


 Research
 Immunology—Article

Donor-Derived CD19-Targeted T Cell Infusion Eliminates B Cell Acute Lymphoblastic Leukemia Minimal Residual Disease with No Response to Donor Lymphocytes after Allogeneic Hematopoietic Stem Cell Transplantation



Yifei Cheng^{a,b,#}, Yuhong Chen^{a,b,#}, Chenhua Yan^{a,b}, Yu Wang^{a,b}, Xiangyu Zhao^{a,b}, Yao Chen^{a,b}, Wei Han^{a,b}, Lanping Xu^{a,b}, Xiaohui Zhang^{a,b}, Kaiyan Liu^{a,b}, Shasha Wang^{a,b}, Lungji Chang^c, Lei Xiao^d, Xiaojun Huang^{a,b,*}

^a Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Peking University Institute of Hematology, Peking University People's Hospital, Beijing 100044, China

^b Collaborative Innovation Center of Hematology, Peking University, Beijing 100084, China

^c Shenzhen Geno-Immune Medical Institute, Shenzhen 518057, China

^d Innovative Cellular Therapeutics Co., Ltd., Shanghai 201499, China

A R T I C L E I N F O

Article history:

Received 7 April 2018

Revised 23 December 2018

Accepted 26 December 2018

Available online 11 January 2019

Keywords:

Donor-derived CD19-targeted T cell infusion
 Hematopoietic stem cell transplantation
 B cell acute lymphoblastic leukemia
 Minimal residual disease

A B S T R A C T

Leukemia relapse is still the leading cause of treatment failure after allogeneic hematopoietic stem cell transplantation (allo-HSCT) for B cell acute lymphoblastic leukemia (B-ALL). Relapsed patients with B-ALL after allo-HSCT have a very short median survival. Minimal residual disease (MRD) is predictive of forthcoming hematological relapse after hematopoietic stem cell transplantation (HSCT); furthermore, eliminating MRD effectively prevents relapse. Donor lymphoblastic infusion (DLI) is the main established approach to treat B-ALL with MRD after allo-HSCT. However, about one-third of patients with MRD are non-responsive to DLI and their prognosis worsens. Although donor-derived cluster of differentiation (CD)19-directed chimeric antigen receptor-modified (CAR) T cells (CART19s) can potentially cure leukemia, the efficiency and safety of infusions with these cells have not yet been investigated in patients with MRD after HSCT. Between September 2014 and February 2018, six patients each received one or more infusions of CART19s from HSCT donors. Five (83.33%) achieved MRD-negative remission, and one case was not responsive to the administration of CAR T cells. Three of the six patients are currently alive without leukemia. No patient developed acute graft-versus-host disease (aGVHD), and no patient died of cytokine release syndrome. Donor-derived CAR T cell infusions seem to be an effective and safe intervention for patients with MRD in B-ALL after allo-HSCT and for those who were not responsive to DLI.

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1. Introduction

B cell acute lymphoblastic leukemia (B-ALL) can be cured by allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1,2]. Hematological relapse is a common cause of treatment failure in patients with B-ALL after allo-HSCT, and clinical outcomes for these patients are dismal. Relapsed adult patients with B-ALL after allo-HSCT had a median survival time of 5.5 months, and

relapsed children with B-ALL after allo-HSCT had a three-year event-free survival (EFS) of 15% and an overall survival (OS) of 20% [3,4].

Minimal residual disease (MRD) after hematopoietic stem cell transplantation (HSCT) is a reliable marker for impending hematological relapse and can thus serve as a trigger for pre-emptive therapy [5–7]. In general, reduction/withdrawal of the immunosuppressant, the administration of interferon, and donor lymphoblastic infusion (DLI) are the main established approaches to prevent relapse in patients with B-ALL and positive MRD after allo-HSCT [8–10]. Several studies have investigated the effectiveness of interferon and DLI in treating MRD in B-ALL after allo-HSCT. DLI is an

* Corresponding author.

E-mail address: xjhrm@medmail.com.cn (X. Huang).

