REGULAR ARTICLE

blood advances

Posttransplant cyclophosphamide as GVHD prophylaxis in patients receiving mismatched unrelated HCT: the PHYLOS trial

Anna Maria Raiola,[1] Benedetto Bruno,[2] Antonio Maria Risitano,[3] Federico Mosna,[4] Irene Maria Cavattoni,[4] Francesco Onida,[5,6] Giorgia Saporiti,[6] Francesca Patriarca,[7] Marta Lisa Battista,[7] Vincenzo Pavone,[8] Anna Mele,[8] Patrizia Chiusolo,[9,10] Simona Sica,[9,10] Barbara Loteta,[11] Carmen di Grazia,[1] Angelo Michele Carella,[12] Dalila Salvatore,[12] Enrico Morello,[13,14] Alessandro Leoni,[13,14] Luisa Giaccone,[2] Paolo Bernasconi,[15] Elisabetta Terruzzi,[16] Nicola Mordini,[17] Carlo Borghero,[18] Francesco Zallio,[19] Mario Luppi,[20] Anna Grassi,[21] Attilio Olivieri,[22] Eugenia Piras,[23] Nicoletta Sacchi,[24] Giovannino Ciccone,[25] Anna Castiglione,[25] Eliana Degrandi,[26] Emanuele Angelucci,[1] Massimo Martino,[11] and Francesca Bonifazi[27]

[1]Department of Hematology and Cellular Therapy, Istituto di Ricovero E Cura A Carattere Scientifico, Ospedale Policlinico San Martino, Genoa, Italy; [2]Division of Hematology, Azienda Ospedaliera Universitaria Citta' della Salute e della Scienza di Torino, and Department of Biotechnologies and Health Sciences, University of Torino, Turin, Italy; [3]Hematology and Bone Marrow Transplant Unit, Azienda Ospedaliera di Rilievo Nazionale San Giuseppe Moscati Avellino, Avellino, Italy; [4]Hematology and Bone Marrow Transplantation Unit, Hospital of Bolzano (SABES-Azienda Sanitaria dell'Alto Adige), Teaching Hospital of Paracelsus Medical University, Bolzano, Italy; [5]Hematology and Bone Marrow Transplantation Unit, Azienda Socio Sanitaria Territoriale Fatebenefratelli-Sacco, University of Milan, Milan, Italy; [6]Centro Trapianti Midollo Osseo e Terapie Cellulari-Struttura Complessa Ematologia, Fondazione Istituo di Ricovero E Cura A Carattere Scientifico, Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [7]Division of Hematology, S. Maria della Misericordia Hospital, Dipartimento di Medicina, Università di Udine, Azienda Sanitaria Universitaria Friuli Centrale, Udine, Italy; [8]Unità Operativa Complessa Ematologia con Trapianto, Azienda Ospedale Car Panico Tricase (Lecce), Tricase, Italy; [9]Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica del Sacro Cuore, Rome, Italy; [10]Dipartimento di Scienze di laboratorio ed Ematologiche, Fondazione Policlinico A. Gemelli Istituo di Ricovero E Cura A Carattere Scientifico, Rome, Italy; [11]Stem Cell Transplantation and Cellular Therapies Unit, Grande Ospedale Metropolitano, "Bianchi-Melacrino-Morelli," Reggio Calabria, Italy; [12]Department of Hematology and Stem Cell Transplant Unit, Fondazione Istituo di Ricovero E Cura A Carattere Scientifico Casa Sollievo della Sofferenza, Foggia, Italy; [13]Blood Diseases and Cell Therapies Unit, Bone Marrow Transplant Unit, "Azienda Socio Sanitaria Territoriale-Spedali Civili" Hospital of Brescia, Brescia, Italy; [14]Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; [15]Hematopoietic Stem Cell Unit, Hematology Department, Fondazione Istituo di Ricovero E Cura A Carattere Scientifico Policlinico San Matteo, Pavia, Italy; [16]Haematology Unit, Istituo di Ricovero E Cura A Carattere Scientifico San Gerardo, Monza, Italy; [17]Division of Hematology, Azienda Ospedaliera S. Croce e Carle, Cuneo, Italy; [18]Hematology Department, "San Bortolo" Hospital, Vicenza, Italy; [19]Hematology Department, SS Antonio and Biagio and C. Arrigo Hospital, Alessandria, Italy; [20]Ematologia, Dipartimento di Scienze Mediche e Chirurgiche Materno-Infantili e dell'Adulto, Università degli Studi di Modena e Reggio Emilia, Modena, Italy; [21]Hematology-Bone Marrow Transplantation Unit Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Italy; [22]Clinica di Ematologia, Azienda Ospedaliero Universitaria delle Marche, Ancona, Italy; [23]Hematology Unit, Businco Hospital, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy; [24]Italian Bone Marrow Donor Registry, E.O. Galliera Hospitals, Genoa, Italy; [25]Unit of Clinical Epidemiology, Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino e Centro Prevenzione Oncologica Piemonte, Turin, Italy; [26]Trial Office Gruppo Italiano per il Trapianto di Midollo Osseo, cellule staminali emopoietiche e terapia Cellulare, Bologna, Italy; [27]Istituo di Ricovero E Cura A Carattere Scientifico Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Key Points

- PPTCy-based GVHD prophylaxis leads to a low rate of aGVHD, a low incidence of NRM and acceptable relapse rate after HSCT from MMUD

Posttransplant high-dose cyclophosphamide (PTCy) is effective in overcoming the negative impact of HLA disparity in the haploidentical setting. In light of these results, we investigated the efficacy of PTCy, in improving clinical outcomes of hematopoietic stem cell transplantation (HSCT) from a mismatched unrelated donor (MMUD) in patients with acute myeloid malignancies by reducing the incidence and severity of acute graft-versus-host disease (aGVHD). A prospective, single-arm, phase 2 study (PHYLOS) was conducted by the Gruppo Italiano Trapianto di Midollo Osseo. The ethical committees of the participating centers approved the study (EURODRACT 2017-003530-85). A total of 77 consecutive patients (acute myeloid leukemia: 64; myelodysplastic syndrome: 13) were enrolled at 26 Italian transplant centers (January 2020-November 2022). Median age of the patients was 53 (range, 19-65) years. The 100-day cumulative incidence of grades 2 to 4 aGVHD was 18.2% (95% CI,

Submitted 24 October 2024; accepted 8 January 2025; prepublished online on *Blood Advances* First Edition 10 February 2025. https://doi.org/10.1182/bloodadvances.2024015173.

