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CAR-T therapy in acute lymphoblastic leukemia (ALL): current clinical applications and beyond

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Abstract. Chimeric antigen receptor T-cell (CAR-T) therapy has revolutionized the treatment landscape for acute lymphoblastic leukemia (ALL), particularly in relapsed and refractory (r/r) B-cell ALL. This review explores the efficacy of this therapy targeting CD19 and CD22, addressing the challenges of antigen escape, treatment durability, and manufacturing delays. Innovative strategies, including bispecific CAR-T and FasT CAR-T technology, promise to overcome these limitations by improving production timelines and reducing relapse rates. The review further highlights the potential of combining CAR-T therapy with radiation or sequential targeting to enhance long-term outcomes in high-risk patients. Although CAR-T therapy has demonstrated significant clinical success, ongoing research is essential to improve persistence, reduce toxicity, and expand its applicability across broader patient populations.

Keywords: CAR-T therapy, ALL, CD19, CD22.

1. Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder marked by the uncontrolled growth and accumulation of lymphoid progenitor cells in the blood and bone marrow [1]. While only about 20% of ALL cases occur in adults, it is the most common pediatric cancer, accounting for nearly a quarter of all new pediatric cancer diagnoses. The highest incidence is seen in children aged 1 to 4 years [2]. B-cell ALL makes up around 75% of adult cases and is biologically distinct from T-cell ALL, resulting in differing treatment strategies and outcomes. Refractory/relapsed (r/r) B-cell ALL refers to cases where patients do not respond to treatment or relapse, which remains a key focus of research.

Traditionally, the treatment of ALL has relied heavily on intensive chemotherapy regimens aimed at inducing complete remission (CR). However, these treatments are associated with significant toxicity and high induction-related mortality, particularly among older patients. Despite advancements in chemotherapy, the modest success rates and important side effects have prompted a shift toward developing novel therapies.

Chimeric antigen receptor (CAR) T-cell therapy is recognized as a promising strategy for the treatment of ALL [3]. Since 2017, the FDA has approved six CAR-T cell therapies for hematologic cancers [4]. However, challenges like antigen escape have led to treatment failures. To address this, strategies such as dual antigen targeting are under investigation [5].

Despite the considerable benefits of CAR-T therapy, challenges related to safety, efficacy, and specificity persist. This review summarized recent related advancements, addresses ongoing challenges, and explores potential strategies to enhance the effectiveness of this groundbreaking treatment.

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2. Current advancements

2.1. FasT CAR-T platform

CD19 is the most extensively used and well-developed target in CAR-T treatments for B-ALL, with CD19-directed CAR-T treatments showing a remarkable CR rate of 70% to 90%. However, the current manufacture of CAR-T remains time-consuming and typically requires 7-14 days. This delay can be critical, as patients may experience rapid disease progression and even death before receiving treatment, underscoring the urgent need to accelerate manufacturing timelines while maintaining therapeutic efficacy [6].

A novel platform called the FasT CAR-T (F-CAR-T) has been developed to address the challenge, enabling next-day CAR-T cell production [7]. Preclinical studies indicated that F-CAR-T cells targeting CD19 display enhanced proliferation and tumor-elimination abilities, which also involve a longer manufacturing process. The novel therapy demonstrated a favorable safety profile with 24 % of patients. F-CAR-T showed a promising future in clinical applications with a current 100% manufacturing success rate and a significantly expedited procedure. This acceleration may reduce the burden on patients by shortening hospital stays and increasing the success remission rates among those with severe diseases. Additionally, studies have indicated that the F-CAR-T technology can successfully produce dual-target (CD19/CD22) cells within 24 hours.

