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Advances in the Research and Application of CAR-T Cell Therapy in Tumor Therapy

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Abstract: Cancer is a frightening thing in people's eyes. With the change of people's living habits, eating habits, living environment, etc., more and more various diseases haunt us. It is even said that the improvement of the level of technology has enabled us to discover diseases that had not been discovered before. Tumor, there are good and bad points, but also easy to absorb human nutrition, damage people's lives. Therefore, cancer therapy research is related to people's hope of anti-cancer treatment. With the continuous improvement of gene editing technology, the research of CAR-T cell therapy (CT) has made remarkable progress. CAR-T CT, or chimeric antigen receptor T CT, is a revolutionary approach to cancer treatment. It is based on genetic engineering technology, by modifying the patient's own T cells, so that they have the ability to specifically recognize and attack tumor cells. Although chemotherapy and radiotherapy can inhibit the growth of tumors to a certain extent, they are often accompanied by serious side effects such as nausea, vomiting, fatigue, and greater damage to normal cells. In contrast, CAR-T CT has higher targeting and lower side effects. It can achieve the purpose of treating tumors without damaging normal cells. This paper analyzes the role of CAR-T CT in hematologic tumors and solid tumors, so as to provide further evidence for tumor treatment. This paper investigates and analyzes the basic changes of myeloma patients before and after receiving CT and the status of carcinoembryonic antigen, cytokeratin 19 fragment and neuron-specific enolase in lung cancer patients. The blood pressure values of myeloma patients varied from 110 to 130 during the test. Lung cancer patients had smaller fluctuations in neuron-specific enolase, and there was some independence between this tumor marker and the effect of CAR-T CT. The effect of CT on the expansion of B-cell mature antigen in blood is weaker than that in bone marrow.

1. Introduction

The success of CAR-T CT is inseparable from its unique mechanism of action. By genetically modifying T cells, they can specifically detect antigens on the surface of tumor cells, thereby activating immune defense mechanisms that directly kill tumor cells. At the same time, these modified T cells can further spread in the body, forming a durable anti-tumor immune response,

effectively preventing tumor recurrence and metastasis. CAR-T CT has proven highly effective in clinical trials of blood tumors such as acute lymphoblastic leukemia and non-Hodgkin lymphoma, and some patients have even achieved long-term disease-free survival. At present, clinical trials on solid tumors are still in the exploratory stage, but through precise target selection and optimized CAR design, the CT has also achieved encouraging results in the treatment of solid tumors such as lung cancer and breast cancer.

In the 21st century, scientists in the United States successfully prepared CAR-T cells for the first time and demonstrated their powerful anti-tumor activity in a mouse model. This breakthrough research has laid a solid foundation for the clinical application. In the following years, the research of CT has entered a stage of rapid development. A number of clinical trials have been conducted, involving many types of cancer treatment. Among them, the most representative is the treatment of blood tumors. However, the development of CT has not been smooth sailing. In this paper, we need to consider improving the activity of CAR-T cells.

In this paper, the basic principle and development of CT were briefly reviewed. Secondly, this paper analyzed the results of other scholars' research on CAR-T, and then put forward the research intention of this paper. Then, according to the survey objects, survey results, detection indicators and statistical analysis, the situation of patients treated by this method was statistically analyzed. After that, this paper visually displayed the survey results and analyzed the data to get the relevant conclusions. Finally, CT was discussed and summarized in this paper.

