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Current Status and Challenges of CAR-T Cell Therapy in the Treatment of Hematological Malignancies

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Abstract. In the last few decades, T-cell therapy has received wide attention. The previous articles discussed its clinical application a lot, but rarely connected its mechanism with the limitation. More specifically, it has made stage achievements in the medical intervention of hematological malignancies in terms of improving the five-year survival rate and reducing the probability of recurrence. The conclusion generated from in-depth analyzed the article and summarized the connections among different viewpoints. The aim of this article is systematically and concisely explaining the mechanism of chimeric antigen receptor T-cell (CAR-T) therapy, explain its clinical application and effect and the current limitations. In the research, it is found that the limitations come from the basic mechanism of recognition. This is also why CAR-T cell therapeutic effect shows differences between solid tumors and hematological malignancies. This is also why his treatment effect will be affected by different types. In summary, this study is pointed out that the development focus of CAR-T in the future will shift from simply improving the killing ability to improving durability, reducing toxicity, overcoming immune escape, and achieving more accurate and individualized treatment.

Keywords: CAR-T cell therapy, hematological malignancies, clinical application

1. Introduction

Hematological malignancies constitute an important burden of cancer diseases around the world, among which non-Hodgkin's lymphoma and leukemia have a high number of new cases and a burden of death. There are about 1.31 million new cases of malignant tumors in the blood system worldwide every year. However, although traditional treatment does make the survival of patients with hematological malignancies longer and longer, there is still a huge survival gap between different blood tumors due to different ages, causes and treatment methods. Moreover, the same type of hematological malignancies will also have different five-year survival rate due to different subtypes. Therefore, it is necessary to study new therapies is to improve the prognosis of patients with recurrent/treatment-resistant hematological malignancies after the failure of traditional treatment. CAR-T therapy, antibody–drug conjugates (ADCs), and new targeted drugs have begun to be studied. For CAR-T cell therapy, compared with other types of immune cells, T cells exhibit potent cytotoxicity, robust proliferative capacity, and long-term persistence, while also possessing the ability to form immunological memory. Therefore, one key challenge is how to solve the

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problem of t-cell recognition to fight the immune escape of tumors. CAR through extracellular antigen-binding domain, allowing T cells to identify specific surface antigens tumor cells directly. After antigen binding, receptor clustering occurs on CAR-T cell membrane. The intracellular CD35 domain harbours multiple immunoreceptor tyrosine-based activation motifs (ITAMs). Upon cross-linking, ITAMs undergo tyrosine phosphorylation, recruiting downstream signalling kinases, triggering a cascade of intracellular signalling events. These signals boost the activation of T-cell. However, through the mechanism, CAR-T cell therapy contains limitations in several aspects. The most classic is that CAR-T needs to rely on specific tumor surface antigens. But tumor cells are highly heterogeneous and plastic. Under the pressure of treatment, some tumor cells may reduce/lose the target antigen, resulting in the inability of CAR-T to recognize and tumor recurrence. CAR-T cell therapy will appear antigen-negative relapse. Additionally, antigens need to stimulate CAR-T cell for a long time after entering the tumor which is one of the causes of T-cell exhaustion. Therefore, the proliferation ability and cytotoxicity of T cells will decrease. This is also why many studies are now concentrating on CAR-T cell state and metabolic reprogramming. In the future, the direction of research will also be biased towards to find ways to enhance the therapeutic outcome of solid- tumour-targeted CAR-T cell therapy, reduce the corresponding side effects and improve the mechanism to achieve the better prognosis effect.

