A myeloablative fractionated busulfan conditioning regimen with post-transplant cyclophosphamide in HLA-matched and haploidentical transplantation: results of a phase II study

Myeloablative conditioning (MAC) is associated with superior outcomes than reduced-intensity conditioning in patients undergoing allogeneic hematopoietic stem cell transplantation (HCT).[1,2] MAC is associated with a higher risk of non-relapse mortality (NRM) and is often avoided in older patients and those with comorbidities. Therefore, we studied a novel schedule of delivering pretransplant conditioning chemotherapy in a fractionated manner, in which busulfan is administered over a period of 2 weeks, rather than the traditional dosing on 4 consecutive days in recipients of grafts from matched donors. We showed that this fractionated myeloablative busulfan and fludarabine (Bu-Flu) regimen was safe in patients up to 75 years of age,[3-7] whose NRM was 4-6% at day 100.[3] However, the NRM increased to 20-24% at 1 year,[3] most of which was attributed to graft-*versus*-host disease (GvHD)-related deaths.[3,8] The GvHD prophylaxis in that study included tacrolimus and methotrexate. Thus, we questioned whether outcomes could be improved with post-transplantation cyclophosphamide (PTCy). It was also unknown whether this strategy could be employed in the haploidentical HCT setting. We therefore added PTCy to our myeloablative fractionated Bu-Flu regimen and included patients with haploidentical donors in addition to HLA-matched donors.

This was an open-label, non-randomized, phase II clinical trial (ClinicalTrials.gov identifier NCT02861417) that assessed the safety and efficacy of fractionated Bu-Flu conditioning with PTCy-based GvHD prophylaxis (Figure 1). The eligibility criteria included patients 12-65 years of age with any hematologic malignancy who had an HLA-matched (unrelated or sibling) or haploidentical donor and had adequate organ function. Fifty-five patients were enrolled in the first three groups of this study between August 2016 and June 2018 and are reported here (Table 1). By the end of the study period, 34 patients were alive, including 14 in the haploidentical and 20 in the HLA-matched group. With a median follow-up of 37.6 months (range, 25.3-47.8 months), the estimated 2-year overall survival was 65.5% (95% confidence interval [95% CI]: 54-79.3%) in the entire cohort, 53.8% (95% CI: 37.7-76.9%) in the haploidentical group, and 75.9% (95% CI: 61.8-93.2%) in the HLA-matched group (Figure 2A). Twenty-eight patients were alive without disease progression, including 12 in the haploidentical and 16 in the HLA-matched group. The overall 2-year progression-free survival rate was 54.5% (95% CI: 42.9-69.4%); 46.2% (95% CI: 30.5-69.9) in the haploidentical and 62.1% (95% CI: 46.7-82.5%) in the HLA-matched group.

Fourteen patients (5 in the haploidentical group, 9 in the HLA-matched group) experienced a relapse of the underlying malignancy. The cumulative incidence of relapse at 2 years was 23.6% (95% CI: 12.3-35%); 19.2% (95% CI: 3.6-34.9%) in the haploidentical and 27.6% (95% CI: 10.9-44.2%) in the HLA-matched group (Figure 2B). Among the 39 patients with myeloid malignancies, 1/16 patients (6.25%) in the haploidentical group relapsed, compared to 6/23 patients (26%) in the matched group.

