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Donor hematopoietic stem cell/lymphocyte as maintenance treatment after CAR T-cell therapy in B-cell acute lymphoblastic leukemia relapsed after stem cell transplant

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Abstract

Background: Maintaining the efficacy of anti-CD19 chimeric antigen receptor modified (CAR) T-cell therapy in patients with B-cell acute lymphoblastic leukemia (B-ALL) relapsed after transplantation is an urgent problem.

Methods: Overall 22 B-ALL patients who relapsed after allogenic hematopoietic stem cell transplantation (allo-HSCT) received anti-CD19-CAR T-cell therapy. Patients who responded to CAR-T cell therapy received donor hematopoietic stem cell infusion (DSI) or donor lymphocyte infusion (DLI) as maintenance therapy. We compared the clinical responses, acute graft versus host disease (aGVHD), expansion of CAR-T cells, and adverse events between two groups.

Results: CR or CR with incomplete count recovery was achieved in 19 patients (86.4%). After DSI/DLI therapy, grade I-II of aGVHD was observed in 4 patients (36.4%) in DSI group. Only one patient developed grade II aGVHD in DLI group. The peaks of CAR T-cells in DSI group were higher than in DLI group. IL-6 and TNF-α levels increased again in 9 of 11 patients after DSI but not in DLI group. Progression-free survival and overall survival were higher in DSI group than in DLI group at 365 days.

Conclusions: For B-ALL patients who relapsed after allo-HSCT, DSI is a feasible maintenance therapy if CR is obtained with CAR-T cell therapy.

Trial registration:

ChiCTR-ONN-16009862 *Registered14November2016,https://www.chictr.org.cn/showproj.aspx?proj=16756*

ChiCTR1800019622 *Registered24November2018,https://www.chictr.org.cn/showproj.aspx?proj=33185*

Introduction

Allogeneic hematopoietic stem cell transplant (allo-HSCT) for the therapy of B-cell acute lymphoblastic leukemia (B-ALL) has been an effective treatment and improved the long-term survival significantly [1, 2]. However, some patients still relapse with a very poor prognosis[1]. Refractory B-ALL and its progression are the major causes of death in these patients[3]. Salvage chemotherapy, second allo-HSCT, and donor lymphocyte infusion (DLI) are considered salvage treatments for patients with B-ALL who relapsed after allo-HSCT. However, all these salvage treatments have very low complete response (CR) rates and poor results [4–7].

Anti-CD19 chimeric antigen receptor modified (anti-CD19-CAR) T cell immunotherapy has shown satisfactory effects in patients with relapsed/refractory (R/R) B-ALL [8–10]. However, the recurrence rate after anti-CD19-CAR T-cell therapy is approximately 34% [11]. Bridging allo-HSCT after anti-CD19-CAR T-cell therapy in R/R B-ALL patients could improve progression-free survival (PFS) without increasing the risk of severe acute graft versus host disease (aGVHD) [12]. Donor-derived anti-CD19-CAR T-cell therapy has also shown a very high CR rate and surprising results in B-ALL patients who relapse after allo-HSCT, without serious aGVHD in most studies [13–15]. It is thus a promising, safe, and effective option for B-ALL patients relapse after allo-HSCT and might be superior to DLI [16].

However, B-ALL patients who relapsed after allo-HSCT still experienced relapse after anti-CD19-CAR T-cell therapy. Therefore, maintaining the efficacy of CAR-T therapy in these patients and avoiding relapse is an urgent problem to be solved. In this study, we explored the efficacy of donor hematopoietic stem cell infusion (DSI) therapy and DLI therapy as a maintenance therapy after B-ALL patients achieved CR following humanized anti-CD19-CAR T-cell therapy, which was administered when they relapsed after allo-HSCT.

Patients And Methods

Patient characteristics

Twenty-two B-ALL patients who relapsed after allo-HSCT between January 2019 and August 2020 were enrolled in our study. All patients showed high CD19 expression on their B-ALL cells upon flow cytometry (FCM) analysis. All these patients were enrolled in a clinical trial of anti-CD19 chimeric antigen receptor-modified (anti-CD19-CAR) T cell therapy (*ChiCTR16009862*and *ChiCTR1800019622*). They all provided informed consent before enrollment. From the date of anti-CD19-CAR T-cell infusion, follow-up was carried out up to the cutoff date or the date of death. The cutoff date was November 30, 2021.

Anti-CD19-CAR T-cell therapy

All the donors of the 22 B-ALL patients provided their peripheral blood mononuclear cells (PBMCs) for this anti-CD19-CAR T-cell therapy. Lymphodepletion chemotherapy comprised fludarabine (30mg/m^2) and cyclophosphamide (400 mg/m^2) from day -4 to day -2. All donor-derived anti-CD19-CAR T-cells were infused on day 0 (1x106 cells/kg) in all B-ALL patients.

Maintenance therapy with Donor hematopoietic stem cell infusion (DSI) therapy and donor lymphocyte infusion (DLI) therapy

Approximately 60 days after anti-CD19-CAR T-cell infusion, when the adverse events (AEs) and the subsequent aGVHD disappeared, patients who obtained CR or CR with incomplete count recovery (Cri) and had previously preserved frozen stem cells, received DSI (DSI group) as maintenance therapy. The other patients who reached CR/CRi received DLI (DLI group) as maintenance therapy. The T cells in DLI therapy were not previously frozen, but were donor peripheral blood T cells that had not been mobilized using granulocyte colony stimulating factor (G-CSF). The interval time between the two DSI/DLI

therapies was generally one month, unless the patients developed serious aGVHD. Moreover, one patient who reached CR/CRi received a second allo-HSCT following DLI therapy (Figure 1). One patient who did not reach CR/CRi after anti-CD19-CAR T-cell therapy received DSI as a salvage therapy. Two other patients who did not reach CR/CRi died because of disease progression within a short time.

Clinical response criteria and donor chimerism analysis

The therapy response in this study was evaluated at 14 days, 28 days, and monthly thereafter. The detection methods included bone marrow (BM) morphology and BM flow cytometry. Disease status was defined as CR, CRi, and no remission (NR). In this study, from the date of anti-CD19-CAR T-cell infusion, follow-up was performed until the patient died. We then evaluated progression-free survival (PFS) and overall survival (OS) after anti-CD19-CAR T-cell therapy.

The changes of donor chimerism in BM were analyzed by fluorescence-labeled multiple PCR amplification of short-tandem repeat (STR) on day14 after anti-CD19-CAR T-cell infusion.