2.2. CD22 CAR-T therapy

Post-treatment relapse rates remain high in some CD19 CAR-T cases and have become an urgent objective for the next stage of development [8]. With the CD19 treatments, it is expected to notice that some patients do not respond, and up to 55% of patients who achieved CR relapsed within a year. CD19 antigen loss and mutation were frequently observed, highlighting the need to develop more effective therapeutic methods. CD22 CAR-T treatment represents a candidate used to treat adult and pediatric patients with r/r B-ALL who experienced treatment failure from previous CD19 CAR-T. Among the 37 enrolled patients, while a high CR rate of 70.5% was achieved, only a mile or no cytokine release syndrome (CRS) and neurotoxicity occurred. More importantly, the association between relapsed patients and CD22 antigen loss or mutation was not observed when CD22 expression on leukemia cells was examined. Evidence from this study suggested a promising efficacy of CD22 in achieving remission. Still, results indicated that follow-up bridging therapy may be necessary to remain in remission since a large proportion of patients without subsequent treatment experience relapse.

However, the long-term efficacy of CD22-targeted CAR-T and applicability still require further research before larger-scale clinical application. Investigating a larger sample of relapsed cases after CD19 CAR-T therapy is highly suggested to identify the possibility of CD22 downregulation. The persistence of CAR-T cells is critical for preventing relapse after therapies and has always been challenging to improve. In this study, no normal B-cells were detected in patients in sustained remission, indicating a relatively long duration of CD22 cells despite limitations caused by the FCM detection. The mechanism associated with the cell duration is a key objective for future research.

2.3. Bispecific CAR-T cells

The clinical outcomes and relapsing rate after treatment are sometimes limited by only targeting a single target [9]. Novel bispecific CAR-T cells were developed and performed in a phase 1 clinical trial (NCT03185494) for adult patients with r/r B-ALL. Six eligible adult patients were treated with autologous CD19/CD22 CAR-T cells, with results showing 100% remission without the occurrence of minimal disease residue (MDR). Cerebrospinal fluid (CSF) assessments showed evidence of cell interactions with the central nervous system (CNS). Still, only low-grade CRS was observed in patients, which ultimately resolved back to baseline level, and no neurotoxicity was observed, suggesting an acceptable toxicity level of CD19/CD22 CAR-T. Relapse occurred in two patients for various reasons other than antigen loss, demonstrating the loss of efficacy after infusion, highlighting the importance of

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improving persistence. This study also enhanced the understanding of the importance of incorporating scFv sequence in CAR-T cell functionality and improve the safety profile.

Dual antigen targeting is a promising strategy to address antigen loss or downregulation, a key cause of treatment failure [5]. In a phase 1 study involving 15 patients with r/r B-ALL, researchers tested AUTO3, autologous transduced T-cells co-expressing anti-CD19 and anti-CD22 CARs (NCT03289455). Results indicated that single or split dosing did not directly affect the incidence of post-treatment adverse events. However, more studies with larger and more even samples are needed to offer a more comprehensive perspective. The reasons still need to be considered in this study, as well as the inability to assess the correlation between dual targeting and cell exhaustion and conflicting results observed in the quantitative PCR test. A perspective assumption suggested that varied manufacturing approaches may cause different product phenotypes, leading to a limited persistence in vivo. The lack of long-term efficacy remains a challenge in many related trials, indicating that more specific issues must be addressed despite this strategy's tentative recognized feasibility and safety profile.

2.4. Sequential combination of CD19 and CD22

While single-target CAR-T treatments have demonstrated high CR rates in many trials for r/r B-ALL, achieving long-term remission remains difficult for most patients. In a trial aimed at extending relapse-free survival, post-transplant patients with r/r B-ALL expressing both CD19 and CD22 antigens on lymphoblasts received sequential CD19 and CD22 CAR-T treatment [10]. Of the 27 patients who initially received CD19 CAR-T, 85% achieved CR, and 21 of them subsequently received CD22 CAR-T, with 14 attaining CR. Although adverse events and disease progression led to some treatment failures and deaths, the trial indicated that the combination therapies holds promise for improving long-term survival. The study also suggested that this sequential combination strategy may be effective for patients with single-site extramedullary disease (EMD).

