# Donor-derived Anti-CD19 CAR T cells GC007g for relapsed or refractory B-cell acute lymphoblastic leukemia after allogeneic HSCT: a phase 1 trial

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## Summary

**Background** Although chimeric antigen receptor-modified T cells (CAR T) cell therapy has been widely reported in improving the outcomes of B-cell acute lymphoblastic leukemia (B-ALL), less research about the feasibility and safety of donor-derived CAR T after allogeneic hematopoietic stem cell transplantation (allo-HSCT) was reported.

**Methods** This phase 1 clinical trial aims to evaluate safety and efficacy of donor-derived anti-CD19 CAR T cells (GC007g) in B-ALL patients who relapsed after allo-HSCT. This trial is registered with ClinicalTrials.gov, NCT04516551.

**Findings** Between 15 March 2021 and 19 May 2022, fifteen patients were screened, three patients were excluded due to withdraw of consent, donor's reason, and death, respectively. Patients received donor-derived CAR T cells infusions at $6 \times 10^5$/kg (n = 3) or $2 \times 10^6$/kg (n = 6) dose level. The median time from HSCT to relapse was 185 days (range, 81–2063). The median age of patients was 31 years (range 21–48). Seven patients (77.8%) had BCR-ABL fusion gene. CAR T cells expanded in vivo and the median time to reach $C_{max}$ was 9 days (range, 7–11). One patient had hyperbilirubinemia after GC007g infusion which was defined as a dose-limiting toxicity. All patients experienced CRS and hematological adverse events. Three patients had acute graft-versus-host-disease (grade I, n = 1; grade II, n = 1; grade IV, n = 1) and all resolved after treatment. They received CAR T cells from matched sister, haploidentical matched father and sisiter, respectively. At 28 days after infusion, all patients achieved complete remission with/without incomplete hematologic recovery (CRi/CR) with undetectable MRD. At a median follow-up of 475 days (range 322–732), seven patients remained in CR/CRi while two had CD19-negative relapse. The overall response rates (ORR) were 100% (9/9), 88.9% (8/9), and 75% (6/8) at 3 month, 6 month, and 12 month, respectively. The 1-year progression-free and overall survival were 77.8% and 85.7%, respectively.

**Interpretation** GC007g expanded and induced durable remission in patients with B-ALL relapsed after allo-HSCT, with manageable safety profiles.

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Keywords: Donor-derived CAR T; B-cell acute lymphoblastic leukemia; Allogeneic hematopoietic stem cell transplantation; Safety

## Research in context

### Evidence before this study

We searched PubMed for full-text clinical trials written in English published up to March 10, 2023, to identify papers on donor-derived CAR T after allo-HSCT. The search terms used were "(donor-derived) AND (CAR-T)", and "(CAR-T) AND (post-HSCT)". The search revealed a scarcity of prospective clinical trials of donor-derived CAR-T cells administered in patients who relapsed after allo-HSCT.

### Added value of this study

To the best of our knowledge, this is the first phase I clinical trial to evaluate the safety and efficacy of donor-derived CAR-T cells in patients who relapsed after allo-HSCT. In this study, we provide support for the feasibility and efficacy of this donor-derived CAR-T cells product, and identified the appropriate dose for a phase II trial in future.

### Implications of all the available evidence

Our study findings provide support for the safety and efficacy of donor-derived CAR-T cells in patients who relapsed after allo-HSCT, which warrant further large-scale and multi-centre clinical trials evaluating it.

## Introduction

Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most effective and potentially curative treatments for patients with B-cell acute lymphoblastic leukemia (B-ALL), 26–66% patients experience relapse after allo-HSCT.[1–3] The prognosis of post-transplantation relapse is dismal and current treatment options remain limited, with an average survival of less than 6 months and estimated 5-year survival rates of approximately 10%.[4] It is thus critical to develop advanced strategies that can effectively eradicate B-ALL after post-HSCT relapse.

In the past decade, chimeric antigen receptor-modified T cells (CAR T) therapies have emerged as a new strategy for cancer treatment. CD19-targeted CAR T cell therapy has shown promising results in which the complete remission (CR) rates as high as 70%–90% in treating relapsed or refractory (R/R) B-ALL.[5–7] Despite the potent antitumor efficacy, production of autologous CAR T cells can be challenging particularly in some heavily-pretreated patients with profound lymphopenia or rapidly progressing disease. Furthermore, the biological characteristics of autologous T cells are also adversely affected by the previous therapies.[8] Therefore, a donor-derived CAR T product would be an attractive option for patients who relapsed from previous allo-HSCT, due to the healthy T cells of the donor. Meanwhile, as the patients' immune systems have been reconstituted with donor-derived cells, the allogeneic CAR T cells are less likely to get rejected which may allow for long-term persistence.

