains can provide more signal

stimulus, so that they enhance their activation, survival, and proliferation abilities. Comparatively, the third generation may have more long-lasting effects and more durable proliferation abilities.

The NFAT domain is now present in the fourth generation of CAR-T-therapy, commonly denoted to as T cells for universal cytokine-mediated killing (TRUCKs). Both these NFAT and TCR signalings promote the production of IL-2 and other immune cytokines, thus influencing T cell proliferation and differentiation. In a continuous development process, the introduction of more costimulatory domains is improving efficacy, specificity, and safety [10].

4. CAR-T clinical trials of different types of cancer

B-cell lymphoma involves a type of cancer in which lymphoid tissues and cells are targeted, and it also includes non-Hodgkin lymphoma and Hodgkin lymphoma. Some of the common antigens on the surface of B-cell lymphoma cells that CAR-T cell therapy targets include CD19. In designing the CAR-T cells from the antigen structure of the CD19 cells, this kills the cancer cells through binding and releasing cytotoxins. Several clinical trials have shown that CD19 CAR-T cell therapy lead to a remarkable totaldiminution rate and long-term maintained diminution among patients with reverted or obstinate B-cell lymphoma [11].

In addition, many more clinical trials reached a similar conclusion, and overall, CAR-T cell therapy in patients with relapsed or refractory B-cell lymphoma has resulted in high rates of wholeretort and extent of response. However, efficacy depends on individual differences and other factors; thus, not all treated patients can be found to have the same results. Moreover, CAR-T cell therapy might cause somedrawbacks: cytolytic syndrome and neurotoxicity [12]. Therefore, when executing CAR-T cell treatment, patients have to be treated with monitoring to ensure efficacy under clinical conditions. These studies give credence to the fact that CAR-T cell therapy offers immense probability for the treatment of B-cell lymphoma and can contribute to better survival rate.

Acute lymphoblastic leukemia is a hematopoietic malignancy, and the majority of the recipients usually fall in the category of children and young adults. At times, ALL treatment may pose problems like drug resistance and relapse. High therapeutic response in patients diagnosed with reverted or obstinate ALL has been noted in several clinical trials regarding CAR-T cell therapy. One of the most famous CAR-T cell therapy cases among patients with acute lymphoblastic leukemia is Emily Whitehead. She was a 6-year-old girl at that time who could not control leukemia after receiving many treatments in chemotherapy. She was then joined in a clinical trial using the use of CD19 CAR-T cell therapy as a modality of treatment. She totallyresponded to the treatment, and follow-up tests have been indicative that she has remained well [13]. Her case is illustrative of what CAR-T cell therapy promises in being a fronting-edge treatment modality for refractory leukemia.

Multiple myeloma is a hematologic malignancy in the bone marrow, composed of plasma cells. Unfortunately, it usually has the features of high resistance to treatment and recurrence. The BCMA is a specific antigen for plasma cell tumors—the target antigen of CAR-T cell therapy for the treatment of MM. Preclinical and early clinical trials demonstrated that BCMA CAR-T cell therapy had emerged with satisfactory efficacy in patients diagnosed with refractory or relapsed multiple myeloma [14]. One such specific example was the clinical trial called LEGEND. However, some of the other adverse effects also include cytolytic syndrome, more commonly known as CRS, and neurotoxicity. These are toxicities that must be closely monitored and managed by a professional medical provider in order to ensure maximum safety of treatment.

5. Limitations of CAR-T cell therapy

As one of the advanced personalized therapies, CAR-T therapy still faces many challenges. One major challenge facing the use of this treatment is the possibility of serious side effects. Examples of serious side effects linked to CAR-T cell therapy include cytokines released syndrome and neurotoxicity. Cytokine release syndrome is one of the most polular and severe drawbacks of CAR-T therapy. Activation of CAR-T cells may lead to IL-6 and TNF release, thereby triggering an immune response. The resulting cytokine release may cause an exaggerated immune response and an ensuing inflammatory

reaction with a spectrum of severe symptoms like high fever, chills, hypotension, dyspnea, and even tissue and organ damage. While severe CRS may cause life-threatening organ defects, another important side effect is neurotoxicity. It may activate CAR-T cells *in vivo*, leading to inflammation of the nervous system acting on nontarget tissues and causing neurotoxic side effects after CAR-T therapy, including cognitive impairment, headache, altered consciousness, increased intracranial pressure, and other neurological symptoms. In rare cases, neurotoxicity may be severe enough to cause seizures, aphasia, coma, etc. [16,17].

