Fludarabine and Exposure-Targeted Busulfan Compares Favorably with Busulfan/Cyclophosphamide-Based Regimens in Pediatric Hematopoietic Cell Transplantation: Maintaining Efficacy with Less Toxicity


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Abstract

Busulfan (Bu) is used as a myeloablative agent in conditioning regimens before allogeneic hematopoietic cell transplantation (allo-HCT). In line with strategies explored in adults, patient outcomes may be optimized by replacing cyclophosphamide (Cy) with or without melphalan (Mel) with fludarabine (Flu). We compared outcomes in 2 consecutive cohorts of HCT recipients with a nonmalignant HCT indication, a myeloid malignancy or a lymphoid malignancy, or a lymphoid malignancy with a contraindication for total body irradiation (TBI). Between 2009 and 2012, 64 children received Flu + Bu at a target dose of 80-95 mg·h/L and between 2005 and 2008, 50 children received Bu targeted to 74-80 mg·h/L + Cy. In the latter group, Mel was added for patients with myeloid malignancy (n = 12). Possible confounding effects of calendar time were studied in 69 patients receiving a myeloablative dose of TBI between 2005 and 2012. Estimated 2-year survival and event-free survival were 82% and 78%, respectively, in the FluBu arm and 78% and 72%, respectively, in the BuCy (Mel) arm (P = not significant). Compared with the BuCy (Mel) arm, less toxicity was noted in the FluBu arm, with lower rates of acute (noninfectious) lung injury (16% versus 36%; P = .007), veno-occlusive disease (3% versus 28%; P = .003), chronic graft-versus-host disease (9% versus 26%; P = .047), adenovirus infection (3% versus 32%; P = .001), and human herpesvirus 6 infection reactivation (21% versus 44%; P = .005). Furthermore, the median duration of neutropenia was shorter in the FluBu arm (11 days versus 22 days; P < .001), and the patients in this arm required fewer transfusions. Our data indicate that Flu (160 mg/m²) with targeted myeloablative Bu (90 mg·h/L) is less toxic than and equally effective as BuCy (Mel) in patients with similar indications for allo-HCT.

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Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative treatment for a variety of diseases; however, its use is limited by the risk of graft failure, relapse of malignant disease, transplantation-related complications/mortality, and late effects. Busulfan (Bu) is the backbone of most chemotherapy-based conditioning regimens, and previous studies have shown a wide variability among children’s responses to Bu-based conditioning before allo-HCT [1-5]. In a previous study, our group demonstrated that a first step in optimizing a conditioning regimen is to target i.v. Bu to an optimal exposure of 78 mg·h/L (±5 mg·h/L) in combination with cyclophosphamide (Cy) [6].

Even with individualization of the Bu dose, the toxicity (early and late) of the conditioning regimen remains a major concern. In line with strategies explored in adult transplantation, the next step in further optimizing the pediatric conditioning regimen may be to replace the alkylating agent Cy with the nucleoside analog fludarabine (Flu) as an immuno-suppressive agent in the conditioning regimen. Because both Bu and Cy use glutathione S-transferase (GST) in drug metabolism, a combination of these drugs results in GST depletion, thereby increasing the risk of toxicity, whereas Flu does not cause GST depletion [7,8]. In addition, the FluBu combination may act synergistically on apoptosis of target cells [9].

Most clinical studies in adult patients using this combination have shown promising results. Compared with BuCy, FluBu has been associated with reduced toxicity (ie, lower rates of veno-occlusive disease [VOD] and graft-versus-host disease [GVHD]) and with improved outcomes [10-13]. A recent study by Lee et al. [14] did not show a favorable effect of FluBu, however. That study did not use dose targeting of Bu, which might have led to low and variable Bu exposures.
possibly accounting for the low donor chimerism reported with the FluBu regimen [14]. Data on the use of FluBu in children are limited [15-18]. Apart from a phase 1 study reported by Lee et al. [16], pediatric studies have studied low-dose FluBu in the setting of nonmyeloablative or reduced-intensity conditioning regimens. Little data have been published on the use of high-dose, myeloablative FluBu [19-21], and conditioning regimens have not yet been compared. Horn et al. [20] studied high-dose FluBu, but closed the study prematurely owing to a high incidence of graft failure. Switching from antithymocyte globulin (ATG) to alemtuzumab has been shown to increase the rate of engraftment [19].

