Biol Blood Marrow Transplant 25 (2019) 1395–1406

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Haploidentical

Donor-Specific Anti-HLA Antibodies in Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide: Risk of Graft Failure, Poor Graft Function, and Impact on Outcomes

Stefania Bramanti[1,#], Valeria Calafiore[1,*,#], Elena Longhi[2], Jacopo Mariotti[1], Loretta Crespiatico[2], Barbara Sarina[1], Chiara De Philippis[1], Angela Nocco[2], Armando Santoro[1], Luca Castagna[1]

[1] *Bone Marrow Unit, Humanitas Cancer Center, Rozzano, Milan, Italy*
[2] *Laboratory of Transplant Immunology, IRCCS Ca 'Granda Foundation Maggiore Policlinico Hospital Milan, Italy*

Article history:
Received 23 December 2018
Accepted 18 February 2019

Key Words:
Haploidentical stem cell transplantation
Post-transplantation cyclophosphamide
Donor-specific anti-HLA antibodies
Graft failure
Poor graft function

A B S T R A C T

The presence of donor-specific anti-HLA antibodies (DSA) is associated with a 10-fold increased risk of graft failure in haploidentical stem cell transplantation (haplo-SCT). Consensus guidelines from the European Society for Blood and Marrow Transplantation set a mean fluorescence intensity (MFI) >1000 as a cutoff for DSA positivity. In the absence of an alternative donor, it is recommended that patients undergo desensitization therapy, especially with high DSA levels (>5000 MFI). The aim of this study was to analyze the impact of DSA on risk of graft failure and poor graft function, as well as on major outcomes in a consecutive cohort of patients who were systematically screened for DSA before haplo-SCT. A total of 141 consecutive patients were candidates for unmanipulated haplo-SCT with post-transplantation cyclophosphamide (PT-Cy) at our center between January 2012 and January 2018, and 135 were analyzed for the presence of HLA antibodies. Of these 134 patients underwent haplo-SCT. HLA antibodies were detected in 40 patients, including 19 with DSA and 21 without DSA. Ten of the 19 patients with DSA underwent transplantation using that donor, whereas 2 underwent a desensitization program before transplantation. Only 2 patients experienced primary graft failure (1.4 %), both of whom were without DSA. Twenty patients developed a poor graft function (15%). The 3-year overall survival (OS), 3-year progression-free survival (PFS), and 1-year nonrelapse mortality (NRM) were analyzed according to the presence or absence of DSA. No statistically significant difference was found. No impact of the presence of DSA on the risk of developing graft failure and poor graft function was revealed. Major outcomes of transplantation were analyzed separately in patients with poor graft function and those with good graft function. The 3-year OS, 3-year PFS, and 1-year NRM in good graft function and poor graft function populations were 62% versus 20% ($P < .0001$), 53% versus 20% ($P < .0001$), and 12% versus 40% ($P = .009$), respectively. The presence of low-level DSA in the absence of desensitization did not correlate with the risk of developing graft failure and poor graft function. Patients who experienced poor graft function had worse outcomes than patients with good graft function.

INTRODUCTION

Allogeneic stem cell transplantation (SCT) is a curative option for many hematologic malignancies. The probability of finding an HLA-identical donor in the family is only 25%. Despite the existence of a worldwide registry of HLA-typed volunteer donors, approximately 40% of patients do not identify any HLA-identical donor at the proper time for the clinical need. Alternative donors include partially matched unrelated donors, haploidentical related donors, and cord blood units [1]. Post-transplantation cyclophosphamide (PT-Cy) is an attractive approach for crossing the HLA barrier in unmanipulated haploidentical SCT (haplo-SCT) because the treatment is cheap, strikingly effective, and requires no special expertise beyond chemotherapy administration [2].

Nonetheless, in haplo-SCT, the HLA disparity between the donor and recipient can lead to an intense bidirectional alloreactivity, not only in the graft-versus-host direction, but also in the host-versus-graft direction, leading to a higher predisposition for developing primary graft failure. The mortality rate in case of graft failure is high owing to infectious complications, and salvage rescue could include a second allogeneic SCT [3]. The incidence of graft failure in the setting of PT-Cy-based haplo-HSCT, using either a myeloablative conditioning (MAC) or nonmyeloablative (NMA) conditioning regimen, ranges from 0 to 30% [4]. In haplo-SCT, in vivo T cell depletion using

Financial disclosure: See Acknowledgments on page 1406.
* Correspondence and reprint requests: Valeria Calafiore, Via Alessandro Manzoni n. 56, 20089 Rozzano Milan, Italy.
E-mail address: valeriacalaf@gmail.com (V. Calafiore).
S.B. and V.C. are co-first authors.

https://doi.org/10.1016/j.bbmt.2019.02.020
1083-8791/

PT-Cy overcomes T cell- and NK cell-mediated graft rejection, and in this situation, antibody-mediated rejection appears to be one of the principal mechanisms of primary graft failure. The presence of DSA against the unshared haplotype in the recipient was associated with a 10-fold increased risk of engraftment failure [5]. In adult patients with hematologic malignancies, the prevalence of anti-HLA antibodies can be up to 40%; however, not all of these anti-HLA antibodies are directed against donor HLA antigens. In haplo-SCT, the prevalence of DSA may range between 10% and 21% [5-8]. The prevalence of DSA is lower in male recipients (5%) compared with female recipients (86%) [9], because pregnancy is a cause of these antibodies.

Another complication following haplo-SCT is the occurrence of poor graft function. Graft function may be poor as a result of slow or incomplete recovery of blood counts (primary poor graft function) or decreasing blood counts after a successful hematopoietic engraftment (secondary poor graft function) [10]. The main causes of poor graft function are conditioning, graft-versus-host disease (GVHD), bacterial sepsis, viral infections (eg, cytomegalovirus [CMV], human herpesvirus 6) [11] and the use of myelotoxic drugs [12]. Definitions of poor graft function are extremely heterogeneous, and there are no specific cutoff values for hemoglobin, platelets, and white blood cells [13-15]. For these reasons, poor graft function incidence varies from 5% to 27% among patients undergoing allo-SCT [10,16,17]. Poor graft function is associated with considerable morbidity and mortality related to infections and hemorrhagic complications [14].

The aim of the present single-center retrospective study was to analyze the rate of graft failure and the occurrence of poor graft function according to DSA status in a cohort of patients with hematologic malignancy treated with haplo-SCT with PT-Cy.

METHODS

Patients and Donors

All consecutive patients evaluated for haplo-SCT at the Bone Marrow Unit of Humanitas Cancer Center in Rozzano, Italy between January 2012 and January 2018 were retrospectively reviewed. All patients provided informed consent for the retrospective collection of their data.

According to our institutional policy, patients who are candidates for transplantation underwent simultaneous sibling, unrelated, and haploidentical donor searches. Potential haploidentical donors considered included both first-degree and non-first-degree relatives (ie, brothers, aunts, uncles, cousins, nieces, nephews, grandchildren, parents, and children).

Haploidentical donor selection was done first based on the absence of DSA. Other factors taken into account were patient and donor CMV serostatus, blood group (avoiding major and minor incompatibilities only in cases of bone marrow as source), and sex (avoiding a female donor with previous gestation for a male recipient), age, weight ratio between donor and recipient (avoiding donors weighing $\geq$20 kg less than the recipient). The presence of DSA was considered an exclusion criterion for potential donors, except in patients with no other donor options.

HLA Typing

HLA typing was performed for 2 different peripheral blood samples. In aplastic patients, a buccal swab served as the DNA source. DNA was isolated with a Qiagen EZ1 instrument (Qiagen, Hilden, Germany), and purity and concentration were evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 260 and 280 nm. HLA-A, -B, -C, -DRB1, and -DQB1 low-resolution typing was performed by Luminex technology using sequence-specific oligonucleotide probes and locus-specific primers (LabType SSO, Lambda Array Beads Multi-Analyte System; One Lambda, Canoga Park, CA) [18]. High-resolution typing was performed by Sanger sequence-based typing. HLA-A, -B, -C, -DRB1, and -DQB1 loci were first amplified with locus- and group-specific primers (HLAssure SE B Locus SBT Kit; Texas BioGene, Richardson, TX) to separate the 2 alleles. Exons 2, 3, and 4 for class I and exon 2 for class II were then sequenced in isolation and in both directions. When necessary to exclude ambiguities, extra exons were amplified and sequenced. Sequences were processed with an Applied Biosystems 3130 Genetic Analyzer (Thermo Fisher Scientific) and analyzed with AccuType software (Nova Biosoft, Norman, OK).

