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Infections after Allogenic Transplant with Post-Transplant Cyclophosphamide: Impact of Donor HLA Matching

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A B S T R A C T

Incidence and outcome of infections after allogeneic hematopoietic stem cell transplantation (HSCT) with post-transplant cyclophosphamide (PT-Cy) as graft-versus-host disease (GVHD) prophylaxis are largely unknown. Study aims were to estimate the incidence of pre-engraftment bloodstream infections (PE-BSIs) and viral infections (VIs; cytomegalovirus [CMV], adenovirus [ADV], human herpes virus 6 [HHV6], and BK-polyomavirus hemorrhagic-cystitis [BKPyV-HC]), their predictive factors, and infection-related mortality (IRM) after HSCT with PT-Cy. We analyzed 235 patients: 62%, 21%, and 17% received haploidentical (haplo), matched-unrelated donor (MUD), and matched-related donor, respectively. Overall, 72 patients had 77 PE-BSI episodes at a median time of 13 days after HSCT: cumulative incidence function (CIF) at 28 days was 32%, without differences among donor types (P = .988). By multivariate analysis, CIF of PE-BSI was higher in patients with severe neutropenia before HSCT (adjusted hazard ratio [AHR] = 2.90) and in multidrug-resistant Gram-negative bacteria rectal carriers (AHR = 2.68). IRM at 30 days was 5%, without differences by donor type (P = .106). Overall, 208 patients experienced ≥1 VIs (first occurrence among CMV, HHV6, ADV, BKPyV-HC) at a median time of 20 days after HSCT: CIF at 90 days was 91%, significantly higher in MUD and haplo (P = .0089). By multivariate analysis, also acute GVHD grade ≥2 (AHR = 1.32) and host/donor CMV-serology mismatch (positive/positive versus negative/negative: AHR = 2.95, positive/negative versus negative/negative: AHR = 2.41, negative/positive versus negative/negative: AHR = 2.35) affected VIs occurrence. IRM at 180 days was 8%, without differences among donor types (P = .106). In conclusion, study results did not show a significant impact of donor type on PE-BSI incidence; conversely, MUD and haploidentical transplants retained a higher occurrence of VIs in the early phase after HSCT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapy for many malignant and nonmalignant hematologic disorders [1,2]. Although matched-related donor (MRD) and matched-unrelated donor (MUD) are considered the ideal sources of hematopoietic stem cells, many patients lack timely access to a suitable matched donor, especially in the context of aggressive diseases.

Unmanipulated haploidentical (haplo) HSCT with post-transplant cyclophosphamide (PT-Cy) represents a valid option for patients who do not have a HLA-matched donor [3,4]. Haplo-HSCT with PT-Cy originally relied on nonmyeloablative conditioning and bone marrow as graft source [5]. Although associated with low rates of graft-versus-host disease (GVHD), such transplantation strategy was limited by relatively high relapse rates and infectious complications. Different groups have reported encouraging outcomes using peripheral blood stem cell (PBSC) grafts and myeloablative conditioning regimens [6-9]. Recently, the PT-Cy platform has also been extended to MRD and MUD [10,11].

Bloodstream infections (BSIs) are frequent and life-threatening complications in HSCT recipients, particularly during neutropenia [12-14]. BSIs affect from 16% to 40% patients [12-16], with an associated mortality ranging from <5% in case of Gram-positive bacteria (GPB) to 40% in case of multidrug-resistant (MDR) *Pseudomonas aeruginosa* (*Pa*) and 64% in carbapenem-resistant (CR) *Klebsiella pneumoniae* (*Kp*) infections among allogeneic HSCT recipients [12,14,17,18]. The knowledge of current epidemiology and incidence according to transplantation protocols is

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fundamental for deciding the most appropriate empirical therapy at every site [19].

The incidence and outcome of infections associated with haplo-HSCT with PT-Cy remain to be determined. Series published so far, the largest on 104 patients, reported an infection-related mortality (IRM) between 9% and 20% [9,20-23]. Despite a high rate of viral infections (VIs) in the early period [9], anti-infection immunity was well preserved with PT-Cy approach [21], resulting in low rates of invasive fungal diseases (IFDs) and bacterial infections, as reported in a retrospective analysis by Ciurea et al. [24] in comparison to a historical cohort who received $CD34^+$ selected haplo-HSCT and anti-thymocyte globulin.

The aim of this study was to collect a comprehensive report of incidence, risk factors, and mortality of pre-engraftment BSIs (PE-BSIs) and double-strand DNA (ds-DNA) VIs after HSCT with the PT-Cy platform among different donor sources.

METHODS

Patients and Data

We include in our analysis a cohort of consecutive adult patients who underwent PBSC allogeneic HSCT with PT-Cy as GVHD prophylaxis from January 2013 to December 2017 at our institution. Patients were treated according to institutional programs upon written informed consent for transplant procedures and for use of medical records within the noninterventional "ALMON study," approved by San Raffaele Institutional Ethical Committee on October 19, 2007.

The primary objective was to estimate and compare the cumulative incidence of PE-BSIs and ds-DNA VIs among donor sources (MRD, MUD, haplo).

The secondary objectives included the evaluation of risk factors for PE-BSIs and ds-DNA VIs and the assessment of IRM.

Baseline was the date of HSCT. Follow-up was censored at the date of the occurrence of the event of interest or competing event or last available visit, whichever occurred first.

We collected patients' age, sex, diagnosis, diseases status at HSCT, hematopoietic cell transplant comorbidity index, cytomegalovirus (CMV) recipient/donor (R/D) serology, MDR–Gram-negative bacteria (GNB) colonization, presence of neutropenia before HSCT, conditioning type, donor type, neutrophil engraftment, presence of GVHD, disease relapse, overall survival, and cause of death.

Data on BSI episodes occurring from the beginning of conditioning to neutrophil engraftment and on antibiotic susceptibilities were obtained from the electronic records of the microbiology service and cross-checked with patients' charts.

Details on BK-polyomavirus hemorrhagic cystitis (BKPyV-HC), CMV, human herpes virus 6 (HHV6), and adenovirus (ADV) infections were collected from transplant to last available visit. Also, data on IFD were recorded.

Definitions

For common skin contaminants, BSIs were diagnosed if ≥2 consecutive blood cultures were positive for the same species. PE-BSIs were defined as the isolation of a pathogen from ≥1 blood culture of a neutropenic patient since the beginning of conditioning to engraftment. BSIs were considered polymicrobial if ≥2 pathogens were isolated from a single blood culture.

Neutropenia was defined as an absolute neutrophil count (ANC) <500 cells/mm^3 and engraftment as the first of 3 consecutive days with ANC >500 cells/mm^3.

Underlying diseases were classified as follows: myeloid disorders (acute myeloid leukemia, myelodysplastic syndrome), chronic myeloproliferative disorders (chronic myeloid leukemia, idiopathic myelofibrosis, myeloproliferative disorders), lymphoid disorders (acute lymphoblastic leukemia, non-Hodgkin lymphoma, chronic lymphocytic leukemia, Hodgkin lymphoma, and multiple myeloma), and other disorders.

Donors were divided into MRD, MUD, and haplo.

Acute and chronic GVHD were defined and scored according to Harris et al. [25] criteria and National Institutes of Health consensus criteria [26], respectively.

Patients were defined MDR-GNB carriers if they had a positive rectal swab within 30 days before transplant.

Neutropenia before HSCT was defined for patients with ANC <500 cells/mm^3 the day of transplant for at least 7 days before, taking into account if the conditioning regimen had started in aplasia.

