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ORIGINAL ARTICLE

Graft CD8 T-cell-based risk system predicts survival in antithymocyte globulin-based myeloablative haploidentical peripheral blood stem cell transplantation

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Abstract

Objective. This study investigated the cellular composition of peripheral blood grafts for anti-thymocyte globulin (ATG)-based myeloablative haploidentical haematopoietic stem cell transplantation (haplo-HSCT). **Methods.** Clinical characteristics were retrospectively evaluated in a training cohort with ATG-based myeloablative haplo-HSCT between January 2016 and February 2020 and confirmed in a validation cohort between March 2020 and June 2021. **Results.** A higher dose of graft $CD8^+$ T cells ($\geq 0.85 \times 10^8$ kg^{-1}) was significantly improved overall survival (OS; hazard ratio [HR], 1.750; $P = 0.002$) and disease-free survival (DFS; HR, 1.751; $P < 0.001$) in the training cohort, according to multivariate Cox regression analysis. Higher doses of mononuclear cells (MNCs) demonstrated better OS (HR, 1.517; $P = 0.038$) and DFS (HR, 1.532; $P = 0.027$). Older patient age (> 46 years), older donor age (≥ 50 years) and a higher refined disease risk index (rDRI) were also related to OS. A graft $CD8^+$ T-cell risk system based on graft $CD8^+$ T-cell dose, donor age and rDRI was constructed using a nomogram model after LASSO Cox regression analysis. It showed acceptable discrimination, with a C-index of 0.62 and 0.63, respectively. Graft $CD8^+$ T-cell dose was negatively correlated with donor age ($P < 0.001$) and positively correlated with a higher lymphocyte percentage in the peripheral blood before mobilisation ($P < 0.001$). **Conclusion.** A higher $CD8^+$ T-cell dose in peripheral blood-derived grafts improves patients' survival with ATG-based myeloablative haplo-HSCT. Younger donors with higher lymphocyte percentages improved patients' survival with an intermediate rDRI risk.

Keywords: $CD8^+$ T cells, graft, haematopoietic stem cell transplantation, haploidentical donor, myeloablative conditioning regimen

INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) is a well-established curative strategy for various malignant diseases.[1,2] Haematopoietic stem cell transplantation with human leukocyte antigen (HLA)-haploidentical donors has outcomes equal to those with HLA-matched sibling donors.[3] Optimal donor selection should be considered because of the easy accessibility of several haploidentical donors for an HSCT recipient.[4,5] Graft cell composition from different donors is heterogeneous and plays pivotal roles in engraftment kinetics, immune reconstitution and clinical outcomes following HSCT.[6] The number of $CD34^+$ cells in a graft is significantly associated with engraftment and survival after HSCT.[7,8] The cellular composition of grafts other than $CD34^+$ cells also affects HSCT clinical outcomes.[9–11]

However, different cellular populations in the grafts result in distinct clinical outcomes after HSCT. A higher dose of $CD3^+$ T cells in the peripheral blood grafts of haploidentical HSCT (haplo-HSCT) reportedly increases the risk of acute graft-*versus*-host disease (GVHD).[12] In contrast, the number of $CD8^+$ T cells in the graft did not influence the acute GVHD in this study.[12] Notably, a large study by the Center for International Blood and Marrow Transplant Research (CIBMTR) uncovered that the number of $CD3^+$ T cells in peripheral blood-derived grafts did not influence the risk of acute and chronic GVHD when using HLA-matched sibling donors or 8/8-matched unrelated donors by multivariate analysis.[11] In anti-thymocyte globulin (ATG)-based haplo-HSCT, the CD3/CD4 ratio and dose of $CD8^+$ cells were found to be independent factors for predicting severe acute GVHD.[13] Additionally, discrepant clinical outcomes associated with graft composition can be observed with different GVHD prophylaxis protocols. Nikoloudis *et al*. found that a high CD4/CD8 ratio in peripheral blood stem cells adversely impacted survival in the context of mycophenolate- or post-transplant cyclophosphamide (PTCy)-based GVHD prophylaxis rather than methotrexate-based GVHD prophylaxis.[9]

Generally, identification of the optimal graft composition is complicated by the donor type, GVHD prophylaxis and graft sources. Hence, to investigate the impact of the cellular composition of peripheral blood-derived grafts on the clinical outcomes after ATG-based myeloablative haplo-HSCT, we evaluated the clinical data in a training cohort and confirmed it in a validation cohort.

RESULTS

Characteristics of the haplo-HSCT recipients

The baseline clinical and laboratory characteristics of the training and validation cohorts are summarised in Table 1. In total, 750 haplo-HSCT recipients who received myeloablative conditioning regimens were enrolled: 528 in the training cohort and 222 in the validation cohort. The median follow-up time was 1297 (7–2354) days for the training cohort and 489 (2–847) days for the validation cohort ($P < 0.001$). There were significant differences between the training and validation cohorts in terms of patient age ($P < 0.001$), rDRI ($P = 0.005$), donor age ($P = 0.048$), donor sex ($P = 0.028$), donor–recipient relationship ($P < 0.001$), ATG type for GVHD prophylaxis ($P < 0.001$) and various cellular compositions in peripheral blood grafts. Patient age, underlying disease and donor–recipient ABO blood compatibility did not differ significantly between the cohorts. The optimal cut-off values for cellular composition in the graft are shown in Supplementary table 1.

Clinical outcomes in the training cohort

The cumulative incidence of grade II–IV acute GVHD and grade III–IV acute GVHD at 100 days was 28.2% (95% confidence interval [CI], 24.4–32.1%) and 14.3% (95% CI, 9.7–15.3%), respectively. The 2-year cumulative incidence of total chronic GVHD and moderate-to-severe chronic GVHD was 37.5% (95% CI, 33.3–41.7%) and 9.8% (95% CI, 7.4–12.6%), respectively. The probabilities of OS and DFS at 2 years were 76.5% (95% CI, 73.0–80.0%) and 72.9% (95% CI, 69.2–76.6%), respectively. The two-year cumulative incidence of relapse and non-relapse mortality (NRM) at 2 years was 19.1% (95% CI, 15.9–22.6%) and 8.0% (95% CI, 5.8–10.5%), respectively.