DSA Analysis: Solid-Phase Immunoassays

The presence of DSA was tested using the Luminex technology. EDTA-treated serum samples from the recipients were analyzed for class I and class II IgG HLA antibodies using the commercially available LABScreen Single Antigen Beads Class I and Class II Assay Kit (One Lambda) that consists of beads with 1 HLA molecule attached (either class I or class II), referred to as single antigen beads. The procedure was performed according to the manufacturer's instructions and then analyzed on a LABScan200 flow analyzer (One Lambda). Luminex 100 IS version 2.3 software (Luminex Corporation, Austin, TX) was used for data acquisition, and data analysis was done with HLA Fusion software 4.2 (One Lambda). Results were interpreted using MFI values. All samples with an MFI value >1000 were considered positive.

The C1-q assay allows the detection of only complement-fixing HLA-specific antibodies. Class I and class II C1q-SAB assays (One Lambda) were performed in serum samples according to the manufacturer's directions. In brief, heat-inactivated serum (56 °C for 30 minutes) was spiked with 150 mg/mL purified human C1q in Hepes buffer (One Lambda) to ensure equal functional amounts of C1q per sample. Single antigen beads were added to the mixture and incubated for 20 minutes at room temperature, followed by the addition of phycoerythrin-conjugated anti-human C1q. Beads were washed twice and analyzed on a LABScan200 flow analyzer (One Lambda). The C1q antibody MFI threshold for positivity was 1000.

Cell-Based Assays

Patient sera were crossmatched against lymphocytes from potential donors at the time of pretransplantation screening. The crossmatch (XM) techniques used were standard complement- dependent cytotoxicity crossmatch (CDCXM) and flow cytometry crossmatch (FCXM) tests.

CDCXM

CDCXM assays were performed according to EFI standards for histocompatibility and immunogenetics testing. In brief, Dynabeads (LubioScience, Zurich, Switzerland) (HLA class I and class II; www.lifetechnologies.com) were used to isolate $CD8^+$ T cells and $CD19^+$ B cells from peripheral blood according to the manufacturer's instructions. Duplicate CDC reactions were performed using Terasaki trays (One Lambda) by incubating 1 μL of isolated donor cells with 1 μL of recipient serum. CDCXM was performed on untreated serum and in serum with added dithiothreitol to identify any positivity due to the presence of IgM antibodies.

Using fluorescence microscopy, cytotoxicity was determined by enumerating dead cells (results are reported using the internationally used National Institutes of Health cell death scoring rate) after incubation with 5 μL of rabbit complement. Dead cells are identified with FluoroQuench fluorescent dye (acridine orange .3 mg/mL and ethidiumbromide 1 mg/mL; One Lambda). A positive CDC crossmatch was assessed when >20% of cells died after incubation with raw sera.

FCXM

In the FCXM assays, 50 μL of recipient serum and 30 μL of donor lymphocytes (3×10^5 cells) were incubated for 30 min at 4 °C to facilitate DSA antibody binding. The cells were washed twice with 2 mL of PBS1X and then centrifuged at $500 \times g$ for 5 minutes. Subsequently, 20 μL of anti-human immunoglobulin (anti-IgG-FITC) were added to the cells, followed by incubation at 4 °C in the dark to bind the attached DSA. Cells were washed twice more, after which an antibody cocktail (5 μL of anti-CD3-PeCy 5.5 and 5 μL of anti-CD19-APC) was added to the cells. Cells were then washed once in 1 mL of PBS1X and resuspended in .2 mL of PBS1X before acquisition by flow cytometry. Three-color flow cytometry analysis was performed with a FACS-Canto instrument (BD Biosciences, San Jose, CA). Lymphocytes were gated on the basis of their forward- and side-scatter characteristics. The median channel fluorescence for anti-human IgG F(ab)' FITC was quantified on $CD3^+$ T cells and $CD19^+$ B cells. Optimization experiment crossmatch data were expressed as the median channel fluorescence shift (MCFS) from the negative control serum. Validation experiment crossmatch data were expressed as MCFS from the 3 SD cutoff, determined using the negative patient serum FCXM median channel fluorescence values. The positive FCXM cutoffs used by our laboratory were >87 MCFS for T cells and >120 MCFS for B cells. A positive crossmatch was identified when the sample median fluorescence intensity exceeded that of negative control values by 3 SD.

Conditioning Regimen and GVHD Prophylaxis

The NMA conditioning regimen comprised Cy 14.5 mg/kg on days -5 and -6, fludarabine 30 mg/m^2 from day -6 to day -2, and low-dose total body irradiation (TBI; 2 Gy) on day -1. The MAC regimen comprised thiotepa 5 mg/kg on days -6 and -5, fludarabine 50 mg/m^2 on days -5 to -3, and busulfan 3.2 mg/kg on days -4 and -2. The RIC regimen comprised (1) thiotepa 10 mg/kg on day -6, cyclophosphamide 30 to 60 mg/kg on day -5, fludarabine 30 mg/m2 on days -5 to -2, and low-dose TBI (2 Gy) on day -1; (2) thiotepa 5 mg/kg on days -6 and -5, fludarabine 50 mg/m2 on days -5 to -3, i.v. busulfan 3.2 mg/kg on days -4 and -3; or (3) thiotepa 5 mg/kg on day -6,

fludarabine 50 mg/m2 on days -5 to -3, and busulfan 3.2 mg/kg on days -4 and -3. Only 5 patients received a sequential conditioning regimen because of refractory disease.

GVHD prophylaxis consisted of PT-Cy 50 mg/kg administered on days +3 and +4, tacrolimus (FK) or cyclosporine A (CSA), and mycophenolate mofetil (MMF) started on day +5. A continuous infusion of FK (total dose of 1 mg) or CSA (3 mg/kg) was administered during hospitalization and then switched to oral formulation after discharge. The dosage was adjusted based on the respective range of activity of these 2 drugs. MMF was administered at total dose of 45 mg/kg/day orally until day +35. The dose of FK/CSA was progressively reduced by day +100 to day +180. Patients received granulocyte colony-stimulating factor (G-CSF) starting on day +5.

Supportive Care

Patients received prophylaxis against bacterial, viral, and fungal agents. Monitoring for CMV and Epstein-Barr virus reactivation by PCR was performed twice weekly during the early period (day +15 to day +100) and then weekly thereafter (from day +100 to day +180).

RBC transfusions were performed in patients with symptomatic anemia or a hemoglobin level <8 g/dL. Platelet transfusions were performed considering a threshold level of 10,000 platelets/μL; a higher threshold (20,000 platelets/μL) was considered in patients with bleeding or fever.

Desensitization Regimen

Patients with an MFI level between 1000 to 3000 and/or a negative CDCXM or FCXM test were not desensitized. The desensitization protocol used was based on the Johns Hopkins University experience [19] and consisted of alternate days of plasma exchange with 1 volume of albumin exchange and polyvalent i.v. immunoglobulin at 500 mg/kg (days -14, -12, -10, -8, and -1), i.v. FK at 1 mg or CSA at 3 mg/kg/day as a continuous infusion, and MMF at 1 g twice daily (from day -14 to day -8) [20]. If the DSA level on day -1 was not negative, a postinfusion dose of rituximab was given.

Definitions

Neutrophil engraftment was defined as the first of 3 consecutive days when the absolute neutrophil count achieved $.5 \times 10^9$/L without G-CSF stimulation. Platelet engraftment was defined as the first of 7 consecutive days when the platelet count was $\geq 20 \times 10^9$ L^{-1}, independent of platelet transfusion.

