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Second Allogeneic Hematopoietic Cell Transplantation Following Graft Failure in Children

Eun Sang Yi,[1] **Hee Young Ju**,[2] **Ji Won Lee**,[2] **Ki Woong Sung**,[2] **Jin Hur**,[3,4,5] **and Keon Hee Yoo**[2,6,7]

[1]Department of Pediatrics, Korea University Guro Hospital, Korea University College of Medicine, Seoul, Korea
[2]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
[3]Department of Convergence Medicine, Pusan National University School of Medicine, Yangsan, Korea
[4]PNU GRAND Convergence Medical Science Education Research Center, Pusan National University School of Medicine, Yangsan, Korea
[5]Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, Korea
[6]Department of Health Science and Technology, SAIHST, Sungkyunkwan University, Seoul, Korea
[7]Cell & Gene Therapy Institute, Samsung Medical Center, Seoul, Korea

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Address for Correspondence:
Keon Hee Yoo, MD, PhD
Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea.
Email: hema2170@skku.edu

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ORCID iDs
Eun Sang Yi
https://orcid.org/0000-0001-8214-2106
Hee Young Ju
https://orcid.org/0000-0001-6744-0412
Ji Won Lee
https://orcid.org/0000-0003-0084-1304
Ki Woong Sung
https://orcid.org/0000-0001-5989-4772
Jin Hur
https://orcid.org/0000-0003-0692-0150

ABSTRACT

Background: Graft failure (GF) is a major complication of allogeneic hematopoietic cell transplantation (allo-HCT). Secondary transplantation has been recognized as a potential curative intervention.
Methods: This study aimed to investigate the characteristics and outcomes of salvage transplantation by analyzing the patients who underwent a second HCT for GF following the initial allo-HCT between 1998 and 2020.
Results: Overall, 23 recipients were identified, including 14 and 9 individuals with primary and secondary GF, respectively. Nine recipients underwent a second transplant from the same donor. Familial mismatched donors predominated in the second HCT (86.9%), with reduced-intensity conditioning as the prevailing approach (60.9%). Neutrophil engraftment occurred in 17 patients (73.9%) following the second HCT at a median of 17 days (range: 9–58 days) post-transplantation. However, secondary GF subsequently occurred in 5 patients, and successful engraftment following salvage transplantation was achieved in 12 (52.2%) patients. In the entire study population, the estimated 5-year probability of overall survival (OS) and treatment-related mortality (TRM) were 30.4% and 58.5%, respectively. Among patients who achieved successful engraftment following a second transplantation, the OS and TRM rates were 41.7% and 33.3%, respectively, indicating a trend toward better OS and significantly lower TRM compared to those with GF. Notably, 17 patients died, with infection being the most common cause (n = 12), irrespective of the engraftment status.
Conclusion: A successful engraftment following a second allo-HCT reduced the TRM; however, the OS remained suboptimal. The effective control of infectious diseases remains crucial for patients with GF, regardless of the engraftment status following salvage transplantation.

Keywords: Engraftment; Transplantation; Haematopoietic Stem Cells; Children; Treatment-Related Mortality

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Keon Hee Yoo
https://orcid.org/0000-0002-5980-7912

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Disclosure
The authors have no potential conflicts of interest to disclose.

Author Contributions
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INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is the treatment of choice for numerous non-malignant and malignant diseases in children.[1] Despite the progressive advancements in allo-HCT outcomes in pediatric patients, graft failure (GF) persists as a formidable and potentially life-threatening complication.[2] GF is defined as either the absence of initial engraftment of donor cells (primary GF) or the subsequent loss of donor cells following initial engraftment (secondary GF).[1,3] GF makes patients vulnerable to infection, hemorrhage, and disease recurrence because of prolonged aplasia, ultimately increasing morbidity and mortality.[4-7]

The standard protocol for the management of patients with GF remains lacking, and several approaches have been proposed to treat GF, including growth factors or re-transplantation. Although a second transplantation is a potential therapeutic approach, not all patients can undergo salvage transplantation when experiencing GF.[8] Additionally, information regarding the efficacy of a second transplantation in pediatric populations is limited in the literature, and consensus on the optimal procedural approach for secondary transplantation is lacking.

Over the last few decades, studies have endeavored to elucidate the therapeutic efficacy of a second transplant in HCT recipients with GF. However, most studies were surveys, case series, or cohort-based studies, with a limited scope and depth of information. Notably, information on the outcomes of salvage transplantation following GF, particularly in the pediatric population, is scanty, and most previous studies have predominantly focused on adult cohorts.[3-5,7,9-11] Consequently, contemporary studies delineating the therapeutic outcomes of a second HCT for GF in pediatric patients are necessary.