Previous studies have shown high CR rates of 79–89% with donor-derived CAR T cells therapy.[9,10] A recent study of donor-derived CD19 CAR T cells in pediatric B-ALL reported comparable efficacy and toxicity when compared with their data from a clinical trial on autologous CAR T cells.[11] However, concerns remain in the potential risk of graft-versus-host disease (GvHD) caused by donor-derived CAR T cells and the durability of CAR T cells.[12,13] Here, we conduct this phase 1 clinical trial to evaluate the safety and efficacy of a donor-derived anti-CD19 CAR T cells, GC007g, for R/RB-ALL patients who relapsed after allo-HSCT.

## Methods

### Participants

Patients age 18–70 years, diagnosed with B-ALL who relapsed after allo-HSCT with Eastern Cooperative Oncology Group status ≤1, lymphocyte count $\geq 1 \times 10^8$ cells per L, absolute neutrophil count $\geq 1 \times 10^9$ cells per L, platelet count $\geq 50 \times 10^9$ cells per L, without uncontrollable infections, or organ failure were deemed eligible. Patients were required to have ≥5% blasts with CD19 expression in bone marrow. For patients with BCR-ABL fusion gene were required to be resistant to at least two kinds of Tyrosine Kinase Inhibitors (TKI) or have ABL kinase mutations which were resistant to currently available TKIs. Patients were excluded if they previously received autologous CD19-CAR T cells. Patients who had central nervous system leukemia, a history of grade III-IV aGvHD or severe cGvHD were excluded.

### Study design

This is a single-arm, phase 1 study of donor-derived anti-CD19 CAR T cells (GC007g) which was conducted in two centers in China. The Ethics Review Committee at each study site approved the protocol. Patients and donors were required to provide written consent. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The CAR is composed of a mouse FMC63 anti-CD19 single-chain variable fragment (scFv), a GM-CSF R

signal peptide, a CD28 hinge and transmembrane domain, a CD28 costimulatory domain, and a CD3ζ activation domain. A FLAG tag (DYKDDDDK) was inserted into CD19-CAR between the scFv and the hinge region for detection by flow cytometry. Considering the potential contamination and difficult of manufacturing a drug product from heavily treated patients with profound lymphopenia and rapidly progressing leukemia, donor-derived T cells was preferred. Peripheral blood mononuclear cells (PBMCs) were obtained from donors by leukapheresis. T cells were isolated with Dynabeads CD3/CD28 CTS (Thermo Fisher Scientific) according to the manufacturer's instructions and transduced with lentiviral vectors carrying the CD19-CAR construct. CAR-T cells were cultured in X–VIVO 15 medium (Lonza) containing recombinant IL-2 for 7 days before harvest. After passing all tests required for release, cryopreserved products were transported to the hospital for infusion.

Bridging therapies were allowed but restrict to drugs which were used previously. Patients received lymphodepletion regimen of intravenous fludarabine 30 mg/$m^2$/d and cyclophosphamide 300 mg/$m^2$/d on days −5, −4 and −3 before infusion. Patients were consecutively assigned to receive a single dose of GC007g infusion at 2 doses levels (DL) of $6 \times 10^5$/kg CAR T cells or $2 \times 10^6$/kg CAR T cells. No anti-tumor treatment was given after CAR T cells infusion unless disease recurrence.

### Study end points and assessments

The primary objective of the study was to evaluate the safety including the incidence of dose-limiting toxicity (DLT) and adverse event (AE). DLT was determined within 28 days and defined as (1) ≥ grade 3 neurotoxicity or cytokine release syndrome (CRS) lasting more than 7 days or (2) other ≥ grade 2 non-hematological adverse events lasting more than 28 days. Adverse events were documented, and severity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0. CRS and neurotoxicity were graded according to the American Society for Transplantation and Cellular Therapy criteria[14] aGvHD were graded according to Glucksberg criteria[15] while cGvHD NIH criteria.[16]

Secondary endpoints included investigator-assessed ORR (CR + CR with incomplete count recovery [CRi]), ORR with negative minimal residual disease (MRD-negative, determined by flow cytometry) within 12 weeks, duration of remission (DOR), progression-free survival (PFS), overall survival (OS). These were based on National Comprehensive Cancer Network guidelines, version 1.2020. Secondary endpoints also included levels of CAR T cells and cytokines in blood, bone marrow and cerebrospinal fluid (CSF) (in necessary). Additional details of endpoints and specific procedures can be found in the protocol.

### Statistical analysis

The sample size was based on 3 + 3 design principles to determine maximum tolerated dose. Descriptive statistics included means with standard deviations or median with ranges for continuous variables and frequencies or percentages for categorical variables. PFS and OS were estimated using the Kaplan–Meier method. The chi-square statistic or Fisher exact test was used for comparisons between categorical variables, and the Mann–Whitney U test was used for continuous variables. Statistical analyses were performed using SAS 9.4. This study was registered with ClinicalTrials.gov, NCT04516551.

### Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors have access to the entire dataset. The decision to submit for publication was made by Y. L., L. G., J. L., X. Z., W. L., and H. H., and all authors agreed with the publication.

