Chimeric Antigen Receptor T Cell Therapy for Acute Leukemia

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Abstract

The worldwide use of CD19 chimeric antigen receptor (CAR)-T cells has increased the response rate in patients with refractory or relapsed B-cell acute lymphoblastic leukemia. Clinical practice has become much safer with the help of immunotherapy-related toxicity management guidelines, such as the ASTCT consensus grading system. Tocilizumab and steroids are the major interventions for controlling cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). New drugs and interventions for uncontrolled CRS and ICANS, including JAK1/2 inhibitors, have also been investigated. The combination of ruxolitinib and steroids effectively controlled severe CRS without impeding CAR-T cell expansion. Patients with refractory CNS3 status and CNS masses were excluded from the clinical trials because of the high risk of severe ICANS. Intracranial injections of steroids and Ommaya capsule implantation were effective. For some heavily treated patients, the difficulties in CAR-T cell manufacturing and expansion may be resolved by combination with blinatumumab. Relapse is a major concern after CAR-T therapy, and combination interventions, such as allogeneic stem cell transplantation, dual-target CAR-T cell therapies, and sequential CD19/22 CAR-T infusion, have been investigated in many centers. For T-lineage-targeted CAR-T therapies, the CAR T-cell fratricide can be overcome using many techniques. The efficacy and safety of CD7+ CAR-T cell therapy have been widely reported in recent years. A high response rate can be achieved when the immune reconstitution is prolonged. Infections, particularly viral reactivations, should be carefully monitored, as relapses are another potential issue. Switching targets and eliminating residual CD7+ CAR-T cells in the blood are key points for patients who relapse after CD7+ CAR-T cell therapy. CAR-T cell therapies for AML have not been investigated in a large-scale cohort, except for CD19-positive AML with the *AML1-ETO* fusion gene.

Introduction

Over the past decade, chimeric antigen receptor (CAR)-T cell therapies have been widely used in B-cell acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma. Long-term survival of refractory or relapsed (r/r) B-ALL was significantly improved with this novel treatment[1-3]. The event-free survival (EFS) and overall survival (OS) for 53 r/r B-ALL adults who received 19-28z CAR-T cells were reported as 6.1 and 12.9 months[2]. The outcome of pediatric and young adult patients is much better, with a 3-year OS and EFS rates of 63% and 44%[4]. Nowadays, CD19 CAR-T products are most popular in clinical trials and applications, and the CAR vector is widely available as a second-generation lentivirus vector consisting of a CD 28 or 4-1BB co-stimulatory domain. Other targets such as CD22, CD20, bispecific CD19/22, CD7, or CD5 for the T-lineage, and CD33 or CD371 for AML are at an exploratory stage.

With the worldwide clinical applications of CAR-T therapies, especially the clinical experiences with CD19 CAR-T cell therapies, three aspects are key to their success. These three aspects are safety management, efficiency enhancement, and relapse rate reduction.

Safety Management

Clinical Practice Guidelines for management of immunotherapy-related toxicities

In the early phase of clinical trials of CD19 CAR-T cell therapies, life-threatening adverse events (AEs) were frequently observed, which impaired worldwide applications[5]. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are observed as major adverse events in the acute phase. Recently, various grading systems have been applied to manage CRS and ICANS. Early clinical trials modified the CTCAE v4.03 grading system, further refinements were achieved, one of which is now commonly referred to as the Lee criteria[6]. To harmonize the definitions and grading systems for CRS and neurotoxicity, the American Society for Transplantation and Cellular Therapy (ASTCT) grading system was recommended to uniformly categorize the severity and management of the toxicities. Immunotherapy-related toxicity management guidelines have made clinical practice safer. Thus, the ASTCT grading system is easily applicable. Although a comparative study showed inconsistent final grades across all grading systems, the ASTCT grading system was quite similar to the other grading systems[7]. The ASTCT grading system was proposed as a unified grading system that can avoid confusion and clarify the CAR-T cell-specific treatment.

