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Incidence and impact of invasive fungal infection comparing post-transplant cyclophosphamide with cyclosporine plus methotrexate GVHD prophylaxis in allogeneic HSCT

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ABSTRACT

Background: In recent years, haploidentical hematopoietic stem cell transplantation (haploHSCT) with post-transplant cyclophosphamide (PTCy) has become increasingly prevalent. However, the precise impact of invasive fungal disease (IFD) in relation to graft-versus-host disease (GVHD) prophylaxis and donor type remains to be elucidated.

Methods: In this study, we analyzed data from 580 HSCT patients, comprising 80 patients who received haploidentical grafts and 500 patients who received grafts from other donor types. PTCy was exclusively administered to haploidentical HSCT recipients, while cyclosporine A (CsA) in combination with short-course methotrexate (scMTX) was used for patients receiving grafts from other donors.

Results: The IFD rate by PTCy and CsA plus scMTX was 15 % and 15.6 %, respectively. At 6 months and 1 year post-transplant, the cumulative incidence of IFD was 9.4 % and 14.8 % for the PTCy group, and 7.9 % and 12.3 % for the CsA plus scMTX group, respectively. Both groups exhibited poor survival outcomes associated with IFD. Identified risk factors for IFD included age $\geq$ 45 years, disease relapse, and grade III-IV acute GVHD. *Aspergillus* spp. and *Candida* spp. were the most commonly isolated pathogens. High rate of cytomegalovirus reactivation was also noticed in PTCy or CsA plus scMTX group, but not a risk factor for IFD.

Conclusion: The similar IFD rate between haploHSCT with PTCy and others with CsA plus scMTX was documented, with *Aspergillus* spp. and *Candida* spp. as the most common pathogens. Further research is needed to investigate IFD following haploHSCT with PTCy and to explore differences with other types of allogeneic HSCT.

1. Introduction

The strategies of allogeneic hematopoietic stem cell transplantation (alloHSCT) and supportive care have made significant advances over the past few decades, thereby improving the survival rates of patients with hematological diseases.[1,2] AlloHSCT remains a major potentially curative therapy for many hematological disorders. However, the challenges associated with finding an appropriate donor match and the significant toxicities involved remain substantial barriers.[1] Geographical and ethnic disparities have made access to matched donors increasingly difficult, prompting the exploration of alternative donor sources such as haploidentical HSCT (haploHSCT).[3]

As a result, standard graft-versus-host disease (GVHD) prophylaxis, typically involving cyclosporine A (CsA) in combination with short-course methotrexate (scMTX), with or without the addition of antithymocyte globulin (ATG), has played a pivotal role in reducing the risks of graft failure and GVHD in alloHSCT, including those involving HLA matched sibling donors (MSD) and matched unrelated donors (MUD).[4,5]In recent years, haploHSCT with post-transplant cyclophosphamide (PTCy) has gained increasing prominence. PTCy selectively eliminates donor alloreactive T-cells, facilitating recovery of CD4 and $CD8^+$ T cells.[6–9] However, compared to MSD or MUD, haploHSCT with

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PTCy prophylaxis is associated with slower rates of immune reconstitution, potentially increasing the risk of infectious complications, although it does not significantly affect rates of non-relapse mortality.[10–13] Previous studies have indicated that PTCy may elevate the risk of bacterial infections during the pre-engraftment phase.[14,15] Additionally, higher incidences of cytomegalovirus (CMV), BK virus, and respiratory viral infections have been observed in patients undergoing haploHSCT with PTCy prophylaxis.[16,17] Furthermore, fungal infections appear to occur more frequently in haploHSCT with PTCy cohorts when mold-active prophylaxis is not administered, necessitating further investigation into the specific role of PTCy in this context.[14–16]

Our study retrospectively investigated and specifically evaluated fungal complications following haploHSCT with PTCy, without ex vivo T-cell depletion, in comparison to MSD, MUD, and other alternative donor approaches using CsA plus scMTX in a large cohort of 580 patients.

2. Patients and methods

2.1. Patients

We included adult patients who underwent alloHSCT for hematologic disorders at the Blood and Marrow Transplant Center of Taipei Veterans General Hospital from 2003 to 2023. We conducted a retrospective review of pre-transplant characteristics, transplant-related information, and post-transplant clinical data. This included variables such as age, gender, disease diagnosis, disease status, comorbidities, donor type, conditioning regimen, graft source, acute and chronic graft-versus-host disease (aGVHD and cGVHD) prior to the first event of invasive fungal disease (IFD), as well as the CMV status of both recipient and donor, and CMV viremia before IFD. The study was approved by the Institutional Review Board of Taipei Veterans General Hospital and adhered to the Helsinki Declaration of 1975, as revised in 2008.

2.2. Transplant details and GVHD evaluation

Stem cells were obtained from matched sibling donors (MSDs), matched unrelated donors (MUDs), mismatched unrelated donors (MMUDs), haploidentical donors, and umbilical cord blood. HLA typing for 6–10 alleles (HLA-A, -B, -DR, with or without HLA-C and HLA-DQ) was performed to determine the degree of disparity between patients and donors. The most commonly used myeloablative conditioning regimens included busulfan (3.2 mg/kg/day for 4 days) plus cyclophosphamide (60 mg/kg/day for 2 days), or total body irradiation (TBI) at 12 Gy plus cyclophosphamide (60 mg/kg/day for 2 days). For older, less fit, or more complicated patients, fludarabine-based reduced-intensity conditioning regimens were employed.

