Changing donors improves outcomes of second transplantation in patients who experienced graft failure after first allogeneic stem cell transplantation

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Abstract

A second transplantation is almost the only salvage for patients encountering graft failure (GF) following first allogeneic stem cell transplantation. However, there were no standard protocols for second transplantations, and the role of changing donors remained controversial. We retrospectively studied 272 consecutive patients from 18 Chinese centers undergoing second transplantations due to GF, aiming to assess the impact of changing donors and the factors affecting second transplantation outcomes. The primary endpoint was neutrophil engraftment. Other endpoints included platelet engraftment, graft-*versus*-host disease (GvHD), transplant-related mortality (TRM), relapse, and survival. Of the 272 patients, 193 (71.0%) patients experienced primary GF, and 70.6% (192) used a different second donor. Neutrophil engraftment was achieved in 218 (86.3%) patients by day (d)28, and platelet engraftment was achieved in 164 (70.0%) patients by d100. The 3-year cumulative incidence of acute GvHD, chronic GvHD, relapse, and TRM were 43.5%, 27.8%, 15.6%, and 44.6%, respectively. The 1-year and 3-year overall survival (OS) were 56.1% and 49.5%, respectively. Compared to using the same donor, changing donors significantly improved neutrophil engraftment (92.4% *vs*. 71.4%, *P*<0.001) and platelet engraftment (76.9% *vs*. 51.8%, *P*<0.001), 1-year TRM (34.8% *vs*. 56.3%, *P*<0.001), and OS (61.9% *vs*. 42.7%, *P*<0.001). Subgroup analysis confirmed engraftment benefit of changing donor in primary GF (*P*<0.001), but not in secondary GF (*P*=0.346). This is the largest multicenter study of second transplantations for GF, suggesting that changing donors might be critical for engraftment and survival after second transplantation.

Introduction

Graft failure (GF) is a rare yet potentially lethal complication following allogeneic stem cell transplantation (SCT), with its prevalence particularly marked in cord blood (CB) or haploidentical SCT (haplo-SCT).[1-4] A second transplantation is critical to patient survival,[5,6] though there remains no standard protocol concerning conditioning regimens, donor selection, graft-*versus*-host disease (GvHD) prophylaxis, or other pivotal factors.[7-9] Most of the existing literature comprises retrospective summaries, offering limited insight into the efficacy of specific techniques. Currently, the outcomes of a second transplantation are far from satisfactory, with neutrophil engraftment ranging from 58% to 100%, and 1-year overall survival (OS) ranging from 11% to 66%.[10-13] Therefore, there is a pressing need to refine and optimize the protocols for second transplantation to improve patient outcomes.

Recently, we developed an innovative protocol for second haplo-SCT to manage GF after first haplo-SCT, demonstrating encouraging results with 100% engraftment and 60% OS,[14] which was further validated by an updated follow-up study.[15] This novel strategy is different from prior protocols in three key elements: 1) a mini-intensity conditioning regimen based on fludarabine (Flu) and cyclophosphamide (Cy); 2) the intentional selection of a different second donor; and 3) rapid re-transplantation as soon as GF has been identified. It seems that the efficacy of this approach might be largely attributed to changing donors. Nonetheless, the role of changing donors in second transplantations was controversial in previous literature. Therefore, the current study aims to investigate determinants of second transplant outcomes, with an emphasis on the implications of changing donors.

Methods

Patients

From January 2000 to December 2023, consecutive patients who received second transplantations due to GF from 18 transplant centers in China were retrospectively studied. The study was approved by the ethics committee of Peking University People's Hospital. The last follow-up date was March 31, 2024.

Definitions

The primary endpoint was neutrophil engraftment by day (d)28 post second transplantation. Secondary endpoints included platelet engraftment by d100, acute GvHD (aGvHD) by d100, and chronic GvHD (cGvHD), transplant-related mortality (TRM), relapse, and survival after one and three years.

Neutrophil engraftment was defined as the first of three consecutive days with neutrophil count ≥0.5×10^9/L. Platelet engraftment was the first of seven consecutive days with platelet count ≥20×10^9/L without transfusion. Complete donor chimerism was defined as having ≥95% of hematopoietic cells originating from the donor, determined using single nucleotide polymorphism (SNP) and/or fluorescence *in situ* hybridization (FISH). Primary GF was failure to achieve neutrophil engraftment by d28 for haploidentical (HID), matched related (MRD) or unrelated (URD) donors, or d42 for CB recipients. Secondary GF was two or three lineage cytopenias following initial engraftment, without any discernible causes such as disease relapse, infections, or drugs. HID refers to relatives sharing one chromosome 6 with variable non-shared HLA haplotype.[16] GvHD was diagnosed and graded by National Institutes of Health criteria.[17,18] For GvHD prophylaxis, combinations of calcineurin inhibitors (cyclosporine A [CsA] or FK506), mycophenolate mofetil (MMF), and anti-thymocyte globulin (ATG) or basiliximab were defined as intensified regimens.

Second transplantation protocols

Preconditioning and GvHD prophylaxis were heterogenous following institutional guidelines based on transplant types. For patients with donor specific antibody (DSA) median fluorescence intensity (MFI) >2,000-5,000, center-specific desensitization such as rituximab, plasma exchange / immunoadsorption, intravenous immunoglobulin, or combinatorial immunosuppression was applied. Antimicrobial prophylaxis included antiviral (e.g., acyclovir), antifungal (e.g., posaconazole, trimethoprim-sulfamethoxazole), and antibacterial (e.g., fluoroquinolones) approaches and followed institutional protocols.

Statistical analysis

Mann-Whitney U and χ^2 tests were applied for comparison of continuous and categorical variables. Death was a competing event for GvHD and relapse. Relapse was a competing event for TRM. OS and disease-free survival (DFS) were estimated with the Kaplan-Meier method. Variables with $P<0.1$ were included in the multivariate analysis. Statistical significance was defined as $P<0.05$. Analyses were conducted with SPSS (version 23.0; Chicago, IL, USA) and R software.

Results

Patient characteristics

A total of 272 patients from 18 centers were analyzed. First transplant donors included HID (54.0%), CB (29.8%), MRD (9.2%), and URD (7.0%). DSA was positive in 21.1% of patients pre-first transplantation, with a median MFI of 7,178 (range, 246-18,039) (Table 1).

Most patients (71.0%) experienced primary GF. The median interval between transplants was 55 (range, 18-2,592) days, differing between primary GF (41 days, range 18-765) and

secondary GF (195 days, range 43-2,592, *P*<0.001). A different donor was used in 192 (70.6%) patients. DSA was positive in 14.3% of patients before second transplantations, with a median MFI of 2,152 (range, 246-19,682). Preconditioning regimens for second transplantations were heterogeneous, among which a Flu and Cy-based regimen was the most commonly used (29.8%). The combination of CsA plus MMF was the most frequently adopted regimen for GvHD prophylaxis (22.4%) (Table 2).

Outcomes of second transplantations

Engraftment

Neutrophil engraftment was achieved in 218 (86.3%, 95% CI: 82.0-90.6) patients by d28, and in 225 (90.6%, 95% CI: 86.7-94.5) patients by d60. Platelet engraftment was achieved in 164 (70.0%, 95% CI: 63.7-76.3) by d100. The median time for neutrophil and platelet engraftment was 13 (range, 7-50) days and 16 (range, 7-200) days, respectively (Figure 1).

