Study of the Application of CAR-T Cell Therapy in Acute Lymphocytic Leukemia

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Abstract. Currently, the acute lymphocytic leukemia (ALL), which is a severe blood cancer that mostly found in children, can applied with the most updated CAR-T cell therapy for the cure. ALL incidence rate is around fifteen in one hundred thousand people, which is just lower than the incidence rate of Chronic Lymphocytic Leukemia. And the ALL occupied one-fifth of the leukemia cases. With the application of CAR-T cell therapy, ALL can be cured with higher long-term survival rate. But the CAR-T cell therapy brings some negative impact towards patient body like Cytokine Release Syndrome (CRS). The limitations and adverse actions of the therapy needs to be overcome or minimized to achieve the best prospect of treating patients. In this paper, the first part will briefly introduce present situations of ALL and the CAR-T cell therapy, and the following parts will explain mechanism of CAR-T cell therapy, limitations of the therapy on this disease, and future development of the therapy.

Keywords: CAR-T cell therapy, Acute Lymphocytic Leukemia, Anti-CD19.

1. Introduction

ALL is a typical blood cancer which affect hematopoietic system, it can be commonly found in child and rarely be found in adults. ALL facing unique clinical difficulties in both adult and children. In adults, ALL is unusual and it always associated with poor medical prognosis. Patients who under 60 years old experience a 5-year total survival rate between 30% to 40%, whereas those elder patients with more than 60 years old only have a survival rate of less than 15%. Prognoses for relapsed diseases are exceedingly bleak, typically resulting in an average survival duration of only 6 months. In contrast, ALL is the most prevalent cancer and the significant cause of children death cases by cancer.

In cases of refractory or relapsed conditions, the primary objective of therapy has traditionally revolved around achieving remission of a sufficient period to facilitate allogeneic hematopoietic stem cell transplantation (alloHSCT). Nonetheless, it's worth noting that merely 25% (the mid-point of 10% and 40%) of adult patients grappling with refractory or relapsed ailments successfully attain full remission through salvage chemotherapy. Furthermore, the 5-year survival rate without the disease was settled between 10% to 20%. In contrast, a significant proportion of children afflicted by acute lymphocytic leukemia experience a lasting response following high-dose chemotherapy. But there are around 15-20% of these children may eventually experience a relapse [1].

The cause of ALL is still unknown, but it might be related to genetic and environmental factors. Leukemia cells originate from progenitor B or T cells inside the bone marrow, which abnormally proliferate and accumulate in the bone marrow to inhibit hematopoietic, causing the reduction of neutrophil and platelets. Also, ALL associated with syndrome like anemia and lesion of tissues by infiltrate into other tissues such as meninges, thymus, liver, spleen,lymph nodes, etc.

In recent decades, more and more therapies were discovered to treat diseases like ALL. The most common therapies that applied with ALL are chemotherapy, radiotherapy, and alloSCT. Apart from those therapies, the CAR-T cell therapy shows its ability on treating the ALL.

CAR-T cell therapy is a revolutionary drug to enhance immunity as one of the immune therapy against cancer development with the characteristic like being innovative, have high specificity and able to self-replicating. By using genetic modification to transfer particular domains with antigen recognizing function and genetic materials that used for sending signals into T cell in order to obtain

the T cell activation. So that the CAR-T cells is activated when binding with the specific antigen on the surface of cancer to release both cytokine and perforin to kill cancer cells. Currently, the cost of this treatment is extremely expensive, which is not only due to its specificity to every individual, but also the troubles that facing in the manufacturing. CAR-T treatment also brings side effects towards the patient body as it works for eliminating cancer cells, as the CAR-T cell therapy facing challenges on fighting with solid tumors, those side reactions become more obvious.

This paper mainly focus on the application of CAR-T cell therapy on ALL, the mechanism of CAR-T cells on fighting against ALL,the limitations and side effects of CAR-T, and to discuss possible development of CAR-T cell therapy in the future.

2. Mechanism of CAR-T cell therapy

Chimeric Antigen Receptors (CARs) are antigens that designed to modify T-Cells to target the cancerous cells that express specific antigens on their plasma membrane [2]. After the modification and expansion, those CAR-T cells are injected into patients' bodies to fight against tumors. The modified T cells express CARs on their plasma membrane to assist the detection and attachment of CAR-T cells to specific antigens which located on plasma membrane of cancerous cells via cell signaling.

