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ORIGINAL ARTICLE

Reduced intensity conditioning using intravenous busulfan, fludarabine and rabbit ATG for children with nonmalignant disorders and CML

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The major problems with busulfan/cyclophosphamide (Bu/Cy)-containing conditioning regimens are acute toxicities and graft failure. To decrease acute toxicities, we have prospectively evaluated a reduced intensity conditioning (RIC) regimen using targeted dosing of i.v. busulfan, fludarabine, and rabbit ATG (Bu/Flu/rATG) in children with diagnoses that historically would have been conditioned with Bu/Cy regimens. Nineteen pediatric patients were enrolled in the study. The donors included HLA-matched and one antigen-mismatched unrelated volunteers (*n* = 11), unrelated cord blood (*n* = 1), and related donors (*n* = 7). Four patients developed graft failure, which occurred between 1 and 8.5 months post transplant. All four of them underwent a second transplantation and 3/4 are alive without evidence of disease. The mean follow-up of living patients is 29.5 ± s.d. 11 months. Despite excellent 2-year post-transplant overall survival (89 ± s.d.7%) and event-free survival (74 ± s.d.10%), the study was closed prematurely due to high graft failure rate (21%). Receiving a transplant from a mismatched unrelated donor was identified as a risk factor for graft failure. The Bu/Flu/rATG RIC regimen was very well tolerated, resulted in excellent overall survival, and provided sustained engraftment in patients undergoing transplant from matched sibling and unrelated donors. However, it did not provide sustained engraftment in the majority of children with nonmalignancies undergoing mismatched unrelated donor transplants.
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Introduction

Hematopoietic stem cell transplantation is the treatment of choice for nonmalignant disorders, such as congenital cytopenias, hemoglobinopathies, immune deficiencies, and metabolic disorders, such as Hurler's syndrome.[1–4] Establishment of normal donor hematopoiesis in these disorders reverses the disease or halts disease progression. However, limitations of transplant include toxicities of conditioning regimens, graft rejection, and GVHD. Although reduced intensity conditioning (RIC) regimens have been studied extensively in adults with a variety of disorders, the reports on their use in children with nonmalignant disorders are scarce. With the goal of avoiding TBI and its deleterious effects on growth and neurocognitive development in young children, in the past, Bu/Cy was used as a backbone in the conditioning of children with nonmalignant disorders and myeloid malignancies.[1,2,5,6] The main complications of Bu/Cy conditioning included toxicities, such as veno-occlusive liver disease (VOD), mucositis, long-term effects on gonads and hair, and failure of engraftment, in particular in an unrelated donor setting.[7–10] In a previous study, we showed that oral busulfan, when targeted to the level >600 ng/ml, with Cy and horse ATG resulted in engraftment rate of more than 90%; however, with significant hepatic and mucosal toxicity.[11] In an effort to minimize toxicity, we combined targeted intravenous busulfan with a new immunosuppressive antimetabolite (fludarabine) and a more potent anti-thymocyte globulin – rATG (Thymoglobulin, SangStat), and studied it prospectively in a pediatric patient population.[12,13] We describe the engraftment rate, chimerism, immune reconstitution, and toxicities of this reduced intensity conditioning regimen when used in children with a variety of nonmalignant disorders, myelodysplastic syndrome (MDS) and CML.

Study design

Between September 2000 and June 2004, 19 pediatric patients with nonmalignant disorders, CML and MDS, were enrolled in a prospective phase II study conducted at the University of California, San Francisco and University of Wisconsin, Madison. The study was approved by the Committee on Human Research and Cancer Center

Protocol Review Committee at each participating institution, and informed consent was obtained from all study participants.

Donors and stem cell sources
The best available donor based on HLA-A, B, C, and DRβ1 high-resolution typing was selected. Stem cell sources included bone marrow (BM), peripheral blood, and cord blood. BM was requested as a stem cell source for patients with nonmalignant conditions, and PBSC for patients with malignancies; however, donor's preference for donation of BM or peripheral blood was respected.