Graft failure was defined as no appearance or complete loss of donor-derived neutrophils after transplantation using chimerism analysis, excluding progressive hematologic malignancy. Poor graft function was defined using cutoff values and criteria reported in a recent study published by Sun et al [21]: persistent neutropenia (neutrophils $<.5 \times 10^9$/L), thrombocytopenia (platelets $<20 \times 10^9$/L), and/or hemoglobin <7 g/L for at least 3 consecutive days by day +28 post-transplantation; transfusion dependence; association with hypoplastic/aplastic bone marrow; and complete donor chimerism without concurrent GVHD or disease relapse. Secondary poor graft function was defined using the same criteria but occurring after initial engraftment. Concomitant infections were excluded from the analysis.

Chimerism was evaluated within day +100 via microsatellite PCR detection of peripheral blood chimerism for total leukocyte in cases of sex-matched pairs. For sex-mismatched couples, chimerism status was performed by FISH analysis. Mixed donor chimerism was defined as >5% but <95% donor; full donor chimerism was defined as >95% donor.

Statistical Analyses

All clinical data were analyzed retrospectively using SPSS software (IBM, Armonk, NY). Categorical variables were reported as proportion; continuous variables, as median and range. The cumulative incidences of acute GVHD and chronic GVHD were estimated, with death without GVHD as the competing event [22]. OS and PFS were calculated using the Kaplan-Meier method [23]. In the calculation of NRM, disease relapse and progression were considered competing events, whereas NRM was a competing event to establish the cumulative incidence of relapse or progression. Outcomes (and respective 95% confidence intervals [CIs]) were calculated from the date of transplantation. The incidence of primary poor graft function was calculated by the proportion of poor graft function in the total cohort of patients alive by day +28 after haplo-SCT. Univariate analysis for comparing crude incidence rates was performed using the chi-square test or Fisher's exact test. Risk factors with $P < .20$ in univariate analysis were chosen for further evaluation by multivariate logistic regression.

RESULTS

Patient characteristics are summarized in Table 1. A total of 141 patients underwent haplo-SCT with PT-Cy between January 2012 and January 2018, of whom 134 (96%) were screened for DSA. All samples with an MFI value >1000 were considered positive. Forty patients (30%) were HLA antibody-positive, 21 (52%) were non-DSA-positive, and 19 (48%) were DSA-positive (Figure 1).

Antibodies against HLA molecules were most frequently detected in female patients compared with male patients (63% versus 38%; $P = .0149$) and in patients who underwent allo-SCT for acute leukemia/myelodysplastic syndrome (58% versus 33%; $P = .0081$). Consistently, DSA was significantly more frequent in female patients (79% versus 38%; $P = .0012$); 80% of the DSA-positive females were parous, and 58% of these patients were offspring to mother. Most of the DSA-positive patients had antibodies against class I HLA antigens (71%), and fewer had antibodies against class II HLA antigens (21%). Characteristics of the DSA-positive patients are presented in Table 2.

No therapeutic measures have been taken for patients with anti-HLA antibodies who did not react with donor specificities (non-DSA-positive group) [24]. Among the 19 DSA-positive patients, 9 performed haplo-SCT from an alternative haploidentical donor without DSA, and they were included in non DSA group; the other 10 patients underwent transplantation with that donor despite DSA. Patients with MFI level between 1000 and 3000 and/or a negative CDCXM or FCXM test were not desensitized. Desensitization treatment was performed in 2 patients. Both patients presented with MFI values >10,000, and one of them also had a positive CDCXM. DSA reduction to a low MFI level (1000 to 3000) was achieved in 1 of 2 patients by the end of the desensitization regimen. The other patient did not achieve DSA reduction after desensitization; she underwent transplantation with active disease and died of sepsis in aplasia at day +12 post-transplantation. Among the 8 patients who were not desensitized despite DSA positivity, 6 had an MFI between 1000 and 3000, and the other 2 had a moderate/high DSA level between 3000 and 10,000 MFI, with negative CDCXM results. Notably, only 2 of these patients had received a MAC regimen before transplantation.

Additional Tests in DSA-Positive Patients

The CDCXM assay was performed in 11 of the 19 DSA-positive patients and was positive in 3 out of 5 patients with an MFI >10,000 and negative in the 1 patient with 5000<MFI<10,000, the 3 patients with MFI <3000, and in the 2 patients with 3000<MFI<5000. The CDCXM test was concordant with MFI level only in negative cases. The FCXM assay was performed in 5 patients and was positive in the 2 patients with MFI >5000 and negative in the 3 patients with MFI <3000. C1q was tested in only 1 patient and was negative.

Outcomes of All Patients

Overall, the median duration of follow-up was 28 months (range, 1.2 to 74 months). The 3-year OS was 50% (95% CI, 41% to 59%), 3-year PFS was 44% (95% CI, 35% to 53%), and 1-year NRM was 20% (95% CI, 13% to 27%). The cumulative incidences of grade II-IV and grade III-IV acute GVHD were 24% (95% CI, 17% to 31%) and 3% (95% CI, 1% to 8%), respectively. The cumulative incidence of chronic GVHD necessitating immunosuppressive treatment at 2 years was 6% (95% CI, 3% to 11%). The 100-day incidence of CMV infection was 58% (95% CI, 49% to 67%).

Outcomes of DSA-Positive Patients

Characteristics and outcomes of the DSA-positive patients are summarized in Table 3. The 3-year OS was 54% (95% CI, 44% to 63%) for the non-DSA-positive group and 20% (95% CI, 1% to 57%) for the DSA-positive group ($P = .20$); the 3-year PFS for the 2 groups was 47% (95% CI, 37% to 56%) and 25% (95% CI,

Table 1
Patient Characteristics

Characteristic	HLA Ab-Negative	HLA Ab-Positive	
		Non-DSA	DSA
No. of patients	94	30	10
Age, yr, median (range)	50.5 (20-72)	57 (24-70)	50 (36-70)
Female sex, n (%)	36 (38)	17 (57)	8 (80)
Diagnosis, n (%)			
MDS/acute leukemia	31 (33)	15 (50)	8 (80)
Lymphoma	59 (63)	14 (47)	2 (20)
MM	3 (3)	1 (3)	0
MFI	1 (1)	0	0
DRI, n (%)			
Low	3 (3)	1 (3)	0
Intermediate	71 (76)	21 (70)	4 (40)
High	17 (18)	6 (20)	6 (60)
Very high	3 (3)	2 (7)	0
HCT-CI, n (%)			
0-2	80 (85)	17 (57)	6 (60)
≥3	14 (15)	13 (43)	4 (40)
CMV mismatch, n (%)			
D^+/R^+	60 (64)	21 (70)	6 (60)
D^+/R^-	12 (13)	1 (3)	1 (10)
D^-/R^+	18 (19)	6 (20)	2 (20)
D^-/R^-	4 (4)	2 (7)	1 (10)
ABO mismatch, n (%)			
Compatible	63 (67)	17 (57)	7 (70)
Minor	18 (19)	4 (13)	1 (10)
Major	9 (10)	7 (23)	1 (10)
Major and minor	4 (4)	2 (7)	1 (10)
Donor-recipient relationship, n (%)			
Sibling	35 (37)	12 (40)	5 (50)
Offspring to mother	13 (14)	5 (17)	4 (40)
Offspring to father	21 (23)	5 (17)	1 (10)
Mother to offspring	5 (5)	1 (3)	0
Father to offspring	15 (16)	1 (3)	0
Others	5 (5)	6 (20)	0
HLA mismatch in HVG vector, n (%)			
0	60 (64)	23 (77)	4 (40)
1	18 (19)	6 (20)	3 (30)
2	14 (15)	0	3 (30)
≥3	2 (2)	1 (3)	0
Stem cell source, n (%)			
PBSCs	44 (47)	15 (50)	6 (60)
BM	50 (53)	15 (50)	4 (40)
Conditioning regimen, n (%)			
MAC	8 (8)	6 (20)	3 (30)
RIC	46 (49)	14 (47)	6 (60)
NMA	40 (43)	10 (33)	1 (10)

MDS indicates myelodysplastic syndrome; MM, multiple myeloma; HCT-CI, Hematopoietic cell transplantation comorbidity index; D, donor; R, recipient; HVG, host versus graft; PBSCs, peripheral blood stem cells; BM, bone marrow.