Observation of aGVHD after anti-CD19-CAR T-cell therapy and DSI/DLI therapy

The occurrence and extent of aGVHD were observed from the day of anti-CD19-CAR T-cell infusion to the date of disappearance of aGVHD or death. GVHD was classified using the Glucksberg and Seattle classical scales [17, 18]. After anti-CD19-CAR T-cell therapy or DSI/DLI therapy, five patients received JAK1/JAK2 kinase inhibitor (ruxolitinib) to treat grade II-III aGVHD.

Adverse events (AEs) of anti-CD19-CAR T-cell therapy and DSI/DLI therapy

AEs were observed for 28 days following anti-CD19-CAR T-cell infusion and for 100 days following DSI/DLI. The cytokine release syndrome (CRS) grade was determined according to the National Cancer Institute Common Terminology Criteria for AE v4.03 [19]. Neurotoxicity syndrome was determined according to immune effector cell-associated neurotoxic syndrome (ICANS) [20].

The proportions of anti-CD19-CAR T-cell in the peripheral blood CD3+ T cells were determined on days 0, 4, 7, 14, 21, 28, and 56 after CAR-T cell infusion by FCM, and on days 28, 42,and 56 after DSI/DLI therapy. Anti-CD19-CAR gene expression was detected simultaneously using quantitative polymerase chain reaction (qPCR).

Cytokine levels in peripheral blood, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), were measured on days 0, 4, 7, 14, 21, and 28 after CAR-T cell infusion and on days 28, 42, and 56 after DSI/DLI therapy using respective enzyme-linked immunosorbent assays.

Follow-up

From the date of anti-CD19-CAR T-cell infusion, follow-up was performed until the cutoff date or the date of death. Progression-free survival (PFS) and overall survival (OS) were calculated from the date of anti-CD19-CAR T-cell infusion.

Statistical analysis

Data are expressed as the mean + SE. CRS and ICANS were grouped and compared using the Mann-Whitney rank test. Non-normal distribution data are expressed as median and interquartile range (IQR). The Pearson correlation coefficient was used to evaluate the correlation between different factors. The PFS and OS probabilities were estimated using the Kaplan-Meier method and were compared using the log-rank test. All statistical analyses were computed using SPSS (version 17.0). Statistical significance was set at $P< 0.05$.

Results

Patient characteristics

Overall, 22 B-ALL patients who relapsed after allo-HSCT, then received anti-CD19-CAR T-cell therapy were included in this study. The detailed characteristics of the patients are shown in Table 1. The median time from allo-HSCT to relapse was 13 months (IQR 7–15 months). The median proportion of leukemia cells was 44.7% (IQR 32.8–58.4%) in BM and 37.2% (IQR 25.8–45.5%) in the peripheral blood (PB) when patients were enrolled. The median percentage of donor BM chimerism at enrollment was 55.9%(IQR 48.2–71.3%). Patients had no GVHD or central nervous system disease at enrollment. Moreover, patients had not previously received blinatumomab or CD19-CAR T-cell therapy.

Clinical responses and donor chimerism analysis after anti-CD19-CAR T-cell therapy

In the first evaluation of therapy response on day 14 after anti-CD19-CAR T-cell infusion, 19 patients (19/22, 86.4%) achieved CR/CRi. Of these, 6(6/19, 31.6%) achieved CR, and 13 patients (13/19, 68.4%) achieved CRi. Seventeen patients (17/19, 89.5%) achieved minimal residual disease (MRD)-negative responses, whereas two patients were evaluated as CR/CRi with MRD-positive response (Pt_{DSI} 5,11).The other three patients were evaluated as NR owing to the progression of leukemia.

After 14 days of anti-CD19-CAR T-cell infusion, the donor chimerism in BM increased from 56.3 ± 22.9% to 99.9 ± 0.2% in the 19 patients who achieved CR/CRi. The donor chimerism in a patient evaluated as NR with extramedullary leukemia was 99.0%, whereas it was 10.7% and 29.2% in the other two

patients who were evaluated as NR. Before or after anti-CD19-CAR T- cell therapy, there was no difference in donor chimerism in the DSI and DLI groups (P_{before}=0.056 and P_{after}=0.828).

Maintenance therapy with DSI/DLI following the anti-CD19-CAR T-cell therapy

Of the patients achieving CR/CRi, 11 patients (Pt_{DSI} 1-11) who had previously preserved frozen stem cells received DSI, whereas the other 8 patients (Pt_{DLI} 1-8) received unfrozen DLI maintenance therapy. Only one patient (Pt_{DLI} 7) received a second allo-HSCT after DLI. In the DSI group, six patients received one DSI treatment, whereas five received two DSI treatments. In the DLI group, three patients received one DLI treatment, whereas four patients received DLI twice and one patient received DLI thrice. As all patients received DSI/DLI for different times, we only compared the changes in various indicators after the first DSI/DLI therapy.

The median number of donor CD34+ cells infused in these patients was 1.3 (IQR 1.2–1.4)×10^5 cells/kg and that of CD3+ T cells was 2.2 (IQR 1.9–2.3)×10^7 cells/kg in DSI therapy. In the DLI group, the median number of donor CD3+ T cells infused was2.4(IQR 2.2–2.7)×10^7 cells/kg. There was no difference in the number of CD3+ T cell infusions between the two groups (*P*=0.351). The median percentage of CD19+ cells in the donor product in the DSI group was 0.02±0.03%, whereas it was 2.20±0.71% in the DLI group.

One patient who did not achieve CR/CRi with the anti-CD19-CAR T-cell therapy received DSI once (Pt 3). The number of donor CD34+ cells and CD3+ cells infused was1.4×10^5cells/kg and1.9×10^7 cells/kg, respectively.

aGVHD after anti-CD19-CAR T-cell therapy and after DSI/DLI therapy

After anti-CD19-CAR T-cell therapy, aGVHD was observed in 6 patients (6/11, 54.6%) from 19 to 48 days before DSI, and it was observed in 4 patients (4/8, 50.00%) before DLI. Only one patient (Pt_{DSI} 5) developed grade III aGVHD, whereas no patient developed grade III-IV aGVHD in the DLI group. There was no difference in the grade of aGVHD between the two groups (*P*=0.732).

In the DSI group, aGVHD was observed in four patients (4/11, 36.4%) from 32 to 56 days after the first DSI. These four patients (Pt_{DSI}1, 2, 4, and 6) developed grade I-II aGVHD. Only one patient (Pt_{DLI} 3) developed grade II aGVHD after 52 days of the first DLI. There was no difference in aGVHD between the two groups after DSI/DLI therapy (*P*=0.636). None of the patients in the DSI and DLI groups developed grade III-IV aGVHD. Pt 3 did not develop any grade of aGVHD after DSI. None of the patients died because of aGVHD in our study.