A univariate analysis of the factors associated with clinical outcomes is shown in Supplementary table 2. Table 2 presents the independent factors related to the clinical outcomes according to the multivariate analysis. A high CD4/CD8 ratio (≥ 1.44) was an independent risk factor for acute GVHD. Compared with ATG-T, ATG-F significantly increased the acute and chronic GVHD risk. In the multivariate

Table 1. Baseline characteristics for patients with myeloablative haplo-HSCT

Characteristics	Training cohort, *n* (%)	Validation cohort, *n* (%)	*P*-value
No.	528	222	
Patient age, median (range), years	31 (15–62)	41 (15–63)	< 0.001
Patients gender, female/male	225/303	110/112	0.081
Underlying disease			0.154
AML	245 (46.4)	105 (47.3)	
MDS	43 (8.1)	22 (9.9)	
ALL	188 (35.6)	84 (37.8)	
Others	52 (9.8)	11 (5.0)	
rDRI			0.005
Low	29 (5.5)	7 (3.2)	
Intermediate	334 (63.3)	120 (54.1)	
High	131 (24.8)	83 (37.4)	
Very high	34 (6.4)	12 (5.4)	
Donor age, median (range), years	34 (10–59)	30 (10–65)	0.048
Donor gender, female/male	194/334	63/159	0.028
Donor-patient relationship			< 0.001
Parents	187 (35.4)	64 (28.8)	
Children	155 (29.4)	110 (49.5)	
Siblings	159 (30.1)	35 (15.8)	
Other relatives	27 (5.1)	13 (5.9)	
Donor–patient ABO blood compatibility			0.284
Compatible	286 (54.2)	107 (48.2)	
Major-incompatibility	103 (19.5)	50 (22.5)	
Minor-incompatibility	113 (21.4)	48 (21.6)	
Bidirectional-incompatibility	26 (4.9)	17 (7.7)	
Graft cellular compositions, median (IQR)			
CD34 cells, $\times 10^6$ kg^{-1}	5.59 (3.93–7.79)	6.23 (4.86–7.73)	0.006
MNC, $\times 10^8$ kg^{-1}	14.36 (10.80–19.43)	12.20 (9.60–18.00)	0.002
CD3, $\times 10^8$ kg^{-1}	1.86 (1.20–2.58)	1.29 (0.63–1.97)	< 0.001
CD4, $\times 10^8$ kg^{-1}	0.94 (0.55–1.33)	0.70 (0.28–1.07)	< 0.001
CD8, $\times 10^8$ kg^{-1}	0.78 (0.48–1.08)	0.53 (0.28–0.85)	< 0.001
CD19, $\times 10^8$ kg^{-1}	0.39 (0.22–0.59)	0.14 (0.05–0.42)	< 0.001
NK, $\times 10^8$ kg^{-1}	0.32 (0.22–0.46)	0.26 (0.11–0.41)	< 0.001
CD4:CD8, $\times 10^8$ kg^{-1}	1.16 (0.87–1.51)	1.09 (0.86–1.48)	0.547
ATG type			< 0.001
ATG-T	255 (48.3)	217 (97.7)	
ATG-F	273 (51.7)	5 (2.3)	
Median follow-up, median (range), days	1297 (7–2354)	489 (2–847)	< 0.001

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; haplo-HSCT, haploidentical haematopoietic stem cell transplantation; IQR, interquartile range; MDS, myelodysplastic syndromes; rDRI, refined disease risk index.

Cox regression analysis, the independent risk factors associated with worse OS were older donors (≥ 50 years), older recipients (> 46 years), a lower dose of CD8$^+$ T cells in the graft (< 0.85 × 10^8 kg^{-1}), lower dose of MNCs in the graft (< 9.30 × 10^8 kg^{-1}), a lower dose of NK cells in the graft (< 0.18 × 10^8 kg^{-1}) and a higher risk of rDRI. However, donor age and NK cell count in the graft had no impact on DFS following haplo-HSCT in the multivariate Cox regression analysis.

Nomogram model for graft immune cell-based risk system

Based on the results of the least absolute shrinkage and selection operator (LASSO) analysis, rDRI, donor age and CD8$^+$ T cells in the graft were incorporated into a graft CD8$^+$ T-cell-based risk system (gCD8RS) *via* the nomogram model to predict OS (Figure 1). Subsequently, haplo-HSCT recipients were classified into high- and low-risk

Table 2. Multivariate analysis for clinical outcomes of myeloablative haplo-HSCT in the training cohort

Variables for clinical outcomes	HR	95% CI	*P*
OS			
Patient age (ref. ≤ 46)	1.571	0.432–0.939	0.023
Donor age (ref. < 50)	1.801	0.371–0.830	0.004
rDRI			
High	Ref.		
Low	0.367	0.131–1.030	0.057
Intermediate	0.715	0.491–1.040	0.079
Very high	2.267	1.341–3.832	0.002
CD8 T cells (ref. high dose)	1.750	1.227–2.496	0.002
MNC (ref. high dose)	1.517	1.023–2.239	0.038
NK cells (ref. high dose)	1.530	1.025–2.285	0.037
DFS			
rDRI			
High	Ref.		
Low	0.327	0.117–0.911	0.032
Intermediate	0.760	0.530–1.091	0.136
Very high	2.440	1.470–4.053	< 0.001
CD8 T-cells (ref. high dose)	1.751	1.257–2.440	< 0.001
MNC (ref. high dose)	1.532	1.049–2.239	0.027
Relapse			
Donor age (ref. < 50)	1.599	1.008–2.536	0.046
rDRI			
High	Ref.		
Low	0.191	0.045–0.811	0.025
Intermediate	0.543	0.362–0.815	0.003
Very high	2.032	1.092–3.782	0.025
NRM			
Patient age (ref. ≤ 46)	1.980	1.088–3.590	0.025
rDRI			
High	Ref.		
Low	1.170	0.226–6.090	0.850
Intermediate	1.940	0.866–4.350	0.110
Very high	2.910	1.016–8.350	0.047
MNC (ref. high dose)	2.070	1.080–3.960	0.028
NK (ref. high dose)	2.570	1.368–4.830	0.003
Grade II-IV acute GVHD			
ATG type (ref. ATG-T)	2.322	1.635–3.299	< 0.001
CD4:CD8 ratio (ref. high dose)	0.648	0.464–0.906	0.011
Grade II-IV acute GVHD			
ATG type (ref. ATG-T)	2.296	1.342–3.926	0.002
CD4:CD8 ratio (ref. high dose)	0.414	0.254–0.676	< 0.001
Mild-to-severe chronic GVHD			
ATG type (ref. ATG-T)	2.250	1.655–3.050	< 0.001
Moderate-to-severe chronic GVHD			
Donor relationship (ref. non-sibling donor)	2.44	1.390–4.260	0.002
ATG type (ref. ATG-T)	22.63	5.49–93.24	< 0.001