METHODS

Study patients

A retrospective chart review involving children who underwent more than one allo-HCT at Samsung Medical Center, Seoul, Korea, between 1998 and 2020 was conducted. Throughout the study period, 578 and 71 patients underwent the first and second allo-HCT, respectively. Among the 71 patients who underwent second allo-HCT, GF was the primary indication in 30 patients. Of these 30 patients, one patient who underwent the initial allo-HCT at another medical institution was excluded. Also, four patients with an inter-transplantation interval exceeding 6 months were excluded. Finally, two patients who showed autologous recovery following the first allo-HCT were excluded to ensure a focus on the efficacy of salvage transplantation for primary or secondary GF. These exclusions resulted in a final cohort of 23 patients. The patient demographics and clinical information were assessed during the initial and subsequent transplantations.

Definitions

GF was defined as the absence of donor-derived neutrophil recovery or the necessity for either a boost from the same donor or a second transplantation owing to inadequate neutrophil recovery. Primary GF was defined as the absence of initial engraftment of donor cells, while secondary GF was defined as the loss of donor cells following successful initial engraftment.[1,3]

Hematopoietic reconstitution was characterized based on the neutrophil recovery in the estimated time until the first of three consecutive days post-transplantation, when an absolute neutrophil count of at least 0.5×10^9/L was attained. The diagnosis and grading of acute and chronic graft-versus-host disease (GVHD) were predicted based on established clinical criteria.[12,13] Post-transplant engraftment was assessed using bone marrow (BM) or peripheral blood (PB) specimens at 28, 56, 100, 180, and 365 days post-transplantation, employing sex chromosome fluorescence in situ hybridization or short tandem repeat-based chimerism analysis. Complete donor chimerism was defined as the presence of > 99% DNA in the recipient's sample from the donor.[14] Mixed chimerism was considered when 1–99% of the recipient DNA was derived from the donor.[15]

The selection of the conditioning regimen and the administration of antithymocyte globulin (ATG) were determined by the treating physician, based on the patient's clinical condition. Conditioning treatment intensity was defined as described previously.[16] Myeloablative conditioning (MAC) was defined as the documented dosages of myeloablative agents, including ≥ 5 Gy single dose or ≥ 8 Gy fractionated total body irradiation, ≥ 9 mg/kg busulfan, or ≥ 150 mg/m^2 melphalan.[16] The treatment protocols comprising ATG and/or other immunosuppressants were categorized as minimally intensive regimens. All other modalities were categorized as non-myeloablative (NMA) or reduced-intensity conditioning (RIC) regimens.

Statistics

The clinical characteristics of the study population and outcomes following the initial and subsequent transplantation were evaluated using the Fisher's exact test for categorical variables and the two-sample *t*-test or Mann-Whitney test for continuous variables. The overall survival (OS) was calculated from the date of the first hematopoietic stem cell (HSC) infusion at the second transplantation until death from any cause using the Kaplan-Meier method. Treatment-related mortality (TRM) was defined as death resulting from causes unrelated to the underlying disease, or death during remission. The differences in the survival rates among the distinct groups were compared using log-rank tests.

Ethics statement

This study was approved by the Institutional Review Board (IRB) of Samsung Medical Center (SMC) (IRB No. SMC 2024-01-165), and the requirement for informed consent was waived.

RESULTS

Characteristics of the study patients

Among the 578 patients who underwent their first allo-HCT at our center during the study period, 29 (5.0%) patients underwent a second allo-HCT because of GF. Upon disease classification, 15 of 414 patients (3.6%) with malignant disorders and 14 of 164 patients (8.5%) with non-malignant disorders were included ($P = 0.020$). Based on the transplant period, 21 of 305 patients (6.9%) between 1998 and 2009 and 8 of 273 (2.9%) patients between 2010 and 2020 received a second allo-HCT due to GF ($P = 0.035$).

The characteristics of the 23 patients included in this analysis are summarized in **Table 1**. Detailed information on the characteristics and outcomes of each patient is provided in **Supplementary Table 1**. Of the 23 patients, 14 and 9 presented with primary and secondary

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Table 1. Characteristics of the study patients

Characteristics	Patients (n = 23)
Type of GF	
Primary	14 (60.9)
Secondary	9 (39.1)
Age at first allo-HCT, yr	7.3 (0.3–14.9)
Male	18 (78.3)
Interval between the 1st and 2nd HCTs, day	51 (26–181)
Same donor between the 1st and 2nd HCTs	9 (39.1)
Underlying disease	
ALL	2 (8.7)
AML	7 (30.4)
JMML or CML	3 (13.0)
Aplastic anemia	2 (8.7)
Fanconi anemia	2 (8.7)
PID or metabolic disorder	6 (26.1)
Neuroblastoma	1 (4.3)
Year of 1st HCT	
1998–2009	16 (69.6)
2010–2020	7 (30.4)

The values are presented as the number of patients (%) or median (range).
GF = graft failure, allo-HCT = allogeneic hematopoietic cell transplantation, ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, JMML = juvenile myelomonocytic leukemia, CML = chronic myeloid leukemia, PID = primary immunodeficiency.