CMV-seropositive recipients were defined as having a high risk of disease according to the European Society for Blood and Marrow Transplantation (EBMT) criteria [27-30]. CMV end-organ disease was classified according to guidelines [31], and viremia leading to pre-emptive therapy (PET) was defined as clinically significant infection (CS-CMVi). HHV6 infections were categorized as end-organ disease according to guidelines [32], including reactivation with cutaneous rash and/or delayed engraftment, or asymptomatic reactivation leading to antiviral treatment. ADV infections and BKPyV-HC were defined according to international consensus document and European Conference on Infections in Leukaemia (ECIL) guidelines [33-35]. IFD was classified according to European Organisation for Research and Treatment of Cancer (EORTC) criteria [36]. Transplant Related Mortality (TRM) was defined as the time from transplant to death by a transplant-related cause without relapse/recurrence. IRM was defined as the time from transplant to death by an infectious cause, without relapse/recurrence or GVHD.

BSI-attributable mortality was defined, in the subgroup of patients with PE-BSIs, as the mortality at day 7 (day 0 as the day of positive blood culture). For the analysis of survival after each BSI episode to define the impact of etiology on early mortality, we analyzed patients with at least 1 GNB PE-BSI episode (single-species GNB BSI, polymicrobial BSI with at least 1 GNB) and GPB PE-BSI episodes (single-species GPB BSI, polymicrobial BSI sustained only by GPB).

Transplantation-Related Procedures

Transplantations were performed according to institutional guidelines [10,21]. Conditioning regimens were treosulfan based, and PT-Cy was used for in vivo T cell depletion. All patients received a conditioning regimen based on treosulfan (14 g/m^2/d) on days −6 to −4 and fludarabine (30 mg/m^2/d) on days −6 to −2; 81% received an intensified conditioning with addition of melphalan (70 mg/m^2/d) on days −3 and −2 or thiotepa 5 mg/kg/d on days −3 and −2.

Graft source was PBSCs, mobilized with subcutaneous granulocyte colony-stimulating factor and collected by leukapheresis and infused without any ex vivo manipulation.

Postgrafting immunosuppression consisted of PT-Cy (50 mg/kg/d) on day 3 and day 4.

GVHD prophylaxis protocols were calcineurin-inhibitor free, based on sirolimus, withdrawn between months 3 and 6 after HSCT in the absence of GVHD or relapse; mofetil mycophenolate was added for 30 days if the donor was not an MRD.

Infection Prophylaxis, Monitoring, and Treatment

Anti-infectious prophylaxis was administered according to institutional protocols, based on international recommendations [37-42]: from the onset of conditioning, patients received levofloxacin 500 mg/d until engraftment and acyclovir 800 mg bid until 365 days.

Surveillance cultures through a rectal swab were performed at admission and weekly thereafter. In MDR-GNB carriers, the empiric therapy of febrile neutropenia was designed to target such a strain; a de-escalation strategy was gradually introduced starting in 2013.

CMV and HHV6 infections were monitored weekly until 100 days, while ADV detection was performed at clinical suspicion. PET with ganciclovir (GCV) or foscarnet (FOS) was started when plasmatic CMVDNA was ≥1000 copies/mL or increased >0.5 log. For HHV6 and ADV infections, a specific treatment was started according to physicians' clinical judgment. Further details are provided in the Supplemental Methods.

Statistical Analysis

Patients' characteristics are described as median (interquartile range, IQR) for continuous variables or proportions for categorical variables. Distributions of continuous variables were compared using the Kruskal-Wallis test; differences between proportions were tested by the chi-square or Fisher exact test.

The cumulative incidence function (CIF) of any PE-BSIs or GNB PE-BSIs or GPB PE-BSIs was calculated according to donor type with Gray's method [43]; 95% confidence intervals (CIs) for survival probabilities and cumulative incidence were calculated accounting for competing risks of pre-engraftment death, engraftment, and retransplantation.

The CIF of ds-DNA VIs was calculated according to the donor type with death and retransplantation as competing events. VIs of interest were BKPyV-HC and any positive viremia for CMV, HHV6, and ADV; they were individually considered or grouped according to the following classification: ≥1 VIs (the first occurrence among CMV, HHV6, ADV, and BKPyV-HC was retained in the analysis) or none.

CIFs were also estimated for TRM and IRM. Disease relapse/progression was competing risk for TRM; relapse/progression, GVHD grade ≥2, and death from any other cause were competing risks for IRM.

Univariate and multivariate Fine-Gray subdistribution hazard models were applied to estimate the relative change in the rate of the occurrence of PE-BSIs or GNB PE-BSIs or ≥1 VIs associated with age, sex, type of donor, a priori baseline factors known to have a potential effect on each outcome, and other baseline covariates with $P < .2$ at univariate analysis. Hazard ratios (HRs) with the corresponding 95% CIs were reported.

All statistical tests were 2-sided at the 5% level and were performed using SAS statistical software version 9.4 (Statistical Analyses System, Cary, NC).

RESULTS

Patients' Characteristics and Differences Based on Donor Type

Overall, 235 patients with a median age of 50 years (IQR = 37 to 62) were analyzed. Median follow-up after HSCT was 276 days (IQR = 137 to 580). Graft source was PBSCs; median $CD34^+$ and $CD3^+$ cell doses were 6.00×10^6/kg and 2.19×10^8/kg, respectively. Donors were MRD for 40 (17%), MUD for 50 (21%), and haplo for 145 (62%) patients. Conditioning was myeloablative conditioning in 184 (78%) and reduced-intensity conditioning in 51 (22%) patients.

Patients' characteristics are reported in Table 1.

All patients were affected by high-risk hematologic malignancies, mainly acute myeloid leukemia (n = 129); 11% and 2% received a second or a third HSCT, respectively. At HSCT, 55% of patients were not in complete remission; according to Disease Risk Index [44], patients at first HSCT were stratified as very high (n = 17), high (n = 88), intermediate (n = 91), and low (n = 7) risk (1 patient affected by a benign disorder was not classifiable). The majority of the cohort (96%) achieved neutrophil engraftment at a median time of 20 days (IQR = 17 to 24); acute GVHD (aGVHD) grade ≥2 and relapse occurred in 32% and 27%, respectively, without differences according to donor type. Before HSCT, 28% of recipients had severe neutropenia and 8% were MDR-GNB carriers (details about pathogens are reported in Table 2).

Incidence, Etiology, and Risk Factors of Pre-engraftment BSI

Overall, 72 patients had 77 PE-BSI episodes during aplasia (median time to the first PE-BSI was of 13 days [IQR = 7 to 17] among patients who developed ≥1 BSIs): the estimated CIF at 28 days was 32% (95% CI, 26% to 38%); no differences were observed in CIF among different donor types (30% versus 34% versus 32% in MRD, MUD, and haplo, respectively; Gray's test: $P = .988$). The time to engraftment among patients with and without PE-BSIs was 20 days (17 to 25) and 20 days (17 to 24), respectively ($P = .393$).

CIFs of GNB PE-BSIs and GPB PE-BSIs by donor type are shown in Figure 1.

PE-BSI episodes were sustained by single-species GNB and single-species GPB in 35% (27/77) and 51% (39/77) of cases, respectively, whereas 13% (10/77) were polymicrobial and 1% (1/77) were sustained by nontuberculous mycobacteria. Among 87 isolated pathogens, the most represented GNBs were *Escherichia coli* and *K pneumoniae*, while the most common GPBs were coagulase-negative staphylococci and *Enterococcus*. Details about etiology and antimicrobial resistance are reported in Table 2.

Among MDR-GNB carriers, 5 of 9 patients colonized by CR-*Kp* developed PE-BSIs sustained by the same pathogen and 3 of 5 patients experienced 2 episodes of CR-*Kp* PE-BSIs, while no episodes of CR-*Kp* PE-BSIs occurred in noncarriers; 1 of 2 patients colonized by *Stenotrophomonas maltophilia* and CR-*Enterobacter*, respectively, developed PE-BSIs sustained by the same pathogen.