Expansion of anti-CD19-CAR T-cell

The proportion of anti-CD19-CAR T-cell was detected by FCM at 0, 4, 7, 14, 21, 28, and 56 days after anti-CD19-CAR T-cell infusion and at 28, 42, and 56 days after the first DSI/DLI therapy (Figure 2 a). The median peak of the anti-CD19-CAR T-cell in CD3+ T cells in peripheral blood was 34.9±14.0% on day 8.3±2.7 of anti-CD19-CAR T-cell therapy in all 22 patients. In the process of anti-CD19-CAR T-cell therapy, there was no difference in the expansion peaks of anti-CD19-CAR T-cell in the DSI and DLI groups(*P*=0.124) (Figure2 b). From 28–56 days after DSI therapy, the expansion of anti-CD19-CAR T-cell increased again in 9 of 11 patients(Figure 2 a). The expansion peaks of anti-CD19-CAR T-cell in the DSI group were higher than those in the DLI group after DSI/DLI therapy (*P*<0.0001) (Figure 2 c).

In all patients with aGVHD after CAR-T cell therapy or DSI/DLI therapy, there was no difference in the expansion peaks of anti-CD19-CAR T-cell in the period of CAR-T cell therapy and in the period of DSI/DLI therapy between the DSI and DLI groups ($P_{CAR\text{-}T}$=0.609 and $P_{DSI/DLI}$=0.903)(Figure 2 d e).

In the period of CAR-T cell therapy, there was no difference in the expansion peaks of the anti-CD19-CAR T-cell between patients with and without aGVHD(*P*=0.474) (Figure 2 f). The mean peak of anti-CD19-CAR T-cell in patients with aGVHD in the period of aGVHD before DSI/DLI therapy was 5.4±3.9%, and was not higher than that the mean peak of anti-CD19-CAR T-cells in patients without aGVHD at 28 days after CAR-T cell infusion (3.2±1.4%) (*P*=0.128) (Figure 2 g).

Anti-CD19-CAR DNA changes in anti-CD19-CAR T-cell therapy and DSI/DLI therapy

In the two groups of patients, the copies of anti-CD19-CAR DNA were detected at 0, 4, 7, 14, 21, 28, and 56 days after CAR-T cell infusion and at 28, 42, and 56 days after the first DSI/DLI therapy (Figure 2h). In the process of anti-CD19-CAR T-cell therapy, there was no difference in the median peak of anti-CD19-CAR DNA in the DSI and DLI groups(*P*=0.070) (Figure 2i). From 28–56 days after the first DSI/DLI therapy, the median anti-CD19-CAR DNA peaks in the DSI group increased again after DSI therapy, but not in the DLI group (*P*<0.0001) (Figure 2j).

In all patients with aGVHD after CAR-T cell therapy or DSI/DLI therapy, there was no difference in the mean peak of anti-CD19-CAR DNA in the period of CAR-T cell therapy and in the period of DSI/DLI therapy between the two groups ($P_{CAR\text{-}T}$=0.973 and $P_{DSI/DLI}$=0.599) (Figure2 kl). In anti-CD19-CAR T-cell therapy and in DSI/DLI therapy, the mean peak of anti-CD19-CAR DNA showed the same trend as that of the mean peak of anti-CD19-CAR T-cells between patients with and without aGVHD(P=0.199 and P=0.059) (Figure 2 mn).

Cytokine levels in anti-CD19-CAR T-cell therapy and DSI/DLI

In the anti-CD19-CAR T-cell therapy, the cytokines reached their peaks at 7 to 10 days after anti-CD19-CAR T-cell infusion, and then declined from 14 to 18 days after infusion (Figure3 a).There was no difference in the peaks of IL-6 and TNF-α in the two groups in anti-CD19-CAR T-cell therapy and in the period

of aGVHD before DSI/DLI therapy (Figure 3 b c). However, following DSI/DLI therapy, the IL-6 and TNF-α levels increased again in 9 of the 11 patients in the DSI group. All 4 patients who developed aGVHD after DSI therapy were included in these 9 patients. However, the levels of the two cytokines did not increase again in the DLI group after DLI (Figure 3 d).

Observation of AEs upon anti-CD19-CAR T-cell therapy and DSI/DLI therapy

In anti-CD19-CAR T-cell therapy, the clinical symptoms were similar to those reported in our previous studies[21]. These AEs resolved 14–18 days after anti-CD19-CAR T-cell infusion. There was no difference in the incidence of AEs between the two groups during this period (Table 2). In DSI/DLI therapy, at 28-56 days post DSI/DLI treatment, AEs reappeared in the two groups. There was no difference in the incidence of AEs between the two groups during this period as well (Table 2). The duration of AEs in the DSI/DLI treatment ranged from 7-15 days.

Eighteen patients who obtained CR/CRi (18/22, 81.8%) had grade 3-4 hematological toxicity after anti-CD19-CAR T-cell infusion. The other four patients only had grade 2 hematological toxicity. The hematological toxicity in all patients was recovered prior to their subsequent DSI/DLI therapy.

In anti-CD19-CAR T-cell therapy, 17 (17/22, 77.3%) patients were diagnosed with grade 0-2 of CRS, whereas 5 (5/22, 22.7%) patients were diagnosed with grade 3-4 of CRS. Only one patient in the DSI group and two patients in the DLI group developed grade 1 ICANS during this period. There were no differences in the grades of CRS and ICANS between the two groups in anti-CD19-CAR T-cell therapy (Figure 3 e f). No CRS or ICANS-related deaths were observed in our study.

Antipyretic drugs and methylprednisolone were administered to overcome AEs. Only four patients (Pt_{DSI} 3,5 and Pt_{DLI} 2,4) who developed grade 3-4 CRS received tocilizumab after anti-CD19-CAR T-cell therapy. None of the patients received tocilizumab after DSI/DLI therapy.