In this prospective clinical study, we aimed to reduce the toxicity of the conditioning regimen in pediatric allo-HCT for nonmalignant indications, myeloid malignancy, or lymphoid malignancy with a contraindication for total body irradiation, while maintaining myeloablation and efficacy. We compared the outcomes of 64 pediatric patients included in a prospective study receiving a FluBu conditioning regimen between 2009 and 2012 with a recent historical cohort of 50 pediatric patients receiving BuCy (+ melphalan [Mel] in myeloid malignancies) between 2005 and 2008 in nonmalignant and (mainly) myeloid malignant indications for HCT. The FluBu regimen compared favorably with the BuCy-based regimen, demonstrating similar efficacy with less toxicity.

**PATIENTS AND METHODS**

**Study Design**

This prospective study was performed in the pediatric HCT unit of the University Medical Center Utrecht and was approved by the institutional Ethical Committee. Written informed consent was obtained from all participating patients or their legal representatives before allo-HCT. HCT data were collected prospectively in the TRASUS database [22], and were captured from the database on June 17, 2013, for this analysis.

Patients were prospectively recruited to 2 consecutive conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period. Possible calendar time effects were evaluated using a separate dataset of pediatric patients who received TBI as conditioning regimens over an 8-year period.

**Conditioning Regimens and Patient Inclusion**

Patients with a nonmalignant indication (eg, hemoglobinopathies, primary immune deficiencies, metabolic diseases), myeloid malignancy, or lymphoid malignancy with a contraindication for TBI received a Bu-based myeloablative conditioning regimen. TBI was contraindicated in patients with previous craniospinal radiation, poor cardiac function (eg, ejection fraction <30%), or compromised lung function (eg, forced expiratory volume in 1 second <80%). Patients with acute lymphoblastic leukemia (ALL) generally received a TBI-based conditioning regimen.

**BuCy(Mel)**

Between 2005 and 2009, BuCy(Mel) was the standard conditioning regimen in our center (based on either national or international protocols) for all nonmalignant indications, myeloid malignancy, and lymphoid malignancy with a contraindication for TBI. The initial dose of Bu (Busilvec; Pierre Fabre Medicament, Boulogne, France) was 120 mg/m² for all nonmalignant indications, myeloid malignancies, and lymphoid malignancies assuming a similar therapeutic environment in the calendar time periods for patients receiving BuCy(Mel) or FluBu and those receiving TBI.

**FluBu**

Patients were included between 2009 and 2012. Flu (Fludara; Sanofi) 40 mg/m² was given 1 hour before a once-daily 3-hour infusion of Bu. Starting in 2010, the Bu dose was adjusted to a body weight–dependent dosing regimen described by Bartelink et al. [30]. Bu dose targeting was based on therapeutic drug monitoring to achieve an AUC day 0-4 of 80-95 mg h/L (= 5400 µM min/day). Based on reports in adult transplantation, a higher target exposure of Bu was chosen when used in combination with Flu [30,31,32]. Serotherapy with ATG 10 mg/kg was administered to unrelated donor graft recipients from day 5 to day 2 and to cord blood recipients from day 8 to day 5. It was anticipated that the earlier administration of ATG in the cord blood recipients would shorten the period of profound T cell depletion, owing to less in vivo T cell depletion.

