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TRANSPLANTATION: RESEARCH ARTICLE

Reduced-toxicity conditioning regimen with busulfan, fludarabine, rATG, and 400 cGy TBI in pediatric patients undergoing hematopoietic stem cell transplant for high-risk hematologic malignancies

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Abstract

Background: Myeloablative conditioning regimens decrease the risk of relapse in pediatric patients undergoing allogeneic hematopoietic stem cell transplant (HCT) for hematologic malignancies, but cause significant toxicities

Procedure: This prospective study evaluated the use of a reduced-toxicity, myeloablative regimen with dose-adjusted busulfan, fludarabine, antithymocyte globulin and 400 cGy of total body irradiation in 40 patients < 21 years of age undergoing HCT for high-risk leukemias. Busulfan pharmacokinetics were measured to target 4000 μmol*min/day in the first 30 patients; this was increased to 5000 μmol*min/day in the subsequent 10 in efforts to further decrease relapse risk

Results: Overall survival at two- and five-years post-HCT was 67% and 51%, respectively. Relapse occurred in 11 patients (28%) at a median of seven months and was the leading cause of death. Transplant-related mortality was 8% and 13% at 100 days and one-year post-HCT, respectively. Trends toward improved survival were seen in patients transplanted for myeloid disease using bone marrow as stem cell source who achieved a busulfan AUC > 4000 μmol*min/day with two-year relapse-free survival approaching 80%

Conclusions: This conditioning regimen is safe and effective in patients with high-risk leukemias, particularly myeloid disease. Larger studies are needed to compare its safety and efficacy to other myeloablative regimens in this population.

KEYWORDS
pediatric leukemia, reduced-toxicity conditioning regimen, stem cell transplant

1 | INTRODUCTION

Allogeneic hematopoietic stem cell transplant (HCT) is curative for some pediatric patients with high-risk hematologic malignancies.

Abbreviations: > Gr2, greater than grade II; aGVHD, acute graft-versus-host disease; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; BM, bone marrow; Bu, busulfan; cGVHD, chronic graft-versus-host disease; CIBMTR, Center for International Blood and Marrow Transplant Research; Cy, cyclophosphamide; EBMT, European Society for Blood and Marrow Transplantation; Flu, fludarabine; GVHD, graft-versus-host disease; HCT, hematopoietic stem cell transplant; MAC, myeloablative conditioning; MRD, minimal residual disease; OS, overall survival; PBSC, peripheral blood stem cells; PK, pharmacokinetics; RD1, regimen dose #1; RFS, relapse-free survival; TBI, total body irradiation; TD, test dose; TRM, transplant-related mortality.

Despite decades of investigation, the optimal conditioning regimen for HCT remains elusive. Myeloablative conditioning (MAC) regimens are favored in young, otherwise healthy patients given the potential decreased risk of post-HCT relapse, yet carry a higher risk for toxicities. Prospective, randomized trials in adults comparing busulfan (Bu) chemotherapy-based regimens to total body irradiation (TBI)-based regimens in patients with a variety of hematologic malignancies showed variable results,[1–4] likely attributed to the heterogeneity of malignancies included and variability in Bu administration. Subsequent multicenter, retrospective studies conducted by the European Society for Blood and Marrow Transplantation (EBMT) and Center for International Blood and Marrow Transplant Research (CIBMTR) seem to favor Bu-based regimens especially in myeloid malignancies.[5–7] To achieve the goal of avoiding toxicities of high-dose TBI without compromising efficacy, further studies focused on comparing different Bu-based regimens. Again, prospective studies showed varying results comparing myeloablative Bu/fludarabine (Bu/Flu) with Bu/cyclophosphamide (Bu/Cy), but a meta-analysis concluded that these regimens overall afford similar efficacy with lower toxicity seen with Bu/Flu.[8] However, for acute lymphoblastic leukemia (ALL), retrospective analyses have consistently shown superiority of TBI-based regimens to chemotherapy-based regimens.[9,10]

The research in pediatrics has shown similar results. Children's Cancer Group trials using TBI/Cy (CCG 251 and CCG 213) versus Bu/Cy (CCG 2891) in pediatric patients with acute myeloid leukemia (AML) show comparable outcomes,[11] consistent with a review by the EBMT.[12] An International Bone Marrow Transplant Registry study demonstrated superior overall survival (OS) and relapse-free survival (RFS) with TBI/Cy, compared with Bu/Cy among patients < 20 years of age with ALL.[13] The Pediatric Blood and Marrow Transplant Consortium confirmed these findings in a randomized controlled trial of myeloablative Bu- versus TBI-based regimens in the same population.[14] Thus, myeloablative Bu-based regimens are favored in pediatric patients with AML and TBI-based regimens in those with ALL, but there is still significant variability in exact regimens used.

