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RESEARCH

Reduced-dose donor lymphocyte infusion is a viable therapeutic strategy for Epstein–Barr virus-related post-transplant lymphoproliferative disease after hematopoietic stem cell transplantation: a single-center experience

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Abstract

Post-transplant lymphoproliferative disease (PTLD) is a life-threatening complication of hematopoietic stem cell transplantation caused by Epstein–Barr virus (EBV) reactivation due to immunosuppression. Frontline treatment includes the reduction of immunosuppressive therapy and administration of rituximab. However, the incidence of EBV-related PTLD (EBV$^+$ PTLD) continues to increase, and patient prognosis remains poor. In this retrospective study, we designed an exploratory treatment strategy for PTLD using designated reduced-dose donor lymphocyte infusion (DLI) (CD3 + T cells: 5×10^4/kg) for majority patients (11/14). We further analyzed the data of 27 patients with PTLD who underwent transplantation at our institutions. Our therapeutic strategy effectively treated PTLD. In this study, the DLI cohort demonstrated higher overall response and complete remission rates than rituximab monotherapy after two-week intervention. Additionally, the DLI group had a markedly higher 1-year overall survival (OS) than the rituximab group. Similarly, the reduced-dosage DLI group had a significantly higher 1-year OS than the conventional-dosage group. These results indicate that varied treatments (rituximab vs DLI) and DLI dosages (conventional vs reduced) had significant impact on OS. Finally, the reduced-dosage DLI group had a lower risk of non-relapse mortality and acute graft versus host disease than the conventional-dosage group. This study demonstrates that reduced-dosage DLI is a promising treatment for EBV$^+$ PTLD.

Keywords Immunosuppression · Post-transplant lymphoproliferative disease · Hematopoietic stem cell transplantation · Rituximab · Lymphocyte infusion

Introduction

Post-transplant lymphoproliferative disease (PTLD) is an aggressive and life-threatening hematologic complication that can occur as a result of immunosuppression after

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hematopoietic stem cell transplantation (HSCT). PTLD after allogeneic HSCT (allo-HSCT) tends to be more aggressive than that subsequent to solid organ transplantation. In addition, it is typically characterized by early dissemination and high mortality. Nearly, all PTLD cases are Epstein–Barr virus (EBV)-positive (EBV^+). These cases occur as a result of EBV activation in EBV-negative patients who receive a transplant from EBV^+ donors or due to EBV reactivation in previously infected patients following transplantation [1–5]. Recently, the incidence of EBV-related PTLD (EBV^+ PTLD) continues to increase caused by several risk factors, including more unrelated or human leukocyte antigen mismatched transplant, cord blood transplant, T cell depletion in vivo [3].

PTLD typically emerges 60–90 days post-HSCT. Most cases occur between one through six months post-HSCT [6, 7]. EBV-related PTLD has a mortality rate of 50–90% [3, 8]. It has several pathology subtypes harboring different clinical outcomes: reactive hyperplasia, polymorphic, monomorphic, as well as classical Hodgkin lymphoma types. Reactive hyperplasia is a benign condition characterized by the proliferation of normal lymphoid tissue and has a favorable prognosis. Polymorphic PTLD shows clonal or oligoclonal lymphoid populations but does not meet the criteria for classification as a specific lymphoma type. Contrastingly, monomorphic PTLD includes B cell neoplasms such as diffuse large B cell lymphoma, Burkitt lymphoma, and plasma cell myeloma. T/NK cell neoplasms include peripheral T cell lymphoma and hepatosplenic T cell lymphoma; however, these are less common [9, 10].

Multiple factors associated with EBV^+ PTLD post-allo-HSCT have been identified. These include T cell depletion, receipt of anti-thymocyte globulin (ATG), age (age > 50 years), reduced intensity conditioning, EBV serology mismatch, second HSCT, pre-transplant splenectomy, HLA mismatch and haploidentical transplant, infusion of mesenchymal stromal cells, grades II–IV acute graft versus host disease (aGVHD), and cytomegalovirus (CMV) reactivation [11–15]. Clinical practice guidelines recommend rituximab with or without reduction in immunosuppression as frontline treatment for EBV^+ PTLD. This treatment should be initiated as soon as possible, owing to the risk of developing lymphoma and multiple-organ impairment. Rituximab monotherapy administered weekly for up to four doses demonstrated positive outcomes in almost 70% of patients. However, reduction of immunosuppression is rarely successful as the sole intervention in PTLD following HSCT. In addition, it may increase the risk of or GVHD. Thus, it should be combined with rituximab administration [16]. Patients who do not respond well to rituximab have poor outcomes and result in limited treatment options. Up to 50% of patients with EBV^+ PTLD post-HCT may respond poorly to rituximab-containing treatments [17, 18]. In such instances, second-line therapy options such as donor lymphocyte infusion (DLI), EBV-specific cytotoxic T lymphocytes (EBV-CTL), or chemotherapy ± rituximab should be considered [19, 20]. Hence, we retrospectively investigated the effect and safety with reduced-dose DLI (CD3 + T cells, 5×10^4/kg) against EBV^+ PTLD, which will pave a way to build up an optimized therapy for PLLD.

Materials and methods

Patients

A retrospective analysis was conducted to evaluate the treatment outcomes of 27 patients with post-HSCT EBV^+PTLD diagnosed from 2018 to 2023 at Department of Hematology, the second Hospital of Dalian Medical University in China (SH-DMU). The underlying diseases in patients received allogeneic HSCT included severe aplastic anemia (SAA), acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), myelodysplastic syndrome (MDS), and congenital hemophagocytic syndrome. The patient outcomes were followed up until the end of July 2024. This study was approved by the Ethics Committee of SH-DMU in China.

