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Once Daily i.v. Busulfan and Fludarabine (i.v. Bu-Flu) Compares Favorably with i.v. Busulfan and Cyclophosphamide (i.v. BuCy2) as Pretransplant Conditioning Therapy in AML/MDS

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ABSTRACT

We postulated that fludarabine (Flu) instead of cyclophosphamide (Cy) combined with i.v. busulfan (Bu) as preconditioning for allogeneic hematopoietic stem cell transplantation (HSCT) would improve safety and retain antileukemic efficacy. Sixty-seven patients received BuCy2, and subsequently, 148 patients received Bu-Flu. We used a Bayesian method to compare outcomes between these nonrandomized patients. The groups had comparable pretreatment characteristics, except that Bu-Flu patients were older (46 versus 39 years, $P < .01$), more often had unrelated donors (47.3% versus 20.9%, $P < .0003$), and had shorter median follow-up (39.7 versus 74.6 months). To account for improved supportive care and other unidentified factors that may affect outcome ("period" effects), 78 acute myelogenous leukemia (AML) patients receiving Melphalan-Flu (MF), treated in parallel during this time (1997-2004) were used to estimate the period effect. The MF patients' outcomes worsened during this period. Therefore, the period effect is unlikely to explain the greatly improved outcome with Bu-Flu. Patients transplanted with Bu-Flu in the first complete remission (CR1) had a 3-year overall survival and event-free-survival (EFS) of 78% and 74%, respectively, whereas CR1 patients younger than age 41 had a 3-year EFS of 83%. These results support replacing BuCy ± ATG with Bu-Flu ± rabbit-antithymocyte globulin (ATG), and warrant a prospective comparison between allogeneic HSCT and conventional induction/consolidation chemotherapy for AML in CR1.

KEY WORDS

i.v. Busulfan • Fludarabine • Cyclophosphamide • AML • MDS • Allogeneic stem cell transplantation

INTRODUCTION

Introduction of i.v. Busulfan (i.v. Bu) as an alternative to oral Bu [1] rekindled interest in optimizing the conditioning regimen to improve treatment outcome after allogeneic hematopoietic stem cell transplantation (HSCT) for myelogenous leukemia [2-5]. Recent studies with i.v. Bu and cyclophosphamide (Cy) suggested a lower incidence of serious hepatic veno-occlusive disease (VOD) and other treatment-related serious adverse events compared to what would be expected after oral BuCy2 [6,7]. These risks are of particular concern because typically Bu is combined with other agent(s), for example, Cy, known to cause VOD [8,9]. However, not only regimen-related toxicity, but also engraftment and acute graft-versus-host disease (aGVHD) may be influenced by variable systemic exposure [6,7,10,11] and the relative timing of the individual cytotoxic agent(s) in a high-dose chemotherapy combination [12-14]. This is especially true when alkylating agents with partly overlapping dose-limiting toxicities are combined in myeloablative pretransplant conditioning therapy. We thus decided to combine i.v. Bu with an immunosuppressive agent having very limited hepatotoxic potential, fludarabine

(Flu). A Bu-Flu combination has several appealing features; Flu is likely as immunosuppressive as Cy [15] and, when timed appropriately, it synergistically promotes Bu-induced cytotoxicity through interference with repair of XRT- and alkylator-induced DNA-damage [16]. Further, Flu does not cause VOD, and its long plasma half-life encourages once daily administration. We recently reported safety and outcome data after HSCT for acute myelogenous leukemia (AML)/myelodysplastic syndrome (MDS) with a myeloablative, once daily i.v. Bu-Flu regimen [17], and similarly encouraging data have been obtained in patients undergoing allogeneic HSCT for a variety of hematologic malignancies [18,19]. These early reports demonstrate i.v. Bu-Flu combinations to be safe and efficacious, resulting in low treatment-related mortality (TRM) because of, at least in part, highly reproducible intra- and interpatient systemic Bu exposure [17,18,20,21]. Although the available safety and efficacy data appear promising, there was apprehension about possibly suboptimal antileukemic efficacy of Flu compared with Cy, particularly in patients with active leukemia at the time of transplant [4]. Ideally, this question would be addressed through a comparison of BuCy2 and Bu-Flu in a randomized phase III study, stratifying patients for clinical disease stage and other prognostic factors. However, aside from the large number of patients and long time needed to complete such a study, it would also be fraught with uncertainty as to whether fixed dose Bu delivery is optimal; an ongoing study at the M.D. Anderson Cancer Center is comparing fixed-dose Bu with drug delivery based on patient-specific pharmacokinetic (PK) information. Further comparisons of clinical outcome and systemic Bu exposure suggest the presence of an optimal therapeutic interval for i..v. Bu in combination with either Cy or Flu [11,22]. Because this issue is unresolved, and PK-guided dosing currently is being refined, it is premature to begin a long-term study of fixed dose Bu-Flu versus BuCy2.