**Supportive Care**

Cytomegalovirus (CMV) prophylaxis, consisting of cyclosporine A (trough level, 200-250 µg/L) in all patients, remained the same throughout the study period. In recipients of an unrelated bone marrow transplant, methotrexate was added on days +1, +3, and +6 after HCT, and in unrelated cord blood recipients, prednisolone was added up to day +28 after HCT. Patients included between 2005 and 2009 who received BuCy(Mel) (n = 6) also received defibrotide as VOD prophylaxis as part of a trial [34]. VOD was treated with defibrotide 25 mg/kg/day, given in 4 divided doses.

Antimicrobial prophylaxis was standard for all patients. Ciprofloxacin was given starting at the initiation of conditioning and continuing until neutropenia resolved. Pneumocystis carinii pneumonia prophylaxis was started once neutropenia was resolved with co-trimoxazole 30 mg/kg (maximum dose, 960 mg) 3 times per week until a CD4+ cell count >200/µL was achieved. Patients who were herpes simplex virus seropositive received 500 mg/m² valacyclovir until day +12 or until a CD4+ cell count >200/µL was achieved. Standard antifungal prophylaxis consisted of fluconazole administered from the start of conditioning up to the resolution of neutropenia (neutrophils >2000/µL). Voriconazole prophylaxis was the treatment of choice in patients with unexplained fever with negative bacterial cultures during neutropenia persisting for longer than 72 hours. In the event of evidence suggesting fungal infection other than with Aspergillus, or invasive yeast infection, liposomal amphotericin-B (Ambisome) was given.

**Primary and Secondary Endpoints and Definitions**

Primary study endpoints were overall survival (OS), event-free survival (EFS), relapse-free survival in malignant diseases), relapse, and nonrelapse mortality (NRM). OS was defined as the time from transplantation to death; EFS, as survival from transplantation to last contact, autologous reconstitution (defined as documented ≤10% donor-derived engraftment), or graft failure (defined as a lack of neutrophil recovery or transient engraftment of donor cells after transplantation and/or a requirement for a second transplantation); and chronic GVHD (cGVHD) was defined as the time from transplantation to death unrelated to underlying disease. All surviving patients were censored at date of last contact.

Secondary endpoints were acute GVHD (aGVHD), diagnosed and graded according to the scheme of Glucksberg et al. [36]; chronic GVHD (cGVHD); viral infections; and viral reactivation. aGVHD, as defined in the presence of acute bilateral pulmonary infiltrates with cough, dyspnea, and hypoxemia in the absence of infection. Viral reactivation was defined as a viral load <1000 cp/mL adenovirus, human herpesvirus 6 [HHV6], cytomegalovirus [CMV], and Epstein-Barr virus [EBV]. Viral load was checked weekly up to 4 months after HCT; in the event of low-level reactivation (>100 cp/mL), levels were checked twice weekly.
Other endpoints were neutrophil engraftment (at day 60), defined as the first day of achieving a neutrophil count of >0.5 $\times$ 10$^9$/L for 3 consecutive days, and thrombocyte engraftment (at day 180), achieving a count of ≥ 20 $\times$ 10$^9$/L, for 7 consecutive days. In addition, the duration of neutropenia, defined as days of a neutrophil count < 0.5 $\times$ 10$^9$/L between the time of HCT and neutrophil engraftment, was recorded. In addition, the number of erythrocyte and thrombocyte transfusions was noted. Chimerism was defined as complete response; MSD, matched sibling donor; UCB, umbilical cord blood; UBM, unrelated bone marrow.

### Statistical Analysis

The duration of follow-up was defined as the time to the last assessment for surviving patients or death. To analyze risk factors for outcomes, we considered patient-related factors (eg, age at date of transplantation, sex, treatment-related mortality [TRM] risk), disease (ie, malignant or nonmalignant disease), and donor (ie, source, donor relationship, and HLA disparity). The association between these factors and primary and secondary endpoints were analyzed using Cox proportional hazards regression analysis. The duration of neutropenia, defined as days of a neutrophil count < 0.5 $\times$ 10$^9$/L between the time of HCT and neutrophil engraftment, was recorded. In addition, the number of erythrocyte and thrombocyte transfusions was noted. Chimerism was defined as complete response. MSD, matched sibling donor; UCB, umbilical cord blood; UBM, unrelated bone marrow.