Deidentified individual participant data that underlie the reported results will be made available for a period of 1 year after the publication date. Deidentified patient data are available on request from the corresponding author, Anna Maria Raiola (annamaria.raiola@hsanmartino.it). If available, data will be provided electronically in Excel worksheets, within a reasonable timeframe. The study protocol is included as a data supplement available with the online version of this article.

The full-text version of this article contains a data supplement.

10.6-27.6) and of grades 3 to 4 was 6.5% (95% CI, 3.1-15.1). Seventy-one patients (92%) had full-donor chimerism with complete neutrophil engraftment by day +30. One-year cumulative incidence of chronic GVHD was 13.4% (95% CI, 6.9-22.1). One-year cumulative incidence of nonrelapse mortality was 9.1% (95% CI, 4.0-16.9), and the relapse rate was 23.8% (95% CI, 14.9-33.9). One-year overall survival and graft relapse-free survival were 78.6% (95% CI, 67.4-86.3) and 55.3% (95% CI, 43.4-65.7), respectively. Our study in a homogeneous patient cohort suggests that PTCy leads to a low rate of aGVHD and improves clinical outcomes of HSCT from MMUD. This trial was registered at www.clinicaltrials.gov as #NCT03270748.

Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment option for several malignant and nonmalignant hematologic diseases, and HLA disparity remains one of the most important factors affecting long-term survival. Indeed, outcomes are inferior with HLA-mismatched unrelated donors (MMUDs).[1-3]

Single HLA mismatches at HLA-A, -B, -C, or -DRB1 locus (7/8 HLA matched) are associated with an increased risk of severe acute graft-versus-host disease (aGVHD), resulting in increased nonrelapse mortality (NRM) and decreased overall survival (OS) when compared with matched related or unrelated donor HCT.[4-10] Strategies to facilitate the use of MMUDs are relevant for patients from ethnic minorities or in the presence of familial disease. The probability of finding a matched unrelated donor can vary between 16% and 75%, depending primarily on the patient's ethnic background.[11] To overcome HLA diversity and intensify immunosuppression, in vivo T-cell depletion (thymoglobulin/antilymphocyte or alemtuzumab) is used as GVHD prophylaxis for MMUD HCT, but a shared schedule of treatment in homogenous group of patients is lacking and results in terms of acute and chronic GVHD (cGVHD) are heterogeneous.[12-16]

Posttransplant high-dose cyclophosphamide (PTCy) is effective in overcoming the negative impact of HLA disparity with familial haploidentical donors.[17-19] Indeed, when combined with a calcineurin inhibitor and mycophenolate, PTCy led to acceptable rates of engraftment, GVHD, and survival.[20-22] Several studies have been published on the use of PTCy in matched sibling and unrelated donor as GVHD prophylaxis, in combination or as a single agent.[23-27]

Results from 2 nonrandomized phase 2 studies suggest that PTCy alone may not be sufficient to prevent GVHD after a transplant with peripheral blood stem cells (PBSCs) from an HLA identical donor (grades 2-4 GVHD 45%) supporting the concept that combination with another immunosuppressive drug may optimize the effect, at least in the PBSC HCT setting.[28,29]

In contrast, limited data are available in the context of transplant from MMUD with a few retrospective[30-33] or prospective studies[34-36] that included heterogeneous populations. Two European Bone Marrow Transplantation (EBMT) retrospective studies compared PTCy and anti-thymocyte globulin (ATG) in this setting, and both reported better outcomes with PTCy.[37,38] Therefore, we investigated the efficacy and safety of PTCy, combined with calcineurin inhibitor and mycophenolate mofetil, in the context of a homogeneous group of 1 antigen/allele mismatched (7/8) unrelated HCT in patients with myeloid malignancies after a myeloablative conditioning (MAC) regimen.

Patients and methods

Study design and eligibility

PHYLOS (NCT03270748 EURODRACT 2017-003530-85) is a prospective, phase 2, multicenter, single-arm, open-label study promoted by Gruppo Italiano per il Trapianto di Midollo Osseo, cellule staminali e terapia cellulare (GITMO).

Eligibility criteria for a first allogeneic HCT were an age of 18 to 65 years, a diagnosis of acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) in complete remission (CR) at transplant, an Eastern Cooperative Oncology Group performance status <2, and an adequate cardiac, liver, pulmonary, and renal function. Patients were excluded if they had a suitable HLA-identical sibling or MUD available. Donor searches were carried out by the Italian Bone Marrow Donor Registry. High-resolution HLA typing was performed for all donor-recipient pairs matching for HLA-A, -B, -C, -DRB1, and -DQB1. Any single antigen or allele mismatch at HLA-A, -B, -C, or -DRB1 was defined as 7/8. The presence of any other mismatches (HLA DQB1 and DPB1) has been reported.

Treatment plan

As conditioning regimen, all patients received busulfan 0.8 mg/kg 4 times per day in 2-hour infusions for 4 consecutive days (16 doses from day −6 to day −3) and fludarabine 40 mg/m^2 per day for 4 consecutive days (from day −6 to day −3) for a total dose of 160 mg/m^2. GVHD prophylaxis consisted of cyclophosphamide 50 mg/kg per day on days +3 and +4, cyclosporine or tacrolimus (continuous IV infusion and then oral) starting from day +5, with the dose adjusted to maintain a therapeutic level, to be continued for at least 100 days, and mycophenolate mofetil (15 mg/kg every 12 hours) starting from day +5 to day +35 (supplemental Figure 1). Patients received either granulocyte colony-stimulating factor–mobilized peripheral blood cells or bone marrow (BM) on day 0. AB0 incompatibility was managed as per institutional procedures.

Supportive care

Granulocyte growth factor was given from day +5 to neutrophil engraftment. Mesna was administrated to reduce the risk of hemorrhagic cystitis. Antimicrobial prophylaxis was administered according to institutional practice guidelines.