These authors contributed equally to this work.

important option for the relapse of B-ALL after HSCT. However, response rates after DLI have been low. Yan et al. [11] found that approximately 64% patients with relapsed acute leukemia after haplo-identical hematopoietic stem cell transplantation (haplo-HSCT) were able to achieve complete remission (CR) after DLI, and that the one-year disease-free survival (DFS) rate was 36%. Yan et al. [12] also found that MRD- and graft-versus-host disease (GVHD)-guided multiple consolidation chemotherapy and DLI reduced the cumulative incidence of relapse (CIR) and increased leukemia-free survival (LFS) and the survival rate in patients who had relapsed after allo-HSCT for acute leukemia, in comparison with the controls. However, approximately one-third of patients who were non-responsive to DLI had worse prognoses, and innovative approaches are urgently needed to improve the OS of these patients.

Leukemic cells can be eliminated efficiently by cluster of differentiation (CD)19-directed chimeric antigen receptor-modified (CAR) T cells (CART19s) in relapsed patients treated with chemotherapy; they also play an important role in patients after HSCT. Historically, CART19s can be developed from either the patient or a donor. Although autologous CART19s can exhibit potent anti-leukemia activity before or after HSCT without GVHD, it may be difficult to obtain sufficient high-quality T cells from patients with previous HSCT, those having undergone chemotherapy, and those with disease recurrence. Donor-derived CART19s wield potent graft-versus-leukemia (GVL) activity without affecting the cytotoxic activity from CART19s [13], and the safety and effectiveness of donor-derived CART19s were the focus of attention in this study. Brudno et al. [14] evaluated the effect of CART19s in morphologically relapsed patients after human leukocyte antigen (HLA)-matched sibling donor and unrelated donor allogeneic blood or marrow transplantation with the absence of GVHD. Subsequently, donor-derived CD19-targeted T cell infusions induced MRD-negative remission in patients with relapsed B-ALL who had no response to DLI after haplo-HSCT [15].

These clinical studies indicate that donor-derived CART19s can eliminate leukemic cells in relapsed patients after HSCT, even in patients with no response to DLI. Preliminary results also indicate that donor-derived anti-CD19 CAR T cell infusion is safe for patients with relapsed B cell malignancies after HSCT. Whether or not donor-derived CAR T cell infusions can be used as an effective intervention to treat MRD in B cell malignancies after HSCT has not been determined. In the present study, we evaluated preparatory clinical outcomes following donor-derived CAR T cell infusion in patients with MRD of B cell lineage malignancies who were non-responsive to DLI after allo-HSCT.

2. Methods

2.1. Patients

Patients who had received a CAR T cell infusion between September 2014 and February 2018 at the Peking University Institute of Hematology were enrolled in the present study if they met the following criteria: ① They were diagnosed with B-ALL; ② they had MRD after allo-HSCT, their MRD was defined as morphologic remission, and they received positive results on any MRD test, including flow cytometry, *WT1* expression, and fusion gene detection by quantitative real-time polymerase chain reaction (qPCR) in patients with detectable fusion genes; ③ they showed no response to one or more DLIs (no response was defined as MRD-positive based on testing of bone marrow aspirate 25–30 d after DLI); and ④ in these patients, CD19 expression on leukemic cells was confirmed by flow cytometry.

The protocol was evaluated and allowed by the Peking University People's Hospital review board. All enrolled patients gave informed consent in accordance with the Declaration of Helsinki.

2.2. Haplo-HSCT procedure

The haplo-HSCT procedure, including the conditioning regimen, GVHD prophylaxis, stem cell collection, and supportive care, is described in our previous report [16]. The following drugs were administered during the conditioning regimen: cytosine arabinoside ($4 \text{ g} \cdot (\text{m}^2 \cdot \text{d})^{-1}$ for 2 d); busulfan (Bu) ($3.2 \text{ mg} \cdot (\text{kg} \cdot \text{d})^{-1}$ for 3 d); cyclophosphamide (Cy) ($1.8 \text{ g} \cdot (\text{m}^2 \cdot \text{d})^{-1}$ for 2 d); semustine ($250 \text{ mg} \cdot \text{m}^{-2}$ for 1 d); and anti-human thymocyte immunoglobulin ($2.5 \text{ mg} \cdot (\text{kg} \cdot \text{d})^{-1}$ for 4 d; Thymoglobulin, Genzyme Corporation, Boston, MA, USA). All patients received granulocyte colony-stimulating factor-mobilized bone marrow cells plus peripheral blood stem cells. All transplant recipients received cyclosporine A (CsA), mycophenolate mofetil, and short-term methotrexate (MTX) for prophylaxis against acute post-transplantation GVHD.

