CAR-T Cell Therapy Targeted Non-Hodgkin's Lymphoma: Clinical Development and Challenge

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Abstract. Non-Hodgkin's lymphoma incidence has been rising worldwide in last few years. And the dismal prognosis following treatment to cure relapsed or resistant T-cell lymphoma and B-cell malignancies. Today, this therapy has already achieved incredible results with the field of non-Hodgkin's lymphoma. It becomes a new pillar in the treatment of cancer and shows great promise. Recently, there have been a number of new clinical studies and technology discoveries that have led to significant clinical responses to this therapy in certain subpopulations of Non-Hodgkin's Lymphoma. However, there are still many structural and functional challenges that limit the therapeutic efficacy of this therapy. Therefore, new strategies and ideas are needed to overcome the current challenges. The most recent developments and clinical trial findings on this therapy's effectiveness in treating NHL are covered in this review, as well as discussing the limitations of this therapy and the current solutions and strategies to address them.

Keywords: CAR-T cell therapy, Non-Hodgkin's Lymphoma, Development, Challenge.

1. Introduction

Non-Hodgkin's Lymphoma is a diverse lymphoid malignancy. They are classified as B-cell, T-cell, or Null-cell due to the WHO classification. The incidence of NHL has steadily increased globally in recent years. Especially for certain subtypes, with B-NHL being more common, accounting for about 80-85% of all NHL cases, and T-NHL accounting for about 10-15% [1]. The occurrence of NHL is a multistep process in which various risk considerations contribute. These include genetic abnormalities, immune system dysregulation, infectious diseases (e.g., Epstein-Barr virus and HCV), medications, autoimmune diseases, and certain lifestyle factors (e.g., smoking and obesity) [1]. There are notable differences in the clinical outcome of NHL between adolescents and adults [2]. Moreover, projected demographic changes suggest a significant increase in new cases of NHL globally. Therefore, in order to treat NHL, advanced and innovative strategies are still needed to design more powerful CAR-T cell therapy.

While for decades the only way to treat cancer was to remove the tumor, the emergence of this therapy has revolutionized the former treatment of NHL by producing an effective and long-lasting clinical response during treatment. In recent years, this therapy has attracted attention because of its remarkable breakthroughs. For example, CRISPR-Cas9 technology, CD 19 as well as CD 22 targeted CAR-T cell therapy are prospective during treating B-NHL. Anti-CD4 CARTs, anti-CD5 CARTs and some other therapies show significant results in treating TCL. However, at present, this therapy still faces many challenges during treatment [3].

This review discusses the latest innovations of this therapy in the treating non-Hodgkin lymphoma family of tumors, as well as the limitations and possible solutions.

2. Background

The main components compromise four main parts: the extracellular antigen recognition structural domains, the hinge regions, the transmembrane structural domains, and the intracellular signaling structural domains [3].

The Extracellular Antigen Recognition Domain is responsible for recognizing and binding specific antigens located on the surface of targeted cells. It's usually made using a single-chain varying segment from the antibody or other antigen-binding protein [3].

The hinge region can connect extracellular antigen recognition domain to transmembrane domain. Hinge regions are commonly used to derive from the amino acid sequences [3]. The choice of hinge region influences the persistency of these CAR-T cells and their interactions to Fc γ receptor.

The transmembrane structural domain can anchor the CAR to the T cells membrane. It acts as a key character in CAR-T cell, including CAR expression levels, stability, signaling, the formation of synapse, and dimerization with endogenous signaling molecules. These transmembrane structural regions are usually obtained from native proteins such as CD3 ζ, CD4, CD8 α or CD28 [3].

Intracellular signaling domains are responsible for transmitting activation signals to T cells upon antigen recognition. The most commonly used signaling domain is derived from CD3 ζ. It includes an immunoreceptor tyrosine-based activation motif (ITAM) [3]. 2nd generation CARs contain one or multiple costimulatory structural domains linked to the CD3 ζ structural domain to enhance T cell activation and persistence [3].

This therapy is an immunotherapy that can genetically modify immune cells in order to express CARs, which enable these cells to recognize tumor-associated antigens and thus specifically target cancer cells. The process of this therapy involves few phases. Primary, T-cells can be obtained from either patients or a donors' blood sample. They are genetically modified by using viral vectors to express CAR, and then the CAR-engineered immune cells are cultivated in the laboratory until the desired cell count is achieved [4]. Eventually, these cells are implanted back to the patient's body to combat the cancerous cells [4]. Before the infusion, lymphatic clearance is usually performed to improve cell inoculation. To date, several kinds of this therapy have been registered by regulatory authorities such as the US Food and FDA and the EC [5]. More than 500 clinical trials around the world are currently investigating the benefits of this therapy in cancer [5].