For patients with severe aplastic anemia (SAA), the conditioning regimen generally consisted of cyclophosphamide (50 mg/kg/day for 4 days) and low-dose TBI (2–5Gy) with or without rabbit ATG (2.5 mg/kg/day for 2–3 days). GVHD prophylaxis for haploHSCT involved high-dose cyclophosphamide (50 mg/kg) administered on days +3 and + 4, combined with an anti-calcineurin agent and mycophenolate mofetil (MMF) starting on day +5. The standard GVHD prophylaxis involving CsA plus scMTX was described in our previous report.[4,18]

The severity of aGVHD was assessed according to the grading system developed by Glucksberg and Thomas,[19,20] while cGVHD severity was evaluated using the NIH scoring system, which categorizes the condition as mild, moderate, or severe, or classifies it as limited or extensive.[21,22] Patients with aGVHD exceeding functional overall Grade II, extensive cGVHD, or alloimmune lung disease typically received high-dose methylprednisolone (1–2 mg/kg/day). Anti-fungal prophylaxis was also administered during high-dose steroid therapy.

2.3. Antifungal prophylaxis, monitoring, and treatment

All patients received antifungal prophylaxis, which included either echinocandins (micafungin, anidulafungin, or caspofungin) or azoles (fluconazole or voriconazole). Pneumocystis jirovecii pneumonia prophylaxis was administered with trimethoprim-sulfamethoxazole. Universal antibacterial prophylaxis was not employed during HSCT. Levofloxacin was initiated when patients met neutropenic criteria, defined as an absolute neutrophil count of $<500 \times 10^9$ cells/L, and continued until neutrophil recovery.

In our study, we included proven and probable IFD. The diagnosis of individual fungal pathogens for proven and probable IFD was made according to the current definitions of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG).[23,24] Since October 2009, a galactomannan (GM) antigen assay has been performed at our hospital for patients with clinical suspicion of IFD. Proven IFD was defined by the demonstration of fungal elements through microscopic examination or positive fungal culture results from diseased tissue or blood. According to clinical GM antigen assay at our hospital, we adopt galactomannan antigen index >0.5 in plasma/serum and/or galactomannan antigen >0.8 in bronchoalveolar lavage fluid as the positive finding of invasive aspergillosis.[23] Detailed methodologies were described in our published report.[18]

2.4. Study endpoints and statistical analysis

Median values and ranges were used to describe continuous variables, while percentages and frequencies were used for categorical variables. Differences in clinical characteristics between HSCT patients receiving CsA plus scMTX or PTCy prophylaxis were compared using the Pearson chi-square test or Fisher's exact test as appropriate. The cumulative incidence of IFD was calculated considering death as a competing risk. Survival differences were assessed using the Kaplan-Meier method and log-rank test.

Composite endpoints included grades III-IV aGVHD, cGVHD requiring systemic treatment, relapse, or death, with outcomes defined asGVHD-free/relapse-free survival (GRFS).[25] Possible risk factors for IFD were analyzed retrospectively using Cox proportional hazard models. Factors showing statistical significance ($P < 0.05$) in univariate analysis were included in multivariate analysis. Results are reported as hazard ratios with corresponding 95 % confidence intervals (CIs). Statistical analyses were performed using IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and STATA version 14 (College Station, TX, USA).

3. Results

3.1. Characteristics of patients with alloHSCT

In a total of 580 patients who underwent alloHSCT, the median age at alloHSCT was 43 years (IQR: 31–53). The median follow-up time after alloHSCT was 17.9 months. The primary hematologic diseases necessitating alloHSCT were acute myeloid leukemia (AML) (40.2 %) and acute lymphoblastic leukemia (ALL) (19.7 %). Donor sources included MSD (37.9 %), MUD (45.4 %), haploidentical donors (13.8 %), and others (MMUD and umbilical cord blood) (2.9 %).

GVHD prophylaxis with CsA plus scMTX ($n = 500$, 86.2 %) was used for alloHSCT with MSD, MUD, and other donors, while PTCy ($n = 80$, 13.8 %) was applied for haploHSCT. Patients receiving CsA plus scMTX GVHD prophylaxis mainly underwent myeloablative conditioning (MAC) regimens, whereas non-myeloablative/reduced intensity conditioning (Non-MAC/RIC) regimens were used for haploHSCT with PTCy prophylaxis.

In our cohort, 493 donors (85 %) were CMV IgG positive. Regarding CMV serostatus of donor-recipient pairs, the most common scenario was positive CMV IgG in both donors and recipients (82.4 %).The incidence

of grade III-IV aGVHD was 17.9 %, while 12.4 % of patients experienced extensive cGVHD requiring steroid therapy. A total of 329 patients (56.7 %) experienced CMV reactivation before developing IFD. Detailed characteristics are summarized in Table 1.

3.2. Incidence of IFD and outcome

In our study, post-transplant IFD occurred in 90 patients (15.5 %). Of these, 78 out of 500 patients (15.6 %) who received CsA plus scMTX and 12 out of 80 patients (15 %) who received PTCy prophylaxis developed IFD (Table 3). The cumulative incidence of IFD at 6 months and 1 year was 9.4 % and 14.8 %, respectively, for the PTCy prophylaxis, and 7.9 % and 12.3 %, respectively, for the CsA plus scMTX prophylaxis. The cumulative incidence of IFD had no difference between the two GVHD prophylaxis (Fig. 2).

Patients with post-transplant IFD had significantly worse overall survival (OS), disease-free survival (DFS), and GRFS compared to those without IFD (all $P < 0.001$; Fig. 1a–c). Among patients with IFD, those receiving CsA plus scMTX or PTCy prophylaxis had poorer survival outcomes than those without IFD (all $P < 0.001$; Fig. 3a–c). For patients without IFD, OS and DFS were similar between the CsA plus scMTX and PTCy prophylaxis. The patients without IFD who received PTCy prophylaxis had significantly better GRFS compared to those receiving CsA plus scMTX prophylaxis ($P = 0.005$; Fig. 3c).