The attachment between the CAR-T cell and the cancerous cell triggers the intracellular signaling of the CAR-T cells. Therefore the modified cells are activated to release cytotoxic to destroy cancer cells. This anti-cancer mechanism is similar to the signaling pathways that normal T-cells. After the injection of CAR-T cells into the site of tumor inside the body, the CAR-T cells will attach to the cancer cells by binding with both scFv and CAR receptors. CAR-T cells are able to recognize various tumor antigens like BCMA, CD19, CD20, CD30, and other type of antigens, the CD19 antigen is being studied the most among various tumor antigens [2]. The CAR-T cell experience structural modifications and then be activated after binding with tumor antigens. Cytotoxic effector proteins like perforin and granulysin are released to kill cancer cells by create transmembrane pores on the membrane, and release cytokines into the nearby tissues to gather endogenous immune cells to kill the cancer cells. Phagocytes will remove and digest the dead cancer cells just after the cancer cell death. At the same time, memory T cells will be differentiated from the T cells to provide long-term anti-cancer effect.

3. Current Clinical Trials of CAR-T Cell Therapy in ALL

ALL is a kind of blood cancer that can be found in both children and adults. In the case of ALL, lymphoid progenitor cells that located inside the bloodstream, bone marrow, or extramedullary sites experience a malignant transformation and proliferate rapidly, ultimately developing into cancerous cells. [3].

There were several treatments that used to treat ALL, but some treatments brings intense side effects such as Graft versus Host Disease (GvHD) in the past, which associated with fever, vomit, purging, damage of liver, and even the decrease of white blood cell and platelets. Thus, finding some new and effective methods to apply in the treatment becomes crucial. In the past few years, the use of CAR-T cell therapy in curing ALL have received a lot of attention and bringing new series of methods because of the safety of the treatment [4]. The anti-CD19 target is CAR with highest efficiency for treating ALL, where CD19 antigen is an important biomarker that mostly expressed in B-cell acute lymphocytic leukemia (B-ALL) [4]. Other promising targets include anti-CD20 and light chains of immunoglobulin [5].

As the CAR-T cell therapy is developing, there are three generations of the therapy (Fig.1). The first type of CAR-T cell only included a CD3ζ chain and it cannot provide any potential anti-tumor effects [5]. Therefore, this generation only be used for a short time. This inspired researchers to advance, which lead to the discovery of the next generation of CAR, which is the second generation

in the series. Although the second generation CAR-T cell with either CD28 or 4-1BB targets has a higher efficacy, the combination of the two targets may bring a better efficacy and lead to the appearance of the third generation of CAR-T cell [6].

The therapy has notably enhanced the perspective of children and adults dealing with recurrent or resistant diseases. Maude and colleagues conducted an extensive study involving a cohort of 30 individuals consist of both children and adults to collect more data to investigate rates of complete remission and the endurance of such remission, and also the long-term insistence of CAR-T cells (which categorized as CTL019 cells). In this phase I/IIA study, 15 patients who had received alloSCT and two blinatumomab-resistant patients were assessed. Achieving 78% overall survival rate (95% CI, 65 to 95) and a 68% event-free survival rate (95% CI, 50 to 92), and the durable remission was gained within 2 years. [6]. These results of the experiment were obtained from the initial studies, which only contain small groups of patient samples who had chemotherapy-resistant disease, that showed impressive reactions to CAR-T cell treatment.

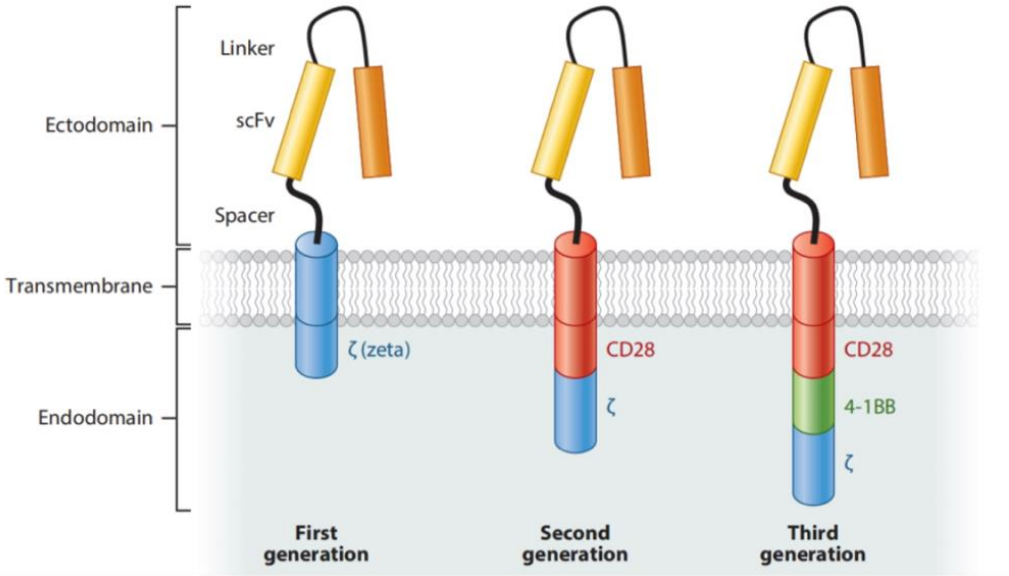

Figure 1. Three generations of chimeric antigen receptors (CARs).

