Use of chimeric antigen receptor T cells in allogeneic hematopoietic stem cell transplantation

Jun Liu¹, Xi Zhang¹, Jiang F Zhong² & Cheng Zhang^{*,1}

¹Department of Hematology, Xinqiao Hospital, Army Medical University, Chongqing, People's Republic of China

²Division of Periodontology, Diagnostic Sciences & Dental Hygiene, & Division of Biomedical Sciences, Herman Ostrow School of Dentistry, University of Southern California, Los Angeles, CA, USA

*Author for correspondence: Tel.: +86 023 6877 4209; chzhang2014@163.com

The chimeric antigen receptor T (CAR-T) cells play an antileukemia role, and can be used to treat or prevent relapse by targeting minimal residual disease for patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the infusion of allogeneic CAR-T cells may also cause graft-versus-host disease, which limited their applications during and after allo-HSCT. In this review, we discuss the clinical trials that applying CAR-T cells before allo-HSCT and the use of donor-derived CAR-T cells as conditioning regimen during allo-HSCT. At last, we analyzed the effect of donor-derived CAR-T cells on preventive infusion after allo-HSCT.

First draft submitted: 29 June 2017; Accepted for publication: 25 October 2018; Published online: 7 December 2018

Keywords: acute lymphoblastic leukemia • acute myeloid leukemia • allogeneic CAR-T cells • allogeneic hematopoietic stem cell transplantation • CAR-T cells • conditioning regimen • donor-derived CAR-T cells • hematological malignancies • preventive infusion

The use of chimeric antigen receptor T (CAR-T) cells is an excellent example of applying basic research concept to clinical treatment. The CAR-T cells have shown a strong function in antileukemia [1,2]. With the advancement of technology, there are four generations of CAR-T cells available now [3]. At present, the second generation is extensively used in the treatment of hematological malignancies.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most effective methods to treat refractory/relapsed acute lymphoblastic leukemia (ALL) [4]. The high relapse rate has been the main cause of death for patients with ALL after allo-HSCT [5,6]. And how to decrease the relapse rate after allo-HSCT is an important matter in treating ALL. Applications of CAR-T cells have resulted in good outcomes in refractory/relapsed ALL [7]. However, the adverse events, especially cytokine release syndrome (CRS), still need to be addressed for the application of CAR-T cells in clinical treatments [8–10]. CAR-T cells can treat or prevent relapse by targeting minimal residual disease (MRD) for B-cells malignancies. However, the infusion of allogeneic CAR-T cells could also cause the graft-versus-host disease (GVHD), which limits the use of CAR-T cells during and after allo-HSCT. The outcome is very poor for patients with refractory/relapsed hematological malignancies undergone allo-HSCT without reaching complete remission (CR) [11]. In this review, we discuss using CAR-T cells with allo-HSCT for treating refractory/relapsed B-cells malignancies, especial for B-cell ALL. Further, we discuss the use of donor-derived CAR-T cells as a part of conditioning regimen during allo-HSCT. Finally, we discuss the role of donor-derived CAR-T cells on preventive infusion after allo-HSCT for preventing relapse and improving long-term survival of patients.

Production of allogeneic CAR-T cells

The production of allogeneic CAR-T cells needs several steps. The first step is leukapheresis of leukocytes from the donors. Then, the T cells are separated from the leukocytes [12]. The third step is the separation of CD4/CD8 T-cell subsets by using specific markers or antibody–bead conjugates. Then, the T cells were activated by co-culturing

with anti-CD3/anti-CD28 monoclonal antibodies coated beads; autologous antigen-presenting cells from donors; anti-CD3 antibodies in combination with feeder cells and growth factors, such as IL-2 [13,14].

CAR-T cells as a bridge of allogeneic transplantation

Many studies showed that using CAR-T cells can achieve approximately 70–90% CR in relapsed/refractory B-cell malignancies [15–20]. However, CAR-T cells only can survive in the body for a short period of time, some of the patients relapsed quickly [21,22]. In our center, a total of 14 patients with refractory/relapsed B-ALL were treated with CD19-CAR-T cells. Among them, seven cases use autologous CAR-T cells and seven cases use cells from the donors. Eight patients achieved CR, but three of these patients relapsed within 3 months (Zhang C *et al.*, UNPUBLISHED DATA). It is noteworthy that we have treated a case of B-ALL patients with CNS recurrence. After the treatment of CAR-T cells, the patient remains negative for CNS leukemia. Only one patient died on day 10. The main adverse reactions were granulocyte deficiency, anemia, thrombocytopenia and thrombocytopenia. Thus, further studies should be carried out to improve the long-term outcomes.