### OS and EFS

Two-year estimated OS and EFS were 82% and 78%, respectively, for the FluBu cohort and 78% and 72%, respectively, for the BuCy(Mel) cohort ($P < .059$) (Figure 1A and B). The number of relapses in patients with malignant disease was not significantly different between the 2 cohorts ($P = .361$) (Figure 1C). Moreover, NRM in all patients did not differ between the 2 cohorts ($P = .57$). Results of the univariate analysis are presented in the Appendix. Table 2 presents the significant multivariate predictors of OS and EFS, as well as the various toxicity endpoints. After adjustment for differences in baseline characteristics between the treatment cohorts, anticipated TRM risk (based on European Group for Blood and Marrow Transplantation comorbidity risk score $[39,40]$) and diagnosis remained significant independent predictors of long-term OS and EFS. After excluding myeloid malignancies and infant ALL (ie, the diseases with an indication for Mel), the 2-year estimated OS and EFS were 91% and 89%, respectively, for the FluBu cohort and 71% and 66%, respectively, for the BuCy cohort ($P = .059$ and .036, respectively; Appendix, bottom row).

### RESULTS

#### Patient Characteristics

Between 2005 and 2012, 64 patients received FluBu, 50 received BuCy(Mel), and 69 received TBI conditioning before HCT. Patient age ranged from 2 months to 19 years. The patients in the BuCy(Mel) and FluBu cohorts were comparable in terms of age and indication for HCT (Table 1). The FluBu cohort included more male patients and demonstrated a trend toward greater use of umbilical cord blood ($P = .076$) as a donor source. Because the FluBu cohort was treated in the more recent period, the duration of follow-up was shorter in this cohort. Furthermore, Bu exposure was higher in the FluBu cohort, given the higher target exposure in this conditioning regimen.

#### Secondary Endpoints

Cumulative incidence curves for the secondary endpoints are shown in Figure 2. In univariate analysis, the FluBu...
conditioning regimen predicted a lower risk of VOD, acute lung toxicity, and cGVHD, as shown in the Appendix. Table 3 lists the significant multivariate predictors of the secondary endpoints. After adjustment for differences in baseline characteristics, FluBu and nonmalignant disease were associated with a lower risk of acute lung toxicity, VOD, and cGVHD. There was no impact of the conditioning regimen on the endpoint aGVHD ($P = .886$).

The FluBu conditioning regimen was the sole predictor of a lower risk of viral reactivation (HHV6 and adenovirus), as shown in Figure 3 and Table 2. The incidence of CMV and EBV reactivation was not significantly different between the 2 cohorts ($P = .363$ and .155, respectively). All patients in the BuCy(Mel) and FluBu cohorts had full donor chimerism at day +60 as well as at the latest follow-up time point. After excluding myeloid malignancies (AML, MDS, and juvenile monomyocytic leukemia) and infant ALL (indication for Mel), the association between the conditioning regimen and the secondary endpoints remained the same in the 2 cohorts (Appendix, bottom row), with lower toxicity in the FluBu cohort.

**Other Endpoints**

The probability of neutrophil engraftment at day 60 was 98% in both cohorts. Probability of thrombocyte engraftment (platelets >50 ×10^9/L) at day +180 was higher in the FluBu cohort (93% versus 82%; $P = .005$). In addition, time to thrombocyte engraftment and duration of neutropenia were significantly longer in the FluBu cohort compared with the BuCy(Mel) cohort (Table 3). In addition, the patients treated with FluBu required significantly fewer erythrocyte transfusions (median, 2 versus 5; $P = .009$) and thrombocyte transfusions (median, 5 versus 12; $P = .001$) after HCT.