Graft-versus-host disease

The cumulative incidences of grade 2 to 4 and grade 3 to 4 aGvHD by d100 were 33.4% (95% CI: 26.8-39.8) and 20.3% (95% CI: 14.4-26.1), respectively (Figure 1). cGvHD developed in 36 patients, including 10 cases of moderate-to-severe cGvHD. Cumulative cGvHD incidence was 23.9% (95% CI: 16.8-31.0) at one year and 27.8% (95% CI: 17.7-32.5) at three years. Corresponding incidence for moderate to severe cGvHD was 6.2% (95% CI: 3.5-7.3) at one year and 7.5% (95% CI: 5.1-9.9) at three years.

Infections

111 (40.8%) patients developed cytomegalovirus (CMV) reactivation, and 26 (9.56%) developed Epstein-Barr virus (EBV) reactivation. Median time to reactivation was 28 days (range, 1-1,156) for CMV and 41.5 days (range, 11-411) for EBV. The cumulative incidence of CMV and EBV reactivation on d100 were 43.9% (95% CI: 37.6-50.6) and 10% (95% CI: 6.1-13.9), respectively (Figure 1).

Relapse

In 183 patients with hematologic malignancies, 15 experienced disease relapse, which was the cause of death in 11 of them. The cumulative incidence of relapse for one year and three years was 9.9% (95% CI: 4.2-15.6) and 15.6% (95% CI: 7.4-23.8), respectively (Figure 1).

Survival

The median follow-up for survivors was 604 (range, 13-4,061) days after second transplantation. 123 patients died, with main causes including infections (52.8%), multi-organ dysfunction (11.4%), GvHD (8.94%), and relapse (8.94%). Seven (5.69%) patients died from GF (*Online Supplementary Table S1*). The cumulative incidence of TRM at 30 days, 100 days, one year, and three years was 11.1% (95% CI: 7.4-14.8), 27.2% (95% CI: 21.9-32.5), 41.2% (95% CI: 35.1-47.2), and 44.6% (95% CI: 38.0-51.0), respectively. The 1-year and 3-year OS was 56.1% (95% CI: 50.1-62.3) and 49.5% (95% CI: 42.9-56.3), respectively (Figure 1).

Subgroup analysis for transplant outcomes

Patients with aplastic anemia (AA) showed advantages in platelet engraftment and survival compared to hematologic malignancies (*Online Supplementary Figure S1*). Patients

Table 1. Patient characteristics of the first transplantations.

Variables	Number (%)
Age, years, median (range)	26 (3-67)
Sex, male	128 (59.3)
Disease	
AA	87 (32.0)
AML	75 (27.6)
ALL	50 (18.4)
MDS	34 (12.5)
CML	10 (3.7)
CMML	7 (2.6)
PMF	2 (0.7)
NHL	2 (0.7)
Other	5 (1.8)
Donor age, years, median (range)	34 (2-64)
Donor sex, male	164 (61.7)
Donor type	
MRD	25 (9.2)
URD	19 (7.0)
CB	81 (29.8)
Haplo	147 (54.0)
DSA prior to 1st transplant	
Available	161 (59.2)
Positive	34 (21.1)
MFI, median (range)	7,178 (246-18,039)
Conditioning regimen	
Bu/Cy/ATG	112 (41.2)
TBI-based	42 (15.4)
Bu/Cy/Flu	36 (13.2)
Bu/Cy/Flu/ATG	23 (8.5)
Bu/Cy	15 (5.5)
Bu/Flu/ATG	11 (4.0)
Cy/ATG	10 (3.7)
Other	22 (8.1)
Graft	
BM+PB	105 (39.0)
PB	83 (30.9)
CB	81 (30.1)
MNC, 10^8/kg, median (range)	7.69 (0.22-24.2)
CD34, 10^6/kg, median (range)	2.77 (0.14-15.1)

AA: aplastic anemia; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; ATG: anti-thymocyte globulin; BM: bone marrow; Bu: busulfan; CB: cord blood; CML: chronic myeloid leukemia; CMML: chronic myelomonocytic leukemia; Cy: cyclophosphamide; DSA: donor specific antibody; Flu: fludarabine; Haplo: haploidentical transplantation; MDS: myelodysplastic syndrome; MFI: median fluorescence intensity; MNC: mononucleated cell; MRD: matched related donor; NHL: non-Hodgkin lymphoma; PB: peripheral blood; PMF: primary myelofibrosis; TBI: total body irradiation; URD: unrelated donor.

Table 2. Patient characteristics of the second transplantations.

Variables	Number (%)
GF type	
Primary	193 (71.0)
Secondary	79 (29.0)
Chimerism	
Full donor	80 (31.5)
Mix	97 (38.2)
Full recipient	77 (30.3)
Time from 1st Tx to 2nd Tx , days, median (range)	55 (18-2,592)
Changing donor	
Yes	192 (70.6)
No	80 (39.4)
Donor age, years, median (range)	35 (2-68)
Donor sex, male	155 (57.8)
2nd donor type	
MRD	21 (7.7)
URD	16 (5.9)
CB	19 (7.0)
Haplo	216 (79.4)
2nd donor ABO type	
Match	152 (55.9)
Minor mismatch	55 (20.2)
Major mismatch	43 (15.8)
Bidirectional mismatch	22 (8.1)
DSA prior to 2nd Tx	
Available	154 (56.6)
Positive	22 (14.3)
MFI, median (range)	2,512 (246-19,682)
Conditioning	
Flu/Cy	81 (29.8)
TBI/Flu/Cy	50 (18.4)
Bu/Cy/Flu	29 (10.7)
Flu	23 (8.5)
Bu/Cy	13 (4.8)
Bu/Flu	10 (3.7)
Bu	9 (3.3)
TBI/Flu	9 (3.3)
Cy	8 (2.9)
TBI/Cy	6 (2.2)
Other	34 (12.5)
Graft	
BM+PB	141 (51.8)
PB	110 (40.4)
CB	21 (7.7)
MNC, 10^8/kg, median (range)	9.36 (0.28-30.7)
CD34, 10^6/kg, median (range)	4.50 (0.16-22.1)
GvHD prophylaxis	
CSA+MMF	61 (22.4)
CSA+MMF+ATG	53 (19.5)
CSA+MMF+CD25	49 (18.0)
CSA+MMF+MTX+ATG	45 (16.5)
FK506+MMF+MTX+ATG	16 (5.9)
CSA+MMF+MTX	9 (3.3)
CSA+MMF+Cy+ATG	9 (3.3)
FK506+MMF+ATG	4 (1.5)
CSA+MMF+CD25+PtCy	3 (1.1)
CSA+ATG+PtCy	3 (1.1)
Other	20 (7.4)

encountering secondary GF engrafted and survived better than primary GF (*Online Supplementary Figure S2*), but none of the above were significant in multivariate analysis. No significant disparities were observed in engraftment or survival concerning first transplant source (CB *vs.* others; *Online Supplementary Figure S3*) or chimerism status (*Online Supplementary Figure S4*).

Primary GF patients were further stratified by possible GF etiologies, with 24 with positive DSA (Group A), 110 DSA-negative with full recipient or mixed chimerism indicative of T-cell mediated rejection (Group B), and 42 DSA-negative with full donor chimerism suggesting non-immune etiologies (Group C). Comparative analysis revealed inferior platelet engraftment, TRM, and OS in Group C, while neutrophil engraftment was similar across groups (*Online Supplementary Figure S5*).