Study design

No patient received immunosuppression tapering at the diagnosis of PTLD. The treatment methods were mainly divided into three modes (Fig. 1): 1. The initial treatment was rituximab monotherapy at 375 mg/m^2 once a week. The body temperature began to decrease, and the palpable lymph nodes gradually shrank within one week after medication, and then, the original treatment continued for 2–4 times totally. 2. The disease progressed after treatment with rituximab, resulting in discontinuation of this treatment. Three patients were then switched to DLI at an infusion dose of 5×10^6/kg of CD3 + T cells. 3. DLI was used for initial treatment, 5×10^6/kg CD3 + T cells once a week for one patient, and 5×10^5/kg once a week for two patients, 5×10^4/kg CD3 + T cells twice a week for eleven patients. The efficacy was evaluated after 2 weeks, and treatment was stopped after complete response (CR). The original dose of DLI in patients with partial remission (PR) was continued, and patients with progressive disease (PD) were switched to other regimens. Considering that the incidence of aGVHD was high when we used 5×10^6/kg DLI based upon the conventional dose internationally [21], we tried to give a reduced dose of DLI and have planned to perform prospective RCT trial (ChiCTR2400084399) in the future.

The primary endpoint of this study was the CR rate at 2 weeks post-intervention. Secondary endpoints included:

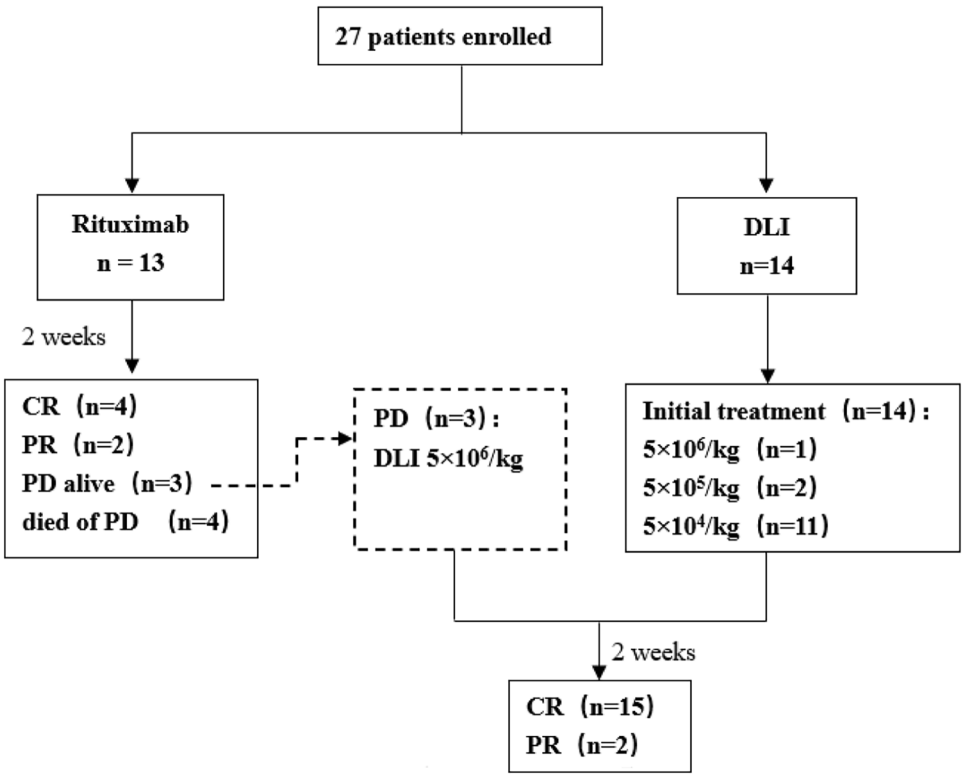

Fig. 1 Flow diagram of the study procedure

overall response rate (ORR), PR, and PD at 2 weeks in both groups; 1-year OS following different treatment methods; and cumulative incidence of NRM and aGVHD.

Definitions

Diagnosed EBV$^+$ PTLD refers to the discovery of evidence of EBV infection in lymph nodes or tissue samples, as well as the destruction of lymph node structures or the discovery of monoclonal cell populations. A suspected diagnosis refers to EBV-DNAemia accompanied by significant lymph node enlargement, hepatosplenomegaly, or other extranodal organ involvement that can be determined as a suspected diagnosis after excluding other causes [11]. There is no international definition for low, medium, and high doses of DLI. The EBMT consensus reduces the dose of DLI for treating leukemia recurrence to 1×10^7/kg and that for preventing leukemia recurrence to 1×10^6/kg [22]. The dosage is not uniform in the treatment of PTLD. Previous studies have used 2×10^5/kg–5.7×10^7/kg [21–23]. In this study, 5×10^4/kg was the lowest dose used to treat PTLD; therefore, we designated it as reduced-dose DLI. Overall survival (OS) was estimated from the time of allo-HSCT until death from any cause, and patients who were still alive at the final follow-up were censored at that point. Patients alive and relapse-free at final follow-up were censored at that point. Non-relapse mortality (NRM) was defined as death from any cause other than disease progression or relapse.

Allogeneic HSCT procedure

All 24 haplo-HSCT patients underwent transplantation with Beijing protocol except 3 MSD-HSCT, a myeloablative (MA) conditioning of modified BU/CY conditioning as follows: 2 g/m^2, q12h cytarabine for 2 days (qd for fully matched donor); 3.2 mg/kg/day busulfan for 4 days; 1.8 g/m^2 cyclophosphamide for 3 days; and 2.5 mg/kg/d ATG for 4 days (2 days for fully matched donor) [24]. Enhanced MA regimens, including the administration of decitabine, idarubicin, and clarithromycin, were given based on the risk of primary disease and disease status before transplantation [25]. SAA application of FLU/CY conditioning combined with ATG for the removal of T cells in vivo involved the administration of 30 mg/m^2 fludarabine for 4 days, 30–50 mg/kg cyclophosphamide for 2 days, and 2.5 mg/kg/d ATG for 4 days [26]. Full-matched siblings donor transplantation was performed using tacrolimus combined with short-term methotrexate. Mycophenolate mofetil was also added for haploidentical and unrelated donor transplantation.

PTLD treatment response evaluation criteria

Our center uses computer tomography (CT) or ultrasound to examine lymph node size, and the remission criteria were set according to the criteria of the International Working Group on Lymphoma [27]. The lymph node reduction area was the evaluation target organ, and treatment response was grouped into CR, PR, disease stability, and disease progression. However, the existing guidelines do not recommend a time for post-treatment evaluation. Thus, our center uses 2 weeks as the evaluation time for CR rate.