3.3. Risk factors for IFD

In risk factors for IFD, we examined several variables, including age, gender, GVHD prophylaxis, ATG-containing or myeloablative conditioning, disease relapse, grade III-IV aGVHD, extensive cGVHD, CMV reactivation, and echinocandin prophylaxis. The significant risk factors were associated with IFD, including age ≥ 45 years (HR: 1.55; 95 % CI: 1.02–2.35; $P = 0.04$), relapse after HSCT (HR: 1.85; 95 % CI: 1.19–2.88; $P = 0.007$), and grade III-IV aGVHD (HR: 2.14; 95 % CI: 1.31–3.51; $P = 0.003$). Detailed results are presented in Table 2.

3.4. Characteristics and microbiology of IFD

In the CsA plus scMTX prophylaxis ($n = 500$), invasive mold infection (IMI) developed in eighteen patients (8.2 %) out of 220 matched sibling HSCTs and thirty-one patients (11.8 %) out of 263 matched unrelated HSCTs. Conversely, in the PTCy prophylaxis ($n = 80$), seven patients (8.8 %) were diagnosed with IMI following haploHSCT. For invasive yeast infection (IYI), nine patients (4.1 %) with matched sibling HSCT, nineteen patients (7.2 %) with matched unrelated HSCT, and five patients (6.3 %) with haploHSCT were identified. Although the infection rates of either IMI or IYI were relatively high in patients undergoing HSCT from MUD, no statistically significant difference was observed among different donor types.

In the GVHD prophylaxis with CsA plus scMTX, the majority of patients received fluconazole anti-fungal prophylaxis ($n = 412$, 82.4 %), whereas 43 patients (53.8 %) in the PTCy group received echinocandin prophylaxis ($P < 0.001$). Additionally, a higher incidence of CMV viremia was noted in the PTCy prophylaxis compared to the CsA plus scMTX prophylaxis (77.5 % vs. 53.4 %, $P < 0.001$). The high risk with donor CMV IgG (−) to recipient CMV IgG (+) for CMV reactivation attributed to the serostatus of donor-recipient pairs, was identified in more patients in the PTCy prophylaxis than in the CsA plus scMTX group (47.5 % vs. 8.8 %, $P < 0.001$; Table 3).

Compared to the CsA plus scMTX group, the incidence of grade III-IV aGVHD (8.8 %, $P = 0.021$) and extensive cGVHD (3.8 %, $P = 0.011$) was significantly lower in the PTCy group. The majority of IFD cases were due to IMI. In the CsA plus scMTX group, *Aspergillus* spp. and *Penicillium* spp. were the most frequently isolated pathogens at the time of IMI. *Aspergillus* spp. was the predominant pathogen identified in the PTCy group. In cases of IYI in both the CsA plus scMTX and PTCy groups,

Table 1
Baseline characteristics of adult patients receiving allogeneic hematopoietic stem cell transplantation (n = 580)[a].

Characteristics (%)	Total (n = 580)	CsA + scMTX (n = 500)	PTCy (n = 80)	p value
Age at SCT, years	43 [IQR: 31–53]	42 [IQR:31–52]	56 [IQR:40–64]	<0.001
Sex, male	329 (56.7)	286 (57.2)	43 (53.8)	0.563
Diagnosis				
Acute myeloid leukemia	233 (40.2)	192 (38.4)	41 (51.3)	0.030
Acute lymphoblastic leukemia	114 (19.7)	101 (20.2)	13 (16.3)	0.409
Myelodysplastic syndrome	30 (5.2)	24 (4.8)	6 (7.5)	0.311
Non-Hodgkin lymphoma	58 (10)	54 (10.8)	4 (5)	0.108
Multiple myeloma	23 (4)	23 (4.6)	0	ns
Hodgkin lymphoma	6 (1)	6 (1.2)	0	ns
T-cell lymphoma	29 (5)	23 (4.6)	6 (7.5)	0.269
Severe aplastic anemia	54 (9.3)	49 (9.8)	5 (6.3)	0.310
Chronic myeloid leukemia	14 (2.4)	14 (2.8)	0	ns
Chronic lymphoid leukemia	4 (0.7)	4 (0.8)	0	ns
Myelofibrosis	10 (1.7)	7 (1.4)	3 (3.8)	ns
Acute mixed phenotype leukemia	3 (0.5)	2 (0.4)	1 (1.3)	ns
Chronic myelomonocytic leukemia	2 (0.3)	1 (0.2)	1 (1.3)	ns
Donor sources				ns
Matched sibling donors	220 (37.9)	220 (44)	0	
Matched unrelated donors	263 (45.4)	263 (52.6)	0	
Haploidentical donors	80 (13.8)	0	80 (100)	
Others	17 (2.9)	17 (3.4)	0	
GVHD prophylaxis				ns
CsA + scMTX	500 (86.2)	500 (100)	0	
PTCy	80 (13.8)	0	80 (100)	
Conditioning regimens				<0.001
Myeloablative	335 (57.8)	327 (65.4)	8 (10)	
Non-Myeloablative/ Reduced Intensity	245 (42.2)	173 (34.6)	72 (90)	
CMV IgG of donors				<0.001
Positive	493 (85)	453 (90.6)	40 (50)	
Negative	87 (15)	47 (9.4)	40 (50)	
Donor/Recipient CMV serostatus				<0.001
Donor CMV IgG (+)/Recipient CMV IgG (+)	478 (82.4)	438 (87.6)	40 (50)	<0.001
Donor CMV IgG (−)/Recipient CMV IgG (+)	82 (14.1)	44 (8.8)	38 (47.5)	<0.001
Donor CMV IgG (+)/Recipient CMV IgG (−)	15 (2.6)	15 (3)	0	ns
Donor CMV IgG (−)/Recipient CMV IgG(−)	5 (0.9)	3 (0.6)	2 (2.5)	ns
GVHD status				
Acute GVHD	203 (35)	188 (37.6)	15 (18.8)	0.001
Acute GVHD, grade III-IV	104 (17.9)	97 (19.4)	7 (8.8)	0.021