2. Related Work

CAR-T CT has achieved remarkable results in the treatment of various blood tumors. In the treatment of acute lymphoblastic leukemia, the efficiency of CT is higher, and the survival of patients has been significantly extended. For some tumor types, CT carries a risk of recurrence. To this end, Cappell KM reviewed the efficacy persistence, recurrence rate, and safety of CAR-T CT, collecting and analyzing data from published studies on the long-term effects of CAR-T CT. They summarized the long-term efficacy of CT in a variety of tumors [1]. Johnson P C et al. introduced the basic principle and development history of CAR-T CT, analyzed its advantages and limitations, and proposed to further improve the design of CAR-T cells through gene editing technology [2]. By analyzing the research data of CAR-T CT in autoimmune diseases, Schett G summarized the preliminary application results of CAR-T CT [3]. Yang J revealed the interaction mechanism of CAR-T cells in the tumor microenvironment, and successfully used multidimensional omics data to improve the efficacy of CAR-T cells in tumor therapy [4]. The efficacy of CAR-T CT in the treatment of solid tumors is not ideal. Albelda SM analyzed the research data of CAR-T CT in the treatment of solid tumors, optimized the drug delivery mode, and combined with other treatment methods to improve its efficacy [5]. Mackensen A. investigated the use of anti-CD19 CAR-T CT in refractory systemic lupus erythematosus. However, it is necessary to expand the sample size and extend the follow-up time to further verify its long-term efficacy [6]. CAR-T CT may cause serious toxic reactions in tumor treatment. Chohan K L discussed the balance between the efficacy and safety of this therapy [7]. Regarding how patient selection can be done more effectively to improve the success rate of CAR-T CT, whether bridging therapy is effective for all patients, and how the side effects of lymphatic clearance can be managed, Amini L focuses on the preparation stages of CAR-T CT: patient selection, bridging therapy, and lymphatic clearance. He described the criteria and considerations for patient selection before treatment, discussed the role of bridging therapy, and studied the effect of lymphatic clearance [8]. Kaczanowska S et al. studied the immune determinants of GD2 CT in patients with solid tumors and revealed the immune mechanism affecting the expansion of CAR-T cells in patients with solid tumors [9]. In order to explore the

long-term effect of GD2-CAR-T CT on patients with H3K27M mutated diffuse midline glioma, Majzner R G reported the efficacy of CT and discussed how this therapy affected the survival rate and quality of life of patients [10]. Juluri KR investigated the association of severe cytokine release syndrome with hematological toxicity after CD19 CAR-T CT. He found a significant relationship between severe cytokine release syndrome and hematological toxicity [11]. The preparation of the therapy is complex and costly, which limits its wide clinical application. Because each person's immune system is different, CT can trigger immune-related adverse reactions. Therefore, how to reduce side effects while ensuring the treatment effect is the focus of CT to be discussed. This paper needs to conduct investigation and statistics on the experimental data of this therapy, and display the results through visual form.

3. Data and Methods of CAR-T Cell Therapy

3.1 Research Object

In this survey, 30 patients diagnosed with myeloma and lung cancer and receiving CT at local cancer hospitals from June 2021 to January 2023 were selected as specific investigation objects. In this paper, the investigated patients were divided into three states: progression, recurrence and refractory, and the patients were judged as low risk, standard risk and high risk by evaluation method.

The exclusion criteria were: weight less than 30 kg; pregnancy reaction was positive; subjects had been treated with steroid drugs in the past 4 weeks; participants had participated in other somatic CT studies within the past four weeks; estimated survival time < 2 months.

Inclusion criteria were: any disease diagnosed as recurrent or refractory: CD19-positive myeloma, lung cancer; absolute lymphocyte count in blood ≧600/μl; no infection with human immunodeficiency virus, human T-lymphocytophilic virus, syphilis, or any other active virus; under 70 years of age; no serious heart, liver or kidney disease.

3.2 Survey of the Overall Clinical Results of CAR-T CT

This paper investigated the clinical outcome data of CT, as shown in Table 1. After rigorous clinical trials and data analysis, Kymirah, Yescarta, Carvykti and other therapies have been confirmed to have significant effects in the treatment of malignant hematologic tumors [12-13]. Kymirah, a single-chain antibody drug targeting CD19, and its intracellular signaling domain of CD3, has shown good efficacy in the treatment of follicular lymphoma, with a complete response rate of 69.1% and an overall response rate of 86.2%. Yescarta showed a higher response rate of 83% in the treatment of large B-cell lymphoma than the conventional standard treatment group, which consisted of two to three rounds of chemical immunotherapy followed by high-dose chemotherapy and autologous stem cell transplantation in patients who responded well. In addition, the complete response rate in the Yescarta group was 62.5%. Carvykti is a CAR-T cell immunotherapy targeting B cell maturation antigen, which is primarily used to treat multiple myeloma. The overall response rate in clinical trials was as high as 97% and the complete response rate was 79%.

