

Targeting CD19 and CD22 with CAR-T cell therapy in acute lymphoid leukemia

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Abstract. Acute lymphoid leukemia (ALL) was usually occurred in children. It accounted for 75% of all acute leukemias. In other words, it accounted for 35 percent of all cancers in the same age group, especially Caucasian children. CAR-T cell therapy was an resultful treatment for ALL patients. CARs were artificial receptors. Their function was primarily to target lymphocytes, most commonly T cells, recognizing and eliminating cells that express specifically targeted antigens. There were some targets for CAR-T, such as CD19 and CD22. When treated with common methods, the prognosis of recurrent ALL after allotransplantation was frustrating. In specific the cell of CAR-T with CD19, B-ALL treatment was highly successful, 70 to 94% in a complete response (CR). therapy with CD19 and CD22 was targeted when treating refractory/relapse(R/R) B-ALL achieved high CR, most of patients couldn't maintain enduring remission. This therapy was a huge success in curing leukemia, up to 60% of patients with this therapy relapse. To extend the period of recurrence-free survival, people targeted CD22 or CD19 antigen expression, respectively, treating relapse B-ALL patient after transplant treatment. The CR rate in single therapy with the first round achieved 85%. After that, among patients who completed CAR-T with CD19 and CD22, 88.5% and 67.5% were OS and EFS, respectively, between 12 and 18 months. The new strategy significantly improved treatment outcomes. In the future, the key of the research was shift to strategies with minimal cytotoxic chemotherapy and hematopoietic stem cell transplantation, and we hope can treat tumor successfully.

Keywords: CAR-T Cell Therapy, CD19, CD22, Acute Lymphoid Leukemia.

1. Introduction

CAR-T cell therapy treatment was ground-breaking, because it produced remarkable resultful and enduring clinical reaction. CARs were a kind of man-made receptors. The function of them were main targeted lymphocytes and the most common one was T-cell, recognizing and removing cell which expressed cell of special targeting antigen. CARs combined targeting antigen with cell surface expression, independent of MHC receptor, therefore producing intense T-cell activation and strong anti-tumor response [1]. CARs were made of modular fusion protein, include extracellular target binding domain with common coming from single stranded variable fragment of antibody, interval area, transmembrane area and intracellular signal. T-cell with expression CARs from gene transfer technology engineering could through SCFV combine area to recognize targeting antigen specially [2]. Acute lymphoid leukemia was a common disease of malignancy tumor for children. It usually occurred among healthy people. The research showed only seldom patient discovered susceptibility factor, such as exposed environment, heredity susceptibility and so on. There were three majors in ALL, chromosome abnormality, something involving lymphoid differentiation and genetic changing in precursor cell proliferation [3]. ALL was a kind of malignant. It was originated from single Hematopoietic precursor impact B-cell or T-cell. Cells could gain a many of genetic changes with some possible destroying common progress, and it leaded to block proliferation in differentiation and transformed cell [4].

There were most ALL case that happened in children. In the US, the morbidity of patient with range zero to fourteen years old was 3 to 4/100,000. The morbidity of patient who was above fifteen years was 1/100,000. In children, ALL took up 75 percent in all acute leukemia. In another way, it occupied 35 percent in the same age group with all kinds of cancer. The high incidence rate in age

group 2 to 5 which took low proportion in adult group. It was more common in adult acute myelogenous leukemia (AMLS) and chronic lymphocytic leukemia. There were slight male advantages all age groups, but the morbidity of white children was significantly on the high side [5]. ALL showed us bimodal distribution, the first peak value appeared about 5 years, the second one appeared about 50 years. Although ALL main impact children, it took up 20 percent in adult leukemia and made up 75 percent case [6]. In children group, ALL was highly curable. The long-term survival rate was above 90 percent. It was main due to risk-oriented treatment, optimizing chemotherapy dose and timetable in developing last 40 years. By contrast the same treatment strategy in adult group only produced long term survival rate with 35percent to 45percent [7].