(continued on next page)

Table 1 (*continued*)

Characteristics (%)	Total (n = 580)	CsA + scMTX (n = 500)	PTCy (n = 80)	p value
Chronic GVHD	204 (35.2)	201 (40.2)	3 (3.8)	<0.001
Extensive GVHD	72 (12.4)	69 (13.8)	3 (3.8)	0.011
CMV reactivation before IFD	329 (56.7)	267 (53.4)	62 (77.5)	<0.001

SCT, stem cell transplantation; GVHD, graft-versus-host disease; CsA + scMTX, Cyclosporine and short course methotrexate; PTCy, post-transplant cyclophosphamide; CMV, cytomegalovirus.
GVHD, graft versus host disease; IFD, invasive fungal disease; ns, not-significant.
[a] Values are reported as median [IQR (interquartile-range)] or *n* (%).

Table 2
Risk factors for IFD.

Predictive variables	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	p value	HR (95 % CI)	p value
Age ≥ 45 years	1.58 (1.05–2.40)	0.030	1.55 (1.02–2.35)	0.040
Sex (male)	1.13 (0.74–1.72)	0.566		
PTCy prophylaxis	1.22 (0.66–2.26)	0.522		
ATG-containing conditioning	1.26 (0.83–1.92)	0.276		
Myeloablative conditioning	0.96 (0.63–1.46)	0.846		
Relapse after SCT	2.09 (1.35–3.24)	0.001	1.85 (1.19–2.88)	0.007
Grade III-IV acute GVHD	2.36 (1.45–3.85)	0.001	2.14 (1.31–3.51)	0.003
Extensive chronic GVHD	0.79 (0.44–1.43)	0.436		
CMV reactivation before IFD	1.26 (0.82–1.92)	0.295		
Echinocandin prophylaxis	1.53 (0.96–2.46)	0.076		

HR, hazard ratio; CI, confidence interval; IFD, invasive fungal disease.
PTCy, post-transplant cyclophosphamide; ATG, Anti-thymocyte globulin; SCT, stem cell transplantation; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

Table 3
Characteristics of IFD in different GVHD prophylaxis.

	CsA + scMTX group (n = 500)			PTCy group (n = 80)	p value
Transplant type	MSD (n = 220)	MUD (n = 263)	Others (n = 17)	Haplo (n = 80)	
Mold, n (%)[a]	18 (8.2 %)	31 (11.8 %)	1 (5.9 %)	7 (8.8 %)	0.530
Yeast, n (%)[a]	9 (4.1 %)	19 (7.2 %)	2 (11.8 %)	5 (6.3 %)	0.375
Total IFD, n (%)	78 (15.6 %)			12 (15 %)	0.891
Proven IFD	26 (5.2 %)			6 (7.5 %)	
Probable IFD	52 (10.4 %)			6 (7.5 %)	
Anti-fungal prophylaxis Echinocandins/ Fluconazole	84/412[b] (16.8 %/82.4 %)			43/35[c] (53.8 %/43.8 %)	<0.001
CMV viremia before IFD, n (%)	267 (53.4 %)			62 (77.5 %)	<0.001
(D)CMV IgG(−)/(R) CMV IgG (+)	44/500 (8.8 %)			38/80 (47.5 %)	<0.001
Grade III-IV acute GVHD, n (%)	97 (19.4 %)			7 (8.8 %)	0.021
Extensive chronic GVHD, n (%)	69 (13.8 %)			3 (3.8 %)	0.011
Pathogenic species of mold, n[a]					
Aspergillus spp.	34			7	
Mucorales	3			0	
Penicillium spp.	7			0	
Acremonium spp.	1			0	
Unidentified	7			0	
Pathogenic species of yeast, n[a]					
Candida spp	22			4	
Trichosporon spp.	3			1	
Cryptococcus spp.	3			0	
Rhodotorula spp.	1			0	
Unidentified	2			0	

IFD, invasive fungal disease; GVHD, graft-versus-host disease; CsA + scMTX, Cyclosporine and short course methotrexate; PTCy, post-transplant cyclophosphamide; (D) CMV, donor cytomegalovirus; (R) CMV, recipient cytomegalovirus; MSD, matched sibling donor; MUD, matched unrelated donor; Haplo, haploidentical.
[a] : Number of episodes of infection.
[b] : one patient with Amphotericin B and three patients with voriconazole.
[c] : two patients with voriconazole.

Candida spp. was the most commonly isolated pathogen at IFD. Characteristics and microbiology of IFD were described in Table 3.

4. Discussion

With the advancement of haploHSCT, several strategies have been developed over the last decade to overcome HLA barriers and mismatches.[7,26–28] PTCy has improved outcomes by reducing the high incidence of graft rejection and GVHD associated with earlier haploHSCT experiences.[26–28] Currently, haploHSCT with PTCy has become a viable alternative for patients lacking MSD or MUD. However, it is crucial to determine whether PTCy increases the risk of infections.

PTCy has been associated with an increased risk of bacterial infections, particularly pre-engraftment bacteremia. Multidrug-resistant gram-negative bacteria have been identified as major causes of infection-related deaths.[14,15,29] Studies have reported a bacterial infection rate exceeding 40 %, with bloodstream infections being the most frequent.[14,15,29] CMV remains the most frequent viral infection after HSCT. T-replete haploHSCT recipients have been reported as a higher risk of CMV reactivation compared to recipients of other alternative donor types.[14–17,30–32] Additionally, fungal infections appear to be more prevalent in patients receiving PTCy, although the exact role of GVHD prophylaxis and donor type requires further investigation.[14–16] Our study identified a similar infection rate and cumulative incidence of IFD within one year in both haploHSCT with PTCy and other HSCT with CsA plus scMTX prophylaxis. IFD significantly contributed to worse OS, DFS, and GRFS. IMIs were the major pathogenic species of IFD, primarily caused by *Aspergillus* spp., followed by *Candida* spp. The risk factors for IFD included age ≥ 45 years, disease relapse, and grade III-IV aGVHD.