**Calendar Time Effects in the TBI Cohort**

A myeloablative dose of TBI was used for conditioning in patients with ALL (n = 61; 88%), AML (n = 3; 4%), and non-Hodgkin lymphoma (n = 5; 8%) undergoing allo-HCT. Patient characteristics are summarized in Table 4. The TBI cohort demonstrated no significant calendar time effect for the primary endpoints OS and EFS or for any of the secondary endpoints (Table 4). In addition, there was no overall trend toward an effect on the incidence of toxicity.

**DISCUSSION**

This study shows a favorable effect of FluBu compared with BuCy(Mel) on the outcomes of allo-HCT in a variety of pediatric malignant and nonmalignant diseases. Despite the greater Bu exposure in the FluBu cohort compared with the BuCy cohort, the FluBu regimen was less toxic and associated with a shorter neutropenic period and fewer blood transfusions, while maintaining equivalent efficacy. These results are in line with observations in adults, which show a low toxicity profile in favor of the FluBu cohort with equivalent or even improved efficacy [10,11,13,33,41]; however, Horwitz et al. [42] prematurely closed a study of dual cord blood transplants using FluBu owing to a high number of graft failures, and Lee et al. [14] reported lower donor chimerism in patients treated with Flu combined with untargeted Bu compared with BuCy(Mel)-treated patients (44% versus 97.2%; $P < .001$).

The low engraftment in these 2 studies could be related to the lack of serotherapy (ATG or Campath) in recipients of unrelated donor transplants owing to lower target AUC (median, 69.3 mg·h/L in the Horwitz et al. study [42]) or an untargeted, lower, and variable Bu exposure (in the Lee et al. study [14]). A pediatric study by Horn et al. [20] also found a high incidence of graft failure, possibly related to the low target exposure of Bu. Thus, we propose maintaining therapeutic drug monitoring and targeting to a higher Bu exposure of 80 to 95 mg·h/L when using FluBu in children.

**Table 2**

<table>
<thead>
<tr>
<th>Multivariate Predictors of Survival and EFS and Toxicity Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endpoint</strong></td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>OS</td>
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<tr>
<td></td>
</tr>
<tr>
<td>EFS</td>
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<td></td>
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<tr>
<td>VOD</td>
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<tr>
<td>IPS</td>
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<tr>
<td></td>
</tr>
<tr>
<td>cGVHD</td>
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<tr>
<td>Adenovirus</td>
</tr>
<tr>
<td>HHV6</td>
</tr>
</tbody>
</table>

Comparisons between the FluBu and BuCy(Mel) cohorts using the Mann-Whitney U test.
This high target AUC is similar to that used for adults at M.D. Anderson Cancer Center [32,43]. Although the follow-up period of in our FluBu cohort may be short to allow optimal assessment of efficacy, the high rate of donor engraftment is promising across a wide range of diagnoses, including metabolic disorders, where donor chimerism can be difficult to achieve. This is the first pediatric study to use myeloablative Bu exposure and to compare outcomes after FluBu with BuCy(Mel) as a conditioning regimen.

The optimal approach for comparing different conditioning regimens is to perform a prospective randomized trial as comparisons of single-arm, consecutive trials may suffer from confounding effects of (un)known factors, including improved supportive care, introduction of new antifungals, change in the timing of ATG administration, and increased experience of the nursing and medical staff. The use of defined clinical endpoints and prospective inclusion diminished assessor bias as much as possible. In this study, possible confounding effects of calendar time were assessed by exploiting the data from the TBI cohort, a program that remained unchanged from 2005 to 2011. The relatively small size of the TBI cohort ($n = 68$) might have limited the study’s power to fully address this limitation, however. Because of the relatively small sample size, it was not possible to fully assess differences in outcomes among various subgroups within the malignant and nonmalignant groups. Furthermore, propensity scores were used to adjust for possible group selection of the use of a FluBu or BuCy(Mel) conditioning regimen. Although this study may have some limitations, these cannot account for the promising results of less VOD, less chronic GVHD, shorter duration of neutropenia, and fewer transfusions seen with the use of FluBu as myeloablative conditioning, despite the higher Bu exposure targeted in this cohort.