Novel conditioning regimens continue to be developed with the goal of maintaining efficacy while reducing toxicity. Addition of 400 cGy TBI to myeloablative Bu/Flu/antithymocyte globulin (ATG) has been shown to improve survival outcomes in adults with AML and ALL.[15–17] Given the efficacy of TBI in patients with leukemia, we hypothesized that this regimen (Bu/Flu/ATG/TBI) would allow for decreased short- and long-term toxicity compared with standard MAC regimens while maintaining disease control. In the current study, we prospectively explored the safety and efficacy of this reduced-toxicity, MAC regimen in children and adolescents receiving HCT for high-risk hematologic malignancies.

2 | MATERIALS AND METHODS

2.1 | Study participants

Participants included patients ≤ 21 years of age receiving HCT for hematologic malignancies who were treated at Lurie Children's Hospital. Eligible diagnoses included both myeloid (AML, chronic myeloid leukemia, myelodysplastic syndrome) and lymphoid (ALL) disease. Patients were excluded if they had a history of prior HCT. The Institutional Review Board of Lurie Children's Hospital approved protocol NCT00679536. Written consent was obtained from participants or participant guardians and assent was obtained when required.

2.2 | Donor selection

HLA matching was performed at the allele level with high-resolution typing. Unrelated donors were prospectively typed for HLA-A, HLA-B, HLA-C, and HLA-DRB1. Up to two allele/antigen-level mismatches were allowed. The protocol allowed for either HLA-identical siblings or unrelated donor grafts. Initially, stem cell source was restricted to peripheral blood stem cells (PBSC), but given excellent rates of engraftment, the protocol was amended to also include bone marrow (BM) in 2014. Age of donor, CMV serostatus, gender, and parity were considered in the donor selection as per our standard operating procedure. All donors met the standard infectious disease criteria at our institution and study-specific infectious disease criteria prior to study entry. Matched unrelated donors included those who were either 8/8 or 7/8 HLA-matched to the patient; matched related donors were all HLA-identical siblings.

2.3 | Pre-HCT disease status

All patients underwent BM and cerebrospinal fluid evaluation with assessment of minimal residual disease (MRD) prior to HCT. For patients with ALL, the cutoff for MRD positivity by flow cytometry was 0.01%, whereas for AML it was 0.1%, based on standard testing at our institution.

2.4 | Treatment protocol

The myeloablative reduced-toxicity conditioning regimen consisted of (1) Flu 30 mg/m^2/day over 1 hour on days −6 to days −2, (2) Bu i.v. over 3 hours on days −5 through −2 to target an area under the curve (AUC) of 4000 μmol *min/day with a test dose of 0.8 mg/kg i.v. on day −10 or −9, (3) thymoglobulin (rabbit ATG) 1.5 mg/kg/day on days −4 to −2, and (4) 400 cGy of TBI given in two fractions on day −1. After the first 30 patients were treated, target AUC for Bu was increased to 5000 μmol*min/day. Bu was given within 30 minutes after finishing Flu given synergistic interaction with Flu.[18,19] Additional central nervous system therapy was allowed at the discretion of the treating provider. One patient received thiotepa 10 mg/kg i.v. on day −1 instead of TBI per the treating physician's discretion given young age (28 months). In the last 10 patients, ATG was omitted in patients with HLA-identical sibling donors.[20]

2.5 | Busulfan pharmacokinetics (PK)

PK samples were drawn after a test dose of Bu (0.8 mg/kg) and confirmed on regimen dose one on day −5. Samples were processed by the Pharmacokinetics Lab at the Fred Hutchinson Cancer Research Center.[21]

2.6 | Graft-versus-host disease (GVHD) prophylaxis and treatment

Cyclosporine A (CSA) or tacrolimus given as an i.v. infusion starting on day −1 and continued until an oral formulation could be administered, and mycophenolate mofetil were used as acute GVHD (aGVHD) prophylaxis. GVHD was diagnosed and graded according to accepted criteria and treated according to institutional guidelines.[22,23]

2.7 | Supportive care

Isolation, infection prophylaxis and treatment, antiemetics, and blood product support were provided per institutional protocol. IgG levels were drawn every 2 weeks, and 400 mg/kg IVIG was given for hypogammaglobulinemia (IgG < 400 mg/dL). Patients were screened for EBV, CMV, and Adenovirus weekly at least through day 100. Patients received one dose of 0.05 mg/kg (maximum of 2 mg) i.v. lorazepam prior to each dose of Bu as seizure prophylaxis.