groups according to total points in the gCD8RS model using the R software with *'Survminer:: surv_cutpoint'* package, with a cut-off point of 69.

Patients with high-risk gCD8RS had significantly shorter OS ($P = 0.036$) and DFS ($P = 0.015$) than those with low-risk gCD8RS (Figure 2). The C-index of the gCD8RS for 2-year OS in the training cohort by bootstrap resampling was 0.65 (95% CI, 0.61–0.69). The calibration curve displayed good concordance between the predicted and actual survival rates in gCD8RS (Figure 3). The C-indices for the validation and entire cohorts were 0.62 (95% CI, 0.54–0.70) and 0.63 (95% CI, 0.59–0.67), respectively. Good consistency between the predicted and actual survival rates was observed in the validation and entire cohorts (Figure 3). The area under the curve (AUC) values at 2 years for gCD8PS were 0.672 (95% CI, 0.621–0.728), 0.613 (95% CI, 0.485–0.741) and 0.670 (95% CI, 0.623–0.717) for the training, validation and entire cohorts, respectively (Figure 3). The 2-year decision curve analysis (DCA) demonstrated that the gCD8RS model had a better net benefit within a range of tolerable threshold probabilities in the training, validation and entire cohorts (Figure 4).

Impact of CD8 T cells in the peripheral blood-derived graft on haplo-HSCT outcomes

According to a previous study regarding to the relationship between donor characterisation and the outcome of haplo-HSCT,[14] the entire cohort was divided into two subgroups based on the donor age of 38 years. In a subgroup of haplo-HSCT recipients with younger donors (< 38 years, $N = 441$), compared to patients with a low CD8 T-cell dosage, those with a higher dosage had a better OS (82.7%; 95% CI, 77.5–88.2% *vs*. 74.0%; 95% CI, 68.3–80.2%, $P = 0.031$), DFS (77.8%; 95% CI, 72.2–83.8% *vs*. 68.4%; 95% CI, 62.5–74.8%, $P = 0.009$) and decreased CIR (13.2%; 95% CI, 8.9–18.3% *vs*. 22.0%; 95% CI, 16.8–27.7%, $P = 0.009$) at 2 years (Supplementary figure 1). With respect to haplo-HSCT recipients with older donors (≥ 38 years, $N = 309$), patients with a higher dosage had a better OS (82.2%; 95% CI, 74.4–90.8% *vs*. 73.2%; 95% CI, 67.5–79.5%, $P = 0.037$), DFS (82.3%; 95% CI, 74.6–90.9% *vs*. 68.9%; 95% CI, 62.9–75.3%, $P = 0.027$) and decreased NRM rate (3.5%; 95% CI, 0.9–9.1% *vs*. 10.8%; 95% CI, 7.1–15.3%, $P = 0.028$) (Supplementary figure 2).

In the subgroup of 454 patients with intermediate risk of rDRI, high dose of CD8 T cells increased the OS (87.5%; 95% CI, 82.7–92.7% *vs*. 76.2%; 95% CI, 71.1–81.6%, $P = 0.003$) and DFS (85.2%; 95% CI, 80.1–90.8% *vs*. 72.4%; 95% CI, 67.2–78.0%, $P < 0.001$), and reduced CIR (7.1%;