2.3. HLA-matched sibling donor HSCT procedure

All patients were treated with a modified BuCy2 regimen consisting of the following: hydroxyurea ($80 \text{ mg} \cdot \text{kg}^{-1}$, orally in two doses on day -10); Ara-C ($2 \text{ g} \cdot \text{m}^{-2}$, intravenous injection on day -9); and Bu ($3.2 \text{ mg} \cdot (\text{kg} \cdot \text{d})^{-1}$ administered intravenously on days -8 to -6), Cy ($1.8 \text{ g} \cdot (\text{m}^2 \cdot \text{d})^{-1}$, days -5 to -4), and semustine ($250 \text{ mg} \cdot \text{m}^{-2}$, day -3). For GVHD prophylaxis, CsA was used as described above; mycophenolate mofetil was discontinued on the day of myeloid recovery, and MTX was administered on days 1, 3, and 6 [17].

2.4. DLI procedure

In the present study, DLI consisted of an infusion of granulocyte colony-stimulating factor-mobilized peripheral blood stem cells after chemotherapy and administration of immunosuppressive agent post-infusion to prevent GVHD [18]. The median dose of mononucleated cells was $1 \times 10^8 \text{ kg}^{-1}$. After DLI, all patients received immunosuppressive agents such as CsA to prevent GVHD for 4–6 weeks at the discretion of the attending physicians (usually depending on the patient's GVHD status after DLI). The initial dose of CsA was $2.5 \text{ mg} \cdot (\text{kg} \cdot \text{d})^{-1}$, and the dose was adjusted to maintain a plasma concentration of 150–250 ng·mL $^{-1}$. No response to DLI was defined as the detection of MRD one month after DLI.

2.5. Cell production

We produced anti-CD19 CAR T cells derived from peripheral blood mononuclear cells (PBMCs) from the allogeneic transplant donors of each patient via apheresis or peripheral blood. The duration of CAR T cell production was 5–15 d. T cells were activated and modified to express the *4SCAR19* gene after PBMCs were obtained. On days -5 to -7 , PBMCs were activated and enriched for T cells, followed by *4SCAR19* lentiviral transduction. Prior to CART19 infusion, the CART19s for each patient were subjected to a fluorescence-activated cell-sorting (FACS) analysis of transduction efficiency and *in vitro* cytotoxicity assays. In addition, possible fungal, bacterial, mycoplasma, chlamydia, and endotoxin contamination was evaluated during CART19 cultures. The other details of CART19s are provided in the Appendix A.

2.6. CAR T cell infusion protocol

Patients were administered a conditioning treatment for lymphodepletion. Most received a fludarabine- or cyclophosphamide-based conditioning treatment according to the tumor burden and the discretion of the attending physicians (details are described in Section 3.2). CART19s were transfused directly to patients without premedication; the dosages and characteristics of the infused cells

are provided in Section 3.4. Patients received the second CAR T cell infusion dose when one of the following criteria was fulfilled: ① Positive results of MRD were detected in the patients after the first infusion; or ② the patients suffered from morphological relapse.

2.7. Toxicity of the infusion of CAR T cells and GVHD

Toxicity was graded using the common criteria for cytokine release syndrome (CRS) after CAR T cell infusion [19,20]. The possibility of infection was considered in all patients presenting with CRS symptoms after obtaining the appropriate cultures and initiating empiric antibiotic treatment.

GVHD was diagnosed as acute or chronic according to the clinical features of the affected organs, and acute graft-versus-host disease (aGVHD) was graded according to previously published criteria [21]. Primary therapy consisting of methylprednisolone or a dexamethasone equivalent was administered when GVHD could not be ruled out. Methylprednisolone was administered intravenously at a dose of 1–2 mg.(kg.d)⁻¹. CsA was administered and then adjusted to maintain a blood concentration greater than 150 ng.mL⁻¹ in patients whose GVHD was not controlled.

2.8. Response evaluation

A bone marrow examination was performed on the 15th and/or 30th day after CAR T cell infusion to assess the clinical effect, or sooner if clinically indicated. Subsequently, bone marrow examination was performed every 1–3 months. A bone marrow smear and MRD detection by flow cytometry, fusion gene detection by qPCR, and measurement of *WT1* gene expression levels were performed for all patients.