2.8 | Engraftment and donor chimerism

Neutrophil engraftment was defined as an absolute neutrophil count > 500/uL for three consecutive days. Platelet engraftment was defined as > 20 000/uL at least seven days after the last platelet transfusion.[24] Primary graft failure was defined as the absence of neutrophil engraftment by day +28. Secondary graft failure was defined as the loss of a previously functioning graft resulting in cytopenia of at least two blood cell lineages. Donor chimerism analysis was evaluated for total, T and myeloid cells at engraftment, and at 3, 6, 9, and 12 months post-HCT. Full donor chimerism was defined as ≥ 97% donor DNA as determined by magnetic cell separation and fragment analysis of short tandem repeats; mixed chimerism was defined as < 97% donor DNA. Adequate immune reconstitution was defined as CD3 > 400/uL, CD4 > 200/uL, and IgG > 400 mg/dL.

2.9 | Study outcomes

The primary outcome of the study was the safety and toxicity of this regimen with OS at day +100 post-transplant. Secondary outcomes included two- and five-year RFS and OS and incidences of greater than grade II (> Gr2) aGVHD and chronic GVHD (cGVHD). OS was defined as the time from HCT to death; RFS was defined as the time from HCT to relapse or death.

2.10 | Statistical analysis

Descriptive statistics were conducted to summarize variables related to patient, disease, and transplant characteristics. The distribution of patient and transplant characteristics between patients with underlying myeloid versus lymphoid disease and those receiving PRBC vs BM were compared using chi-square tests for categorical variables and Wilcoxon signed-rank tests for continuous variables. The percentage of having each of the safety and toxicity measures was calculated. The incidences of transplant-related mortality (TRM) and GVHD were calculated from the number of GVHD cases divided by the number of total patients. Kaplan-Meier estimators were used to estimate the probability of OS and RFS. Wilcoxon tests were performed to examine whether OS differed between myeloid and lymphoid disease. Cox proportional-hazard models were used to compare OS by GVHD and by donor type (related vs unrelated). Logistic regression analysis was conducted to predict the probability of aGVHD by donor age in decades, and the probability of death and the probability of relapse or death by Bu AUC levels. A significance level was set as 0.05. All analyses were conducted using SAS 9.4 (Cary, NC).

3 | RESULTS

3.1 | Patient and transplantation characteristics

A total of 40 patients (18 males and 22 females) were treated with a median age of 12 years (range, 2-19) at the time of transplant (Table 1). Thirty-one had myeloid disease (AML, 24; myelodysplastic syndrome, 5; chronic myeloid leukemia, 2) and nine were treated for lymphoid disease. Four patients had positive MRD pre-HCT (three AML, one ALL). Stem cell source was peripheral blood in 25 and BM in 15. There were no significant differences in baseline characteristics between patients transplanted for myeloid versus lymphoid disease, except that a greater portion of patients with lymphoid disease received matched related donor HCT ($P = 0.02$).

3.2 | Engraftment

All patients engrafted neutrophils at a median of 11 days (range, 10-14) and 13 days (range, 11-25) in patients receiving PBSC and BM grafts, respectively ($P = 0.01$) (Table 2). Platelet recovery occurred in 36 patients at a median time of 16 days (range, 10-46) and 18 days (15-57) in patients receiving PBSC and BM, respectively ($P = 0.05$). Four were platelet-dependent through day 100 in the setting of severe GVHD and/or early TRM. Full donor chimerism in all subsets was seen at engraftment in 23 (58%), whereas 17 showed mixed chimerism (1