2. Main body

2.1. The basic mechanism of CAR-T cell therapy

The CAR- T cell treatment mainly has two major mechanisms about T cell signalling, that is, core recognition mechanism and intracellular signal transduction mechanism. The core recognition mechanism is based on CAR, which are fusion proteins recombinantly engineered, expressed on the membrane of genetically modified T lymphocytes. From a structural perspective, the extracellular region comprises a single chain variable fragment(scFv) obtained from monoclonal antibodies to achieve more accurate and specific recognition of tumour associated antigens presented on the surface of cells. CAR structure also has the hinge and transmembrane domain which provides flexibility and stability, enabling efficient interaction of the scFv and its corresponding target antigen whilst securing the receptor to the T-cell membrane. Moreover, the intracellular region may contain co-stimulatory signaling motifs, including CD28 or 4-1BB, that augment T-cell activation, expansion, durability and overall anti-tumour efficacy. Unlike endogenous T-cell receptors that is strictly governed by major histocompatibility complex (MHC) presentation, CAR-mediated antigen recognition functions in anMHC independently. Such a peculiar mechanism enables the genetically engineered T cells to avoid common tumour immune escape mechanisms due to MHC down-regulation, whereby it extended the range of tumour targeting and enhancing the reliability of tumour cell detection [1, 2]. Furthermore, the intracellular signal transduction pathway will be activated after the antigen-scFv binding leads to clustering of CAR complex at the plasma membrane of CAR-T cell. The cytoplasmic domain of CD3-ζincludes multiple ITAMs. When receptors cluster, these ITAMs get phosphorylated on tyrosine residues to attract downstream signalling kinases and start the subsequent intracellular signalling cascades. These co-stimulatory domains also enhance these signals as well as induce metabolic changes and enduring T cell activation. These events eventually lead to T cell expansion, secretion of cytokine and expression of cytotoxic molecules effector functions leading to the recognition and destruction of malignant cells displaying the cognate antigen [2]. Thus, the coupling of antigen specific recognition with

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intracellular signal activation allows CAR-T cell to translate tumour antigen binding in an orchestrated and powerful cellular immune response.

2.2. Current clinical application in hematological malignancies

It is now recognised that CAR-T cell therapy represents an innovative treatment modality for hematological malignancies, which showed the greatest success so far in B-cell leukemias/lymphomas, such as B-cell acute lymphoblastic leukemia (B-ALL) and B-cell non-Hodgkin lymphoma (B-NHL). Therefore, CAR-T has become a key therapeutic option for some of the patients with hard-to-treat diseases, especially when traditional therapy is not effective enough [3-5]. Genetically modified to express a synthetic CAR, CAR-T cells are a subset of T lymphocytes [4, 5]. With this mechanism, CAR-Ts are capable of direct recognition and elimination of malignant cells that display the respective target antigen. Among all of these established CAR-T target, CD19 is the most frequently utilized antigen against B-cell malignancy [3, 4]. CD19 directed CAR-T cells has shown robust clinic activity against B-cell malignancies, particularly B-ALL and B-NHL [3-5].

In addition, proper target selection is an important factor in CAR-based immunotherapy, and B-lineage-specific antigens have yielded encouraging clinical outcomes, highlighting the importance of selecting suitable antigens for CAR therapies [6]. The clinical relevance of the latter approach becomes apparent especially for patients suffering from R/R disease, who may not benefit from further disease control with conventional chemotherapy or other standard therapy [3-5]. Thus, CD19 CAR-T treatment significantly altered the therapeutic scenario in selected patients with B-cell leukemia and lymphoma. But, the widespread expression of CD19 in normal B cells also implies that therapy results in B-cell aplasia and hypogammaglobulinaemia, which could lead to higher incidence of infections after CAR-T treatment [7]. Therefore, while CD19 is a favourable target for therapy, its expression in normal B cells also highlights a difficulty for selective tumour targeting. The clinical utilisation of CAR-T therapy has been further extended from CD19 positive B cell malignancy with the establishment of other tumor associated targets. The B cell maturation antigen (BCMA) is one of them, especially for the therapy of multiple myeloma (MM) [3, 4]. The BCMA targeted CAR-T cell therapies showed encouraging clinical activity in patients with heavily pretreated and/or RR MM, thus extending application of CAR-T strategies toward plasma cell neoplasia [3, 4]. Moreover, development of CD19- and BCMA-targeted CAR-T agents illustrate the need for choosing tumor associated antigen with sufficient expression in malignant cells, which is accessible to targeting via modified T-cells [3, 4]. The significance of target selection is also supported by research focusing on distinct antigenic targets, with modified CAR constructs being further refined to recognise and eliminate tumour cells with enhanced potency [6]. All these developments made CAR-T a very relevant tool in the contemporary armamentarium to treat some types of hematologic cancers Despite these major advances in the clinic, it remains challenging to diversify the implementation of CAR-T treatment to other blood malignancies. AML, for example is hard to address using CAR-T cells because there is a lack of really tumour specific antigens. Many putative AML-specific targets are also presented by non-malignant haematopoietic cells, thus increasing the probability for on-target, off-tumour toxicity and damage of normal tissue [4, 7, 8]. Similarly, controlling T-cell malignancies such as T-cell acute lymphoblastic leukemia (T-ALL), is particularly challenging, since malignant T cells can have the same surface antigens that are shared by healthy T cells, targeting of those antigens may lead to fratricide whereby CAR-T cells kill off other T-cells that also express the targeted antigen [7, 8]. The efficacy, target selection and CAR-T-treatment-related safety in AML or T-cell malignancies have not yet reached the maturity level in B-cell malignancies. However, clinical studies continue expanding the spectrum of hematological