Factors associated with second transplant outcomes

Multivariate analysis indicated changing donors (HR 0.624, *P*=0.039), and a younger second donor (HR 0.668, *P*=0.019) were related to better neutrophil engraftment, whilst changing donors (HR 0.559, *P*=0.035), younger recipients (HR 0.597, *P*=0.011), and higher $CD34^+$ doses (HR 0.688, *P*=0.034) improved platelet engraftment. Superior TRM and OS were observed in younger recipients (TRM: HR 0.560, *P*=0.030; OS: HR 0.610, *P*=0.046), patients who changed donors (TRM: HR 0.431, *P*=0.006; OS: HR 0.405, *P*=0.004), and first transplant from MRD/HID. Compared to using MRD as second donors, grafting from URD, HID, and CB were risk factors for aGvHD (URD: HR 10.13, *P*=0.033; CB: HR 8.789, *P*=0.045; HID: HR 10.28, *P*=0.023). Recipient age was the only risk factor for cGvHD (< median *vs.* ≥ median, HR 0.458, *P*=0.028) (Table 3).

Donor specific antibody positivity (N=22) before the second transplant showed borderline inferior neutrophil (77.3% *vs.* 93.2%, *P*=0.062) and platelet engraftment (50.0% *vs.* 77.3%, *P*=0.051), while 1-year OS was comparable (53.8% [95% CI: 32.6-75.0] *vs.* 63.8% [95% CI: 55.0-72.6], *P*=0.311). Although with limited cases, subgroup analysis stratified by MFI thresholds (≥2,512 *vs.* <2,512) revealed DSA-high patients had impaired platelet engraftment compared to DSA-low patients (25.0% *vs.* 75.0%, *P*=0.046), while differences in neutrophil engraftment (55.6% *vs.* 88.9%, *P*=0.114) and survival (55.6% *vs.* 55.6%, *P*=0.100) were non-significant.

As the most commonly used preconditioning regimen, Flu/Cy had no impact on engraftment (neutrophil engraftment, *P*=0.081; platelet engraftment, *P*=0.843) or survival (*P*=0.659) (Table 3). Also, in patients conditioned with Flu/Cy with or without low-dose total body irradiation (TBI),

ATG: anti-thymocyte globulin; BM: bone marrow; Bu: busulfan; CB: cord blood; CSA: cyclosporine A; Cy: cyclophosphamide; DSA: donor specific antibody; Flu: fludarabine; GF: graft failure; GvHD: graft-*versus*-host disease; Haplo: haploidentical transplantation; MFI: median fluorescence intensity; MMF: mycophenolate mofetil; MNC: mononucleated cell; MRD: matched related donor; MTX: methotrexate; PB: peripheral blood; PtCy: post-transplant cyclophosphamide; TBI: total body irradiation; Tx: transplantation; URD: unrelated donor.

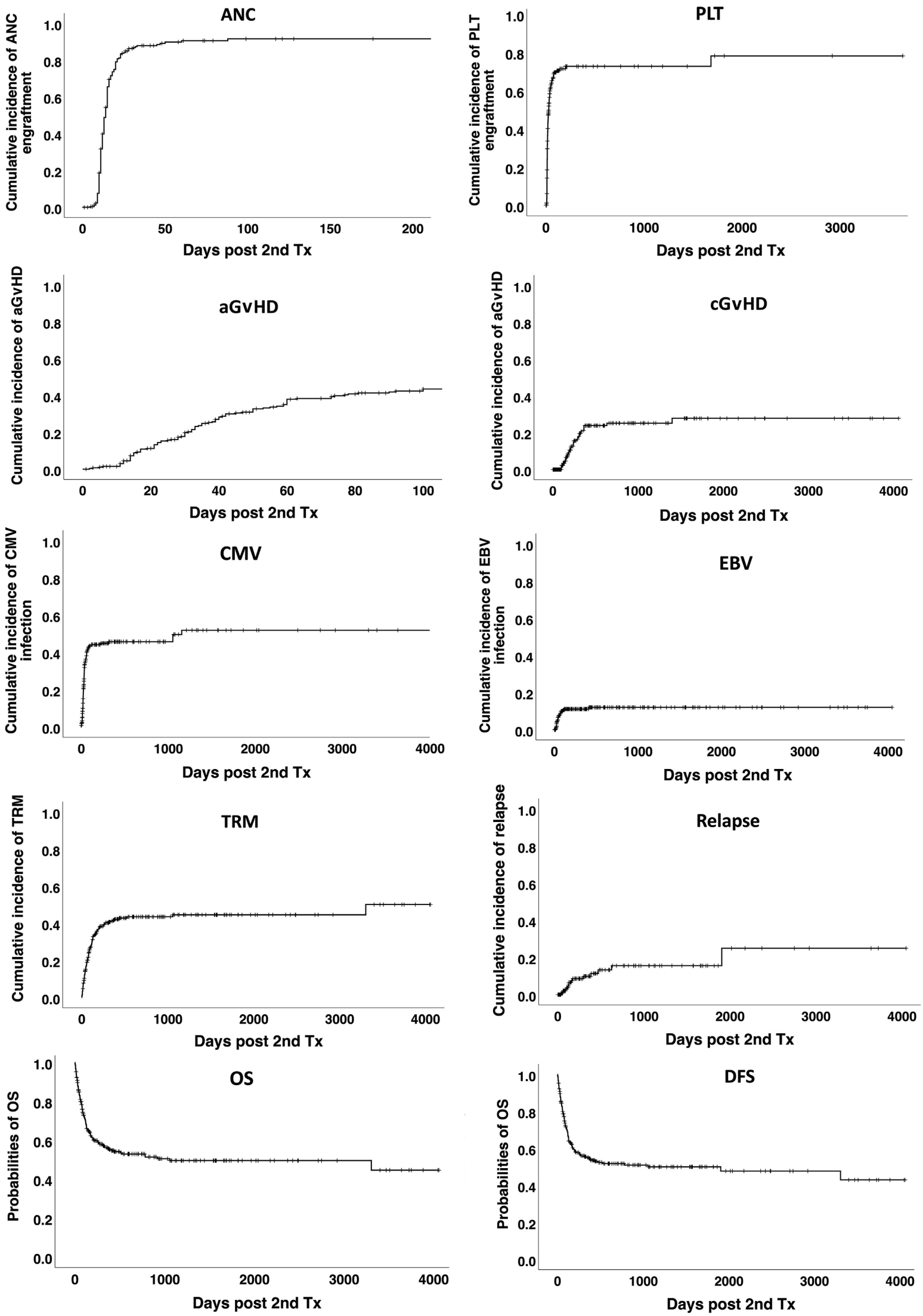

Figure 1. Outcomes of the 2nd transplantations for the entire cohort. ANC: absolute neutrophil count; aGvHD: acute graft-*versus*-host disease; cGvHD: chronic GvHD; CMV: cytomegalovirus; DFS: disease-free survival; EBV: Epstein-Barr virus; OS: overall survival; PLT: platelet; TRM: transplant-related mortality; Tx: transplant.

Table 3. Univariate and multivariate analysis for 2nd transplant outcomes in all patients.