GF, respectively. The median age of the study population was 7.3 years, with a male predominance (78.3%). The median interval between the first and second transplantations was 51 days (range: 26–181 days). Notably, nine patients underwent allo-HCT from the same donor during both transplantations. Leukemia was the most common underlying disease, with predominantly acute myeloid leukemia (AML) in seven patients, followed by acute lymphoblastic leukemia, juvenile myelomonocytic leukemia, and chronic myeloid leukemia in two, two, and one patient, respectively. Non-malignant disorders were the next most prevalent diseases, comprising five patients with primary immunodeficiencies, four with aplastic anemia (AA) or Fanconi anemia, and one with an inherited metabolic disorder. Additionally, one patient presented with a neuroblastoma. Approximately 70% (n = 16) of the patients underwent their first transplantation before 2010, and the remaining seven patients received transplantation thereafter.

Transplant characteristics

The characteristics of both allo-HCTs in patients are compared in **Table 2**. In the initial allo-HCT, the most common donor type was unrelated donor (URD), accounting for 52.2% of the donors, and the most prevalent stem cell source was cord blood (CB), accounting for 47.8%. Conversely, in subsequent allo-HCT, the majority of recipients (86.9%) received HSCs from familial mismatched donors (FMMDs), and five patients received CD34-selected cells from FMMDs in conjunction with CB. Among the 20 recipients from FMMDs for their second allo-HCT, the CD34 positive selection, T-cell receptor alpha/beta depletion, and post-transplant cyclophosphamide (PT-Cy) techniques were employed in 12, 1, and 1 patient, respectively. No significant disparities were observed in the total nucleated cell dose and CD34+ cell dose between the two transplantation groups. MAC regimens were used more frequently in the first allo-HCT than the RIC or NMA regimens. Conversely, the RIC and NMA regimens were more prevalent in the second allo-HCT group. Notably, 5 of the 23 patients received minimally intensive conditioning therapy or underwent transplantation without conditioning.

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Table 2. Comparison of the patient characteristics between the first and second allogeneic HCTs

Characteristics	First HCT, No. (%)	Second HCT, No. (%)	*P* value
Donor type			0.001
MSD	2 (8.7)	2 (8.7)	
URD	12 (52.2)	1 (4.3)	
FMMD	9 (39.1)	20 (86.9)	
Multiple source			
FMMD only	9	15	
FMMD + CB	0	5	
T-Cell manipulation			
CD34 selection	4 (44.4)	12 (60.0)	
PT-Cy	1 (11.1)	1 (5.0)	
TCR alpha/beta depletion	2 (22.2)	1 (50.0)	
Stem cell source			< 0.001
BM	3 (13.0)	4 (17.4)	
PB	9 (39.1)	13 (56.5)	
CB	11 (47.8)	0	
Combined	0	6 (26.1)	
TNC dose[a]			
PB or BM	7.3 (6.6–12.9)	9.5 (3.0–19.1)	
CB	4.6 (2.7–10.8)	4.0 (2.2–9.3)	
CD34 cell dose[b]			
PB or BM	6.7 (4.0–11.2)	6.8 (0.5–17.6)	
CB			
Intensity of conditioning			0.010
MAC	14 (60.9)	4 (17.4)	
TBI-based	2	2	
Busulfan-based	12	2	
RIC or NMA	7 (30.4)	14 (60.9)	
Flu-containing	1	6	
TBI-containing	3	3	
Alkylating agent-containing	7	12	
None or minimal	2 (8.7)	5 (21.7)	
GVHD prophylaxis			0.507
CSA ± MMF	14 (60.9)	8 (34.8)	
CSA + MTX and/or corticosteroid	5 (21.7)	9 (39.1)	
FK506 + MMF or MTX	1 (4.3)	2 (8.7)	
Others	1 (4.3)	1 (4.3)	
None	2 (8.7)	3 (13.0)	
Use of ATG	15 (65.2)	18 (78.3)	0.326

The values are presented as the number of patients (%) or median (range).
HCT = hematopoietic cell transplantation, MSD = matched sibling donor, URD = unrelated donor, FMMD = familial mismatched donor, CB = cord blood, PT-Cy = post-transplant cyclophosphamide, TCR = T-cell receptor, BM = bone marrow, PB = peripheral blood, TNC = total nucleated cell, MAC = myeloablative conditioning, TBI = total body irradiation, RIC = reduced-intensity conditioning, NMA = nonmyeloablative, Flu = fludarabine, GVHD = graft-versus-host disease, CSA = cyclosporine A, MMF = mycophenolate mofetil, MTX = methotrexate, ATG = anti-thymocyte globulin.
[a]Unit: PB or BM ($\times 10^8$/kg); CB ($\times 10^7$/kg).
[b]Unit: PB or BM ($\times 10^6$/kg); CB ($\times 10^5$/kg).