By multivariate analysis, after adjustment for age, sex, year of HSCT, donor type, and disease status at HSCT, patients with neutropenia before HSCT (adjusted HR [AHR] = 2.90; 95% CI, 1.54 to 5.44) and MDR-GNB carriers [AHR = 2.68, 95%CI = 1.25-5.75] had an effect on the rate of any PE-BSIs. These covariates were confirmed as independent factors also affecting the rate of GNB PE-BSIs (Table 3).

Graft source did not influence PE-BSI occurrence (data not shown).

Incidence and Risk Factors of ds-DNA Viral Infections

Overall, 208 patients experienced ≥1 VIs: the estimated CIF at 90 days was 91% (95% CI, 86% to 94%), and the median time to the first VI was 20 days (IQR = 16 to 26). The cumulative incidence was significantly higher in MUD and haplo than in MRD transplants (98% versus 93% versus 74% in MUD, haplo, and MRD, respectively; Gray's test: $P = .0089$) (Figure 2).

CMV infection occurred in 144 patients (61%), almost exclusively in CMV-seropositive recipients (142/144); the time to CMV infection was 34 days (IQR = 19 to 54), and the estimated CIF according to donor type is shown in Figure 2. However, 14% (20/144) of patients developed end-organ disease: 4 patients with probable pneumonia received GCV, 2 patients with encephalitis (1 probable, 1 possible) received GCV plus FOS, 9 of 12 patients affected by possible gastrointestinal disease received GCV or FOS, and the remaining 3 of 12 did not require specific therapy; finally, 2 patients received GCV for proven colitis and possible hepatitis. Forty-two percent (61/144) and 44% (63/144), respectively, experienced CS-CMVi and low-level viremia (<1000 copies/mL) not requiring PET. Among 144 patients with CMV infection, 31% experienced a second one.

HHV6 infection occurred in 179 patients (76%); time to HHV6 infection was 24 days (IQR = 19 to 26). HHV6 end-organ disease occurred in 14% (25/179): 4 patients with proven encephalitis received GCV plus FOS and 2 with possible encephalitis received GCV, 1 patient was treated with GCV for possible pneumonia, 15 of 18 patients affected by possible gastrointestinal disease received GCV or FOS, and 3 of 18 did not require treatment. Overall, 8% (14/179) and 21% (38/179) of patients received GCV or FOS for reactivation with cutaneous rash and/or delayed engraftment and asymptomatic reactivation, respectively; the remaining 57% with asymptomatic viremia (102/179) did not require treatment.

Further details on CMV and HHV6 infections according to donor type are reported in Supplementary Table S1.

BKPyV-HC occurred in 36 patients (15%) at a median time of 32 days (IQR = 14 to 45); half of them (18/36) received intravenous and/or intrabladder cidofovir.

ADV infection developed in 14 patients (6%) at a median time of 50 days (IQR = 18 to 86): 6 of 14 with systemic reactivation (1/6 treated with cidofovir) and 8 of 14 with possible end-organ disease (6/8 treated with cidofovir).

By multivariate analysis (Table 4), after adjustment for age, sex, year of HSCT, and aGVHD grade ≥2 (AHR = 1.32; 95% CI, 1.01 to 1.74), having received haplo (AHR = 2.00; 95% CI, 1.37 to 3.12) or MUD (AHR = 2.04; 95% CI, 1.29 to 3.21) transplant and unfavorable host/donor (H/D) CMV serology (positive/positive versus negative/negative: AHR = 2.95, 95% CI, 1.55 to 5.63; positive/negative versus negative/negative: AHR = 2.41, 95% CI, 1.23 to 4.73; neg/positive versus negative/negative: AHR = 2.35, 95% CI, 1.07 to 5.19) were factors having an effect on the rate of ≥1 VI occurrences.

The occurrence of VI was not influenced by graft source (data not shown).

The incidence of IFD was also analyzed and reported in Supplementary Table S2.

Infection-Related Mortality after HSCT

Overall, IRM at 30 days and 180 days was 4% (95% CI, 2% to 7%) and 8% (95% CI, 5% to 12%), respectively, with no differences by donor type (Gray's test: $P = .149$), even if in the

Table 1
Baseline and Follow-up Characteristics of Patients Who Underwent Hematopoietic Cell Transplantation with PT-Cy Platform

Patients' Characteristics	Overall Population (n = 235)	MRD (n = 40)	MUD (n = 50)	Haplo (n = 145)	*P* Value
Baseline					
Age at HSCT, yr, median (IQR)	49.6 (37.0-62.0)	48.1 (40.9-59.4)	50.6 (37.4-57.0)	51.6 (36.4-63.1)	.863
Male sex	147 (63)	25 (63)	33 (66)	89 (62)	.844
Year of HSCT, median (range)	2016 (2013-2017)	2016 (2014-2017)	2016 (2015-2017)	2015 (2013-2017)	**<.0001**
2013-2015	109 (46)	15 (38)	9 (18)	85 (59)	<.0001
2016-2017	126 (54)	25 (62)	41 (82)	60 (41)	
ANC ≤500 for ≥7 days before HSCT	66 (28)	10 (25)	5 (10)	51 (35)	**.003**
Diagnosis,*					.411
Myeloid disorders	169 (72)	29 (72)	34 (68)	106 (73)	
Lymphoid disorders	65 (27)	11 (28)	15 (30)	39 (27)	
Other disorders	1 (1)	0 (0)	1 (2)	0 (0)	
Time from diagnosis to HSCT					.064
>12 months	93 (40)	10 (25)	18 (36)	65 (45)	
≤12 months	142 (60)	30 (75)	32 (64)	80 (55)	
Disease phase at HSCT					**.001**
>CR1	40 (17)	1 (2)	9 (18)	30 (21)	
CR1	63 (27)	16 (40)	20 (40)	27 (19)	
Active disease	131 (55)	23 (58)	20 (40)	88 (61)	
Not applicable	1 (1)	0	1 (2)	0	
HCT-CI					.244
0-2	110 (47)	20 (50)	28 (56)	62 (43)	
≥3	125 (53)	20 (50)	22 (44)	83 (57)	
Conditioning regimen					.286
Myeloablative conditioning	184 (78)	35 (88)	39 (78)	110 (76)	
Reduced-intensity conditioning	51 (22)	5 (12)	11 (22)	35 (24)	
MDR-GNB rectal carrier within 30 days before HSCT	18 (8)	3 (8)	1 (2)	14 (10)	.214
Number of HSCTs					**.001**
First allogenic HSCT	204 (87)	39 (98)	50 (100)	115 (79)	
Second allogenic HSCT	27 (11)	1 (2)	0	26 (18)	
Third allogenic HSCT	4 (2)	0	0	4 (3)	
GVHD prophylaxis†					.387
PT-Cy/sirolimus/(MMF)	231	40 (100)	50 (100)	141 (98)	
PT-Cy/cyclosporine A/MMF	3	0	0	3 (2)	
R/D CMV					.131
Negative/negative	17 (7)	2 (5)	5 (10)	10 (7)	
Positive/negative	59 (25)	5 (12)	16 (32)	38 (26)	
Positive/positive	146 (62)	31 (77)	24 (48)	91 (63)	
Negative/positive	13 (6)	2 (5)	5 (10)	6 (4)	
CMV risk among R-positive patients					<.0001
High	170 (72)	6 (15)	19 (38)	145 (100)	
Low	65 (28)	34 (85)	31 (62)	0 (0)	
Antifungal prophylaxis					<.0001
Primary antimolds	182 (77)	23 (57)	48 (96)	111 (76)	
Primary antiyeast	17 (7)	12 (30)	1 (2)	4 (3)	
Secondary antimolds	35 (15)	4 (10)	1 (2)	30 (21)	
Secondary antiyeast	1 (1)	1 (3)	0 (0)	0 (0)	
Follow-up					
Follow-up, days, median (IQR)	276 (137-580)	289 (197-577)	316 (174-531)	259 (114-618)	.579
ANC engraftment	225 (96)	39 (98)	50 (100)	136 (94)	.144
Time to engraftment, days, median (IQR)	20 (17-24)	20 (16-24)	22 (19-29)	19 (17-24)	**.046**
aGVHD grade ≥2	76/235 (32)	6/40 (15)	12/50 (24)	58/145 (40)	.250
Time to GVHD among patients who developed aGVHD, days, median (IQR)	24 (15-41)	38 (28-47)	25 (20-44)	21 (14-35)	.061
Relapse	64 (27)	13 (33)	7 (14)	44 (30)	.058
Time to relapse among patients who had relapse, days, median (IQR)	120 (63-202)	97 (63-129)	102 (73-282)	136 (63-214)	.540

Values are presented as number (%) unless otherwise indicated.
Significant values are in bold.
HCT-CI indicates hematopoietic cell transplantation-comorbidity index; MMF, mofetil mycophenolate.