Variables	ANC engraftment				Platelet engraftment				aGvHD				cGvHD				TRM				OS			
	Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate	
	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*
M *vs.* F recipient	1.062	0.652	-	-	0.860	0.339	-	-	0.901	0.604	-	-	1.129	0.716	-	-	1.188	0.375	-	-	0.876	0.473	-	-
<Mdn *vs.* ≥Mdn recipient age in yr	0.717	0.014	-	-	0.611	0.002	0.597	0.011	0.785	0.947	-	-	2.514	0.008	2.185	0.028	0.613	0.011	0.560	0.030	0.627	0.011	0.610	0.046
AA *vs.* other	1.010	0.771	-	-	1.054	0.183	-	-	1.307	0.223	-	-	2.291	0.032	-	-	0.633	0.035	-	-	1.861	0.004	-	-
1st donor type																								
MRD	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000		1.000		1.000		1.000	
URD	1.029	0.932	-	-	1.595	0.203	-	-	3.128	0.040	-	-	0.568	0.488	-	-	2.101	0.141	3.596	0.033	2.093	0.120	4.230	0.015
CB	1.174	0.508	-	-	1.681	0.049	-	-	2.032	0.147	-	-	0.515	0.233	-	-	1.707	0.202	3.208	0.044	1.728	0.163	4.181	0.014
Haplo	0.620	0.038	-	-	2.128	0.003	-	-	2.888	0.023	-	-	0.995	0.992	-	-	2.081	0.066	1.867	0.177	2.055	0.054	2.053	0.117
Primary *vs.* secondary GF type	1.613	0.524	-	-	1.708	<0.001	-	-	0.724	0.155	-	-	1.505	0.227	-	-	1.658	0.028	-	-	1.642	0.023	-	-
Chimerism																								
Full donor	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-
Mixed	1.276	0.175	-	-	0.864	0.470	-	-	0.696	0.153	-	-	0.868	0.716	-	-	0.702	0.127	-	-	0.690	0.101	-	-
Full recipient	1.111	0.521	-	-	0.684	0.066	-	-	1.026	0.918	-	-	0.513	0.145	-	-	0.651	0.086	-	-	0.759	0.234	-	-
<Mdn *vs.* ≥Mdn time from 1st to 2nd Tx in days	0.994	0.963	-	-	1.302	0.090	-	-	0.763	0.175	-	-	1.635	0.157	-	-	1.171	0.408	-	-	0.834	0.317	-	-
Neg. *vs.* pos. DSA prior to 2nd Tx	0.615	0.062	-	-	0.537	0.051	-	-	0.671	0.354	-	-	0.714	0.649	-	-	0.790	0.542	-	-	1.421	0.314	-	-
Relapse *vs.* CR prior to 2nd Tx	0.066	0.797	-	-	1.317	0.485	-	-	0.627	0.433	-	-	0.043	0.379	-	-	1.208	0.715	-	-	0.902	0.824	-	-
Change donor, no *vs.* yes	1.745	<0.001	1.603	0.039	2.006	<0.001	1.788	0.035	1.135	0.577	-	-	0.600	0.149	-	-	2.074	<0.001	2.320	0.006	1.881	<0.001	2.469	0.004
<Mdn *vs.* >Mdn 2nd donor age in yr	0.744	0.057	0.668	0.019	0.814	0.217	-	-	1.480	0.069	-	-	1.171	0.657	-	-	0.714	0.107	-	-	0.699	0.071	-	-
M *vs.* F 2nd donor	0.244	0.852	-	-	0.907	0.538	-	-	0.896	0.592	-	-	1.126	0.723	-	-	0.772	0.176	-	-	1.223	0.269	-	-

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Variables	ANC engraftment				Platelet engraftment				aGvHD				cGvHD				TRM				OS			
	Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate	
	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*
2nd donor type																								
MRD	1.000	-	-	-	1.000	-	-	-	1.000	-	1.000	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-
URD	1.297	0.451	-	-	0.913	0.806	-	-	11.669	0.022	10.125	0.033	1.036	0.970	-	-	1.008	0.989	-	-	0.902	0.857	-	-
CB	3.058	0.002	-	-	3.623	0.003	-	-	9.983	0.031	8.789	0.045	1.222	0.827	-	-	1.733	0.276	-	-	1.588	0.342	-	-
Haplo	1.427	0.142	-	-	1.712	0.038	-	-	11.849	0.014	10.275	0.023	1.285	0.680	-	-	1.463	0.332	-	-	1.471	0.294	-	-
Matched/min mismatched *vs.* maj/ bidirectional mismatched ABO of 2nd Tx	0.855	0.457	-	-	0.406	0.197	-	-	0.788	0.464	-	-	1.023	0.783	-	-	0.445	0.282	-	-	0.798	0.457	-	-
FC *vs.* other conditioning of 2nd Tx	0.966	0.081	-	-	1.034	0.843	-	-	1.014	0.951	-	-	0.790	0.509	-	-	1.063	0.770	-	-	0.917	0.659	-	-
Graft source of 2nd Tx																								
PB+BM	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000		-	-
PB	1.859	0.047	-	-	1.362	0.058	-	-	0.734	0.148	-	-	1.611	0.166	-	-	1.424	0.077	-	-	1.303	0.162	-	-
CB	3.021	0.002	-	-	2.770	0.006	-	-	0.935	0.858	-	-	1.205	0.803	-	-	1.598	0.176	-	-	1.406	0.319	-	-
<Mdn *vs.* ≥Mdn infused MNC	1.646	<0.001	-	-	1.820	<0.001	-	-	1.050	0.809	-	-	1.208	0.580	-	-	1.629	0.012	-	-	1.493	0.030	-	-
Infused CD34 cells	1.495	0.006	-	-	1.851	<0.001	1.453	0.034	0.948	0.804	-	-	1.727	0.142	-	-	1.701	0.013	-	-	1.938	0.001	-	-
Non-intensified *vs.* intensified GvHD prophylaxis for 2nd Tx	1.754	<0.001	-	-	2.125	<0.001	-	-	1.256	0.345	-	-	0.699	0.284	-	-	2.037	<0.001	-	-	1.957	<0.001	-	-
2nd Tx pre *vs.* post Oct 2019	1.473	0.004	-	-	1.762	<0.001	-	-	1.143	0.500	-	-	0.265	0.683	-	-	0.706	0.072	-	-	0.720	0.074	-	-

AA: aplastic anemia; aGvHD: acute graft-*versus*-host disease; ANC: absolute neutrophil count; BM: bone marrow; CB: cord blood; cGvHD: chronic GvHD; CR: complete remission; DSA: donor specific antibody; F: female; FC: fludarabine/cyclophosphamide; GF: graft failure; Haplo: haploidentical transplantation; HR: hazard ratio; M: male; maj: major; Mdn: median; min: minor; MNC: mononucleated cells; MRD: matched related donor; neg: negative; OS: overall survival; PB: peripheral blood; pos: positive; TRM: transplant-related mortality; Tx: transplantation; URD: unrelated donor; yr: years.

no significant differences were observed in engraftment (neutrophil engraftment, *P*=0.227; platelet engraftment, *P*=0.151) or survival (*P*=0.761) compared to using alternative conditioning regimens.