Reducing leukemia burden and decreasing the risk of severe CRS

Most patients experienced CRS within the first 8 weeks after infusion, with the onset occurring around the first 2 weeks from infusion[7, 8]. Neurotoxicity was not expected to occur in most patients, but neurologic events occurring less than 8 weeks post-infusion may be correlated with CRS severity and a history of pre-infusion neurologic events[8]. Tocilizumab and steroids are major interventions to control CRS and ICANS in the acute phase. Our study and others have reported that leukemia burden was associated with severe CRS[2, 8, 9]. Reducing the leukemia burden before infusion is considered to decrease the risk for patients who have a high leukemia burden and are suspected of experiencing severe CRS. Therefore, bridging chemotherapy is a viable treatment option. However, most patients recommended CD19 CAR-T cell therapy are refractory to chemotherapy. Bridging chemotherapy may be ineffective and may result in prolonged cytopenia. Steroid-based bridging chemotherapies can also suppress T cell function and influence autologous CAR-T cell production and expansion. Blinatumomab is a bispecific anti-CD19/CD3 T-cell engaging antibody (bispecific T-cell engager [BiTE]), which is reported as having high efficacy in r/r B-ALL. Two aspects of this drug make it an ideal bridging regimen for CAR-T cell therapies in the real world[10-12]. Considering its high efficiency in CD19+ B-cells and lack of suppression of T cells, it can be effective in reducing the leukemia burden and preserving autologous T cells for CAR-T cell manufacturing. In addition, due to its short half-life in the blood, its anti-tumor effect and adverse events would stop after stopping continuous injections. After a marked reduction in the leukemia burden, patients would be quickly prepared for leukopoiesis and CAR-T cell manufacture. Short-term use of BiTEs (less than 2 weeks) may also reduce the risk of CD19 loss. For example, a 9-year-old boy with r/r B-ALL in our center, who had no chance for autologous CD19 CAR-T cell therapies, succeeded in CAR-T cell manufacturing and achieved complete remission post-infusion with the help of one injection of BiTEs.

New drugs and interventions for AEs management

However, some cases of severe CRS and ICANS cannot be well controlled despite tocilizumab and steroid interventions. Therefore, new drugs and interventions are currently being investigated. In our early study, Ruxolitinib, a JAK1/2 inhibitor, can be effective for controlling severe steroid-refractory CRS, showing no impeding CAR-T cell expansion in vivo[13]. Refractory CNS3 status and CNS mass are excluded from clinical trials because of the high risk of severe ICANS[8]. Our and other studies showed that Intracranial injection with steroids can be effective for severe ICANS[14, 15]. Recently Ommaya capsule implantation is considerable and investigated in our and other clinical trials for r/r B-ALL and T-ALL patients with CNS mass[16].

Enhancing Efficiency

Good CAR-T cell expansion is important for successful treatment. However, the activity of autologous CAR T cells varies, particularly in heavily treated patients. Many clinicians have attempted interventions to improve CAR-T cell expansion and efficiency. One of these interventions was blinatumomab, which can robustly induce CD22 CAR-T cell expansion in peripheral blood as reported[17]. In our clinical experience, blinatumomab could be a good choice for patients with leukemia progression before CAR-T cell expansion. However, long-term use of blinatumomab is associated

with an increased risk of CD19 loss and relapse. Most CD19 CAR-T cells exhibit peak expansion within 1-month post-infusion. In patients with extramedullary diseases (EMDs), disease progression is easily observed 3 months post-infusion if EMDs are not eliminated by CAR-T cells. Sequential CAR-T cell infusion and dual-target CAR-T cell therapies have been established to enhance efficiency[18-20]. Our group discovered that modifying the timing of sequential CD19/22/20 CAR-T cells enhanced the anti-tumor synergistic effects. Previous CAR-T cell re-expansion by sequential CAR-T cell infusion was easily observed, and the patient responded well to this modified sequential CAR-T cell infusion. Further clinical interventions, such as PD1 inhibitors, ibrutinib, and enhanced CAR vectors with IL-15 over-expression, which intentionally improve CAR-T cell expansion and efficiency, are currently being investigated[21-23].