These cells have been designed so that they can recognize specific antigens as well as active T cells activation and achieve antitumor activity. The selection of each component has an impact on the function of this cell, persistence, cytokine production, and tumor infiltration. Numerous studies aim to optimize the construction of these cells, enhancing their efficacy and minimizing toxicity.

3. Current CAR-T therapy for NHL

3.1. B-NHL

This therapy has demonstrated encouraging conclusions in the management of B-NHL. There is a recent paper investigated this therapy in patients with TP53 alterations, which are associated with inferior prognosis in refractory/relapse B-NHL [6]. The patients underwent treatment with a CAR T-cell blend that targeted both CD19 and CD22. This treatment was administered either alone or together with ASCT [6]. TP53 mutations were detected in 123 of the patients enrolled, at the same time they were confirmed in 60. The findings indicate this therapy alone led to ORR of 87.1% and CRR of 45.2% in patients with TP53 alterations [6]. The average time to overall PFS was 14.8 months, whilst OS after 24 months was 56.3% [6]. Similar ORR, PFS and OS were also observed in people who had TP53 alterations as well as patients without TP53 alterations. When this therapy was combined with ASCT, increased ORR, CRR, PFS, and OS were achieved [6]. The top ORR and CRR rates achieved in these individuals were 92.9% and 82.1%, correspondingly. Patients with TP53 mutations had 24 months PFS and OS rates approximating to 77.5% and 89.3%, respectively [6]. The combined approach additionally projected an enhanced overall survival rate through multivariate analysis. CAR-T immunotherapy exhibits efficacy in managing resistant/recurrent aggressive B-NHL carrying TP53 mutations. Moreover, the long-term prognosis of these patients can be further upgraded by introducing ASCT.

Through a meta-analysis concluded 14 studies and 405 patients diagnosed with any r/r B-NHL until July 2022. The efficacy and security of CAR-T therapies with CD22 and CD19 were evaluated

for the first time in r/r B-NHL [7]. This study demonstrated encouraging outcomes for this therapy in treating NHL. The efficacy of this treatment for those with r/r-B-NHL was assessed by the meta-analysis [7]. The results showed that the OR and CR were high in NHL patients. The one-year OS was 70% and PFS was 49%, respectively [7]. In NHL patients, the OR rate was 85% and the CR rate was 57% [7]. The meta-analysis also found that this therapy had a tolerable safety profile. Furthermore, the study suggested that this therapy is much more effective compared to single-targeting CAR-T cell therapies. This treatment also has reasonable safety potential in treating r/r B-NHL [7]. It is important to note, however, that these results are on the basic outcomes of the meta-analysis and the responses of individual patients may be different [7]. This meta-analysis demonstrated that this therapy was highly efficient in people with B cell malignancies with tolerable adverse effects.

A recent clinical trial was conducted for the assessment of the non-viral method of treating r/r B-NHL using PD-1-integrated CD19 targeting CAR-T cells. This technology is able to effectively limit the poisoning of this therapy and significantly optimize treatment [8]. These cells have been successfully developed using CRISPR-Cas9 technology. This approach eliminates the need for viral vectors during CAR-T cell production, solves problems associated with insertion mutagenesis and viral DNA immunoreactivity [8]. This method is innovative as it combines a non-viral production procedure with the advantages of precise genome editing, which not only simplifies the manufacturing process, but also shortens the preparation time and reduces the production cost [8]. An improved of this cell was developed by combining the anti-CD19 CAR with PD1 [8]. These cells comprise a significant amount of memory T cells and exhibit an exceptional capacity to eliminate tumor cells in xenograft models [8]. Additionally, PD1 disruption enhanced the anti-cancer immune responses of these cells, allowing more effective tumor cell eradication even there is a low PD-L1 expression [8]. In a clinical trial aimed at treating r/r B-NHL, administering these cells yielded a substantial complete remission rate (87.5%) and sustained response in eight patients with no serious adverse events [8]. Significantly, the therapy proved effective with a small infusion dose as well as a limited percentage of CAR+ cells.

This therapy has shown many successes in treating B-NHL and is already in use, but more trials should continue to be conducted to find out some of the existing problems.

3.2. TCL

CAR-T therapy for TCL is an evolving therapeutic approach that offers great promise in treating r/r TCL. This therapy modifies T-cells so that these cells can express a CAR, and his receptor recognizes specific antigens on cancer cells and targets them for treatment. This therapy has shown great results in treating various hematologic malignancies, including TCL. Some clinical trials now are investigating the use of these cells to target different antigens in TCL.