Conditioning regimen
The conditioning regimen consisted of i.v. busulfan from day −9 to −6, total of 16 doses targeting continuous steady-state concentration of 600 ng/ml for patients with nonmalignant conditions and 900 ng/ml for patients with CML and MDS. Busulfan dose was determined based on a test dose (0.5 mg/kg) done on day −13, pharmacokinetic studies were carried out on day −9 and if any modifications in dose was made, pharmacokinetic studies were repeated on day −7. The fludarabine dose was 40 mg/m^2/day given from day −5 to −2 (total dose 160 mg/m^2), and rATG (Thymoglobulin) dose was 0.5 mg/kg/day on day −4, and 2.5 mg/kg/day on days −3 to −1 (total dose 8 mg/kg). G-CSF was started on day +21 if ANC was $<500/\mu$l at that time.

GVHD prophylaxis
GVHD prophylaxis consisted of CsA and MTX. CsA started on day −1 at 3 mg/kg/day and continued until day +50 (trough was maintained between 150 and 250 ng/ml). From September 2000 until September 2002, CsA taper started on day +50 in all patients. In September 2002, the protocol was changed for patients with mixed chimerism. If patients remained mixed chimeras, CsA was continued until day +180, when a slow taper with careful monitoring of chimerism status began. MTX was given at 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. In patients with cord blood transplants, methylprednisolone was used instead of MTX and given at 1 mg/kg/day on days 0–7, 2 mg/kg/day on days 8–21, and subsequently it was tapered by 0.2 mg/kg/week. The protocol did not provide any recommendations regarding use of DLI.

Supportive care
Seizure prophylaxis was used, initially with phenytoin and later with lorazepam. Prophylaxis with acyclovir, fluconazole, co-trimoxazole, and intravenous immunoglobulin was used until normal T cell proliferative responses were confirmed.

Complications were graded using CTCAE v2.0 and v3.0 criteria. GVHD and VOD were graded using published criteria.[14,15]

Chimerism testing
Chimerism was evaluated by short tandem repeat (STR) DNA analysis of peripheral blood if ANC was $>500/\mu$l, or BM if WBC recovery was not adequate. Testing was carried out at the 1, 3, 6, 9, 12, 15, 18, and 24 months for patients with full donor engraftment, and monthly during the first year and every 3 months thereafter for patients with mixed chimerism. Subset analyses were also performed (CD3, CD14/15, and CD19) on specimens collected during or after 2003. Post transplant mixed chimerism was defined as the presence of any host cells in whole blood or cell subsets at the level of detection by STR (usually 1%).

Immune reconstitution
T and B cell immunity was measured at 3-month intervals. Bcell immunity was measured by determining IgM levels (normal >40 mg/dl) and anti-A and anti-B IgM isohemagglutinin titers (normal >1:8). T and B cell lymphocyte subsets were quantified by flow cytometric analysis using fluorochrome-conjugated monoclonal antibodies to human cell-surface antigens (CD3, CD4, CD8, CD19, CD56) and a FACS analyzer (Becton Dickinson, Mountain View, CA, USA). Lymphocyte blastogenesis to mitogens, including PHA, pokeweed, and concavalin A, and lymphocyte blastogenesis to antigens (Candida and Tetanus) was performed at Mayo Medical Laboratories and was compared to control.

Statistical analysis
For statistical analysis, descriptive statistics were used. Survival was calculated using Kaplan–Meier analysis from the time of transplant until the last contact for living patients, and event-free survival (EFS) was calculated from the day of transplant until the event (death or second transplant). We used a two-tailed *t*-test for comparison of continuous variables and χ^2 with two-sided *P*-values for comparison of categorical variables.

All data analyses and tests were performed using the SPSS statistical program (SPSS Inc., Chicago, IL, USA).

Results

The median age of children treated on this study was 5 years (range 0.6–18.5 years). Table 1 summarizes patients' characteristics. The diagnoses were as follows: Wiskott–Aldrich syndrome ($n=2$), α-mannosidosis ($n=1$), SCID ($n=2$), Hurler's syndrome ($n=1$), congenital neutropenia ($n=1$), β-thalassemia ($n=2$), aplastic anemia ($n=2$), chronic granulomatous disease ($n=3$), adrenal leukodystrophy ($n=1$), congenital thrombocytopenia ($n=1$), CML ($n=2$), and MDS ($n=1$). None of our patients received previous chemotherapy; however, two patients with SCID and RAG2 deficiency underwent a previous T cell-depleted haplocompatible transplant after conditioning with fludarabine, and were enrolled in this study due to failure of engraftment. The stem cell source was BM ($n=13$), peripheral blood ($n=4$), combined related cord blood and BM ($n=1$), and unrelated cord blood ($n=1$). Twelve patients received a transplant from unrelated donors; in eight, the transplant was fully matched at HLA-A, -B, -C, and -Drβ-1 locus; and in four, there was a one-antigen mismatch at A-, B-, or C-locus, by high-resolution HLA