Statistical analyses

Data were analyzed using STATA/SE 15.1 software (STATA Corp, College Station, Texas, USA). ORR, CR, and PR were analyzed using Fisher's precision probability test. The Kaplan–Meier method was used to estimate OS, and the Log-rank test was used to determine statistical significance. NRM and cumulative incidence of relapse were estimated as competing risks for each other. Relapse and death were estimated as competing risks of aGVHD. The reverse Kaplan–Meier method was used to calculate median follow-up duration. Prognostic variables for OS were evaluated through univariate and multivariate analyses using Cox's proportional hazards regression analysis. Variables exhibiting statistical significance at $p < 0.1$ in the univariate analysis were included in the multivariate analysis to adjust for potential confounding effects; $p < 0.05$ was considered statistically significant. The following variables were evaluated: sex, age at allo-HSCT (< 40 years vs. ≥ 40 years), donor sex (female donor to male recipient vs. others), disease type (others vs SAA vs AML/MDS vs ALL), disease characteristics (malignant vs benign), treatment mode (rituximab vs DLI), DLI dosage (reduced vs normal dosage), donor age (> 60 vs. < 60), LDH level (high vs normal), and PTLD lesion (multiple sites vs one site).

Table 1 Baseline patient characteristics ($n = 27$)

Characteristic	Cases		p value
	Rituximab ($n = 13$)	DLI ($n = 14$)	
Age at HSCT (median, y)	30(6–52)	38(9–54)	
Sex			0.50
Male	3	6	
Female	10	8	
Diagnosis			0.77
Benign	4	4	
Malignant	9	10	
Donor type			0.95
Haplo	11	13	
Matched	2	1	
Donor gender			0.83
Male	6	6	
Female	7	8	
Stem cell Source			
BM + PB	10	2	0.004
PB	3	12	
Conditioning regimen			0.56
Standard	8	6	
Intensive	5	8	
GVHD prevent			0.64
CSA based	2	2	
FK506 based	11	12	
Antithymocyte globulin use			NA
Yes	13	14	
No	0	0	
Donor EBV-IgG			NA
Positive	13	14	
Negative	0	0	

AA aplastic anemia; *AL* acute leukemia; *MDS* myelodysplastic syndrome; *HLH* hemophagocytic syndrome; *BM* bone marrow; *PB* peripheral blood; *CSA* cyclosporine A; *FK506* tacrolimus; *NA* not available

Results

Patient and treatment characteristics

Patient demographics and baseline disease characteristics are shown in Table 1. The study comprised a cohort of 27 patients with EBV$^+$ PTLD. Of these, 18 were men with a median age of 30 (range 6–54) years, whereas nine were women with a median age of 42 (range 9–50) years. The overall median age was 34 (range 6–54) years, and the median PTLD onset time was 54 (range 30–65) days post-allo-HSCT. At the time of transplantation, 48.1%, 29.6%, and 18.5% of patients were classified as having AL, SAA, and MDS, respectively. Forty percent of patients had male donors, whereas 60% had female donors. The stem cell sources were bone marrow plus peripheral stem cell (PBSC), solely PBSC in 56%, 44%, respectively. Seventy percent of patients underwent standard MA conditioning regimens, whereas 30% underwent intensive MA regimens. Most patients (85%) received a tacrolimus-based GVHD prophylaxis mode, and 15% were cyclosporine-based. Furthermore, most patients (89%) underwent haploidentical HSCT. ATG was used in all 27 patients. Finally, all 27 PTLD patients showed donor EBV-IgG positivity without detectable EBV DNA.

Clinical and laboratory profiles of patients with PTLD

The clinical characteristics of the 27 patients are summarized in Table 2. Twenty-three cases were probable diagnose, and four were proven diagnose according to the European ECIL-6 diagnostic criteria. All 27 patients had lymph node enlargement and EBV activation. Eight cases involved extranodal organs, including one case in the central nervous system, five cases with simultaneous enlargement of the liver and spleen, and six cases with tonsils involvement. Furthermore, five cases underwent lymph node biopsy. Of these, four cases were pathologically diagnosed as PTLD, two were diagnosed as monotypic PTLD, two were diagnosed as polymorphic PTLD, and one case did not undergo immunohistochemical examination due to the absence of lymphoid tissue in the tissue specimens. Parameters such as neutrophils, platelets, and liver function were simultaneously monitored to facilitate understanding of laboratory changes in PTLD patients.

Table 2 Clinical and laboratory profiles of PTLD Patients (n = 27)

Characteristic	Cases		*p* value
	Rituximab ($n=13$)	DLI ($n=14$)	
Nodal			0.72
Only	2	3	
≥ 2 sites	8	14	
ECOG			0.89
0–2	8	10	
3–4	3	6	
Extranodal sites			0.75
CNS	1	0	
Liver	3	5	
Spleen	3	5	
Lung	1	0	
Waldeyer ring	3	2	
LDH			0.49
Elevate	10	8	
Normal	3	6	
Neutropenia			0.08
1–2 level	6	12	
3–4 level	7	2	
Thrombocytopenia			0.68
1–2 level	5	2	
3–4 level	14	6	
ALT			0.06
< 2 times	5	5	
≥ 2 times	2	0	
正常	4	11	
AST			0.24
< 2 times	4	5	
≥ 2 times	2	1	
Normal	10	5	
TB			
< 2 times	1	2	0.01
≥ 2 times	2	2	
Normal	8	12	

ECOG Eastern Cooperative Oncology Group performance, *CNS* central nervous system, *LDH* lactate dehydrogenase, *ALT* alanine transaminase, *AST* aspartate transaminase, *TB* total bilirubin

PTLD treatment diagram

The 27 patients were divided into two groups as shown in Fig. 1. Thirteen patients were initially treated with rituximab monotherapy at a dose of 375 mg/m^2 once a week. Patients whose body temperature began to decrease and palpable lymph nodes gradually shrank within one week continued the original treatment plan for 2–4 times totally. Three patients discontinued rituximab treatment after one week due to achievement of PD and then switched to treatment of DLI at a dose of 5×10^6 CD3$^+$ T cells/kg once a week for two weeks. DLI was employed as initial treatment in 14 patients, encompassing 1 patient receiving 5×10^6/kg once per week for 3 weeks, 2 receiving 5×10^5/kg once a week for two and three times, respectively, and 11 received reduced-dose DLI at 5×10^4 CD3$^+$ T cells/kg twice per week for a median of 4 (range 3–5 times) times within 2 weeks. Considering that the three patients whose PTLD progressed with rituximab treatment further received DLI salvage treatment, thus, the DLI group comprised a total of 17 patients.

