

CAR-T therapy in Acute lymphocytic leukemia

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Abstract. Acute lymphocytic leukemia is a serious threat to people's health. For a long time, the main therapy to cure the cancers include surgery and chemotherapy, but the traditional therapy still has some limitations and the side effect after treatment will seriously affect the quality of life. CAR-T has gradually become a new treatment to cure ALL. CAR-T cell which is through gene engineered T cell to carry the scFV. Then the engineered T cell can specific recognition the antigen and kill the tumor cell. But with the application of CAR-T, there are more and more problems show up, such as the safety, efficiency and relapse. This review discussed the mechanism and applications of CAR-T in acute lymphocytic leukemia and introduce the advantage of CAR-T and some limitations and future development.

Keywords: CAR-T, Therapy, Cancer

1. Introduction

Acute lymphocytic leukemia, also called ALL, is a type of bone marrow and blood cancer that affects the white blood cell. The word "Acute" presents this type of cancer will grow fast if it is not treated. Acute lymphoblastic leukemia occurs in all age groups, most commonly in children and also in adults. According to the American Cancer Society's (ACS), With 75% of leukemia diagnoses in patients under the age of 20, ALL is the most prevalent kind of leukemia in children and adolescents. The greatest risk of ALL is in children under the age of five. After the age of 50, when children grow into adults, the overall risk of ALL rises once more. Adults make up for 4 out of 10 patients with ALL [1].

L1 type of ALL accounts for around 20 percent of adult cases and over 80 percent of child cases. They are mainly small and regular cells and nucleoli; there is little cytoplasm and vacuoles are not obvious. Large cells are dominant, with different sizes, irregular nucleoli, one or several larger nucleoli, more cell mass and indefinite vacuoles. In L3 type, 1 % cases for this particular type. Large cells are dominant, with uniform cell size, regular nucleoli, one or more nucleoli, more cell mass, obvious vacuoles and honeycomb shape [2]. However, the World Health Organization (WHO) revised this version and published a new version that is classified by immunophenotypic. B-cell type is the most common subtype in childhood acute lymphoblastic leukemia, accounting for about 85% to 90% of cases. According to the different differentiation degree of B lymphocytes expressing different differentiation antigens. T-cell type accounts for about 15% of acute lymphoblastic leukemia in children and has unique clinical, immunological, cytogenetic and molecular biological features that are different from those of B-cell type [3].

Three steps of treatment are often used: induction, consolidation, and maintenance. Typically, the complete course of treatment lasts two years, with the maintenance phase taking up the majority of that time. There are typically four standard treatments for ALL. Targeted therapy attracts specific targets by the drugs [4]. Long-term chemotherapy is typically the main course of treatment for adult ALL. A complete remission will occur in 80 to 90 percent of individuals at some point during these therapies. This indicates that their bone marrow is no longer home to leukemia cells. The overall cure rate is around 40%, although roughly half of these individuals will experience a relapse.

In clinical practice, conventional chemotherapy is known as hyper-CVAD, which is a high-dose treatment. It consists of four drugs: cyclophosphamide (CTX), vincristine (VCR), adriamycin, and dacarbazine. According to the study [5], 74% of patients who used the combination therapy and did not receive an allogeneic HSCT were still alive at five years. Although the cure rate is high enough, there are still some issues that need to be addressed. The first is drug tolerance. A subset of patients can develop drug resistance after receiving combination therapy. In addition, elderly people cannot receive high doses of combination therapy. The problems of suboptimal efficacy, ease of recurrence and drug resistance are still not fundamentally addressed.

At this point, CAR-T therapy can be a good solution to this problem. CAR T is the in vitro modification of T cells from patients. T cells are extracted from the patient's body and genetic engineering techniques are used to modify T cells from the patient to express a receptor that specifically recognizes the surface targets of tumor cells - CAR - and then infused back into the patient to exert anti-tumor effects. In ALL, uncontrolled early B cells are predominantly found in the periphery. The fact that acute lymphoblastic leukemia is scattered around and that normal B cells express CD19 means that almost all CD19 CAR-T cells will be constantly exposed to antigens that drive cytokine production and thus are in an activated state.

However, unlike solid tumors, only a few CAR-Ts are exposed to the tumor, while the vast majority will not even encounter their cognate antibodies, so most CAR-T cells will be in a resting state. At the same time, the microenvironment of solid tumors is usually immunosuppressive, forcing CAR-T cells to fight a vicious battle against the forces of evil [6].