End point definitions

The primary end point was cumulative incidence of aGVHD, grades 2 to 4, at 100 days after MMUD transplants with PTCy in patients with AML (CR1 or CR2) or MDS. aGVHD was scored according to the Glucksberg criteria. Secondary efficacy end points were as follows: 1-year OS, 1-year cumulative incidence of cGVHD and graft failure at 100 days and at 1 year, 1-year GVHD-free and relapse-free survival (GRFS), time to hematologic reconstitution (neutrophil and platelet recovery) at 30 days from transplant, time and causes of NRM, cumulative incidence and time of disease relapse, incidence of infectious complications, and time to immune reconstitution. OS was defined as the time from transplant to the date of death regardless of the cause. The duration of follow-up was at least 1 year for all the enrolled patients alive at data cutoff. cGVHD was defined and scored according to the National Institutes of Health consensus criteria. Time to neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $>0.5 \times 10^9$/L after transplantation. Time to platelet engraftment was defined as the first of 3 consecutive days with a platelet count $>20 \times 10^9$/L without transfusion. Graft failure was defined as lack of donor-derived engraftment (<95% donor chimerism) by day 30. NRM was defined as death without evidence of disease. Relapse was defined by either morphological evidence of AML or MDS consistent with pretransplant features. Pretransplant minimal residual disease (MRD) was defined as any evidence of a defined abnormality using conventional cytogenetics, fluorescence in situ hybridization techniques, cytofluorimetric analysis, or molecular probes. GRFS was defined as occurrence of grades 3 to 4 aGVHD or cGVHD requiring systemic immunosuppressive treatment, disease relapse, or death from any cause during the first 12 months after hematopoietic stem cell transplantation (HSCT). Safety assessments included reports of adverse events graded according to the Common Terminology Criteria Adverse Events (version 4.03: 14 June 2010).

Statistical analysis

This was a single-arm phase 2 trial based on Fleming single-stage design. The MMUD transplant reference rate of aGVHD (grades 2-4) was assumed to be 50%.[13,14] We hypothesized to observe an absolute 15% aGVHD reduction with this protocol, from an expected 50% to 35%. A total of 78 patients were required to test this hypothesis with a 1-sided significance level of 0.05 and a power of 85%. According to the sample size, 31 was the maximum number of patients who may develop aGVHD to reject the null hypothesis.

A safety interim analysis was planned after the first 30 enrolled patients on the incidence of grades 2 to 4 GVHD. An independent panel of experts not involved in the study has monitored the study safety.

Patient, disease, and transplantation characteristics were summarized using descriptive statistics. Categorical variables are reported as absolute value and percentage, whereas quantitative variables are described as median value and range.

Cumulative incidence of relapse, NRM, aGVHD, and cGVHD was calculated using the Fine and Gray method, considering the respective competitive risks (death in remission for relapse, disease recurrence for NRM, and death for GVHD). One patient was censored at the data of second transplant.

Ethical consideration

The study was conducted according to Good Clinical Practice and the Declaration of Helsinki and was approved by the ethical committee of all participating centers. All patients provided written informed consent.

Results

Patient characteristics

Between 15 January 2020 and 10 November 2022, a total of 81 patients from 26 GITMO centers were screened. Four patients were excluded because they did not meet the inclusion criteria; 77 patients were available for the final analysis: 64 AML and 13 MDS. The baseline demographic disease and transplantation-related characteristics are reported in Table 1. Median age was 53 (range, 19-65) years, and 45 patients were males (5.4%). Of 64 patients with AML, 13 (20.3%) were classified as high risk according to ELN 2022 (European Leukemia NET), 15 (23.4%) had not achieved CR after first induction, and 28 (43.7%) had a positive MRD at transplant (specific molecular transcript or immunophenotype). The source of hematopoietic stem cells was peripheral blood for 72 patients (93.5%) and BM for 5 patients (6.5%). The interim analysis on the incidence of grades 2 to 4 aGVHD after the first 30 patients did not have a higher-than-expected risk of developing GVHD and/or a higher incidence of transplant-related mortality.

Primary end point: aGVHD

At day 100, cumulative incidence of grades 2 to 4 aGVHD was 18.2% (95% CI, 10.6-27.6; 90% CI, 1.6-25.9) (14 patients) (Figure1A; Table 2). Furthermore, 5 patients, 8 patients, and 1 patient had 1, 2, and 3 organs involved (5 only gut; 8 skin and gut; and 1 gut, skin, and liver), respectively. The gut was always an involved organ (14/14). Within 100 days from transplant, 5 patients (6.5%, 95% CI, 3.1; 15.1%) developed grades 3 to 4 aGVHD (Figure 1B) and 17 patients grade 1; 6 of them received a therapeutic dose of steroid.

Engraftment

There were 5 patients who failed to achieve engraftment and died while having cytopenia, and 1 patient had autologous recovery and underwent a second transplant from a different donor. Furthermore, 71 patients (92%) had full-donor chimerism by day +30 as tested on unmanipulated BM and selected peripheral blood $CD3^+$ cells, including 4 of 5 patients transplanted from BM. The median time (days) to neutrophil recovery was +17 (range, 10-35), and the median time to platelet recovery was +22 (range, 11-137). Cumulative incidence of engraftment was 88.3% for neutrophils on day +30 and 83.2% for platelets on day +60 (Figure 2).

cGVHD

The 1-year cumulative incidence of cGVHD and moderate/severe cGVHD was 13.4% (95% CI, 6.9-22.1) and 9.4% (95% CI, 4.1-17.2) (10 and 7 patients), respectively (Figure 1C).