(Not original. Adapted from [5])

Seven CR patients received no further treatment, and 3 of them remained in remission at 6, 6.6, and 14 months after infusion. Eleven CR patients were promptly bridged to transplantation, and 8 of them remained in remission at 4.6 to 13.3 months after transplantation, resulted in 1-year leukemia-free survival rate of 71.6% (95% CI, 44.2-99.0). CD22 antigen loss or mutation was not observed to be associated with relapsed patients.

In a study conducted by Pan, J. et al, modified T-cells with CD20 domain on CAR was administered to 34 child and adult patients who had experienced relapse or refractory (r/r) to previous CD19 CAR-T cell therapy. Among the 30 patients evaluated 30 days after infusion, 24 of them (80% of the tested patients) achieved either complete remission or complete remission with incomplete count recovery. This success rate accounted for 70.5% of all participated patients. Notably, most patients only encountered mild cases of CRS, which is less harmful compared with adverse effect of CD19 CAR-T cells) and neurotoxicity.

For the patients who achieved complete remission, 7 of them received no further treatment, and 3 patients maintained their remission at 6, 6.6, and 14 months after the injection with CD20 CAR-T cells. 11 patients with complete remission were rapidly transitioned to alloHSCT, and 8 of 11 patients remained in remission at 4.6 to 13.3 months after transplantation. This resulted in the survival rate without leukemia in one year is around 71.6% (95% CI, 44.2-99.0). Also, it is important to mentioned that the loss or mutation of CD22 antigen was not observed to be associated with patients who experienced relapse.

4. Limatation and Side Effects of The Therapy

There are various limitations of CAR-T cell therapy that should be focused on, for example on-target off-tumor effect, antigen escape, CAR-T cell associated toxicities, and the challenge in manufacturing. Those are the limitations that involved in this paper, there are still other limitations of this immunotherapy technique.

4.1. Antigen Escape

Antigen escape is a phenomenon that the malignant lymphoid cell in ALL patients may stop to express the targeted antigen after the first intake of the therapy, and the tumor resistance has been built from the use of single antigen targeting CAR such as the first two generation of the CAR-T cell therapy. Under such a condition, the CAR-T cells lose their target, and they will be non-functioning towards the ALL development [7]. The tumor is able to continually growing without threaten from the CAR-T cells. The Alpha-fetoprotein (AFP) is a biomarker that indicate the proliferate of cancer cells, and it should be checked regularly. If the level of AFP becomes extreme larger than the average, then it is a high probability that the tumor is growing or infiltrating to other tissues without the control of the single antigen targeting CAR-T cell therapy. The CAR-T cells that able to recognize multiple tumor antigens can be applied to those patients to find the cure.

4.2. On-target Off-tumor Effect

Since the malignant lymphoid cells are mutated from the normal lymphoid cells, the antigens located on the plasma membrane of lymphoid cells from both ALL patient and health people are similar to each other. Thus, antigen selection is an important step in the CAR design, and this step might limit the “on-target off-tumor” effect from distinguish abnormal ALL antigens from the normal antigens. If the chosen antigen is found on both ALL and normal cells, then the normal tissues and blood vessels would be attacked by the CAR-T cells and the human body will receive high level of toxicities which leads to the damage of tissue or even death. One possible approach to address the challenge of targeting antigens present on both tumor and normal tissues is by focusing on tumor-specific post-translational modifications (PTM). The PTM consist of cutting the RNA to form the 5’ end and 3’ -OH end, and then applying enzymes to modify the RNA sequence. Therefore the CAR-T cells will only recognize cancer cells instead of normal cells. Unfortunately, the first generation of TAG72-CAR-T cells shows no anti-cancer response against colorectal cancer, the second generation of TAG72-CAR-T cells and other PTMs that able to restrict tumor growth are now being explored [8].

4.3. CAR-T Cell Associated Toxicities

The discovery of CAR-T cell therapy is a revolutionary development in the cancer treatment, but this therapy is not being permitted as the first-line treatment. CAR-T cell therapy brings severe side effects and toxicities to the body, in some cases the patient were died because of it [7]. The CRS is one of the adverse syndrome of CAR-T cell therapy. In this condition, the immune system is activated and release inflammatory cytokines in the blood stream, which may cause fever, muscle pain, low blood pressure, edema, high heart rate and even hepatic failure. The data from patients with either Acute Lymphocytic Leukemia or Lymphoma (ALL/LBL) treated with CAR-T therapy, almost event-free survival rate patients suffered from weaker side effects and around 23-46% of the patients showed symptoms like extreme supraphysiologic cytokine production and numerous in-vivo T cell production [9]. Currently there is no CAR-T cell therapy with none toxicity that passed the clinical trial.