## Results

### Patients

Between 15 March 2021 and 19 May 2022, 12 patients were enrolled and their donors underwent leukapheresis. Three patients were excluded due to withdraw of consent, donor's reason, and death, respectively. All patients received bridging therapies. Three patients received TKI, two received TKI plus low-dose chemotherapy, four received more intensive chemotherapy. Nine patients successfully received GC007g infusion at $6 \times 10^5$/kg (DL1, n = 3) or $2 \times 10^6$/kg (DL2, n = 6), with a median time from leukapheresis to infusion of 33 (range, 30–74) days (Fig. 1). In the group of DL1, T cells were derived from matched brother, haploidentical matched father and brother, respectively. In the group of DL2, T cells were derived from related matched sibling donors (MSD) (n = 3) and related haploidentical matched donors (father, n = 1; daughter, n = 1; sister, n = 2). More detailed data of the drug product characteristics was provided in the Supplementary Table S1. There was no difference on the dose of CAR T cells infused between fully matched and haploidentical matched donors. The median age of patients was 31 years (range 21–48). Seven patients (77.8%) had BCR-ABL fusion gene and two had ABL1 kinase domain mutation. Six (66.7%) patients relapsed after haploidentical HSCT while three (33.3%) relapsed after related MSD HSCT. The median time form HSCT to relapse was 185 days (range, 81–2063). No patients had extramedullary disease involvement. At screening, patients had median 82% (6–93%) blast cells in the bone marrow (Table 1). More detailed data on patient characteristics and donor type was shown in the Supplementary Table S2.

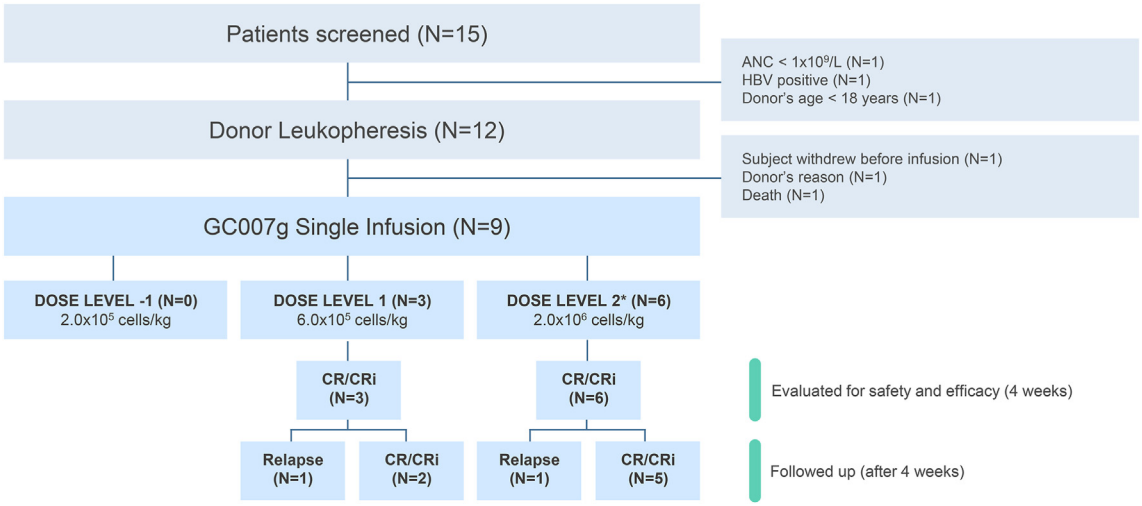


***Fig. 1:*** Consort diagram. The sample size was based on 3 + 3 design with 2 dose group (DL1: $6 \times 10^5$ CAR + T Cells/kg and DL2: $2 \times 10^6$ CAR + T Cells/kg).

## Safety

DLT was reported in one patient (DL2) who developed hyperbilirubinemia four days after GC007g CAR T cell infusion. He was diagnosed with grade IV GvHD and serum bilirubin level decreased to normal after treatment of glucocorticoid, cyclosporin A, and ruxolitinib.

All patients experienced CRS. Grade 1–2 CRS were reported in 8 of 9 patients (89%) and a grade 3 CRS was reported in 1 patient (11%, DL2 group). No grade 4 CRS was observed. The median time to CRS onset after infusion was 4 days (range 0–6) and the median duration of CRS symptoms was 5 days (range 1–10). Fever and hypotension were common symptoms of CRS. No immune effector cell associated neurotoxicity syndrome was observed. For CRS management, three patients were treated with tocilizumab, one patient with glucocorticoids and four patients with tocilizumab plus glucocorticoids. All the symptoms of CRS were rapidly relieved after treatment.