This review focused on the clinical application of CAR-T therapy on B-ALL and T-ALL and discuss the untoward action of CAR-T and solutions.

2. Mechanism of CAR-T therapy

A synthetic structure called a CAR vis a structural domain known as a scFv. A transmembrane structural domain structural domain connects these two modules to create the first-generation CAR, which is the most basic type of CAR.

In 2002, second-generation CAR-T therapies were introduced. The second generation of CAR-T therapy added co-stimulatory receptor signaling, which allowed CAR-T cells to expand efficiently and develop a good killing effect on tumors while accurately finding cancer cells. This makes second-generation CAR-T cells significantly more capable of activation than first-generation CAR-T cells, and it also leads to unexpected therapeutic results in clinical trials. Because the transfected viral vectors used in second-generation CAR-T cells are mostly retroviruses, which can only accommodate and carry a limited number of fragments [7].

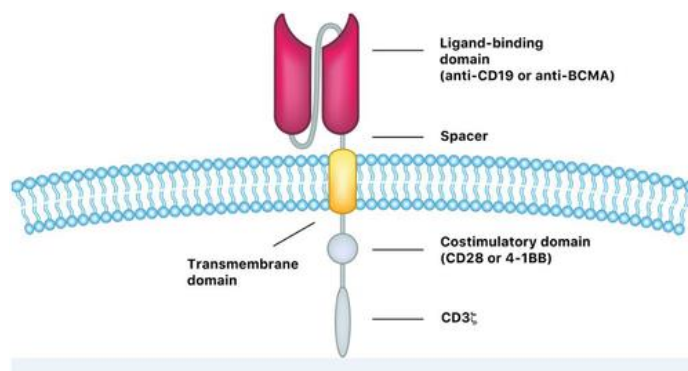


Figure 1. Mechanism of CAR T therapy [8].

3. Structure of CAR T and the clinical CAR T medicines

There are four main components of CARs [9]. Binding through junctions to form single-chain variable fragments (scFv) antigen, but too high an amount can lead to the weakening of T cells

induced to express CAR. Hinge region gives scFv flexibility and can be utilized to improve target epitope identification in otherwise inaccessible locations by anchoring CARs to the T cell membrane. Additionally, CAR expression, signaling, and intensity activation output are impacted by hinge length and composition. Better access to glycosylated antigens or membrane-proximal epitopes is made possible by longer spacers. Targeting distal membrane epitopes is more effective with shorter hinges [10]. Intracellular region is the functional segment of the receptor, the most basic component of which is CD3 ζ . The intracellular region of some CAR molecules also contains multiple co-stimulatory molecules.

4. Application of CAR-T

According to statistics, 85% of childhood ALL patients belong to B-lineage lymphocytic leukemia. Since T cells require co-stimulation for full activation, second generation CARs have been designed with additional intracellular structural domains (usually from CD-28 or 4-1BB) that deliver a "second signal" upon receptor binding.

In acute lymphoblastic leukemia, the uncontrolled early B cells (blasts) are mainly found in the periphery. These cells typically express high levels of CD19, a cell surface antigen that is present in almost all cells of the B-cell lineage but not elsewhere. CD 19 expressed in normal B cell, the patients show positive result in a short term even though B Cellular aplasia with hypogammaglobulinemia has targeted, non-tumorigenic toxicity. The safety and effectiveness of CD19 CAR-T treatment have been showed by a number of early experiments and later bigger experiments. The first commercially medicine, tisagenlecleucel, was developed after larger clinical experiments got approval by FDA. 75 kids and teenagers were given CD19 CAR-T cells in the authorized ELIANA experiment. The remission rate at three months was 81%, and patients who responded to treatment were MRD negative. EFS and OS rates at one year were 50% and 76% [11].

In T-ALL but not in B-ALL, CD7 is another transmembrane protein that is expressed. Malignant T-cell lines and primary tumors have been developed and treated using CD7-based CAR T-cell cancer treatments in T-AL xenograft models. The findings show that CD7-tagged CAR T cells have strong anticancer efficacy. The effectiveness of CAR T-cell treatment against CD7-positive hematologic malignancies in pediatric, adult, and geriatric patients is being assessed in a new clinical trial. It serves as an adhesion protein in conjunction with CD31 or as a multifunctional extracellular enzyme catabolite in conjunction with NAD⁺ and NADP. HTLV-1+ T-cell leukemia is effectively eradicated. The outcomes showed that CAR T-38 cells have powerful and targeted anti-tumor activity [12].