Univariate analysis using October 2019 (median transplant date) as the temporal demarcation point demonstrated trends toward reduced TRM (HR=0.706, *P*=0.072) and improved OS (HR=0.720, *P*=0.074), though these trends did not reach statistical significance in multivariate analysis (Table 3). Other variables, including primary diseases, GF types, chimerism, and the transplant interval had no association with outcomes, demonstrated by multivariate analysis.

The impact of changing donors in second transplantation outcomes

Details of donor type change are summarized in *Online Supplementary Table S2*. Across the entire cohort, changing donor showed improved neutrophil (92.4% [95% CI: 88.3-96.5] *vs*. 71.4% [95% CI: 61.8-82.0], *P*<0.001) and platelet engraftment (76.9% [95% CI: 70.2-83.6] *vs*. 51.8% [95% CI: 38.5-65.1], *P*<0.001), reduced 1-year TRM (34.8% [95% CI: 27.7-41.9] *vs*. 56.3% [95% CI: 45.1-67.5], *P*<0.001), and superior 1-year OS (61.9% [95% CI: 54.6-69.2] *vs*. 42.7% [95% CI: 31.5-51.9], *P*<0.001). Rates of aGvHD, cGvHD were comparable (Figure 2). Multivariate analysis confirmed changing donors was related to better neutrophil and platelet engraftment, TRM, and OS, but not to aGvHD or cGvHD (Table 3).

Subgroup analysis based on donor types of the first transplantations

As switching to a different donor is inevitable for patients with first transplants from CB, we evaluated the impact of changing donors in first transplants with MRD, URD, and HID. In this cohort, changing donors improved neutrophil engraftment (94.7% [95% CI: 90.2-99.2] *vs*. 71.4% [95% CI: 60.8-82.0], *P*<0.001), platelet engraftment (83.0% [95% CI: 74.3-91.1] *vs*. 51.8% [95% CI: 38.5-65.1], *P*<0.001), TRM (31.9% [95% CI: 22.9-40.9] *vs*. 56.3% [95% CI: 45.1-67.5], *P*<0.001), and OS (65.3% [95% CI: 56.1-74.5] *vs*. 42.7% [95% CI: 31.5-53.9], *P*<0.001) (*Online Supplementary Figure S6*). Multivariate analysis reinforced benefits of switching donors in neutrophil engraftment (HR 0.632, *P*=0.034), platelet engraftment (HR 0.525, *P*=0.035), and TRM (HR 0.428, *P*=0.008) (*Online Supplementary Table S3*).

Among the 147 patients with first HID transplants, 82 used different second donors, including 66 HID, 10 URD, 4 CB, and 2 MRD. Changing donors exhibited better neutrophil engraftment (96.3% [95% CI: 89.0-99.9] *vs*. 66.5% [95% CI: 54.2-78.8], *P*<0.001), platelet engraftment (78.5% [95% CI: 68.7-88.3] *vs*. 45.0% [95% CI: 29.7-60.3], *P*<0.001), 1-year OS (67.0% [95% CI: 56.2-77.8] *vs* 35.7% [95% CI: 23.5-47.9], *P*<0.001), and TRM (30.8% [95% CI: 20.2-41.4] *vs*. 63.1% [95% CI: 50.9-75.3], *P*<0.001) (*Online Supplementary Figure S7*), confirmed by multivariate analysis (*Online Supplementary Table S4*). Similar benefits evolved in 131 patients receiving two HID transplants, in which switching to a different HID demonstrated better engraftment, OS, and TRM (*Online Supplementary Figure S8*).

Subgroup analysis based on primary diseases

In AA patients (N=87), changing donors resulted in better platelet engraftment (86.3% [95% CI: 76.9-95.7] *vs*. 53.9% [95% CI: 30.8-77.0], *P*=0.028) and OS (74.2% [95% CI: 63.2-85.2] *vs*. 53.1% [95% CI: 30.8-75.4], *P*=0.028), with comparable neutrophil engraftment (*Online Supplementary Figure S9*). Multivariate analysis linked changing donors to OS (HR 0.400, *P*=0.047), second donor age to neutrophil engraftment (≥ median *vs*. < median, HR 2.392, *P*=0.006), and CB as second donors to platelet engraftment (HR 9.709, *P*=0.028) (*Online Supplementary Table S5*).

In patients with hematologic malignancies (N=181), changing donors also demonstrated superior engraftment (neutrophil engraftment: 91.8% [95% CI: 85.3-96.3] *vs*. 66.0% [95% CI: 52.9-79.1], *P*<0.001; platelet engraftment: 81.3% [95% CI: 62.5-80.1] *vs*. 51.8% [95% CI: 34.6-69.0], *P*=0.003) and survival (1-year OS: 56.6% [95% CI: 47.2-66.0] *vs*. 37.9% [95% CI: 25.2-50.6], *P*=0.005), with comparable risks of relapse (*Online Supplementary Figure S10*). Multivariate analysis confirmed the association between changing donors and platelet engraftment (HR 0.558, *P*=0.043), OS (HR 0.594, *P*=0.030), and DFS (HR 0.585, *P*=0.040). Primary GF was also a risk factor for survival compared to secondary GF (HR 1.727, *P*=0.045) (*Online Supplementary Table S6*).

Subgroup analysis based on GF types

In 193 patients encountering primary GF, 143 used different donors, resulting in better neutrophil (91.8% [95% CI: 85.7-95.9] *vs*. 61.9% [95% CI: 47.6-76.2], *P*<0.001) and platelet engraftment (73.4% [95% CI: 65.4-81.4] *vs*. 35.7% [95% CI: 19.6-51.8], *P*<0.001), 1-year OS (58.3% [95% CI: 49.9-66.7] *vs*. 28.8% [95% CI: 16.1-41.5], *P*<0.001), and TRM (37.3% [95% CI: 29.1-45.5] *vs*. 69.9% [95% CI: 57.0-82.8], *P*<0.001) (*Online Supplementary Figure S11*). Multivariate analysis demonstrated the significance of changing donors in platelet engraftment (HR 0.451, *P*=0.026) and OS (HR 0.573, *P*=0.025) (*Online Supplementary Table S7*). Transplant interval had no impact on engraftment or survival. Regression analysis in subgroups by GF etiologies also indicated significance of changing donors in neutrophil (HR=0.372, *P*=0.002) and platelet (HR=0.419, *P*=0.033) engraftment in DSA-negative patients with full recipient/mixed chimerism (group B), though patient numbers in the other two groups were limited (*Online Supplementary Tables S8-10*).

In 79 patients encountering secondary GF, 49 used a different donor. In contrast to those with primary GF, no differences were observed between using the same or a different donor regarding neutrophil engraftment (96.9% [95% CI: 91.4-99.9] *vs*. 88.0% [95% CI: 75.3-99.9], *P*=0.346) and platelet engraftment (86.8% [95% CI: 76.6-97.0] *vs*. 80.2% [95% CI: 61.8-98.6], *P*=0.259), 1-year TRM (27.4% [95%

CI: 14.1-40.7] *vs*. 31.5% [95% CI: 14.4-48.6], *P*=0.299), and OS (72.6% [95% CI: 59.3-85.9] *vs*. 68.5% [95% CI: 51.4-85.6], *P*=0.424) in secondary GF (*Online Supplementary Figure S12*). Cox analysis only linked recipient age (< median *vs*. ≥ median, HR 0.388, *P*=0.007) and first donor type (URD: HR 6.289, *P*=0.007; HID: HR 2.623, *P*=0.023) to platelet engraftment but failed to link any other risk factors to engraftment or survival (*Online Supplementary Table S11*). Transplant interval demonstrated no association with engraftment or survival in this cohort, but critical data regarding the diagnosis-to-transplant interval following secondary GF were unavailable for analysis.