Three patients had acute graft-versus-host-disease (aGvHD) (grade I, n = 1; garde II, n = 1; grade IV, n = 1). They received CAR T cells from matched sister, haploidentical matched father and sisiter, respectively. None of these three patients had a history of GvHD during previous allo-HSCT. Two of them received GC007g infusion at $6 \times 10^5$/kg (DL1) while one patient received GC007g infusion at $2 \times 10^6$/kg (DL2). However, one of them experienced grade I aGvHD (skin and eyes) before lymphodepletion regimen (day-6) and proceeded to grade IV aGvHD (gastrointestinal and liver) at day 5. The symptoms of aGvHD occurred at a median of 5 days (range 3–32) post CAR T cells infusion, with a median duration of 15 days (range 7–44). And all patients relieved after administering methylprednisolone (n = 2) and ruxolitinib (n = 1). No chronic GvHD occurred during follow-up.

Grade 3 or greater hematological adverse events were observed in all patients, including neutropenia (n = 9), lymphocytopenia (n = 7), thrombocytopenia (n = 2), and anemia (n = 3). Infections occurred in 5 (55.5%) patients. Two patients experienced viremia with BK virus (n = 1) and cytomegalovirus (n = 1) respectively. Severe AEs of pneumonia occurred in two patients and both relieved after treatment. Other AEs (≥ grade 3) observed in ≥20% of patients were hypokalemia (n = 3), elevated glutamyltransferase (n = 4), elevated alanine aminotransferase (n = 3), and elevated aspartate aminotransferase (n = 2) (Table 2).

## Efficacy

All patients responded to treatment. One patient achieved CR and eight patients achieved CRi at 28 days after infusion, all with undetectable MRD. At a median follow-up of 475 days (range, 322–732), seven patients remained in CR/CRi while two had CD19-negative relapse (Fig. 2A; Supplementary Figure S1). One patient (Patient 04) relapsed at 175 days after infusion and died of DIC after dasatinib and ponatinib treatment. Another (Patient 02) relapsed at 259 day post-infusion, and decided to seek CAR T cell therapy followed by allo-HSCT at another institution. This patient has been alive till the cut-off date. A positive MRD by flow cytometry was reported in Patient 05 at 3 months after CAR T cells infusion, which turned to negative 6 months later without any further treatment. In all patients, ORR was 100% (9/9), 88.9% (8/9), and 75% (4/6) at 3 month, 6 month, and 12 month, respectively. The 1-year PFS and OS rate estimates were 77.8% and

| Characteristic | DL1: 6 × $10^5$/kg (n = 3) | DL2: 2 × $10^6$/kg (n = 6) | All patients (n = 9) |
|---|---|---|---|
| **Median age (range) y** | 35 (21–48) | 31 (27–46) | 31 (21–48) |
| **Male sex (%)** | 2 (67) | 3 (50) | 5 (56) |
| **ECOG performance status (%)** | | | |
| 0 | 3 (100) | 4 (67) | 7 (78) |
| 1 | 0 | 2 (33) | 2 (22) |
| **Disease type, N (%)** | | | |
| Common-B-ALL | 2 (67) | 5 (83) | 7 (78) |
| Pro-B-ALL | 1 (33) | 1 (17) | 2 (22) |
| **Philadelphia chromosome–positive (%)** | 1 (33) | 6 (100) | 7 (78) |
| **B-Blast cells CD20 + (%)** | 2 (67) | 2 (33) | 4 (44) |
| **Median time since diagnosis (range) months** | 10 (7–30) | 34 (8–84) | 15 (7–84) |
| **Median time from allogeneic HSCT to relapse (range) days** | 93 (81–282) | 809 (150–2063) | 185 (81–2063) |
| **Median time from allogeneic HSCT to infusion (range) days** | 139 (139–383) | 930 (208–2412) | 352 (139–2412) |
| **Median time from leukapheresis to infusion (range) days** | 31 (30–32) | 37 (31–74) | 33 (30–74) |
| **Previous lines of therapy (except allogeneic HSCT, %)** | | | |
| 1 | 1 (33) | 2 (33) | 3 (33) |
| 2 | 1 (33) | 2 (33) | 3 (33) |
| 3 | 1 (33) | 2 (33) | 3 (33) |
| **Type of allogeneic HSCT (%)** | | | |
| Matched sibling donor | 2 (67) | 1 (17%) | 3 (33) |
| Haploidentical donor | 1 (33) | 5 (83) | 6 (67) |
| **Number of previous allogeneic HSCT (%)** | | | |
| 1 | 2 (67) | 6 (100) | 8 (89) |
| 2 | 1 (33) | 0 | 1 (11) |
| **Median percentage of BM Blast (range)** | | | |
| At screening | 79 (56–82) | 89 (6–93) | 82 (6–93) |
| After lymphodepletion | 13 (2–92) | 0 (0–34) | 1 (0–92) |

DL: dose level; HSCT: hematopoietic stem cell transplantation; BM: bone marrow.

***Table 1:*** **Baseline characteristics of 9 treated patients and subgroups.**

85.7%, respectively (Fig. 2B). The median duration of DOR, PFS and OS was not reached.