Table 1 Characteristics of patients

N	*Age*	*Diagnosis*	*Donor*	*Source*	*Cell*[a] *dose*	*GVHD*	*Follow-up (months)*	*Donor's cells (whole blood) (%)*	*Donor's cells (CD3+)(%)*	*Outcome*
1	1.1	Wiskott–Aldrich	MUD	BM	N/A	Acute Gr. I	8	100	Not available	Died from CMV
2	0.6	Wiskott–Aldrich	UCB	CB	0.78	No	49	100	Not available	Alive, DF
3	18.5	Aplastic anemia	MMUD	BM	1.9	No	23	1	Not available	Graft loss at 1 month, alive after second BMT
4	8.1	Aplastic anemia	MUD	PB	6.5	No	22	100	100	Alive, DF
5	9.1	Myelodysplastic syndrome	MR	PB	6.2	No	21	100	100	Alive, DF
6	7.6	Adrenal leukodystrophy	MMUD	PB	4.1	N/A	3	18	7	Graft loss at 1.3 months, died during second BMT
7	5.1	Mannosidosis	MMUD	BM	2.9	No	46	17	23	Graft loss at 8.5 months, alive after second BMT
8	1.1	SCID	MUD	BM	6.3	No	38	35	95	Alive, DF
9	1.1	SCID	MUD	BM	6.0	No	38	38	99	Alive, DF
10	5.9	CGD	MR	BM	5.8	No	39	97	89	Alive, DF
11	2.2	Hurler's syndrome	MUD	BM	6.2	Acute Gr. II chronic (skin)	35	99	91	Alive, DF
12	7.2	Thalassemia	MR+ RCB	BM CB	2.3 0.04	 No	24	99	96	Alive, DF
13	14.3	Congenital neutropenia	MR	BM	2.3	No	23	78	27	Alive, DF
14	1.4	CGD	MR	BM	6.0	No	21	16	48	Alive, DF
15	3.7	CGD	MUD	PB	6.7	Acute Gr. II chronic (skin, mouth)	16	100	100	Alive, DF
16	1.1	Congenital thrombocytopenia	MUD	BM	6.1	No	12	62	52	Alive, DF
17	13.2	CML	MR	BM	5.4	Acute Gr. I chronic (liver)	24	100	100	Alive, DF
18	3.1	CML	MUD	BM	5.6	No	38	95	92	Alive, DF
19	7.5	Thalassemia	MR	BM	5.6	No	28	42	6	Graft loss at 6 months, alive after second BMT

UCB = unrelated cord blood; MUD = matched unrelated donor; MMUD = mismatched unrelated donor; MR = matched related donor; RCB = related cord blood; BM = bone marrow; PB = peripheral blood; CB = cord blood; Gr = grade; CGD = chronic granulomatous disease.
[a]Cell dose for bone marrow and cord blood = $N \times 10^8$/kg nucleated cells/kg of recipient weight, cell dose for peripheral blood stem cells = $N \times 10^6$/kg CD34+ cells.
Donor's cells refers to the last test done on this study. All patients who are currently alive after the second myeloablative transplant have 100% donor's cells.