Some researchers showed that CAR-T cells treatment with allo-HSCT may be the best way to treat relapsed/refractory B-cell malignancies. Zhang *et al.* had showed that the patients with relapsed/refractory B-cell malignancies showed no sign of relapse if they received CAR-T cells treatment followed by allo-HSCT at the last follow-up [23]. In personal communication, Tong also showed that the survival is better for patients with refractory/relapsed B-cells ALL who received allo-HSCT after CD19-CAR-T cells treatment compared with the patients only received CAR-T cells [24].

However, it is controversial whether patients with refractory/relapsed B-cell malignancies should receive allo-HSCT after CAR-T cells. A recent study showed that there is no significant difference between patients who underwent allo-HSCT and those who did not (79 vs 80%) after CAR-T cells treatment in terms of 6-month overall survival [25]. The 6-months overall survival rates were 64% in patients who did not receive allo-HSCT after CD19-CAR-T cells treatment, and 70% in patients who underwent allo-HSCT post-CAR-T cells treatment without statistical difference.

A few studies showed good outcomes for relapsed/refractory B-cell malignancies for those who received CAR-T cells treatment followed by allo-HSCT. However, there are some concerns on these studies. First, all of these studies were not randomized clinical trials and the follow-up time is short, along with a small case number, thus make it difficult to draw the definitive conclusions. Second, the controlled trials should be performed to compare the outcomes of CAR-T cells treatment followed with or without allo-HSCT for a longer follow-up time.

CAR-T cells as conditioning regimen

After allo-HSCT, disease relapse remains a significant cause of treatment failure in acute leukemia [26]. It is crucial to eliminate the leukemia cells as much as possible during allo-HSCT. Although myeloablative conditioning can eliminate most leukemia cells, procedure-related toxicities restrict its use, especially for older and weak patients with high leukemia burden [27,28]. Reduced-intensity conditioning allo-HSCT has less procedure-related toxicities and provides adequate immunosuppression. But the relapse incidence remains high [29–32]. Thus, it is vital to know how to further eliminate MRD and prevent the relapse in these patients.

Although the CAR-T cells have been used in relapsed/relapse B cells malignancies with good outcomes, the outcome is still poor for nonresponse patient treated by donor lymphocyte infusion (DLI) after allo-HSCT [33]. The outcome is also poor for a patient with a higher burden of disease [34]. In addition, it is very difficult to collect sufficient T cells for the construction of CAR-T cells from patients who have an extremely high leukemia burden [23,35].

One study showed that the donor-derived anti-CD123-41BB-CAR-T cells have graft-versus-leukemia (GVL) effect after infusion in an acute myeloid leukemia xenograft model with NSG mice [36]. In this study, the CAR was constructed with a third-generation self-inactivating lentiviral vector plasmid, pRRL-SIN-CMV-eGFP-WPRE, in which the cytomegalovirus promoter was replaced by the EF-1 α promoter. The study showed that CAR-T cells have GVL effect [36]. In another murine haploidentical HSCT model, donor-derived CD19-CAR-T cells constructed with retroviral vector can induce GVL effect and diminish GVHD effect at day 1 [37].

A recent study systematically analyzed GVL effect and GVHD complication for 72 patients with relapsed ALL after allo-HSCT and received donor-derived CAR-T cells [38]. In this study, only 5 of 72 patients (6.9%) developed slight GVHD without other serious side effects such as CRS and CNS toxicity. The authors indicated that donor-derived CAR-T cells are highly effective for relapse prophylaxis, salvaging relapse or MRD clearance.

Table 1. Clinical trial registrations on allogeneic chimeric antigen receptor T cell in allogeneic transplantation.