Treatment response assessment

The primary endpoint of this study was the CR rate at 2 weeks post-intervention. The DLI cohort demonstrated higher CR (94.1%) than the rituximab group (40%) after two-week intervention (Table 3, Fig. 2A). The DLI cohort also exhibited higher ORR (100%) than the rituximab group (60%) in the secondary endpoints (Table 3, Fig. 2B). In addition, a lower PD rate was observed in

Table 3 Response assessment post-2-week treatment

	Rituximab ($n=10$)	DLI ($n=17$)	Odds ratio (95 CI)	*P*
ORR	60% (6/10)	100% (17/17)	0.000–0.893	0.035
CR	40% (4/10)	94.1% (16/17)	0.0053–0.594	0.0237
PR	20% (2/10)	5.8% (1/17)	0.2444–39.5	0.58
PD	40% (4/10)	0	0.00–0.3448	0.0039

ORR overall response rate, *CR* complete remission, *PR* partial remission, *PD* progressive disease

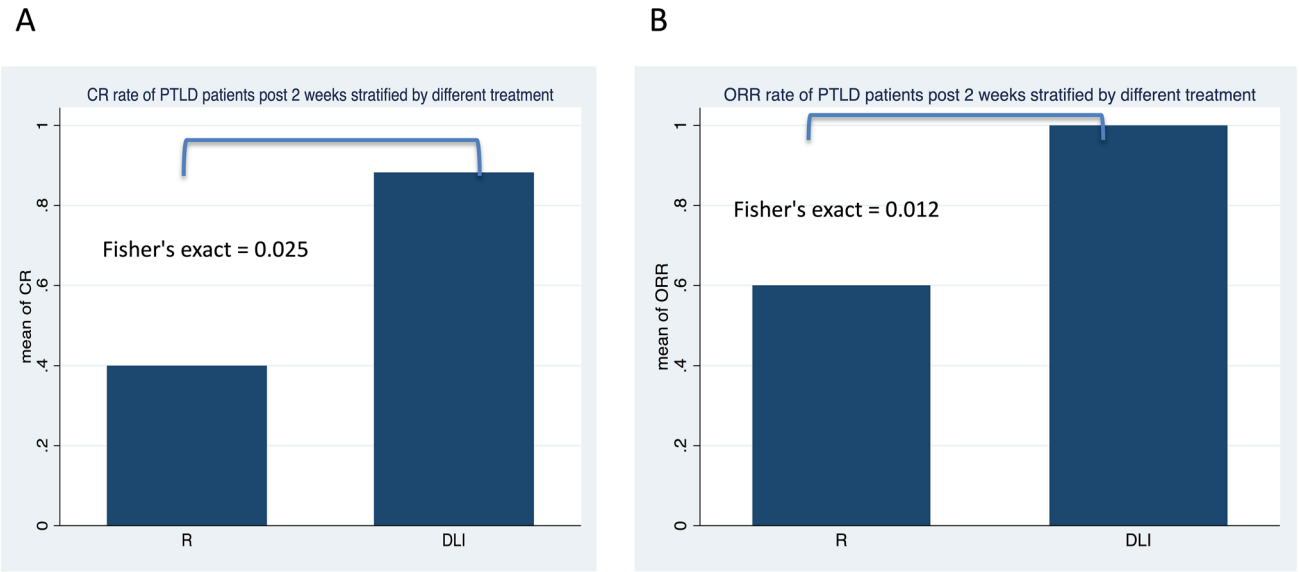

Fig. 2 CR and ORR assessment post-2-week treatment

the DLI cohort than in the rituximab cohort (0 vs 40%). The six patients who received conventional doses of DLI (5×10^5–5×10^6 CD3 + T cells/kg) exhibited a CR rate of 100% after 2 weeks. Similarly, the 11 patients who received reduced-dose DLI (5×10^4/kg) twice a week exhibited a 2-week CR rate of 90.9%. The secondary endpoint ORR also showed significant advantages in the reduced-dose DLI group ($P = 0.035$). The treatment flowchart and response outcomes of all 27 patients with PTLD are comprehensively described in Fig. 3.