Engraftment and outcomes following second transplantation

Table 3 shows the outcomes of the second transplantation. Neutrophil engraftment was observed in 17 patients (73.9%) following the second allo-HCT at a median of 17 days post-transplantation, with a range of 9 to 58 days. Notably, five of these patients experienced secondary GF, and successful engraftment was achieved in 12 patients (52.2%) following the second transplantation. Primary GF persisted in five patients even after undergoing a second transplantation, whereas recovery of the autologous BM occurred in one patient. Subsequently, four patients underwent a third allo-HCT. Of these, three required re-transplantation due to persistent GF following the second HCT, while one patient with

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Table 3. Outcomes following second transplantation

Outcomes	Patients (N = 23)
Engraftment	
Yes	12 (52.2)
No	11
Primary GF	5 (21.7)
Secondary GF	5 (21.7)
Autologous recovery	1 (4.3)
Neutrophil recovery, day[a]	17 (9–58)
Platelet recovery, day[b]	35 (19–49)
VOD[c]	5 (41.7)
Acute GVHD[c]	
Grade II–IV	7 (58.3)
Grade III–IV	5 (41.7)
Chronic GVHD[c,d]	
Limited	1 (11.1)
Extensive	1 (11.1)
CMV infection[c]	1 (11.1)

The values are presented as median (range) or number of patients (%).
GF = graft failure, VOD = veno-occlusive disease, GVHD = graft-versus-host disease, CMV = cytomegalovirus.
[a]Seventeen patients who exhibited neutrophil recovery following the second transplantation were included.
[b]Eight patients who eventually achieved engraftment and platelet recovery were included.
[c]Twelve patients who achieved engraftment following the second transplantation were included.
[d]Two patients who died before 100 days following the second transplantation were excluded.

AML underwent re-transplantation due to the development of myelodysplastic syndrome after autologous recovery. Notably, following the third transplantation, successful engraftment was achieved in all three patients with persistent GF following the second allo-HCT, with two patients receiving HSCs from the same FMMD and one patient from the CB. A univariate analysis of patient characteristics did not reveal any significant association with engraftment following the second allo-HCT (**Supplementary Table 2**).

The incidence of veno-occlusive disease (VOD), GVHD, and cytomegalovirus (CMV) infection was investigated in 12 patients who achieved successful engraftment following second allo-HCT. Of these patients, five experienced VOD and seven (58.3%) had grade II–IV acute GVHD, with an incidence of grade III or IV GVHD reaching 41.7%. Limited or extensive chronic GVHD manifested in one patient each. Additionally, CMV infection occurred in 9 of the 12 patients. Among all study patients, VOD and CMV infection occurred in 7 patients (30.4%) and 16 patients (69.6%), respectively, following the second HCT.

Among the 12 patients, complete donor chimerism was observed in nine patients at the last evaluation, whereas three patients exhibited mixed donor chimerism. Notably, all four patients who underwent a third allogeneic HCT achieved complete donor chimerism at the final evaluation.

Survival analysis and causes of mortality

The 5-year probabilities of OS and TRM were 30.4% and 58.2%, respectively, for the entire study population (**Figs. 1A** and **2A**). The survival outcomes did not differ significantly between the patients presenting with primary and secondary GF following initial transplantation (**Figs. 1B** and **2B**). Moreover, no significant differences in OS or TRM rates were observed based on the underlying disease (malignant vs. non-malignant) (**Figs. 1C** and **2C**) and donor type (same donor vs. non-same donor) (**Figs. 1D** and **2D**). The 5-year OS and TRM rates for patients who achieved successful engraftment following the second allo-HCT were 41.7% and 33.3%, respectively, and these were 18.2% and 81.8%, respectively, in those with failed

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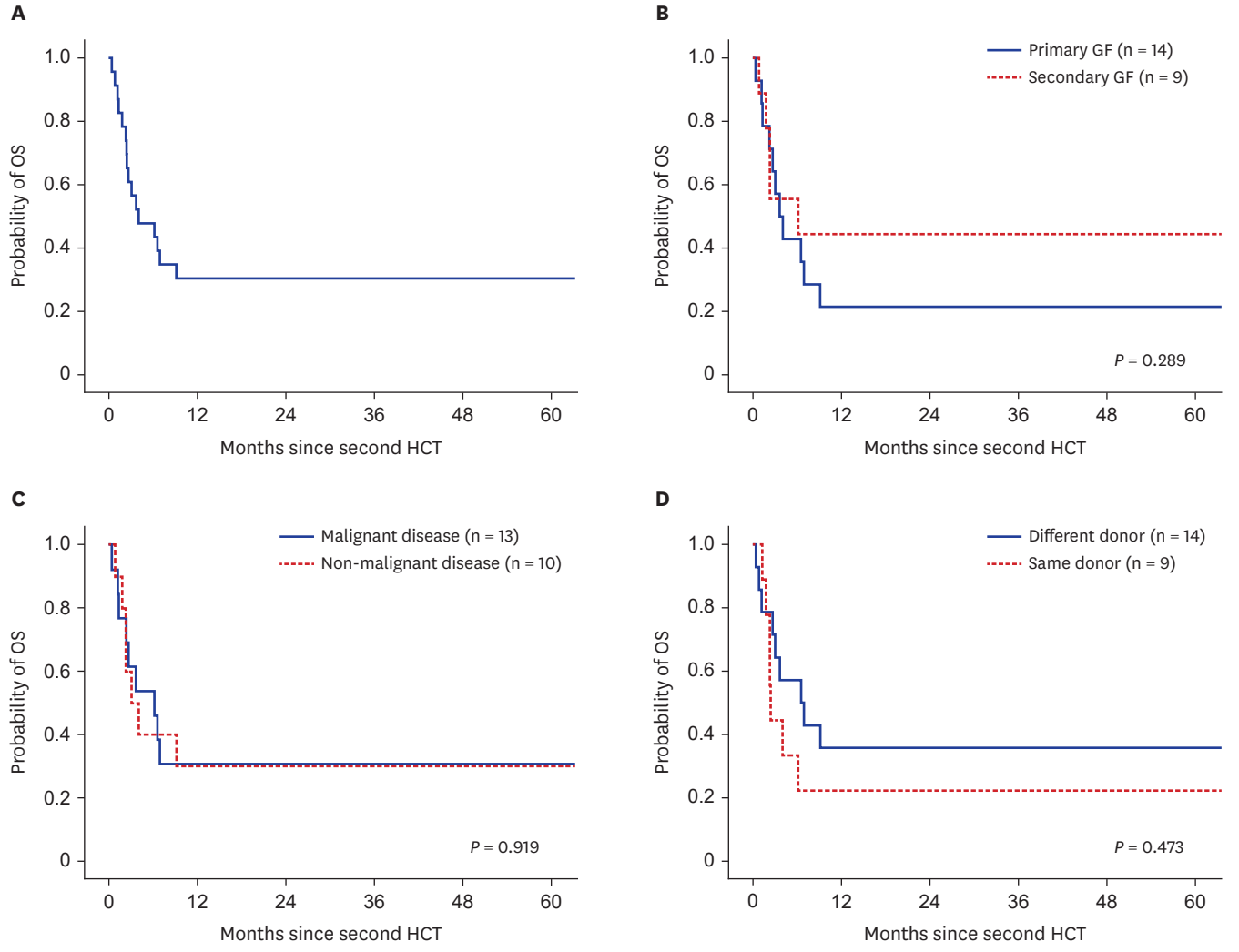