3.2.1. Anti-CD4 CART

CD4 can be presented in T-lymphocytes and also on CD4-positive malignancies. In the case of TCL, preclinical research has suggested CD4 targeted CAR-T cells can be efficient when eliminating lymphoma cells [9]. Nevertheless, these cells could trigger CD4 T cell dysplasia as well, ultimately resulting in an HIV/AIDS-like syndrome [9]. Current clinical trials are researching the use of these cells to treat patients suffering from CD4+ T-cell leukemia/lymphoma [10].

3.2.2. Anti-CD5 CARTs

CD5 can express in both normal T lymphocytes and T cell malignancies. Preclinical research has shown that CD5-targeted cells are efficacious for TCL [10]. There is a study published by Rayne H. Rouce and his colleagues report a finding that investigated the beneficial outcomes in autologous T-cells when expressing this therapy in treating r/r mature TCL [11]. 14 patients were enrolled, and nine patients were treating with this therapy. The findings demonstrate that CD5.CAR-T cells endured in every patient for 9 months, with a temporary drop in peripheral T-cell numbers [11]. 4 of the 9 patients had a response, permitting 3 to advance in alloHSCT [11]. The findings imply that these cells can

generate anti-tumour responses in individuals with relapsed/refractory TCL, enabling them to move forward with alloHSCT without notable T-cell aplasia.

3.2.3. Anti-CD30 CAR T cells

CD30 can express when activating lymphocyte subsets and is a common surface molecule in TCL. CD30 targeted therapy is currently used to treat a patient with multiple relapses of EATL [12]. This person was participating in a Phase 1b trial and received this therapy. Although the patient developed grade 1 CRS, it resolved on its own without specific treatment [12]. Inflammatory cytokines increased in tandem via the growth by CD30 CAR-T cells. However, following a three-month injection, these cells can no longer be detected in patients' blood. Disease assessment using PET/CT scans showed complete regression of lymphomas at various sites after six weeks of CD30 CAR-T cell therapy [12]. Imaging over the next 24 months showed sustained remission. The patient remained clinically stable more than 30 months after treatment [12]. This case report demonstrates that this therapy can induce long term remission in patients with multiple relapses of EATL and suggests further clinical trials to investigate its potential in this patient population.

A recent study looked into using bispecific CAR-T cells to treat TCL by targeting both CD30 and CD5. These bispecific cells showed better efficacy and specificity against TCL as compared to CAR-T cells with solely one target [9]. Using an LDH assay, the tumor-specific knockdown activities of T-cells. It was been assessed at various effector-to-target ratios in order to evaluate the cytotoxicity of bispecific T cells [9]. An ELISpot assay was also used to assess how to produce IFN-gamma by CAR-T cells [9]. Flow cytometry evaluated how to express target antigens (CD30 as well as CD5) on neoplastic cells and the T cell phenotype. Using a xenograft mouse model, in vivo tests were carried out wherein NCG mice were given injections with Karpas-299 cells then afterwards subjected to transduced CAR-T cell treatment [9]. Compared to other CAR-T designs, this study demonstrated that these cells more successfully prevented the growth of cancer.

The studies aim to discuss the efficacy and sustainability of this therapy responses in treating TCL, and to deliver tailored treatment options for patients suffering from relapsed/refractory TCL.

4. Side Effects and Current challenges

This therapy has showed encouraging findings during the management in NHL. However, certain restrictions still exist:

4.1. Manufacturing complexity

The treatment includes the gathering of T cells from patients, genetic modification of said cells in the laboratory to express CARs and reinfusing the modified cells into the patient. This process is complex as well as time-consuming and requires specialized facilities and expertise [13].

4.2. Cost

It is expensive, largely due to its complex manufacturing process and the personalized nature of the treatment. This therapy requires the CAR-T cells be produced for each patient [13]. The high cost of this therapy has limited its accessibility to a wider population.

4.3. Toxicity

This therapy can cause serious side effects. The severity and incidence of these toxicities vary by CAR design, target antigen, and tumor type. CRS is characterized by the release of inflammatory cytokines and can cause fever, hypotension, and organ dysfunction [13]. Neurotoxicity can manifest as confusion, seizures, and other neurologic symptoms [13]. Efforts are ongoing to enhance comprehension and control of these toxicities, in order to increase the safety of this treatment.

4.4. limited targeted antigens

It refers to the evolution of tumor resistance to the CAR-T cells due to absence or down-regulation of the target antigens on the tumor cells [3]. For instance, in individuals that have r/r ALL who underwent therapy with these cells targeting CD19, a significant proportion of patients have down-regulated or lost expression of the CD19 antigen, leading to disease relapse [3]. Not all cancers express target antigens suitable for this therapy, which limits the applicability of the therapy to some patients.