The pattern of toxicity (lung, hepatic, and cGVHD) and more rapid thrombocyte engraftment with FluBu suggests a reduction in endothelial damage [44]. The low risk of FluBu toxicity is most likely related to the nonoverlapping organ toxicity of the 2 drugs, with Flu not dependent on hepatic

![Figure 2](image.png)

**Figure 2.** Kaplan-Meier estimates for the probabilities of VOD (A), aGVHD (B), cGVHD (C), and IPS (D) by treatment cohort in 50 patients treated with BuCy(Mel) (---) and in 64 patients treated with FluBu (--). The numbers in parentheses indicate number of events and cohort size.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BuCy(Mel)</th>
<th>FluBu</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of neutropenia, d, median (range)</td>
<td>22 (6-95)</td>
<td>11 (5-102)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Time to neutrophil engraftment, d, median (range)</td>
<td>21 (9-95)</td>
<td>17 (12-54)</td>
<td>.118</td>
</tr>
<tr>
<td>Time to thrombocyte engraftment, d, median (range)</td>
<td>56 (14-177)</td>
<td>40 (18-166)</td>
<td>.005</td>
</tr>
<tr>
<td>Erythrocyte transfusions, n, median (range)</td>
<td>5 (0-15)</td>
<td>2 (0-35)</td>
<td>.009</td>
</tr>
<tr>
<td>Thrombocyte transfusions, n, median (range)</td>
<td>12 (2-45)</td>
<td>5 (0-58)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Comparisons between the FluBu and BuCy(Mel) cohorts using the Mann-Whitney U test.
glutathione stores for detoxification. By decreasing the levels of glutathione, toxic Cy metabolites and Bu may have a synergistic toxic effect [7,8]. This effect is more pronounced when BuCy is combined with Mel [6,45]. Of the 12 patients who received BuCyMel, 6 who were enrolled between 2007 and 2009 received defibrotide as prophylaxis. Defibrotide might have decreased the incidence of VOD in these patients, as described by Corbacioglu et al. [34]. Thus, without the use of defibrotide, the cumulative incidence of VOD in the BuCy(Mel) cohort would have been even greater and the difference between the cohorts larger. The subgroup analysis (excluding patients with myeloid malignancy from both cohorts, who would have received Mel in the BuCy conditioning) demonstrated that the lower toxicity profile of FluBu was not solely related to dropping Mel from the conditioning.

Changing the conditioning regimen from BuCy(Mel) to FluBu in patients with similar indications for HCT significantly reduced the risk of HHV6 and adenovirus infection and shortened the neutropenic period in these pediatric patients, effects that have not been reported previously to our knowledge. Our group previously reported that HHV6 reactivation is significantly associated with serious transplantation-related morbidity and mortality [46,47]. Thus, reducing the incidence of HHV6 reactivation is critical to optimizing HCT outcomes. The earlier administration of ATG (from day –8 to day –5) in cord blood recipients might have contributed to the lower incidence of these viral reactivations, possibly providing less in vivo T cell depletion and subsequently resulting in better T cell reconstitution.

In contrast to the unchanged incidence of CMV and EBV infections seen in the present study, some previous studies reported that Flu-containing conditioning regimens were associated with higher incidences of opportunistic infections, such as CMV and EBV, after transplantation because of the immunosuppressive effect of Flu [48,49]. This effect of low rates of viral reactivation after cord blood transplantation was previously described by Chiesa et al. [50] in a series of pediatric cord blood recipients without ATG in the conditioning regimen. Shortening the period of chemotherapy administration from 7 to 9 days (days –9 to –2) in the BuCy(Mel) cohort to 4 days (days –5 to –2) in the FluBu cohort, the low toxicity profile of FluBu might have resulted in the shorter neutropenic period and rapid cell engraftment in the FluBu cohort.