Although there were some improvements in the treatment for ALL and the survival rate were improved remarkable in past 50 years. In the field of Cancer immunotherapy and children acute lymphoid leukemia was a landmark thing. In august, the tisagenlecleucel in Novartis was approved by FDA. This was a kind of CAR-T cell product with a CD19 target. For patients under 25 years of age with relapsed/refractory ALL. Tisagenlecleucel isa chimeric antigen receptor (CAR) T-cell and gene therapy. It is also the first FDA-approved treatment for childhood cancer in recent years [8]. However, there were no massage introducing development and mechanism about the treatment using in ALL.

The passage includes the recent research paper and express them more. The passage discusses more about application for this treatment in acute lymphoid leukemia treatment. It introduced the mechanism about how CAR-T therapy and how they work in patient body. It also talks about the targeting CD19, it was a remarkable event for ALL treatment at that time. The passage shows more about the foundation of the CD19. It had a high recurrence rate and we found another method to replace CD19. That was multi-targeting CD19/CD22 and it had more good effects. It can avoid the high recurrence rate. At last, I analysis the limitation of the CAR-T cell treatment and explain my hope for this treatment

2. CAR-T cell used in ALL

CARs were a kind of T-cell in genetic engineering. It expressed an immune globulin in antigen binding area. Connected with the part of transduction cellular receptor signal in T-cell passed by membrane area. The basic structure of CARs included activate area in T-cell, hinge area out of cell, transmembrane regions and the part of antigen recognition out of cell from antibody. The second generation of CAR-T cell had coding for costimulatory area, such as CD27 and the family number of tumor necrosis factor receptor, such as CD137. This area could provide better product cell factor and breed, and it could enhance the persistance of CAR-T cell. The third generation of CAR-T cell had two Costimulatory area, using Costimulatory area and structural domain, such as CD27or CD134 [9].

CAR-T cell combined specificity antigen of antibody and cell toxicity potency of T cell. Getting through used of original T cell in patient body and also used in vitro genetic engineering specially recognition tumor antigen out of cell with the power of inborn and adaptive immunity. Usually activate complete T cell relied on two activate signal at least. The first one was endogenous T cell receptor (TCR) through by homologous peptide The second one was main organization Compatibility complex (MHC) management pass by subunit CD3.And passed by any number mechanism providing other costimulatory signal include CD28/CD80 or 4-1BB/4-1BBL. In a condition with no second signal, peptide called complexus MHC take part in it may lead to T-cell no power [8].

Through targeting CD19, CD20 or CD30, they showed a hopeful result. In special CD19 CAR-T cell therapy, the treatment of B-ALL achieved a big successful and the complete remission reached between 70 and 94 percent.

3. The treatment with CAR-T cells target CD19

Cd19 became the target of ALL immunological therapy due to CD19 can almost common expressing on B lymphocyte. The thinking of Using B lymphocyte to make a adoptive immunity treatment for leukemia emerged in the 1990s. Clonal T-cell antigen acceptor with after zeta-chain , Eshhar and other people imagined the first Chimeric antigen receptor. In order to improve the Anti-tumor efficacy, people through embellished much molecule configurations for the production. So many organizations such as MSKCC, FHCRC and so on, already exploited CAR-T products. And they also began to clinic test for B-cell malignant tumor anti-CD19 treatment. In 2012, after successful Preliminary clinical, University of Pennsylvania (UPenn) contributed to league of research with a pharmaceuticals company, developing CAR-T cell for commercialize. CTL019, which was called tisagenlecleucel (Tisacel). It was one of CAR-T cell therapy method ratified by US Food and Drug Administration. The preliminary result of CTL019 in ALL was published in 2013. From that time, many experiments of CAR-T product with targeting for different adaptation were under way, and achieved satisfactory results [9].

The second part was the mechanism of reappear. There were two patterns after CAR reappear in B-ALL. The first one was positive relapse CD19, another was negative relapse. The main mechanism of positive relapse CD19 was poor durability, however, CD19 could still stay on the surface of B-cell and tested by flow cytometry. For B-cell negative reappear, CD19 absent, it led to the tumor get away from CAR recognition and remove, although CAR-T cell exit. The next part was positive reappear. It had relationship with limitation development low-efficiency CARs, less reaction CARs for patient and regenerator of B-cell [10]. According to clinical experience, 10 percent to 20 percent ALL patient had negative relapse after CD19 CAR-T cell treatment. The next part was gene mutation. In the recently research, gene CD19 had exon between 1 and 13, including exon 1 to 4 coding structural domain and exon 5 to 13 coding transmembrane structural domain. Orlando and other people did more research. The result showed selectivity cut was one of the reasons when CD19 gene mutation [10].