Survival after the anti-CD19-CAR T-cell therapy and DSI/DLI therapy

By November 30, 2021, 14 patients survived without leukemia. Anti-CD19-CAR T-cell therapy and DSI/DLI therapy, second allo-HSCT, response to anti-CD19-CAR T-cell therapy, occurrence of aGVHD, PFS and OS, and cause of death are shown in Figure 4a. The rates of PFS and OS at 180 days were 90.91% and 90.91%, respectively, and those at 365 days were 63.64% and 63.64%, respectively, in the DSI group. The PFS and OS rates at 180 days were 50.50% and 50.50% and those at 365 days were 12.50% and 12.50%, respectively, in the DLI group. Although the rates of PFS and OS in the DSI group were higher at 180 days, there was no difference in the rate of PFS or OS between the two groups at 180 days (P_{PFS}=0.064 and P_{OS}=0.057). The PFS and OS rates in the DSI group were higher than those in the DLI group at 365 days(P_{PFS}=0.010 and P_{OS}=0.009)(Figure 4 b c).Pt 1,2,and 3 did not respond to anti-CD19-CAR T-cell therapy and died of leukemia. Pt_{DSI} 11 and Pt_{DLI} 6 died of disease recurrence with negative CD19 expression, whereas Pt_{DLI} 5 and 8 died of disease recurrence with positive CD19 expression. Pt_{DSI} 2 died of cytomegalovirus infection at 720 days post anti-CD19-CAR T-cell infusion and 620 days after the second DSI therapy. Pt_{DLI} 4 died of sudden cardiac death at 83 days after anti-CD19-CAR T-cell infusion and 22 days after DLI therapy without aGVHD.

Discussion

Relapse after allo-HSCT remains a major issue in B-ALL treatment failure. In particular, high-risk B-ALL patients are still at a high rate of recurrence after allo-HSCT, with a very short survival [1, 22, 23]. Owing to the poor efficacy of salvage chemotherapy, some studies have selected DLI therapy for patients with B-ALL who relapsed after allo-HSCT [24–27]. This could induce durable remission by enhancing the graft versus leukemia (GVL) effect [28]. The efficacy of DLI varies among different types of leukemia. DLI therapy was more effective in chronic myeloid leukemia (CML) relapse after allo-HSCT than in ALL relapse after allo-HSCT, with CR rates of 70–80% and less than 40%, respectively [29,30]. Moreover, DLI has been found more potent in AML than in ALL [31]. However, the GVL effect of DLI therapy usually leads to different levels of aGVHD. Infusion of large numbers of donor T cells might result in serious aGVHD or even therapy-related mortality [32,24]. Compared with DLI therapy, few studies have assessed DSI therapy after allo-HSCT. Regrettably, despite severe aGVHD, the prognosis of DLI therapy remains unsatisfactory, especially in patients with ALL who relapse after allo-HSCT.

Donor anti-CD19-CAR T-cell therapy is an alternative salvage therapy for patients with B-ALL who relapse after allo-HSCT. Compared with DLI therapy and other conventional therapies, anti-CD19-CAR T-cell therapy is relatively safe and effective. Allogeneic anti-CD19-CAR T-cell could clear leukemia cells directly, they have also been found to have GVL effects without serious aGVHD in most studies [14,15,33–35]. However, maintaining the efficacy of anti-CD19-CAR T-cell therapy after allo-HSCT remains an urgent problem. Our previous study showed that DSI and DLI could be used as maintenance treatments after anti-CD19-CAR T-cell therapy for patients with B-ALL who had relapsed after allo-HSCT. DSI induced an increased proportion of anti-CD19-CAR T-cell and an increased level of anti-CD19-CAR DNA expression with mild aGVHD. This might lead to further clearance of minimal residual disease (MRD) and longer PFS [36]. Notably, our DSI or DLI therapy was initiated when the patients with B-ALL who relapsed after allo-HSCT had obtained a CR from their anti-CD19-CAR T-cell therapy without relapse.

DLI therapy in patients with complete donor chimerism without MRD was defined as prophylactic DLI therapy (pro-DLI), which could obviously reduce the relapse rate [37–39]. Therefore, early DLI therapy after allo-HSCT may be a more reasonable and effective therapy. All patients with B-ALL who relapsed after allo-HSCT, and were enrolled in our study, obtained CR from their anti-CD19-CAR T-cell therapy. DSI or DLI therapy was administered 60 days after anti-CD19-CAR T cell therapy to prevent their disease progression. After 1–3 times of DSI or DLI therapy, the PFS and OS were higher in the DSI group than in the DLI group at 365 days. Thus, further studies with increased number of cases are needed to confirm these observations. However, DSI maintenance therapy has the potential to present better survival in patients who relapsed after allo-HSCT and obtained CR from anti-CD19-CAR T-cell therapy.

We observed the side effects of the two types of maintenance treatments and found no difference in the grade of aGVHD in the DSI and DLI groups during the anti-CD19-CAR T-cell therapy and the DSI/DLI therapy. After DSI therapy, the expansion of anti-CD19-CAR T-cell increased again in 9 of the 11 patients in DSI group. The peaks of the anti-CD19-CAR T-cell were higher than those in DLI group at the same time. This suggests that DSI therapy might facilitate the re-amplification of anti-CD19-CAR T-cell. The levels of IL-6 and TNF-α also increased again in DSI group after DSI. However, whether the re-amplification of anti-CD19-CAR T-cell is related to better PFS and OS of patients in the DSI group requires further studies.

Abbreviations

B-ALL:B-cell acute lymphoblastic leukemia; **allo-HSCT**: Allogeneic hematopoietic stem cell transplant; **CR**: complete response; **CRi**:complete response with incomplete count recovery;**NR**:no remission; **DLI**: donor lymphocyte infusion; **anti-CD19-CAR**: Anti-CD19 chimeric antigen receptor modified; **PFS**: progression free survival; GVL: graft versus leukiemia; **GVHD**: graft versus host disease; **DSI**: donor hematopoietic stem cell infusion; **OS**: overall survival;LFS:leukemia free survival ;**FCM**: flow cytometry; **PBMCs**: peripheral blood mononuclear cells; **STR**: short-tandem repeats; AEs: Adverse events; **CRS**: Cytokine release syndrome; **ICANS**: Immune effector cell associated neurotoxic syndrome; **qPCR**: quantitative polymerase chain reaction; **IL-6**: interleukin-6; **TNF-α**: tumor necrosis factor-α; **IQR**: interquartile range; **PB**: peripheral blood; **CNSL**: central nervous system leukemia; **MRD**: minimal residual disease; **CML**: chronic myeloid leukemia; **pro-DLI**: prophylactic DLI therapy.

Declarations

Authors' contributions

LQ performed the study, analyzed the data, designed the figures, and wrote the paper. DHB, LMJ, WJ and JEL performed clinical works. YJJ provided materials. YJJ provided technical support for cell therapy. ZHB,LCC did the statistical analysis. DQ and ZRL supervised the study, designed the clinical trial, and analyzed the data. LQ wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of the Tianjin First Center Hospital (Tianjin, China). (Approved No. of ethic committee: 2015002X and 2018N105KY).