* Myeloid disorders: acute myeloid leukemia, myelodysplastic syndrome, chronic myeloproliferative diseases (chronic myelogenous leukemia, idiopathic myelofibrosis, myeloproliferative neoplasm); lymphoid disorders: acute lymphoblastic leukemia, Hodgkin lymphoma, aggressive non-Hodgkin lymphoma, indolent non-Hodgkin lymphoma, multiple myeloma, chronic lymphocytic leukemia; other disorders: chronic granulomatous disease.

† One patient died because of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* BSI before receiving GVHD prophylaxis.

Table 2
Etiology of PE-BSIs and of Gastrointestinal Colonization Revealed by Active Surveillance through Rectal Swabs

Characteristic	Total Isolates from Blood, n = 87
Gram-positive bacteria	**52**
Staphylococcus spp.	34
Staphylococcus aureus	1
• Resistance to methicillin	0 of 1 (0%)
• Resistance to levofloxacin	0 of 1 (0%)
Coagulase negative	33
• Resistance to methicillin	33 of 33 (100%)
• Resistance to levofloxacin	33 of 33 (100%)
Enterococcus spp.	12
Enterococcus faecalis	3
Enterococcus faecium	9
• Enterococci resistant to vancomycin	3 of 12 (25%)
Viridans streptococci	4
Other Gram-positive bacteria*	2
Gram-negative bacteria	**34**
• Gram negative bacteria resistant to piperacillin/tazobactam	24 of 34 (71%)
• Gram negative bacteria resistant to carbapenems	10 of 34 (29%)
• Gram negative bacteria resistant to fluoroquinolones	28 of 34 (82%)
Escherichia coli	18
• *Escherichia coli* ESBL producing	10 of 18 (56%)
• *Escherichia coli* carbapenemase producing	0 of 18 (0%)
Other Enterobacteriaceae	11
Klebsiella pneumoniae	9
• *Klebsiella pneumoniae* ESBL producing	0 of 9 (0%)
• *Klebsiella pneumoniae* carbapenemase producing	8 of 9 (89%)
Enterobacter spp.	2
• *Enterobacter* spp. ESBL producing	0 of 2 (0%)
• *Enterobacter* spp. carbapenemase producing	1 of 2 (50%)
Pseudomonas aeruginosa	0
Other Gram-negative bacteria†	5
Nontuberculous mycobacteria‡	**1**
	Total Isolates from Rectal Swab, n = 20
Gram-negative bacteria	**20**
Escherichia coli	3
□ *Escherichia coli* ESBL producing	1 of 3 (33%)
□ *Escherichia coli* resistant to carbapenems	2 of 3 (67%)
Other Enterobacteriaceae	12
• *Klebsiella pneumoniae* carbapenemase producing	9 of 12 (75%)
□ Colistin resistant	3 of 9 (33%)
• *Enterobacter* spp. resistant to carbapenems	2 of 12 (17%)
• *Citrobacter* spp. resistant to carbapenems	1 of 12 (8%)
Pseudomonas aeruginosa resistant to carbapenems	3
Stenotrophomonas maltophilia	2

* Other Gram-positive bacteria: 1 *Granulicatella adiacens*, 1 *Rothia mucilanosa*.
† Other Gram-negative bacteria: 1 *Stenotrophomonas maltophilia*, 1 *Pseudomonas alcaligens*, 1 *Acidovorax radicis*, 2 *Sphingomonas paucimobilis*.
‡ Nontuberculosis mycobacteria: 1 *Mycobacterium mucogenicum*.

postengraftment phase it was slightly higher in haplo (Figure 3). Among patients who died by IRM, 23% had a poor graft function (80% received stem cell boost before death; 2 experienced >1 CR-*Kp* PE-BSI leading to graft failure, and 2 others developed ≥1 VIs), and 14% had chronic GVHD.

IRM at 30 days among recipients who developed GNB PE-BSIs (n = 29) and GPB PE-BSIs (n = 42) was 14% and 7%, respectively, while patients who did not develop PE-BSIs (n = 164) had an IRM of 2% (Gray's test: $P = .010$) (Figure 3). BSI-attributable mortality after at least 1 GNB PE-BSI episode and GPB PE-BSI episode was 6% (2/33) and 0% (0/43), respectively, while CR-*Kp* BSI-attributable mortality was 13% (1/8).

IRM at 180 days among recipients who developed CMV end-organ disease was 15%, slightly higher (although not statistically different) than those with CS-CMVi and without CS-CMVi (viremia not requiring PET and no reactivation) (Figure 3). TRM at 180 days was 14% (95% CI, 10% to 19%) and higher in a haploidentical setting (5%, 8%, and 19% in MRD, MUD, and haplo, respectively) (Gray's test: $P = .021$). Among CMV-seropositive recipients, CMV serostatus of the donor did not affect TRM at 180 days (14% and 12% in positive/negative and positive/positive, respectively) (Gray's test: $P = .191$).

DISCUSSION

This study drew a complete picture of infections after allogenic HSCT with PT-Cy GVHD prophylaxis, in both a matched (MRD, MUD) and a mismatched (haplo) donor setting.

Regarding bacterial infections in the pre-engraftment phase, the main findings of this study of 235 HSCT recipients can be summarized as follow: (1) incidence of PE-BSIs was not affected by donor type, and it was significantly increased in patients with neutropenia before HSCT and in MDR-GNB carriers; (2) PE-BSIs had a negative impact on 30 days IRM; and (3) BSI-attributable mortality was influenced exclusively by GNB etiology.

Our cumulative incidence of PE-BSIs of 32% is similar to those previously reported, which range from 16% to 40% [12-18,45,46]. Few studies identified risk factors for PE-BSIs, including duration of neutropenia, severity of mucosal damage, type of conditioning, graft source, age, and active underlying disease [17,45]. Risk factors for GPB or GNB BSIs were largely unknown. A retrospective study on 553 allogeneic HSCTs reported a 30% rate of PE-BSIs with a 7-day mortality of 5%, 43%, and 75% for any etiology, CR-*Enterobacteriaceae*, and MDR-*Pa*, respectively; donor type (MUD, haplo) and age emerged as risk factors for GNB PE-BSIs [46]. A prospective study showed an incidence of GNB PE-BSIs in allogeneic HSCTs of 19% with a 30-day mortality of 14%, significantly higher for CR-*Kp* and MDR-*Pa*; risk factors for GNB PE-BSIs were cord blood and mismatch transplants, age, severe neutropenia, and underlying disease [47]. In our cohort, the rate of PE-BSIs was not influenced by age, disease status at HSCT, and donor type, while the main risk factors were neutropenia and colonization by MDR-GNB. The role of pretransplant neutropenia likely depends on poor neutrophil function or low response to chemotherapies of patients affected by high-risk diseases, leading to the performance of urgent transplant during ongoing aplasia, mainly from a family donor. Such heavily pretreated patients tend also to acquire MDR-GNB colonization, and it significantly affects the risk of PE-BSIs as emerged in other studies [18,47,48]. Our goal was to analyze a cohort who received transplant with a homogeneous GVHD prophylaxis: in this context, HLA mismatch did confer neither a greater risk of PE-BSIs nor a higher 30-day IRM. Mikulska et al. [46] reported a high risk of PE-BSIs (38%) in haplo-HSCT with PT-Cy and, in

