Biol Blood Marrow Transplant 20 (2014) 345–353

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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Article history:
Received 27 September 2013
Accepted 29 November 2013

Key Words:
Busulfan
Fludarabine
Hematopoietic cell transplantation
Pediatrics

A B S T R A C T

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 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/m^2) 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.

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 immunosuppressive 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,

Financial disclosure: See Acknowledgments on page 351.

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1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.11.027

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 TRIASUS 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 myeloablative conditioning in our program between 2005 and 2011, assuming a similar therapeutic environment in the calendar time periods for the patients receiving BuCy(Mel) or FluBu and those receiving TBI.

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 malignancies, and lymphoid malignancies with a contraindication for TBI. The initial dose of Bu (Busilvex; Pierre Fabre Medicament, Boulogne, France) was 120 mg/m^2 in patients age >1 year and 80 mg/m^2 in those age <1 year. Bu was administered in a 3-hour infusion once daily, with dose targeting based on therapeutic drug monitoring to a total area under the curve (AUC_{day0-4}) of 74-82 mg·h/L (4400-4900 μM·min/day), as described previously [6,17,18,23-25]. In short, the AUC was based on 3 to 6 blood samples obtained between 5 minutes and 7 hours after the end of Bu infusion on day 1, using a single-compartment model with linear pharmacokinetics established by Cremers and coworkers [26-28]. Empirical Bayesian pharmacokinetic parameter estimates (ie, clearance and volume of distribution) were estimated using the MwPharm pharmacokinetic software package [29]. The Bu dose was adjusted only when the AUC differed by >10% from the target AUC. Evaluation of the AUC after dose adjustment was performed on the next day and at day +4. All values were used to calculate the total Bu exposure.

Cy was dosed either 60 mg/kg for 2 days in patients with malignant disease or 50 mg/kg for 4 days in those with nonmalignant disease. Cy was administered at least 24 hours after Bu. Mel was added in patients with myeloid malignancy (ie, myelodysplastic syndrome [MDS] or acute myelogenous leukemia [AML]) and in those with infant ALL. Melphalan was dosed at 140 mg/m^2 once daily after 2 days of Cy therapy. Serotherapy with ATG (Thymoglobulin; Sanofi, Cambridge, MA, USA) 10 mg/kg was administered to all recipients of an unrelated donor graft over 4 days (day −5 to day −2).

FluBu

Patients were included between 2009 and 2012. Flu (Fludara; Sanofi) 40 mg/m^2 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 an AUC_{day0-4} 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 [10,31-33]. 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.

TBI

Patients received a cumulative TBI dose of 12 Gy (6 × 2 Gy). These patients were included as the TBI cohort only between 2005 and 2011, because after 2011, the Dutch national conditioning regimen for patients with ALL was changed to a TBI-free regimen containing clofarabine (CloFluBu). These patients were not included in the FluBu cohort. The patients who received TBI were included solely to study possible calendar time effects and had a similar therapeutic environment as the patients receiving BuCy(Mel) or FluBu in the time periods studied.

Supportive Care

GVHD 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 2007 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/m^2 (val)acyclovir until day +28 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 >500/L for 3 days). From 2008 onward, patients at high risk for fungal infections were given voriconazole as prophylaxis [35].

Empirical antifungal therapy with voriconazole (trough level targeted to 2-5 μg/L) was initiated 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), with relapse and death considered events. NRM 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); VOD, according to Bearman et al. [37]; acute noninfectious lung injury (ie, idiopathic pneumonia syndrome [IPS]); and viral reactivation. IPS was defined as 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.