This Clinical trials is registered at http://www.chictr.org.cn/index.aspx as *ChiCTR-ONN-16009862* and http://www.chictr.org.cn/index.aspx as *ChiCTR1800019622*.

The patients agreed to participate in our Clinical trials. They agreed to the use of their data for our study.

Consent for publication

Not applicable.

Conflict of interest: The authors declare no potential conflicts of interest.

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Tables

Table 1. Patients baseline characteristics

Patient	Age	Gender	Diagnosis	Donor Type	GVHD before relapse	Time from transplant to relapse	Blasts in BM (%)	Blasts in PB (%)	Donor chimerism (%)	DSI/DLI CD3+ ($\times10^7$ cells/kg)	CD34+ ($\times10^5$ cells/kg)	Therapy after relapse
Pt_{DSI} 1	53	M	B- ALL	Haplo-HSCT (5/10)	aGVHD (I) cGVHD	21 months	66.2	45.5	20.55	**DSI** 2.34	1.35	No
Pt_{DSI} 2	44	F	B-ALL (Ph+)	MMUDT(9/10)	No	48 months	80.4	59.6	11.82	**DSI** 2.14	1.16	TKI
Pt_{DSI} 3	58	F	B-ALL	MSDT	No	25 months	48.2	39.4	18.56	**DSI** 1.95	1.42	No
Pt_{DSI} 4	10	M	B-ALL	MSDT	aGVHD (I)	9 months	32.8	36.8	71.25	**DSI** 2.59	1.55	One course
Pt_{DSI} 5	23	M	B-ALL	Haplo-HSCT (5/10)	cGVHD	7 months	45.2	40.5	41.88	**DSI** 1.95	1.14	No
Pt_{DSI} 6	20	M	B-ALL	Haplo-HSCT (5/10)	aGVHD (I)	15 months	40.2	35.8	52.34	**DSI** 2.78	1.43	No
Pt_{DSI} 7	38	F	B-ALL	MSDT	No	19 months	60.5	51.5	48.23	**DSI** 2.01	1.35	No
Pt_{DSI} 8	52	M	B-ALL	MSDT	No	18 months	24	19.8	79.48	**DSI** 2.24	1.45	No
Pt_{DSI} 9	43	F	B-ALL (Ph+)	Haplo-HSCT (5/10)	aGVHD (I)	13 months	66.4	52.2	46.14	**DSI** 1.89	0.98	TKI
Pt_{DSI} 10	56	M	B-ALL	MSDT	No	11 months	20.8	19.8	68.9	**DSI** 1.67	1.01	No
Pt_{DSI} 11	11	M	B-ALL	Haplo-HSCT (5/10)	No	6 months	65.5	60.4	65.12	**DSI** 2.21	1.26	No
Pt_{DLI} 1	58	F	B-ALL	Haplo-HSCT (8/10)	No	7 months	15.6	12.8	80.17	**DLI** 2.12	-	No
Pt_{DLI} 2	38	F	B-ALL	Haplo-HSCT (5/10)	aGVHD (I)	4 months	68.8	49.6	36.46	**DLI** 2.66	-	No
Pt_{DLI} 3	19	M	B-ALL	MSDT	No	21 months	40.8	38.4	65.27	**DLI** 1.73	-	No
Pt_{DLI} 4	22	F	B-ALL (Ph+)	Haplo-HSCT (5/10)	No	7 months	48.6	40.6	44.89	**DLI** 2.23	-	TKI
Pt_{DLI} 5	55	M	B-ALL	Haplo-HSCT (5/10)	aGVHD (I)	5 months	28.6	25.5	72.23	**DLI** 2.91	-	No
Pt_{DLI} 6	9	M	B-ALL	MSDT	No	11 months	22.8	18.6	68.26	**DLI** 2.62	-	No
Pt_{DLI} 7	34	F	B-ALL	MSDT	aGVHD (I)	16 months	1.48	1.0	89.1	**DLI** 1.91	-	No
Pt_{DLI} 8	21	F	B-ALL	Haplo-HSCT (5/10)	No	5 months	82.1	78.6	88.34	**DLI** 2.65	-	No
Pt 1	16	M	B-ALL	MUDT	aGVHD (II)	4 months	58.4	25.8	23.12	-	-	Two courses
Pt 2	56	M	B-ALL	MSDT	aGVHD (I)	9 months	52.4	56.4	52.08	-	-	Two courses
Pt 3	22	M	B-ALL	Haplo-HSCT (5/10)	aGVHD (II)	15 months	12.8	10.6	85.16	**DSI** 1.94	1.35	No

HLA-matched sibling donor transplantation:*MSDT.* HLA-matched unrelated donor transplantation:*MUDT.* HLA-mismatched unrelated donor transplantation:*MMUDT.* Haploid donor transplantation:*Haplo-HSCT.* Tyrosine kinase inhibitor:*TKI.* Donor hematopoietic stem cell infusion: *DSI.* Donor lymphocyte infusion:*DLI.*