typing (two received unrelated BM, one peripheral blood, and one cord blood). Seven patients received fully matched related transplants. Patients undergoing a BMT received 4.6 (mean) $\pm$ (s.d.) 1.8×10^8/kg of nucleated cells; for those receiving PBSC, the mean cell dose was 5.9 (mean) $\pm$ 1.2 (s.d.) $\times 10^6$/kg CD34+ cells, and the one patient with cord blood transplant received 0.74×10^8/kg of nucleated cells. Median time to engraftment (ANC > 500/μl) was 20 days (range 16–28). All patients engrafted initially with donor's cells; however, 4/19 subsequently rejected the donor cells at 1, 1.3, 6, and 8.5 months post transplant. All patients who rejected the transplant had busulfan targeted at 600 ng/ml. Patients who rejected their graft underwent a second transplant and three of the four are alive, disease free. Two out of 19 patients died, one from toxicities of the second transplant and another one from lung CMV disease (CMV infection was present prior to transplant in this patient). The cumulative EFS for this group of patients was 74% $\pm$ s.d.10% (events include death or need for 2nd transplant), and cumulative overall survival was 89 $\pm$ s.d.7%. The median follow-up of all living patients was 24.8 (range 12.3–49) months.

Withdrawal of immunosuppression and DLI

Withdrawal of immunosuppression followed several patterns. In a group of patients treated during the early course of the study, CsA was tapered between day +50 and 6 months post transplant (patients #1, 2, 7, 8, 9, and 19). Patients undergoing transplant after September 2002 with full donor chimerism and without GVHD had CsA tapered between 4 and 8 months after transplant (patients #4 and 5). Patients with mixed chimerism had CsA tapered between 7 and 15 months after transplant (patient #10, 12, 13, 14, 16, and 18). Patients with GVHD remained on immunosuppression until 14 and 35 months post transplant (patients #11 and #17), and one patient continues on immunosuppression at 16 months post transplant for cGVHD (patient #15). Patients with early graft rejection (#3 and #6) were on immunosuppression at the time of rejection, and patients with late rejection (#7, #19) developed increasing host chimerism as CsA was being tapered, and rejected the graft shortly after discontinuation of CsA.

At the treating physician's discretion, patient #14 received two donor lymphocyte infusions at 4 and 6 months after transplant, due to the change in whole blood chimerism from 71% (at 1.3 months post transplant) to 53% (at 6 months post transplant). The patient was still on CsA at the time of DLI. There was no apparent change in whole blood chimerism after DLI, and CD3+ chimerism continued the upward trend, present prior to DLI.

Chimerism evaluation and risk factors for graft loss and mixed chimerism

Four of 19 patients rejected the graft, 6/19 achieved 100% donor engraftment, and 9/19 remained mixed chimeras at the time of the last follow-up. The percentage of donor cells on the first whole blood chimerism test done at the time of engraftment (ANC > 500/μl) was 99.5 $\pm$ 1% (mean $\pm$ s.d.) for patients who achieved full donor chimerism; 86.4 $\pm$ 12.5% for those who stayed mixed chimeras at the last follow up, and 87.2 $\pm$ 10.4% for those who rejected their graft. These means were not statistically different and chimerism testing at the time of engraftment could not predict future graft loss or state of mixed chimerism. At the last follow-up, 6/15 patients with durable engraftment had 100% donor cells in whole blood and CD3+ cell subset, and nine patients had mixed chimerism. Chimerism status at the last follow-up for each individual patient is presented in Table 1. Figures 1 and 2 illustrate trends of chimerism in whole blood and CD3+ cell subsets. Patients are grouped in 'mixed chimerism', 'graft loss', and 'full donor' based on their status at the last follow-up. Figures 1 and 2 illustrate the trend of decrease of donor's whole blood and CD3+ cells over time in patients who lost their graft, compared to those who achieved stable mixed chimerism in whom donor's cells remained stable (Figure 1 – whole blood chimerism) or gradually increased over time (Figure 2 – CD3+ cell chimerism). Of note, two of our patients have stable mixed chimerism with CD3+ being less than 50% at the last follow-up (patients #13 and #14). Graft rejection

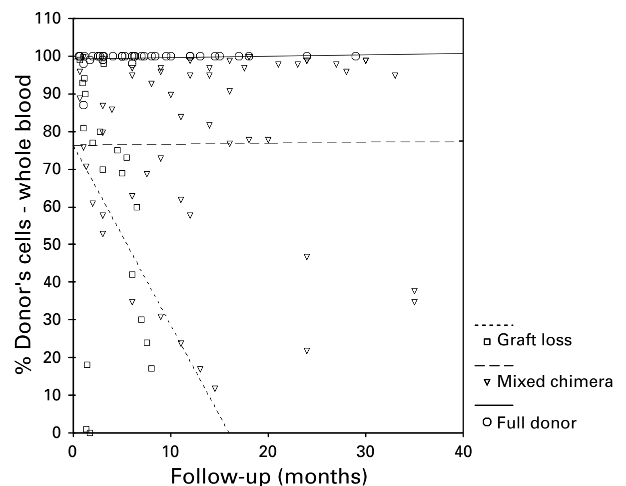

Figure 1 Chimerism trend – whole blood.