MRD-negative CR was defined as morphologic remission and negative results on all MRD tests, including *WT1* expression, flow cytometry, and fusion gene detection by qPCR in patients with detectable fusion genes.

2.9. Statistical analysis

The patient characteristics were evaluated using descriptive statistics. MRD rates were compared using Fisher's exact test, and qualitative variables were assessed using Student's *t*-test. All analyses were performed using SPSS software version 19 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of patients

Six B-ALL (three females, three males; age range: 12–46 years old) with MRD after allo-HSCT (four haplo-HSCT and two HLA-matched sibling donor HSCT cases) were enrolled in this study. All patients experienced MRD and were non-responsive to at least one course of DLI (Table 1). Five patients received more than one infusion each (total infusions: 15), and the median CAR T cell infusion dose was 1.51 × 10⁸ cells.kg⁻¹ (range: 2 × 10⁷–3.66 × 10⁸ cells.kg⁻¹). All patients were observed until February 2018.

3.2. CR rate and clinical results

Five of six (83.33%) patients achieved MRD-negative CR after the first infusion, and five patients remained healthy for a median of 3 months (range 2–17 months) after CAR T cell infusion. Patients 1–4 developed recurrent MRD. Patient 1 received a second infusion, but failed to achieve remission. Patient 2 received a second infusion and again achieved remission, but suffered MRD recurrence 2 months later. Subsequently, patient 2 was administered four infusions of CART19s and failed to attain remission. Patients 3 and 4 received second infusions and continue to live without leukemia. Patient 5 did not respond to the first and the second CAR T cell infusion, developed morphologic relapse, and then died of leukemia. Patient 6 achieved remission and continues to live without disease. All details are summarized in Tables 2 and 3.

3.3. Graft-versus-host disease

No patient developed aGVHD during the follow-up CAR T cell infusion.

3.4. Cytokine release syndrome

The toxicities observed after CAR T cell infusion, which included fever, hypotension, hypoxemia, and elevated alanine transaminase (ALT), were classified as CRS after ruling out other causes. CRS was scored on the basis of a revised grading system [21]. The toxicities that occurred in each patient are listed in Table 4. Six patients experienced a total of five (83.33%) CRS events, and these five events occurred after the first infusion (grade 1, two events; grade 2, one event; grade 3, two events).

One patient required medication intervention (tocilizumab): Patient 6 developed a rash, fever, and hypotension on the 8th

Table 1
Characteristics of patients.

Patient	Age (years old)	Sex	Donor/type of transplant	Malignancy	Detection of MRD	Status before CAR T cell infusion	DLI before CAR T cell infusion	Frequency of CAR T cell infusion
1	16	M	Father/haplo-HSCT (4/6)	B-ALL ph+ (T3151) CNSL TL	FCM (0.02%), <i>WT1</i> , <i>BCR/ABL</i> = 1.4%	MRD+, CNSL	6	1 + 1
2	12	F	Father/haplo-HSCT (3/6)	B-ALL, <i>TEL/AML1</i> +	FCM, <i>WT1</i>	MRD+	1	1 + 5
3	40	M	Brother/haplo-HSCT (4/6)	B-ALL ph+ CNSL	FCM, <i>WT1</i> , <i>BCR/ABL</i>	MRD+, CNSL	2	1 + 1
4	28	F	Father/haplo-HSCT (3/6) The second HSCT, CP3	CML lymphoblastic crisis	FCM, <i>WT1</i> , <i>BCR/ABL</i>	MRD+	2	1 + 1
5	18	M	Sibling (6/6)	B-ALL	FCM, <i>WT1</i>	MRD+	2	1 + 1
6	46	F	Sibling (6/6)	B-ALL ph+	FCM, <i>WT1</i> , <i>BCR/ABL</i>	MRD+	1	1

CML: chronic myelogenous leukemia; CNSL: central nervous system leukemia; F: female; FCM: flow cytometry; M: male; ph+: philadelphia chromosome-positive; sibling: HLA-matched sibling donor; TL: testicular leukemia.

Table 2

The first infusion of CAR T cells and patient outcomes.