### Clinical pharmacology

CAR T cells expansion and persistence was measured by CAR gene copy number and by flow cytometry in blood marrow and peripheral blood. CAR T cells expanded in all patients. The median time to reach maximum CAR T cell expansion (Cmax) was 9 days (range, 7–11) and Cmax was 54025 copies/μgDNA (range, 17,239–490,338) (Fig. 3A) in blood. CAR gene copies were still detectable in 67% (6/9), 67% (4/6) and 100% (2/2) in blood of patients at months 6, 12 and 24, respectively. No statistically significant difference in the CAR T persistence was observed between MSD and haploidentical donor derived CAR T ($p = 0.27$). In the two patients with CD19-negative relapse, one patient had detectable CAR T cells at the time of relapse while another patient wasn't detected. The maximum CAR T cell expansion was comparable between patients who developed aGVHD or not ($p = 0.9$). There was no difference in the dose of untrasduced T cells infused between patients who developed aGVHD or not ($p > 0.99$). The peak CAR T expansion was significantly higher in patients who had higher level of MRD (>1%) than those with lower lever of MRD (≤1%) after lymphodepletion (Fig. 3B). The Cmax was not apparently different between patient subgroups according to the severity of CRS, CAR T cells dose, GvHD, donor type, and time interval between CAR T and HSCT (Fig. 3B).

All patients experienced B-cell aplasia (BCA). The median onset of BCA was day 4 (range: 3–16). Five patients showed persistent BCA at the last follow-up. The median duration of BCA of other four patients was 208 days (range: 49–279 days).

## Discussion

Allogeneic CAR T cell products hold great potential to expand the repertoire of T cell donor selection and increase the accessibility of CAR T therapy. This registrational clinical trial has shown that donor-derived CAR T therapy post-HSCT is safe and can mediate potent antitumor effect, suggesting that donor-derived anti-CD19 CAR T therapy is a clinically feasible approach for patients who relapsed after Allo-HSCT.

| TEAE | DL1: 6 × $10^5$/kg (n = 3) | | DL2: 2 × $10^6$/kg (n = 6) | | All patients (n = 9) | |
|---|---|---|---|---|---|---|
| | Any grade | Grade ≥3 | Any grade | Grade ≥3 | Any grade | Grade ≥3 |
| **CRS** | 3 (100%) | 1 (33%) | 6 (100%) | 0 | 9 (100%) | 1 (11%) |
| **aGvHD** | 1 (33%) | 0 | 2 (33%) | 1 (17%) | 3 (33%) | 1 (11%) |
| Skin | 0 | 0 | 2 (33%) | 0 | 2 (22%) | 0 |
| Intestinal | 1 (33%) | 0 | 1 (17%) | 1 (17%) | 2 (22%) | 0 |
| Liver | 0 | 0 | 1 (17%) | 1 (17%) | 1 (11%) | 1 (11%) |
| Eyes | 0 | 0 | 1 (17%) | 0 | 1 (11%) | 0 |
| **Any TEAE** | 3 (100%) | 3 (100%) | 6 (100%) | 6 (100%) | 9 (100%) | 9 (100%) |
| **Hematological** | | | | | | |
| Neutrophil count decreased | 3.00 | 3 (100%) | 6 (100%) | 6 (100%) | 9 (100%) | 9 (100%) |
| White blood cell decreased | 2 (67%) | 1 (33%) | 6 (100%) | 4 (67%) | 8 (89%) | 5 (56%) |
| Lymphocyte count decreased | 2 (67%) | 2 (67%) | 6 (100%) | 5 (83%) | 8 (89%) | 7 (78%) |
| Platelet count decreased | 1 (33%) | 1 (33%) | 4 (67%) | 1 (17%) | 5 (56%) | 2 (22%) |
| Anemia | 1 (33%) | 1 (33%) | 3 (50%) | 2 (33%) | 4 (44%) | 3 (33%) |
| **Infection** | | | | | | |
| Pneumonia | 2 (67%) | 2 (67%) | 1 (17%) | 0 | 3 (33%) | 2 (22%) |
| BK virus infection | 1 (33%) | 0 | 0 | 0 | 1 (11%) | 0 |
| Cytomegalovirus | 1 (33%) | 0 | 0 | 0 | 1 (11%) | 0 |
| **Gastrointestinal disorders** | | | | | | |
| Diarrhea | 2 (67%) | 0.00 | 3 (50%) | 0 | 5 (56%) | 0 |
| GGT increased | 3 (100%) | 2 (67%) | 5 (83%) | 2 (33%) | 8 (89%) | 4 (44%) |
| ALT increased | 3 (100%) | 1 (33%) | 5 (83%) | 2 (33%) | 8 (89%) | 3 (33%) |
| AST increased | 3 (100%) | 0.00 | 5 (83%) | 2 (33%) | 8 (89%) | 2 (22%) |
| LDH increased | 3 (100%) | 0.00 | 6 (100%) | 0 | 9 (100%) | 0 |
| ALP increased | 2 (67%) | 1 (33%) | 3 (50%) | 0 | 5 (56%) | 1 (11%) |
| Bilirubin increased | 2 (67%) | 0 | 1 (17%) | 1 (17%) | 3 (33%) | 1 (11%) |
| **Metabolism and nutrition disorders** | | | | | | |
| Hypoalbuminemia | 2 (67%) | 0 | 4 (67%) | 0 | 6 (67%) | 0 |
| Hypophosphatemia | 1 (33%) | 0 | 2 (33%) | 0 | 3 (33%) | 0 |
| Hypocalcemia | 2 (67%) | 0 | 1 (17%) | 0 | 3 (33%) | 0 |
| Hypokalemia | 2 (67%) | 2 (67%) | 3 (50%) | 0 | 6 (67%) | 2 (22%) |
| Hypertriglyceridemia | 0 | 0 | 5 (83%) | 1 (17%) | 5 (56%) | 1 (11%) |
| Cholesterol high | 1 (33%) | 0 | 5 (83%) | 0 | 6 (67%) | 0 |
| Fibrinogen decreased | 2 (67%) | 1 (33%) | 1 (17%) | 0 | 3 (33%) | 1 (11%) |