1% to 63%), respectively ($P = .40$), and the 1-year NRM for the 2 groups was 20% (95% CI, 13% to 27%) and 0%, respectively ($P = .40$) (Figure 2).

Engraftment

The cumulative incidence of day +30 neutrophil engraftment was 100% in the DSA- positive group and 96% in the non-DSA-positive group ($P = .50$). The cumulative incidence of day +30 platelet engraftment in the 2 groups was 100% and 94%, respectively ($P = .90$) (Figure 3).

Graft Failure

The overall incidence of graft failure was 1.4%. None of the 9 evaluable patients who underwent allo-SCT with DSA (9/10) developed graft failure. Both patients who experienced graft failure underwent a second haplo-SCT from the same donor, and both engrafted. One patient died at day +63

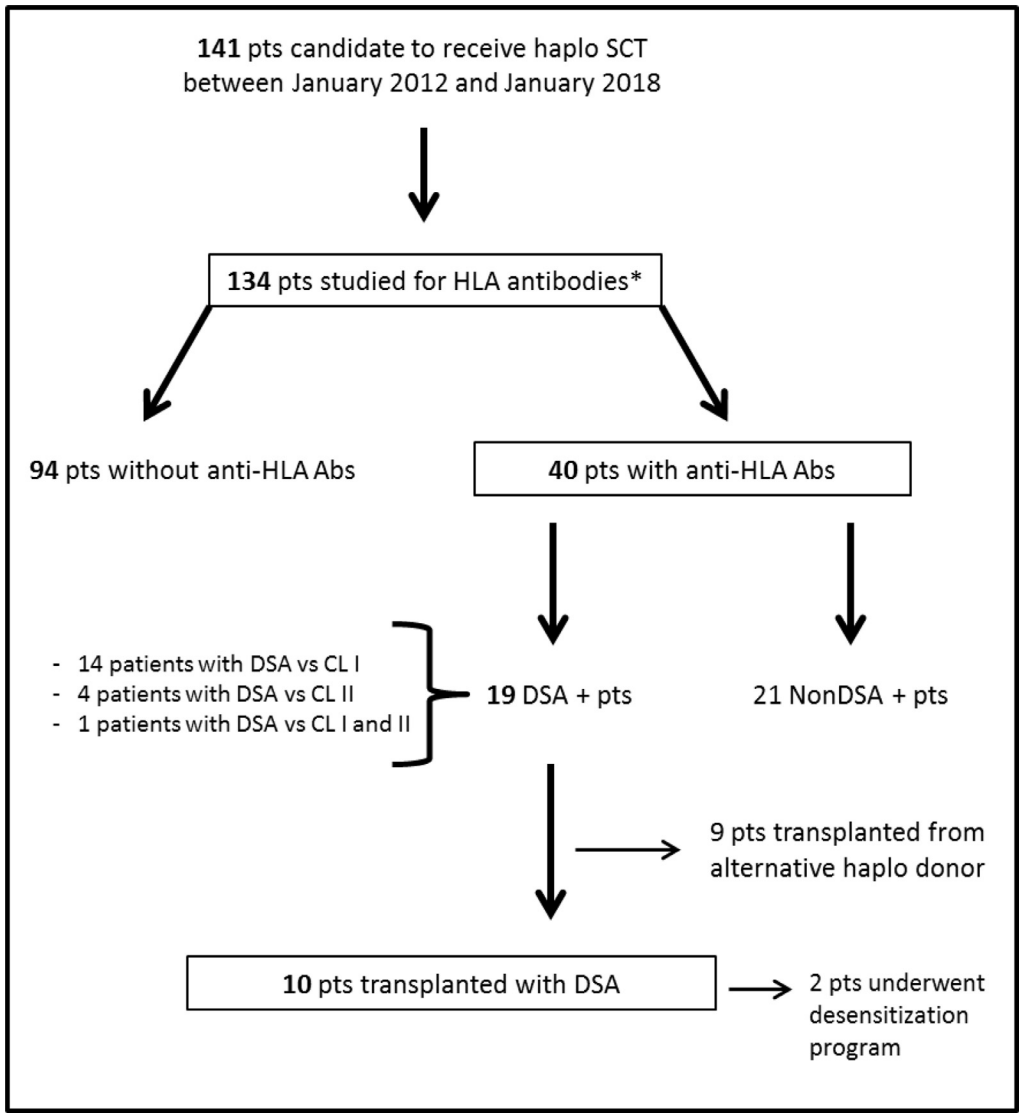

Figure 1. Flowchart of the study. *One patient was not considered because transplanted from MUD for Haplo donor ineligibility. CL I, class I HLA antigens; CL II, class II HLA antigens; DSA+, DSA-positive patients; MUD, matched unrelated donor; nonDSA+, non-DSA-positive patients.

due to multiorgan failure, and the other one was alive at the time of this report.

Primary Poor Graft Function

Twenty patients (16%) had primary poor graft function, and 69 patients had good graft function. According to the intensity of conditioning regimen, primary poor graft function occurred in 7% of the patients receiving an MAC regimen, in 23% of those receiving an RIC regimen, and in 29% of those receiving an NMA conditioning regimen. Factors influencing the risk of developing primary poor graft function, taking into account donor- and recipient-related variables and transplantation-related variables, are summarized in Table 4.

In univariate analysis, DSA positivity was not associated with an increased risk of primary poor graft function ($P = .50$), whereas there was a trend toward a higher incidence of poor graft function for patients with a high Disease Risk Index (DRI) ($P = .09$). On multivariate analysis, patients with a high DRI were 2.5 times more likely than other patients to develop primary poor graft function ($P = .08$).

Compared with patients with good graft function, those with primary poor graft function had poorer survival. The 3-year OS was 62% (95% CI, 48% to 73%) in the good graft function group versus 20% (95% CI, 6% to 39%) in the poor graft function group ($P < .0001$). The 3-year PFS was 53% (95% CI, 39% to 65%) in the good graft function group versus 20% (95% CI, 6% to 39%) in the poor graft function group ($P < .0001$). One-year NRM was 12% (95% CI, 5% to 21%) in the good graft function group versus 40% (95% CI, 18% to 61%) in the poor graft function group ($P = .009$) (Figure 4).

Multivariate analysis confirmed that poor graft function and high DRI had the most significant impact on OS (hazard ratio [HR], 3.5 [$P < .0001$] and 2.5 [$P = .005$], respectively) and PFS (HR, 2.7 [$P < .002$] and 2.0 [$P = .02$], respectively). Patients with poor graft function were 3.5 times more likely to have worse OS and 2.7 times more likely to have worse PFS compared with patients with good graft function; patients with high DRI were 2.5 times more likely to have worse OS and 2 times more likely to have worse PFS compared with the other patients. Poor graft function also had a significant impact

Table 2
Characteristics of DSA-Positive Patients at Pretransplantation Screening

Patient	Sex	Age, yr	Parous	Disease	Target HLA Loci of DSA	MFI at Screening	CDC	FCXM	Alternative Donor
1	Female	49	Yes	AML	A2 B41	11,000 15,100	Nd	Nd	No
2	Female	47	Yes	NHL	A1 B57 DR7	12,720 8180 1500	Nd	Nd	Yes
3	Female	48	Yes	MDS	DQB2 DR07	10,850 12,600	Nd	Nd	Yes
4	Female	26	No	HL	B8	10,000	Nd	Nd	Yes
5	Female	57	Yes	AML	A25	22,259	T/B positive	Nd	No
6	Male	69	NA	AML	A11	4500	T/B negative	Nd	No
7	Female	45	No	AML	B18	1221	Nd	Nd	No
8	Female	36	Yes	HL	B57	1152	Nd	Nd	No
9	Male	67	NA	AML	DQB7	18,766	T/B negative	Nd	Yes
10	Female	70	Yes	AML	DQB1	13,150	T/B negative	Nd	No
11	Male	70	NA	NHL	B8	14,103	T negative B positive	Nd	Yes
12	Female	30	No	HL	B44	3692	Nd	Nd	Yes
13	Female	48	Yes	AML	B18	2320	Nd	Nd	No
14	Female	70	Yes	AML	B7	4725	T/B negative	Nd	Yes
15	Female	41	Yes	HL	B41	1587	T/B negative	$CD3^-$ $CD19^-$	No
16	Male	51	NA	MDS	B50	1350	T/B negative	$CD3^-$ $CD19^-$	No
17	Female	52	Yes	AML	DQB7	2726	T/B negative	$CD3^-$ $CD19^-$	No
18	Female	55	Yes	AML	B27 C1	13,507 7999	T negative B positive	$CD3^+$ $CD19^+$	Yes
19	Female	59	Yes	AML	B55	6938	T/B negative	$CD3^+$ $CD19^+$	Yes

AML indicates acute myelogenous leukemia; Nd, not determined; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; NA, not applicable.

on NRM (HR, 5.0; $P = .001$); patients who developed poor graft function had a 5-fold greater risk of NRM compared with patients with good graft function.