Previous studies have reported the rate of IFD in PTCy haploHSCT ranging between 10 % and 18 %.[30,33] In a large cohort of haploHSCT with PTCy following RIC (80.2 %) and MAC (19.2 %) reported by Fayard et al., fungal infections were diagnosed in 78 out of 381 patients (20.5 %), with a median occurrence of 20 days post-haploHSCT.[14] Invasive aspergillosis was the most common infection (43.6 %), followed by invasive candidiasis (33.3 %) and pneumocystosis (12.8 %). Esquirol et al.[15] described IFD in 41 out of 236 haploHSCT patients (17 %) with PTCy following RIC (68 %) and MAC (32 %), including 10 % with possible, probable, or proven invasive aspergillosis. The incidence of IFD was 4 % before day +31 and 7 % after day +31, resulting in a 3-year incidence of 11 %.The exact role of PTCy in IFD requires further investigation, as the main limitations are identifying differences between compared groups in terms of patient characteristics, donor type, and GVHD prophylaxis. One study reported that within one year after transplant, the proportion of patients who experienced IFD was

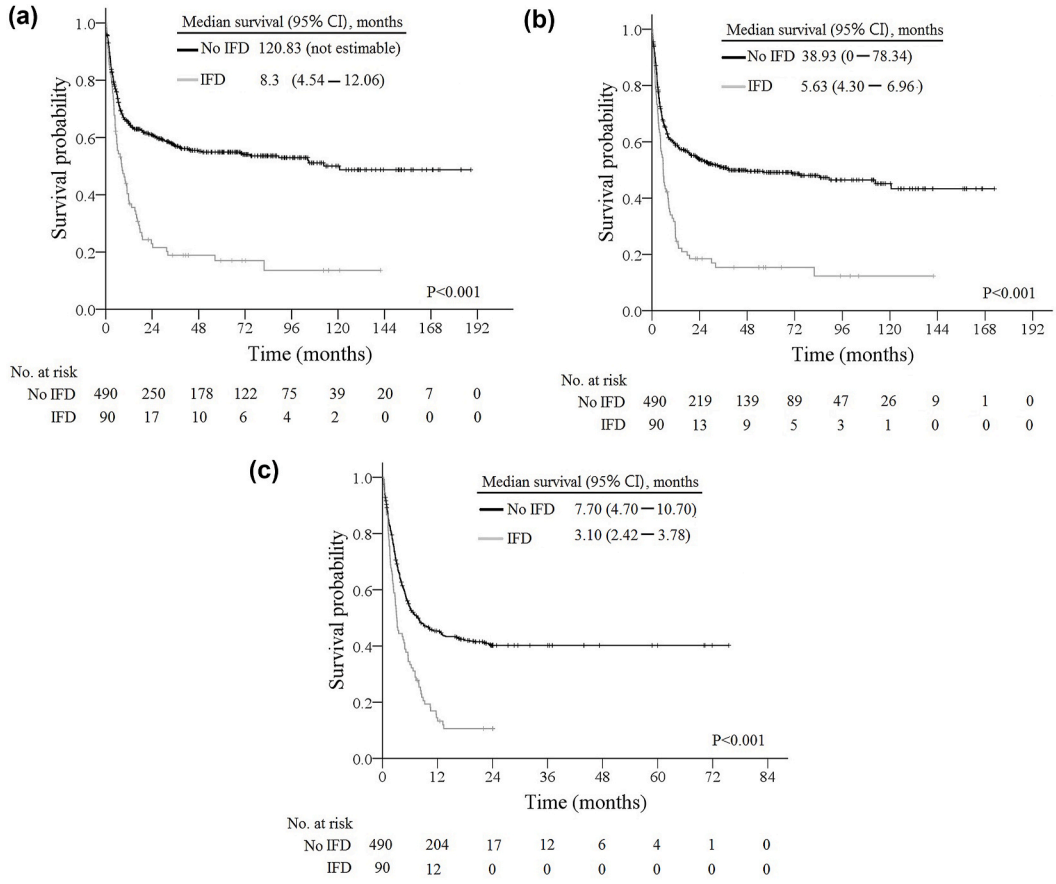

Fig. 1. The survival of 580 HSCT patients with IFD. (a) OS, (b) DFS, (c) GRFS.

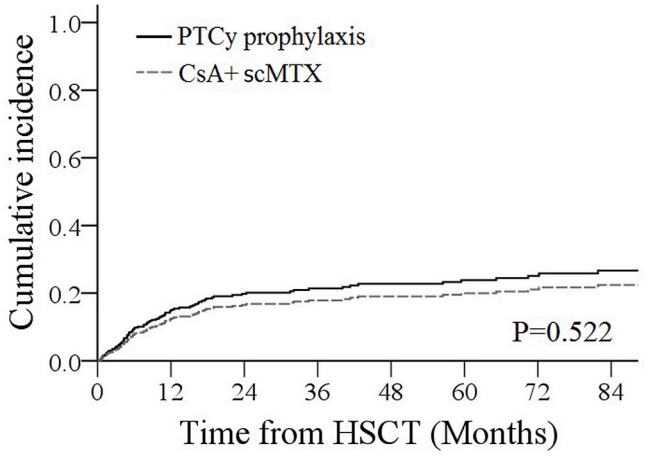

Fig. 2. The cumulative incidence of IFD between PTCy group and CsA plus scMTX group.

comparable between the two groups, occurring in 18.2 % of haploidentical patients versus 12.3 % of non-haploidentical patients (P = 0.44).[34] Another study found that PTCy in matched donors showed a similar incidence of IFD compared to the same donors with other GVHD prevention protocols.[17] In our cohort, we also found no significant difference in the IFD rate between the CsA plus scMTX group and the PTCy group. Invasive aspergillosis was the most common infection, followed by invasive candidiasis. The cumulative incidence of IFD within 1 year was similar between PTCy and CsA plus scMTX prophylaxis. For fungal prophylaxis, 53.8 % of patients in the PTCy group received echinocandins, while 82.4 % of patients in the CsA plus scMTX group received fluconazole prophylaxis. The change in fungal prophylaxis might have provided potential activity against *Candida* spp. and *Aspergillus* spp., leading to the similar IFD rates observed in different GVHD prophylaxis.