Now that we and others have shown that FluBu is a safe and a low-toxicity regimen in pediatric and adult patients, the next step may be a randomized controlled trial to confirm our results. A randomized trial is underway in a more

Table 4
Effects of Calendar Time on Primary and Secondary Outcomes in TBI-Treated Patients

<table>
<thead>
<tr>
<th>Year</th>
<th>P Value</th>
<th>HR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-2008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009-2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at treatment, yr</td>
<td>8.6 (1.6-18)</td>
<td>9.6 (2.1-18)</td>
</tr>
<tr>
<td>Follow-up, mo, median (range)</td>
<td>43 (0.1-105)</td>
<td>10 (0.1-46)</td>
</tr>
<tr>
<td>Sex, male/female, n</td>
<td>26, 16</td>
<td>21, 6</td>
</tr>
<tr>
<td>Transplantation number 1, 2, 3, n</td>
<td>39, 3, 0</td>
<td>25, 1, 1</td>
</tr>
<tr>
<td>Malignant disease (CR1, CR2, CR-2), n</td>
<td>13, 25, 4</td>
<td>10, 14, 2</td>
</tr>
<tr>
<td>AML, n</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ALL, n</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td>Other, n</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Nonmalignant diseases, CMV status of recipient +, n</td>
<td>7, 35</td>
<td>1, 26</td>
</tr>
<tr>
<td>EBV status of recipient +, n</td>
<td>7, 35</td>
<td>9, 18</td>
</tr>
<tr>
<td>Donor, MSD, UCB, UBM, n</td>
<td>15, 16, 11</td>
<td>8, 8, 11</td>
</tr>
<tr>
<td>HLA matched/ mismatched, n</td>
<td>26/16</td>
<td>18/9</td>
</tr>
<tr>
<td>Two-year EFS, n (%)</td>
<td>16 (65)</td>
<td>8 (65)</td>
</tr>
<tr>
<td>Two year OS, n (%)</td>
<td>15 (67)</td>
<td>8 (65)</td>
</tr>
<tr>
<td>cGVHD (&gt;1), n (%)</td>
<td>9 (20)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>aGVHD, n (%)</td>
<td>14 (30)</td>
<td>9 (39)</td>
</tr>
<tr>
<td>I. injury, n (%)</td>
<td>3 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HHV6, n (%)</td>
<td>14 (30)</td>
<td>5 (22)</td>
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<tr>
<td>EBV, n (%)</td>
<td>4 (9)</td>
<td>2 (9)</td>
</tr>
<tr>
<td>CMV, n (%)</td>
<td>7 (15)</td>
<td>7 (30)</td>
</tr>
<tr>
<td>Adenovirus, n (%)</td>
<td>6 (13)</td>
<td>4 (17)</td>
</tr>
</tbody>
</table>

* Comparison of the FluBu and BuCy(Mel) cohorts using the Mann-Whitney U test for patient characteristics and Cox regression for the time-to-event analysis.

1 The risk of TRM was calculated for each patient based on a comorbidity risk score, subdividing the cohort into <20% or >20% TRM risk [40,41].

