

CAR-T Therapy in Diffuse Large B-Cell Lymphoma

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Abstract. CAR-T is a genetically engineered autologous T cell as a "live drug" targeting CD19. As a new tumor treatment method in recent years, CAR-T has been quite effective in DLBCL treatment. In the process of tumor treatment, the tumor appeared immune escape, so modern scientists modified T cells into CART through experiments. The common targets of CAR-T are CD19, BCMA, etc., and the appearance of targets has been reduced to a certain extent. The emergence of immune escape, and the application of dual-target CAR-T further mitigated this problem. However, when CAR-T therapy is implemented in vivo, it is affected by the tumor micro environment, which inhibits its treatment effect. This article discusses the role and positive influence of multi-target cart in the treatment of NHL discuss and explore the negative influence of its efficacy in the micro environment.

Keywords: Chimeric antigen receptor (CAR)-T cell; diffuse large B-cell lymphoma (DLBCL) ; immunotherapy.

1. Introduction

As the highest frequency of non-Hodgkin lymphoma (NHL), the Diffuse large B-cell lymphoma (DLBCL) accounts for about 30% to 40% in NHL [1]. Symptoms of lymphoma include both systemic and local symptoms. Systemic symptoms include weight loss, fever without explanation, fatigue, itchy skin and night sweats. Local symptoms vary depending on the primary and affected site of the lesion, lymphoma can be primary in the body Any organ or tissue of the body, usually divided into primary lymph nodes and lymph nodes. It is a tumor with a very aggressive clinical course and significant morphological heterogeneity. As an immune therapeutic strategy, chimeric antigen receptor (CAR)-T cell therapy is currently effective method for NHL. Genetically engineered autologous T cells act as targets for "living drugs." In clinical terms, CAR T therapy has shown good results, by introducing artificially designed CAR molecules through T cells, and making T cells bind to specific antigens on the surface for treatment. CAR-T therapy in recent years in NHL to promote disease remission rate of 59% to 82% [2], at the same time, CAR-T also brings the current problem that still needs to be overcome, cytokines comprehensive release sign CRS. After CAR T is injected, a large number of cytokines are activated to cause inflammation. The adverse reactions of CRS are divided into six levels in clinical practice. Although there are ways to inhibit and alleviate them, they still pose a risk to life. This article will focus on the application and challenges of CAR-T in NHL.

2. The Structure of CAR

CAR is a modified receptor molecule that gives cytotoxic T lymphocytes (CTL) specificity for target antigens, boosting their ability to kill cancer cells. Therefore, the design of CAR molecules has become a key link in CAR T therapy. The CAR is divided in four parts including the antigen binding domain and spacer domain outside of cell, transmembrane domain, and signal domain inside of cell.

The antigen-binding domain forms a specific single strand fragment variable (scFv) through connection by a flexible splice. The scFv specified targets tumor antigens as the basis for CAR T treatment in effectiveness and safety. Tumor-associated antigen (TAA) is a major target of immunotherapy and is highly expressed on the surface of tumor cells and at lower levels in healthy tissues. Therefore, the specificity of scFv for CAR molecular design is critical to target tumor cells without life-threatening toxicity to health tissues. Multiple receptor-ligand interactions fundamentally

determine the function of CAR molecules. In order to recognize antigens specifically on tumor cells and activate T cells, the moderate affinity of CAR molecules is necessary. The affinity should be enough to induce T cell activation and killing effect on tumor cells, but it should not be so high as to cause toxicity to tissues with low expression of antigens or kill normal cells caused by overactivation [3].

The spacer domain is the extracellular structure of CAR extending from the binding domain to the transmembrane domain, which is capable for providing flexibility to overcome spatial barriers and contribute to length to bind targeting epitopes on tumor cells. That means the length and composition of spacer domain can affect flexibility, signalling, and ultimately the function of the CAR molecule. Therefore, a correct understanding of the extracellular spacer domain is also necessary to effectively target tumor antigen and make effects better of CAR T therapy.