CAR-T therapy also faces the problem of low durability of treatment effect. CAR-T therapy shows a potent antitumor activity in some individuals, but the treatment effect cannot last for a long time, and cancer cells may relapse again. The reasons for poor durability are various. Through the modification of the patient's immune system, CAR-T therapy effectively directs an immune response towards attacking cancer cells, which is its mechanism of action. Certain tumor cells can hide from immune recognition by altering their surface antigen expression or other mechanisms to prevent identification and attack by CAR T cells. These tumor cells appear to have escaped immune surveillance, allowing them to regrow and spread at subsequent times [18]. Second, CAR-T cells may become inactivated *in vivo*. Although CAR-T cells have potent killing capacity when injected into patients, their function may gradually fail over time. Possible reasons include fatigue and attrition due to prolonged initiation of CAR-T cells, as well as decreased activity of CAR-T cells under the influence of inhibitory factors or immunosuppressive environment [19].

The poor durability of treatment indeed partially emanates from the complexity of the tumor microenvironment. It may have immunosuppressive factors and/or drug resistance mechanisms, things that the microenvironment surrounding the tumor cells can possess to let the tumor cells evade the attack by CAR-T cells. If this happens, there would be a decline in treatment efficacy and tumor recurrence. In addition, CAR-T also faces prominent problems due to its cumbersome production process, high cost, and long production duration [3].

6. Future directions of CAR-T cell therapy

Considering these problems, CAR-T cell therapy also has an ostensible direction. First is combination therapy. Combination therapy refers to the ways of improving therapeutic efficacy and durability by integrating CAR-T therapy with other treatments. One combination commonly used in therapy is CAR-T combined with an immunomodulatory agent: an interleukin-2 receptor agonist, which can further activate or surge the activity of the CAR-T therapy to enhance tumor killing. The other is to cooperate CAR-T with radiotherapy, chemotherapy or other immunotherapy, such as PD-1 inhibitors, etc., using different mechanisms to maximize the anti-tumor effect, prolong the durability, and lower the risk of recurrence [20]. Secondly, multiple CAR constructs can be introduced to mark other tumor antigens to improve the efficiency of CAR-T cells to detect and kill cancer cells, and overcome the immune escape mechanism of tumor cells. Additionally, personalized CAR-T cell design can also be carried out according to the individual immune system situation and the unique characteristics of their tumors, so as to more accurately select the precise treatment for each patient's condition.

The development and progress of CAR-T is inseparable from technological innovation, and new technologies also provide more development opportunities for CAR-T therapy. With continued research and innovation, CAR-T therapy will offer a more dependable choice for patients with various types of cancer in the future.

7. Conclusion

In this review, the background, current status, limitations and future directions of CAR-T therapy are discussed. As a personalized immunotherapy, it has made outstanding results by altering the patient's immune system, it enables the immune system to effectively destroy cancer cells. At present, CAR-T therapy has shown significant therapeutic effects in some hematological tumors such as acute leukemia for patients with refractory tumors.

Despite this, CAR-T therapy is still facing numerous limitations and challenges. Their use for patients is restricted due to the occurrence of short comings, such as cytokine release syndrome and neurotoxicity. In addition, the durability and tolerance of the therapeutic effect are still a problem, and some tumor cells can evade immune surveillance or change the expression of surface antigens, leading to ineffective therapy and tumor recurrence. Additionally, it is complex and expensive to manufacture, limiting its broad use in clinical application.

Through continued research and innovation, we will be refining the design and engineering of CAR-T cells, overcoming the immune escape mechanism, refining the durability of CAR-T therapy, and developing more economical production methods of CAR-T therapy. Enhancing the effectiveness of CAR-T therapy can be achieved through the development of personalized treatment approaches and the strategic amalgamation with other therapeutic modalities. CAR-T is on the right path, with the goal of improving the durability and safety of treatment, reducing side effects, and expanding its uses in solid tumors.

In summary, CAR-T therapy offers a new direction for cancer treatment. We eagerly anticipate the continued development of CAR-T therapy and look forward to its greater breakthroughs in clinical trials, bringing better treatment effects and quality of life to patients.

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