Table 1. Patients, disease, and transplant characteristics

Patients	N = 77
Age, median (range), y	53 (19-65)
Patient sex, n (%)	
Male/female	45 (58.4%)/32 (41.6%)
ECOG, n (%)	
0	64 (83.1%)
>0	11 (14.3%)
Unknown	2 (2.6%)
HCT-CI, n (%)	
0-2	64 (83.1%)
≥3	13 (16.9%)
Disease	
AML, n (%)	64 (83.1%)
WHO classification, n (%)	
AML with recurrent genetic abnormalities	16 (25.0%)
AML with myelodysplasia-related changes	15 (23.4%)
Therapy-related myeloid neoplasm	4 (6.3%)
AML, NOS	28 (43.8%)
Myeloid sarcoma	1 (1.6%)
Cytogenetic risk (ELN 2022) , n (%)	
Favorable	14 (21.9%)
Intermediate	37 (57.8%)
Adverse	13 (20.3%)
Molecular markers	
FLT 3	15
NPM1	19
IDH1/IDH2	8
ASXL1	3
AML1/ETO RUNX1::RUNX1T1	2
BCR::ABL1	2
No CR postinduction therapy	15 (23.4%)
≥2 CR	11 (17%)
MRD positive at transplant, n (%)	
Molecular markers	14/64 (21%)
NPM	11 (17%)
AML1/ETO	2 (3%)
bcr/abl	1 (1%)
IF	14/64 (21%)
MDS, n (%)	13 (16.9%)
WHO classification, n (%)	
MDS with single lineage dysplasia	2 (15.4%)
MDS with multilineage dysplasia	2 (15.4%)
MDS with excess blasts	6 (46.1%)
MDS with isolated del(5q)	1 (7.7%)
MDS, unclassifiable	2 (15.4%)
HSCT up-front	5
Transplant and donors	
Diagnosis: transplant, median (range), d	202 (115-1482)
Year of the transplant, n (%)	
2020	21 (27.3%)
2021	32 (41.6%)
2022	24 (31.2%)
Conditioning Bu4Flu, n (%)	77 (100%)
Donor age, median (range), y	29 (18-62)
Donor sex, n (%)	
Male/female	51 (66.2%)/26 (33.8%)
Donor/recipient sex mismatch, n (%)	
Male/male	31 (40.2%)
Male/female	14 (18.2%)
Female/male	20 (26.0%)
Female/female	12 (15.6%)
CMV serostatus, n (%)	
+/+	34 (44.1%)
−/+	19 (24.7%)
+/−	9 (11.7%)
−/−	6 (7.8%)
Missing	9 (11.7%)
Stem cell source, n (%)	
PB	72 (93.5%)
BM	5 (6.5%)
Cell dose	
PB median (range), $CD34^+$, $\times 10^6$/kg	5.9 (2.2-12.2)
BM median (range), $CD34^+$, $\times 10^6$/kg	2.3 (1.4-5.3)
Donor HLA mismatches 7/8, n (%)	77 (100%)
HLA class I mismatch (A, B, C)	72 (93.5%)
HLA class II mismatch (DRB1)	3 (4%)
Missing	2 (2.5%)

CMV, cytomegalovirus; ECOG, Eastern Cooperative Oncology Group; HCT-CI, hematopoietic cell transplantation–comorbidity index; IF, immunophenotyping; NOS, not otherwise specified; PB, peripheral blood; WHO, World Health Organization.

Relapse incidence

The 1-year cumulative incidence of relapse rate was 23.8% (18 patients) (95% CI, 14.9-33.9) (Figure 1D). Relapse was the cause of death in 9 patients (12%). Considering only the AML cohort, 15 of 64 patients relapsed at 1 year.

NRM

The 1-year cumulative incidence of NRM was 9.1% (7 patients) (95% CI, 4.0-16.9) (Figure 1E). The causes of death were sepsis (n = 3), pneumonia (n = 2), and multi-organ failure (n = 2).

OS and GRFS

The 1-year GRFS and OS were 55.3% (95% CI, 43.4-65.7) and 78.6% (95% CI, 67.4-86.3), respectively (Figure 1F-G).

The overall outcomes are outlined in Table 2.