TABLE 1 Patient and transplant details

	Total	%	AML/MDS/CML	%	ALL	%	*P* value
Number	40	100	31	77	9	23	
Age							0.28
< 10 y	15	37	13	42	2	22	
≥ 10 y	25	63	18	58	7	78	
Gender							0.97
Male	18	45	14	45	4	44	
Female	22	55	17	55	5	56	
Ethnicity							0.87
White	20	50	15	48	5	56	
Hispanic	16	40	13	42	3	33	
Black	3	8	2	7	1	11	
Asian	1	2	1	3	0	0	
CR (*n* = 33)[a]							0.39
1	24	73	19	79	5	56	
2	7	21	4	17	3	33	
3	2	6	1	4	1	11	
MRD Pre-HCT (*n* = 33)[a]							0.91
Negative	29	88	21	88	8	89	
Positive	4	12	3	12	1	11	
Donor source							0.02
Related	14	35	8	26	6	67	
Unrelated	26	65	23	74	3	33	
Recipient CMV status							0.45
Negative	29	72	22	71	7	78	
Positive	11	28	9	29	2	22	
Stem cell source							0.77
PBSC	25	62	19	61	6	67	
BM	15	38	12	39	3	33	
GVHD prophylaxis							0.14
CSA + MMF	30	75	21	68	9	100	
Tacro + MMF	10	25	10	32	0	0	
Median MNC/kg (10^8) (range)	4 (0.91-13.46)		4 (0.91-12.83)		3.26 (1.9-13.46)		0.63
Median CD34/kg (10^6) (range)	6 (0.97-29.53)		6 (0.97-29.53)		6.63 (4-13.91)		0.19

Note. For continuous variables, *P* values for Wilcoxon tests were presented. For categorical variables, *P* values for chi-square tests were presented.
Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CML, chronic myeloid leukemia; CR, complete remission; HCT, hematopoietic stem cell transplant; kg, kilogram; MDS, myelodysplastic syndrome; MNC, mononuclear cells; MRD, minimal residual disease; PBSC, peripheral blood stem cells.
[a]CR# and MRD status only reported for patients with acute leukemia.

total, 13 T-cell and 3 total and T-cell). There was a trend toward full donor chimerism at engraftment in patients receiving PBSC (72%) versus BM (47%) ($P = 0.10$). Mixed T-cell chimerism at engraftment was seen in 16 of 38 evaluable patients: 7 PBSC and 8 BM ($P = 0.28$) without a significant difference based on disease type. Subsequent analyses (at minimum day 100 and 1-year post-HCT) showed conversion to full donor chimerism in patients who remained in CR.

3.3 | Toxicities and GVHD

In the first 100 days post-HCT, 8 patients (20%) experienced bacteremia and 21 patients (53%) had a viral reactivation (CMV, 12; BK, 12, Varicella, 1; Adenovirus, 1; no EBV viremia) (Table 2). Although five patients (13%) were treated for invasive fungal infection post-HCT, only one patient (3%) had proven fungal disease. All patients experienced oral mucositis necessitating supplemental nutrition. Sinusoidal obstruction syndrome was seen in four patients (10%): one moderate and three severe requiring defibrotide, without any correlation to achieved Bu AUC.

> Gr2 aGVHD was seen in nine patients (23%): six PBSC and three BM ($P = 0.5$), with an increased risk with increasing donor age by decade (Figure 1). Sixteen patients (40%) developed cGVHD, of which nine were mild/limited. Occurrence of > Gr2 aGVHD was associated with inferior OS ($P = 0.01$), but cGVHD was not. Related donor transplants had lower incidence of combined acute and chronic GVHD

TABLE 2 Transplant outcomes

	Total ($n = 40$)	%	PBSC ($n = 25$)	%	BM ($n = 15$)	%	*P* value
Median neutrophil engraftment, days (range)	12 (10-25)		11 (10-14)		13 (11-25)		0.01
Median PLT engraftment, days (range)	16 (10-57)		16 (10-46)		18 (15-57)		0.05
Viral infection	12	30	6	24	6	40	0.29
CMV viremia	12	30	9	36	3	20	0.29
BK viremia/hemorrhagic cystitis							
VOD	4	10	2	8	2	13	0.59
aGVHD (grade III-IV)	9	22	6	24	3	20	0.77
cGVHD	16	40	12	48	4	27	0.18
• Limited	9	22.5	7	28	2	13	
• Extensive	7	17.5	5	20	2	13	
Mixed T-cell chimerism (< 97%) at engraftment ($n = 38$)	16	42	7	30	9	60	0.10
TRM at day 100	3	7	2	8	1	7	0.88
Days to discharge ($n = 36$), median (range)	29 (19-183)		26 (19-120)		35 (22-183)		0.14
Cause of death	10	25	8	32	2	13	0.64
Primary disease	5	12.5	4	16	1	7	
GVHD	2	5	1	4	1	7	
Infection							

Note. For continuous variables, *P* values for Wilcoxon tests were presented. For categorical variables, *P* values for chi-square tests were presented.
Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; PLT, platelet; TRM, transplant-related mortality; VOD, veno-occlusive disease.