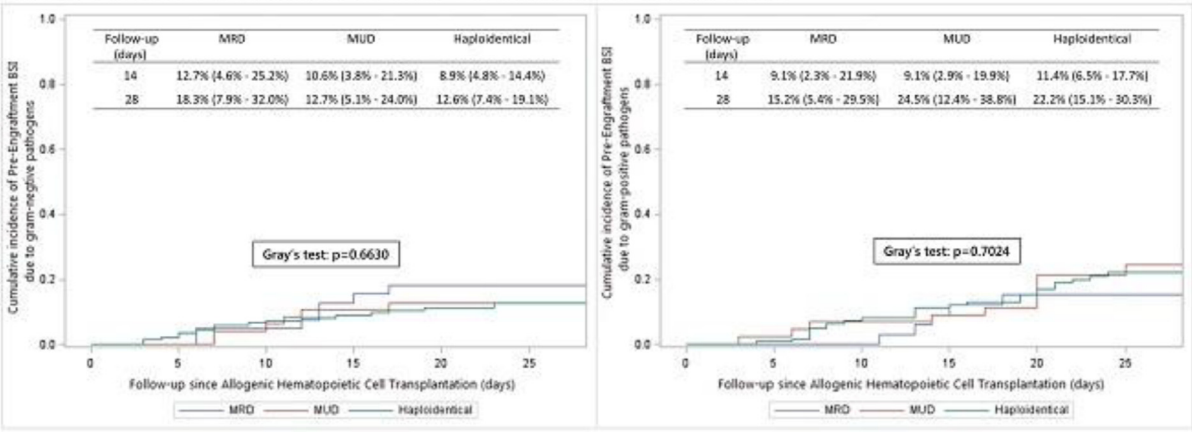

Figure 1. Cumulative incidence of Gram-negative and Gram-positive PE-BSIs according to the type of donor.

half of patients, a busulfan-based conditioning or a full-dose total body irradiation: the timing of BSI onset, approximately 3 to 5 days after cyclophosphamide infusion, and the etiology, mainly intestinal GNB and viridans streptococci, suggested a role of extensive mucosal damage caused by PT-Cy. In our population, using a homogeneous, reduced-toxicity, treosulfan-based conditioning, the mucosal damage was superimposable among donor groups, thus translating to similar rates of PE-BSIs; moreover, PE-BSI onset, approximately 13 days after PBSC infusion, did not support a main role in terms of mucosal damage of PT-Cy, administered in our protocol on days 3 and 4 after HSCT, suggesting instead a later mucosal toxicity of conditioning regimen with double-alkylating agents.

Table 3
Univariate and Multivariate Fine-Gray Models to Assess Baseline Factors with an Effect on the Rate of Any or Gram-Negative Bacteria PE-BSIs

Characteristic at HSCT	Risk Categories	Univariate Analysis		Multivariate Analysis			
		Crude HR of Any PE-BSI (95% CI)	*P* Value	Adjusted HR of Any PE-BSI (95% CI)	*P* Value	Adjusted HR of GNB PE-BSI (95% CI)	*P* Value
Age	Per 3 years older	1.023 (0.976-1.072)	.337	1.010 (0.959-1.063)	.716	0.943 (0.866-1.027)	.178
	>50 vs ≤50 years	1.153 (0.730-1.822)	.541				
Sex	Female vs male	0.897 (0.555-1.451)	.659	0.877 (0.524-1.467)	.616	0.767 (0.340-1.730)	.523
Year of HSCT	Per 2 more recent years	0.833 (0.606-1.144)	.258	0.942 (0.628-1.411)	.770	1.024 (0.515-2.036)	.947
	>2015 vs ≤2015	0.674 (0.428-1.062)	**.089**				
ANC ≤500 for ≥7 days before HSCT	Yes vs no	2.721 (1.707-4.337)	<**.0001**	2.895 (1.542-5.435)	**.0009**	4.866 (1.992-11.89)	**.0005**
Diagnosis*	Lymphoid disorders vs myeloid disorders	1.300 (0.799-2.114)	.291	Not included	—	Not included	—
MDR-GNB rectal carrier within 30 days before HSCT	Yes vs no	3.069 (1.543-6.104)	**.0014**	2.683 (1.253-5.749)	**.011**	3.885 (1.288-11.72)	**.016**
Conditioning regimen				Not included	—	Not included	—
	RIC vs MAC	0.687 (0.372-1.269)	.231				
Number of allogenic HSCTs			.488	Not included	—	Not included	—
	Second vs first	1.494 (0.764-2.921)	.241				
	Third vs first	0.834 (0.125-5.579)	.852				
Type of donor			.967		.496		.367
	Haploidentical vs MRD	1.086 (0.580-2.034)	.797	0.929 (0.480-1.801)	.828	0.656 (0.255-1.688)	.382
	MUD vs MRD	0.987 (0.566-1.720)	.853	1.493 (0.758-2.944)	.387	1.307 (0.417-4.099)	.646
Disease phase at HSCT							
	Active disease vs >CR1/CR1	1.642 (1.028-2.621)	**.038**	0.886 (0.483-1.624)	.694	1.074 (0.432-2.674)	.877

RIC indicates reduced-intensity conditioning.
Significant values are in bold.
* The only patient with other disorders was excluded from this calculation.

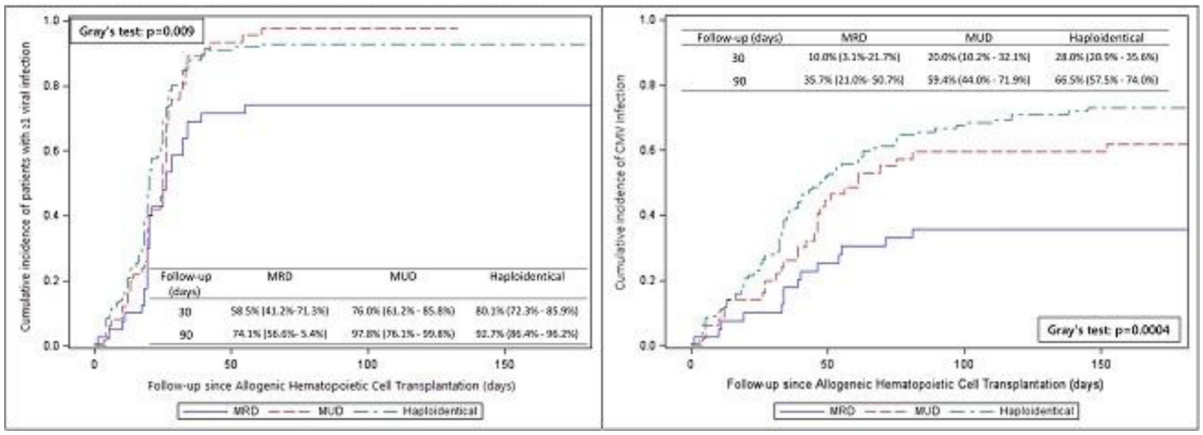

Figure 2. Cumulative incidence function of patients with ≥1 VIs (first occurrence among BKPyV-hemorrhagic cystitis and any positive viremia for CMV, HHV6, and ADV) or with CMV infection (end-organ disease or clinically significant infection leading to pre-emptive therapy or viremia not requiring pre-emptive therapy).