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malignancies amenable for CAR-T therapy. Molecular/cellular engineered strategies are under investigation aiming at identifying additional appropriate targets, improve activity of CAR-T cell, and diminish treatment related toxicity [4, 7, 8]. Novel approaches including the generation of CAR-T cells in vivo rather than ex vivo are also under investigation. One recent study employs targeted lipid nanoparticles (LNPs), which can be employed to transport mRNA coding for a CAR directly into circulating T cells in vivo, thus transiently reprogramming T cells to functional CAR-T cells [9]. These methods could possibly make the manufacturing procedure simpler, as well as give us a more flexible way of performing CAR-T therapy. In general, current clinic evidences indicate that the best result of CAR-T treatment is in B-cell hematologic malignancies and MM, while its use in AML, T-cell malignancies, and other disease is a major focus for future research efforts [5, 9].

2.3. Major clinical challenges of CAR-T therapy and strategies to overcome existing challenges

In spite of the impressive clinical response rate achieved with CAR-T cells against a few haematologic malignancies, their use has been hampered due to toxicity associated with the treatment, drug resistance, production intricacy and challenges to target non-B cell tumors [5]. Such problems are now being considered as a major factor for further development and clinical application of CAR-T treatment. Thus, current studies have been focusing more on increasing the safety, persistence, specificity alongside functional activity of CAR-T cells by advancing cell and molecular engineering. Furthermore, CAR-T cells may have limited persistence in the body and can develop T-cell fatigue and exhaustion, which may make tumour elimination more difficult and contribute to repeated recurrence [10]. One key challenge is that potentially serious, even life-threatening toxicities can arise after CAR-T cell infusion. The side effects are correlated with CAR-T therapy include Cytokine Release Syndrome (CRS)and Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) [3, 7]. Other toxicities have also been recognised clinically in practice such as Immune Effector Cell-Associated Hematotoxicity (ICAHT) and immune effector cell associated haemophagocytic lymphohistiocytosis like syndrome (IEC-HS) [7]. Prolonged B-cell depletion and hypogammaglobulinaemia can also make one susceptible to infection, contributing to an additional clinical burden during treatment and follow-up [7]. Despite the improvement on supportive care and use of immunosuppressive therapy, making the CAR-T treatment safer and more feasible, there has not yet been a universal algorithm to treat all the CAR-T-related toxicities [7], therefore, ongoing prospective assessment and developing less toxic CAR-T product are also crucial approaches in enhancing the treatment's safety [7].

An alternative important drawback is the emergence of resistance and recurrence of disease following a transient response to treatment. Some possible mechanisms have been suggested, including tumor antigen evasion, low CAR-T cell longevity, and interaction with an immunoinhibitory tumour microenvironment [8]. Restricted CAR-T cell persistence and T-cell fatigue or exhaustion may subsequently reduce the ability of CAR-T cells to eradicate tumour cells effectively [10]. Antigen escape is a particular problem since the CAR-T cell depends upon the recognition of certain tumor-associated antigen. As a result, the loss or alteration of target antigens may impair the capacity of CAR-T cells to recognise and kill cancer cell [3, 8]. As a countermeasure, researchers are discovering other molecular targets and devising new engineering strategies for boosting CAR-T cell therapeutic performance and reducing chances for treatment resistance or recurrence [3, 8]. Similarly, modifying the CAR protein and T cells, as well as fine-tuning the crosstalk between T cells and additional constituents of the tumour microenvironment, are being explored to improve CAR-T cell therapy [10]. These methods try to avoid the failure of treatment caused by the loss of single target antigen and augment the efficacy of