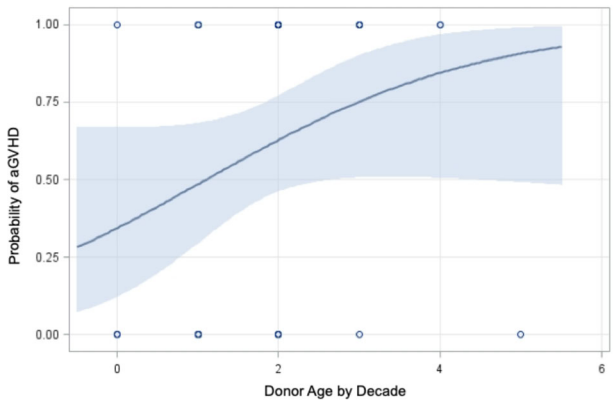

FIGURE 1 Logistic regression analysis showing risk of aGVHD according to donor age by decade with 95% confidence limits

($P = 0.06$). Incidence of cGVHD in patients receiving PBSC was nearly double that in patients receiving BM as a stem cell source, although this did not reach statistical significance (48% vs 27%, $P = 0.18$).

3.4 | Overall survival

The OS was 67% and 51% at 2 and 5 years post-HCT, respectively (Figure 2A). Patients with ALL showed a trend toward lower two- and five-year OS of 56% and 37%, respectively, versus 70% and 55% for myeloid disease ($P = 0.18$) (Figure 2B). Cause of death was relapse of primary disease in 10 (ALL, 3; myeloid disease, 7), GVHD in five and infection in two. The 100-day and 1-year TRM were reasonable at 8% and 13%, respectively, with no difference in incidence between patients who received PBSC or BM. Univariate and multivariate analyses showed no patient or transplant characteristic that had a significant effect on OS, yet there was a trend toward worse OS in patients with lymphoid disease, older age (≥ 10 vs < 10 years), and those receiving PBSC. Median follow-up of surviving patients was 1.98 years (range, 1.02-9.05).

3.5 | Relapse-free survival

The RFS was 54% and 47% at two and five years post-HCT, respectively, with a median time to relapse of 230 days (Figure 2C). The RFS for patients transplanted for lymphoid disease was 33% at both two and five years, versus 61% and 51% for myeloid disease ($P = 0.077$) (Figure 2D). Among the 11 patients who relapsed post-HCT, one- and two-year post-relapse survival was 27% and 18%, respectively. There were no relapses ≥ 2 years post-HCT. There was no significant effect of age at HCT (< 10 vs ≥ 10 years), disease type, stem cell source, pre-HCT MRD, GVHD prophylaxis regimen, post-HCT CMV viremia, mixed T-cell chimera at engraftment or use of ATG on RFS (Figure 3). Two-year RFS was 79% for patients transplanted for myeloid disease