Fig. 1. OS following second allogeneic HCT in the study population (**A**), according to the type of graft failure (**B**), the underlying disease (**C**), and the donor type (**D**).
OS = overall survival, HCT = hematopoietic cell transplantation, GF = graft failure.

engraftments (**Fig. 3**). No significant difference was observed in the OS between patients with successful and failed engraftments following the second transplantation ($P = 0.129$). However, the TRM was significantly higher in patients with failed engraftments than in those with successful engraftments following the second transplantation ($P = 0.030$).

Notably, 17 patients died, and the causes of death are presented in **Table 4**. Among the 12 patients who achieved successful engraftment following the second transplantation, 8 ultimately died, along with 9 of the 11 patients who did not attain successful engraftment. Mortality was caused by infection in most patients (n = 12), followed by relapse (n = 3), lung GVHD (n = 1), and undefined multi-organ failure (n = 1). Among the patients who achieved successful engraftment following the second allo-HCT, the causes of mortality were as follows: infection in four patients, relapse in three patients, and lung GVHD in one patient.

Infection was the most prevalent cause of death, irrespective of the engraftment status. The types of infections observed were sepsis combined with pneumonia in three, sepsis alone in three, and pneumonia alone in six. Bacterial sepsis was identified exclusively in

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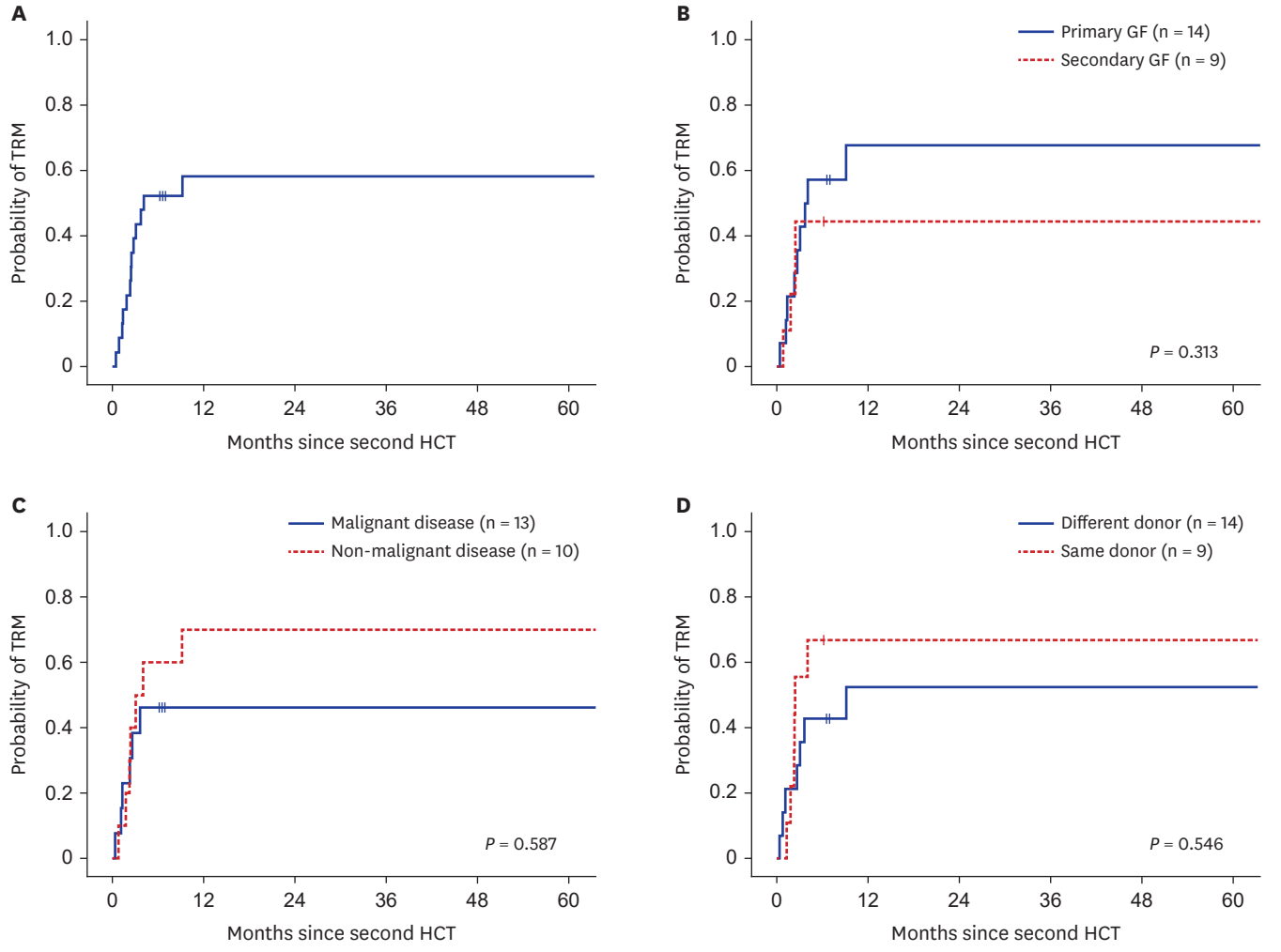

Fig. 2. Cumulative incidence of TRM following second allogeneic HCT in the study population (**A**), according to the type of GF (**B**), the underlying disease (**C**), and the donor type (**D**).
TRM = treatment-related mortality, HCT = hematopoietic cell transplantation, GF = graft failure.

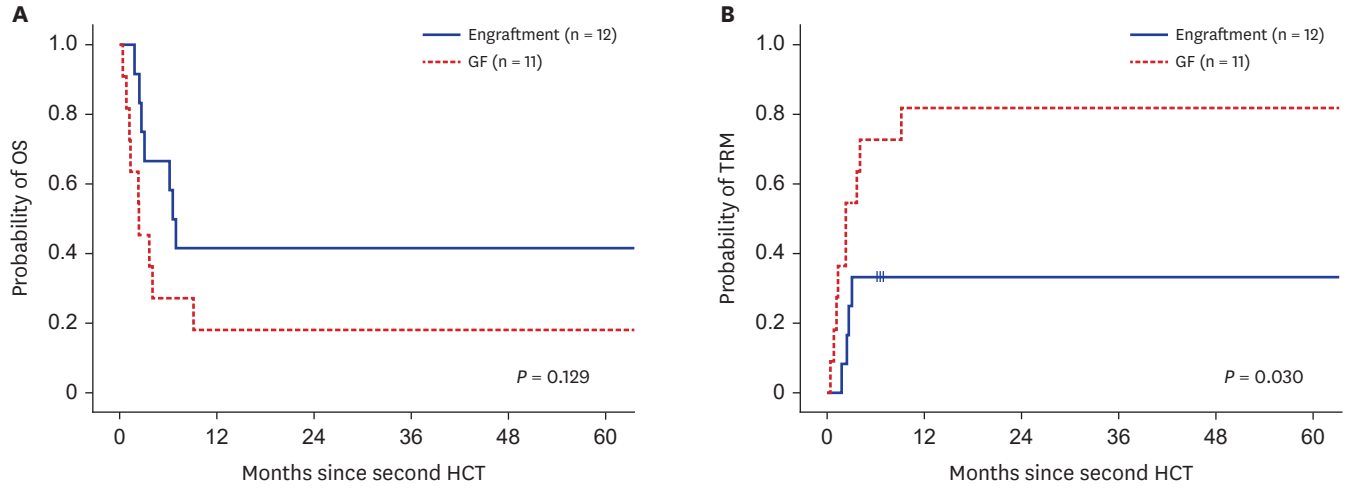

Fig. 3. OS (**A**) and cumulative incidence of treatment-related mortality (**B**) following second allogeneic HCT according to the engraftment state.
OS = overall survival, HCT = hematopoietic cell transplantation.