DISCUSSION

Because each patient shares exactly 1 HLA haplotype with each biological parent or child and one-half of his or her siblings, a haploidentical donor can be identified rapidly in nearly all cases. PT-Cy administered after unmanipulated haploidentical stem cell infusion overcomes the alloreactivity barrier due to mismatched haplotype. This platform, developed by John Hopkins University in Baltimore, is mostly used in Europe and the United States because a high-rate of stable engraftment can be documented together with a low risk of GVHD. Nonetheless, primary graft failure remains a complication associated with very poor outcome owing to increased transplantation-related mortality. One of the most important factors in graft failure is the presence of DSA in the recipient, which is associated with a 10-fold increased risk of graft failure in all hematopoietic cell transplantations with an HLA-mismatched donor [5,24-27].

In the original clinical report of Luznik et al [28], the graft failure rate was 13% with autologous reconstitution in most cases. The presence of DSA increased the risk of primary graft failure, presumably due to antibody-mediated rejection. Several groups confirmed the clear association between DSA and primary graft failure in haplo-SCT [5] as well as in hematopoietic stem cell transplantation with other HLA-mismatched donor [24-26,29]. An M.D. Anderson Cancer Center group described the association between DSA and graft failure in both T cell-depleted and T cell-replete haplo-SCT. Twenty-two of 122 patients (18%) had DSA, of whom 12 received desensitization and 3 did not respond and underwent transplantation despite DSA; 32% of DSA-positive patients had primary graft failure, compared with only 4% of patients without DSA ($P < .001$). Among the DSA-positive patients, all graft failures occurred in those with a DSA level >5000 MFI pretransplantation ($P = .004$); the median DSA level at transplantation was 10,055 MFI for patients who failed to engraft versus 2065 MFI for those who engrafted [9].

The John Hopkins group evaluated 243 recipients and 957 donors, and 87% were mismatched relatives. DSA were detected in 43 recipients (14.5%). The presence of DSA was considered an exclusion criterion for potential donors, except for those patients without a suitable alternative donor. Nine patients without another available donor underwent desensitization. Eight patients with a DSA reduction to negative (MFI <500) or weak (1000<MFI<3000) levels proceeded to transplantation and achieved full donor chimerism [19]. Leffell et al [30] published an update of previous study, with 6 additional patients successfully desensitized before undergoing haplo-SCT with PT-Cy. The choice to desensitize depends on the starting DSA level (MFI) correlated with FCXM and CDCXM assay results as well as on other risk factors, such as an increase in DSA level before the initiation of desensitization or the presence of multiple low DSA levels; definitely DSA-positive patients with weak MFI levels proceeded to transplantation without desensitization and achieved engraftment [30].

In the study of Ciurea et al [9], C1q-positive patients with high MFI levels (>5000) were at high risk of graft failure, demonstrating that this condition should be avoided before haplo-SCT.

Table 3
DSA-Positive Transplantations

Patient	Disease	Conditioning Regimen	Source	$CD34^+ \times 10^6$	Pre-SCT MFI	Cell- Based Assays	Desensitization	Neutrophil Recovery	Platelet Recovery	Graft Failure
1	AML	MAC	PBSCs	5.00	15,100 11,000	/	Yes	19	21	No
5*	AML	RIC	PBSCs	5.00	22,259	CDC^+	Yes	NA	NA	NA
6	AML	RIC	PBSCs	2.49	4500	CDC^-	No	22	30	No
7	AML	RIC	PBSCs	5.00	1221	/	No	16	NA	No
8	HL	RIC	BM	1.49	1152	/	No	19	21	No
10	AML	RIC	BM	2.90	13,150	CDC^-	No	22	34	No
13	AML	MAC	PBSCs	3.50	2320	CDC^-	No	24	32	No
15	HL	Baltimore	BM	7.00	1587	CDC^- $FCXM^-$	No	16	21	No
16	MDS	MAC	PBSCs	6.00	1350	CDC^-	No	21	21	No
17	AML	Sequential	BM	7.30	2726	CDC^- $FCXM^-$	No	28	51	No

* This patient died on day +12 and thus was not evaluable for graft failure.

In 2018, the European Society for Blood and Marrow Transplantation published consensus guidelines for the detection and treatment of DSA in the haploidentical setting. The panel of experts recommended testing DSA using the Luminex platform and/or cell-based assays in all haploidentical donor transplant recipients, and to consider >1000 MFI as indicating DSA positivity. In the absence of an alternative donor, it is recommended that patients undergo desensitization therapy, especially with high DSA levels (>5000 MFI). C1q testing and/or cell-based assays are needed to further assess the risk of allograft rejection. The choice of desensitization protocol may be based on local experience [31].

In the present study, we retrospectively analyzed a consecutive series of patients undergoing MAC or RIC/NMA haplo-SCT with PT-Cy evaluated for the presence of DSA in a pretransplantation workup. We compared the DSA-positive group with the non-DSA-positive group (including both anti-HLA antibody-negative and non-DSA-positive patients) in terms of outcomes.

On the basis of pretransplantation screening among 19 DSA-positive patients, 9 patients (47%) with a suitable alternative donor without DSA against changed to a haploidentical donor, whereas 10 patients (53%) with an MFI level <3000 and/or crossmatch test negativity underwent transplantation without a donor change. Two patients with a high MFI level (>10,000) and no alternative donor underwent desensitization; 1 of them was CDCXM-positive. The failure to lower DSA level in crossmatch-positive patients is likely due to the high strength of the antibody, which it makes ineffective against the desensitization strategy adopted, as reported previously [32]. Comparing those patients with other patients without DSA, we found that the engraftment rate was superimposable between the 2 groups. Therefore, DSA had no significant impact on the timing of engraftment.

The overall incidence of primary graft failure was very low (1.4%), and no graft failure occurred in the 9 evaluable patients who underwent allo-SCT with DSA, even though 8 of these patients did not undergo any desensitization treatment. Our graft failure rate was lower compared with that in similar studies on haplo-SCT with PT-Cy using an RIC or NMA conditioning regimen [6,33,34], in which the graft failure rate ranged from 4% to 13%,. The lowest incidence of primary graft failure (0 to .7%) was observed with an MAC regimen [35,36]; likely, a more intensive conditioning regimen allowed a reduction of alloimmunization, overcoming the antibody- mediated graft rejection mechanism. Pretransplantation DSA screening was not routinely done in all studies, whereas our study population was carefully evaluated before transplantation, which likely contributed to our lower incidence of graft failure. Concerning the risk of graft failure in the presence of DSA, these data suggest that the presence of DSA should not be considered an absolute barrier to haplo-SCT for patients with a low DSA level and no alternative donor options and for whom the indication for allogenic transplantation is lifesaving. However, there are no defined criteria to establish when DSA-positive patients can safety undergo transplantation.