TEAE: DL: dose level; aGvHD: cGvHD; GGT: gamma-glutamyl transpeptidase; ALT: alanine transaminase; AST: aspartate transaminase; LDH: lactate Dehydrogenase; ALP: alkaline phosphatase.

*Table 2:* **Adverse events.**

All patients in this clinical trial achieved MRD-negative CR/CRi with GC007g treatment, and CAR T cell expansion was detected in all patients, with 4 patients exhibiting CAR T persistence of over 300 days. These results suggested that donor-derived CAR T cells can successfully expand and eliminate leukemia cells in vivo. Furthermore, no statistically significant difference in the CAR T persistence was observed between MSD and haploidentical donor derived CAR T. Notably, a retrospective study suggested that the time to relapse post-HSCT can predict the outcome of CAR T therapy, and patients who relapsed within 6 months post-HSCT showed an OS rate as low as 16%.[17] Another reported predictive marker for allogeneic CAR T efficacy was pre-treatment tumor burden.[17] Additionally, high preinfusion disease burden was associated with CD19-relapse.[18] In this study, two patients relapsed after CAR T therapy. The time from allo-HSCT to relapse was 93 and 2063 days, respectively. The tumor burden of the two relapsed patients were relatively high as 92% and 34% before lymphodepletion. However, the potential biomarkers for GC007g clinical outcome need to be further elucidated in a larger cohort of patients in the next stages of clinical studies.

B-ALL is amongst the most intensively studied diseases using CAR T treatment, revealing that antigen loss and escape account for a big proportion of relapse.[19] The introduction of additional targets, such as CD20 and CD22, allows for broader targeting of leukemia cells with the expectation to overcome antigen-negative