Patient	Chemotherapy before infusion	Chimeric	CAR T cells infused (kg)	CRS	Evaluation of MRD	GVHD	Glu	Virus	Bas	MR time (month)	Clinical outcome	Survival time after infusion (month)
1	CODP	—	1.10×10^8	2	CR	—	—	Herpes simplex (12th month)	—	5	Relapse (death)	12
2	Cy	Full chimera	2.00×10^7	3	CR	—	—	—	—	2	Relapse (death)	12
3	COMP	Full chimera	2.24×10^8	1	CR	—	—	—	—	3	MR (CNSL-CR)	15 (alive)
4	None	Full chimera	9.00×10^7	1	CR	—	—	—	—	4	MR	16 (alive)
5	CHOP	—	1.30×10^8	—	NR 0.07% (3%)	—	—	—	—	—	Relapse (death)	5
6	Flu + Cy	Full chimera	3.66×10^8	3 ^a	CR	—	—	—	—	17	MR	17 (alive)

Bas: basiliximab; CHOP: cyclophosphamide ($750 \text{ mg}\cdot\text{m}^{-2}$ on day 1) + vincristine ($1.4 \text{ mg}\cdot\text{m}^{-2}$ on days 1, 8, 15, 21) + adriamycin ($50 \text{ mg}\cdot\text{m}^{-2}$ on day 1) + prednisone ($60 \text{ mg}\cdot\text{m}^{-2}$ on days 1–21); CODP: cyclophosphamide ($750 \text{ mg}\cdot\text{m}^{-2}$ on day 1) + vincristine ($1.8 \text{ mg}\cdot\text{m}^{-2}$ on days 1, 8, 15, 21) + daunorubicin ($40\text{--}60 \text{ mg}\cdot\text{m}^{-2}$ on days 1–3) + prednisone ($60 \text{ mg}\cdot\text{m}^{-2}$ on days 1–21); COMP: cyclophosphamide ($400 \text{ mg}\cdot\text{m}^{-2}$ on days 1, 8) + vincristine ($1.4 \text{ mg}\cdot\text{m}^{-2}$ on days 1, 8) + mitoxantrone ($5 \text{ mg}\cdot\text{m}^{-2}$ on days 1–4) + prednisone ($60 \text{ mg}\cdot\text{m}^{-2}$ on days 1–21); Cy: cyclophosphamide ($500 \text{ mg}\cdot\text{m}^{-2}$ on days 1–3); Flu: fludarabine ($30 \text{ mg}\cdot\text{m}^{-2}$ on days 1–3); Glu: glucocorticoid; MR: molecular remission. NR: no response.

^a Patient 6 required medication intervention (tocilizumab $\times 2$ on days 8 and 9).

Table 3

The second infusion of CAR T cells and patient outcomes.

Patient	Chemotherapy	Evaluation before CAR T cell fusion	CAR T cells infused (kg)	CRS	Evaluation	Clinical outcomes
1	CODP	MRD+	Cy 2.60×10^8	0	MRD+	Relapse (death), 7 months
2	Ara-C	MRD+	—	0	MRD+	Relapse (death), 12 months
3	Flu	MRD+, CNSL	8.00×10^7	0	CR	Alive
4	None	Relapse	6.20×10^7	0	CR	Alive
5	Flu + Cy	MRD+	1.70×10^8	0	Relapse	Relapse (death), 3 months

day after infusion and received two doses of tocilizumab on the 8th and 9th days after infusion.

3.5. Peripheral blood CART19s

During four infusion courses, CART19s in the peripheral blood were measured by qPCR on the 7th, 14th, and 21st days after infusion. The number of CART19s in the peripheral blood peaked on the 7th day and decreased by the 21st day after infusion (Table 4).