2 HLA matching was based on high-resolution typing for class I and class II (10 alleles) for bone marrow or peripheral blood stem cell donors. For cord blood donors, intermediate resolution criteria were used on 6 loci (low resolution for loci HLA-A, -B, and -DRB1 by high-resolution typing). One or more allele or antigen mismatches was considered a mismatch.
focused disease population of juvenile monomyelocytic leukemia in the Children's Oncology Group (ClinicalTrials.gov: NCT01824693). Furthermore, future strategies may focus on the use of Fludibu as a conditioning platform for HCT in all diseases and for providing better disease control in malignant diseases. Dose targeting of Bu is critical to improve outcomes of HCT [32,51-54]. Lee et al. [14] showed that combining Flud with untargeted Bu may result in lower donor chimerism. The optimal dosing of and exposure to Bu in children, defined using the BuCy(Mel) regimen [63,55,56], were increased in this study when Bu was used in combination with Flud. In this new FludBu regimen, further fine-tuning of the Bu dose is needed. For instance, in nonmalignant diseases, exposure deescalation can be used to determine the lowest acceptable exposure not be associated with graft failure. More recent studies suggest that optimal dosing of Flud may be essential as well. Long-Boyle et al. [57] reported a correlation between Flud concentrations and TRM, whereas a saturation of intracellular uptake of Flud may reduce efficacy of higher Flud dosing (personal communication, J. Long-Boyle, August 2013). Compared with the Flud dose of 160 mg/m² given over 4 days in the present study, some previous adult studies used higher cumulative Flud doses, up to 250 mg/m² administered over 4 or more consecutive days [12,32,33]. Lee et al. [14] showed that in children, Flud 250 mg/m² and Bu with an AUC_{day0-4} of 72.5-80 mg·h/L are associated with a high incidence of toxicity, suggesting that increased Flud exposure may be the cause of this toxicity, given that the Bu exposure was lower than that in the present study. A reduction of the Bu target AUC to 72-76 mg·h/L decreased toxicity, but the incidence of graft failure remained significant [16]. In contrast, we found a low toxicity rate and excellent engraftment with a regimen of Bu targeted to 80-95 mg·h/L combined with Flud 160 mg·m². Pre-HCT intervention to improve disease control (eg, by adding a third nonalkylating agent, such as gemcitabine or clofarabine) may result in synergistic cytotoxicity, as described previously [58,59]. Clinically, BuFlu + Clo was proven safe in 51 very-high-risk patients with AML/MDS (mainly adults) [60]. Other strategies may include adding low-dose TBI, as explored by Russell and coworkers [61,62]; however, this might not be a good option in pediatric patients, given the association between TBI and severe late effects. Furthermore, post-HCT interventions with either novel agents (eg, sirolimus, histone deacetylase inhibitors, tyrosine kinase inhibitors) or immune interventions (eg, cellular), on a FludBu backbone may be considered [63-67]. Further individualization of the target exposure of Bu, Flud, or other drugs in the conditioning regimen and improved disease control by disease-specific additions also may contribute to safer and more effective allo-HCT.

In conclusion, we have shown that Flud 160 mg/m² combined with i.v. Bu targeted to a myeloablative exposure (80-95 mg·h/L) has a positive impact on post-HCT safety, while maintaining efficacy in pediatric malignant and nonmalignant HCT indications. This favorable toxicity profile of this FludBu regimen merit its consideration as conditioning platform for HCT in all indications in future studies. In addition to a randomized controlled trial to confirm our results, further studies are warranted to fine-tune such a FludBu platform.

**ACKNOWLEDGMENTS**

The authors thank Cuno Uiterwaal and Stephen Shiboski for providing statistical advice and Jen Hibma for editing the manuscript.

**Conflict of interest statement:** There are no conflicts of interest to report.

**Authorship statement:** I.H.B. designed and performed research, analyzed the data, and wrote the manuscript. E.M.L.v.R. performed research, collected data, and wrote the manuscript. C.E.G. contributed to the study design and data collection and management. E.M.v.M. performed research, collected data, and wrote the manuscript. A.D.W. contributed to the study design and data collection and management. B.V. contributed to the study design and data collection. C.A.L. contributed to the study design and data collection. M.B.B. designed research and wrote the manuscript. J.J.B. designed and performed research, analyzed data, and wrote the manuscript.

**Financial disclosure:** The authors have nothing to disclose.

**APPENDIX**

Univariate Predictors of Survival and EFS and Toxicity Endpoints

Fludarabine Bu busulfan Cy cyclophosphamide Mel Melphalan, TRM-riks treatment related mortality risk, MSD matched sibling donor, MFD matched family donor, UCB unrelated cord blood, UBM unrelated bone marrow, OS overall survival, EFS event free survival VOD veno occlusive disease.CMV Cytomegalovirus, EBV Epstein–Barr virus, HHV6 Human Herpesvirus 6

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