Table 1: Complete response rate and overall response rate for treatment of hematologic tumors

	Complete remission rate	Overall remission rate
Kymirah	69	86
Yescarta	62.5	49.6
Carvykti	79	97

3.3 Detection Indicators

Cytokines and peripheral blood flow cytometry were collected on D1, D3, D7, D10, D14, D21, and D28 to determine the number of BCMA CAR-T cells in the peripheral blood (B cells for maturation antigen). Bone marrow flow cytometry was performed on D1, D14, and D28 to determine the number of BCMA CAR-T cells in the bone marrow. Daily changes in body temperature, blood pressure, respiration, pulse and oxygen saturation were statistically analyzed. At the same time, patient baseline data (gender, age, classification, ECG, risk), presence of extramedullary infiltration before BCMA CT, and tumor exposure were collected. The changes of tumor load, cytokines, blood routine, biochemical index, coagulation index and BCMA CAR-T cell count were measured after infusion.

In CT of cancer, carcinoembryonic antigen, cytokeratin 19 fragments, T cell subsets such as neuron-specific enolase, NK cells, and B cells are detected to reflect the status of patient immune function, and to evaluate the impact of CT on patient immune function. In addition, it is also necessary to pay attention to white blood cells, red blood cells, and platelets.

3.4 Genetic Alterations in Gibson Assembly

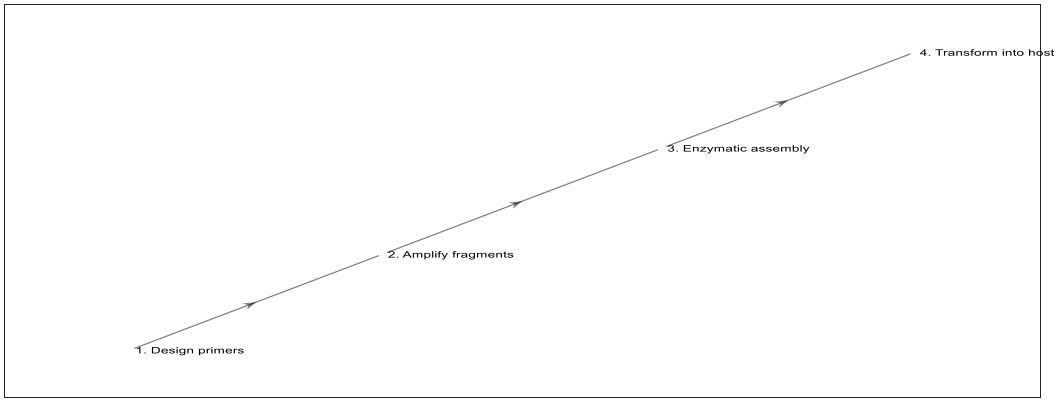

Figure 1: Gibson Assembly assembly method

As shown in Figure 1, Gibson Assembly relies on the connectivity between single-stranded (deoxyribonucleic acid) DNA fragments to achieve molecular assembly. There are four main steps (primer design, amplified fragment, enzyme assembly, and transport host), and three key enzymes are mainly used in the process: T5 exonuclease Photothruster, Phusion DNA polymerase and DNA ligase. First, the DNA fragment to be joined is amplified and specific overlapping sequences are added. The product is then purified to remove the excess primers. The purified product was mixed with T5 exonuclease photopush enzyme, Phusion DNA polymerase and DNA ligase for assembly reaction. The T5 exonuclease photopush enzyme excised the 3 ends of the DNA fragment, and Phusion DNA polymerase filled the excised gap to generate complementary sticky ends. Finally, the assembled DNA molecules are transformed into host cells for cloning and screening.