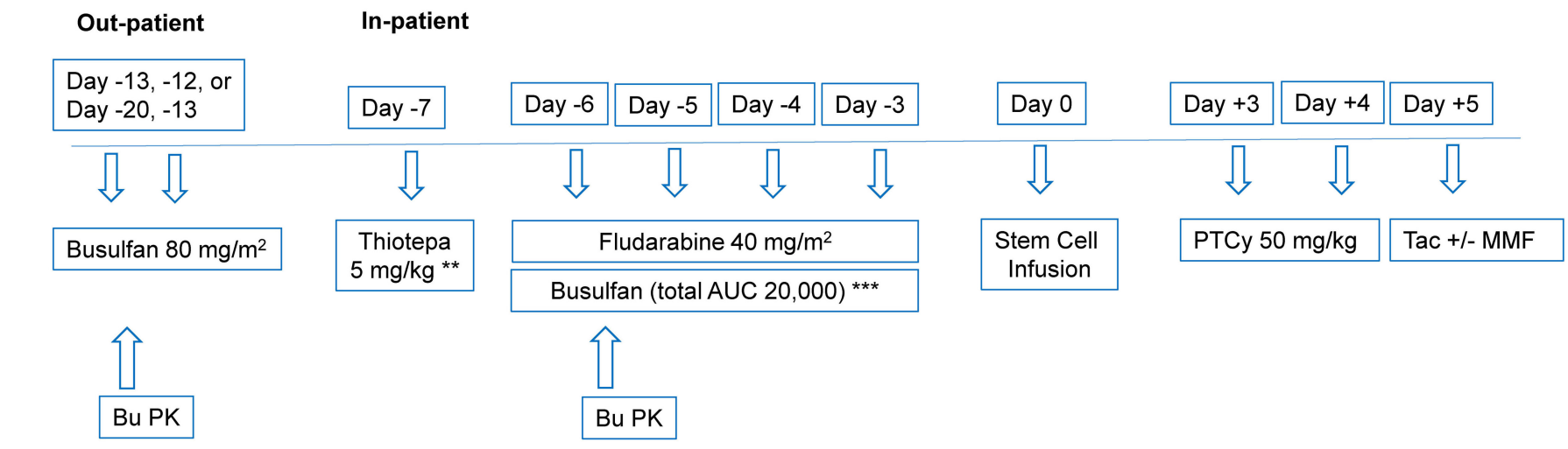
Overall, there were 21 deaths, of which eight were due to recurrence or persistence of the underlying malignancy, four from acute GvHD, six from infections (2 bacterial, 3 viral, and 1 protozoal), and one each from idiopathic pneumonia syndrome, sinusoidal obstruction syndrome, and an unidentified cause. Of note, all infection-related deaths occurred in the haploidentical group. The cumulative incidence of NRM for the entire cohort at 2 years post-HCT was 21.8% (95% CI: 10.8-32.9%). Most of these events occurred in the haploidentical group, with a resultant NRM of 34.6% at 2 years, *versus* 10.3% in the HLA-matched group (Figure 2C).

Forty-seven patients (87%) experienced at least one grade 3 or 4 adverse event within 100 days. The most common adverse event was culture-negative grade 3 neutropenic fever (n=32, 58.2%) that occurred at a median of 3 days post-HCT (interquartile range [IQR] 2-4.5). Many of these early events were likely related to cytokine release syndrome. Twenty-five infection events occurred, which were bacterial (n=13, 23.6%) or viral (n=12, 21.8%). Other common grade 3 or 4 adverse events were pulmonary (n=10, 18.2%) and gastrointestinal, including mucositis (n=14, 25.4%), nausea (n=5, 9.1%), and diarrhea (n=4, 7.3%). There were four (7.3%) cases of sinusoidal obstructive syndrome, one of which was fatal. In addition, nine (16.3%) patients had asymptomatic grade 3 or 4 hyperbilirubinemia. Hemorrhagic cystitis of any grade occurred in 31 (56%) patients at a median of 32 days post-HCT (range, 1-71; IQR 11-42). Of these, 29 (53%) were related to BK virus (grade 1 [n=11, 20%], grade 2 [n=14, 25%], and grade 3 [n=4, 7%]).

Table 1. Patients' baseline characteristics.

Characteristic	
N of patients	55
Age at HCT in years	
Median (range)	47 (15-65)
Interquartile range	40-57
Patients in age group, N (%)	
<50 years	30 (54.5)
≥50 years	25 (45.5)
Sex, N (%)	
Male	34 (61.8)
Female	21 (38.2)
Race, N (%)	
White	32 (58.2)
Black	8 (14.6)
Other	15 (27.2)
Diagnosis, N (%)	
Acute myeloid leukemia/myelodysplastic syndrome	30 (54.5)
Chronic myeloid leukemia/myeloproliferative disorder	9 (16.4)
Acute lymphoblastic leukemia	6 (10.9)
Multiple myeloma	5 (9.1)
Lymphoma	5 (9.1)
Donor, N (%)	
Haploidentical	26 (47.3)
HLA-matched unrelated	18 (32.7)
HLA-matched sibling	11 (20)
Graft source, N (%)	
Peripheral blood progenitor cells	33 (60)
Bone marrow	22 (40)
GvHD prophylaxis, N (%)	
PTCy + tacrolimus	25 (45.5)
PTCy + tacrolimus + MMF *	30 (54.5)
Disease Risk Index, N (%)	
Low	5 (9.1)
Intermediate	36 (65.5)
High	14 (25.4)
HCT-CI, N (%)	
0-2	33 (60)
≥3	22 (40)
Karnofsky performance score, N (%)	
<90	26 (47.3)
90-100	29 (52.7)
Follow-up in months, median (range)	37.6 (25.3-47.8)