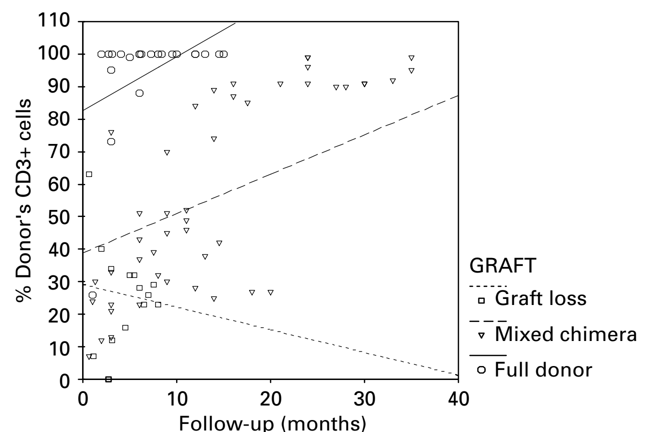

Figure 2 Chimerism trend – $CD3^+$ cells.

Table 2 Time to immune reconstitution after Bu/Flu/rATG conditioning

	Median time (days)	*Range (days)*	N
Absolute lymphocyte count $>1000/\mu l$	158	36–426	15
Absolute CD4 count $>200/\mu l$	178	90–425	15
Normal lymphocyte blastogenesis responses to mitogens and antigens	369	146–656	13
Ig M > 40 mg/dl	180	47–1069	13
Anti-A and anti-B IgM isohemagglutinin titers > 1:8	103	82–1069	12

was more common in patients receiving a mismatched unrelated donor graft (3/4) than in those receiving a fully matched unrelated donor graft (0/8) or a matched related transplant (1/7), (two-sided P for χ^2 test = 0.04). There was no significant difference between time to ANC recovery in patients with full donor chimerism 20.3 ± 3.7 days and those with mixed donor chimerism 21.0 ± 3.8 days, and graft rejection 22.6 ± 5.1 days.

Patients who remained mixed chimeras received primarily BM as a stem cell source (eight received BM, one received BM and cord blood combination); patients with full donor chimerism received peripheral blood (3/6), BM (2/6), and cord blood (1/6). This difference was statistically significant (two-sided P for χ^2 0.04).

Complications and immune reconstitution

Stomatitis grade I (according to CTCAEv3.0 criteria) developed in three patients, grade 2 in four patients, and grade 3 in one patient. Acute GVHD developed in four patients; in two it was grade I, and in two it was grade II. Chronic GVHD developed in 3/15 patients. In one patient cGVHD was limited, involving the liver only and in two patients it was extensive, involving the skin. There were no episodes of liver VOD using McDonald's criteria. CMV reactivation occurred in 4/12 patients at risk for it. Causes of death included CMV in one patient with Wiskott–Aldrich who developed CMV infection prior to transplant and died 8 months after the transplant from lung CMV disease. The second patient, after graft rejection and a second unrelated donor transplant died from multiorgan failure. Immune reconstitution is presented in Table 2. As shown in the Table 2, full T cell reconstitution took longer than B-cell reconstitution. Time to recovery of a CD4 count $>200/\mu l$, time to recovery of absolute lymphocyte count of $>1000/\mu l$, and time to recovery of normal IgM and isohemagglutinin titers was shorter in patients who were full donor chimeras and whose CsA was tapered earlier; however, the difference was not statistically significant (results not shown).