4. Discussion

MRD is a good method to predict hematological relapse after HSCT [22,23], and eliminating MRD can prevent forthcoming relapse. Several measures were adopted to treat MRD. Interferon therapy and chemotherapy followed by DLI constitute an important intervention for relapsed acute lymphoblastic leukemia after HSCT. Mo et al. [24] have indicated that preemptive interferon- α (IFN- α) therapy may be an alternative for MRD-positive patients who cannot receive preemptive DLI after HSCT, based on a comparison of the results of DLI and IFN- α treatment in acute lymphoblastic leukemia patients with positive MRD after HSCT. In this comparison, the one-year probabilities of DFS after intervention were 68.2% and 60.0% for patients in the IFN- α and DLI groups, respectively. Yan et al. [12] have suggested that MRD- and GVHD-guided multiple consolidation chemotherapy and DLI could reduce CIR and increase LFS and survival in comparison with the controls in persons relapsing after allo-HSCT with acute leukemia. The three-year CIR, LFS, and survival post-transplant were 32.4%, 50.3%, and 51.4%, respectively. Ma et al. [25] have reported that 19 patients with a high risk for disease who relapsed after allo-HSCT were treated with DLI and monitored for MRD. Six of these patients (31.58%) showed no leukemic progression after DLI. Thus, it can be seen that the DLI approach was of limited efficacy in treating acute lymphoblastic leukemia and could result in life-threatening GVHD. Moreover, approximately one-third of patients with MRD may be non-responsive to DLI and have the worst prognosis. These patients should be treated using innovative approaches.

CAR-engineered T cells present a novel and promising immunotherapy [26]. Infusion of donor-derived CART19s after HLA-matched sibling donor/unrelated donor transplantation was investigated, and four of five (80%) patients with B-ALL achieved remission without the occurrence of aGVHD [14]. We also conducted a clinical study for donor-derived CD19-targeted T cell infusion and found that this treatment induced MRD-negative remission in relapsed B-ALL with no response to donor lymphocyte infusions after haplo-HSCT. Five of six (83.33%) patients achieved MRD-negative remission; furthermore, CRS and GVHD were controlled [15]. In summary, donor-derived CD19-targeted T cell infusion seems to be effective for relapsed patients with B-ALL after allo-HSCT. However, it remains unknown whether CAR T cell infusions can be effective in patients with MRD who are resistant to DLI after HSCT. In the present study, the results indicated that donor-derived CART19s can also be effective in treating patients with MRD after HSCT. As far as we know, the present study comprises the largest investigation of donor-derived CART19 infusion in relapsed patients with B-ALL after allo-HSCT. The results of this study offer preliminary evidence of the effectiveness of eliminating MRD by means of donor-derived CART19 infusion.

The safety of donor-derived CAR T cell infusion has long been a concern. In *in vitro* experiments, donor CART19s could promote effective GVL activity in the absence of damaging GVHD activity without affecting the cytotoxic activity of CART19s [27]. In the present study, the safety of donor-derived CAR T cell infusions for HSCT recipients was also mainly evaluated with respect to aGVHD and CRS. No patient developed aGVHD, which was the complication of primary concern. The incidence and intensity of GVHD in the present study were less than those resulting from the CAR T cell infusions that were used to treat relapse patients previously [15]. This clinical phenomenon was consistent with the outcome reported by Jacoby et al. [28]. *In vitro*, murine allogeneic CD19 T cells display potent antileukemic activity, but demonstrate potentially lethal GVHD. However, CAR-induced GVHD occurred only with the appearance of leukemic cells. CRS was another major concern. In the present study, no patient died directly of CRS. Moreover, no case of CRS-related cerebral edema occurred, despite the

Table 4
Infused cell characteristics and associated toxicities.

Patient	Stage	CAR T cells infused (kg)	Cell percentage (%)			CD8/CD4 in infused CAR T cells	Content of CART19s detected in peripheral blood (detection time)			CRS symptoms
			CD3 ⁺	CD4 ⁺	CD8 ⁺		CD56 ⁺	1st detection	2nd detection	
1	1st infusion	1.10 × 10 ⁸	88	40	48	11	0.01 (1st DAI)	0.88 (38th DAI)	0 (100th DAI)	Fever, hypotension, dysfunctional blood coagulation
2	2nd infusion	2.60 × 10 ⁸	—	—	—	—	0 (1st DAI)	0 (7th DAI)	0 (21st DAI)	—
3	1st infusion	2.00 × 10 ⁷	—	—	—	—	0.21 (7th DAI)	1.31 (12th DAI)	4.28 (20th DAI)	Fever, hypotension, hypoxemia
3	1st infusion	2.24 × 10 ⁸	—	—	—	—	0 (4th DAI)	1.0 × 10 ⁵ copies (μg·g) ⁻¹ DNA (7th DAI)	0 (20th DAI)	Fever
3	2nd infusion	8.00 × 10 ⁷	—	—	—	—	—	2.0 × 10 ⁵ copies (μg·g) ⁻¹ DNA (7th DAI)	0 (21st DAI)	—
4	1st infusion	9.00 × 10 ⁷	—	—	—	—	0 (4th DAI)	6.0 × 10 ⁵ copies (μg·g) ⁻¹ DNA (7th DAI)	0 (21st DAI)	Fever
4	2nd infusion	6.20 × 10 ⁷	—	—	—	—	0 (1st DAI)	4.6 × 10 ⁵ copies (μg·g) ⁻¹ DNA (7th DAI)	0 (28th DAI)	—
5	1st infusion	1.30 × 10 ⁸	—	—	—	—	0 (1st DAI)	0.12 (7th DAI)	0.05 (21st DAI)	Fever
5	2nd infusion	1.70 × 10 ⁸	—	—	—	—	0 (1st DAI)	0 (7th DAI)	0 (14th DAI)	—
6	1st infusion	3.66 × 10 ⁸	—	—	—	—	0.32 (2nd DAI)	13.86 (7th DAI)	10.87 (30th DAI)	Fever, hypotension, dysfunctional blood coagulation