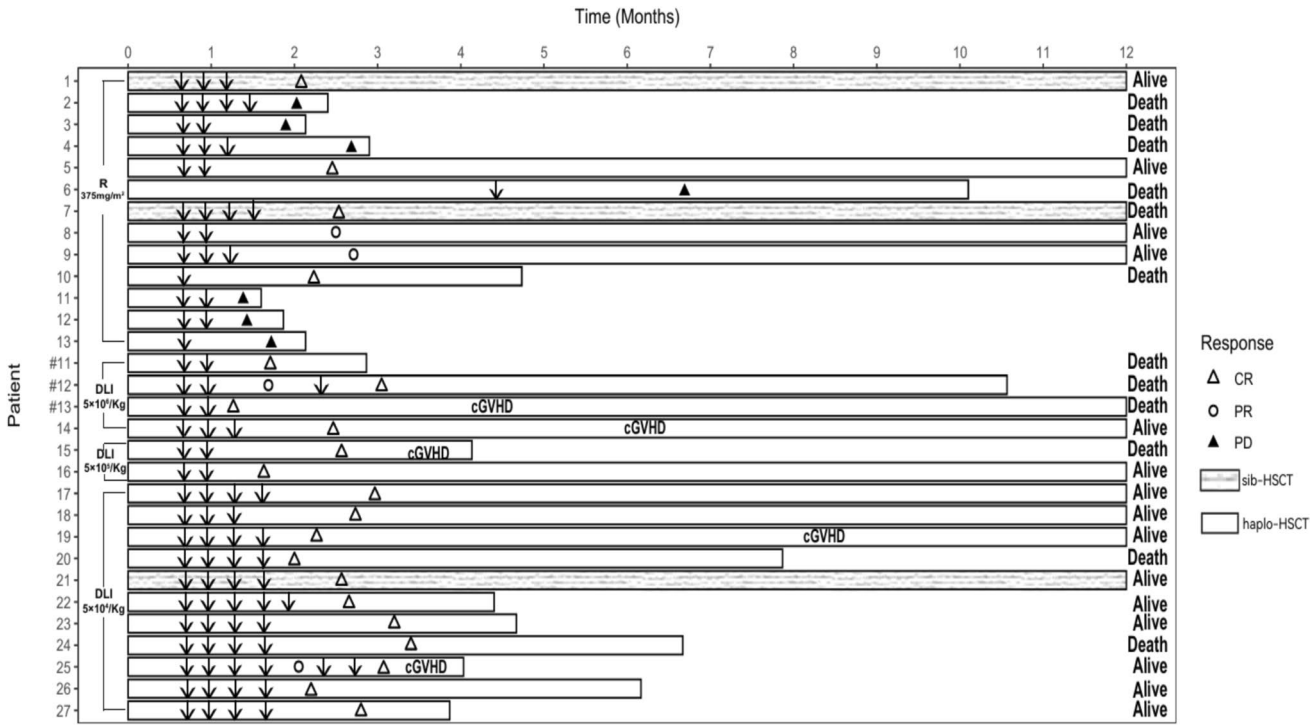

Fig. 3 Treatment and clinical outcome overview of 27 PTLD patients

OS and related risks

The median follow-up duration post-allo-HSCT was 537 (range 64–2234) days. The 1-year OS rates for all 27 patients were 57% (95% confidence interval [CI], 34–75%). Table 4 shows the univariate and multivariate analysis results of risk factors influencing OS. Univariate analysis revealed the following: (1) DLI conferred a significantly more favorable effect on OS (HR 0.22, 95% CI 0.05–1.05, $P=0.03$) than rituximab alone. This result was further substantiated by Kaplan–Meier survival analysis. Figure 4A shows the OS as stratified based on rituximab and DLI, with 1-year OS of 82.5% vs. 38.4% (Log-rank test $p=0.038$). (2) Reduced-dose DLI produced a significantly positive effect on OS (HR 0.14, 95% CI 0.01–1.37, $P=0.05$) than conventional dosage of DLI. This result was further substantiated by Kaplan–Meier survival analysis. Figure 4B shows the OS as stratified based on reduced- and conventional-dose DLI, with 1-year OS of 88.9% vs. 40% (Log-rank test, $p=0.04$). (3) Female donor to male recipient had an inferior effect on OS (HR 5.5, 95% CI 1.39–21.6, $P=0.0097$), which was further substantiated by Kaplan–Meier survival analysis. Figure 4C shows the OS as stratified based on female donor to male recipient and others, with 1-year OS of 27.7% vs. 83.3% (Log-rank test, $p=0.006$). (4) Male patients had poorer OS than female patients. This result was further confirmed via Kaplan–Meier survival analysis (Fig. 4D). However, when this is in multivariate analysis, only DLI treatment (HR 0.27, 95% CI 0.05–1.276, $P=0.05$) and female donor to male recipient (HR 4.76, 95% CI 1.19–18.9, $P=0.02$) had a substantial effect on OS. In addition, we analyzed other major potent risk variables on OS, as shown in Table 4 and Fig. 5. Age at transplantation, disease type, donor age, LDH level, and PTLD lesion sites had no statistically effect on OS.

NRM and aGVHD

Eleven patients died of non-relapse causes (NRM = 40.7%) at 1 year. The major causes of NRM were identified as follows: In the rituximab group, four patients died from PTLD progression despite early rituximab response, one patient died from end-stage TMA, and one patient died due to pneumonia. In the conventional-dosage DLI group, two patients died from GVHD and two died of pneumonia. In the reduced-dosage DLI group, one patient died from pneumonia.

The DLI group had lower NRM than the rituximab group (HR 0.252, 95% CI 0.05–1.18, $P=0.05$; Fig. 6A). The 1-year cumulative incidence of NRM for rituximab vs DLI was 53.8% vs. 17.5%, respectively (Fig. 6A). Although no statically significant difference was observed, the reduced-dosage DLI group had lower NRM tendency than the conventional-dosage group (HR 0.204, 95% CI 0.02–1.82, $P=0.15$; Fig. 6B). This was consistent with the 1-year cumulative incidence of NRM for conventional dosage vs reduced dosage (50% vs. 12.5%) (Fig. 6B). In total, six patients presented with aGVHD. Of these, one, three, and two were in the rituximab, conventional-dosage DLI (two patients died of GVHD), and reduced-dosage DLI groups, respectively. Although no statically difference was observed, the rituximab group had a lower incidence of aGVHD than the DLI group (Fig. 7A). Similarly, the reduced-dose DLI group exhibited a lower incidence of aGVHD than the conventional-dosage group (Fig. 7B).

Table 4 Univariate and multivariate analysis of predictors for OS

Factors		OS					
		Univariate			Multivariate		
		HR	95%CI	*p*	HR	95%CI	*p*
Gender	(Male vs Female)	1.39e + 16	0	0.0002			
Age at HSCT	(> 40 vs ≤ 40)	1.03	0.30–3.55	0.95			
disease type	(Others vs SAA vs AML/MDS vs ALL)	1.32	0.61–2.86	0.48			
Disease type	(Malignant vs benign)	1.13	0.29–4.32	0.85			
Donor Gender	(Female donor to Male recipient vs others)	5.50	1.39–21.6	0.0097	4.76	1.19–18.9	0.02
Treatment	(DLI vs Rituximab)	0.22	0.05–1.05	0.03	0.27	0.05–1.27	0.05
DLI dosage	(Low dosage vs conventional)	0.14	0.01–1.37	0.05			
Donor age	(Above 60 vs below 60)	2.15	0.55–8.36	0.29			
LDH level	(High vs normal)	1.44	0.38–5.48	0.59			
PTLD lesion site	(Multiple sites vs one site)	3.11	0.39–24.4	0.28			