The negative impact of PE-BSIs on 30-day IRM, despite a low BSI-attributable mortality, probably reflects that PE-BSIs lead to systemic complications, particularly in aplastic patients with pretransplant comorbidities, justifying a poorer outcome. In this regard, 53% of our cohort had a hematopoietic cell transplant comorbidity index ≥3.

An important issue in BSI management in neutropenic patients is establishing the risk of BSI-attributable mortality. In our cohort, no patients with GPB PE-BSIs died within 7 days, whereas mortality was 6% in cases of at least 1 GNB PE-BSI, confirming GNB etiology to be the main determinant of BSI-attributable mortality. The low number of events did not allow us to make a reliable conclusion on antimicrobial resistance impact on early mortality, as reported in many studies [18,46,49]. Despite a high proportion of carbapenem resistance among *Kp* isolates from blood in our study (8 out of 9, 89%), it is worth noting that a lot of CR-*Kp* carriers (3 out of 9, 33%) experienced 2 episodes of CR-*Kp* PE-BSI, justifying the above-mentioned overall high rate of carbapenem resistance. Conversely, our incidence of CR-*Kp* PE-BSIs in carriers (5 out of 9, 56%) was comparable to previous reports. Regarding mortality, our CR-*Kp* mortality of 13% was lower than in other cohorts [18,46], underlying the importance of active surveillance for a de-escalation approach at febrile neutropenia in MDR-GNB carriers with an empiric therapy targeting the MDR strain, as recommended by ECIL and common practice at our institution [19,50]. Such an approach makes transplantation feasible also in an endemic country for CR-*Kp* and in high-risk hematologic

Table 4
Univariate and Multivariate Fine-Gray Models to Assess Baseline Factors with an Effect on the Rate of ≥1 VIs (First Occurrence among BKPyV-HC and Any Positive Viremia for CMV, HHV6, and ADV) following HSCT

Characteristic at HCT	Risk Categories	Univariate Analysis		Multivariate Analysis	
		Crude HR of ≥1 VIs (95% CI)	*P* Value	Adjusted HR of ≥1 VIs (95% CI)	*P* Value
Age	Per 3 years older	1.015 (0.988-1.043)	.277	1.015 (0.984-1.046)	.349
	>50 vs ≤50 years	1.166 (0.899-1.513)	.247	—	—
Sex	Female vs male	1.178 (0.897-1.547)	.239	1.250 (0.946-1.651)	.117
Year of HSCT	Per 2 more recent years	1.055 (0.874-1.274)	.578	1.132 (0.919-1.394)	.243
	>2015 vs ≤2015	1.045 (0.803-1.361)	.741	—	—
Conditioning regimen				Not included	—
	Reduced-intensity conditioning vs myeloablative conditioning	0.898 (0.679-1.189)	.454		
Number of allogenic HSCTs			.871	Not included	—
	Second vs first	0.961 (0.634-1.456)	.851		
	Third vs first	0.722 (0.200-2.605)	.619		
Type of donor			.007		.006
	Haploidentical vs MRD	1.939 (1.283-2.929)	.002	2.004 (1.204-3.334)	.008
	MUD vs MRD	1.742 (1.138-2.668)	.011	2.026 (1.285-3.194)	.002
aGVHD ≥2	Yes vs no	1.415 (1.082-1.849)	.011	1.301 (0.942-1.798)	.110
Relapse	Yes vs no	0.943 (0.689-1.291)	.715	Not included	—
R/D CMV			.006		.008
	Positive/positive vs negative/negative	2.841 (1.521-5.307)	.001	2.953 (1.551-5.623)	.001
	Negative/positive vs negative/negative	1.963 (0.854-4.515)	.112	2.351 (1.063-5.198)	.035
	Positive/negative vs negative/negative	2.321 (1.202-4.483)	.012	2.407 (1.226-4.726)	.011
CMV risk	High vs low	1.552 (1.157-2.081)	.003	1.043 (0.642-1.695)	.866

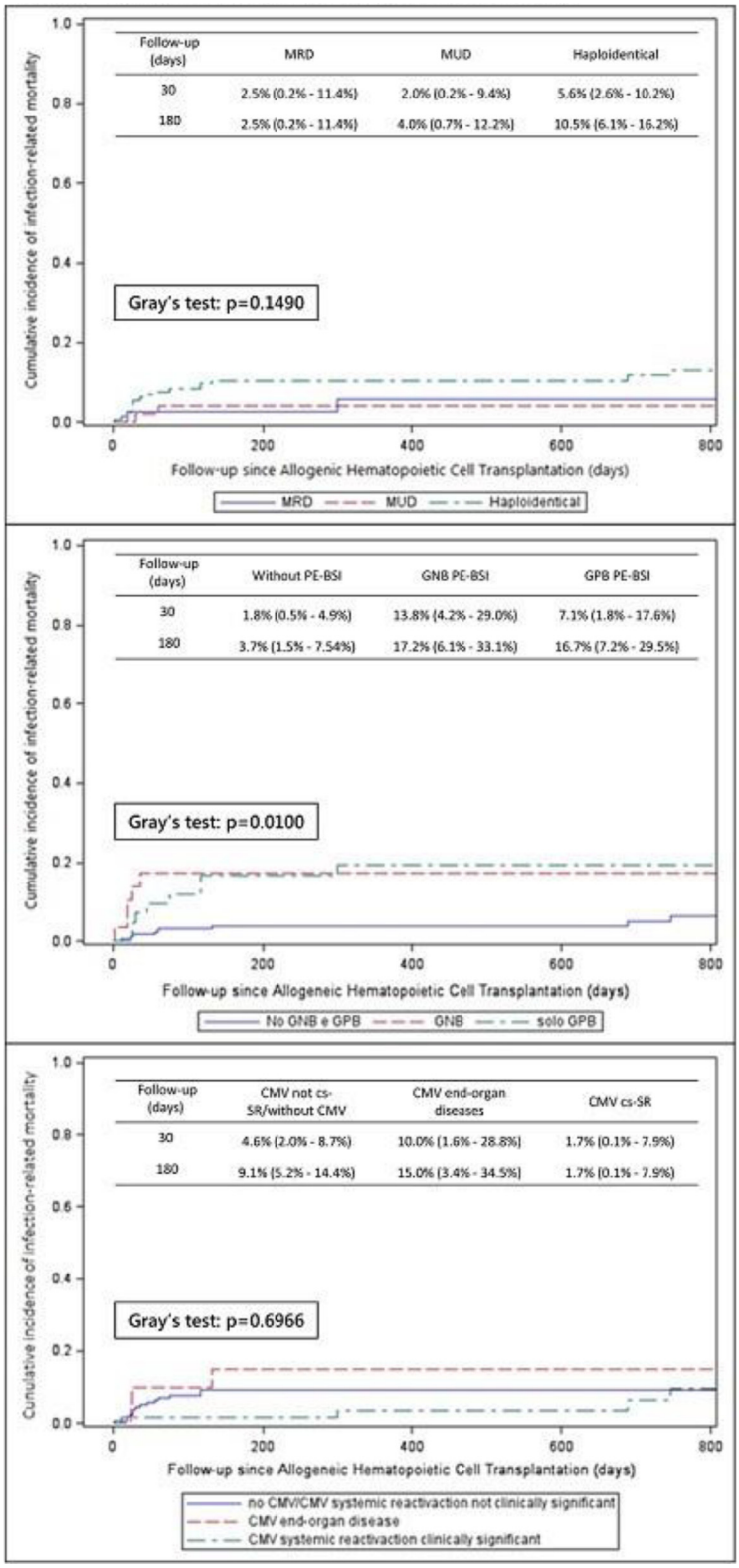

Figure 3. Cumulative incidence of IRM.

patients. However, it is likely an underestimation of MDR-GNB BSI-attributable mortality in our study due to the absence of MDR-*Pa* BSIs in the study period.