This treatment as an emerging immunization therapy, CD19 targeting CAR therapy was a remarkable innovation. Many clinic experiences showed that using CD19 targeting CAR-T cell therapy to cure children and adult could get 70 percent to 90 percent complete remission (CR). However, 30 percent to 60 percent patient relapsed after CAR cell therapy and 10 percent to 20 percent was negative relapsed. Overall, the CD19 targeting was not the best choice for the therapy, we found another target position, it could reduce the relapse rate [10].

4. The treatment with CAR-T cells target both CD19 and CD22

Although Car-T cell therapy was a big successful to treat leukemia, as high as 60 percent treatment after treatment was relapse. The feature of the relapse was losing CD19 antigen. The reason was referred to a lot of different mechanism. Therefore, the designation of losing antigen CAR-T were crying needs

Used white blood cell extraction to collect peripheral blood mononuclear cell from patient (PBMC). Cleaned it well and freezed preservation. Separated T cell from hemameba separation production with thawy and used Dynabeads Human T-activator activate with CD3/CD28. PBMC was fostered in XVIVO 15 substrate with interleukin-2. After original T cell activation, T-cell was transduced encoding CD19/CD22-BB-Z transgenosis lentivirus. After cell expanding 10days, it had quality inspection in CAR-T cell manufacturing process of CAR-T cell [11]. The step of producing double targets CAR-T Cell with CD19 and CD22 showed in figure one.

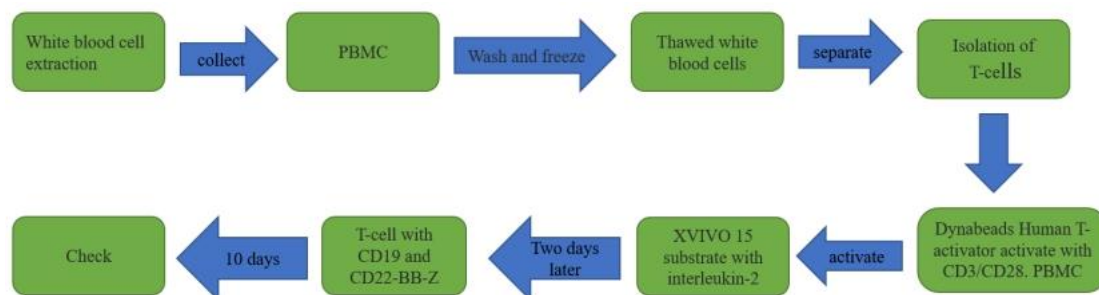


Figure 1. Process in production of CD19/CD22 CAR-T cell

The method to overcome antigen losing after treatment was targeting more antigens at same time on cancer cell. In view of antigen CD22 CAR-T cell also proved clinical effects. This method had more attraction for B-ALL. Some research showed that contrast single antigen targeting. Double antigen or multi-antigen of CARs could product concerted reaction, improving treatment response rate and preventing antigen remove. Supporting clinical date of multi-target method included CAR structure series resistance CD19 to CD20, combine strategy of anti-CD19 and CD123, targeting CD19 and CD22. And some using strategy of clinical experiences were done now. In vitro test, cell second generation CD19/CD22 had a standard CAR construction of three structural domain and structure by EAAAK connexin connect two single stranded variable fragments. Heavy chains and light chains of m917 was connected by sequence 3(GGGS). The product of double targets treatment received wide evaluation in a series of in vitro experiences [11].

Clinic response was more important part. Although single target therapy had a high CR rate which reached 81to 90 percent and had a significant curative effect for (R/R) B-ALL patient. There were 20percent to 50percent patient reappear after six months. In order to conquer the disadvantage of target with single, some group had a therapy with CD19 and CD22. In research, we applied CD19 and CD22 for Relapse in the prognosis for patients with B-ALL is not so good after treatment. CR rate in the first round of single treatment reached 85 percent. Whereafter, in completed CAR-T treatment with CD19 and CD22 patients, the OS and EFS in 12 month and 18 month were 88.5percent, 67.5 percent, respectively. Compare with the result of using single target therapy treat R/R B-ALL, 50 percent to 57 percent patient who not accept CAR-T transplant reappeared in 68 months. This new strategy significantly increased treatment effect. Although we could not compare result with other report directly, these data showed a higher rate in EFS and OS among 12 to 18 years. The most important was these things happened in above 50 percent in adult patient after hematocrit [12].