Another aim of the present analysis was the correlation of DSA with poor graft function. In the context of the haplo-SCT protocol pioneered by the University of Beijing and based on antithymocyte globulin infusion of both peripheral blood and primed bone marrow stem cells (GIAC protocol), DSA appears to be one of risk factors for developing poor graft function. Sun et al [21] retrospectively reviewed 464 patients who underwent unmanipulated haplo-SCT according to the GIAC platform. Twenty-six patients (5.6%) developed primary poor graft function. OS was significantly lower in the patients with primary poor graft function compared to those with good graft function (34.6% versus

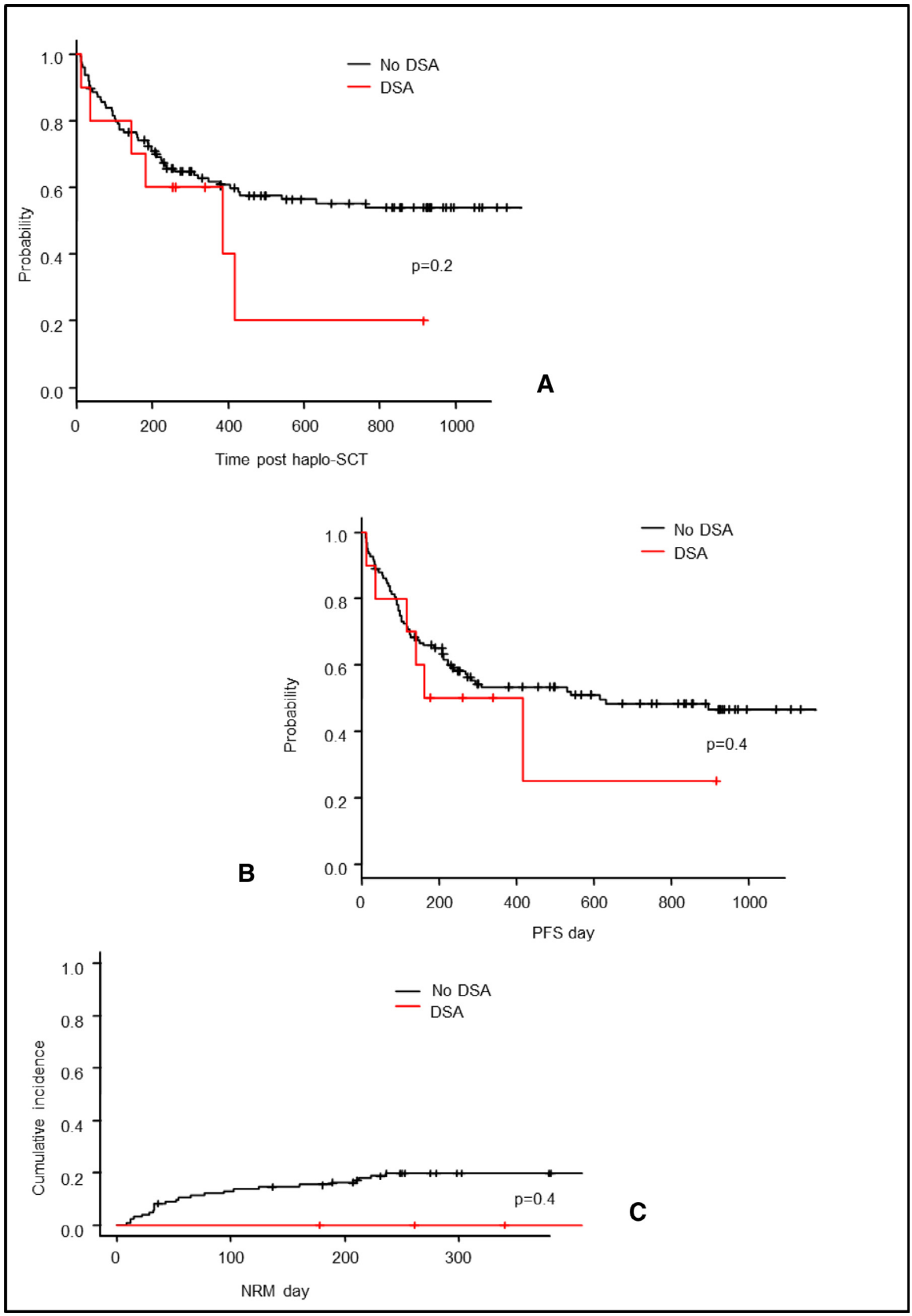

Figure 2. OS (A), PFS (B), and NRM (C) by DSA status.

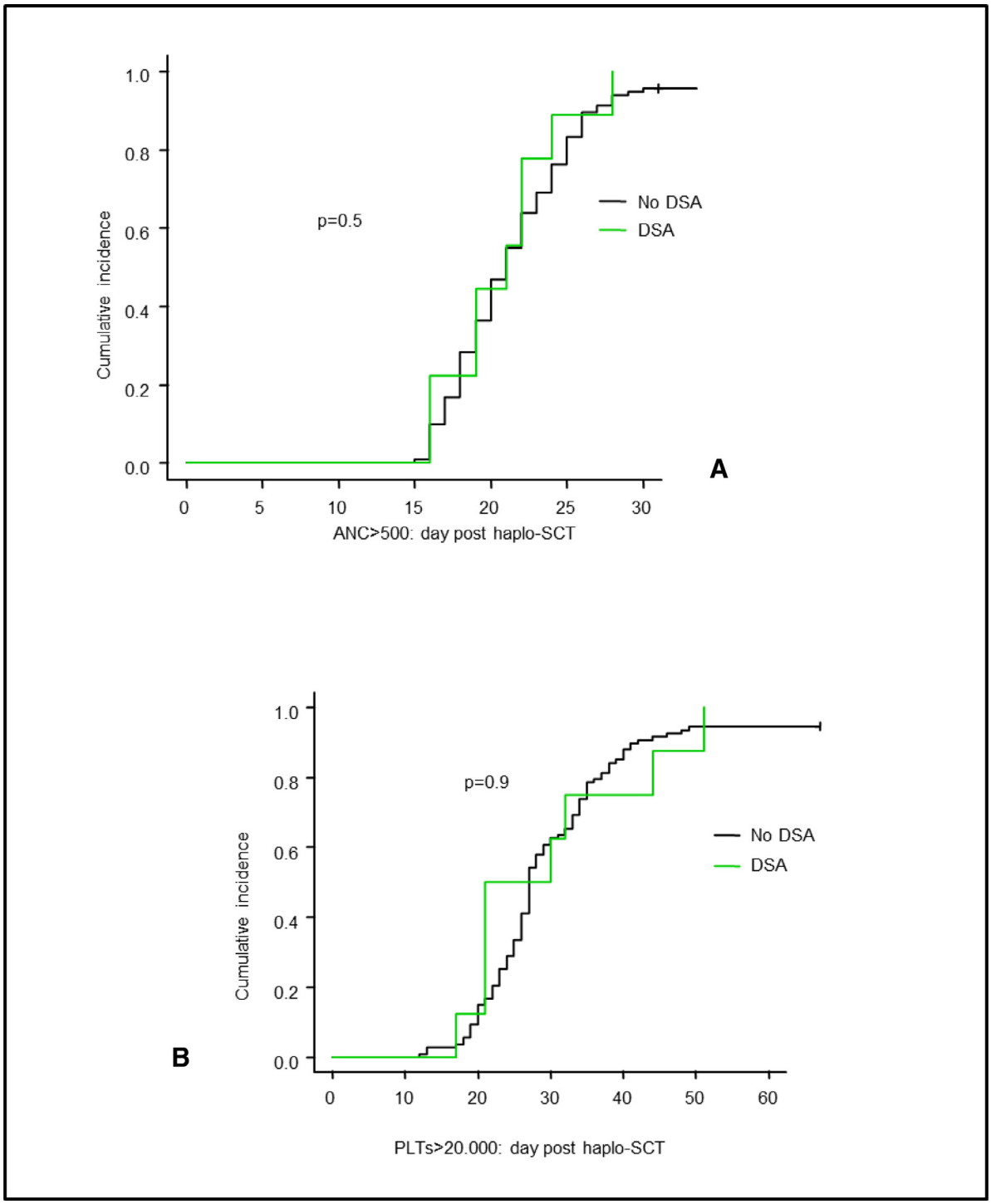

Figure 3. Cumulative incidence of neutrophil (A) and platelet (B) recovery by DSA status. ANC, absolute neutrophil count; PLTs, platelets.