Fungal infections remain a significant cause of morbidity and mortality among patients undergoing alloHSCT.[18,35] The risk factors for developing IFD after HSCT include the type of HSCT, the presence of acute or chronic GVHD, administration of steroids, presence of CMV disease, and antifungal prophylaxis.[18,35,36] In our cohort, compared to patients without IFD, we documented significantly poorer median survival rates in patients with probable/proven IFD, with OS at 8.3 months, DFS at 5.63 months, and GRFS at 3.1 months, respectively (Fig. 1). Among the different GVHD prophylaxis (CsA plus scMTX or PTCy), patients with IFD still exhibited significantly poorer survival outcomes. Interestingly, in patients without IFD, haploHSCT with PTCy showed non-inferior OS and DFS and better GRFS compared to those with CsA plus scMTX. In our cohort, we identified that risk factors such as grade III-IV aGVHD, age $\geq$ 45 years, and disease relapse were significantly associated with the development of IFD. These findings are consistent with previously reported studies. Because the incidence of grade III-IV aGVHD and extensive cGVHD was significantly lower in the PTCy group, the reduced GVHD may provide potential protection against the development of IFD in haploHSCT with PTCy.

Overall, HSCT cohorts with haploidentical donors or PTCy prophylaxis showed significantly higher rates of CMV reactivation, ranging from 42 % to 69 %, with some studies reporting even higher rates when ATG was also used (74–85 %).[14–17,30–32,37] In the largest study, which included 661 transplants with PTCy and 275 with haploidentical donors, CMV reactivation rates were notably higher, as was the risk of fungal infections, other viral infections, and infection-related mortality.[17] IFD

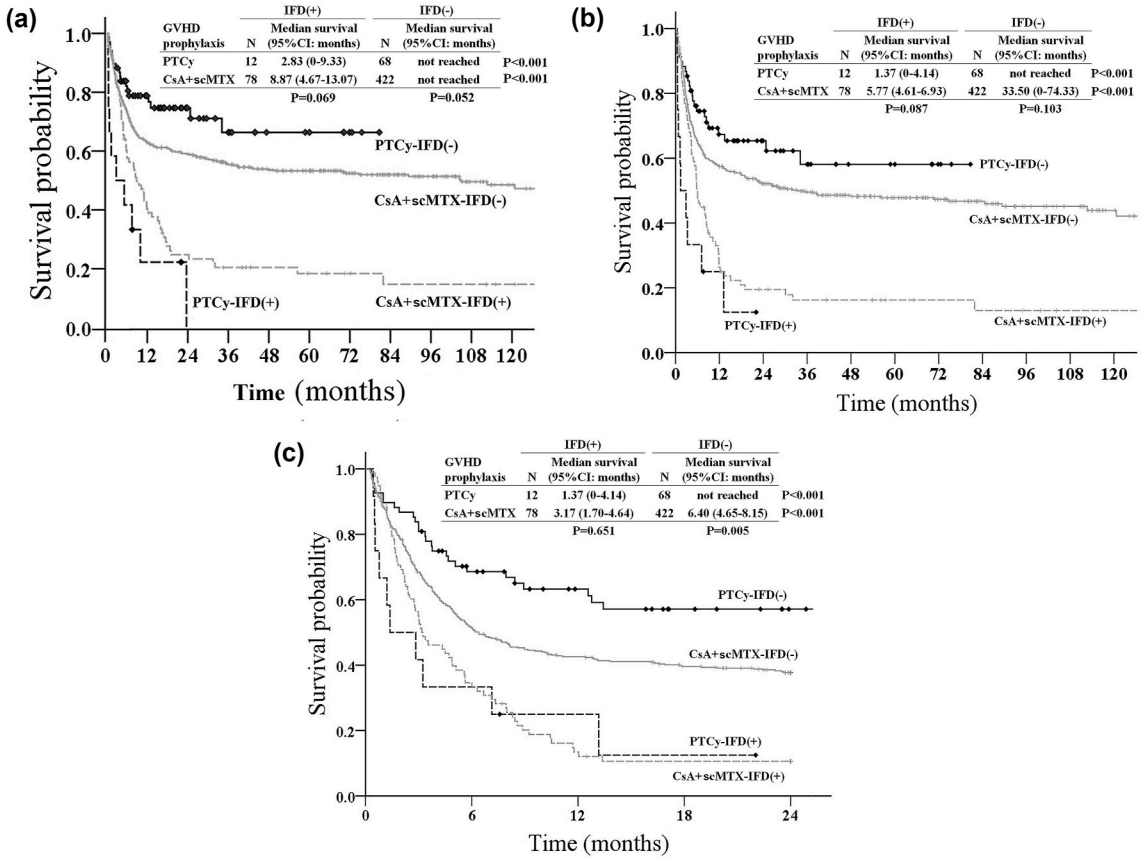

Fig. 3. The survival outcomes of HSCT patients with IFD between the PTCy group and the CsA plus scMTX group. (a) OS, (b) DFS, (c) GRFS.