Reducing Relapse Rate

Two major types of relapses

Relapse is a major concern following CAR-T therapy. Two major types of relapse have been observed in previous studies. One of them is antigen-loss relapse, which has been well reported in many studies[23, 24]. Various genetic mutations of CD19 have been discovered, and a truncated protein with a nonfunctional or absent transmembrane domain is predicted to be the cause of absent CD19 expression on blasts at relapse[24]. CD19 negative clones have been identified and shown to exist before CD19 CAR-T cell infusion by single-cell sequencing[25], which identifies patients with a high risk of CD19 antigen escape and relapse. We also retrospectively analyzed our r/r B-ALL patients who had received CD19 CAR-T cell infusion and tried to find some evidence for CD19 antigen escape[25]. We found that $TP53^{mut}$ patients have a high risk of CD19 antigen loss and failure of CD19 CAR-T cell therapy. Lineage switch (LS) after CD19 CAR-T cell infusion is another type of CD19 antigen loss that is usually observed in mixed phenotype acute leukemia and *MLL*+ rearrangement ALL[26]. Antigen loss is also observed in other CAR-T cell therapies targeting CD22, CD5, and CD 7[27-29]. These phenomena indicate that combination interventions may be necessary for high-risk patients.

The early loss of CAR-T cell persistence is another major type of relapse after CD19 CAR-T cell infusion. CD19+ blasts and B-cell recovery were identified at the time of relapse. This type of relapse was also observed in our CD22 CAR-T cell therapy study[30].

Combination interventions

Many interventions were investigated for CD19+ re-lapse, such as humanized or full human CAR vectors and immune-checkpoint inhibitors among others[5]. To reduce the relapse rate, combination interventions are investigated in our and many other centers, such as allo-geneic stem cell transplantation (allo-HCT), dual-target CAR T cell therapies, and sequential CD19/22 CAR-T infusion[9, 31-34]. Allo-HCT is one of the combination interventions recommended for patients with EMD infiltration, high-risk cytogenetics, and special mutations associated with poor prognoses after CAR-T cell therapies in our group[9, 33]. For children and young adult patients, sequential CD19/22 CAR-T therapy showed promising long-term outcomes in our group. Although, early B-cell recovery indicated a high risk of relapse with this treatment[34].

Salvage therapies after CAR-T therapy

Despite no antigen loss occurring at relapse, anti-CAR immune response is easily observed in the re-infusion of the same CAR vector due to the immuno-genicity of CAR constructs[35]. Humanized or full human CAR constructs can reduce this immunogenicity. However, in our clinical experience, CAR-T cell surveillance after reinfusion of the same CAR-T cell products is much shorter than that during the first infusion. For antigen loss relapse, different targets have been suggested for future CAR-T cell therapies, such as CD22 instead of CD19 or CD5 instead of CD7. For antigen-positive relapse, humanized or full human scFv can be used after the failure of CAR-T therapy using murine scFv. Besides that, the CAR constructs with scFv targeting different extracellular domains have also been considered for salvage treatments after relapse[36, 37].

Cellular Therapies for T-Lineage Targets

There are three major obstacles to the development of T-lineage-targeted CAR-T therapies. One of these is a target candidate for cellular therapies. CD5 and CD7, expressed on most normal and malignant T cells, have been investigated in preclinical and clinical studies[38-41]. The second strategy is to prevent the use of CAR-T cell fratricides. CD5 is expressed on all T cells, and CD7 is expressed on 90-96% of normal T cells and 90-98% of NK cells[38-42]. Blocking these targets on CAR-T cells is necessary to prevent CAR-T cell fratricides. Although nature-selected CD7- normal T cells are reported to prevent CD7 CAR-T cell fratricides[42]. Gene-editing and endoplasmic reticulum (ER) retention techniques are widely used[43, 44]. Our group uses one step for CD7 ER retention and CAR vector transduction for our CD7 CAR-T products to simplify and shorten the manufacturing process[45]. Maksim Mamonkin et al. reported that CD5 expression on normal T cells can self-deregulate

and CD5 CAR-T cells do not need CD5 knockout[46]. However, our group reported that knockout of CD5 expression on T cells also showed high anti-tumor activities and good expansion[47].