82.7%; $P < .001$). The incidence of primary poor graft function was significantly greater in DSA-positive patients compared with DSA-negative patients (31% versus 3.2%; $P = .000$). Chang et al [8] recently showed that poor graft function in patients receiving unmanipulated haplo-SCT according to the GIAC platform was associated with the presence of DSA. Among 345 patients tested, 39 were DSA-positive; the incidence of primary poor graft function was significantly higher in the patients with MFI ≥2000 compared with those with MFI <2000. In our study, the incidence of primary poor graft function was not higher in DSA-positive patients; 20 patients (16%) had primary poor graft function, and only 2 were DSA-positive. When we analyzed risk factors for poor graft function, we found an association only with DRI [37]. However, the incidence of primary poor graft function was different when the intensity of conditioning regimen was taken in account (7% with MAC versus 23% with RIC versus 29% with NMA). The low number of events (n = 20) likely can explain the nonsignificant impact seen on the multivariate analysis.

In our cohort, the patients with primary poor graft function had significantly poorer survival compared with other patients

Table 4
Risk Factors for Poor Graft Function, Univariate and Multivariate Analysis

Variable	Good Graft Function	Poor Graft Function	*P* Value (Univariate)	HR (Multivariate)	*P* Value (Multivariate)
DSA, n (%)					
No	65 (94)	18 (90)	.5		
Yes	4 (6)	2 (10)			
Recipient sex, n (%)					
Female	31 (45)	8 (40)	.8		
Male	38 (55)	12 (60)			
Blood group, n (%)					
No/minor	62 (90)	17 (85)	.7		
Major	7 (10)	3 (15)			
Female→male, n (%)					
No	57 (83)	18 (90)	.7		
Yes	12 (17)	2 (10)			
Child→mother, n (%)					
No	62 (90)	15 (75)	.1	1	.1
Yes	7 (10)	5 (25)		2.6 (.7-9.9)	
Disease type, n (%)					
AML/MDS	22 (32)	7 (35)	.9		
HL	25 (36)	6 (30)			
NHL	14 (20)	5 (25)			
ALL	5 (8)	1 (5)			
MM	3 (4)	1 (5)			
Disease status, n (%)					
CR	42 (61)	9 (45)	.2		
PR	14 (20)	3 (15)			
SD/PD	13 (19)	8 (40)			
HCT-CI, n (%)					
0-2	40 (58)	9 (45)	.3		
≥3	29 (42)	11 (55)			
DRI, n (%)					
Low-intermediate	52 (75)	11 (55)	.09	1	.08
High-very high	17 (25)	9 (45)		2.5 (.9-7.0)	
Graft source, n (%)					
BM	46 (67)	10 (50)	.1	1	.2
PBSCs	23 (33)	10 (50)		1.9 (.6-5.5)	
Conditioning regimen, n (%)					
MAC	13 (19)	1 (5)	.3		
RIC	33 (48)	10 (50)			
NMA	23 (33)	9 (45)			
HLA mismatch in HVG vector, n (%)					
No	49 (71)	11 (55)	.2		
Yes	20 (29)	9 (45)			
Recipient age, yr, median (range)	45 (19-72)	50 (24-70)	.2		
Donor age, yr, median (range)	42 (21-71)	40 (23-66)	.5		
$CD34^+$, median (range)	4.3 (1.2 -8.6)	4.3 (1.6-7.6)	.9		

ALL indicates acute lymphoblastic leukemia; CR, complete remission; PR: partial remission SD, stable disease; PD, progressive disease.

with good graft function (3-year OS, 20% versus 62% [$P < .0001$]; 3-year PFS, 20% versus 53% [$P < .0001$]; 1-year NRM, 40% versus 12% [$P = .009$]). In the multivariate analysis, primary poor graft function and DRI were independent factors associated with poorer outcomes.

This study confirms the importance of including DSA testing using the Luminex platform and cell-based assays as part of the pretransplantation workup for donor selection in patients who are candidates for RIC or NMA PT-Cy haplo-SCT, to reduce the probability of antibody-mediated graft rejection. Our experience suggests that in the presence of low-level DSA or XM test negativity, haplo-SCT can be carried out without desensitization because of the low risk of graft failure and poor graft function. Recent published data support these findings [19,30].

In our study, although outcomes appeared to be poorer in DSA-positive patients compared with non-DSA-positive patients, these differences were not statistically significant (3-year OS, 20% versus 54% [$P = .20$]; 3-year PFS, 25% (1% to 63%) versus 47% (37% to 56%) [$P = .40$]); this is likely due to the limited number of patients and events analyzed.

This study has some limitations relayed to its retrospective nature; the comparison between the DSA-positive and non-DSA-positive groups is not conclusive, because the DSA-positive population is too small relative to the non-DSA-positive

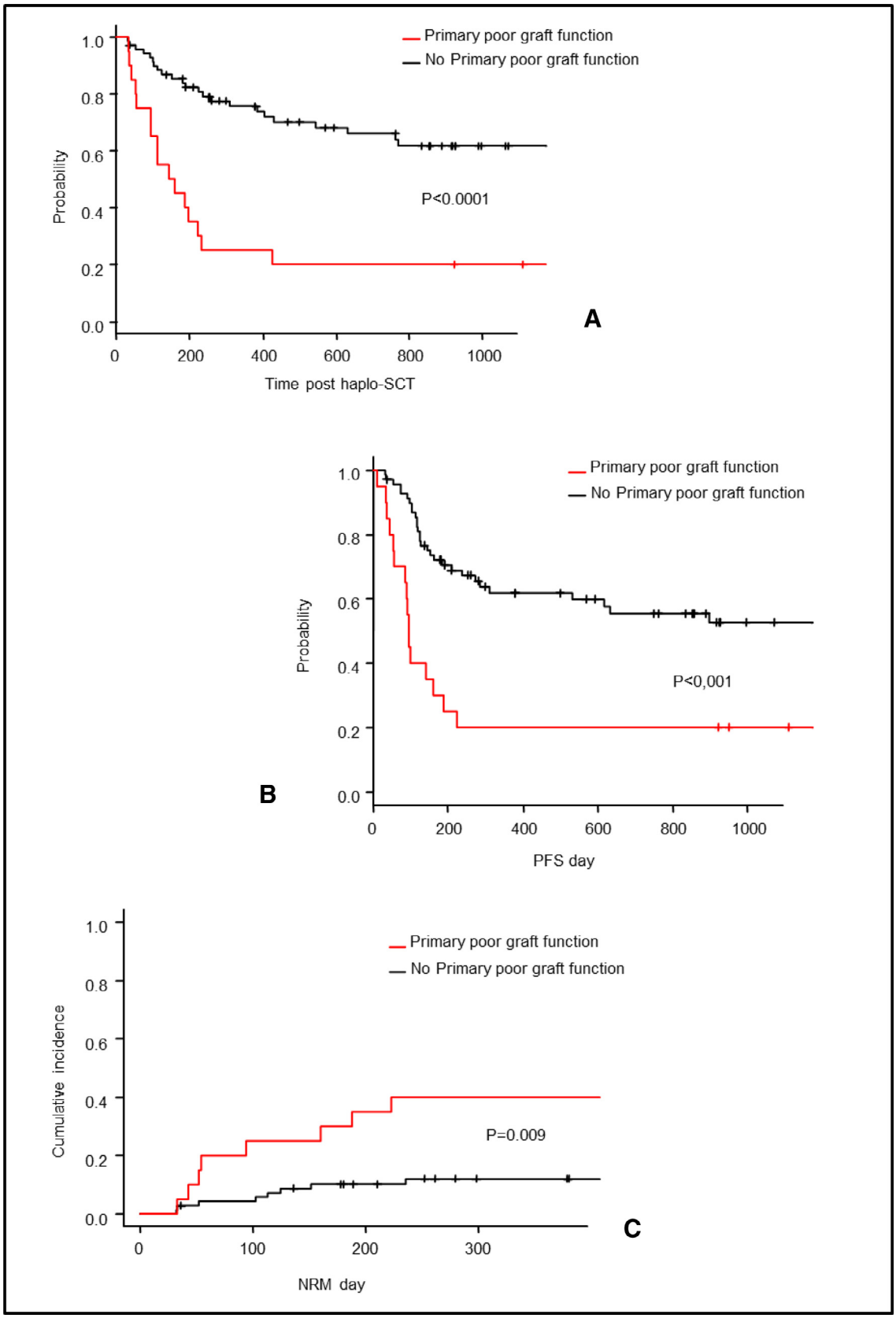

Figure 4. OS (A), PFS (B), and NRM (C), according to primary poor graft function.

population. In addition, FCXM and C1q testing were not available at the time of the study (2012) for the first enrolled patients; consequently, we are not able to better define the laboratory characteristics of patients who did not need desensitization despite DSA positivity regardless of MFI level. However, these findings can support the idea that patients with low-level DSA should not be scheduled for a desensitization procedure, which can possibly entail considerable side effects.