Discussion

We describe a RIC regimen consisting of Bu/Flu/rATG which was used in a variety of pediatric BM failure syndromes, metabolic defects, and CML. Although RIC regimens are commonly used in adults, the reports on their use in children with nonmalignant disorders are scarce. The recent review identified seven published studies on RIC in children which enrolled a total of 97 children, 60 of which had a nonmalignant disorders.[16]

In our study, at a median follow up of 24.8 months, 17 out of 19 patients are alive without evidence of their underlying disease. This conditioning regimen was very well tolerated, with mucositis developing in less than 50% of patients, and no evidence of VOD of the liver. All patients initially engrafted with donor cells; however, 4/19 patients lost the graft 1–8.5 months later. Receiving a mismatched unrelated donor transplant was recognized as a risk factor for graft loss, and initial whole blood chimerism test could not predict durable engraftment.

RIC regimens with lower doses of busulfan (8 mg/kg) have been extensively studied in adults with malignancies undergoing matched sibling transplants as well as matched unrelated donor transplants, and have shown acceptable engraftment rates (90% or higher).[17–19] However, Bu/Flu/ATG regimens, used in children with malignant and nonmalignant disorders, resulted in about 80% engraftment rate.[20,21] In a study that used fludarabine and busulfan targeted at 900 ng/ml for adults with CML and MDS, engraftment was achieved in all patients (26 out of 42 received unrelated donor mobilized blood stem cells).[22] Another study using fludarabine and busulfan (10 mg/kg) and matched sibling mobilized blood stem cells in patients with malignancies indicated that full donor CD3+ engraftment was more readily achieved in patients who received a higher CD34+ cell dose, when compared to those receiving a lower CD34+ cell dose.[23] However, the risk of cGVHD was increased.[23] All patients in our study who lost their graft had busulfan targeted at 600 ng/ml. Given that in patients with nonmalignant disorders, cGVHD is not desirable, the strategies to improve engraftment with current conditioning would include targeting busulfan at a higher level (900 ng/ml), or further intensifying immunosuppression.

The majority of our patients (9/19) remain stable mixed chimeras at their last follow-up, and unlike in patients who lost their grafts late, CD3+ cell chimerism has increased over time in patients with stable mixed chimerism. Withdrawal of immunosuppression after day +180 in patients with mixed chimerism appears to have decreased the risk of late graft rejection. However, our numbers in this group are quite small (six patients underwent delayed withdrawal of immunosuppression and all of them remain stable mixed chimeras). The role of DLI in patients with mixed chimerism and a high proportion of host cells could not be evaluated in this study as only one patient received DLI. Although the numbers are quite small, our data indicate that full donor chimerism was more readily achieved in

patients receiving peripheral blood compared to BM as a stem cell source. The literature supports that finding, as higher doses of CD34+ cells infused correlated with improved CD3+ cell engraftment.[21] Studies also indicate that after RIC with busulfan and fludarabine, the achievement of complete donor chimerism was significantly more likely in patients who received more than two lines of chemotherapy pretransplant.[24] Therefore, it is not surprising to find a high rate of mixed chimerism in our patients, since none received significant chemotherapy pre transplant.

Immune reconstitution in children undergoing unmanipulated unrelated and mismatched related donor transplants receiving different doses of rATG was studied by Duval *et al*.[25] In their study, a higher dose of rATG (median 15.5 mg/kg) was related to significant delay in immune reconstitution. The median time to CD3+ count of $>1000/\mu l$ was 7 months in patients who received 7.5 mg/kg of rATG in Duval's study,[25] compared to 5.3 months in our study. Duval indicated that the time to recovery of T cell immunity was related to the rATG dose. We also noted slower immune reconstitution in patients with mixed chimerism who remained longer on CsA; however, the numbers were too small to reach statistical significance. Despite a long time to the development of normal T cell function (median 1 year), we have not seen an increased number of infections or unusual infections in this patient population.

A recently described RIC regimen using Campath-1H, melphalan, and fludarabine for children with nonmalignant disorders, using a variety of stem cell sources, reported good engraftment rate (87%). However, fatal infectious complications were common, and overall survival was 75%.[26] Del Toro *et al*. reported their results using RIC in 21 children with malignant and nonmalignant disorders. They used three different fludarabine-based regimens and unrelated cord blood and matched family donors. In a subgroup of children with nonmalignant disorders and MDS, they achieved 87% 1-year post transplant overall and EFS.[20] Jacobsohn *et al*.[21] used fludarabine, busulfan, and ATG in 13 children with nonmalignant disorders and achieved engraftment in 10/13 patients (76%). Their 1-year overall survival was 84%. Therefore, our results of 89% overall survival and 74% EFS at 2 years post transplant are in line with other published studies using RIC approach in children with predominantly nonmalignant disorders and a variety of donors.