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Table 4. Causes of mortality according to the engraftment status following second allogeneic hematopoietic cell transplantation

Cause	Engraftment (n = 12)	GF (n = 11)
Total	8	9
Infection	4 (33.3)	8 (72.7)
Relapse	3 (25.0)	
Lung GVHD	1 (8.3)	
Multiorgan failure		1 (9.1)

Values are presented as the number of patients (%) or median (range).
GF = graft failure, GVHD = graft-versus-host disease.

patients who failed to achieve successful engraftment following the second HCT. In patients who achieved engraftment after the second HCT, the pathogens identified included *Candida albicans*, adenovirus, parainfluenza, and human herpesvirus 6. In contrast, in patients who failed the second HCT, the identified pathogens were vancomycin-resistant *Enterococcus*, coagulase-negative *Staphylococcus*, *Klebsiella oxytoca*, CMV, influenza, adenovirus, and *Aspergillus*.

DISCUSSION

We elucidated the characteristics and outcomes of salvage transplantation in children with GF. FMMD transplantation (FMMDT) following conditioning regimens other than MAC, such as RIC or NMA, has emerged as the predominant transplantation strategy. Although a considerable proportion of patients exhibit neutrophil recovery following salvage transplantation, subsequent GF occurrences remain prevalent, and a third transplant is crucial for achieving definitive engraftment in such cases. Despite the efforts to facilitate engraftment, the survival outcomes in our study population were unsatisfactory, with a long-term survival rate of 30%. Notably, infectious diseases are the most common cause of mortality. Our findings provide valuable insights into the infrequently reported practice of GF in pediatric patients. Owing to the lack of comprehensive treatment outcomes in the previous studies for GF in children to the extent of including as many FMMDT as in our study, the results of our FMMDT investigation have substantial clinical significance.

The proportion of patients requiring a second allo-HCT for GF decreased in the latter half of the study period compared to the first half. This decline in GF incidence may be attributed to a better understanding of the optimal transplant cell dosage and composition, human leukocyte antigen matching using high-resolution typing, improved donor selection, conditioning regimens, and increased utilization of peripheral blood stem cells (PBSCs).[17,18] Following a second transplantation to achieve engraftment, 73.9% of the patients exhibited neutrophil recovery. However, the ultimate efficacy of salvage transplantation in conferring survival is only approximately 30%. Moreover, the outcomes did not improve significantly over time following transplantation, and similar results have been observed in previous studies involving adult patients, with a reported cumulative incidence of neutrophil recovery ranging from 46% to 85% and OS ranging from 11% to 61%.[5,10,11,17,19] An observational study using the data from the Center for International Blood and Marrow Transplant Research database on URD transplantations (URDTs) revealed a dismal OS rate particularly in patients with primary GF following initial HCT, indicating a worse prognosis compared to other GF.[17] Additionally, another single-center analysis revealed similar OS rates for primary GF, autologous reconstruction, and secondary GF at 18%, 11%, and 13%, respectively.[18] Our study discerned no significant difference in the outcomes between patients with primary and secondary GF following their initial HCT. Additionally, it is noteworthy that patients who

underwent successful second transplantation demonstrated a trend towards improved OS compared to those whose transplantation failed; however, this trend did not reach statistical significance, likely due to the small sample size of the study.

The predominant cause of mortality among the patients was infection, followed by disease relapse. The failure of donor cells to establish and sustain engraftment lead to a consequential risk of disease recurrence and potentially devastating sequelae of pancytopenia,[20] and consequently, infection emerges as the primary cause of death.[11,21-23] This is likely attributed to the protracted period of severe neutropenia before and after the second transplantation, facilitating the incubation and perpetuation of infectious pathogens.[23] Patients with GF achieve sustained neutrophil recovery following a second allo-HCT and maintain high susceptibility to infectious sequelae even in the absence of GVHD,[21] which was also confirmed in our study. Even with successful engraftment, recipients exhibit significant impairments in both cell-mediated and humoral immunity, leading to prolonged immunosuppression. The recovery of full immune competence can span several months to years. The degree of immunosuppression experienced by patients varies considerably and is influenced by factors, such as the severity of GVHD, the intensity of post-transplant immunosuppression, recipient-related factors (including age, comorbidities, and prior exposure to infections), and graft-related factors.[24]

To minimize infectious complications, the optimal timing of transplantation, conditioning regimen, donor type, and cell source must be carefully tailored to each patient's specific circumstances. Guidelines have been developed to direct strategies for preventing infectious disease complications, encompassing areas such as HSC graft safety, prevention of bacterial, viral, and fungal infections post-transplantation, infection prevention and control in healthcare settings, safe living post-HCT, and recipient vaccination.[24] Adherence to these guidelines to prevent exposure to pathogens and the implementation of appropriate treatment strategies are critical for reducing infection-related morbidity and mortality. Furthermore, while current data is insufficient to recommend universal antimicrobial prophylaxis in children, prophylaxis against bacterial infections may be considered for children with GF due to the high incidence and mortality associated with infectious diseases.