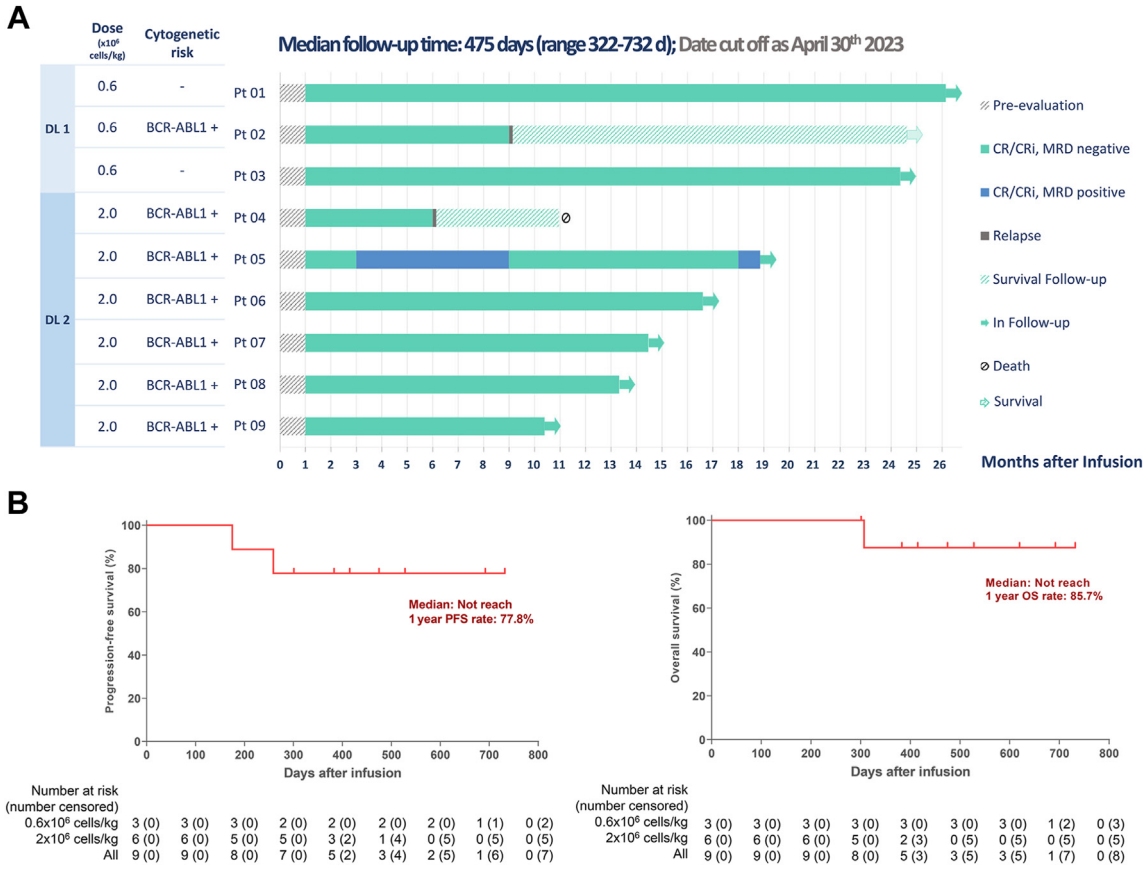


***Fig. 2:*** Clinical response. (A) Swimmer plot of 9 patients showing treatment responses after GC007g infusion. (B) Kaplan–Meier curves of progression-free survival and overall survival.

relapse. A previous report has used sequential infusion of CD19 CART followed by CD22 CAR T cells, resulting in 67% of patients achieving CR for more than 19 months. However, disease relapse still occurred in some patients even after two CAR T cell infusions.[20] Overall, it is critical for future research to identify key targets on leukemia cells relapsed after both CAR T therapy and HSCT, and develop specific therapeutic strategies. Also, it has been unclear about the best strategy to achieve multi-antigen targeting by CAR T cells, especially between a single infusion of dual-targeted cells and sequential infusion of single-targeted cells.[21] Another potential approach is second transplantation, which has been applied in patients who achieved remission after post CAR T infusion. A retrospective study reported 3-year non-relapse mortality was 51.1% and OS was 48.9% in 41 patients after second HSCT following CAR T.[22] In the present study, one patient relapsed and stayed alive after second HSCT. None of the remaining 7 patients received consolidation with allo-HSCT and all of them remained alive in CR. Three of these seven patients showed persistent BCA while four recovered from BCA. Prior HSCT and allogeneic CD19 hCAR T have been reported to achieve durable remission after CAR T treatment alone in a large retrospective research.[23] According to these results, donor-derived CAR T cells in the setting of allo-HSCT seems to induce longer remission. Whether graft-versus-leukemia effect from donor T cells or other mechanism plays a role need to be further explored.

GvHD has been one of the most critical safety concerns in allogeneic CAR T therapy. Previous studies reported preexisting GvHD before CAR T therapy, recent donor lymphocytes infusion, and haploidentical donor were associated with higher risk of post CAR T GvHD.[20,23,24] In present study, three patients developed short-term aGvHD after GC007g infusion. One patient who relapsed from haploidentical HSCT experienced grade I aGvHD before lymphodepletion regimen (day-6) and proceeded to grade IV aGvHD at day 5. The other two patients received CAR T cells from haploidentical and MSD donor and none of them had a history of GvHD. The untrasduced T cells infused and maximum CAR T cell expansion were comparable between patients who developed aGVHD or not. GC007g harbors a CD28 costimulatory signal, which has been shown in a