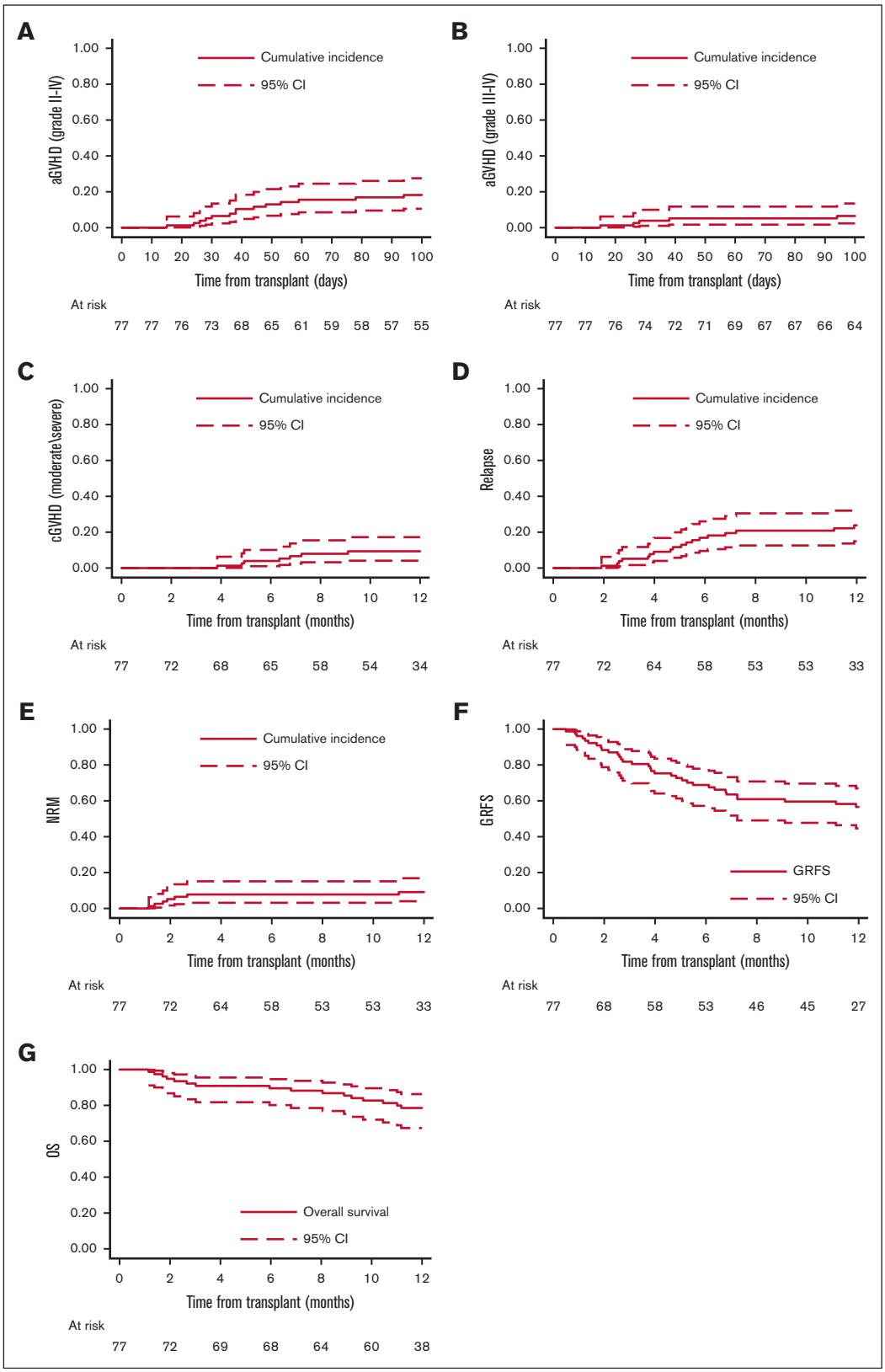

Figure 1. Overall outcomes. (A) Cumulative incidence of aGVHD grades 2 to 4 at 100 days. (B) Cumulative incidence of aGVHD grades 3 to 4 at 100 days. (C) Cumulative incidence of cGVHD moderate/severe at 12 months. (D) Cumulative incidence of relapse at 12 months. (E) Cumulative incidence of NRM at 12 months. (F) GRFS at 12 months. (G) OS at 12 months.

Table 2. End points

	No. of events	Estimate	95% CI, %	
Cumulative incidence of aGVHD at 100 days				
Any grade	28	39.0%	28.1	49.6
Grades 2-4	14	18.2%	10.6	27.6
Grades 3-4	5	6.5%	3.1	15.1
Cumulative incidence of cGVHD at 12 months				
Any grade	10	13.4%	6.9	22.1
Moderate/severe	7	9.4%	4.1	17.2
Cumulative incidence of relapse at 12 months	18	23.8%	14.9	33.9
Cumulative incidence of NRM at 12 months	7	9.1%	4.0	16.9
GRFS at 12 months	33	55.3%	43.4	65.7
OS at 12 months	16	78.6%	67.4	86.3

Toxicity

The most common serious adverse events were infections and infestations (24 events in 21 patients), gastrointestinal disorders (26 events in 18 patients), and blood and lymphatic system disorders (19 events in 14 patients) (Table 3). The most common toxicity was oral mucositis (26 patients). The most frequent infectious complications were of bacterial origin with 18 episodes of bloodstream infections, followed by viral reactivations (only 4 cytomegalovirus reactivations). Cardiovascular events occurred in 3 patients.

Discussion

This multicenter, single-arm phase 2 trial enrolling patients with myeloid malignancies demonstrated that a PTCy-based GVHD prophylaxis in HCT from a MMUD leads to a low rate of aGVHD, with a low incidence of NRM, and acceptable relapse rate. GVHD still represents a major limitation of HSCT in general, especially when there are 1 or more HLA mismatches between donor and recipient.[8,10] Posttransplant administration of cyclophosphamide has proven to be strategic to prevent GVHD, not only in the HLA-haploidentical HCT setting but also in HLA-matched related or unrelated HCT.[17-25,38] Nevertheless, only few systematic studies have confirmed the feasibility and efficacy of PTCy as GVHD prophylaxis in MMUD. Indeed, in an initial prospective study, in the context of non-MAC with BM stem cell source, a PTCy-based GVHD prophylaxis strategy prevented both incidence of graft failure and grades 3 to 4 aGVHD, suggesting a prominent role of PTCy in overcoming HLA differences also in an unrelated setting.[39] These data were confirmed in a subsequent study in the MMUD HCT using PBSC as the stem cell source, in which PTCy was found to lower GVHD and NRM at levels comparable to those observed in transplant from matched unrelated donors.[40] However, both studies enrolled patients with any hematologic malignancies, exploiting mostly non-MAC/reduced intensity conditioning regimens.

The PHYLOS trial aimed to investigate the impact of PTCy in AML and MDS in first or subsequent remission, receiving the same MAC, followed by a peripheral blood (PB) transplant in most patients. We adopted this regimen because in a previous GITMO study on patients with AML in CR at transplant,[41] this conditioning schedule, although still myeloablative, resulted in a lower