Transmembrane domains, anchoring the CAR to T cell membrane, which is probably the least variability. These domains influence the expression levels, stability, signaling transduction and synaptic formation of CAR. It is noteworthy that CD3 ζ transmembrane promotes CAR-mediated T cell activation because of mediating the signal complex formation of CAR dimerization and endogenous T cell receptor (TCR) [4]. The appropriate CAR T cell signaling optimally promoted through linking of intracellular domain to transmembrane domain, for example, using of the commonly CD28 transmembrane domain enhances CAR expression and stability.

Intracellular signaling domains, which are the functional ends of receptors, send signals to T cells and initiate signaling cascades to kill the tumor cells. The TCR is closely related to the CD3 dimer in the cell, which carries an immunoreceptor tyrosine-based activation motif (ITAM), which activates the T cell signal after phosphorylation through the lymphocyt-specific protein tyrosine kinase (LCK) [5].

3. Molecular Design of CAR T-cells

3.1. The Five Generations of CAR

The fifth generation of the CAR has been developed until now because of updating the structure constantly. The first generation of CAR recognizes antigens through an extracellular domain and transduces intracellular signal using a single motif without co-stimulatory molecules, making it unstable in patients and invalid against tumors. As the most widely used CAR in clinical practice, a costimulatory receptor signaling domain, such as 4-1BB, CD28, and OX40, is added into the intracellular signaling domains in the second-generation CAR, which can continue to proliferate and release cytokines to play an anti-tumor role [6]. The third generation CAR designs two co-stimulatory molecules inside of cells to further enhance the killing ability of CAR T cells. The fourth and fifth generations are also improved on the basis of the second generation, including a cytokine transgene expression cassette to mediate pro-inflammatory cytokine secretion or cytokine receptors domain to promote T cell survival and proliferation.

In comparison of the first generation of CAR, the second generation of CAR increased T cells response to activation signals and showed surprising therapeutic effects in clinical treatment. Initially, these CAR T drugs, like Kymriah and Yescarta, were used to relapsed B-cell acute lymphoblastic leukemia (B-ALL) with more than 90% full recovery. Indeed, the CARs from third to fifth generations are currently rare in clinical use because it need to study for a long time about their safety and efficacy (Figure 1).

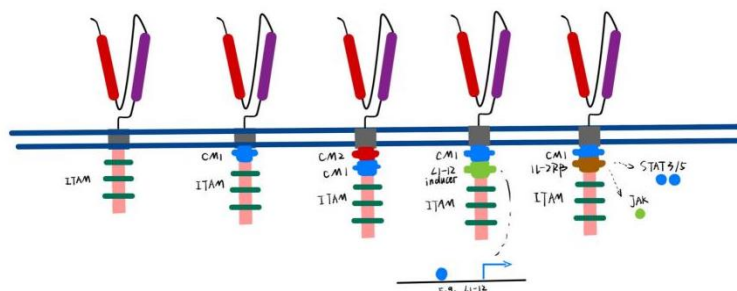


Fig. 1 The CAR structure of five generations

Although all CARs in clinical application effectively promoted the anti-cancer response of T cells, they also had limitations, these restrictions have paved the way for a new generation of CARs that continue to emerge.

3.2. Dual and Three-target CAR

Dual CAR means co-express two independent CAR structures and transmit two independent signals in one single T cell using co-transfection and other methods, which prevent the tumor from escaping in the condition of the tumor target antigen mutation due to tumor heterogeneity. The combination of multi-antigen targeting strategy may counteract potential antigen escape mechanisms because targeting a single specific tumor antigen leads to lower effects with continuing treatment. In contrast to single-target CARs or combined two single-target CARs, dual CARs are more effective in preventing antigen escape and enhancing anti-tumor effects [7]. As the two antigens are co-present, dual CAR T cells exhibit stronger downstream signal transduction. For example, the dual CAR T targeting both CD20 or CD19 and CD3 may improve the outcomes [8]. Notably, although dual CAR can provide more stronger immune pressure on tumor cells, which may also lead to both antigens escape simultaneously. In addition, dual CAR can activate T cells more easily, whereas it is also prone to cause cytokine storms and overkill normal cells with low expression of target antigens. Therefore, it is needed to optimize in structural design and function, effectively performing tumor killing and ensuring safety at the same time. How to combine the two targets of CAR T is also an aspect that scientists need to study in future. Similarly, the three-target CAR T cells enhance T cell activation signaling, extensive tumor antigen coverage, and robust immune synapse formation, but the same shortcomings as dual CAR T are needed to consider. Furthermore, the technical requirements of three CARs are much higher to design a more reasonable gating system, optimizing to recognize precisely tumor and healthy cells [7].