DAI: day after infusion.

inclusion of central nervous system leukemia (CNSL) patients. Therefore, donor-derived CAR T cell infusions seem to be safe for MRD in B-ALL after HSCT.

In the present study, five of six (83.33%) patients with MRD before CAR T cell infusion attained molecular CR after the first infusion, and three patients have survived to the present day. In our previous study, four of five responsive patients showed hematological relapse after 2–7 months [15]. Although the number of cases was less in this study, determining whether the effect of MRD treatment was adequate in relapse patients warranted this evaluation.

5. Summary

Based on clinical observation, we propose that donor-derived CAR T cell infusion is an effective and safe intervention for eliminating MRD in patients with B cell malignancies after HSCT. It was even more impressive that no patient developed GVHD during the period under observation in the present study. However, the mechanism remains unclear. Further experiments and larger scale clinical trials are required to confirm whether CAR T cell infusion can be applied as a first-line intervention measure to eliminate MRD in patients with no response to DLI and in patients with MRD.

Acknowledgement

This work was supported by the Scientific Research Foundation for Capital Medicine Development (2018-2-4084).

Author contributions

Yifei Cheng and Yuhong Chen analyzed the material and wrote the paper. Shasha Wang collected the data. Xiangyu Zhao, Chenhua Yan, Yu Wang, Yao Chen, Wei Han, Lanping Xu, and Xiaohui Zhang performed the research. Lungji Chang and Lei Xiao contributed to cell production. Kaiyan Liu designed the research. Xiaojun Huang designed the research and edited the manuscript.

Compliance with ethics guidelines

The present protocol was evaluated and allowed by the Peking University People's Hospital review board. All enrolled patients gave informed consent in accordance with the Declaration of Helsinki.

Yifei Cheng, Yuhong Chen, Chenhua Yan, Yu Wang, Xiangyu Zhao, Yao Chen, Wei Han, Lanping Xu, Xiaohui Zhang, Kaiyan Liu, Shasha Wang, Lungji Chang, Lei Xiao, and Xiaojun Huang declare that they have no conflict of interest or financial conflicts to disclose.

Nomenclature

- aGVHD acute graft-versus-host disease
- allo-HSCT allogeneic hematopoietic stem cell transplantation
- ALT elevated alanine transaminase
- B-ALL B cell acute lymphoblastic leukemia
- Bu busulfan
- CAR chimeric antigen receptor
- CART19 CD19-directed chimeric antigen receptor-modified T cell
- CD cluster of differentiation
- CIR cumulative incidence of relapse

CR	complete remission
CRS	cytokine release syndrome
CsA	cyclosporine A
Cy	cyclophosphamide
DLI	donor lymphoblastic infusion
EFS	event-free survival
FACS	fluorescence-activated cell-sorting
GVHD	graft-versus-host disease
GVL	graft-versus-leukemia
haplo-HSCT	haploidentical hematopoietic stem cell transplantation
HLA	human leukocyte antigen
HSCT	hematopoietic stem cell transplantation
LFS	leukemia-free survival
MRD	minimal residual disease
MTX	methotrexate
OS	overall survival
PBMC	peripheral blood mononuclear cell
qPCR	quantitative real-time polymerase chain reaction

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.eng.2018.12.006>.

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