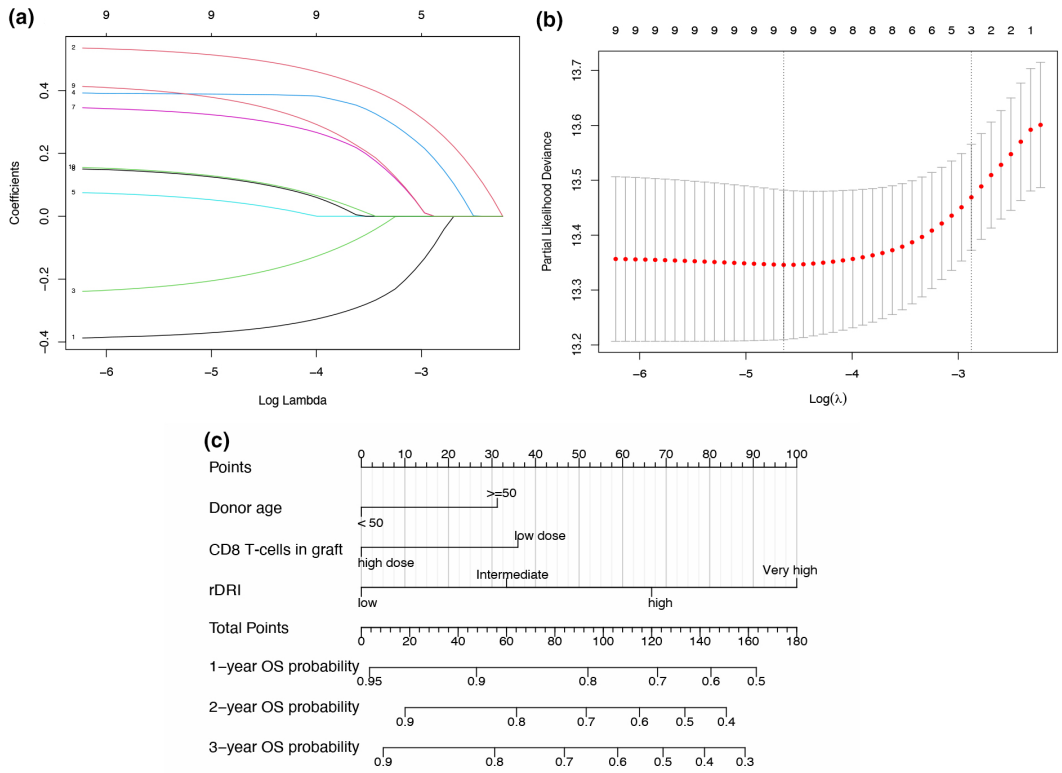

Figure 1. Development of graft CD8$^+$ T-cell-based risk system (gCD8RS). **(a)** The features with nonzero coefficients were selected by optimal lambda. **(b)** The LASSO model was constructed to select the optimal parameters (lambda) and the relationship graph between binomial deviance and log (lambda) was drawn. **(c)** Nomogram model of the gCD8RS.

95% CI, 3.9–11.7% *vs*. 17.7%; 95% CI, 13.4–22.6%, $P = 0.002$) (Supplementary figure 3). In 214 patients with high-risk rDRI, a trend of higher OS and DFS rates and lower CIR was found in patients with a higher dose of CD8$^+$ T cells in grafts (Supplementary figure 4).

In addition, the graft CD8$^+$ T-cell dose was negatively correlated with donor age in the analysis of the entire cohort ($R^2 = 0.03$; $P < 0.001$; Figure 5). As elucidated in the validation cohort, a higher lymphocyte percentage in the peripheral blood before rhG-CSF administration was associated with a higher dose of CD8$^+$ T cells in peripheral blood-derived grafts ($R^2 = 0.06$; $P < 0.001$; Figure 5).

DISCUSSION

In the present study, we analysed the impact of the cellular compositions of haploidentical donor grafts on the outcomes of patients with ATG-based myeloablative HSCT and established a gCD8RS model to predict survival, which might be useful for future donor selection. We found that the counts of CD8$^+$ T cells, NK cells and MNCs correlated with OS. Moreover, a high percentage of lymphocytes in the donor peripheral blood before mobilisation was associated with a high CD8$^+$ T-cell dose in the grafts.

In this study, a high CD8$^+$ T-cell dose ($\geq 0.85 \times 10^8$ kg^{-1}) in the peripheral blood-derived grafts was significantly associated with favorable survival outcomes after ATG-based myeloablative haplo-HSCT. Additionally, a high dose of CD8$^+$ T cells in the grafts decreased the risk of relapse of the underlying disease, although this was not statistically significant in multivariate analysis. A high CD8$^+$ T-cell dose may contribute to the graft-*versus*-leukaemia effect of donor-derived immune cells. It has been reported that early expression of CD94 and loss of CD96 in CD8$^+$ T cells are significant factors for predicting subsequent relapse and survival after HCST.[15] CD8$^+$ T-cell expansion from cord blood grafts is robust and can mediate cytotoxicity to generate an antileukaemic effect.[16] In agreement with our findings, it has been