may be caused by CMV reactivation or as a side effect of antiviral drugs. While several studies have demonstrated that CMV reactivation is a risk factor for IFD, other studies have reported conflicting results.[17,18,38,39] In areas with high CMV seroprevalence, CMV reactivation was not directly associated with the development of IFD.[39]

In our study, more than 95 % of recipients had positive CMV IgG, and 85 % of donors were also seropositive for IgG. A significant difference in CMV reactivation was observed between the CsA plus scMTX group and the PTCy group, with patients receiving PTCy showing a significantly higher rate of CMV reactivation compared to those receiving CsA plus scMTX (77.5 % vs. 53.4 %, $P < 0.001$). The higher CMV reactivation rate in the PTCy group was primarily due to the predominant high risk of donor/recipient CMV serology (D-/R + CMV IgG: 47.5 %, Table 3). The effects of using a CMV-negative donor to CMV-positive patient (D-/R + CMV IgG) include delayed CMV-specific immune reconstitution,[41,42] a higher CMV viral load compared to having a CMV-positive donor,[43] an increased probability of late CMV reactivation,[44] and a higher likelihood of CMV disease.[43] In haploHSCT, younger donors are typically selected, which contributes to the higher prevalence of CMV reactivation in the PTCy group. In our study, CMV reactivation did not contribute to the development of IFD. Additionally, there was no significant difference in the IFD rate between the CsA plus scMTX group and the PTCy group. Our data are consistent with previously reported findings.[18,39] Although PTCy haploHSCT carries a substantially higher risk for CMV infection compared to transplants with CsA plus scMTX, this did not seem to impact IFD rates in our study. Nevertheless, prophylactic anti-CMV antivirals in PTCy GVHD prophylaxis might be warranted due to the high incidence of CMV reactivation.[40]

The main limitation of our study is the differences between the compared groups in terms of donor type and GVHD prophylaxis. In our hospital, PTCy was primarily used for haploHSCT, while CsA plus scMTX was used for MSD, MUD, and other types of transplants. It is challenging to clearly describe IFD based on the specific impact of different donor types in PTCy-based transplants or different GVHD prophylaxis in transplants from the same donor type.

In conclusion, our study provides comprehensive results regarding patient characteristics, risk factors, survival, and details of IFD between the CsA plus scMTX and the PTCy group. Although CMV reactivation was not associated with the development of IFD, anti-CMV prophylaxis should be persistently used beyond day 100 after haploHSCT.

CRediT authorship contribution statement

Yao-Chung Liu: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Ting-An Lin:** Writing – review & editing, Data curation. **Nai-Wen Fan:** Writing – review & editing, Data curation. **Po-Shen Ko:** Writing – review & editing, Data curation. **Hao-Yuan Wang:** Writing – review & editing, Data curation. **Chun-Kuang Tsai:** Writing – review & editing, Data curation. **Sheng-Hsuan Chien:** Writing – review & editing, Data curation. **Chia-Jen Liu:** Writing – review & editing, Software, Data curation. **Liang-Tsai Hsiao:** Writing – review & editing, Data curation.

Declaration of competing interest

All authors have declared no conflict of interest.