4. Clinical Application of CAR T in DLBCL

A lot of data and experiments have proved the feasibility and research of CAR-T. For NHL, the remission rate is still in the 60 to 100 percent range using CAR-T, even though patients have different metrics, which will last a long time in the patient's body to help recovery. Of the 75 patients in Tisgenleucel's study, the overall response rate was 81 percent, 95 percent had some degree of adverse reaction, like CRS [4]. The lower side effects will become an optimistic candidate with the development of CAR-T. But there's no denying that CAR-T has greatly mitigated the NHL.

5. Advantages and Challenges of CAR T-cells Therapy

As immunotherapy of restarting T cell to kill tumor cells, what are the advantages of CAR T cells over immune checkpoint inhibitors (ICIs)? Tumors with high antigen presenting function display an excellent therapeutic effect using ICIs, while cancer cells with low antigen presenting function are easily ignored by endogenous T cells. It is difficult to achieve ideal therapeutic effect on cancer cells with tumors of low antigen presenting function or few lymphocytes infiltrating using ICIs alone. The

combined ICIs and CAR T cell therapy may improve the killing effect of CAR T cells because CAR T cell therapy is hardly affected by antigen presentation function of tumor cells [9].

The reason for failure of CAR T treatment is that immunosuppressive effect of tumor microenvironment (TME) through the upregulation of T-cell surface suppressor receptors. In addition, the influence of MDSCs (Myeloid-derived suppressor cells) on CAR T therapy in TME also need to be considered. MDSCs not only inhibit T cell mediated adaptive immunity, but also regulate innate immunity, decreasing curative effect. The number of MDSCs is significantly increased in patients with DLBCL and can be used as a prognostic indicator after CAR T treatment [5]. Tregs can inhibit T cell activity through release of cytokines or cell-cell contact, which mechanism is not fully understood because Tregs cannot be selectively eliminated. The Tregs selective inhibitors and gene elimination may improve the efficacy [9-10].

It has been found that tumor cells can affect the biological activity and alter the phenotype of macrophages, promoting tumor malignancy. TAMs can promote the matrix remodeling and tumor angiogenesis, accelerating tumor proliferation and metastasis, which can also aid tumor immune escape through the SIP- α /CD47 pathway. Vari et al. found that highly expressed PD-L1 on TAMs participated in directly inhibiting the killing function of PD-1+NK cells in vitro co-culture experiment, demonstrating TAMs-mediated immune escape strategy of DLBCL. Consequently, CAR-T immunity therapy Greatly improved the treatment rate of NHL [11].

6. Summary

Although DLBCL patients have an excellent prognosis with CAR treatment, current therapies need to reduce both acute and long-term toxicities, such as a high incidence of infection, hematologic toxicities, and mucositis. Enhancing CAR-T chemotaxis, migration and infiltration into tumor tissue will improve CAR-T homing and make it effectively reach the deep tumor. In addition to the improvement of CAR-T therapy itself, its application prospects include the combination of drugs, inhibitors related to various cytokines and ICIs to achieve the best therapeutic effect, as well as the lowest recurrence rate and the lowest toxic manifestations. For the future development direction of multi-target CAR-T, finding specific targets and introducing suicide genes are ways to improve their effects and safety.

Author Contribution

All the authors contributed equally, and their names were listed in alphabetical order.

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