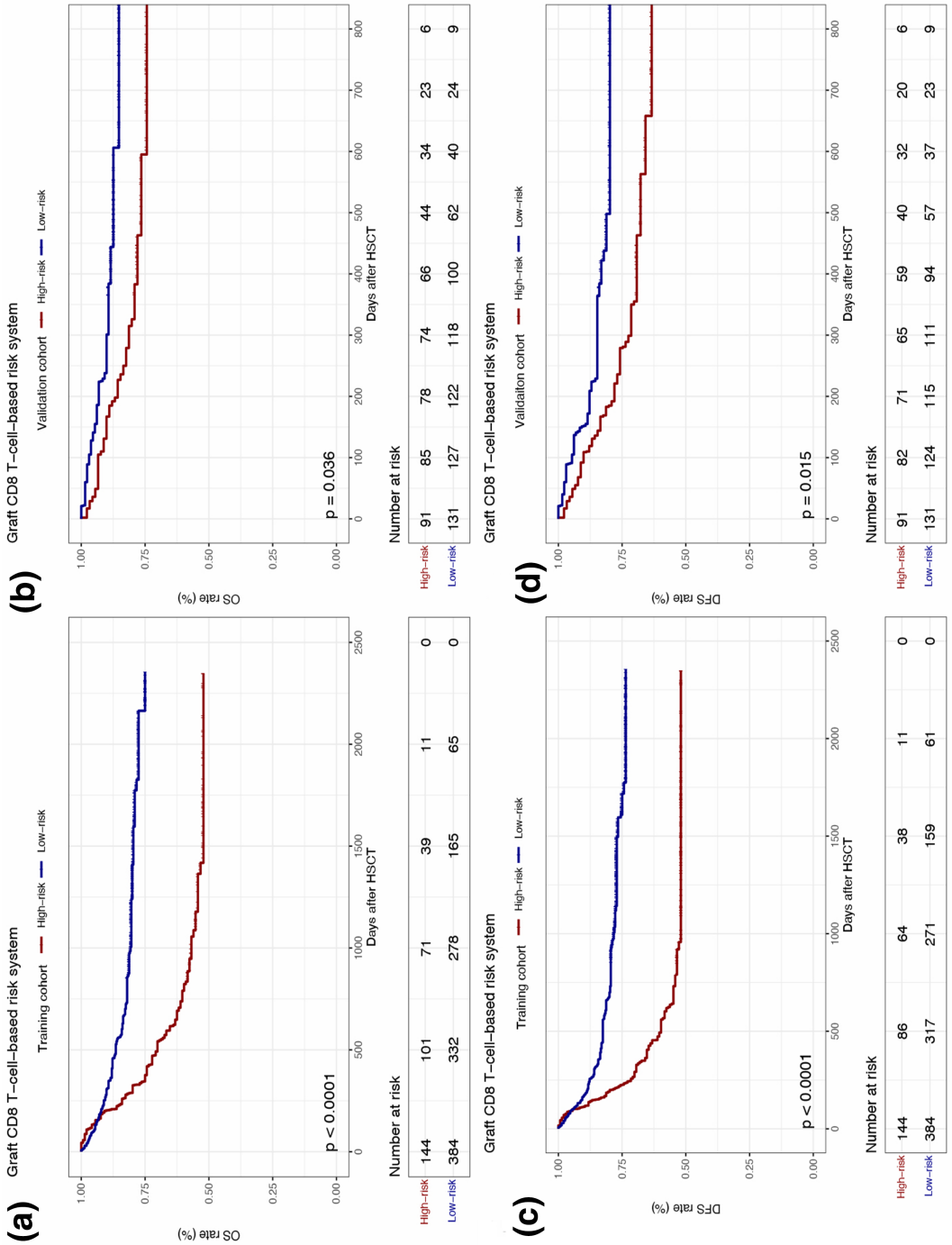

Figure 2. Survival in patients with myeloablative haploidentical haematopoietic stem cell transplantation. **(a)** Probability of OS in recipients with high- and low-risk of gCD8RS in the training cohort. **(b)** Probability of OS in recipients with high- and low-risk of gCD8RS in the validation cohort. **(c)** Probability of DFS in recipients with high- and low-risk of gCD8RS in the training cohort. **(d)** Probability of DFS in recipients with high- and low-risk of gCD8RS in the validation cohort.

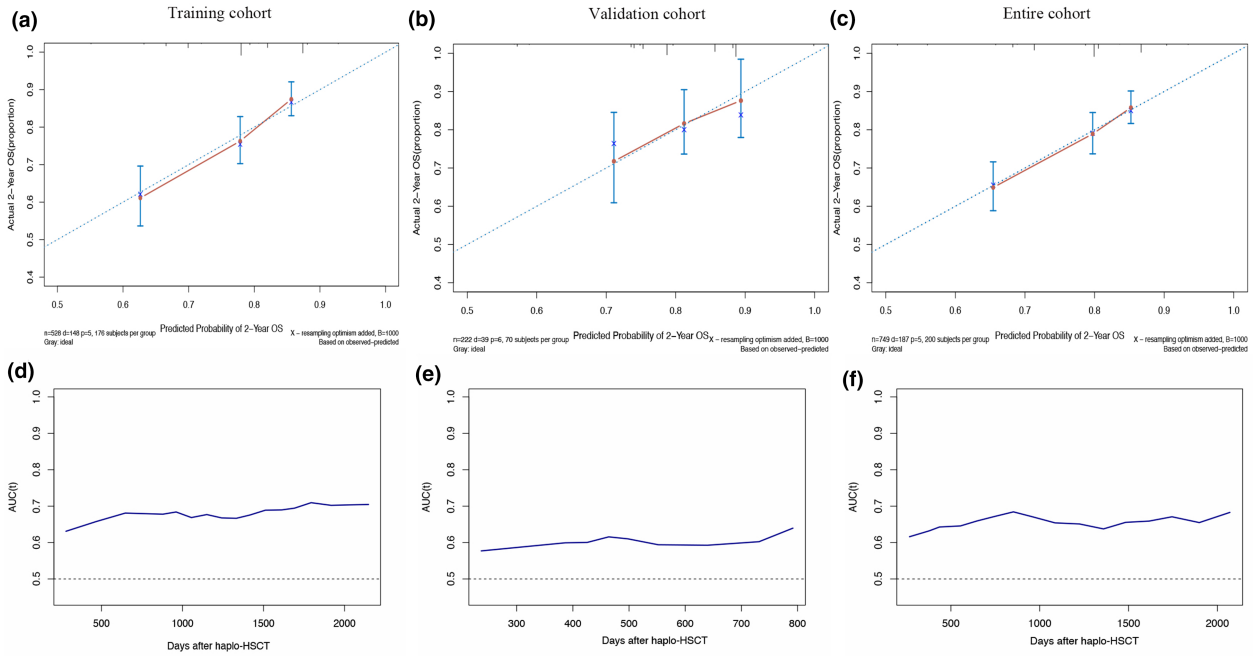

Figure 3. Calibration curves for predicting OS at 2 years after haplo-HSCT and time-dependent AUCs of gCD8RS in the training, validation, and entire cohorts. **(a)** Predicted *vs*. observed probability of 2-year OS in the training cohort. **(b)** Predicted *vs*. observed probability of 2-year OS in the validation cohort. **(c)** Predicted *vs*. observed probability of 2-year OS in the entire cohort. **(d)** Time-dependent AUC of gCD8RS in the training cohort. **(e)** Time-dependent AUC of gCD8RS in the validation cohort. **(f)** Time-dependent AUC of gCD8RS in the entire cohort.

reported that a high $CD8^+$ T-cell dose has an independent influence on OS, DFS and NRM in myeloablative umbilical cord blood transplantation.[17] Furthermore, a previous study indicated that a high graft $CD8^+$ cell dose ($> 0.72 \times 10^8$ kg^{-1}) could predict improved survival after HSCT with reduced-intensity conditioning regimens, which could lower the risk of disease relapse after HSCT,[18] and may be attributed to the differences in the cut-off values of the $CD8^+$ T-cell dose or intensity of the conditioning regimen. Furthermore, the study by Reshef *et al.*[18] predominantly used HLA-matched donors, whereas all patients in our study received haplo-HSCT. Notably, a study consisting of 299 patients who underwent HLA-matched HSCT indicated that a high graft $CD8^+$ cell dose significantly decreased the risk of relapse in the subgroup with unrelated donors.[19] However, Svenberg *et al.*[19] revealed that the graft $CD8^+$ T cells did not affect relapse in HSCT recipients with related donors. Similarly, Cao *et al.*[20] confirmed that the graft $CD8^+$ cell dose had no impact on outcomes after myeloablative HSCT, when conventional HLA-identical related donors were utilised. Notably, these controversial results were observed in the context of HLA-mismatched transplantation using ATG for GVHD prophylaxis but without ATG for HLA-matched-related donors.[17,19] As previously reported, the interaction between the conditioning regimen and ATG affects relapse and OS.[21] Remarkably, different ATG schedules contribute to different early immune reconstitutions, especially $CD8^+$ T-cell reconstitution, influencing the clinical outcomes.[22] In contrast, in haplo-HSCT with PTCy-based GVHD prophylaxis, the clinical outcomes were contingent on the CD4/CD8 ratio in the graft rather than on the counts of $CD8^+$ T cells.[10] A balanced CD4/CD8 ratio (0.85–1.5) was significantly associated with a superior DFS, while a lower CD4/CD8 ratio (≤ 0.85) increased the risk of relapse.[10] A high CD4/CD8 ratio (> 2.42) was associated with overall mortality (hazard ratio [HR], 2.07; $P = 0.04$) in HLA-mismatched HSCT with PTCy-based GVHD prophylaxis.[9]