3.5 Statistical Analysis

If there are two independent sets of samples, the independent sample t test is usually used, based on the t distribution, assuming that the data follows a normal distribution and that the variances of the two groups are equal. The formula is as follows:

$$t = (a_1 - a_2)/(m * \mathrm{sqrt}(1/n_1 + 1/n_2)) \quad (1)$$

Among them, a_1 and a_2 are the mean values of the two groups of samples respectively, and m is the combined standard deviation of the two groups of samples. If the bivariate comes from paired observations from the same set of samples, such as measurements from the same group of patients at different points in time, then paired T-tests should be used. Its formula is as follows (2):

$$t_p = (\bar{a}c - \eta c)/(sc/n) \tag{2}$$

$\bar{a}c$ is the mean of the difference between the paired samples, ηc is the mean of the assumed difference, and sc is the standard deviation. According to the calculated t value, the corresponding P value can be determined.

If the data is a continuous variable and follows a normal distribution, multiple comparisons can be made using one-way ANOVA. It checks that the resources for each group are the same. For categorical variables such as gender and blood type, Chi-square test and Fisher precision test were used for comparison. The Chi-square test is mainly used to compare the difference between the actual observed frequency and the expected frequency. The Fisher accuracy test is used to compare data in a 2x2 table. In the survival analysis, Kaplan-Meier analysis and logarithmic rank test were used to compare the survival time distribution between different groups. Kaplan-Meier analysis estimates the probability of survival at a specific point in time. Logarithmic rank test of survival curve difference between different groups. The indicators required in this paper are relatively complex, so binary Logistic regression model is selected. The Sigmoid function is expressed as:

$$g(z) = 1/(1 + e^{\wedge} - z) \tag{3}$$

Binary logistic regression uses maximum likelihood estimation to fit the model parameters. In order to find the parameters that maximize the likelihood function, the parameter values are iteratively updated using gradient descent until they converge.

4. Result Analysis and Discussion

4.1 Analysis of the Basic Situation of Myeloma Patients

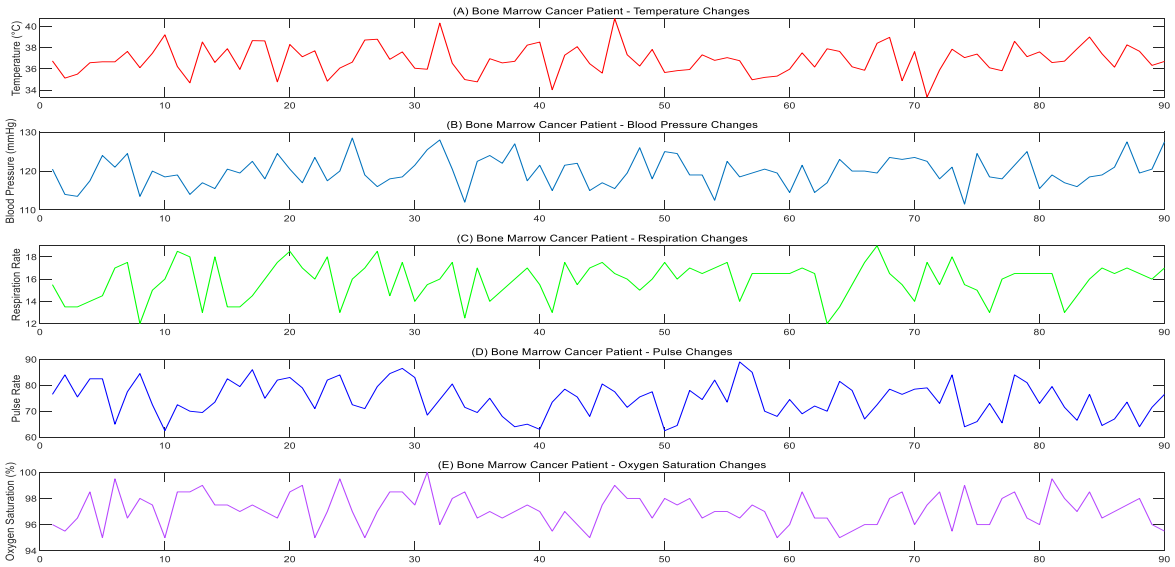

Figure 2: Basic changes of myeloma patients within three months

Figure 2 shows the basic situation of myeloma patients within three months. The patient's body