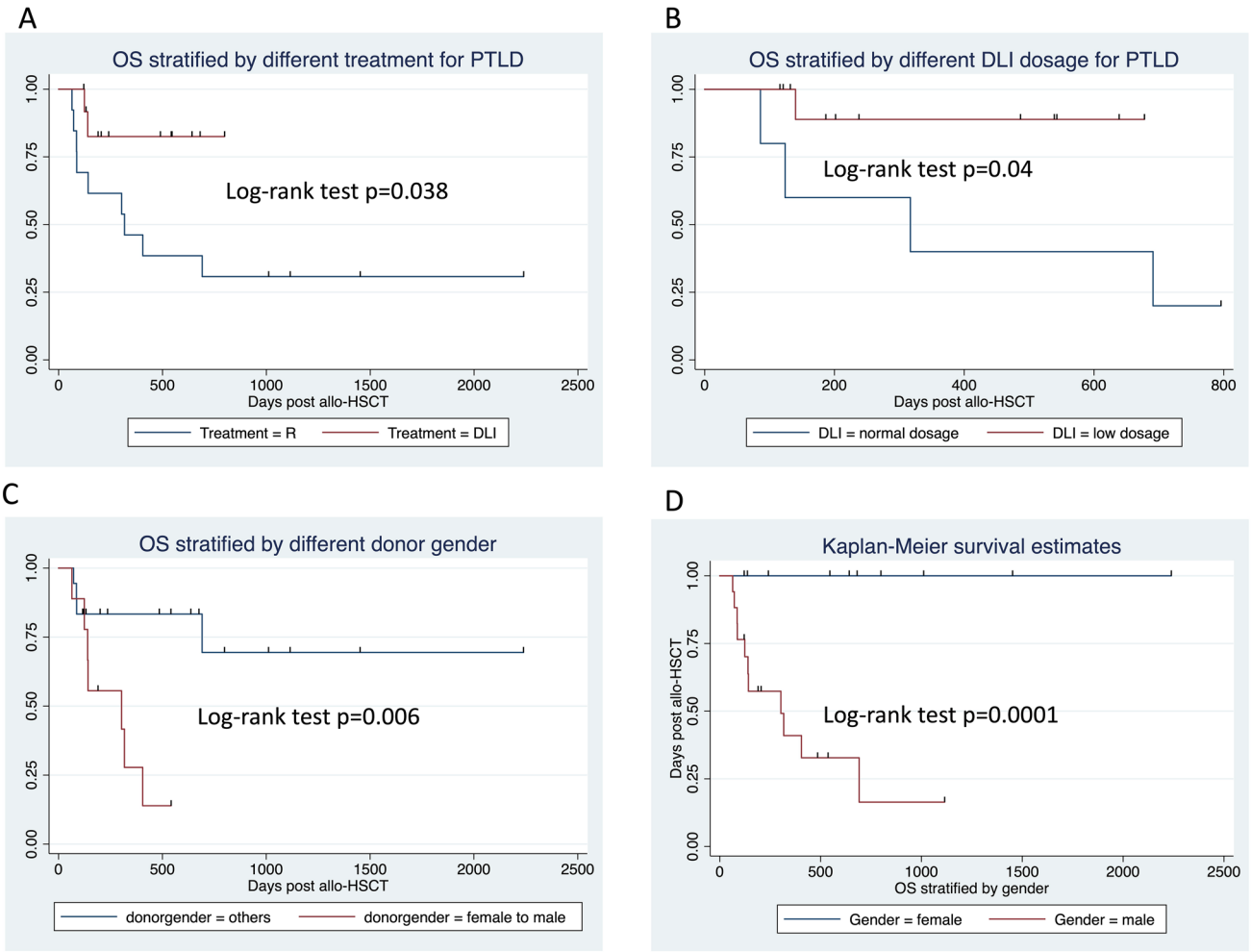

Fig. 4 OS stratified by different treatment, DLI dosage, donor gender, and patient gender

Discussion

EBV$^+$ PTLD is mainly associated with the conditioning regimen in allo-HSCT patients, which causes T cell depletion in vivo. This leads to immune escape and activation of EBV. Infected B cells can rapidly form monoclonal lymphoid proliferation that may also be accompanied by genetic instability and gene mutations. Lymphoma may also occur in certain cases [28]. The objective in PTLD treatment is to clear tumor cells, protect target organ function, and improve remission rates without affecting long-term survival. Further damage to the patient's immune function should be avoided during treatment. DLI is used to prevent or treat leukemia recurrence post-allo-HSCT, to treat PTLD, and to treat the activation of viruses such as EBV, CMV, and adenoviruses post-HSCT [29–31]. DLI enhances the immune response of the recipient by reintroducing T lymphocytes from healthy donors. The donor T cells can recognize and attack B lymphocytes infected with EBV. Furthermore, the donor T cells have natural immunity against EBV due to previous infection [32]. Donor T cells can also directly kill malignant proliferating B cells through cytotoxic effects, consequently providing anti-tumor effects. The infusion of donor lymphocytes facilitates partial restoration of immune function in the recipient, thereby improving the ability of the recipient immune system to recognize and eliminate potential malignant cells [33]. EBV-CTL can be isolated (and expanded in vitro) from EBV-seropositive stem cell donors or third-party donors that can recognize and kill EBV-infected cells, with a response rate of 88.2% in the treatment of EBV$^+$PTCL [34]. Tabelecleucel is an off-the-shelf, allogeneic, EBV-specific T cell immunotherapy consisting of EBV-specific CTLs generated using Good Manufacturing Practice and derived from EBV-seropositive donors that targets and eliminates EBV-expressing cells in an HLA-restricted manner that has a favorable safety profile and shows durable clinical benefit in R/R EBV$^+$ PTLD after HCT or SOT [35]. Nevertheless, the preparation of