Further studies are needed to identify the criteria that make haplo-SCT permissible in the presence of DSA and the actual impact of DSA on long-term outcomes; furthermore, more elucidation of risk factors and mechanisms underlying primary poor graft function is warranted so that effective therapies can be developed.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to disclose.

REFERENCES

1. Henslee-Downey PJ. Allogeneic transplantation across major HLA barriers. *Best Pract Res Clin Haematol*. 2001;14:741–754.
2. Luznik L, O'Donnell PV, Fuchs EJ. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical bone marrow transplantation. *Semin Oncol*. 2012;39:683–693.
3. Mattsson J, Ringdén O, Storb R. Graft failure after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2008;14(suppl 1):165–170.
4. Al-Homsi AS, Roy TS, Cole K, Feng Y, Duffner U. Post-transplant high-dose cyclophosphamide for the prevention of graft-versus-host disease. *Biol Blood Marrow Transplant*. 2015;21:604–611.
5. Ciurea SO, de Lima M, Cano P, et al. High risk of graft failure in patients with anti-HLA antibodies undergoing haploidentical stem-cell transplantation. *Transplantation*. 2009;88:1019–1024.
6. 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.
7. Yoshihara S, Maruya E, Taniguchi K, et al. Risk and prevention of graft failure in patients with preexisting donor-specific HLA antibodies undergoing unmanipulated haploidentical SCT. *Bone Marrow Transplant*. 2012; 47:508–515.
8. Chang YJ, Zhao XY, Xu LP, et al. Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets. *J Hematol Oncol*. 2015;8:84.
9. Ciurea SO, Thall PF, Milton DR, et al. Complement-binding donor-specific anti-HLA antibodies and risk of primary graft failure in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2015;21:1392–1398.
10. Wolff SN. Second hematopoietic stem cell transplantation for the treatment of graft failure, graft rejection, or relapse after allogeneic transplantation. *Bone Marrow Transplant*. 2002;29:545–552.
11. Peralvo J, Bacigalupo A, Pittaluga PA, et al. Poor graft function associated with graft-versus-host disease after allogeneic marrow transplantation. *Bone Marrow Transplant*. 1987;2:279–285.
12. Lin Z, Kong Y, Wang Y, et al. [Analysis of risk factors for secondary cytopenia after allogeneic hematopoietic stem cell transplantation]. *Zhonghua Xue Ye Xue Za Zhi*. 2014;35:4–8. [in Chinese].
13. Mohty M, Faucher C, Chabannon C, et al. $CD34^+$ immunoselected cells for poor graft function following allogeneic BMT. *Cytotherapy*. 2000;2:367–370.
14. Larocca A, Piaggio G, Podesta M, et al. Boost of $CD34^+$-selected peripheral blood cells without further conditioning in patients with poor graft function following allogeneic stem cell transplantation. *Haematologica*. 2006;91:935–940.
15. Kong Y, Chang YJ, Wang YZ, et al. Association of an impaired bone marrow microenvironment with secondary poor graft function after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2013;19:1465–1473.
16. Dominietto A, Raiola AM, van Lint MT, et al. Factors influencing haematological recovery after allogeneic haemopoietic stem cell transplants: graft-versus-host disease, donor type, cytomegalovirus infections and cell dose. *Br J Haematol*. 2001;112:219–227.
17. Lee KH, Lee JH, Choi SJ, et al. Failure of trilineage blood cell reconstitution after initial neutrophil engraftment in patients undergoing allogeneic hematopoietic cell transplantation: frequency and outcomes. *Bone Marrow Transplant*. 2004;33:729–734.
18. Kellar KL, Iannone MA. Multiplexed microsphere-based flow cytometric assays. *Exp Hematol*. 2002;30:1227–1237.
19. Gladstone DE, Zachary AA, Fuchs EJ, et al. Partially mismatched transplantation and human leukocyte antigen donor-specific antibodies. *Biol Blood Marrow Transplant*. 2013;19:647–652.
20. Bramanti S, Nocco A, Mauro E, et al. Desensitization with plasma exchange in a patient with human leukocyte antigen donor-specific antibodies before T cell-replete haploidentical transplantation. *Transfusion*. 2016;56:1096–1100.
21. Sun YQ, He GL, Chang YJ, et al. The incidence, risk factors, and outcomes of primary poor graft function after unmanipulated haploidentical stem cell transplantation. *Ann Hematol*. 2015;94:1699–1705.
22. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695–706.
23. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457–481.
24. Ciurea SO, Thall PF, Wang X, et al. Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. *Blood*. 2011;118:5957–5964.
25. Takanashi M, Atsuta Y, Fujiwara K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood*. 2010;116:2839–2846.
26. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood*. 2010;115:2704–2708.
27. Cutler C, Kim HT, Sun L, et al. Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation. *Blood*. 2011;118:6691–6697.
28. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for haematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14:641–650.
29. Ruggeri A, Rocha V, Masson E, et al. Impact of donor-specific anti-HLA antibodies on graft failure and survival after reduced intensity conditioning-unrelated cord blood transplantation: a Eurocord, Société Francophone d'Histocompatibilité et d'Immunogénétique (SFHI) and Société Française de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC) analysis. *Haematologica*. 2013;98:1154–1160.
30. Leffell MS, Jones RJ, Gladstone DE. Donor HLA-specific Abs: to BMT or not to BMT? *Bone Marrow Transplant*. 2015;50:751–758.
31. Ciurea SO, Cao K, Fernandez-Vina M, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus guidelines for the detection and treatment of donor-specific anti-HLA antibodies (DSA) in haploidentical hematopoietic cell transplantation. *Bone Marrow Transplant*. 2018;53:521–534.
32. Gladstone DE, Bettinotti MP. HLA donor-specific antibodies in allogenic hematopoietic stem cell transplantation: challenges and opportunities. *Hematology Am Soc Hematol Educ Program*. 2017;2017:645–650.
33. Munchel A, Kesserwan C, Symons HJ, et al. Nonmyeloablative, HLA-haploidentical bone marrow transplantation with high-dose, post-transplantation cyclophosphamide. *Pediatr Rep*. 2011;3(suppl 2):e15.
34. 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.
35. Bacigalupo A, Dominietto A, Ghiso A, et al. Unmanipulated haploidentical bone marrow transplantation and post-transplant cyclophosphamide for hematologic malignancies following a myeloablative conditioning: an update. *Bone Marrow Transplant*. 2015;50(suppl 2):S37–S39.
36. Solomon SR, Sizemore CA, Sanacore M, et al. Haploidentical transplantation using T cell-replete peripheral blood stem cells and myeloablative conditioning in patients with high-risk hematologic malignancies who lack conventional donors is well tolerated and produces excellent relapse-free survival: results of a prospective phase II trial. *Biol Blood Marrow Transplant*. 2012;18:1859–1866.
37. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. *Blood*. 2014; 123:3664–3671.