4.5. Replace and Resistance

Despite initial success, some patients may relapse or develop resistance to this therapy over time. This underlines the continuing need for more researches to improve the effectiveness of this therapy [13].

4.6. Tumor Heterogeneity

Tumors may exhibit heterogeneity, meaning that different cells within a tumor may have different antigen expression profiles [13]. This heterogeneity can make it difficult to target all cancer cells with this therapy [13]. Therefore, careful antigen selection is critical to minimize toxicity and ensure efficacy. Strategies such as optimizing target antigen selection and using regional delivery methods have been explored to alleviate this problem [3].

4.7. Lack of persistence

Over time, CAR-T cells may lose their effectiveness due to exhaustion or clearance by the immune system. Researches are underway to upgrade the durability of these cells to overcome limitations.

4.8. Immunosuppressive tumor microenvironment

The existence within the tumor microenvironment of immune cells and factors, like MDSCs, TAMs, Tregs and the immune checkpoint pathway, inhibit these cells functioning as well as limit the anti-tumor immune responses [3]. Combination therapies, such as combine these cells with immunological checkpoint blockade, are being investigated to overcome immunosuppression and improve the efficacy results obtained with CAR-T cells [3].

Although this therapy has demonstrated noteworthy effectiveness at some point, there remain limitations that require attention. Continued research and advances in this therapy are aimed at addressing these limitations and improving the overall efficiency and safety of this treatment.

5. Future expectation

Although many advances in this therapy have been made, and this therapy have already been used in curing NHL. However, treatment in CAR-T cells is still complicated and difficult to generalize due to high cost, manufacturing logistics, and toxicity.

Natural killer cells have an important role in treating B-NHL, although their contributions have been largely disregarded. [9]. Additionally, natural killer cells share cytotoxic mechanisms with T cells, yet have a more diverse safety profile and is better suited to producing allogeneic, ready-to-use products [13]. CAR-NK cell therapy shows greater hope and has more merits. First, these CAR-NK cells are readily available. They can come from a variety of sources [13]. Simultaneously, CAR-NK cells effectively reduce the chance of GVHD [13]. CAR-NK cells do not pose a serious risk of GVHD because they do not recognize and attack healthy tissues based on HLA matching [13]. CAR-NK cells also provide enhanced safety for the treatment. This therapy is unlikely to cause serious limitations such as CRS and neurotoxicity [13]. Additionally, CAR-NK cell therapy allows for broader target antigen recognition, as NK cells are able to recognize a wide range of target antigens, including those expressed on cancer cells [13].

An article published recently discussed the Intelligent controlled CAR-T technologies refer to the development of strategies and tools to modulate CAR-T cells' activities and functions with precision and control. These technologies aim to conquer limitations and difficulties related to this therapy. One approach is to use the on and off switches, which allow reversible management of CAR-T cell activity [14]. These switches can be activated or deactivated through the adding or removing of particular stimulating materials, such as small molecule drugs or light. [14]. On switches enhance the efficacy of CAR-T cells, while off switches help alleviate CRS and neurotoxicity [14]. Another strategy is the use of synthetic Notch receptors, which enable CAR-T cells to recognize dual antigens [14]. Activation of Syn. Notch receptors induces expression of CARs targeting a secondary antigen, enabling prevention of on-target off-tumor toxicity. Syn. Notch circuits also permit localized synthesis of therapeutic payloads including cytokines, chemokines and antibodies with CARs, thereby enhancing CAR-T cell activity and improving the tumor microenvironment [14]. While tunable CAR-T cell therapy offer potential, additional medical studies are necessary to assess their safety and effectiveness. The future evolution of intelligent controlled CAR-T platforms is expected to address current limitations and enhance the therapy's outcomes.

6. Conclusion

This review examines recent discoveries and approaches to use the CAR-T cells as a pioneering form on immunotherapy in curing NHL. These T cells has been engineered to become chimeric receptor expressors, enabling them to target cancer cells. The paper highlights the remarkable results of this therapy in treating B-NHL and TCL. However, this therapy has adverse effects and limitations, including CRS and neurotoxicity and structural functional deficits. This paper concludes with a discussion of future developments in the CAR-T field, including improving efficacy, expanding the range of cancers treated, and making this therapy more accessible and affordable. For example, CAR-NK cell therapy which can be more widely used and have less serious side effects, the intelligent controlled CAR-T technologies which can effectively overcome many limitations. And moving forward, a comprehensive, well-constructed, and centrally organized systematic review and enhancement of current treatments will greatly enhance the results.

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