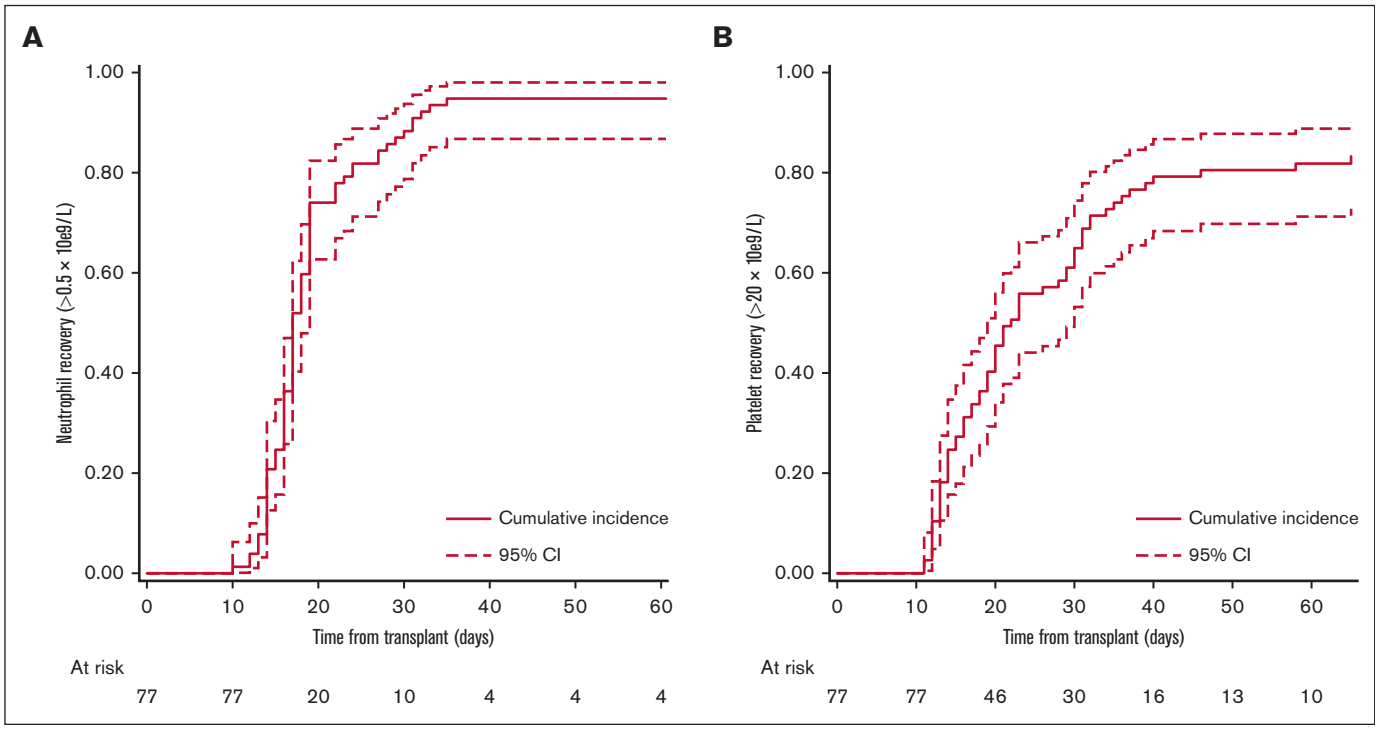

Figure 2. Count recovery. (A) Cumulative incidence of neutrophil recovery. (B) Cumulative incidence of platelet recovery.

Table 3. Toxicity grades 3 to 5 of adverse events until day 360 after HCT

	Grades 3-5	
Toxicity	**n**	**%**
Infections and infestations	21	27.27
Gastrointestinal disorders	18	23.38
Blood and lymphatic system disorders	14	18.18
General disorders	2	2.60
Respiratory, thoracic, and mediastinal disorders	8	10.39
Investigations	6	7.79
Vascular disorders	4	5.19
Metabolism and nutrition disorders	2	2.60
Eye disorders	1	1.30
Cardiac disorders	3	3.90
Musculoskeletal and connective tissue disorders	1	1.30
Renal and urinary disorders	1	1.30
Hepatobiliary disorders	1	1.30
Nervous system disorders	1	1.30
Psychiatric disorders	1	1.30

Adverse events graded according to the Common Terminology Criteria Adverse Events (version 4.03: 14 June 2010).

transplant-related mortality compared with busulfan and cyclophosphamide, even in older patients. Consistently, in our experience, the 1-year cumulative incidence of NRM was 9.2% with infections as the dominant cause of death.

Results from our trial are comparable with those reported in a NMDP prospective study, which reported 1-year rates of NRM, relapse, and OS of 8%, 30%, and 70%, respectively, in patients receiving a MAC.[42] However, in the NMDP study, BM was mandatory as stem cell source, and approximately one-third of patients were matched 4 to 6 of 8, whereas in our study there was no restriction in terms of stem cell source and most patients (72/77) were transplanted from PB (probably due to the ease of collection and an expected faster engraftment). Data on transplant outcomes according to the stem cell source (PB or BM) are lacking in this setting, and our study provides the first and more robust results with PTCy given in the context of a PB-based MMUD HSCT.

Relapse remains an issue, as in our study cumulative incidence was 24% at 1 year, with near one-third of patients harboring high-risk features (biological high-risk, first-line refractory, and/or MRD positive at transplant), comparable to that of previous trials, either prospective or retrospective. Recently, a meta-analysis that included several hundred patients undergoing MMUD HSCT with PTCy reported a pooled relapse rate of 44%.[43] Leukemia relapse after transplantation is a clinical need for patients with AML, especially for those with high-risk features. For this reason, in the future, innovative postengraftment treatments, such as cellular therapies or targeted drugs, should be integrated within the allogeneic HSCT (allo-HSCT) backbone, taking advantage of a possibly better GVHD prophylaxis which allows for a smoother post-HSCT clinical course.

Considering current experiences with MMUD, one might wonder whether a direct head-to-head, possibly randomized comparison between ATG and PTCy as GVHD prophylaxis strategy is possible and eventually needed to set PTCy as a new standard of care for MMUD HSCT.

Some responses come from 2 large retrospective studies that indirectly compared the 2 GVHD prophylaxis strategies. In 2019, an EBMT retrospective study analyzed the results of allo-HSCT from MMUDs in a homogenous population of patients with AML, comparing the outcomes of PTCy vs ATG.[37] This study assigned to PTCy a lower rate of grades 3 to 4 aGVHD and similar rates of grades 2 to 4 aGVHD and any-grade cGVHD as compared with ATG. Furthermore, the authors observed superior survival outcomes in terms of leukemia-free survival and GRFS with the use of PTCy. Penack and EBMT[38] recently published an analysis on 2123 peripheral blood allo-HSCT, from MMUD (9/10 antigen matched) transplanted between January 2018 and June 2021. In their experience, PTCy was associated with an advantage in NRM, 18% vs 24.9% ($P = .028$), and in OS, 65.7% vs 55.7% (<0.001), in comparison to rabbit anti-thymocyte globulin. Progression-free survival was also better with PTCy at 59.1% vs 48.8% for rabbit anti-thymocyte globulin ($P = .001$). Interestingly, the incidence of cGVHD and aGVHD was not significantly different between the 2 groups. These results were confirmed by smaller monocentric experiences,[31,44] but patients analyzed in these studies were heterogenous for conditioning, stem cell source, and phase of disease. Whether these data and the results from prospective, nonrandomized trials (including PHYLOS) are sufficient to consider PTCy as the standard for GVHD prophylaxis with MMUD is not clear.

Our trial has some limitations, such as the absence of a control group, a relatively short follow-up (1 year), and the possible impact of different HLA loci mismatches.

In conclusion, the PHYLOS reveals that PTCy-based MMUD transplants are feasible, safe, and effective, with results superimposable to those of haplo-related HSCT. Available data are not mature enough to conclude whether PTCy should be the preferred GVHD prophylaxis in this setting, but they eventually pave the way for future investigations exploiting the PTCy platform even outside the classical setting of haploidentical HSCT. In the future, the role of abatacept will also be a subject for discussion, due to the very promising results achieved in prospective studies after approval by the US Food and Drug Administration as GVHD prophylaxis in unrelated transplants.[45] This is even more important nowadays, in consideration of the emerging data driving donor selection. Indeed, the choice between a mismatched volunteer donor and a haplo-related donor could be no more hierarchical but inclusive of other factors, such as donor's age, sex mismatch, presence of anti-HLA antibodies, and/or AB0 incompatibility,[46] together with the time required to find a suitable donor and the related costs. Further prospective studies are necessary to better investigate the role of PTCy in different HSCT settings and to dissect the possible impact of specific HLA loci (or even epitopes) or other biological features on GVHD, disease relapse, and finally long-term outcome of HSCT from other than matched related donors.