Graft $CD8^+$ T cells were not an independent factor in the development of GVHD in this study. In line with our findings, several studies have shown that the dose of $CD8^+$ T cells in grafts has no impact on the GVHD.[12,18,19] However, higher numbers of $CD8^+$ T cells in G-CSF-primed peripheral blood grafts could predict severe acute GVHD in haplo-HSCT for patients with acute leukaemia, in which

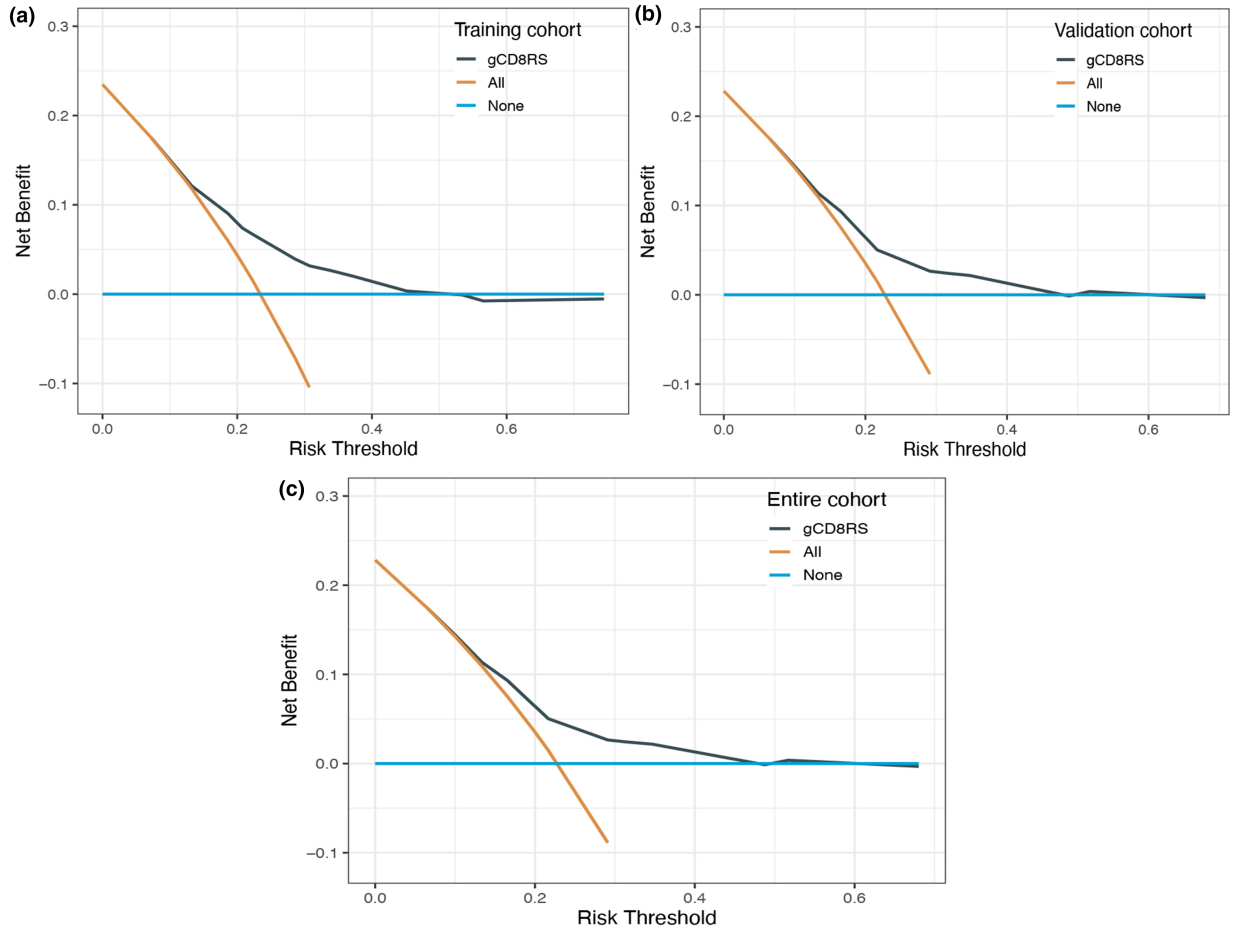

Figure 4. **(a)** The DCA curve in the training cohort. **(b)** The DCA curve in the validation cohort. **(c)** The DCA curve in the entire cohort.