Table 2: The notable adverse events (AEs) in DSI/DLI groups

Events	CAR-T therapy in DSI	CAR-T therapy in DLI	*P* values (In CAR-T)	DSI therapy	DLI therapy	*P* values (DSI/DLI therapy)
General condition						
Temperature ≥38 °C (fever)	11/11(100%)	8/8(100%)	**>0.99**	5/11(45.45%)	2/8(25.00%)	**0.67**
Systolic blood pressure <90 mmHg	2/11(18.18%)	1/8(12.50%)	**1.00**	0/11(0%)	0/8(0%)	**-**
Needing oxygen for SaO_2 >90%	1/11(9.09%)	1/8(12.50%)	**0.81**	0/11(0%)	0/8(0%)	**-**
Organ toxicities						
Cardiac						
Tachycardia	6/11(54.55%)	5/8(62.50%)	**1.00**	3/11(27.27%)	1/8(12.50%)	**0.83**
Arrhythmias	1/11(9.09%)	1/8(12.50%)	**0.812**	0/11(0%)	0/8(0%)	**-**
Myocardial ischemia	3/11(27.27%)	2/8(25.00%)	**1.00**	4/11(36.36%)	1/8(12.50%)	**0.52**
Respiratory						
Hypoxia	3/11(27.27%)	2/8(25.00%)	**1.00**	2/11(18.18%)	0/8(0%)	**0.13**
Dyspnoea	1/11(9.09%)	1/8(12.50%)	**0.81**	0/11(0%)	0/8(0%)	**-**
Cough	3/11(27.27%)	3/8(37.50%)	**1.00**	3/11(27.27%)	0/8(0%)	**0.33**
Pleural effusion	5/11(45.45%)	3/8(37.50%)	**1.00**	4/11(36.36%)	1/8(12.50%)	**0.52**
Gastrointestinal						
Nausea	4/11(36.36%)	2/8(25.00%)	**0.98**	2/11(18.18%)	0/8(0%)	**0.13**
Vomiting	2/11(18.18%)	2/8(25.00%)	**1.00**	2/11(18.18%)	0/8(0%)	**0.13**
Decreased appetite	5/11(45.45%)	3/8(37.50%)	**1.00**	4/11(36.36%)	1/8(12.50%)	**0.52**
Hepatic						
Increased serum ALT, AST	6/11(54.55%)	4/8(50.00%)	**1.00**	7/11(63.64%)	3/8(37.50%)	**0.51**
Increased serum bilirubin levels	1/11(9.09%)	1/8(12.50%)	**0.81**	4/11(36.36%)	1/8(12.50%)	**0.52**
Renal						
Acute kidney injury	3/11(27.27%)	1/8(12.50%)	**0.83**	2/11(18.18%)	0/8(0%)	**0.13**
Coagulopathy						
Disseminated intravascular coagulation	1/11(9.09%)	0/8(0%)	**0.29**	0/11(0%)	0/8(0%)	**-**
Neurological						
Encephalopathy	1/11(9.09%)	1/8(12.50%)	**0.81**	0/11(0%)	0/8(0%)	**-**
Confused state	0/11(0%)	0/8(0%)	**-**	0/11(0%)	0/8(0%)	**-**
Aphasia	0/11(0%)	0/8(0%)	**-**	0/11(0%)	0/8(0%)	**-**
Somnolence	0/11(0%)	1/8(12.50%)	**0.18**	0/11(0%)	0/8(0%)	**-**