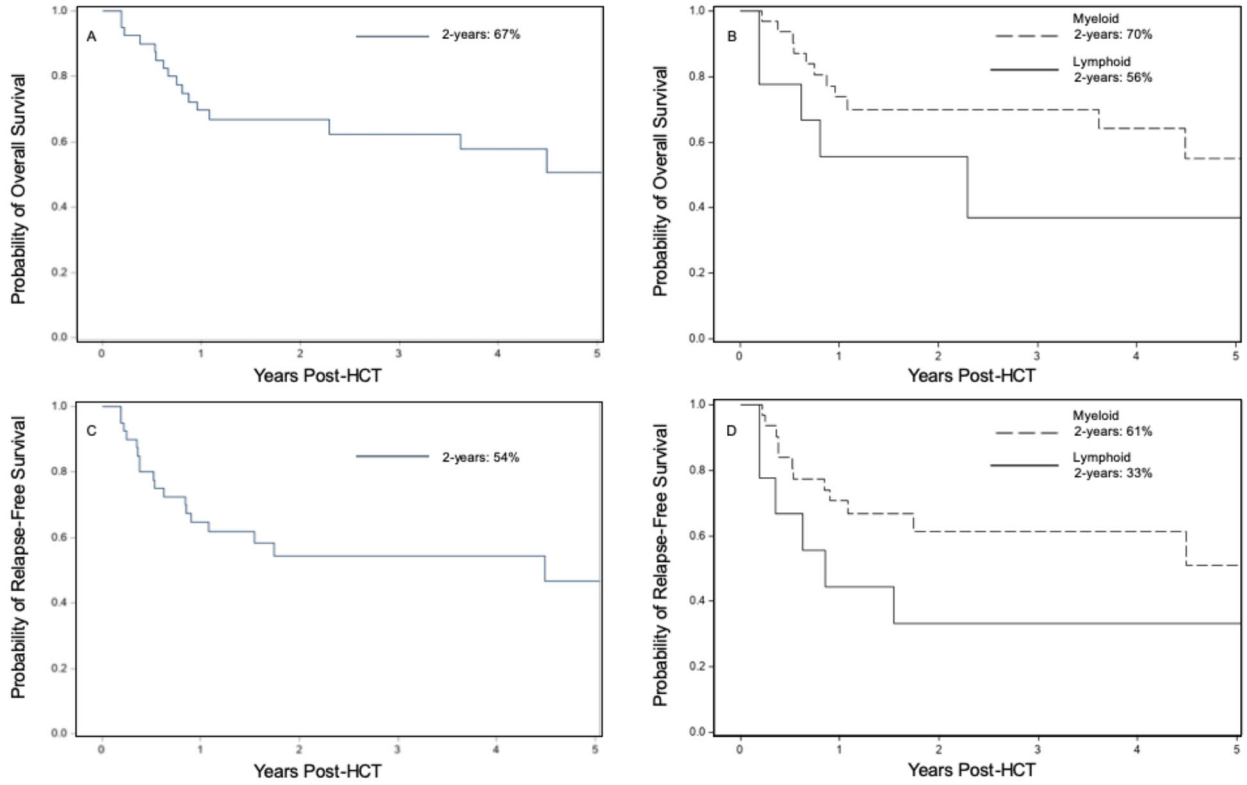

FIGURE 2 Kaplan-Meier estimates for the probability of (A) overall survival, (B) overall survival by disease type, (C) relapse-free survival, and (D) relapse-free survival by disease type

using BM source who achieved a target busulfan AUC of > 4000 ($n = 11$).

3.6 | Busulfan pharmacokinetics (PKs)

All patients had PKs drawn after a planned test dose (TD) on day −10 or −9; 35 of these were repeated after regimen dose #1 (RD1) on day −5. The median TD Bu clearance was 3.4 mL/min/kg (range, 2.25-5.13), and the median AUC was 963 μmol*min (range, 617-1443). Based on these results, RD1 was adjusted in 37 patients (93%) to achieve the target AUC, with increased dosing in 22 and decreased in 15 providing an estimated AUC average exposure within the 3800-4200 μmol*min/day goal in the first 30 patients and 4800-5200 μmol*min goal in subsequent patients. Median RD1 was 3.26 mg/kg (range, 2.27-4.86). RD1 PK confirmation showed that the AUC achieved was within this range for 18 (51%) patients, higher in 4 and lower in 14. Median Bu clearance after RD1 was 3.35 mL/min/kg (range, 2.08-6.16) and the median AUC was 3960 μmol*min (range, 2964-5111). Patients who achieved an AUC within goal range had a median age of 13.2 years versus 9.1 years in those who did not ($P = 0.017$). There was a linear relationship between busulfan AUC and OS/RFS, with increasing Bu AUC correlating with lower risk of death and relapse, respectively, without a clear upper limit of efficacy or toxicity within the AUCs measured (Figure 4).

3.7 | Post HCT late effects

Adequate immune reconstitution was achieved by one-year post-HCT in 21 of the 26 patients who were alive and in remission; all five patients with inadequate immune reconstitution at one year were still on immunosuppressive therapy due to GVHD.

Pulmonary function tests were performed at least once in 24 patients ≥ 1-year post-HCT with a normal FEV1/FVC ratio in all patients. DLCOadj was normal or within 80% of pre-HCT baseline in 11 (46%), < 80% in 12 (50%) and not measured in 1 patient (4%). Only one patient with DLCOadj ≤ 80% had any respiratory symptoms. Cardiac function was normal (EF ≥ 30% and/or SF ≥ 55%) in 24/25 patients with one patient redemonstrating poor function pre-HCT. Further follow-up data on additional post-HCT toxicities were available for 19 patients, and included ovarian insufficiency based on elevated gonadotropins (8), short stature based on growth curves (3), hypothyroidism (3), and learning disabilities (2).