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CAR-T cells and reduce the probability of drug resistance and recurrence. Furthermore, the application of immune checkpoint modulation and the CAR-T-cell-based combination therapy with immune checkpoint inhibitors (ICIs) have also been investigated as potentially more effective strategies for haematological malignancies, with both preclinical and clinical studies being reported [11]. Combination approaches using small-molecule compounds, including protein kinase inhibitors, demethylating drugs and HDAC inhibitors, have also shown promising potential for improving CAR-T cell therapy in both pre-clinical models and clinical trials [12]. For example, by inhibiting excessive kinase signaling, protein kinase inhibitors alleviate CAR-T's functional failure (T-cell exhaustion) and lack of persistence which can help CAR-T cell to maintain a remarkable state. Therefore, the CAR-T durability and anti-tumor activity can be improved.

The choice between appropriate tumour antigens is another major issue for malignancies like AML or T-cell malignancies, although the application of CAR-T therapy showed especially good results with regard to B-cell malignancies, its extension for the treatment of other haematological pathologies appears harder [4, 5, 13]. For such diseases, one important issue consists in finding antigens able to discriminate tumour cells with sufficient precision without excessively affecting normal cells [7, 8, 13]. The importance of appropriate target selection is also highlighted by the clinical success of B-lineage-specific antigens, which has emphasized the need to identify suitable antigens for other CAR therapies [6]. Thus, molecular engineering approaches are under investigation to find other targets as well as to improve the specificity and efficacy of CAR-T cells for disease conditions other than current well-established uses for B-cells [8, 13].

Last but not least is that the traditional manufacture procedure itself has some limitations. The ex vivo CAR-T therapy usually needs to be manufactured individually for every patient, which can complicate therapy, and limit its accessibility and versatility [9]. A recent study developed an alternative approach using targeted LNPs delivering mRNA coding for a CD22 CAR directly into circulating T cells, enabling them to be transiently reprogrammed into functional CAR-T cells in vivo [9]. In the humanized Nalm6 tumor bearing mouse model, this approach inhibited tumour growth suggesting that the ex vivo manufacture of CAR-T cell therapy may be replaced by an in vivo approach [9]. In summary, overcoming toxicity, antigen escape, low CAR-T persistence, tumor microenvironment-associated dysfunction, and manufacturing complexity will be key to improve the safety, longevity, and wider scope for the use of CAR-T treatment [9, 13].

3. Conclusion

In conclusion, A range of haematological malignancies, namely B-cell acute lymphoblastic leukaemia, multiple myeloma and B-cell non-Hodgkin's lymphoma, have gradually been receiving with CAR-T cell therapy. It can recognise tumour-associated antigens autonomously of MHC presentation to directly engage and eradicate malignant cells, and co-stimulatory signals help to stimulate, expand and maintain the survival of T cells. CD19 and BCMA are now targets for clinical application, and and following the failure of alternative therapies, CAR-T cell therapy has begun to be employed to treat patients with relapsed or refractory disease.

Nevertheless, the current application of CAR-T therapy still has some serious problems. Treatment-related toxicities, such as cytokine release syndrome and ICANS, infections due to persistent B-cell depletion and hypogammaglobulinemia, etc., are still serious safety problems. Treatment resistance and disease recurrence maybe caused by interaction with the immune-inhibitory microenvironment of tumour, antigen escape, restricted CAR-T cell persistence and T-cell exhaustion. Another problem with CAR-T therapy for AML and T-cell malignancies is that suitable

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tumour-specific antigens have not yet been identified. In addition, the individualized ex vivo manufacturing process may be more complex to implement and less accessible.

Therefore, the future developments will focus on optimising the treatment-related risk profiles, therapeutic potency, specificity and accessibility of CAR-T cell therapy. Molecular engineering, combination therapy with small-molecule drugs or immune checkpoint blockade, and the discovery of new cancer targets are also being investigated to boost the effect of treatment and overcome resistance. Development of targeted mRNA-LNP technology for in vivo CAR-T cell generation has also provided a route to avoid the ex vivo manufacturing process and may simplify treatment. Although CAR-T therapy has demonstrated some encouraging clinical results for specific blood cancers so far, many problems still need to be solved and more research is required before it can be widely used.

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