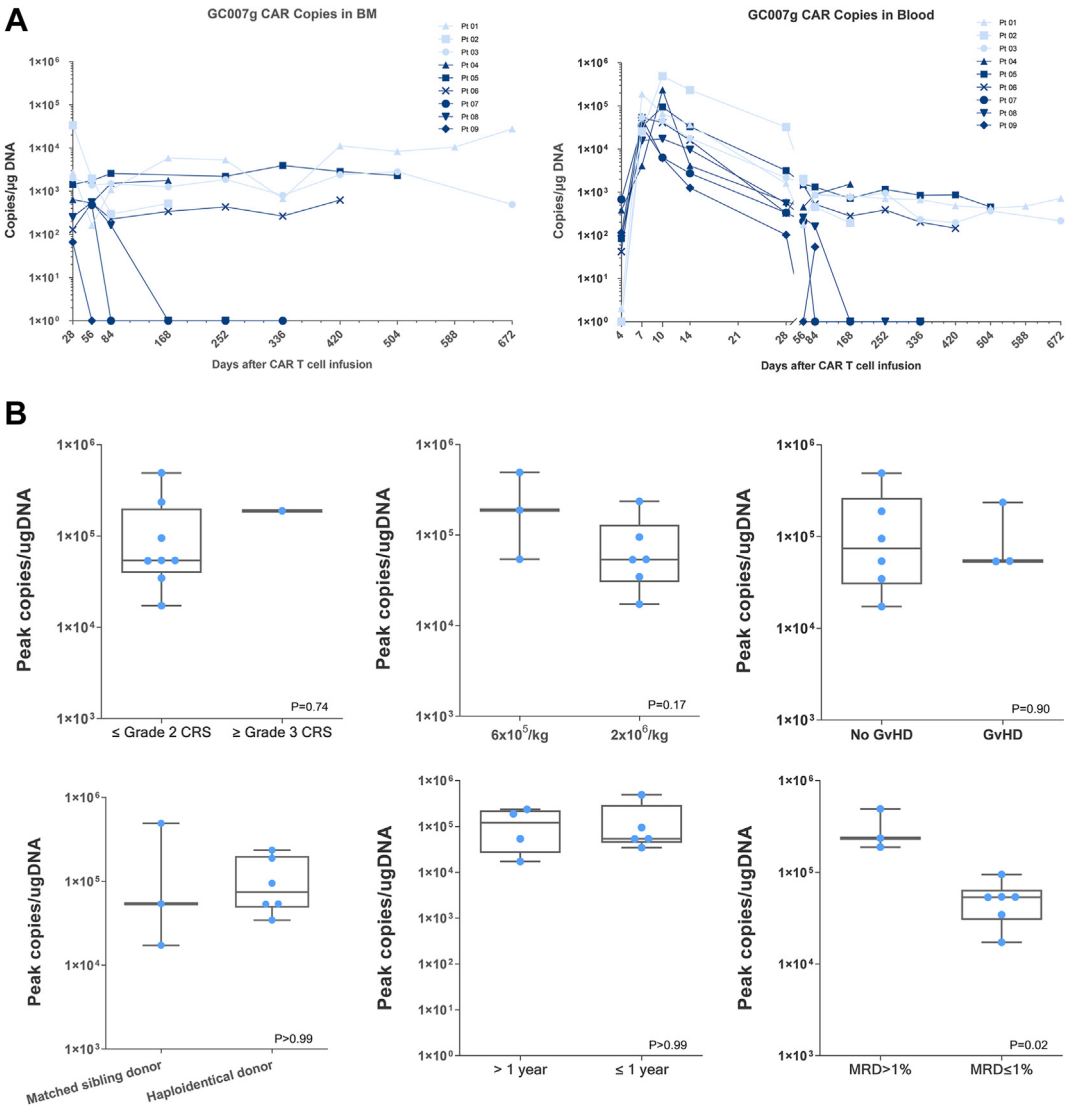


***Fig. 3:*** CAR T cells expansion and persistent. (A) Kinetics of CAR T cells in bone marrow and peripheral blood of individual patients measured by quantitative PCR. (B) The correlation between CAR T $C_{max}$ in bone marrow with CRS severity, infusion dose, occurrence of GvHD, donor type, time interval between allo-HSCT and CAR T, MRD before infusion.

previous study to diminish GvHD potential in animal models compared to the 4-1BB costimulatory domain.[25] In a retrospective study of a small cohort of patients post-HSCT, 4-1BB based CAR T therapy was shown to increase both CR rate and GvHD onset,[26] warranting the future evaluation of costimulatory domain selection in multi-center clinical studies. The number of patients in our study was too small to draw any conclusion.

The quality of CAR T cell products, including the memory-like phenotype and the expansion potential upon antigen stimulation, has been found to closely correlate with the clinical efficacy,[27,28] which also illustrates the necessity to refine the source of T cells. Patients who relapsed from allo-HSCT often suffered from severe immunosuppression and persistent lymphocytopenia. Patient-derived dysfunctional T cells sets challenge for leukapheresis, manufacturing of CAR T product. The use of donor-derived CAR-T cells may potentially overcome these hurdles by providing available healthy T cells and avoid leukemia contamination. A large retrospective study in Germany reported high 2-y relapse rates of 43.2% and OS of 53.2% although high CR rate of 87.7% after autologous CD19-directed CAR T cell therapy.[17] Large cohorts and clinical trials on donor-derived CAR T treatment are lacked. Huang et al. reported the CR rates of allogeneic CAR T cells and

autologous CAR T cells in the HLA-matched transplantation group and haploidentical transplantation group were 100% (3/3), 100% (4/4), 87.5% (7/8) and 75% (3/4).[24] Zhang et al. reported the CR rate and 1-year event-free survival of donor-derived CD19 CAR T were 79% and 43%.[9] The safety and efficacy profiles of this study, together with the previous reports, donor-derived CAR-T cells seems to be a better choice for patients with post-transplant relapse.

In conclusion, we have demonstrated that donor-derived CD19 CAR T cell therapy post-HSCT is safe and effective. The results of this study will lead to further assessment of GC007g in an expansion cohort of B-ALL patients with post-HSCT relapse.

#### Contributors

Y.L., W.L.W.C., X.Z., and H.H. designed the research; Y.L., L.G., J.L., L.W., X.L., Y.L., S.G., L.L., L.Z., Y.Y, X.Z. and H.H. enrolled and treated patients. L.Y., M.W., L.S., and D.W. analyzed the data and wrote the manuscript; and all authors provided patient data and gave final approval for the manuscript. The authors declare no competing financial interests.

#### Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### Declaration of interests

The authors declare that they have no competing interests.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102377.

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