Center	Clinical trial number	Disease	CAR-T type	Status
Xinqiao Hospital, Army Medical University, Chongqing, China	ChiCTR1800015353	ALL	CD19	Recruiting
Affiliated Hospital of Xuzhou Medical University, China	ChiCTR-OIC-17012374	B-cell hematologic malignancies	CD19	Recruiting
Xinqiao Hospital, Army Medical University, Chongqing, China	ChiCTR-OOC-16008447	ALL	CD19	Recruiting
Fred Hutchinson Cancer Research Center	NCT01475058	ALL	CD19	Completed
Affiliated Hospital to Academy of Military Medical Sciences, Beijing Shi, China	NCT03114670	Adult AML	41BB- CD123	Recruiting
Biotherapeutic Department and Hematology Department of Chinese PLA General Hospital, Beijing, China	NCT03463928	B-cell leukemia	Bispecific CD19/22 or CD19	Recruiting
National Cancer Institute, USA	NCT01087294	B-cell cancer	CD19	Recruiting
University of Pennsylvania, USA	NCT01551043	ALL	CD19	Completed

The trials are registered at www.chictr.org.cn/ and ClinicalTrials.gov. Search performed on 3rd September 2018.

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; CAR-T: Chimeric antigen receptor T cell.

Table 2. Chimeric antigen receptor T cells used as conditioning regimens and preventive infusion after allogeneic hematopoietic stem cell transplantation.

Center	Target	No.	Disease	Dose of CAR-T cells	Role of CAR-T cells	GVHD	Serious CRS	Survival at last follow-up
Department of hematology, Xinqiao Hospital, Army Medical University, Chongqing, China	CD19	3	ALL	6.4×10^6 /kg	Conditioning regimens	No	No	100 days
Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas, USA	CD19	2	ALL	9.7×10^7 and 5.8×10^7 , respectively	Preventive infusion	No	No	2 weeks to 8 months
The University of Texas MD Anderson Cancer Center, Houston, Texas, USA	CD19	19	B-cell malignancies	10^6 /m ² to 10^8 /m ²	Preventive infusion	Three patients with GVHD. One died from liver failure	No	12-month OS: 63%

ALL: Acute lymphoblastic leukemia; allo-HSCT: Allogeneic hematopoietic stem cell transplantation; CAR-T cell: Chimeric antigen receptor T cell; CRS: Cytokine-release syndrome; GVHD: Graft-versus-host disease; OS: Overall survival.

Therefore, the donor-CAR-T cells may be safe and effective in allo-HSCT. Recently, Ai *et al.* reported an older patient with refractory ALL, who was treated with low numbers of donor-derived CD19-CAR-T cells and with low dose of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood stem cells [35]. This patient developed serious GVHD and by treating with anti-GVHD drugs, the related complication was controlled. However, this patient died at last because of severe infection on day 31. It is still not clear that whether donor-derived CAR-T cells should be used during allo-HSCT as a part of conditioning regimens because of the related complications, such as CRS and GVHD, especially for patients with high leukemia burden who can collect enough T cell for the construction of CAR-T cells. In our center, a 12-year-old girl with refractory/relapsed ALL was treated with donor-derived CD19-CAR-T cells as a reduced-intensity conditioning regimen for haploidentical transplantation [39]. This patient received a reduced-intensity conditioning regimen, then infusion with equal dose

of the donor-derived CD19-CAR-T cells for 4 days infusion with total dose of $6.4 \times 10^6/\text{kg}$. The granulocyte-mobilized peripheral blood stem cells and granulocyte colony-stimulating factor-mobilized bone marrow was infused on days 1 and 2, respectively. The tacrolimus and mycophenolate mofetil were used on day 1, and the antithymocyte globulin (ATG) was used on days +14 and +15. The highest level of the donor-derived CD19-CAR-T cells in the peripheral blood was reached on day 8 and gradually dropped until undetectable on day +40. The patient completely engrafted without serious complications and is disease free at the last follow-up.