4.4. Challenge on Manufacturing

In the manufacture of CAR-T cell, the process might be affected by many factors. The collection efficiency should be checked at the beginning, components like T-cell count, volume of the blood,

age of patient, disease status, blood quality both can affect the collection efficiency, and those factors can be proven by peripheral blood counts and apheresis parameters [10]. The volume of blood collected should be mentioned from the list, the low volume of blood lead to low T-cell collection efficiency, which cause the failure of production. The detection of adding antigen into the blood is used to rank the immune ability of T-cells, and to figure out whether the T-cell can be applied.

During collection process, vascular access should be able to support the blood flow. But for children, because they have poor venous access and inadequate blood volume, the potential complications may occur during the collection and then induce more severe adverse consequences.

And then in the production process, the examine technique will Freeze, thaw, and carry out viral transduction to the T-cells, which affect activity and function of cells, and further lead to the appearance of effectiveness of CAR-T cells against the cancer, but carry out apoptosis earlier than the normal fresh cells [11].

5. Future Development of CAR-T Cell Therapy on ALL

To achieve better treatment prospect of CAR-T cell therapy on ALL, there are some advices to direct further studies.

5.1. Change the Molecular Structure of CAR

Change the structure of CAR binding domains to make it have weaker affinity towards the targeted antigen might be helpful for reducing the toxicity. When the CAR-T cell attach with the malignant lymphoid cells that have higher antigen density than normal cells, high level of activation can be achieved. In comparison, the normal cells have fewer antigen than cancer cells on there plasma membrane can be protected by only receiving lower toxicity. Modifying the hinge and transmembrane regions to alter the secretion of cytokines by activated CAR-T cells can also achieve the goal of reducing toxicity.

Furthermore, the co-stimulatory domain offers an additional design in CAR with modifiable structural region, allowing customization according to factors such as the tumor type, tumor burden, antigen density, and the target antigen binding domain. More precisely, CAR-T cells that have the 4-1BB antigens on its plasma membrane are associated with reduced toxicity risk, enhanced T cell tolerance and lower peak levels of T cell proliferation. On the other hand, CAR cells with CD28 demonstrate quicker activation but also faster depletion and inadequate persistence.

5.2. Install a Switch on CAR-T Cells

Another potential approach to minimize CAR-T cell toxicity involves the application of "off switch" or gene strategies leads to suicide. These methods aim to selectively diminish CAR-T cell activity when adverse events occur due to secondary inducers. In line with this idea, techniques have been devised to control CAR-T cell function, with examples like inducible Casp9, which achieved the removal of more than 90% of CAR-T cells within 30 minutes in a clinical trial.The most significant drawback of suicide strategies and related methods is to stop the treatment for fast-progressing illnesses immediately. One potential solution involves using dasatinib, which is a tyrosine kinase inhibitor that operates by impeding T cell activation through the inhibition of proximal TCR signaling kinases. In preclinical models, dasatinib demonstrates its ability on suppress the activation of CAR-T cells rapidly and reversibly. This strategy provides a temporary mechanism for curtailing CAR-T cell activity, ultimately facilitating the revival of CAR-T cell therapy once the toxic effects have abated.

6. Conclusion

ALL is one of the most common life-threatening blood cancer that could be found in young population than adults. Currently, ALL associate with high relapse rate and medium 5-years survival rate in adult population and low 5-years survival rate for elders.

CAR T-cell therapy is an innovative and believable therapy with the use of immunity that can be applied on killing malignant lymphoid cells for treating ALL by modifying the T cell receptors. The hybrid receptor of the T cells, CAR, are designed to modify T-cells to target cancerous lymphoid cells which expresses aimed antigen targets on their plasma membrane. CAR-T cells are generated through CAR-T cell treatment has a good and effective effect in the therapy of ALL. Although there are more generation of CAR-T therapy that targets different cancer antigens treatment drugs are still being investigated, it is still a suitable choice to treat ALL. The limitations of the therapy on ALL are closely related to toxicity towards the normal tissue cells and efficiency in producing.

There is a lack of dataset in the clinical trial of ALL, more cases should be added and analsed to enrich the research scale and to visualize the drawback. Further investigation should focus on identify compounds that able to prevent the stoping of antigen production of cancerous lymphoid cells, prolong the life cycle of modified T cells, and reduce the toxicities of the cells as it pass through the blood stream,etc. Also investigate a less harmful CAR-T cell therapy to minize the severe side effect like CRS, so that it might be used as the first-line treatment. The two possible direction of improving CAR-T cell therapy needs to be focused and maybe in the future the CAR-T cell therapy can be applied to eliminate ALL with high efficacy and low toxicity.

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