4 | DISCUSSION

Our study prospectively evaluated the safety and efficacy of a reduced-toxicity, MAC regimen of Bu/Flu/ATG/TBI in pediatric patients. This regimen was overall well tolerated with a reasonable and expected toxicity profile. Engraftment rates were excellent even with BM as

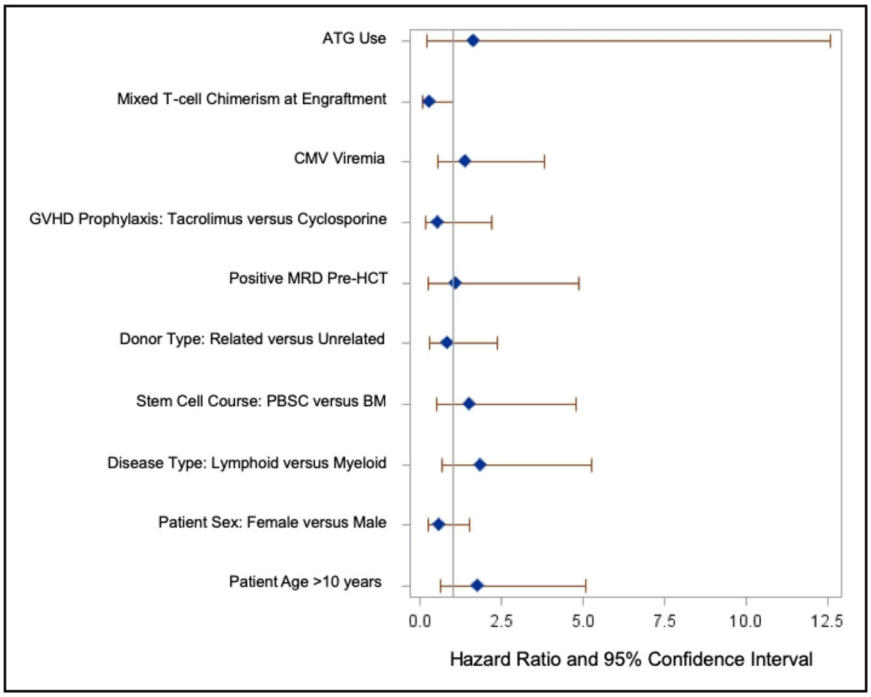

FIGURE 3 Univariate analyses of predictors of relapse-free survival

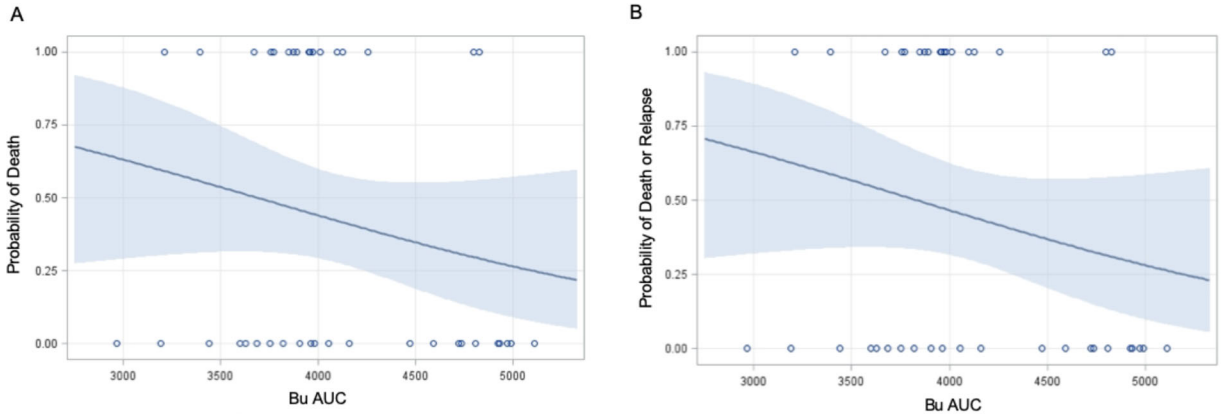

FIGURE 4 Correlation of Bu AUC with (A) risk of death and (B) risk of relapse or death

stem cell source, but relapse rates especially in the lymphoid population were concerning. Late effects of therapy were less significant than those reported in regimens using higher doses of TBI; specifically, no secondary malignancies have been reported. Toxicity seems superior to regimens of Bu/melphalan/Cy, as recently reported data from the BFM reported rates of TRM as high as 31% in patients 12 years and older.[25]