* 25/26 recipients of haploidentical grafts and 4 recipients of grafts from matched unrelated grafts. HCT: hematopoietic cell transplantation; HCT-CI: Hematopoietic Cell Transplantation-Specific Comorbidity Index; HLA: human leukocyte antigen; MMF: mycophenolate mofetil; PTCy: post-transplant cyclophosphamide.

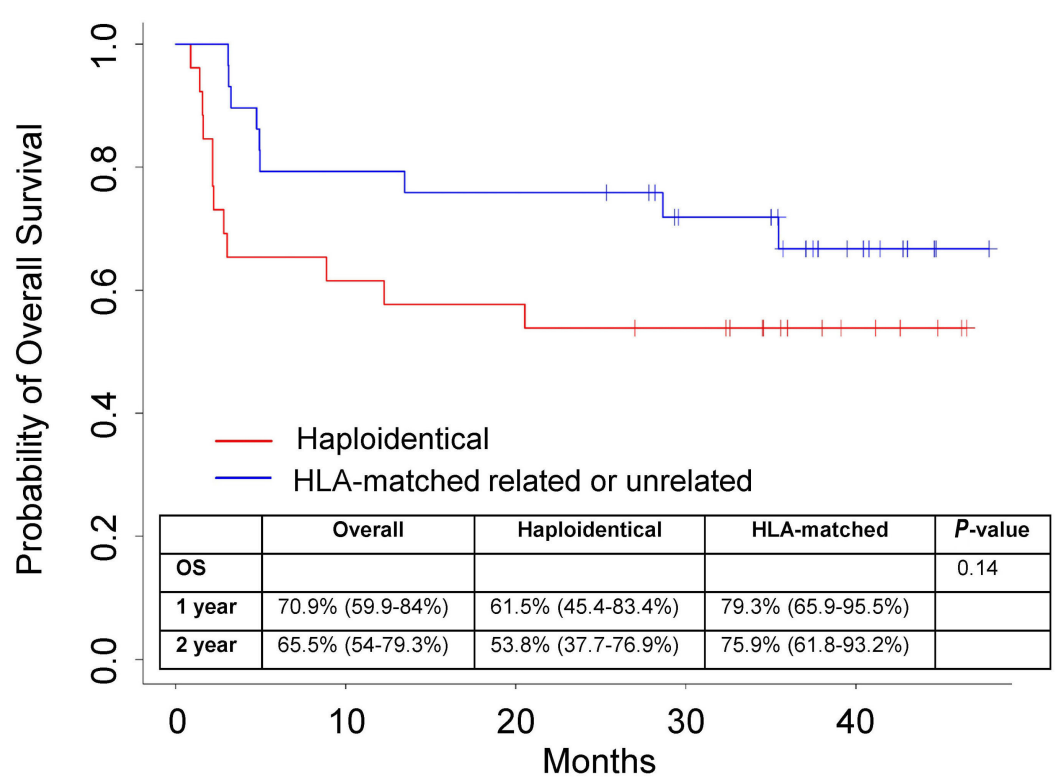


** *Only in the haploidentical group*

*** *Busulfan is administered at the dose calculated to achieve a total (including first two out-patient doses delivered on day -20/-13 and day -13/-12) systemic exposure of 20,000 ± 12% µMol-min based on the PK studies*