The last part was toxicity of CD19/CD22 CAR T cells. Some special negative event had possible with related double specificity double targets therapy. The whole toxicity recovered to the common or foundation line. According to recognized standards to classification Cytokine release syndrome (CRS) from order of severity. Neurovirulence divided class from Immune effector cell associated neurotoxic syndrome. 6 patients were not observed 3 grade or 4 grade CRS. Patient1 had fever in the second day and removed in seven days after cell infection. The patient2 had 5 days low fever from 8th days after injection. The patient3 had fever in 5th days and removed fever in 10th days. Patient 4 had fever after cell therapy 6th days continue for 7 days and it removed.

5. Limitations and future improvement

Today although CAR-T cell was the remarkable treatment in cancer, it also had more disadvantage. At present, the most common Gene transfer strategy was virus technology, such as retrovirus and slow virus. These could lead to permanent transgene expression of coding CAR. However, when CAR-T cell happened significant toxic reaction, these were both adverse. A method without virus was CAR mRNA electroporation, which had feature for passing gene CAR expression. The next step was put the new CAR into patient body. This method had been considered more saft than virus technology. The research people in university of Pennsylvania (upenn) first time assessed solid tumor msln expression which was based on research before clinical. And this research verified the safety

and feasibility. They also observed antineoplastic activity. These supporting the development of solid tumor treatment strategy based on mRNA CAR. We should notice the event, because of transience expression in transgenesis. CAR-T cell mRNA needed to inject many times and it improved the risk of allergic reaction. Based on the security of CAR-T cell in specificity msln, we design anti-msln CAR-T cell with slow virus transduction and we were testing in the clinical experience [13].

With the improvement of innovate and resultful treatments, researches showed such as Ino, blinatumomab and CAR-T cell. Not only therapeutic schedule enlarged, but also the key point was moving to the strategy of the minimum hematopoietic stem cell transplantation and Cytotoxic chemotherapy. However, before these novel drugs got in the best combination and made sure sequence completely. Register of clinical experiences and transfer treatment to level three centers were still very important. With Continuous efforts to optimize Existing treatment options and novel couple, we had reason to optimistic consider adult ALL therapy possibly became the successful story in another tumor.

6. Conclusion

Acute lymphoid leukemia was usually happened in children. In the United States, it occurs in 3-4 out of 100,000 children aged 0-4 years. It affected 1 in 100,000 patients over the age of 15. In children, acute lymphoblastic leukemia accounted for 75% of all acute leukemias. In other words, it accounted for 35 percent of all cancers in same ages. Chimeric antigen receptor was a kind of modular fusion protein. It included target out of cell combine area, interval area, transmembrane area and intracellular area. There were included CD3z connecting with one or two costimulatory molecules. T cell with expression of CAR by gene transfer still could specially recognize target antigen from SCFV binding domain. In specially CD19 CAR-T cell therapy, B-ALL treatment achieved remarkable successfully, complete remission (CR) reached between 70 to 94 percent. A CAR-T cell product targeted CD19. It was suitable for patients under 25 years of age with relapsed/refractory ALL. That was a remarkable thing at that time. Although the single target therapy with CD19 had a high CR rate of 81% to 90% for (R/R) B-ALL patients, the effect was significant. Between 20% and 50% of patients reappeared six months later. To conquer the disadvantage of a single target, some groups were treated with a combination of CD19 and CD22. Some toxicity about double targets CD19and CD22 therapy under the common line. However, there are also some weaknesses about CAR-T cell. The next step was implantation new CAR into patient body. This method was more safety than traditional treatment. The mRNA in CAR-T cell needed injection many times. It improved the risk of anaphylaxis. With the development of new and effective treatment, we believed ALL treatment may make a successful story in another tumor.

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