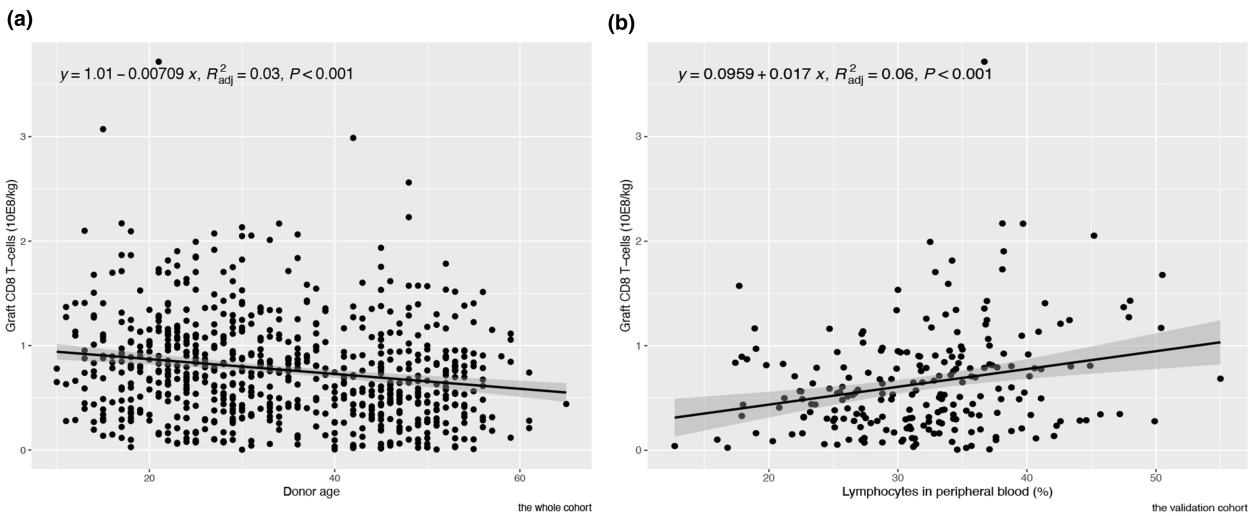

Figure 5. **(a)** The scatter plot of donor age and graft $CD8^+$ T-cell dose. **(b)** A scatter plot of graft CD8 cell dose and circulating lymphocytes in the peripheral blood before mobilisation.

recipients were treated with ATG (2.5 mg kg^{-1} per day, days −5 to −2; Sanofi, France) for GVHD prophylaxis.[13] In haplo-HSCT with 800 cGy total body irradiation, busulfan, fludarabine and ATG-based regimen, a high graft $CD8^+$ T-cell dose was significantly associated with grades II–IV acute GVHD and moderate-to-severe chronic GVHD.[23] The different types and doses of ATG used for GVHD prophylaxis or different regimens could be responsible for these controversial findings. It has been reported that ATG-T rather than ATG-F is associated with delayed immune reconstitution[24] and a lower risk of extensive chronic GVHD.[25] Historically, higher proportions of $CD8^+$ γδ cells in grafts were potentially alloreactive and associated with an increased risk of GVHD after HSCT.[26] Multivariate analysis confirmed that a high CD4/CD8 ratio was an independent factor in the risk of acute GVHD. Concordantly, low levels of $CD8^+CD161^{hi}$ T (MAIT) cells in G-CSF-primed peripheral blood grafts are associated with an increased risk of acute GVHD after haplo-HSCT.[27] The association between a high ratio of CD4/CD8 in grafts and an increased risk of acute GVHD[28] or adverse survival[29] has also been observed previously; however, other studies did not observe the adverse impact of a high CD4/CD8 ratio on clinical outcomes.[19,30] These contradictory results may be attributed to dissimilar GVHD prophylaxis or donor sources at different centers.

We observed that donor age was an independent factor for OS after haplo-HSCT, and there was a trend towards a higher graft $CD8^+$ T-cell dose in younger donors. A previous 10-year follow-up study confirmed that younger donors improved survival in recipients of ATG-based haplo-HSCT.[14] Moreover, in a large cohort of recipients of PTCy-based haplo-HSCT using bone marrow grafts, increasing the donor age by a decade resulted in inferior OS (HR, 1.13; $P = 0.0015$) and DFS (HR, 1.09; $P = 0.015$).[31] However, a recent cohort study of PTCy-based haplo-HSCT using peripheral blood grafts indicated that donor age had no significant impact on survival, which was attributed to the fact that increasing donor age was associated with lower relapse rates and higher NRM.[32] Mariotti *et al.*[33] also confirmed that increasing donor age could reduce the risk of disease relapse (HR, 0.92; $P = 0.001$) and result in a higher NRM and worse OS after T-cell-replete haplo-HSCT with PTCy. However, there is no clear consensus regarding the relationship between donor age and survival in recipients of HSCT. Reshef *et al.*[18] found that favorable survival in recipients with unrelated donors was related to a high $CD8^+$ T-cell dose in grafts and suggested that the counts of $CD8^+$ T cells in grafts rather than the donor age were associated with a potent graft-*versus*-leukaemia effect. They speculated that grafts with a high $CD8^+$ T-cell dose from younger donors would contain a higher number of naïve $CD8^+$ T cells, generating a potent graft-*versus*-leukaemia effect.[34] Additionally, they found that donor age was inversely correlated with the graft $CD8^+$ T-cell dose,[18] which is in accordance with our results. The overall T-cell reservoir decreases with age, and the rapid decrease in $CD8^+$ T cells with age leads to an increase in the CD4/CD8 ratio.[35] A report on donor lymphocyte infusion also found a positive correlation between donor age and the CD4/CD8 ratio.[36]

Despite the relatively large sample size, our study was limited by its retrospective single-centre design and the lack of in-depth information on factors such as naïve $CD8^+$ T cells, which could affect the outcomes. Moreover, the gCD8RS model did not achieve satisfactory ability to predict the clinical outcomes in the validation cohort, which could be attributed to the smaller size and the shorter follow-up period of the validation cohort compared with the training cohort. Moreover, the small number of patients with low or very high risk of rDRI restrained the evaluation of the impact of graft cellular composition on clinical outcomes, and the patient population was highly heterogeneous with regard to the biological characteristics of the underlying disease.

In conclusion, a high dose of $CD8^+$ T cells in peripheral blood grafts was significantly associated with superior survival in patients who underwent ATG-based myeloablative haplo-HSCT, and the gCD8RS model incorporating $CD8^+$ T cells in the graft, donor age and rDRI was capable of predicting survival. Additionally, a haploidentical donor with a younger age and higher circulating lymphocyte frequency would significantly improve the survival of patients with an intermediate risk of rDRI.