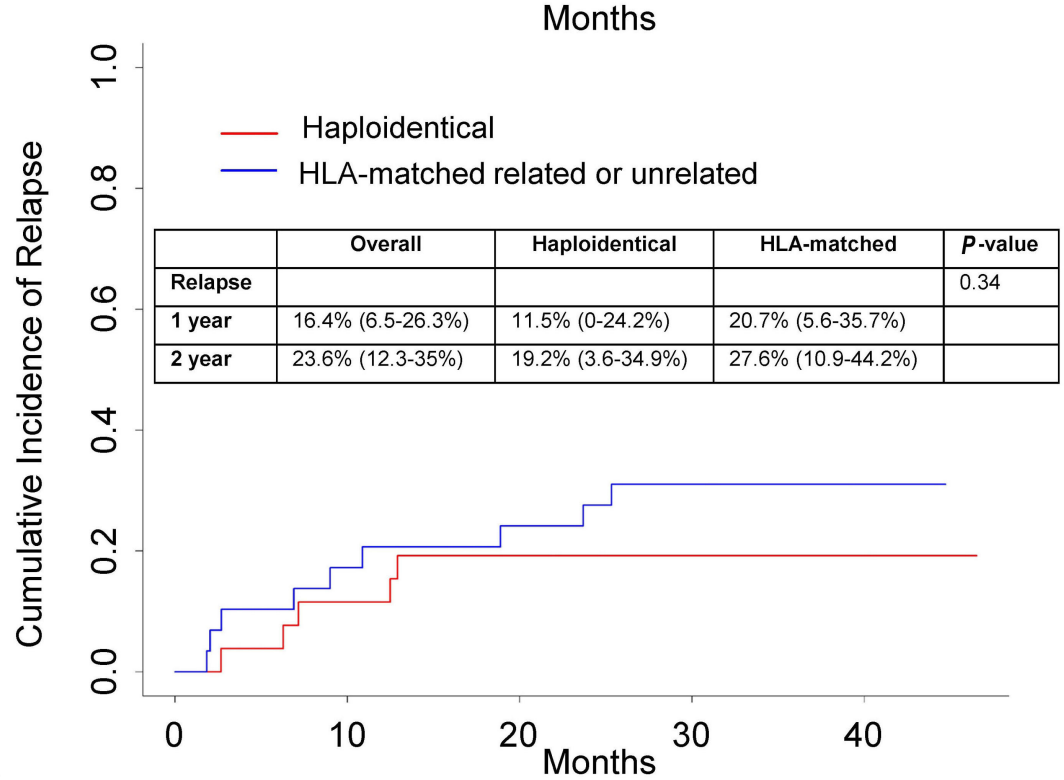
Figure 1. Study schema. Patients received the first two doses of busulfan 80 mg/m^2 intravenously each as an outpatient either on days -13 and -12 (haplo donor n=16 group 1; matched donor n=29 group 2) or on days -20 and -13 (haplo donor n=10 group 3). Inpatient conditioning included busulfan intravenously immediately following each dose of fludarabine 40 mg/m^2 intravenously on day -6 through day -3. Pharmacokinetic analyses were conducted after the first dose of busulfan (day -13 or day -20) and the third dose (day -6), based on which the last two doses of busulfan (days -4 and -3) were adjusted as needed to achieve the total target area under the curve of 20,000 (including outpatient doses). The median total dose of busulfan was 11.29 mg/kg (interquartile range: 9.75-13.37; range, 4.05-20.07). The haploidentical group also received thiotepa 5 mg/kg intravenously on day -7. Day 0 was the day on which the graft was infused. All patients received post-transplant cyclophosphamide 50 mg/kg intravenously on days +3 and +4 and tacrolimus from day +5 for prophylaxis of graft-*versus*-host disease; recipients of haploidentical grafts and later also recipients of grafts from matched unrelated donors also received mycophenolate mofetil from day +5. Bu: busulfan; PK: pharmacokinetics; AUC: area under the curve; PTCy: post-transplant cyclophosphamide; Tac: tacrolimus; MMF: mycophenolate mofetil.