Although our data indicate that Bu/Flu/ rATG is a very well-tolerated conditioning regimen, which results in good engraftment in children with a variety of nonmalignant disorders undergoing matched sibling or fully matched unrelated donor transplant, we would caution against using this regimen in patients undergoing mismatched unrelated donor transplantation and in patients with a history of multiple blood transfusions. For these patients, a more appropriate approach would be using a regimen with intensified immunosuppression, such as to replace rATG with Campath-1H, or to target higher busulfan levels (900 ng/ml). However, both of these approaches are likely to increase transplant-related toxicity.

Conclusion

Targeted intravenous busulfan, fludarabine, and rabbit ATG is a very-well tolerated RIC regimen which resulted in excellent overall survival and sustained engraftment in children undergoing matched related and MUD transplants. However, this conditioning did not provide sustained engraftment in the majority of children undergoing mismatched MUD transplants for nonmalignancies.

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References

1 Walters MC, Patience M, Leisenring W, Eckman JR, Scott JJ, Mentzer WC *et al*. Bone marrow transplantation for sickle cell disease. *N Engl J Med* 1996; **335**: 369–376.
2 Lucarelli G, Clift RA, Galimberti M, Angelucci E, Giardini C, Baronciani D *et al*. Bone marrow transplantation in adult thalassemic patients. *Blood* 1999; **93**: 1164–1167.
3 Peters C, Steward CG, on behalf of the NMDP, IBMTR and the Working Party on Inborn Errors of the EBMT. Hematopoietic cell transplantation for inherited metabolic diseases: an overview of outcomes and practice guidelines. *Bone Marrow Transplant* 2003; **31**: 229–239.
4 Antoine C, Muller S, Cant A, Cavazzana-Calvo M, Veyes P, Vossen J *et al*. Long-term survival and transplantation of hematopoietic stem cells for immunodeficiencies: report of the European experience 1968–1999. *Lancet* 2003; **361**: 553–560.
5 Clift RA, Buckner CD, Thomas ED, Bensinger WI, Bowden R, Brayant E *et al*. Marrow transplantation for chronic myeloid leukemia: a randomized study comparing cyclophosphamide and total body irradiation with busulfan and cyclophosphamide. *Blood* 1994; **84**: 2036–2043.
6 Locatelli F, Pession A, Bonetti F, Maserati E, Prete L, Pedrazzoli P *et al*. Busulfan, cyclophosphamide and melphalan as conditioning regimen for bone marrow transplantation in children with myelodyplastic syndromes. *Leukemia* 1994; **8**: 844–849.
7 Grigg A, McLachlan R, Zaja J, Szer J. Reproductive status in long-term bone marrow transplant survivors receiving busulfan–cyclophosphamide (120 mg/kg). *Bone Marrow Transplant* 2000; **26**: 1089–1095.
8 Ozkaynak MF, Weinberg K, Kohn D, Dender L, Parkman R, Lenarsky C. Hepatic veno-occlusive disease post-bone marrow transplantation in children conditioned with busulfan and cyclophosphamide: incidence, risk factors, and clinical outcome. *Bone Marrow Transplant* 1991; **7**: 467–474.
9 Teinturier C, Hartmann O, Valteau-Couanet D, Benhamou E, Bougneres PF. Ovarian function after autologous bone marrow transplantation in childhood: high-dose busulfan is major cause of ovarian failure. *Bone Marrow Transplant* 1998; **22**: 989–994.
10 Peters C, Balthazor M, Shapiro EG, King RJ, Kollman C, Hegland JD *et al*. Outcome of unrelated donor bone marrow transplantation in 40 children with Hurler syndrome. *Blood* 1996; **87**: 4894–4902.