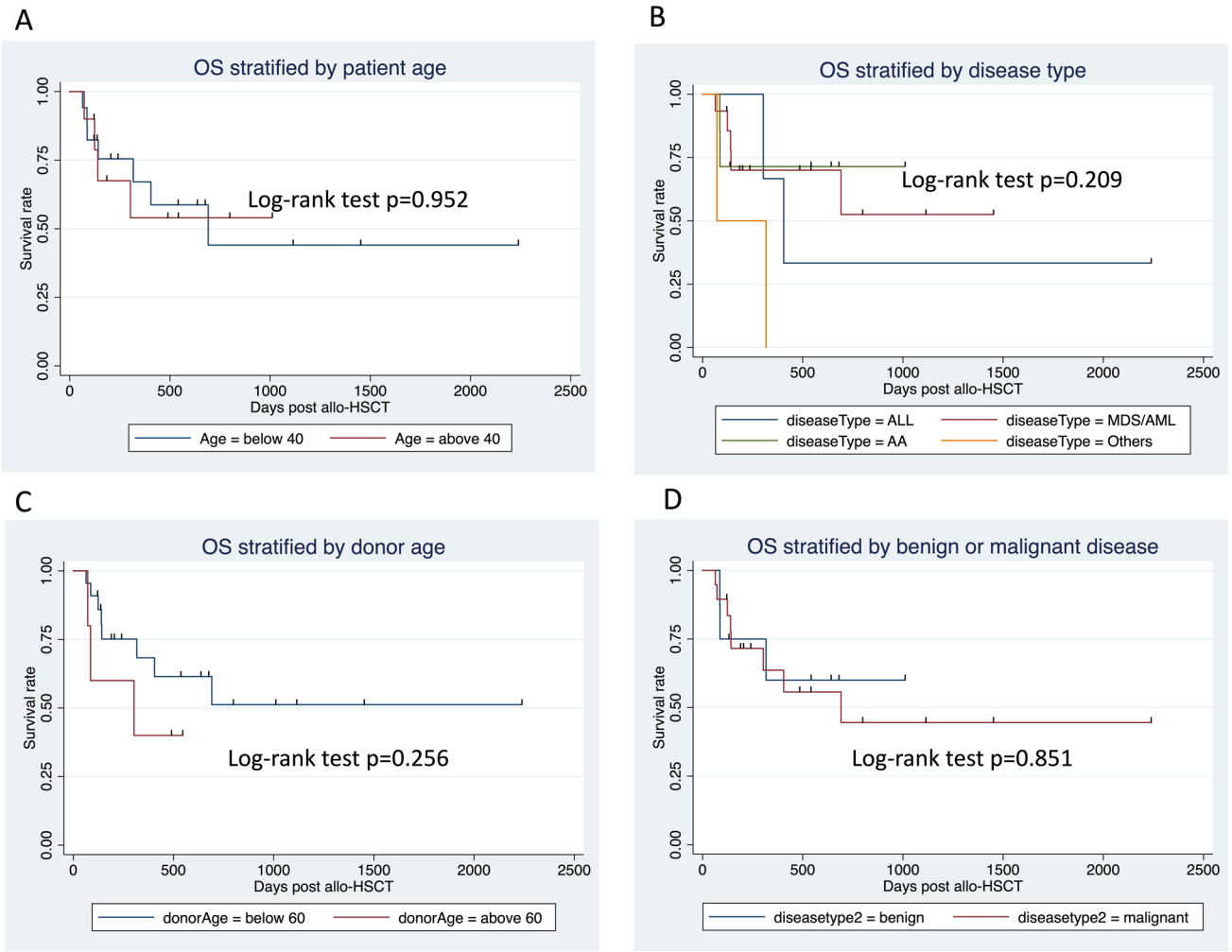

Fig. 5 OS stratified by patient age, disease type, donor age, and disease character

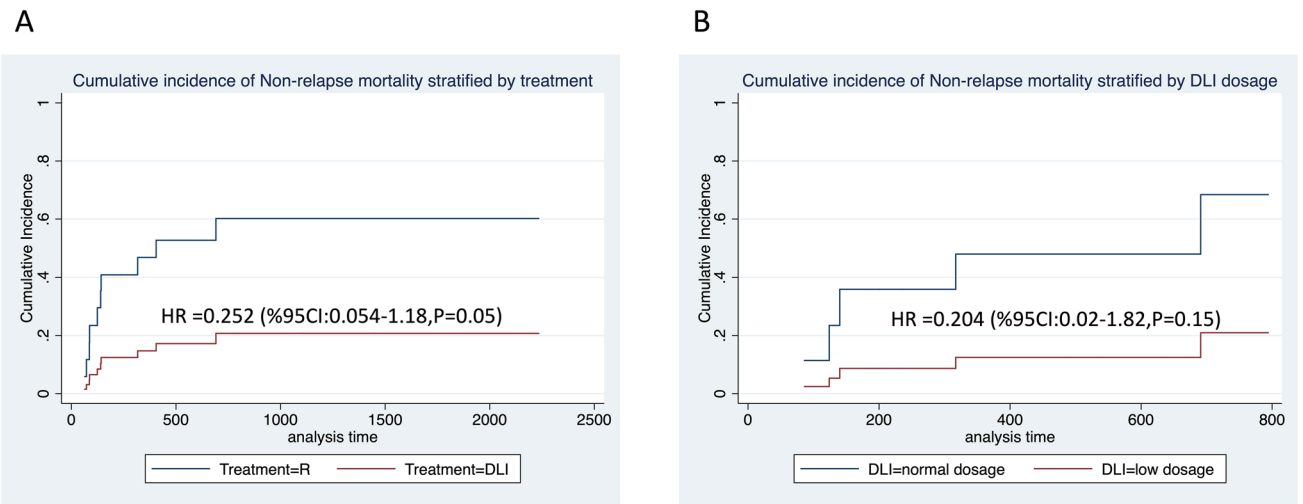

Fig. 6 Cumulative incidence of NRM stratified by different treatment and DLI dosage