Bu/Flu/ATG/TBI was most effective for patients transplanted for myeloid disease with an RFS of > 60%. The higher relapse rate in ALL was also seen in reports by Russell et al. in adults utilizing the same regimen.[15–17] We attribute ALL relapses potentially to a lack of adequate TBI, higher risk ALL patients, and likely lack of graft-versus-leukemia with more related donors in our population. TBI containing regimens for ALL have been shown to be superior to myeloablative Bu-based regimens for pediatric patients with ALL undergoing HCT with three-year RFS of ~55% versus ~33% for Bu-based regimens,[13,14] which was similar to our study. The importance of TBI in patients with ALL undergoing HCT was recently again demonstrated in a large randomized trial that was closed prematurely due to the superiority of the TBI-based regimens.[26] The addition of low-dose TBI did not provide a survival advantage to the Bu MAC regimen in ALL, unlike the AML patients who had improved OS and RFS, and thus this regimen should not be used routinely in patients with ALL. Recent modification of our protocol with limiting the regimen to myeloid diseases, removal of ATG for related donors, increasing the Bu AUC, and utilizing only BM as donor source led to improvement in outcomes with excellent OS and RFS.

A CIBMTR-led retrospective study that compared the toxicities and outcomes of Bu/Flu versus Bu/Cy regimens in > 1700 children

transplanted showed inferior OS for patients with malignancies receiving Bu/Flu (61% vs 71% with Bu/Cy) due to lower rates of salvageability after post-HCT relapse.[27] Our incidence of OS and RFS for myeloid diseases is superior to the above report with two-year OS/RFS approaching 80% with our modified Bu/Flu approach.

Delivery of dose-adjusted Bu levels led to optimized engraftment and decreased the risk of relapse without increasing regimen-related toxicity as pioneered by our team in pediatrics by PK monitoring on a single TD of Bu to determine appropriate therapy doses.[21,28] Our pediatric cohort demonstrated variable Bu clearance, requiring dose adjustments in > 90% patients, with an expected lower mean age of patients who did not achieve target AUCs versus those who did.[21] These data suggest additional PK studies are needed for patients < 10 years of age to achieve optimal targeted Bu levels. Given lack of toxicity and evidence of improved outcomes,[29] we modified our Bu goal AUC to 5000 μmol*min/day and demonstrated a linear improvement in survival with increasing Bu exposure.

There are limitations to this study that must be acknowledged. First, this cohort involves a relatively small number of patients with some heterogeneity in stem cell and donor source. Additionally, the data are too immature to accurately capture late toxicities. Late effects seem to be less significant than those reported with higher doses of TBI, with no secondary malignancies seen, yet these patients warrant further follow-up to ensure that we capture the full spectrum of late effects. Importantly, there is still potential for uncaptured long-term toxicities from the low dose of TBI, including restrictions in growth and development, endocrinopathies, and secondary malignancies. Finally, it is difficult to definitively conclude the specific contribution of low-dose TBI to leukemia control in these patients. Although often avoided due to toxicities, TBI has been shown to have a role in leukemia control for AML patients undergoing HCT.[4] It is possible that improved outcomes seen specifically in our AML patients were related to higher Bu dosing, yet we still believe that the 400 cGy contributes to greater leukemia control in this setting, as has been shown in the adult population.[15–17] Even with these limitations, however, this study remains important in that a standard regimen was used prospectively in a relatively homogeneous group of pediatric patients with 100% engraftment rates and good disease control for patients with myeloid disease.

In conclusion, the reduced-toxicity, myeloablative regimen of dose-adjusted Bu/Flu/ATG/TBI is safe and effective in patients with high-risk leukemias, particularly promising in children transplanted for myeloid leukemias and a target Bu AUC > 4000 μmol*min/day with two-year RFS approaching 80%. Larger prospective trials are warranted using this regimen with a target Bu AUC of 5000 μmol*min/day as a standard in patients with high-risk pediatric myeloid malignancies.

CONFLICTS OF INTEREST

None of the authors have any conflicts of interest in relation to the work described above.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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