Acknowledgments

The authors thank all physicians and nursing staff of Gruppo Italiano Trapianto di Midollo Osseo (GITMO) centers for the

contribution to the study and for clinical care of patients enrolled in this study. The authors also thank the data manager and the trial office of GITMO.

Authorship

Contribution: A.M. Raiola designed the study and wrote the manuscript; B.B., A.M. Risitano, E.A., and F.B. designed the study and reviewed the manuscript; G.C. and A.C. performed statistical analysis; G.C., A.C., and E.D. extracted and analyzed row data updating reference list and sent queries to the participating centers; B.B., A.M. Risitano, F.M., I.M.C., F.O., G.S., F.P., M.L.B., V.P., A.M., P.C., S.S., B.L., C.d.G., A.M.C., D.S., E.M., A.L., L.G., P.B., E.T., N.M., C.B., F.Z., M.L., A.G., A.O., E.P., N.S., and M.M. screened potential eligible patients, enrolled them, and provided follow-up data; and all authors approved the final version of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: A.M. Raiola, 0000-0002-5118-4167; B.B., 0000-0002-7299-6770; A.M. Risitano, 0000-0002-4744-5109; F.O., 0000-0002-2991-3474; V.P., 0000-0003-1712-2744; P.C., 0000-0002-1355-1587; A.M.C., 0000-0002-4967-4204; A.L., 0000-0002-2177-2064; N.M., 0000-0002-4969-1434; A.G., 0000-0002-2806-8336; E.P., 0009-0009-3256-7152; N.S., 0000-0002-7164-401X; G.C., 0000-0001-7644-9574; A.C., 0000-0002-3415-3617; E.A., 0000-0002-6512-6080; M.M., 0000-0002-3987-419X; F.B., 0000-0003-1544-9911.

Correspondence: Anna Maria Raiola, Istituo di Ricovero E Cura A Carattere Scientifico Ospedale Policlinico San Martino, Largo R Benzi 10, 16132 Genoa, Italy; email: annamaria.raiola@hsanmartino.it.

References

1. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110(13):4576-4583.
2. Anasetti C, Beatty PG, Storb R, et al. Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma. *Hum Immunol*. 1990;29(2):79-91.
3. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood*. 2004;104(7):1923-1930.
4. Woolfrey A, Klein JP, Haagenson M, et al. HLA-C antigen mismatch is associated with worse outcome in unrelated donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2011;17(6):885-892.
5. Michallet M, Sobh M, Serrier C, et al. Allogeneic hematopoietic stem cell transplant for hematological malignancies from mismatched 9/10 human leukocyte antigen unrelated donors: comparison with transplants from 10/10 unrelated donors and human leukocyte antigen identical siblings. *Leuk Lymphoma*. 2015;56(4):999-1003.
6. Mediwake H, Curley C, Butler J, et al. Mismatched unrelated donor allogeneic stem cell transplant for high risk haematological malignancy: a single centre experience. *Blood Cancer J*. 2017;7(12):655-664.
7. Kanda J. Effect of HLA mismatch on acute graft-versus-host disease. *Int J Hematol*. 2013;98(3):300-308.
8. Jagasia M, Arora M, Flowers MED, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. *Blood*. 2012;119(1):296-307.
9. Ho VT, Kim HT, Liney D, et al. HLA-C mismatch is associated with inferior survival after unrelated donor non-myeloablative hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2006;37(9):845-850.
10. Saber W, Opie S, Rizzo JD, Zhang MJ, Horowitz MM, Schriber J. Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia. *Blood*. 2012;119(17):3908-3916.
11. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the US registry. *N Engl J Med*. 2014;371(4):339-348.
12. Robin M, Porcher R, Ruggeri A, et al. HLA-mismatched donors in patients with myelodysplastic syndrome: an EBMT registry analysis. *Biol Blood Marrow Transplant*. 2019;25(1):114-120.
13. Bachanova V, Burns LJ, Wang T, et al. Alternative donors extend transplantation for patients with lymphoma who lack an HLA matched donor. *Bone Marrow Transplant*. 2015;50(2):197-203.
14. Verneris MR, Lee SJ, Ahn KW, et al. HLA mismatch is associated with worse outcomes after unrelated donor reduced-intensity conditioning hematopoietic cell transplantation: an analysis from the Center for International Blood and Marrow Transplant Research. *Biol Blood Marrow Transplant*. 2015;21(10):1783-1789.
15. Finke J, Schmoor C, Lang H, Potthoff K, Bertz H. Matched and mismatched allogeneic stem-cell transplantation from unrelated donors using combined graft-versus-host disease prophylaxis including rabbit anti-T lymphocyte globulin. *J Clin Oncol*. 2003;21(3):506-513.
16. Mead AJ, Thomson KJ, Morris EC, et al. HLA-mismatched unrelated donors are a viable alternate graft source for allogeneic transplantation following alemtuzumab-based reduced-intensity conditioning. *Blood*. 2010;115(25):5147-5153.
17. Luznik L, O'Donnell PV, Fuchs EJ. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical bone marrow transplantation. *Semin Oncol*. 2012;39(6):683-693.
18. 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(1):117-122.

19. Kanakry CG, Fuchs EJ, Luznik L. Modern approaches to HLA-haploidentical blood or marrow transplantation. *Nat Rev Clin Oncol*. 2016;13(1):10-24.

20. Kasamon YL, Luznik L, Leffell MS, et al. Nonmyeloablative HLA-haploidentical bone marrow transplantation with high-dose posttransplantation cyclophosphamide: effect of HLA disparity on outcome. *Biol Blood Marrow Transplant*. 2010;16(4):482-489.

21. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641-650.

22. McCurdy SR, Kanakry JA, Showel MM, et al. Risk-stratified outcomes of nonmyeloablative HLA-haploidentical BMT with high-dose posttransplantation cyclophosphamide. *Blood*. 2015;125(19):3024-3031.

23. Luznik L, Bolaños-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. *Blood*. 2010;115(16):3224-3230.

24. Kanakry CG, Bolaños-Meade J, Kasamon YL, et al. Low immunosuppressive burden after HLA-matched related or unrelated BMT using posttransplantation cyclophosphamide. *Blood*. 2017;129(10):1389-1393.