Subgroup analysis based on donor-recipient chimerisms

Seventy-seven patients had full recipient chimerism; for most of these (81.8%), a different donor was chosen for second transplantations. Comparison showed patients grafted from a different donor achieved superior neutrophil reconstitution (92.8% [95% CI: 85.7-99.9] *vs*. 64.3% [95% CI: 39.2-89.4], *P*=0.009) and platelet reconstitution (87.1% [95% CI: 78.3-65.9] *vs*. 52.4% [95% CI: 11.2-93.6], *P*=0.014), and reduced TRM (31.8% [95% CI: 19.8-43.8] *vs*. 64.3% [95% CI: 39.2-89.4], *P*=0.007) (*Online Supplementary Figure S13*). Regression analysis demonstrated associations between changing donors and platelet engraftment (HR 0.328, *P*=0.049). Notably, a reduced 3-year cGvHD was seen in the changing donor group (10.7% [95% CI: 7.0-20.7] *vs*. 77.1% [95% CI: 38.5-99.9], *P*<0.001) and this was confirmed by multivariate analysis (*Online Supplementary Table S12*). In the 80 patients with full donor chimerism, 42 changed

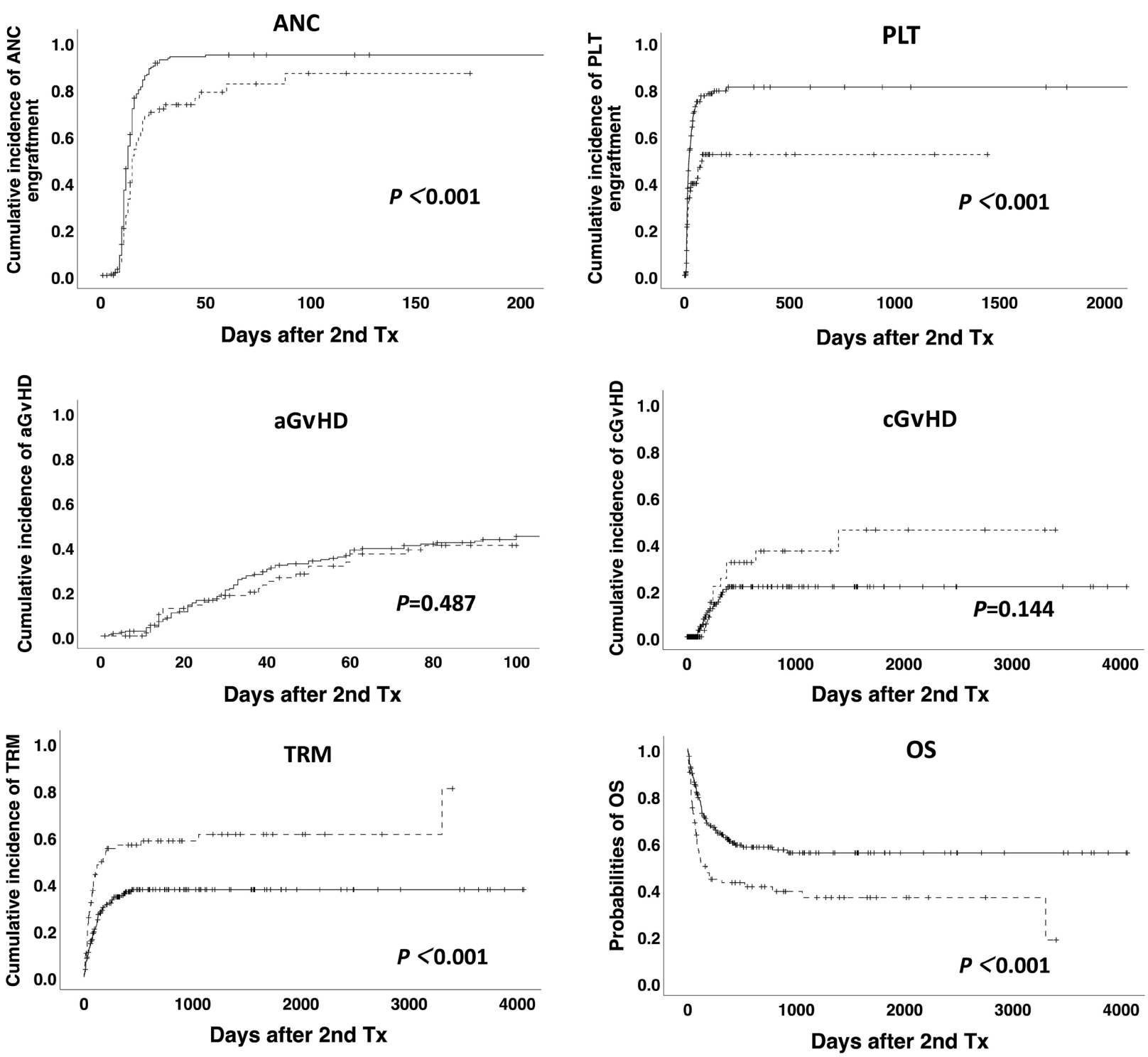

Figure 2. Comparison of the 2nd transplantation outcomes in patients who received grafts from the same or a different donor. Solid line: using a different donor; dashed line: using the same donor. ANC: absolute neutrophil count; aGvHD: acute graft-*versus*-host disease; cGvHD: chronic GvHD; OS: overall survival; PLT: platelet; TRM: transplant-related mortality; Tx: transplant.

to different donors, resulting in better platelet recovery (73.8% [95% CI: 58.7-88.9] *vs*. 46.6% [95% CI: 28.0-65.2], *P*=0.026) and 1-year OS (61.9% [95% CI: 46.4-77.4] *vs*. 39.0% [95% CI: 22.7-55.3], *P*=0.023) (*Online Supplementary Figure S14*). Multivariate analysis confirmed the positive impact of changing donors on platelet recovery (HR 0.432, *P*=0.032) and survival (HR 0.320, *P*=0.036). A Flu/Cy-based preconditioning was related to enhanced neutrophil engraftment (HR 0.393, *P*=0.049), and intensified GvHD prophylaxis was a protective factor for TRM (HR 0.212, *P*=0.008) and OS (HR 0.239, *P*=0.014) (*Online Supplementary Table S13*).

In the 97 patients with mixed chimerism, no statistical significance was observed between using the same or a different donor, although a trend toward better engraftment (neutrophil: 95.7% [95% CI: 90.4-99.9] *vs*. 71.9% [95% CI: 54.2-89.5], *P*=0.052; platelet: 72.3% [95% CI: 60.3-84.3] *vs*. 59.4% [95% CI: 39.0-79.8], *P*=0.140) was shown (*Online Supplementary Figure S15*). Cox analysis identified no influencing factors for transplant outcomes (*Online Supplementary Table S14*).