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References

1. Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med*. 2006;354:1813–1826.
2. Ullmann AJ, Schmidt-Hieber M, Bertz H, et al. Infectious diseases in allogeneic haematopoietic stem cell transplantation: prevention and prophylaxis strategy guidelines. *Ann Hematol*. 2016;95:1435–1455.
3. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. *N Engl J Med*. 2014;371:339–348.
4. Penack O, Marchetti M, Aljurf M, et al. Prophylaxis and management of graft-versus-host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. *Lancet Haematol*. 2024;11:e147–e159.
5. Arai Y, Jo T, Matsui H, Kondo T, Takaori-Kondo A. Efficacy of antithymocyte globulin for allogeneic hematopoietic cell transplantation: a systematic review and meta-analysis. *Leuk Lymphoma*. 2017;58:1840–1848.
6. Kanakry CG, Fuchs EJ, Luznik L. Modern approaches to HLA-haploidentical blood or marrow transplantation. *Nat Rev Clin Oncol*. 2016;13:10–24.
7. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2002;8:377–386.
8. Chang YJ, Huang XJ. Haploidentical stem cell transplantation: antithymocyte globulin-based experience. *Semin Hematol*. 2016;53:82–89.
9. Korbling M, Anderlini P. Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter? *Blood*. 2001;98:2900–2908.
10. Baumeister SHC, Rambaldi B, Shapiro RM, Romee R. Key aspects of the Immunobiology of Haploidentical hematopoietic cell transplantation. *Front Immunol*. 2020;11:191.
11. Atilla E, Atilla PA, Bozdag SC, Demirer T. A review of infectious complications after haploidentical hematopoietic stem cell transplantations. *Infection*. 2017;45:403–411.
12. Chang YJ, Zhao XY, Huo MR, et al. Immune reconstitution following unmanipulated HLA-mismatched/haploidentical transplantation compared with HLA-identical sibling transplantation. *J Clin Immunol*. 2012;32:268–280.
13. Raiola AM, Dominietto A, di Grazia C, et al. Unmanipulated haploidentical transplants compared with other alternative donors and matched sibling grafts. *Biol Blood Marrow Transplant*. 2014;20:1573–1579.
14. Fayard A, Daguenet E, Blaise D, et al. Evaluation of infectious complications after haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide following reduced intensity and myeloablative conditioning: a study on behalf of the francophone Society of Stem Cell Transplantation and Cellular Therapy (SFGM-TC). *Bone Marrow Transplant*. 2019;54:1586–1594.
15. Esquirol A, Pascual MJ, Kwon M, et al. Severe infections and infection-related mortality in a large series of haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide. *Bone Marrow Transplant*. 2021;56:2432–2444.
16. Oltolini C, Greco R, Galli L, et al. Infections after allogenic transplant with post-transplant cyclophosphamide: impact of donor HLA matching. *Biol Blood Marrow Transplant*. 2020;26:1179–1188.
17. Mehta RS, Saliba RM, Ghanem S, et al. Haploidentical versus matched unrelated versus matched sibling donor hematopoietic cell transplantation with post-transplantation cyclophosphamide. *Transplant Cell Ther*. 2022;28:395.e1–395.e11.
18. Liu YC, Chien SH, Fan NW, et al. Incidence and risk factors of probable and proven invasive fungal infection in adult patients receiving allogeneic hematopoietic stem cell transplantation. *J Microbiol Immunol Infect*. 2016;49:567–574.
19. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation*. 1974;18:295–304.
20. Thomas ED, Storb R, Clift RA, et al. *Bone marrow transplant. N Engl J Med*. 1975;292:895–902.
21. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945–956.
22. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204–217.
23. Bassetti M, Azoulay E, Kullberg BJ, et al. EORTC/MSGERC definitions of invasive fungal diseases: summary of activities of the intensive care unit working group. *Clin Infect Dis*. 2021;72:S121–S127.
24. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European organization for research and treatment of cancer and the mycoses study group education and research consortium. *Clin Infect Dis*. 2020;71:1367–1376.
25. Holtan SG, DeFor TE, Lazaryan A, et al. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood*. 2015;125:1333–1338.
26. Solomon SR, Sizemore CA, Sanacore M, et al. Haploidentical transplantation using T cell replete peripheral blood stem cells and myeloablative conditioning in patients with high-risk hematologic malignancies who lack conventional donors is well tolerated and produces excellent relapse-free survival: results of a prospective phase II trial. *Biol Blood Marrow Transplant*. 2012;18:1859–1866.
27. Huang XJ, Liu DH, Liu KY, et al. Haploidentical hematopoietic stem cell transplantation without in vitro T-cell depletion for the treatment of hematological malignancies. *Bone Marrow Transplant*. 2006;38:291–297.
28. Luznik L, O'Donnell P, Symons H, et al. HLA haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14:641–650.
29. Girmenia C, Bertaina C, Piciocchi C, et al. Incidence, risk factors and outcome of pre-engraftment gram-negative bacteremia after allogeneic and autologous hematopoietic stem cell transplantation: an Italian prospective multicenter survey. *Clin Infect Dis*. 2017;65:1884–1896.
30. Slade M, Goldsmith S, Romee R, et al. Epidemiology of infections following haploidentical peripheral blood hematopoietic cell transplantation. *Transpl Infect Dis*. 2017;19, e12629.
31. Goldsmith SR, Abid MB, Auletta JJ, et al. Posttransplant cyclophosphamide is associated with increased cytomegalovirus infection: a CIBMTR analysis. *Blood*. 2021;137:3291–3305.
32. Mehta RS, Saliba RM, Rondon G, et al. Posttransplantation cyclophosphamide versus tacrolimus and methotrexate graft- versus-host disease prophylaxis for HLA-matched donor transplantation. *Transplant Cell Ther*. 2022;28:695.e1–695.e10.
33. El-Cheikh J, Crocchiolo R, Vai A, et al. Comparison of three distinct prophylactic agents against invasive fungal infections in patients undergoing Haplo-identical hematopoietic stem cell transplantation and posttransplant cyclophosphamide. *Mediterr J Hematol Infect Dis*. 2015;7, e2015048.
34. Chang J, Hsiao M, Blodget E, Akhtari M. Increased risk of 100-day and 1-year infection-related mortality and complications in haploidentical stem cell transplantation. *J Blood Med*. 2019;10:135–143.
35. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-6: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis*. 2010;50:1091–1100.
36. Pagano L, Caira M, Nosari A, et al. Fungal infections in recipients of hematopoietic stem cell transplants: results of the SEIFEM B-2004 study—sorveglianza Epidemiologica Infezioni Fungine Nelle Emopatie Maligne. *Clin Infect Dis*. 2007;45:1161–1170.
37. Luo XH, Zhu Y, Chen YT, Shui LP, Liu L. CMV infection and CMV-specific immune reconstitution following haploidentical stem cell transplantation: an update. *Front Immunol*. 2021;12, 732826.
38. Yong MK, Ananda-Rajah M, Cameron PU, et al. Cytomegalovirus reactivation is associated with increased risk of late-onset invasive fungal disease after allogeneic hematopoietic stem cell transplantation: a multicenter study in the current era of viral load monitoring. *Biol Blood Marrow Transplant*. 2017;23:1961–1967.
39. Yeh TJ, Yang CI, Huang CT, et al. Revisit of the association between cytomegalovirus infection and invasive fungal infection after allogeneic hematopoietic stem cell transplantation: a real-world analysis from a high CMV seroprevalence area. *J Fungi (Basel)*. 2022;8:408.
40. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med*. 2017;377:2433–2444.
41. Lilleri D, Fornara C, Revello MG, Gerna G. Human cytomegalovirus-specific memory CD8+ and CD4+ T cell differentiation after primary infection. *J Infect Dis*. 2008;198:536–543.
42. Avetisyan G, Aschan J, Hägglund H, Ringdén O, Ljungman P. Evaluation of intervention strategy based on CMV-specific immune responses after allogeneic SCT. *Bone Marrow Transplant*. 2007;40:865–869.
43. Ljungman P, Perez-Bercoff L, Jonsson J, et al. Risk factors for the development of cytomegalovirus disease after allogeneic stem. *Cell Transplant*. 2006;91:78–83.
44. Ozdemir E, Saliba RM, Champlin RE, et al. Risk factors associated with late cytomegalovirus reactivation after allogeneic stem cell transplantation for hematological malignancies. *Bone Marrow Transplant*. 2007;40:125–136.