Although it is easier to get sufficient T cells from healthy donor in construction of CAR-T cells, the proliferation rate of allogeneic CAR-T cells is slower than that of the autologous CAR-T cells. Therefore, it must be careful when using the immunosuppressors because of their potential effect in killing CAR-T cells and inhibiting the proliferation of CAR-T cells during transplantation. In this study, the immunosuppressors of mycophenolate mofetil and tacrolimus were changed from day 7 to day 1 after the 7th day of the first infusion of CAR-T cells, which was adopted to avoid or alleviate the killing effect and the inhibition of the proliferation of the CAR-T cells [39]. The ATG can also kill and inhibit the proliferation of CAR-T cells. In this protocol, the ATG was used only two-times on days +14 and +15 after 2 weeks of the infusion of CAR-T cells. Then we further applied this treatment in another two patients, which also showed good outcomes (Zhang C *et al.*, UNPUBLISHED DATA). Nevertheless, a trial with larger number of patients is still needed to confirm the curative effect of CAR-T cells.

CAR-T cells in preventing relapse after transplantation

The relapse rate has been high for patients with ALL. Even with allo-HSCT, the long-term survival is still poor for patients with refractory/relapsed ALL [40–42]. So it is vital to prevent relapse for patients with ALL after allo-HSCT.

One of the main methods used to prevent or treat relapse for patients with ALL after allo-HSCT is DLI through the GVL effect [43]. Severe acute GVHD developed in approximately a third of DLI [44]. Besides, the curative effect is very limited for relapse ALL after allo-HSCT or with preventive DLI infusion after transplantation [45–47]. Preventing relapse for patients with ALL remains an unmet clinical challenge after allo-HSCT. Therefore, new treatment strategies are still needed.

Although allogeneic CAR-T cells have been successfully used on patients with refractory/relapsed ALL without serious complications, the long-term survival is still needed to be improved [48]. Pre-emptive therapy should be performed upon the detection of MRD via molecular or immunophenotypic methods [49]. Better detection of MRD can be developed with more advanced molecular analyses, such as single-cell technology and microfluidic devices [50,51]. As a new method of immunotherapy, CAR-T cells could be applied for preventive infusion after allo-HSCT. Recent study showed that between days +55 and +200 after allo-HSCT is the best time for immunotherapy [23].

The infused donor-derived virus-specific T cells expanded *in vivo* can persist long term and display antiviral activity without GVHD after allo-HSCT [52–54]. Therefore, Dotti *et al.* used the donor-derived CD19-CAR-virus-specific T cells to treat B-cell malignancies in a Phase I study in 2003 [55]. In this study, one patient remains in CR for >8 months after CD19-CAR-T cells infusion, and another patient remains in CR for 8 weeks after CAR-T cells treatment. The two patients did not develop GVHD and serious CRS. In this study, whether the patients received immunosuppressors after CAR-T cells infusion is unclear.

Cooper *et al.* reported a Phase I clinical trial for 19 patients with B-cell malignancies who received a CD19-CAR-T cells infusion after allo-HSCT [56]. The CAR-T cells were constructed using the Sleeping Beauty transposon/transposase system to express a CD19-specific CAR. The tacrolimus and minidose methotrexate were used as GVHD prophylaxis for matched-sibling HSCT, and tacrolimus, mycophenolate mofetil and post cyclophosphamide were used for haploidentical HSCT. The drugs of GVHD prophylaxis were tapered and discontinued 6 months after allo-HSCT. The CAR-T cells were administered during 6–12 weeks after allo-HSCT based on a median time occurrence of acute GVHD is 6 weeks after allo-HSCT. The white blood cells in most of these patients were normal ($1.2 \times 10^9/\text{l}$ to $9.2 \times 10^9/\text{l}$) when performed CAR-T cells infusion. The numbers of CAR-T cells were $10^6/\text{m}^2$ to $10^8/\text{m}^2$. The CAR-T cells persisted *in vivo* for 51 days in average, and in one patient, the CAR-T cells still can be detected even after 1 year. No acute infusion or delayed toxicities were developed. Three patients developed GVHD. One of them with grade 1 acute skin GVHD was resolved with topical steroids, one with chronic skin GVHD responded to systemic steroids, and one patient died from liver failure with a component of liver GVHD due to a prior history of drug-induced liver toxicity. The 12-month progression-free and overall survivals rates were 53 and 63%, respectively. This study suggested that CAR-T cells infusion is safe and effective, and the immunosuppressors did not affect the proliferation of CAR-T cells and may inhibit the release of cytokines.