A



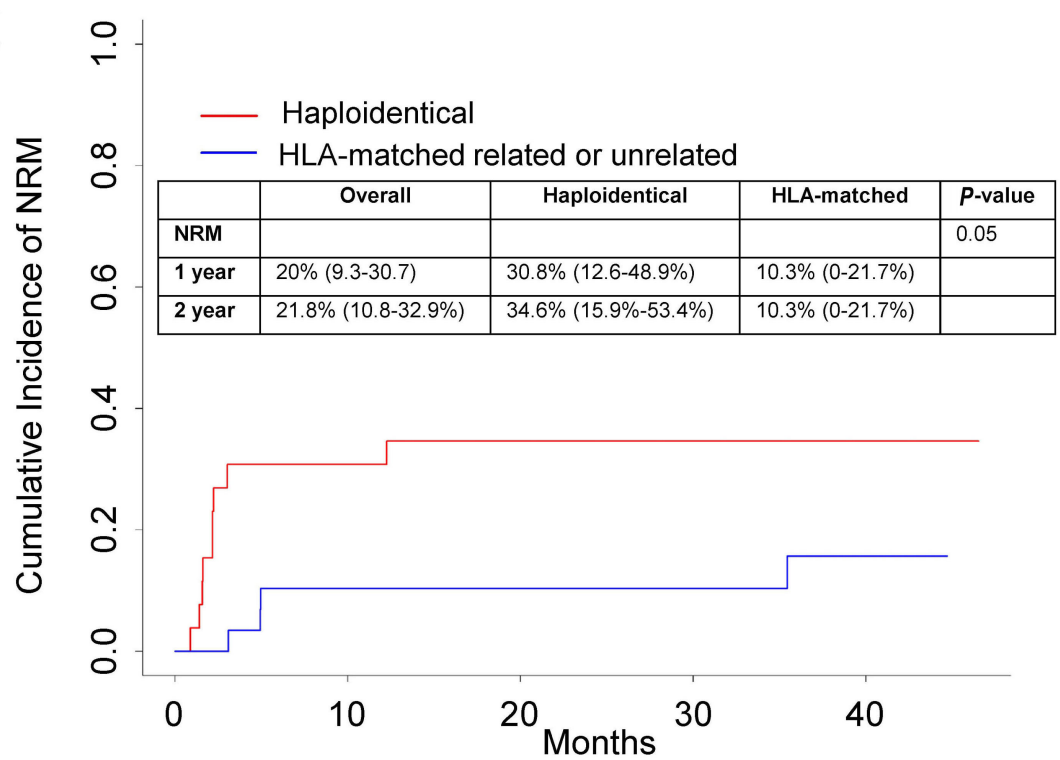
	Overall	Haploidentical	HLA-matched	*P*-value
OS				0.14
1 year	70.9% (59.9-84%)	61.5% (45.4-83.4%)	79.3% (65.9-95.5%)	
2 year	65.5% (54-79.3%)	53.8% (37.7-76.9%)	75.9% (61.8-93.2%)	

B



	Overall	Haploidentical	HLA-matched	*P*-value
Relapse				0.34
1 year	16.4% (6.5-26.3%)	11.5% (0-24.2%)	20.7% (5.6-35.7%)	
2 year	23.6% (12.3-35%)	19.2% (3.6-34.9%)	27.6% (10.9-44.2%)	

C



	Overall	Haploidentical	HLA-matched	*P*-value
NRM				0.05
1 year	20% (9.3-30.7)	30.8% (12.6-48.9%)	10.3% (0-21.7%)	
2 year	21.8% (10.8-32.9%)	34.6% (15.9%-53.4%)	10.3% (0-21.7%)	

Figure 2. Study outcomes. (A) Overall survival, (B) relapse, (C) non-relapse mortality. OS: overall survival; NRM: non-relapse mortality.

No graft failure occurred. The median time to neutrophil engraftment was 17 days (range, 13-39; IQR 15-19); among bone marrow graft recipients it was 18 days (range, 14-39; IQR 16-20) and among recipients of peripheral blood progenitor cell grafts it was 15 days (range, 13-28; IQR 14-18). The median time to platelet engraftment (platelets ≥20x10^9/L, n=49) was 25 days (range, 11-167; IQR 19-38), while the median time to a platelet count ≥50x10^9/L (n=42) was 32 days (range, 15-296; IQR 24-48).

Microsatellite polymorphism analysis on day 30 showed a median of 100% myeloid cells (range, 97-100%; IQR 100-100%) and 100% T cells (range, 0-100%; IQR 98-100%) of donor origin. The median remained unchanged in both myeloid and T-cell compartments at all subsequent time points analyzed (day 100, 6 months, and 1-year post-HCT).

Twenty-one patients (7 in the haploidentical group, 13 in the HLA-matched group) developed grade II-IV acute GvHD by day 100, with a cumulative incidence of 38.2% (95% CI: 25.2-51.2%) and five patients (3 in the haploidentical group, 2 in the HLA-matched group) developed grade III-IV acute GvHD, with a cumulative incidence of 9.1% (95% CI: 1.4-16.8%) at day 100. Eight patients developed chronic GvHD (all extensive; 3 in the haploidentical group, 5 in the HLA-matched group), with a cumulative incidence of 10.9% (95% CI: 2.5-19.3%) at 2 years. Among 22 patients who were alive in remission, the median time to discontinuation of tacrolimus was 254 days (range, 144-742; IQR 178-335).