Figures

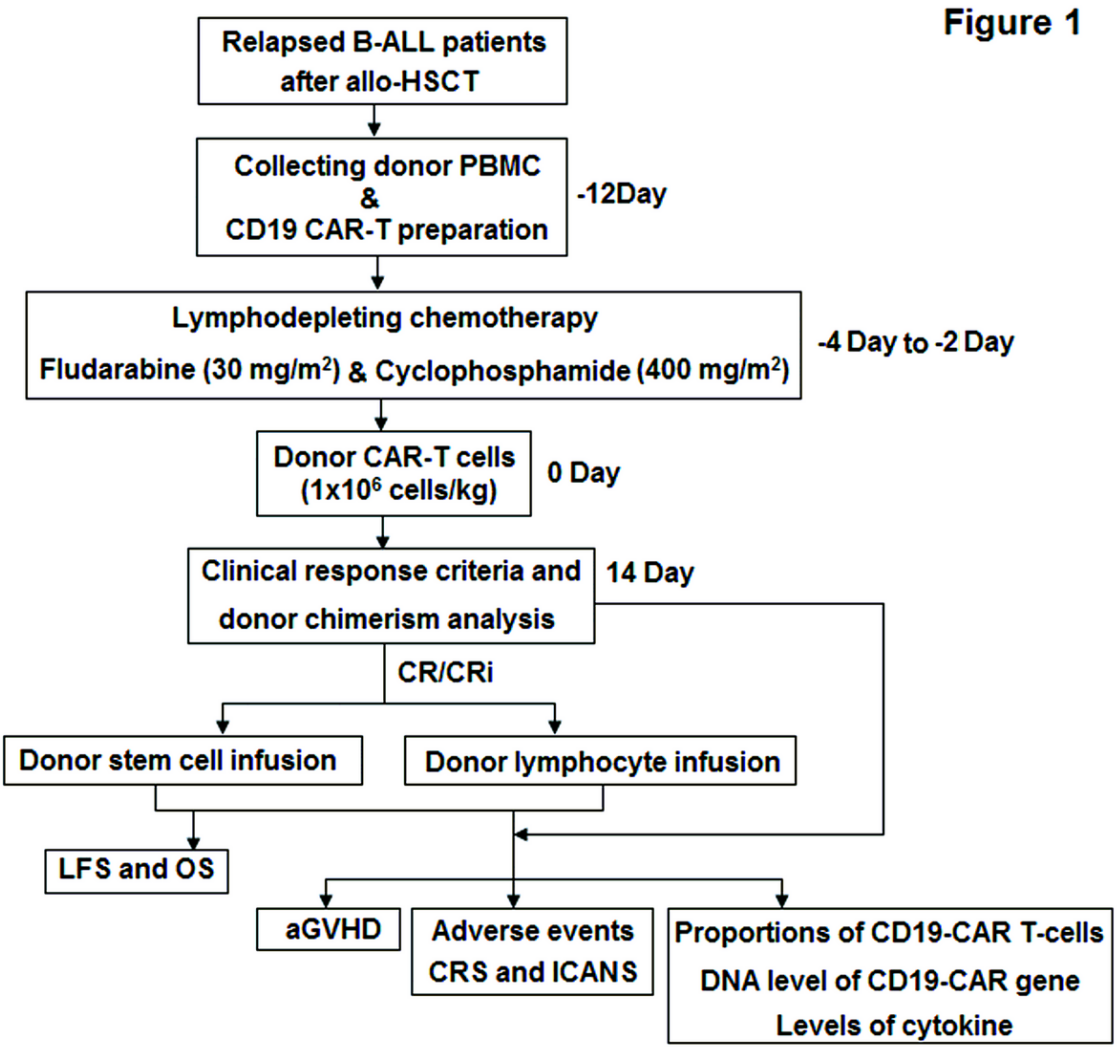

Figure 1

Design process of clinical trials and grouping methods in our study

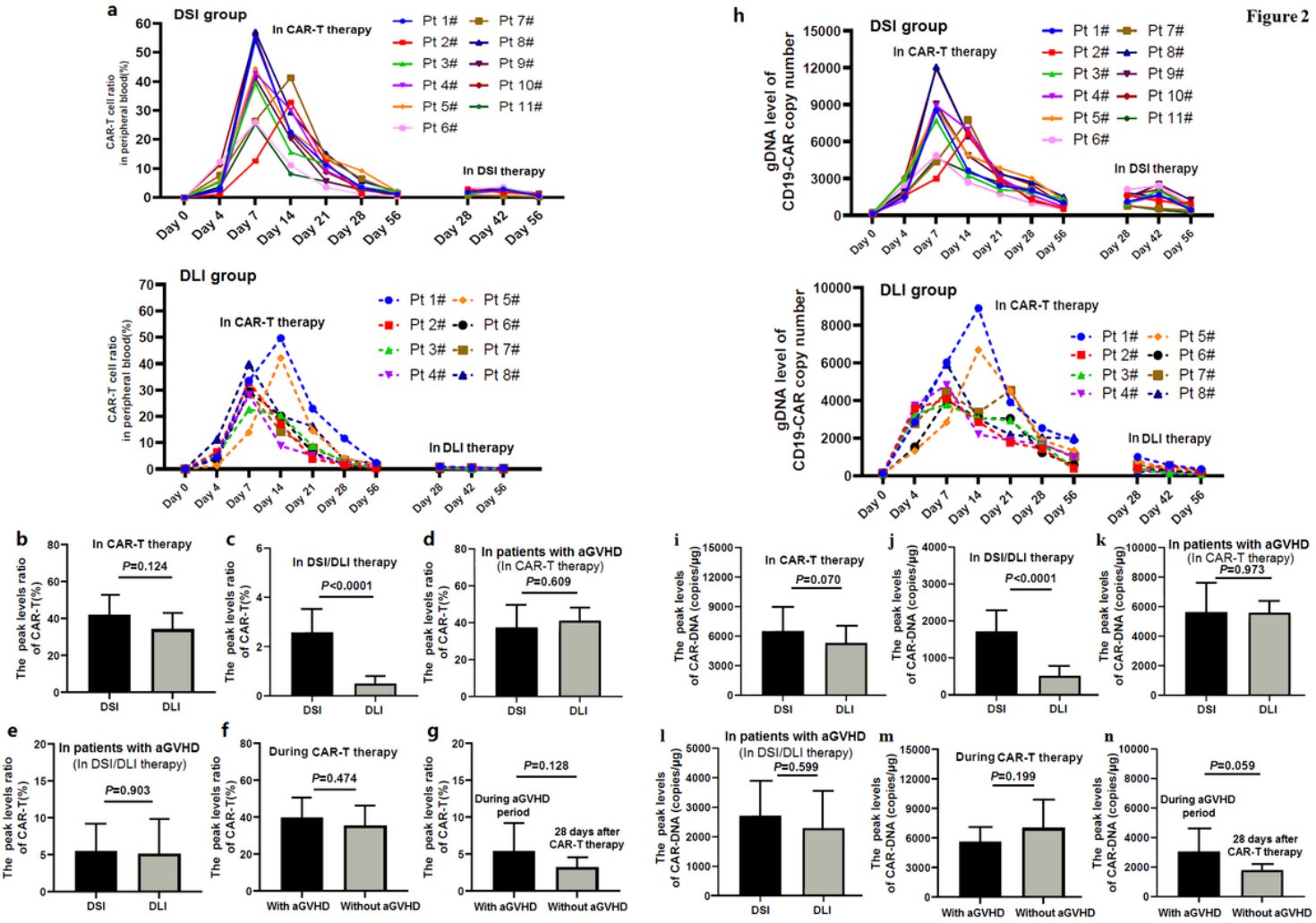

Figure 2

Expansion of anti-CD19-CAR T-cell and anti-CD19-CAR DNA changes upon anti-CD19-CAR T-cell therapy and DSI/DLI therapy

a. The proportion of anti-CD19-CAR T-cell upon anti-CD19-CAR T-cell therapy and DSI/DLI therapy. **b.** There was no difference in the expansion peaks of anti-CD19-CAR T-cell in the two groups with anti-CD19-CAR T-cell therapy. **c.** The peaks of the anti-CD19-CAR T-cell in the DSI group were higher than those in the DLI group. **d. and e.** In patients with aGVHD after CAR-T cell therapy, there was no difference in the expansion peaks of CAR-T cells in the period of CAR-T cell therapy and in the period of aGVHD between the two groups. **f.** There was no difference in the average expansion peaks of the anti-CD19-CAR T-cell in the period of CAR-T cell therapy between the patients with and without aGVHD before DSI/DLI therapy. **g.** There was no difference in the average expansion peaks of the anti-CD19-CAR T-cell at 28 days after CAR-T cell therapy between patients with and without aGVHD. **h.** The average level peaks of anti-CD19-CAR DNA in patients with anti-CD19-CAR-T cell therapy and DSI/DLI therapy. **i.** There was no difference in the average level peaks of anti-CD19-CAR DNA in the two groups in anti-CD19-CAR T-cell therapy. **g.** In DSI/DLI therapy, the average anti-CD19-CAR DNA peaks increased again in the DSI group but not in the DLI group. **k.and l.** In patients with aGVHD after CAR-T cell therapy, there was no difference in the average level peaks of anti-CD19-CAR DNA in the period of CAR-T cell therapy and in the period of aGVHD between the two groups. **m.** There was no difference in the average level peaks of anti-CD19-CAR DNA in the period of CAR-T cell therapy between patients with and without aGVHD before DSI/DLI therapy. **n.** There was no difference in the average level peaks of anti-CD19-CAR DNA at 28 days after CAR-T cell therapy between patients with and without aGVHD.