5. Advantages of CAR-T

Acute lymphocytic leukemia is a serious threat to people's health. For a long time, the main therapy to cure cancers, but these traditional therapies still have some limitations and side effect after treatment will seriously affect the quality of life. With the rapid development of modern oncology, immunology and molecular biology, the rise of tumor immunotherapy has brought a new dawn to the treatment of malignant tumors. CAR-T has gradually become a new treatment to cure ALL.

In contrast with traditional therapy, CAR-T cells have unique specificity and they can identify the targets more accurately and combine with them thus no matter how complex the source of tumor cells is, CAR-T cells can target specific targets and they don't harm the normal tissue and cells. Because of it, its toxicity to the human body is less than other treatments. The choice of antigen is so important to CAR-T cells, in other hand the antigen is the key to the therapy. In CAR-T cells, the potential antigens are having many options, which can be combined with specifically by antibodies in CAR-T so which means the targeting is easy to find and the CAR-T therapy can easy to be used in theory.

In addition, CAR-T cell is through gene engineered T cell to carry the scFV then the engineered T cell can specific recognition the antigen and kill the tumor cell so the engineered T cell can survive in the body for a long time. During engineered T cell exist in the body, if there's relapse once it can

recognize and attack cancer cells in time so CAR-T cells can be treated refractory or relapsed ALL. These patients' cancer progressed rapidly and failed after traditional treatment. The longer CAR-T cells remain in the body, the shorter their time in the treatment process. In general, the patients need to take about two weeks in CAR-T therapy but the traditional chemotherapy needs to at least take three weeks in one session and need to undergo five to six sessions. And because the patients don't use aggressive chemotherapy, most patients can rapid recovery. CAR-T cells are beneficial for both efficiency and toxicity, and they also can combine with chemotherapy or allo-HSCT to reduce the toxicity and increase the cure rate [13].

At last, because of the main function region in T cell, the immune system will be boost and it will do more damage to tumor cells. CAR-T cells can reduce the immune escape of tumor cells by co-stimulating molecular signals.

6. The untoward reaction of CAR-T and solution

Most the targeting antigens in tumor cells will express in normal cells too and maybe the antigens just present a low-level expression in normal cells, which means that it doesn't exist tumor specific antigen (TSA) at present¹. When the CAR-T cells are killing the tumor cells and at the same time, the normal cells will be attacked by CAR-T cells and it leads to injury which is called on-target off-tumor effects. Depending on the organ which has the same antigens as tumor cells be attacked by CAR-T cells, and they can appear different symptoms. In a preclinical animal study, the on-target off-tumor effects are relevant to affinity. The study shows that the mice in which the hepatic tissue is expressed Her2, with high-affinity are more easily cause lethal liver damage than low-affinity mice [14]. At present, the only way to resolve this is that enhance the tumor specificity of CAR-T cells in various ways.

CRS is a kind of general inflammation when the patient received the immunotherapy which include checkpoint inhibitors and CAR-T therapy for cancer or getting infection. After CAR-T therapy, the activated T cell and target cell can release more cytokines than it should and then the immune system overreacts. At last, the cytokines induce the general inflammation. According to the study [15], after the CAR-T cell combine with tumor cell, they can secrete a lot of Interferon- γ (IFN- γ) and then IFN- γ can activate macrophages. Among them, the concentration of IL-6 increased most significantly. In clinically, CRS can present a lot of symptoms (figure 1), such as fever, vomiting, diarrhea, low blood pressure, swelling, confusion and even can become multiple organ failure.