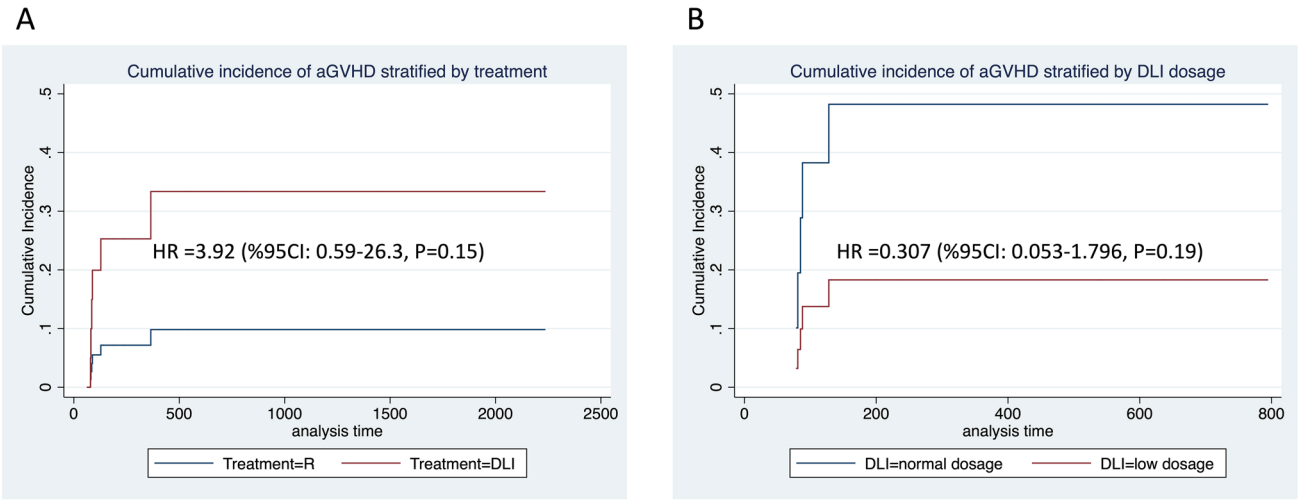

Fig. 7 Cumulative incidence of aGVHD stratified by different treatment and DLI dosage

EBV-CTLs is usually time-consuming and expensive that delays the treatment windows of patients, especially those with rapid disease progression and multiple-organ failure. Rituximab binds to the CD20 antigen on B cells to initiate an immune response that mediates B cell lysis, including complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. This consequently clears pathogenic and regulatory B cells as possible as it can. Therefore, rituximab therapy for PTLD can affect B cell remodeling and increase the risk of infection [36, 37].

A previous study comprising 30 DLI-treated patients with PTLD ($0.1–1\times10^6$ $CD3^+$ T cells/kg) reported a CR rate of 73% (17/30), a grade II-III aGVHD incidence of 14%, and OS rate of 33%. Five, two, and four patients died from PTLD progression, severe GVHD, and infection, respectively, whereas the rest died from primary disease recurrence and interstitial pneumonia [21]. In another study, nine patients with EBV^+PTLD were treated with $1.6–5.7\times10^7$ CD3 + T lymphocytes/kg. Two patients with non-original DLI donors only achieved short-term therapeutic effects, whereas six of the remaining seven patients achieved CR. However, the incidence of aGVHD II-IV was relatively high (57%). Only three patients survived for two years. Three, one, and one patient died from leukemia recurrence, intracranial infection, and gastrointestinal bleeding, respectively, whereas one was lost to follow-up [23]. These results indicate that the current minimum dose for treating PTLD is 1×10^4/kg. These studies further demonstrate the risk of developing aGVHD.

Rituximab monotherapy has been the frontline treatment for PTLD since 2018 in our center. In the present study, 13 patients had a 2-week CR rate of only 30.7%. Four cases of progressive PTLD after R treatment died due to disagreement of DLI treatment or unavailability of donor lymphocyte collection. Of these, three patients progressed but achieved remission after DLI as second-line salvage therapy. However, all three patients developed aGVHD and two died as a result. These results indicate that DLI has a high efficacy rate as a second-line treatment for PTLD. However, the final survival rate decreased due to induction of aGVHD. The side effects of DLI are fatal aGVHD or persistent cGVHD, and no other side effects were observed in our center, such as fever, anaphylaxis, aplasia, and poor engraftment. Thus, the ideal dose of DLI should clear tumor cells without causing aGVHD. However, the threshold for causing aGVHD is to be established not yet. $CD3^+$ T cells at a dose of 1×10^6/kg can cause clinically manifested aGVHD during MSD transplantation, with an incidence rate of approximately 18% reported; the risk of stem cell engraftment failure increases below this threshold [38, 39]. The efficacy of adoptive cell immunotherapy lies in the ratio of effector cells to tumor cells [21]. However, it is impossible to assess the precise tumor burden of PTLD. The reported number of CD3 + T cells internationally is $1\times10^5–5.7\times10^7$/kg.

In our study, firstly we used DLI as the frontline treatment by reducing the therapeutic dose to 5×10^5/kg administered 2–3 times. A remission rate of 100% was observed; however, one patient died from liver GVHD. We then employed DLI administration at a dose as low as 5×10^4/kg twice a week, which led to a complete remission rate of 90.9% within 2 weeks. High CR rate and low complications including low NRM led to better OS in DLI than rituximab group; similarly, better OS in reduced dosage than standard dosage could be seen (Fig. 4A, B). It demonstrates that our designated reduced-dose DLI is both safe and effective. This retrospective study demonstrates that small, repeated doses of DLI infusion can effectively and safely treat EBV^+ PTLD, with less probability for

induction of aGVHD. Moreover, this treatment regimen improved response rates as well as an increasing OS rate. We also found that male donor and female donor to male recipient were associated with poor OS (Fig. 4C, D), and similar phenomenon has been reported in other literature [40].

In the present study, we report on the viability and safety of reduced-dose DLI against PTLD. Our findings demonstrate that continuous optimization of the DLI dose and infusion frequency can maintain the complete remission rate and, meanwhile, ameliorate its impact on immune reconstitution and reduce the incidence of aGVHD. Most importantly, the NRM rate after transplantation decreased in this study, resulting in a more favorable 1-year OS. However, our study had some limitations. The small sample size restricted us from a better estimation of the end points and added statistical bias to our analyses. Furthermore, the challenges associated with the diagnosis and treatment of PTLD in clinical practice warrant further identification of more sensitive biological markers. Finally, the promising effectiveness of PTLD with reduced-dose DLI may be probably attributed to the cytotoxic T cells from healthy donors carrying seropositive immunoglobulin IgG against EBV, which indicate an immunological response capability against EBV infection; therefore, the effectiveness remains undetermined to some extent if with reduced-dose DLI from healthy donors carrying seronegative EBV-IgG. Therefore, large-scale, randomized, controlled, prospective clinical trials are required to explore the clinical outcomes and safety of reduced-dosage DLI in the treatment of EBV^+ PTLD.

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Data availability All data used and analyzed are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Informed consent This article does not contain any studies with human or animal subjects. Informed consent was obtained from all patients included in the study according to the Declaration of Helsinki.

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