Subgroup analysis based on second transplantation conditioning regimens

Preconditioning regimens for second transplantations were heterogeneous, with Flu/Cy-based (29.8%) and TBI-based (18.4%) regimens accounting for half the cases. Among patients receiving Flu/Cy-based regimen, changing donors led to improved 1-year TRM (34.6% [95% CI: 21.7-47.5] *vs*. 60.0% [95% CI: 40.6-79.4], *P*=0.008) and OS (61.5% [95% CI: 48.2-74.8] *vs*. 40.0% [95% CI: 20.6-59.4], *P*=0.033), with a trend toward better platelet engraftment (75.1% [95% CI: 62.6-87.6] *vs*. 51.3% [95% CI: 27.6-75.0], *P*=0.094) (*Online Supplementary Figure S16*). Cox analysis identified changing donors as the only protective factor for TRM (HR 0.373, *P*=0.006) and OS (HR 0.459, *P*=0.034) (*Online Supplementary Table S15*).

In patients receiving TBI-based conditioning, changing donors brought about better platelet engraftment (77.1% [95% CI: 61.8-92.4] *vs*. 41.9% [95% CI: 15.0-68.8], *P*=0.004) and survival (1-year OS: 70.2% [95% CI: 52.8-87.6] *vs*. 40.0% [95% CI: 18.4-61.6], *P*=0.040) (*Online Supplementary Figure S17*). Multivariate analysis revealed an association between changing donors and increased platelet engraftment (HR 0.180, *P*=0.027) and OS (HR 0.157, *P*=0.025) (*Online Supplementary Table S16*).

How to further optimize second transplantation outcomes?

Among 192 patients who changed donors, multivariate analysis showed that patient age (<20 years *vs*. ≥20 years) significantly impacted platelet engraftment (HR 0.615, *P*=0.036), cGvHD (HR 0.438, *P*=0.048), TRM (HR 0.472, *P*=0.015), and OS (HR 0.377, *P*=0.002). Additionally, mononuclear cell (MNC) dose was also a determinant for platelet engraftment (<10.7^10^8/kg *vs*. ≥10.7^10^8/kg, HR 1.564, *P*=0.045). Younger second donors (<36 years *vs*. ≥36 years) were associated with a lower risk of aGvHD (HR 0.411, *P*=0.008). Thus, although with limited data, evidence suggests that selecting a younger second donor and infusing higher MNC doses may potentially improve outcomes in the context of changing donors (Table 4).

We also assessed the effectiveness of HID as the second donors. Both engraftment and survival were comparable between patients grafted with HID and other donor types. Incidences of aGvHD were higher in the HID group (47.4% [95% CI: 40.0-54.8] *vs*. 29.2% [95% CI: 16.3-40.9], *P*=0.029), but cGvHD were similar (*Online Supplementary Figure S18*). These data suggested HID present a viable and effective option for second transplantations despite increased aGvHD risk.

Discussion

A second transplantation usually represents the only salvage for GF;[19] however, the optimized protocols remain undefined. Among variables potentially affecting outcomes, the role of changing donors remains contentious. In the present study, we demonstrated that changing donors is important for the success of a second transplant, impacting both engraftment and survival.

Previous studies have shown GF risk factors on first transplant include bone marrow grafts, myeloproliferative disorders, HLA mismatch, male recipients grafted from female donors, ABO incompatibility, busulfan/Cy conditioning, stem cell cryopreservation, and low Karnofsky/Lansky score.[20] However, few studies have focused on factors predicting GF following second transplantations. Studies in Japan on salvaging CB transplantations demonstrated the combination of calcineurin inhibitors (CNI) and methotrexate (MTX) as immunosuppressive regimens increased the risk of GF. Additional contributing factors include poor disease risk index, conditioning other than Flu/Melphalan (Mel), and the absence of TBI.[21] Cryopreserved CB $CD34^+$ counts <0.8×10^5/kg, Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI) scores ≥3, and non-remission at initial SCT were also possible risk factors.[22] In the present study, we identified changing donors and a younger second donor as protective factors for neutrophil engraftment, and changing donors, younger recipient, and higher CD34 doses as protective factors for platelet engraftment.

In the literature, the effect of changing donors has always been controversial. A Spanish study found no impact of changing donors on engraftment or survival.[5] Similarly, the National Marrow Donor Program (NMDP) study yielded comparable results in URD transplantations.[23] In contrast, a small-scale study involving HID by Giammaco *et al*. reported a GF rate of 30% (4/13) in patients grafted from the same donor, compared to 16% (1/6) in those from different donors, indicating a potential benefit of changing donors.[7]

Table 4. Univariate and multivariate analysis for 2nd transplant outcomes in patients who changed to a different donor.

Variables	ANC engraftment				Platelet engraftment				aGvHD				cGvHD				TRM				OS			
	Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate	
	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*
M *vs.* F recipient	0.836	0.252	-	-	0.673	0.027	-	-	0.889	0.617	-	-	1.404	0.407	-	-	1.147	0.590	-	-	1.072	0.767	-	-
<Mdn *vs.* ≥Mdn recipient age in yr	0.819	0.205	-	-	0.685	0.030	0.615	0.036	1.207	0.413	-	-	0.438	0.048	-	-	0.538	0.002	0.472	0.015	0.454	0.003	0.377	0.002
AA *vs.* other	4.012	0.674	-	-	0.725	0.071	-	-	1.322	0.263	-	-	0.418	0.066	-	-	0.637	0.104	-	-	0.502	0.011	-	-
1st donor type																								
MRD	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-
URD	1.000	1.000	-	-	2.000	0.114	-	-	1.606	0.439	-	-	0.498	0.445	-	-	3.221	0.139	-	-	3.709	0.094	-	-
CB	9.368	0.864	-	-	2.169	0.026	-	-	1.094	0.867	-	-	0.451	0.291	-	-	2.806	0.157	-	-	3.262	0.104	-	-
Haplo	1.000	1.000	-	-	1.862	0.071	-	-	1.617	0.361	-	-	0.769	0.624	-	-	2.029	0.337	-	-	2.540	0.202	-	-
Primary *vs.* secondary GF type	0.031	0.723	-	-	1.565	0.019	-	-	0.935	0.796	-	-	1.385	0.453	-	-	0.662	0.183	-	-	0.663	0.154	-	-
Chimerism																								
Full donor	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-			1.000	-	-	-	1.000	-	-	-
Mixed	0.002	0.723	-	-	1.052	0.834	-	-	0.720	0.309	-	-	1.445	0.456	-	-	0.961	0.904	-	-	0.918	0.790	-	-
Full recipient	0.002	0.736	-	-	0.791	0.326	-	-	1.158	0.628	-	-	0.379	0.133	-	-	0.783	0.479	-	-	0.909	0.947	-	-
<Mdn *vs.* ≥Mdn time from 1st to 2nd Tx in days	1.398	0.237	-	-	1.329	0.104	-	-	1.081	0.738	-	-	1.873	0.148	-	-	1.222	0.419	-	-	0.637	0.443	-	-
Neg. *vs.* pos. DSA prior to 2nd Tx	-	-	-	-	0.812	0.550	-	-	0.610	0.341	-	-	0.042	0.336	-	-	1.082	0.881	-	-	1.240	0.622	-	-
Relapse *vs.* CR prior to 2nd Tx	0.042	0.823	-	-	1.397	0.402	-	-	0.588	0.377	-	-	0.040	0.464	-	-	1.550	0.465	-	-	1.344	0.571	-	-
<Mdn *vs.* >Mdn 2nd donor age in yr	0.015	0.607	-	-	0.782	0.195	-	-	0.411	0.008	-	-	1.532	0.344	-	-	0.542	0.038	-	-	0.521	0.017	-	-
M *vs.* F 2nd donor	0.020	0.642	-	-	1.019	0.916	-	-	0.281	0.771	-	-	1.275	0.553	-	-	0.775	0.310	-	-	0.850	0.486	-	-
2nd donor type																								
MRD	1.000	-	-	-	1.000	-	-	-	1.000	-	-		1.000	-	-	-	1.000	-	-	-	1.000	-	-	-
URD	1.000	1.000	-	-	0.977	0.960	-	-	2.229	0.897	-	-	2.170	0.954	-	-	0.843	0.844	-	-	0.857	0.859	-	-
CB	1.000	1.000	-	-	4.484	0.004	-	-	2.070	0.898	-	-	2.402	0.953	-	-	1.734	0.482	-	-	1.835	0.438	-	-
Haplo	2.918	0.894	-	-	1.580	0.243	-	-	2.459	0.896	-	-	2.331	0.953	-	-	1.074	0.921	-	-	1.316	0.703	-	-

Continued on following page.