2.5. Transcriptional signature and CD19 CAR-T persistent

The persistence of CAR-T cells is a crucial factor for achieving durable remissions. A recent study investigated CAR-T cells from 10 pediatric ALL patients using high-throughput single-cell gene expression and T-cell receptor sequencing [11]. This analysis examined infusion products and serial blood and bone marrow samples collected over a period of up to 5 years post-infusion, identifying specific transcriptional factors associated with CAR-T cell persistence. The results indicate the potential presence of a universal transcriptional signature related to clinically effective, long-lasting CD19 CAR-T cells. If validated, this signature could guide the development of CAR-T therapies that offer more sustained remissions by promoting CAR-T cells' long-term survival and functionality.

3. Treatment in high-risk patients

3.1. CRS and neurotoxicity

The risk of neurotoxicity limits the participation of patients with neurological comorbidities and related diseases in clinical trials. Since the CNS is a common site of relapse in ALL, there is an urgent need for new approaches that enable the safe application of CAR-T therapy in cases involving CNS disease.

Several studies have explored CD19-directed CAR-T therapies for treating relapsed ALL in pediatric patients with CNS involvement [12]. In one study, humanized CD19-directed CAR-T was administered to 12 pediatric patients with CNS leukemia (CNSL) or active neurological comorbidities. Due to high tumor burden, all patients received bridging chemotherapy before CAR-T therapy. The treatment resulted in notable CR rates, with all evaluable patients experiencing remission, whether they had CNSL, BM relapse, or both. Notably, no severe CRS or neurotoxicity occurred, even though 33.3% of patients with bone marrow (BM) relapse and neurological comorbidities developed mild forms of these complications. Cells expanded successfully into the CSF in all patients, with a median persistence of 5.5 months in patients with CNSL and three months in those without CNSL. This trial demonstrated that

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CAR-T can be effective and safe in pediatric patients with CNSL and neurological comorbidities despite the higher tumor burden and increased risks associated with neurological conditions.

A retrospective international study further validated the efficacy of CAR-T therapy in pediatric ALL patients with active CNS involvement [13]. 55 patients were treated with either CD28-based or 4-1BB-based CAR-T cells. Some patients in the CD28-based group also received allo-HSCT after therapy. Both groups experienced CRS and neurotoxicity, with these adverse events being more frequent in the CD28-based cohort. Despite an overall CR rate of 94%, CNS relapse remained a significant challenge.

3.2. Efficacy

Research on the efficacy in patients with high-risk features remains limited. Recently, several studies have begun investigating the use of CAR-T therapy in ALL patients with CNS involvement and other high-risk conditions, such as TP53 mutations and relapse after transplantation. One study assessing the safety and efficacy of CD19 CAR-T in 110 patients with various high-risk characteristics found the therapy to be both feasible and beneficial for these populations [14]. The study also emphasized that consolidative allo-HSCT is highly recommended for improved outcomes.

3.3. Radiation therapy for improvement in CAR-T

The loss of CD19 antigen expression is a frequent cause of relapse after CAR-T therapy. In earlier preclinical studies using pancreatic cancer models, radiation therapy (RT) was employed to prevent the loss of the CAR-T target. This laid a strong foundation for exploring the use of RT in treating B-ALL. RT has been shown to induce the expression of death receptors (DR) on malignant cells, thereby enhancing the tumor-killing capacity of CAR-T.

A study based on a human CD19+ model confirmed that DR upregulation occurs both in vitro and in vivo. Also, low-dose total body irradiation (LD-TBI) extended OS in ALL-bearing mice before CAR-T treatment [15]. These results suggest that RT, particularly in the form of LD-TBI, may improve the effectiveness of CAR-T in B-ALL by increasing the susceptibility of cancer cells to immune-mediated destruction.

4. Conclusion

CAR-T therapy has revolutionized the treatment of ALL, especially in r/r cases. The success of CD19-targeted therapies has spurred innovations such as dual antigen targeting and faster manufacturing techniques like F-CAR-T, which aim to overcome challenges like antigen escape and production delays seen in traditional CAR-T treatments. While these developments show great potential, obstacles persist, particularly in improving CAR-T cell persistence, minimizing toxicity, and preventing relapse. Future research should prioritize optimizing CAR-T cell design, investigating combination therapies, and refining treatment protocols to enhance long-term outcomes. With ongoing innovation, this therapy could redefine the standard of care for ALL and other hematologic cancers.

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