Besides the worrisome finding of a high incidence of CR-*Kp*, the antimicrobial resistance pattern of GNB isolates from blood in our cohort highlighted a high resistance rate also to fluoroquinolones (82%) and piperacillin/tazobactam (71%), leading us to discontinue antibiotic prophylaxis and to reassess the first-line empirical therapy of febrile neutropenia.

Moving to ds-DNA VIs, the main findings of this study are (1) incidence of VIs was very high in early postengraftment phase and significantly increased, as well as in the context of aGVHD grade ≥2 and unfavorable R/D CMV serostatus, also in a MUD and haploidentical setting; (2) CMV and HHV6 end-organ diseases affected a minority of patients; and (3) 180-day TRM in CMV-seropositive recipients was not influenced by donor CMV serostatus.

In this study, the CIF of ≥1 VIs in haplo and MUD transplants at 30 days achieved 80% and 76%, respectively. Although this was a high rate of VIs in the early period, results from different studies [4,9,20,21,24,51] suggested that the PT-Cy approach is well suited to handle the important issue of VIs after HSCT, thanks to a fast and broad immune reconstitution. Cieri et al. [21] showed in a cohort of 40 haplo-HSCTs with PT-Cy a median of 338/μL $CD3^+$ T cells at 30 days and time to $CD4^+$ >200/μL of 41 days. Haplo-HSCT has always been at high risk of VIs because only half of recipients' antigen-presenting cells have expressed the major histocompatibility complex of the donor to successfully recruit T cells and restore a protective immunity. With the PT-Cy platform, the early onset of VIs, the immune reconstitution kinetic of T cells, and the high rate of VIs not only in mismatch-related but also in MUD, could be driven by the T-depleting effect of PT-Cy on donor T cells impairing their ability to interact with recipients' APCs, regardless of HLA mismatch. Based on the mechanism of action of PT-Cy, capable of selectively killing the proliferative alloreactive T cells, it is reasonable to speculate that alloreactivity overlaps with cross-reactivity of antiviral T cells regardless of HLA mismatch degree [21] and of the potential protective effect of sirolimus on CMV reactivation [52].

The proportion of proven-probable end-organ diseases is low (4%) and comparable to literature [53], but it remains largely unknown if these early VIs, also low-level viremia, play a role in triggering other transplant-related complications such as GVHD. Data on VIs need future investigations, including the analysis of virus-specific immune reconstitution.

CMV-seropositive recipients are generally recognized as being at major risk for TRM [54]. However, the impact of donor serostatus on TRM in seropositive recipients receiving PT-Cy is largely unknown. CMV serostatus has a leading role in guiding the choice among multiple potential haploidentical donors; more data on this issue are needed in the context of PT-Cy, mainly considering the introduction of letermovir in clinical practice. A retrospective study conducted by Solomon et al. [55] analyzed 208 patients receiving haplo-HSCT with PT-Cy. Among donor variables associated with inferior survival, they identified the use of a CMV-seronegative donor for a CMV-seropositive patient. Conversely, Cesaro et al. [56] did not find differences in 1-year nonrelapse mortality and overall survival according to donor CMV serostatus in a cohort of 983 CMV-seropositive patients with acute leukemia who received haplo-HSCT with PT-Cy. In our study, donor CMV serostatus did not affect the occurrence of VIs or TRM at 180 days in CMV-seropositive recipients. Therefore, results of our study suggest that, with the PT-Cy platform, not only the high-risk context of R/D positive/negative mismatch but also that of R/D positive/positive status, regardless of donor source, could require letermovir prophylaxis. Larger studies are warranted to better address this issue and the impact of letermovir in allogenic HSCT with PT-Cy GVHD prophylaxis, which took hold after the trial that led to letermovir approval.

Although this study represents a single-center report, its unbiased consecutive patient cohort and the common transplantation platform across different donor options offer conclusions potentially relevant to the definition of donors' choice algorithms.

In conclusion, in our cohort, the risk of PE-BSIs was not affected by donor type within a homogeneous transplant platform. The impact of GNB on mortality highlights the importance of focusing on empiric therapy, tailored on culture results deriving from active surveillance. At our center, which has a high prevalence of extended-spectrum beta-lactamase (ESBL)-producing GNB and CR-*Kp*, this involves using carbapenem or novel drugs (ceftazidime/avibactam, meropenem/vaborbactam). Although we had a high rate of VIs in the early period, these observations suggest that by allowing fast T cell immune recovery, PT-Cy can be an attractive approach to handle the issue of VIs after allogenic HSCT. Future multicenter prospective studies should confirm our results related to the donor source impact on infectious complications in HSCT recipients receiving PT-Cy, as well as explore the impact that letermovir prophylaxis will have on CMV infections.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2020.01.013.

REFERENCES

1. Passweg JR, Baldomero H, Bader P, et al. Is the use of unrelated donor transplantation leveling off in Europe? The 2016 European Society for Blood and Marrow Transplant activity survey report. *Bone Marrow Transplant*. 2018;53:1139–1148.
2. Duarte RF, Labopin M, Bader P, et al. Indications for hematopoietic stem cell transplantation for haematological diseases, solid tumors and immune disorders: current practice in Europe, 2019. *Bone Marrow Transplant*. 2019;54:1525–1552.
3. Ciurea SO, Zhang MJ, Bacigalupo AA, et al. Haploidentical transplant with posttransplant cyclophosphamide vs matched unrelated donor transplant for acute myeloid leukemia. *Blood*. 2015;126:1033–1040.
4. Mussetti A, Greco R, Peccatori J, et al. Post-transplant cyclophosphamide, a promising anti-graft versus host disease prophylaxis: where do we stand? *Expert Rev Hematol*. 2017;10:479–492.
5. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using non-myeloablative conditioning and high-dose, post transplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14:641–650.
6. Raj K, Pagliuca A, Bradstock K, et al. Peripheral blood hematopoietic stem cells for transplantation of hematological diseases from related, haploidentical donors after reduced-intensity conditioning. *Biol Blood Marrow Transplant*. 2014;20:890–895.
7. Bhamidipati PK, DiPersio JF, Stokerl-Goldstein K, et al. Haploidentical transplantation using G-CSF-mobilized T-cell replete PBSCs and post-transplantation CY after non-myeloablative conditioning is safe and is associated with favorable outcomes. *Bone Marrow Transplant*. 2014;49:1124–1126.
8. Castagna L, Crocchiolo R, Furst S, et al. Bone marrow compared with peripheral blood stem cells for haploidentical transplantation with a non-myeloablative conditioning regimen and post-transplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2014;20:724–729.
9. Raiola AM, Dominietto A, Ghiso A, et al. Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. *Biol Blood Marrow Transplant*. 2013;19:117–122.