Variables	ANC engraftment				Platelet engraftment				aGvHD				cGvHD				TRM				OS			
	Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate		Uni-variate		Multi-variate	
	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*
Matched/min mismatched *vs.* maj/bidirectional mismatched ABO of 2nd Tx	0.808	0.219	-	-	0.642	0.187	-	-	0.878	0.506	-	-	0.870	0.735	-	-	0.648	0.215	-	-	0.774	0.265	-	-
FC *vs.* other conditioning of 2nd Tx	0.004	0.594	-	-	1.056	0.777	-	-	1.356	0.833	-	-	0.686	0.371	-	-	0.933	0.801	-	-	0.969	0.901	-	-
Graft source of 2nd Tx																								
PB+BM	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	-
PB	0.026	0.730	-	-	0.635	0.022	-	-	0.888	0.672	-	-	1.449	0.418	-	-	0.564	0.117	-	-	0.533	0.067	-	-
CB	0.026	0.817	-	-	2.874	0.004	-	-	0.988	0.976	-	-	1.192	0.816	-	-	1.618	0.169	-	-	1.413	0.316	-	-
<Mdn *vs.* ≥Mdn infused MNC	6.458	0.611	-	-	1.562	0.013	1.564	0.045	1.063	0.795	-	-	1.193	0.667	-	-	1.381	0.205	-	-	3.509	0.285	-	-
Mdn *vs.* ≥Mdn infused CD34 cells	6.458	0.611	-	-	1.381	0.072	-	-	0.839	0.462	-	-	1.509	0.321	-	-	1.220	0.439	-	-	1.441	0.133	-	-
Non-intensified *vs.* intensified GvHD prophylaxis for 2nd Tx	1.925	0.004	-	-	2.231	0.004	-	-	1.121	0.727	-	-	3.762	0.195	-	-	1.923	0.023	-	-	1.862	0.021	-	-

AA: aplastic anemia; aGvHD: acute graft-*versus*-host disease; ANC: absolute neutrophil count; BM: bone marrow; CB: cord blood; cGvHD: chronic GvHD; CR: complete remission; DSA: donor specific antibody; F: female; FC: fludarabine/cyclophosphamide; GF: graft failure; Haplo: haploidentical transplantation; HR: hazard ratio; M: male; maj: major; Mdn: median; min: minor; MNC: mononucleated cells; MRD: matched related donor; OS: overall survival; PB: peripheral blood; TRM: transplant-related mortality; Tx: transplantation; URD: unrelated donor.

Additionally, research by Kongtim *et al.* suggested that utilizing a different HID in second transplantations resulted in lower TRM.[24]

Unlike the ambiguity present in earlier research, our study strongly suggests changing donors is likely a key determinant of both engraftment and survival. Several reasons may account for this disparity. First, most prior studies had limited sample sizes. Second, the population and transplant regimens may differ markedly between previous studies and our own. Third, all previous research was performed in earlier periods, whereas substantial advancements have occurred in recent years. Last but not least, while previous studies were mainly from CB, after which GF occurred frequently, a significant proportion of transplants in our study was haplo-SCT. So far there has been little research addressing donor change in haplo-SCT, likely attributable to limited studies incorporating HID as a salvage option.[8] Overall, our study demonstrated changing donors is important for the success of the second transplantation.

Notably, when donors are changed, conditioning regimens appear to have less impact. This was initially proposed in our earlier studies, and has since been corroborated by the current analysis involving a much larger cohort. Nevertheless, the heterogeneity of conditioning regimens across centers requires further prospective studies with standardized protocols.

Another interesting finding was that, unlike primary GF, secondary GF outcomes were not associated with changing donor, suggesting distinct mechanisms underlying primary and secondary GF. Prior research had identified possible risk factors for secondary GF to include GvHD and viral infections. CD34 cell exhaustion may play a significant role, as evidenced by good efficacy of boosting CD34 and of thrombopoietin receptor agonists in this context.[25,26] We also observed a weaker correlation between changing donors and engraftment in AA and in mixed donor-recipient chimerism. We still do not have any explanation for these observations but it may be related to relatively small sample sizes within subgroups, requiring further studies for validation.

In addition to changing donors, we identified patient age and first donor type as factors related to OS. Previous research highlighted various elements influencing survival following second transplantation. A Japanese study revealed old age, poor performance status, ongoing antimicrobials, and severe organ dysfunction were associated with inferior OS and TRM.[27] A European group demonstrated advancing age, second remission, low Karnofsky performance status, and myeloablative conditioning pre-first SCT were adverse prognostic factors for TRM, LFS, and OS.[10] There were no prognostic factors reported in URD transplants from NMDP.[23] Despite the general application of prophylaxis, infection remained the major contributor to mortality in our cohort. Although not significant in regression analysis, the trend toward improved TRM and OS in transplants post October 2019 likely reflects advancements in supportive care including antimicrobial strategies. Introduction of novel antimicrobials and diagnostic tools, with immune reconstitution monitoring could offer insights into better infection management.

Our study also proposed a preliminary principle for selecting a different second donor. In line with the established donor selection principles for first transplantations, a younger donor was associated with reduced aGvHD following second transplantations, and higher MNC doses may enhance efficacy. Despite the limited sample size, our study suggests opting for a different, younger second donor and increasing MNC dose could potentially improve second transplant outcomes.

It is important to acknowledge the limitations of our study. Firstly, due to the retrospective, multicenter design, precise documentation of some critical information (e.g., GF diagnosis to salvage transplantation interval, DSA desensitization) was unavailable in some cases, and baseline disparities between groups may exist, highlighting the need for prospective studies. Secondly, the underlying mechanism of changing donor remains unclear. We hypothesize that there may be unidentified rejection mechanisms between initial donor and recipient, and further investigation into donor-recipient immune interactions might provide valuable insights.

In conclusion, our study highlighted the importance of changing donors for successful second transplantations in GF salvage. To our knowledge, this represents the largest multicenter analysis of second transplantations for GF. Nevertheless, the underlying pathogenesis is still unclear and prospective studies are needed.

Disclosures

No conflicts of interest to disclose.

Contributions

X-JH and Y-QS contributed to the study design. RM and Y-QS wrote the manuscript. All authors provided clinical or biological data, and reviewed and approved the manuscript.

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Data-sharing statement

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

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