Approximately 60% of patients underwent a second allo-HCT from a different donor, whereas 40% of patients received grafts from the same donor. Typically, same-family donors are frequently preferred in related donor transplantations compared with URDTs because of the greater availability of familial donors for subsequent donations than URDs.[11] Notably, in cases where GF transpires following a familial donor transplantation, subsequent donation from the same donor is feasible immediately thereafter. Occasionally, the products previously collected and cryopreserved for the initial URDTs may be utilized for a second transplantation.[17] However, the source of transplantation may vary even when the same donor is accessible. A report from the Severe AA Working Party of the European Society for Blood and Marrow Transplantation indicated that the same donor as the first allo-HCT was preferred for 81% of patients for the subsequent donation, and a shift in the choice of HSC source was observed in 56% of patients.[11] Unlike the initial HCT, PBSCs were the preferred HSC source in the second HCT, irrespective of the donor type. Our findings further underscore that PBSCs constitute the most common HSC source in the second HCT. Although identical donors are preferred whenever feasible, the superiority between identical and different donors remains uncertain.[11] One study reported that HCT from a different donor yielded superior odds of engraftment compared with HCT from the same donor,[25]

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whereas another study suggested that the use of the same or alternative donor did not significantly affect patient survival.[21]

FMMDs, known for their high availability in emergency scenarios, have emerged as a viable alternative HSC source,[5,25-27] as observed in our study. CB is also a crucial source for immediate HCT, offering readily available HSCs without necessitating donor manipulation.[10,23,25,28] Additionally, repeated HSC collections from BM or PB can pose risks to volunteer donors, whereas CB is devoid of ethical concerns related to donor involvement.[28] On the other hand, neutrophil and platelet recovery is delayed in cord blood transplantations (CBT) compared to those using BM or PB.[5] A study utilizing Japanese nationwide registry data highlighted that salvage transplantation for GF in patients aged > 15 years exhibited comparable OS and non-relapse mortality between haploidentical donor transplantation using BM or PB compared with CBT.[5] Nevertheless, the applicability of these findings to contemporary transplantation settings, where T-cell manipulation techniques, such as PT-Cy, are more prevalent, may be limited owing to the small sample size of patients receiving PT-Cy. Our study, elucidating the outcomes of 20 pediatric patients who underwent FMMDT following GF, is noteworthy because FMMDT in children following GF has been infrequently reported, except in registry-based studies or case series with a limited number of patients.

In our study, RIC or NMA conditioning was preferred over MAC conditioning in HCTs. In previous studies, variable intensities of conditioning regimens were used.[5,10,22,25,28] In adult studies, RIC or NMA have generally been recommended as the preferred strategy to avoid excessive toxicity, particularly in older or other adult patients with comorbidities who may not tolerate the toxicities associated with MAC.[3,19,22] Additionally, as the BM of patients with primary GF is already hypocellular, salvage HCT does not require MAC,[25] but requires conditioning to a degree that induces adequate eradication of the residual host cells to overcome immunological rejection without causing unacceptable long-term toxicity.[23,26] In cases of graft dysfunction accompanied by significant donor chimerism, it has been suggested that a second HSC boost using the same donor might be administered without cytotoxic conditioning.[29] Although several registry-based studies have reported successful engraftment in patients with GF without conditioning,[17,21] robust evidence supporting this approach, especially in children, is still insufficient. Generally, it is accepted that a second HCT for GF necessitates conditioning that often includes additional cytotoxic therapy.[29] Various RICs[5,23,25,26] exhibit sufficient immunosuppressive effects, enabling stable engraftment. However, the results must be interpreted cautiously in children because several of these studies only included patients aged > 15 years, and the relationship between the intensity or type of conditioning and survival in children has not yet been established. Moreover, the definition of conditioning regimen intensity varies considerably among studies, potentially affecting the study outcomes. We followed the criteria outlined by Bacigalupo et al.,[16] where MAC was defined as a busulfan dose of ≥ 9 mg/kg. However, other investigations have defined MAC using different thresholds, such as a busulfan dose exceeding 6.4 mg/kg[30] or 8 mg/kg.[31,32] Furthermore, the recommended busulfan dose may vary according to the patient's weight.[33]

This study has a few limitations. First, as this was a retrospective study conducted at a single institution, definitive conclusions regarding the optimal strategy for GF or reproducibility in other institutions cannot be drawn. Furthermore, there were no consistent, pre-determined guidelines for selecting conditioning regimens and using ATG. This lack of standardization may introduce bias into the analysis of prognosis related to transplant outcomes. Additionally, the small number of patients included in this study limited the robustness of

the analyses, and consequently, the factors associated with transplant outcomes did not yield statistically significant results.

Despite these limitations, this study provides valuable insights regarding the characteristics and outcomes of salvage transplantation in children with GF. Although the optimal transplant strategy with respect to donor selection, conditioning intensity or type, and timing of the second HCT remains unclear, HSC infusion after RIC or NMA conditioning, regardless of the HSC source or donor type, may serve as an additional option for engraftment and survival in a subset of patients. Our findings indicated that successful engraftment following a second allo-HCT may decrease the TRM. However, meticulous monitoring and the intensive management of infectious diseases remain imperative even in patients with successful engraftment. Multicenter prospective studies employing the same protocol are warranted in the future to explore the optimal strategies for children with GF.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Characteristics and outcomes of transplantation for each patient

Supplementary Table 2

Factors affecting engraftment following the second transplantation

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