In our center, we performed a prospective clinical trial to explore the safety and efficacy of preventive infusion with donor-derived CD19.CAR-T after allo-HSCT at day +60 (ChiCTR1800015353). In this study, the GVHD prophylaxis was continually used according to the standard protocol of allo-HSCT. Our preliminary results showed that the CAR-T cells persisted, and they are safe and effective without serious adverse effects, such as CRS and GVHD. The reason that the infusion of allogeneic CAR-T cells post-transplant seldom induced GVHD may be the continual use of immunosuppressor, and that the donor-derived CAR-T cells are partial tolerance forms because the immune system origins from the donors after hematopoietic reconstitution with full chimerism, which is similar to the patients' autologous CAR-T cells. At present, there are several clinical trials on the use of allogeneic CAR-T cells (Table 1).

Conclusion

Some questions still need to be solved for the use of allogeneic CAR-T cells. The sequential CAR-T cells or dual CAR-T cells should be deeply explored to escape off-target effect and further improve the curative effect and long-term survival. Universal CAR-T cells may be ideal CAR-T cells in the future. New CAR-T cells those can combat relapse, mitigate overactivation and enhance specificity should be produced.

Altogether, the application of CAR-T cells before allo-HSCT showed good outcome for refractory/relapsed B-cells malignancies, suggesting a safe and effective role of CAR-T cells as conditioning regimens (Table 2). However, further randomized and double-blinded clinical trials should be performed to systematically evaluate the role of CAR-T cells in conditioning regimens and preventive infusion after allo-HSCT.

Future perspective

Although allogeneic or donor-derived CAR-T cells showed good outcomes at present, there are still some questions need to be solved. First, the sequential CAR-T cells or dual CAR-T cells such as CD19CD22 CAR-T cells, CD5CD19 CAR-T cells and CD33CD123 CAR-T cells should be deeply explored in order to escape off-target effect and increase long-term survival rate with improved curative effect. Second, although the use of donor-derived allogeneic CAR-T cells showed good outcomes during and after transplantation with little complications, the production period of CAR-T cells is still too long; the universal CAR-T cells that can be used immediately may be one of the best CAR-T cells in future. Third, the new CAR-T cells that can combat relapse, mitigate overactivation and enhance specificity should be developed. A recent study reported a split, universal and programmable CAR system, which simultaneously encompasses multiple critical 'upgrades', such as finely tune T-cell activation strength, sense and logically respond to multiple antigens, and the ability to switch targets without re-engineering the T cells [57]. Due to the persistence and engraftment related to the state of T-cell differentiation, limiting differentiation

Executive summary

Background

- Relapse is still the main factor of death after allogeneic hematopoietic stem cell transplantation (allo-HSCT).
- At present, there are no effective methods to prevent the relapse of disease after allo-HSCT.
- The chimeric antigen receptors T (CAR-T) cells can treat or prevent relapse by targeting minimal residual disease for B cells malignancies; however, whether the CAR-T cells can be safely be used with allo-HSCT is still unclear.

Production of allogeneic CAR-T cells

- The production of allogeneic CAR-T cells needs several steps.
- The allogeneic CAR-T cells are usually produced from the donors.

CAR-T cells as bridge of allogeneic transplantation

- CAR-T cells can make a good outcome for refractory/relapsed B cells malignancies; however, relapse rate is very high.
- It may be the best way to treat relapsed/refractory acute lymphoblastic leukemia with CAR-T cells followed by allo-HSCT.

CAR-T cells as conditioning regimen

- CAR-T cells can be used as a part of conditioning regimens in allo-HSCT.
- The immunosuppressors did not affect the proliferation of CAR-T cells and may inhibit the release of cytokines.

CAR-T cells in preventing relapse after transplantation

- It is safe and effective for preventive infusion of CAR-T cells after allo-HSCT.
- The immunosuppressors did not affect the proliferation of CAR-T cells and may inhibit the release of cytokines by CAR-T cells.

may enhance the efficacy of CAR-T cell therapy. A recent study showed that producing CAR-T cells with less time can improve effector function [58].

Financial & competing interests disclosure

This study was funded by the clinical key foundation of Xinqiao Hospital of Army Military Medical University (No. 2016YLC03). The authors have no other relevant affiliations or financial involvement with any organization of entity with a financial interest in or conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

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