We demonstrated the safety of adding PTCy for GvHD prophylaxis to the fractionated myeloablative Bu-Flu regimen in recipients of haploidentical, as well as HLA-matched grafts. The addition of PTCy was tolerated well and resulted in lower than expected severe acute and chronic GvHD, with a severe grade III-IV acute GvHD rate of about 9% at day 100, and chronic GvHD rate of about 11% at 2 years. Without directly comparing results across studies, the risk of chronic GvHD appeared to be remarkably lower than in our previous fractionated Bu-Flu MAC study with tacrolimus/methotrexate prophylaxis (55% overall, 36% extensive).[3] These results are even more encouraging, as about half of the patients in the current study had a mismatched haploidentical donor, while in a previous study all patients had a matched donor.

As GvHD is the leading cause of NRM that occurs beyond day 100,[3,8] any reduction in GvHD may result in lower NRM and better survival. As expected, we found a lower risk of NRM than in previous studies in the HLA-matched group, with a resultant NRM of about 10% at 2 years *versus* 22% at 1 year with fractionated Bu-Flu MAC and tacrolimus/methotrexate prophylaxis.[3] It was not the intent of the current study to compare these results to those of our previous studies without PTCy prophylaxis in which older patients were also treated,[3-6] so no formal comparisons can be made. Nevertheless, the outcomes from previous studies provide benchmarks to assess baseline risk without PTCy, as the conditioning regimen was identical, and the current study was constructed on the foundation laid by the previous studies with a fractionated Bu-Flu regimen. In this study, the overall survival of the patients with matched donors was 76% at 2 years, which is comparable to that reported with myeloablative regimens in patients with myelodysplastic syndrome or acute myeloid leukemia in complete remission.[1] Whether additional fractionation and lengthening of this regimen with pharmacokinetic monitoring[9] could further reduce toxicity and NRM, and improve survival is being studied in older patients. If successful, it may offer a safe myeloablative alternative for older patients undergoing matched donor transplantation.

Our results also provide another option for a MAC regimen for recipients of haploidentical transplantation, with a 2-year overall survival of 54%. These results appear to be comparable to those of published studies with MAC and are better than those achieved with reduced intensity conditioning.[10] In our study, thiotepa was added in the haploidentical group to intensify immunosuppression and reduce the risk of graft failure. With that, no graft failures occurred. Moreover, thiotepa appeared to be associated with a reduction in relapse risk,[11] especially in patients with myeloid malignancies in our study. The alloreactive immune effects of a haploidentical graft may also be responsible for a lower relapse rate. Both thiotepa and poor immune reconstitution after a haploidentical transplant may have resulted in a higher NRM than that occurring in recipients of transplants from matched donors (35% *versus* 10%, respectively, at 2 years). Reducing the conditioning intensity may reduce NRM and thus improve outcomes further in the haploidentical group. Our ongoing studies are assessing this by examining lower doses of busulfan and/or thiotepa. For instance, we are evaluating the use of a lower dose of busulfan (area under the curve 16,000) with the same dose of thiotepa (5 mg/kg) *versus* the same dose of busulfan (area under the curve 20,000) with a lower dose of thiotepa (2.5 mg/kg).

Thus, the fractionated myeloablative Bu-Flu conditioning regimen with PTCy is tolerated well and leads to a low risk of severe acute and chronic GvHD and NRM, with encouraging survival, especially in those with HLA-matched donors. Further modifications are needed in the haploidentical group to improve outcomes.

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Contributions

UP conceptualized the study design, helped with interpreting the data, and ensured compliance with regulatory requirements for the clinical trial; RB contributed to the data analysis and figures and wrote the statistical section of the manuscript; JK performed the busulfan pharmacokinetic analysis; AMA, GA, QB, CMH, JSI, PK, DM, YN, AO, BO, SS, MHQ, and EJS enrolled patients in the study and monitored clinical responses; BCV helped with laboratory data; REC and BSA enrolled patients in the study and monitored clinical responses and conceptualized the study design; RSM contributed to interpreting the data and wrote the manuscript.

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Data-sharing statement

The data presented in this study are available upon reasonable request to the corresponding author by e-mail.

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