11 Bolinger AM, Zangwill AB, Slattery JT, Risler LJ, Sultan DH, Glidden DV *et al*. Target dose adjustment of busulfan in pediatric patients undergoing bone marrow transplantation. *Bone Marrow Transplant* 2001; **28**: 1013–1018.
12 Kashyap A, Wingard J, Cagnoni P, Roy J, Tarantolo S, Hu W *et al*. Intravenous versus oral busulfan as part of busulfan/cyclophosphamide preparative regimen for allogneic hematopoietic stem cell transplantation: decreased incidence of hepatic venoocclusive disease (HVOD), HVOD-related mortalilty, and overall 100-day mortality. *Biol Blood Marrow Transplant* 2002; **8**: 493–500.
13 Duval M, Pedron B, Rohrlich P, Legrand F, Faye A, Lescoeur B *et al*. Immune reconstitution after haematopoietic transplantation with two different doses of pre-graft antithymocyte globulin. *Bone Marrow Transplant* 2002; **30**: 421–426.
14 Przepiorka D, Wiesdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al*. 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
15 Bearman S, Anderson G, Mori M, Hinds MS, Shulman MM, McDonald GB. Venoocclusive disease of the liver: development of a model for predicting fatal outcome after marrow transplantation. *J Clin Oncol* 1993; **11**: 1729–1736.
16 Satwani P, Harrison L, Morris E, Del Toro G, Cairo MS. Reduced-intensity allogeneic stem cell transplantation in adults and children with malignant and nonmalignant disease: end of the beginning and future challenges. *Biol Blood Marrow Transplant* 2005; **11**: 403–422.
17 Mothy M, Bay JO, Faucher C, Choufi B, Bilger K, Tournilhac O *et al*. Graft-versus-host disease following allogeneic transplantation from HLA-identical siblings with antithymocyte globulin-based reduced-intensity preparative regimen. *Blood* 2003; **102**: 470–476.
18 Nagler A, Aker M, Or R, Naparstek E, Varadi G, Brautbar C *et al*. Low-intensity conditioning is sufficient to ensure engraftment in matched unrelated bone marrow transplantation. *Exp Hematol* 2001; **29**: 362–370.
19 Shaughnessy PJ, Ornstein D, Ririe D, Callander NS, Anderson JE, Pollack MS *et al*. Phase II study of moderate-intensity preparative regimen with allogeneic peripheral blood stem cell transplantation for hematologic disease: the Texas Transplant Consortium experience. *Biol Blood Marrow Transplant* 2002; **8**: 420–428.
20 Del Toro G, Satwani P, Harrison L, Cheung YK, Brigid Bradley M, George D *et al*. A pilot study of reduced intensity conditioning and allogeneic stem cell transplantation from unrelated cord blood and matched family donors in children and adolescent recipients. *Bone Marrow Transplant* 2004; **33**: 613–622.
21 Jacobsohn DA, Duerst R, Tse W, Ketzel M. Reduced intensity hematopoietic stem-cell transplantation for treatment of nonmalignant diseases in children. *Lancet* 2004; **364**: 156–162.
22 Bornhauser M, Storer B, Slattery J, Appelbaum FR, Deeg HJ, Hansen J *et al*. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. *Blood* 2003; **102**: 820–826.
23 Perez-Simon JA, Diez-Campelo M, Martino R, Brunet S, Urbano A, Caballero D *et al*. Impact of CD34+ cell dose on the outcome of patients undergoing reduced-intensity-conditioning allogeneic peripheral blood stem cell transplantation. *Blood* 2003; **102**: 1108–1113.
24 Valcarcel D, Martino R, Caballero D, Mateos MW, Perez-Simon JA, Canals C *et al*. Chimerism analysis following allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning. *Bone Marrow Transplant* 2003; **31**: 387–392.
25 Duval M, Pedron B, Rohrlich P, Legrand F, Faye A, Lescoeur B *et al*. Immune reconstitution after hematopoietic transplantation with two different doses of pre-graft antithymocyte globulin. *Bone Marrow Transplant* 2002; **30**: 421–426.
26 Shenoy S, Grossman WJ, DiPersio J, Yu LC, Wilson D, Barnes YJ *et al*. A novel reduced-intensity stem cell transplant regimen for nonmalignant disorders. *Bone Marrow Transplant* 2005; **35**: 345–352.