temperature peaks in the middle stage (high temperature), and the patient's condition is not very good, even can be said to be life-threatening. But the overall temperature remained in the normal and low-fever range. The patient's breathing rate fluctuates periodically and remains in a normal state most of the time. The pulse rate and oxygen saturation have some fluctuations during the 90-day period, the pulse rate does not exceed 90 and the oxygen saturation does not exceed 100%.

4.2 Analysis of Lung Cancer Patients

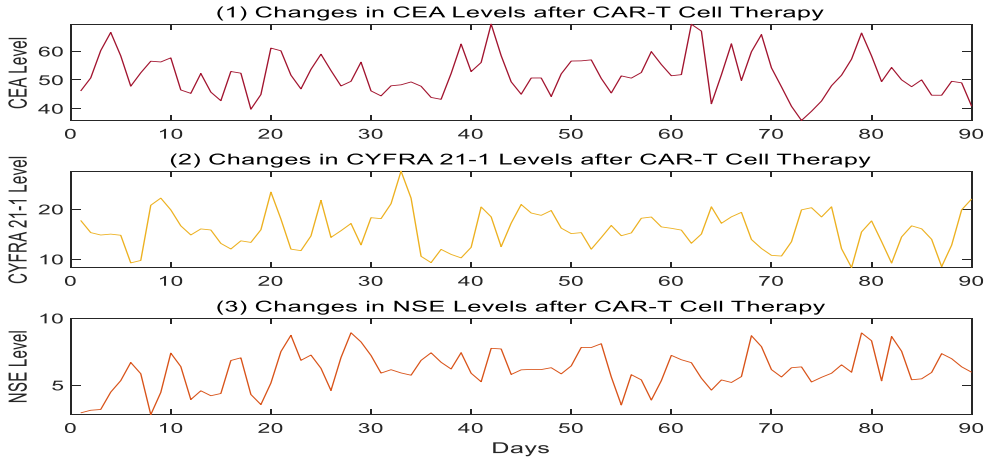

Figure 3: Changes of carcinoembryonic antigen, cytokeratin 19 fragment and neuron-specific enolase in patients with lung cancer

Figure 3 shows the changes in three tumor markers within 90 days of lung cancer patients receiving CAR-T CT. Carcinoembryonic antigen showed a large fluctuation, indicating that the tumor load was reduced after receiving CAR-T CT, and the immune system had a certain degree of response to the tumor. The cytokeratin-19 fragment remains at a relatively stable level, so the effect of CT on lung cancer patients is limited and does not significantly change the level of cytokeratin-19 fragment. These numerical changes indicated that CAR-T CT had a certain degree of tumor control, but had little effect on cytokeratin-19 fragment and neuron-specific enolase.