METHODS

Patients

Clinical and laboratory data of patients with haplo-HSCT were retrospectively collected and analysed from the electronic medical record system at the First Affiliated Hospital of Zhejiang University School of Medicine between January 2016 and June 2021. Patients aged ≤ 15 years, receiving bone marrow stem cells, using reduced-intensity conditioning, lacking information on

graft immune cellular composition or undergoing a second transplantation were excluded. This study was approved by the Ethics Review Committee of the First Affiliated Hospital of the Zhejiang University School of Medicine (approval no. IIT20210351A) and adhered to the Declaration of Helsinki.

Development of a graft cellular composition-based nomogram system

In total, 528 patients who underwent haplo-HSCT between January 2016 and February 2020 were classified into the training cohort. Patients who underwent haplo-HSTC between March 2020 and June 2021 ($N = 222$) were included in the validation cohort. The underlying disease, patient and donor sex, rDRI, donor-recipient relationship, donor–recipient ABO blood type compatibility, ATG type and graft cellular composition were analysed. Univariate and multivariate Cox regression models were used to identify the variables associated with survival after haplo-HSCT. Least absolute shrinkage and selection operator (LASSO) Cox regression analysis was used for variable selection in the nomogram.[37] A 5-fold cross-validation procedure was performed using LASSO Cox regression. Significant variables with $P < 0.05$ in univariate analysis were included in multivariate Cox regression and LASSO Cox regression analyses. Parameters with nonzero coefficients were incorporated into the graft-cellular composition-based nomogram system.

Harrell's concordance index (C-index) was used to evaluate the discriminative ability and predictive accuracy of the nomogram in the training cohort. A calibration curve from 1000 bootstrap replicates was used to assess the conformity between the model-predicted probability and actual conditions. The predictive ability of the nomogram was evaluated using time-dependent receiver operating characteristic (ROC) curves and area under the curve (AUC) in the training, validation and entire cohorts. The net benefit of the novel nomogram was measured with decision curve analysis (DCA). Patients were classified into high- and low-risk groups based on the nomogram risk scores.

Flow cytometry evaluation of graft cellular composition

Mononuclear cells (MNCs), $CD34^+$ cells, $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ and NK cells were counted by multiparameter flow cytometry on freshly mobilised G-CSF-primed peripheral blood grafts on either a FACScan or FACSCalibur cytometry system (Becton Dickinson, San Jose, CA, USA) by the Key Laboratory of Kidney Disease Prevention and Control Technology, Zhejiang Province. The cellular dose was calculated based on the patient's body weight.

Transplantation procedure

Myeloablative conditioning regimens comprising busulfan and cyclophosphamide or the modified regimens based on busulfan and cyclophosphamide were administered to all patients. Graft-*versus*-host disease prophylaxis consisted of cyclosporin A, methotrexate and low-dose mycophenolate mofetil.[38] Rabbit antithymocyte globulin (ATG-T, Thymoglobulin; Genzyme, Cambridge, MA, USA, 6 mg kg^{-1} total dose; days −5 to −2) or Anti-T-lymphocyte globulin (ATG-F; Fresenius, Bad Homburg, Germany, 10 mg kg^{-1} total dose; days −5 to −2) was administered to all patients. Peripheral blood stem cells were mobilised with rhG-CSF (5–7.5 μg kg^{-1} per day; Filgrastim; Kirin, Japan) for 5–6 consecutive days from Day −4, and were harvested with a COBE Blood Cell Separator (Spectra LRS; COBEBCT Inc., Lakewood, CO). All the patients received unmanipulated peripheral blood grafts.

Definitions

Disease risk was retrospectively assessed using rDRI.[39] Acute GVHD and chronic GVHD were defined and graded as previously established.[40,41] Relapse was defined as disease recurrence documented by blast reappearance (> 5%) in bone marrow, or extramedullary locations. Non-relapse mortality was defined as death during continuous remission. Disease-free survival was defined as the time from haplo-HSCT to disease recurrence or death from any cause. Overall survival was defined as the time from haplo-HSCT to death from any cause or the last follow-up.

Statistical analysis

Baseline characteristics were summarised as median and interquartile range (IQR) for the graft cellular composition and median and range for the remaining continuous variables. Continuous variables were compared using the Mann–Whitney *U*-test. The best cut-off values for continuous variables were calculated in R using the '*Survminer*' package. Categorical variables were summarised as frequencies and percentages and evaluated using the chi-squared test. Competing risk regression analysis was used to estimate the cumulative incidence of relapse (CIR), NRM and GVHD *via* R using the cmprsk package.[42] The Kaplan–Meier approach and log-rank test were used to estimate OS and DFS. A Cox regression model for OS and DFS was used in R using the Survival package. The rms package in R was used to plot the nomogram and formulate a calibration curve. The ROC curve with the AUC was constructed using the R with timeROC package. The DCA curve was plotted in R using the ggRCA package. Statistical significance was defined as a two-tailed *P*-value of <0.05. Statistical analyses were conducted using the SPSS 26.0 software (SPSS, Chicago, IL, USA) and R software (http://www.r-project.org).

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AUTHOR CONTRIBUTIONS

Panpan Zhu: Data curation; formal analysis; methodology; writing – original draft. **Luxin Yang:** Data curation; formal analysis; methodology. **Yibo Wu:** Data curation; writing – review and editing. **Jimin Shi:** Resources; supervision. **Xiaoyu Lai:** Data curation; resources. **Lizhen Liu:** Data curation; investigation. **Yishan Ye:** Data curation. **Jian Yu:** Data curation. **Yanmin Zhao:** Data curation. **Xiaolin Yuan:** Data curation. **Huarui Fu:** Data curation. **Zhen Cai:** Data curation. **He Huang:** Data curation; project administration; resources; supervision. **Yi Luo:** Conceptualization; funding acquisition; project administration; writing – review and editing.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

The data sets analysed during this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL

This study was approved by the Ethics Review Committee of the First Affiliated Hospital of the Zhejiang University School of Medicine (approval no. IIT20210351A) and adhered to the Declaration of Helsinki. Trial registration: The trial was registered at www.chictr.org.cn (#ChiCTR2100052071).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.