10. Greco R, Lorentino F, Morelli M, et al. Post-transplantation cyclophosphamide and sirolimus for prevention of GVHD after HLA-matched PBSC transplantation. *Blood*. 2016;128:1528–1531.
11. Ruggeri A, Sun Y, Labopin M, et al. Post-transplant cyclophosphamide versus anti-thymocyte globulin as graft- versus-host disease prophylaxis in haploidentical transplant. *Haematologica*. 2017;102:401–410.
12. Collin BA, Leather HL, Wingard JR, et al. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. *Clin Infect Dis*. 2001;33:947–953.
13. Gudiol C, Garcia-Vidal C, Arnan M, et al. Etiology, clinical features and outcomes of pre-engraftment and post-engraftment bloodstream infection in hematopoietic SCT recipients. *Bone Marrow Transplant*. 2014;49:824–830.
14. Mikulska M, Del Bono V, Raiola AM, et al. Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of Gram-negative rods and increasing antibiotic resistance. *Biol Blood Marrow Transplant*. 2009;15:47–53.
15. Blennow O, Ljungman P, Sparrelid E, et al. Incidence, risk factors, and outcome of bloodstream infections during the pre-engraftment phase in 521 allogeneic hematopoietic stem cell transplantations. *Transpl Infect Dis*. 2014;16:106–114.
16. Kikuchi M, Akahoshi Y, Nakano H, et al. Risk factors for pre- and post-engraftment bloodstream infections after allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis*. 2015;17:56–65.
17. Zheng C, Tang B, Zhu X, et al. Pre-engraftment bloodstream infections in acute leukemia patients undergoing unrelated cord blood transplantation following intensified myeloablative conditioning without ATG. *Ann Hematol*. 2016;96(1):115–124.
18. Girmenia C, Rossolini GM, Piciocchi A, et al. Infections by carbapenem-resistant Klebsiella pneumoniae in SCT recipients: a nationwide retrospective survey from Italy. *Bone Marrow Transplant*. 2015;50:282–288.
19. Averbuch D, Orasch C, Cordonnier C, et al. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European Conference on Infections in Leukemia. *Haematologica*. 2013;98:1826–1835.
20. Crocchiolo R, Bramanti S, Vai A, et al. Infections after T-replete haploidentical transplantation and high-dose cyclophosphamide as graft-versus-host disease prophylaxis. *Transpl Infect Dis*. 2015;17:242–249.
21. Cieri N, Greco R, Crucitti L, et al. Post-transplantation cyclophosphamide and sirolimus after haploidentical hematopoietic stem cell transplantation using a treosulfan-based myeloablative conditioning and peripheral blood stem cells. *Biol Blood Marrow Transplant*. 2015;21:1506–1514.
22. Slade M, Goldsmith S, Romee R, et al. Epidemiology of infections following haploidentical peripheral blood hematopoietic cell transplantation. *Transpl Infect Dis*. 2017;19. https://doi.org/10.1111/tid.12629. Epub 2016 Dec 28.
23. Mohty R, Brissot E, Battipaglia G, et al. Infectious complications after post-transplantation cyclophosphamide and anti-thymocyte globulin-based haploidentical stem cell transplantation. *Br J Hematol*. 2019;187:e64–e68.
24. Ciurea SO, Mulanovich V, Saliba RM, et al. Improved early outcomes using a T cell replete graft compared with t cell depleted haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2012;18:1835–1844.
25. Harris AC, Young R, Devine S, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai Acute GVHD International Consortium. *Biol Blood Marrow Transplant*. 2016;22:4–10.
26. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21:389–401.
27. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. *Hematol Oncol Clin North Am*. 2011;25(1):151–169.
28. Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. *Blood*. 2009;113(23):5711–5719.
29. Ljungman P, Perez-Bercoff L, Jonsson J, et al. Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. *Haematologica*. 2006;91(1):78–83.
30. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med*. 2017;377:2433–2444.
31. Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. *Clin Infect Dis*. 2017;64:87–91.
32. Ljungman P, de la Camara R, Cordonnier C, et al. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. *Bone Marrow Transplantation*. 2008;42:227–240.
33. Sandkovsky U, Vargas L, Florescu DF. Adenovirus: current epidemiology and emerging approaches to prevention and treatment. *Curr Infect Dis Rep*. 2014;16:416.
34. Cesaro S, Dalianis T, Hanssen Rinaldo C, et al. ECIL guidelines for the prevention, diagnosis and treatment of BK polyomavirus-associated hemorrhagic cystitis in hematopoietic stem cell transplant recipients. *J Antimicrob Chemother*. 2018;73:12–21.
35. Styczynski J, Reusser P, Einsele H, et al. Management of HSV, VZV, EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. *Bone Marrow Transplantation*. 2009;43:757–770.
36. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46:1813–1821.
37. Heslop HE. How I treat EBV lympho-proliferation. *Blood*. 2009;114(19):4002–4008.
38. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant*. 2009;15(10):1143–1238.
39. Flowers CR, Seidenfeld J, Bow EJ, et al. Antimicrobial prophylaxis and outpatient management of fever and neutropenia in adults treated for malignancy: American Society of Clinical Oncology clinical practice guideline. *J Clin Oncol*. 2013;31(6):794–810.
40. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by Infectious Disease Society of America. *Clin Infect Dis*. 2011;52(4):e56–e93.
41. Baden LR, Bensinger W, Angarone M, et al. Prevention and treatment of cancer-related infections. *J Natl Compr Canc Netw*. 2012;10(11):1412–1445.
42. EBMT handbook 2008, revised edition 2012. https://www.ebmt.org/education/ebmt-handbook.
43. Fine JP, Gray RJ. A proportional hazards model for the sub-distribution of a competing risk. *J Am Stat Assoc*. 1999;94:496–509.
44. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. *Blood*. 2014;123(23):3664–3671.
45. Almyroudis NG, Fuller A, Jakubowski A, et al. Pre- and post-engraftment bloodstream infection rates and associated mortality in allogeneic hematopoietic stem cell transplant recipients. *Transpl Infect Dis*. 2005;7:11–17.
46. Mikulska M, Raiola AM, Galaverna F, et al. Pre-engraftment bloodstream infections after allogeneic hematopoietic cell transplantation: the impact of T-repleted transplant from haploidentical donors. *Biol Blood Marrow Transplant*. 2018;24(1). 10dth9-118.
47. Girmenia C, Bertaina A, Piciocchi A, et al. Incidence, risk factors and outcome of pre-engraftment Gram-negative bacteremia after allogenic and autologous hematopoietic stem cell transplantation: an Italian prospective multicenter survey. *Clin Infect Dis*. 2017;65:1884–1896.
48. Forcina A, Lorentino F, Marasco V, et al. Clinical impact of pre-transplant multidrug-resistant gram-negative colonization in autologous and allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2018;24:1476–1482.
49. Averbuch D, Tridello G, Hoek J, et al. Antimicrobial resistance in Gram-negative rods causing bacteremia in hematopoietic stem cell transplant recipients: intercontinental prospective study of the Infectious Diseases Working Party of the European Bone Marrow Transplantation group. *Clin Infect Dis*. 2017;65:1819–1828.
50. Forcina A, Baldan R, Marasco V, et al. Control of infectious mortality due to carbapenemase-producing *Klebsiella pneumoniae* in hematopoietic stem cell transplantation. *Bone Marrow Transplantation*. 2017;52:114–119.
51. Luznik L, Jalla S, Engstrom LW, et al. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. *Blood*. 2001;98(12):3456–3464.
52. Piñana JL, Perez-Pitarch A, Guglieri-Lopez B, et al. Sirolimus exposure and the occurrence of cytomegalovirus DNAemia after allogeneic hematopoietic stem cell transplantation. *Am J Transplant*. 2018;18(12):2885–2894.
53. Ljungman P, Perez-Bercoff L, Jonsson J, et al. Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. *Haematologica*. 2006;91(1):78–83.
54. Ljungman P, Brand R, Hoek J, et al. Donor cytomegalovirus status influences the outcome of allogeneic stem cell transplant: a study by the European group for blood and marrow transplantation. *Clin Infect Dis*. 2014;59(4):473–481.
55. Solomon SR, Aubrey MT, Zhang X, et al. Selecting the best donor for haploidentical transplant: impact of HLA, killer cell immunoglobulin-like receptor genotyping, and other clinical variables. *Biol Blood Marrow Transplant*. 2018;24(4):789–798.
56. Cesaro S, Crocchiolo R, Tridello G, et al. Comparable survival using a CMV-matched or a mismatched donor for CMV+ patients undergoing T-replete haplo-HSCT with PT-Cy for acute leukemia: a study of behalf of the infectious diseases and acute leukemia working parties of the EBMT. *Bone Marrow Transplantation*. 2018;53(4):422–430.