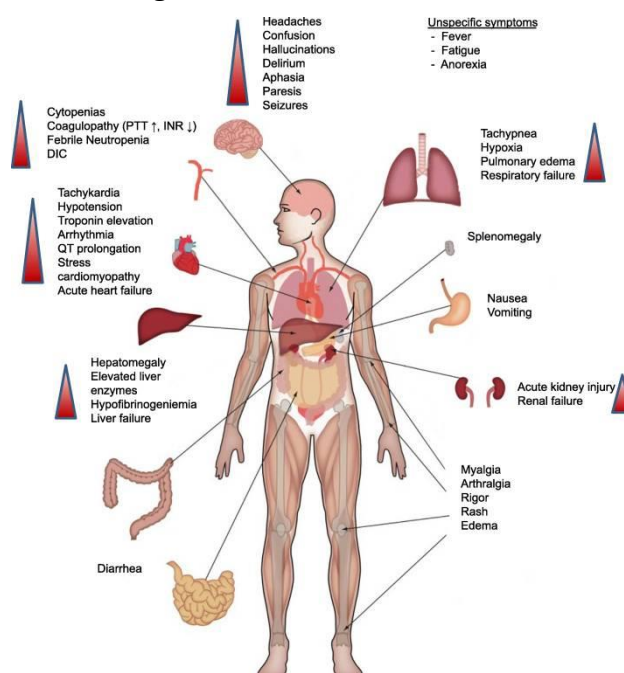


Figure 2. clinical presentation of CRS

After patients receive CAR-T therapy in three weeks, they include temperature higher than 38, systolic pressure less than 90mmHg, hypoxemia, organ toxicity. And the serious cytokine release syndrome(sCRS) means that a fever higher than 38 continues more than three days and at least two kinds of serum cytokines rise to 75 times or one kind of serum cytokines rise to 250 times and have a severely organ toxicity. Depending on different classifications (Table 1), the treatment plan is also different. In general, antipyretic and supportive care are often used for low-level CRS, and at same time prevent infection is also important to CRS. For serious CRS, Tocilizumab which is a anti IL-6-receptor antibody is a general cured method². In addition, appropriate amounts of glucocorticoids can eliminate inflammation. There is a catch. Glucocorticoids can't use too much because it can reduce the effect of CAR-T therapy. In addition to early use drugs to anesisthe CRS, and how to prevent it is also very critical. The doctor need to strictly control the dose of CAR-T and reduce the dose of CAR-T in necessity.

ICANS generally base on clinical symptoms to diagnose. After eliminate the other causes such as infection, the patients appear to aphemia, confusion, and impaired fine motor skills, and in more severe cases, they may show seizures, coma and cerebral oedema. For management of ICANS the most important thing is expectant treatment and give supportive therapy. According to the grade of ICANS, other grades which except grade 1 are use glucocorticoids as routine treatment.

7. Limitation and future development

The first one is waiting time for preparation. The preparation is too long to use for some patient with rapidly progressing disease so how to reduce the preparation time is a critical point to increase the efficiency of CAR-T therapy. Second one is the safety of CAR-T therapy. We discuss some side effect of CAR-T therapy which were mentioned above and these issues greatly impact the safety and efficiency of CAR-T. Maybe we can combine other therapy to reduce the side effect of CAR-T, such as modular CAR-T(modCAR-T) and immune checkpoint molecules. ModCAR-T is different from the original CAR-T, it has a new structure is called adaptor. The adaptor one end combined with CAR-T and the other recognition and combine with the antigen on tumor cell. Thus, modCAR-T can both enhance the specificity of recognition the antigen to reduce the possibility of tumor escape and control the activated state of T cell by manipulating the adaptor [16].

The immune checkpoint molecule is a group of regulatory molecules, immune checkpoint molecules undergoing the role which maintain the tolerance of autoimmune system and the immunologic homeostasis. After PD-1 combine with PD-L1, the activation conjunct can lead to T cell of attenuation and dysfunction. Tumor cell is expression the PD-L1 and it can target the PD-1 then to inhibit the T cell so as to achieve the effect of immune escape. According to the research, immune escape can be inhibited and recover the ability of T cell to kill the tumor cell by blocking axis of PD-1/PD-L1. The strategy of PD-1 regulation combined with CAR-T is mainly aimed at building CAR-T cell which can target PD-1 alone or in combination. The mechanism can divide into two points, building a CAR-T cell and targeting PD-1, which can secrete the PD-1 inhibitor, and indirect enhance the cytotoxicity in T cell and the combined degree of CAR-T with tumor cell.

8. Conclusion

Acute lymphocytic leukemia is a big problem for hematologic malignancies no matter adult or children. In recent years, CAR-T become a newly developed approach for ALL. CAR-T cell include four main domains. CAR-T therapy can aim at the drawback of traditional therapy to resolve it and in the meantime, CAR-T can also increase the treatment efficiency through gene engineered T cell to target CD19 in B-cell and target CD7 and CD38 in T-cell to combine with it and then to induce the tumor cell die. But with the applications of CAR-T, some new problems follow. All of it is a common complication during CAR-T therapy. At present, most of these questions adopt supportive therapy

but how to improve these side effect and prevent it are also a big issue for scientist. In additional, decrease the recurrence rate is also a critical thing to resolve.

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