25. Mielcarek M, Furlong T, O'Donnell PV, et al. Posttransplantation cyclophosphamide for prevention of graft-versus-host disease after HLA-matched mobilized blood cell transplantation. *Blood*. 2016;127(11):1502-1508.

26. Greco R, Lorentino F, Albanese S, et al. Posttransplantation cyclophosphamide- and sirolimus-based graft-versus-host-disease prophylaxis in allogeneic stem cell transplant. *Transplant Cell Ther*. 2021;27(9):776.e1-776.e13.

27. Sun X, Yang J, Cai Y, et al. Low-dose antithymocyte globulin plus low-dose posttransplant cyclophosphamide combined with cyclosporine and mycophenolate mofetil for prevention of graft-versus-host disease after HLA-matched unrelated donor peripheral blood stem cell transplantation. *Bone Marrow Transplant*. 2021;56(10):2423-2431.

28. Bradstock KF, Bilmon I, Kwan J, et al. Single-agent high-dose cyclophosphamide for graft-versus-host disease prophylaxis in human leukocyte antigen-matched reduced-intensity peripheral blood stem cell transplantation results in an unacceptably high rate of severe acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2015;21(5):941-944.

29. Holtick U, Chemnitz J-M, Shimabukuro-Vornhagen A, et al. OCTET-CY: a phase II study to investigate the efficacy of post-transplant cyclophosphamide as sole graft-versus-host prophylaxis after allogeneic peripheral blood stem cell transplantation. *Eur J Haematol*. 2016;96(1):27-35.

30. Mehta RS, Saliba RM, Chen J, et al. Post-transplantation cyclophosphamide versus conventional graft-versus-host disease prophylaxis in mismatched unrelated donor haematopoietic cell transplantation. *Br J Haematol*. 2016;173(3):444-455.

31. Modi D, Kondrat K, Kim S, et al. Post-transplant cyclophosphamide versus thymoglobulin in HLA-mismatched unrelated donor transplant for acute myelogenous leukemia and myelodysplastic syndrome. *Transplant Cell Ther*. 2021;27(9):760-767.

32. Nykolyszyn C, Granata A, Pagliardini T, et al. Posttransplantation cyclophosphamide vs. antithymocyte globulin as GVHD prophylaxis for mismatched unrelated hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2020;55(2):349-355.

33. Paviglianiti A, Ngoya M, Peña M, et al. Graft-versus-host-disease prophylaxis with ATG or PTCY in patients with lymphoproliferative disorders undergoing reduced intensity conditioning regimen HCT from one antigen mismatched unrelated donor. *Bone Marrow Transplant*. 2024;59(5):597-603.

34. Al Malki MM, Tsai N-C, Palmer J, et al. Posttransplant cyclophosphamide as GVHD prophylaxis for peripheral blood stem cell HLA-mismatched unrelated donor transplant. *Blood Adv*. 2021;5(12):2650-2659.

35. Sugita J, Kuroha T, Ishikawa J, et al. Posttransplant cyclophosphamide in unrelated and related peripheral blood stem cell transplantation from HLA-matched and 1 allele mismatched donor. *Bone Marrow Transplant*. 2024;59(3):344-349.

36. Shaw BE, Jimenez-Jimenez AM, Burns LJ, et al. Three-year outcomes in recipients of mismatched unrelated bone marrow donor transplants using post-transplantation cyclophosphamide: follow-up from a National Marrow Donor Program-sponsored prospective clinical trial. *Transplant Cell Ther*. 2023;29(3):208.e1-208.e6.

37. Battipaglia G, Labopin M, Kröger N, et al. Posttransplant cyclophosphamide vs antithymocyte globulin in HLA-mismatched unrelated donor transplantation. *Blood*. 2019;134(11):892-899.

38. Penack O, Abouqateb M, Peczynski C, et al. PTCy versus ATG as graft-versus-host disease prophylaxis in mismatched unrelated stem cell transplantation. *Blood Cancer J*. 2024;14(1):45-48.

39. Kasamon YL, Ambinder RF, Fuchs EJ, et al. Prospective study of nonmyeloablative, HLA-mismatched unrelated BMT with high-dose posttransplantation cyclophosphamide. *Blood Adv*. 2017;1(4):288-292.

40. Rappazzo KC, Zahurak M, Bettinotti M, et al. Nonmyeloablative, HLA-mismatched unrelated peripheral blood transplantation with high-dose post-transplantation cyclophosphamide. *Transplant Cell Ther*. 2021;27(11):909.e1-909.e6.

41. Rambaldi A, Grassi A, Masciulli A, et al. Busulfan plus cyclophosphamide versus busulfan plus fludarabine as a preparative regimen for allogeneic haemopoietic stem-cell transplantation in patients with acute myeloid leukaemia: an open-label, multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2015;16(15):1525-1536.

42. Shaw BE, Jimenez-Jimenez AM, Burns LJ, et al. National Marrow Donor Program-sponsored multicenter, phase II trial of HLA-mismatched unrelated donor bone marrow transplantation using post-transplant cyclophosphamide. *J Clin Oncol*. 2021;39(18):1971-1982.

43. Mushtaq MU, Shahzad M, Tariq E, et al. Outcomes with mismatched unrelated donor allogeneic hematopoietic stem cell transplantation in adults: a systematic review and meta-analysis. *Front Oncol*. 2022;12:1005042.

44. Soltermann Y, Heim D, Medinger M, et al. Reduced dose of post-transplantation cyclophosphamide compared to ATG for graft-versus-host disease prophylaxis in recipients of mismatched unrelated donor hematopoietic cell transplantation: a single-center study. *Ann Hematol*. 2019;98(6):1485-1493.

45. Watkins B, Qayed M, McCracken C, et al. McCrackenPhase II trial of costimulation blockade with abatacept for prevention of acute GVHD. *J Clin Oncol*. 2021;39(17):1865-1877.

46. Nagler A, Labopin M, Swoboda R, et al. Young (< 35 years) haploidentical versus old (≥ 35 years) mismatched unrelated donors and vice versa for allogeneic stem cell transplantation with post-transplant cyclophosphamide in patients with acute myeloid leukemia in first remission: a study on behalf of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2024;59:1552-1562.