Three sources of CAR-T cell products are used in T-lineage CAR-T clinical trials[48]. The advantages and disadvantages of specific sources depend on the leukemia burden, residual functional T cells, patient factors, or previous treatments. The efficacy and safety of CD7 or CD5 CAR-T cell therapies have been widely reported in recent years using different knockout techniques and cell sources[28, 49-52].

Despite the high response rates reported in many clinical trials, short- and long-term safety may be the most important concerns in T-lineage cellular therapies. The CRS and ICANS of newer CAR-T cell therapies are reported to be similar to those of CD19 CAR-T products[28, 45]. Different CAR-T products and T-cell sources would develop different CRS and ICANS, which should be monitored individually. For "off-the-shelf" CAR-T and donor-derived CAR-T products, graft-versus-host disease (GVHD) is a risk. Hematological toxicities, including protracted cytopenia, may also be significant. Most patients who receive CD7 or CD5 CAR-T products need one to three months for hematological recovery. Infections, especially viral infections, during this recovery period could be fatal, and precautionary measures should be taken. The long-term survival of patients is impeded by infections. Our studies showed good CD5 and CD7 CAR-T cell persistence during long-term follow-up. Immune reconstitution was observed; however, the process was very slow. Overall, in our clinical experience, T cell aplasia may be the biggest obstacle to T-lineage cellular therapies. Moreover, when patients have refractory viral infections after bridging transplantation after CD7 or CD5 CAR-T cell infusions, CD7 or CD5 CAR-T cells and the CD7+ or CD5+ T cell ratio must be monitored. Some clinical cases have shown that CD7 or CD5 CAR-T cells can still function after conditioning, causing severe immunosuppression.

Relapse was commonly observed after conducting CD7+ CAR-T cell clinical trials. CD7 negative relapse usually occurs during CD7 CAR-T cell surveillance. When these patients received CD5+ CAR-T cells, we monitored the co-expansion of the two types of CAR-T cells and only one of them eventually dominated. Therefore, we believe that the two types of CAR-T cells targeted and eliminated each other after the mixed infusion. For CD7-negative relapse, switching targets and eliminating residual CD7+ CAR-T cells in the blood are key points for patients who relapse after CD7 + CAR-T cell therapy.

Cellular Therapies for AML

CAR-T cell therapies for AML have not been investigated in large-scale cohorts, except for CD19-positive AML with the AML1-ETO fusion gene[53]. Preliminary results of ongoing CD33 CAR-T cell clinical trials in our group showed that CD33+ cells can be eliminated in peripheral blood post-infusion, with 3 of 4 patients achieving complete remission. However, the number of cases was small, and safety remains a concern.

In summary, B-lineage CAR T-cell therapy is safe and effective for treating r/r B-ALL. Combination therapies can improve the outcomes in high-risk patients. T-lineage-targeted CAR-T cell therapies have controllable safety and high efficacy in the acute phase; however, immune reconstitution is slow. Follow-up with stem cell transplantation for patients who receive T-cell-targeted CAR-T cell therapy is recommended to prevent severe infections. CD7 or CD5 CAR-T cells and CD7+ or CD5+ T cell ratios should be monitored, regardless of transplantation. However, cellular therapies for AML require large-scale cohort studies.

Author Contributions

JP wrote the manuscript and approved the final version.

Conflicts of Interest

The author declares no conflict of interest. Disclosure form provided by the author are available on the website.

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