4.3 Analysis of Changes of B Cell Maturation Antigen

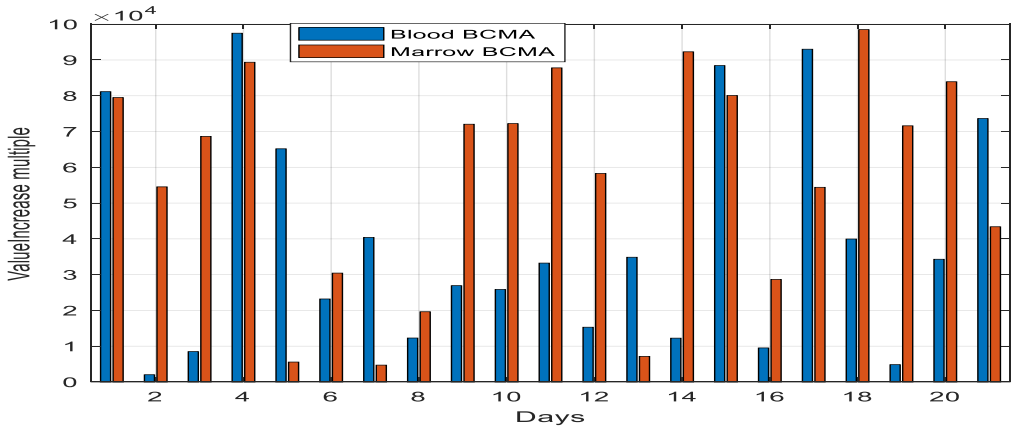

Figure 4: In vivo BCMA amplification after CAR-T cell reinfusion

As shown in Figure 4, in most of the time, the multiplication of bone marrow B cell mature antigen was larger than that of blood B cell mature antigen. Only a few times do they get the

opposite result. The multiplication of BCMA in blood was different from that in bone marrow. This is due to a specific physiological or immune response triggered during treatment, resulting in inconsistent changes in BCMA in the blood and bone marrow. It also suggests that there are some unique biological changes at a particular point in time.

4.4 Discussion

After CAR-T treatment, the three indexes of patients showed a significant decrease trend. In this process, the recovery time of white blood cells and hemoglobin is relatively similar, while the recovery time of platelets is longer. It is worth noting that although the changes in platelets are relatively obvious, they are still within the acceptable range. In contrast, the changes in white blood cells and hemoglobin were not significant, which further confirmed that BCMA is mainly expressed in plasma cells, rather than in normal tissues and organs of the human body. At the end of treatment, bone marrow plasma cell levels were maintained in most patients, while flow cytometry showed that the number of abnormal plasma cells continued to decrease.

The discovery of BCMA has indeed brought a revolutionary breakthrough in the field of immunotherapy for anti-myeloma treatment [14-15]. Immunotherapy fights disease by activating or enhancing the patient's own immune system [16-17]. Through precise treatment, the patient's own immune cells are effectively mobilized to fight against multiple myeloma cells. Target selection and optimization is an important part of CAR-T CT [18-19]. CD19 is a highly expressed antigen in B-cell acute lymphoblastic leukemia and one of the important targets. Therefore, before developing CAR-T products, patients should be strictly screened and judged, pathogen characteristics should be mastered, biological characteristics of targets should be deeply explored, and more reliable treatment methods should be provided for patients [20]. It is prepared by taking T cells from patients and then genetically modifying and expanding the cells. Each step requires precise control to ensure the activity, specificity and persistence of CAR-T cells. In the process of genetic modification, an advanced viral vector system is used to efficiently integrate CAR genes into the T cell genome. The growth curve, activity and purity of the cells were monitored during the phase of cell expansion. In addition to addressing these issues, there are limitations to consider: drug resistance, recurrence problems, adverse reactions. The development of drug resistance is related to the heterogeneity of tumor cells. Cytokine release syndrome is a severe systemic inflammatory response, accompanied by high fever, low blood pressure, and multiple organ failure.

5. Conclusion

CAR-T cell immunotherapy is an effective treatment option for patients with myeloma and lung cancer, even in patients with recurrence and refractory treatment. BCMA CAR-T cell immunotherapy is well tolerated in myeloma patients, and the response of most patients is maintained in a low controllable range. Due to the small number of included cases, short follow-up time, and only BCMA CAR-T cell results investigated in this paper, intuitive and strong comparison with other treatment methods cannot be made. The bias of patient follow-up and data retrieval will have some influence on the results of the study. Therefore, it is necessary to collect more survey data for comparative analysis and demonstration analysis, and the data fidelity should be realized by timely return visit and update.

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