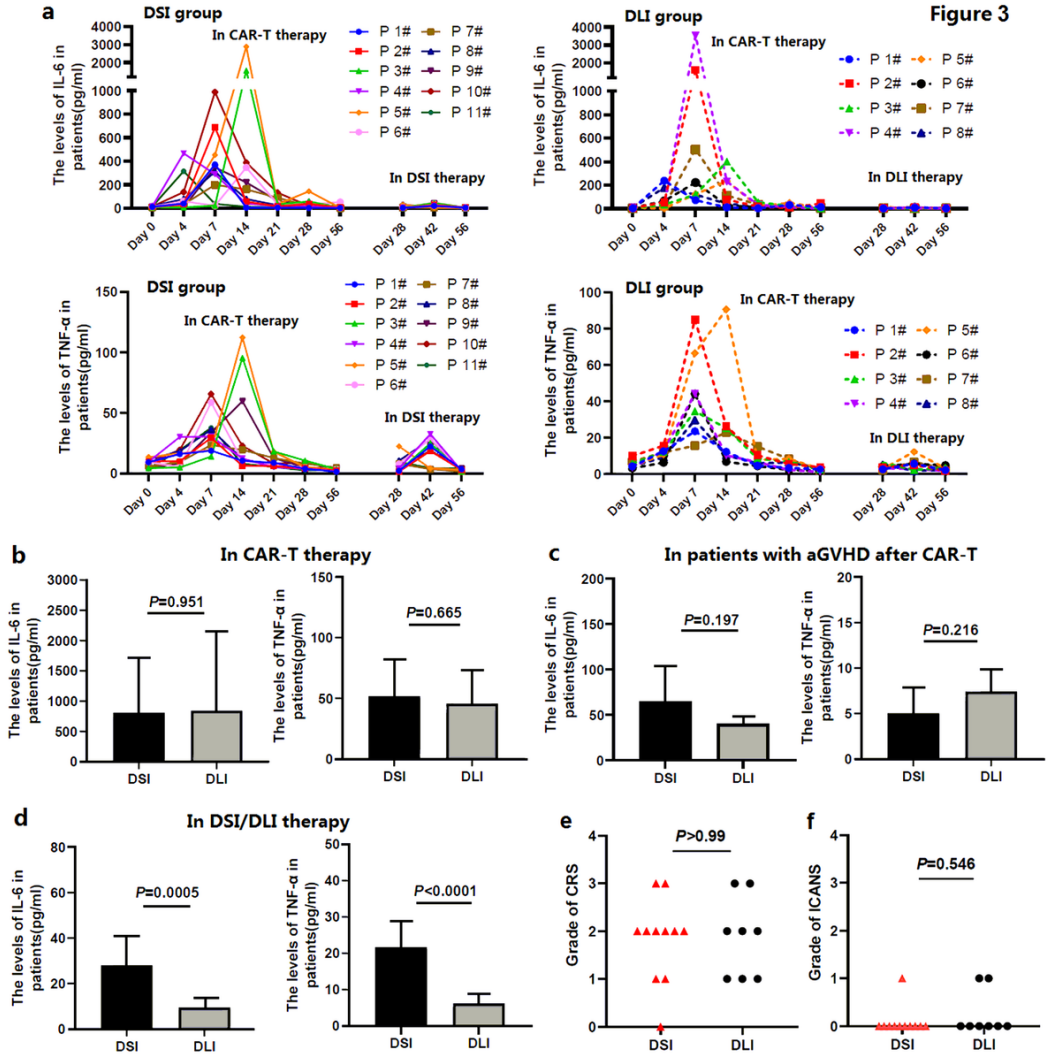

Figure 3

The level of cytokines, cytokine release syndrome (CRS), and immune effector cell-associated neurotoxic syndrome(ICANS) in the therapy. a. The level of IL-6 and TNF-α in the anti-CD19-CAR T-cell therapy and DSI/DLI therapy. **b.** There was no difference in the peaks of IL-6 and TNF-α in the DSI/DLI groups in CAR-T cell therapy. **c.** There were no difference in the peaks of cytokines in patients with aGVHD before DSI/DLI therapy. **d.** IL-6 and TNF-α levels increased again in 9 of 11 patients in the DSI group after DSI but not in the DLI group. **e. and f.** There were no differences in the grades of CRS and ICANS between the two groups in anti-CD19-CAR T-cell therapy.

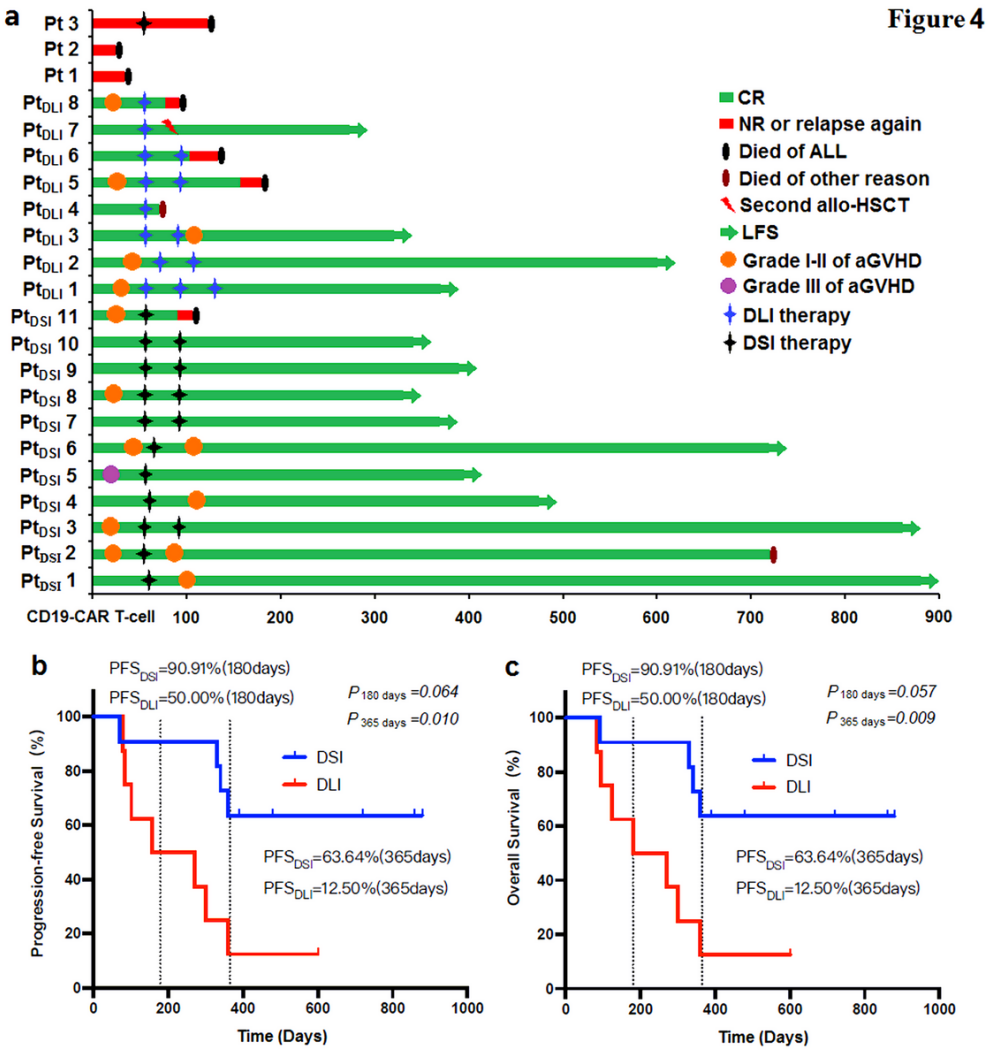

Figure 4

Survival observation for anti-CD19-CAR T-cell therapy and DSI/DLI therapy. a. All treatment processes, occurrence of aGVHD, progression-free survival (PFS) and overall survival (OS), cause of death. **b and c.**The rates of PFS and OS in the DSI group were higher at 180 days, but there was no difference in PFS or OS between the two groups at 180 days. The rates of PFS and OS in the DSI group were higher than those in the DLI group at 365 day