In the present analysis, we compared the outcomes of 67 patients receiving BuCy2 with 148 consecutive patients treated subsequently with the fixed-dose Bu-Flu combination. We observed a remarkable difference in TRM rates between the 2 groups, both within the first 100 days and at 1 year posttransplant. This low early TRM after Bu-Flu was strikingly different from previous experience by both our group and that of others with alternative conditioning regimens [23-28].

However, comparison of Bu-Flu and BuCy2 based on these new data was complicated by several issues. First, there were significant differences in age and other characteristics of the 2 patient populations. Second, and more importantly, patients were not randomized between the 2 conditioning regimens; rather, the programs were executed sequentially during an 8-year period: 1997-2001 for the Bu Cy2 trial and 2001-2005 for the Bu-Flu trial. Consequently, the difference between BuCy2 and Bu-Flu—the "treatment effect"—is confounded by possible differences between the patient groups or therapeutic environments in these 2 time periods that are unrelated to the preparative regimens, including changing practice patterns such as addition of new antibacterial, antifungal, and antiviral antibiotics, introduction of rabbit antithymocyte-globulin (ATG), changing referral patterns, differences in patient characteristics, or the effects of other, unknown variables. We will refer to the composite influence of all such confounding factors as the "period" effect. The statistical problem thus is to compare treatment effects between 2 treatment groups while accounting for the confounding between-trial effects. We will do this, using a Bayesian model and method [2,29,30] that deals with treatment-trial confounding, by estimating the period effect using a separate data set of 78 patients who received pretransplant conditioning therapy with Melphalan and Flu (MF) at M.D. Anderson during the period 1997 to 2004 [26], and assuming that the period effect for the BuCy2 and Bu-Flu patients was the same as for the MF patients. Although this Bayesian approach is not a substitute for a randomized phase III trial, it can be used to obtain a reasonable estimate of treatment effect(s) under the assumption that period effect accounts for between-trial effects. The results may be used to decide whether to compare these regimens in a prospective randomized trial. Additionally, although ongoing studies of individualized, PK-guided i.v. Bu (combined with Flu ± ATG) are completed, our analyses also can be used to support therapeutic decision making in patients with AML/MDS who are considered for allogeneic HSCT using i.v. Bu-based conditioning therapy. Presently, our analyses strongly support (1) Bu-Flu ± ATG as a preferred regimen over i.v. BuCy2 ± ATG, and (2) a prospective comparison of allogeneic HSCT with conventional chemotherapy in first complete remission (CR1) for patients with AML.

PATIENTS AND METHODS

Patient Eligibility

AML patients should have failed initial induction chemotherapy, or have high-risk disease in CR1, characterized by cytogenetics other than translocation (t)(8;21), inversion (inv)16, or t(15;17), or by the need for more than 1 cycle of chemotherapy to achieve CR [31]. Patients beyond CR1 were also eligible. Subjects with MDS were eligible if they had a high International Prognostic Score System (IPSS) score (≥2) [32], or if they progressed after chemotherapy.

The eligibility criteria included acceptable renal (creatinine ≤1.5 mg%) and hepatic function with normal bilirubin, SGPT ≤3 times the upper normal limit, a ZUBROD performance status ≤2, negative

serology for hepatitis B and C, and HIV, LVEF ≥45%, FEV_1, FVC, and DLCO ≥50% of predicted, absence of uncontrolled infection, and no chemotherapy within 30 days prior to entry. A human leukocyte antigen (HLA) compatible related (fully matched or 1-antigen mismatched) or matched unrelated donor (MUD) was required. All patients signed informed consent according to institutional guidelines. One Bu-Flu patient was treated off protocol with institutional review board approval, under a "compassionate plea" mechanism because of chronic renal failure developed after a previous nonmyeloablative transplant. No patients treated after August 2005 were included in this study to allow for comparison of patient populations with a median follow-up >2 years.

Conditioning Regimens

I.V. Bu-Flu. The treatment has been previously described [**17**], and consisted of Flu (Fludara®, Berlex Laboratories, Inc., Montville, NJ) 40 mg/m^2 given over 60 minutes daily for 4 days, each dose immediately followed by i.v. Bu (IV Busulfex® (busulfan) Injection, ESP Pharma, Inc., Edison, NJ), 130 mg/m^2 over 3 hours daily (days –6 to –3).

I.V. BuCy2. This regimen was also previously described [33]. Briefly, i.v. Bu was administered at 0.8 mg/kg (~32 mg/m^2) over 2 hours every 6 hours for 16 doses (days –7 to –4) and Cy was then given at 60 mg/kg i.v. over 1 hour daily for 2 doses (days –3 and –2).

MF. In the MF group the patients were treated with Flu 25 mg/m^2 i.v. daily for 5 days and melphalan 90 mg/m^2 or 70 mg/m^2 daily for 2 days as described by Giralt et al. [26]. Melphalan was given after Flu on the last 2 days of chemotherapy. Day zero was the day of transplantation in all protocols. In late 2004, this program was revised to incorporate gemtuzumab ozogamicin [34]. Patients treated on this revised MF protocol were not included in the current comparison. Patients were eligible for the MF program if they were older than 55 years and/or having at least 1 comorbid condition that made them ineligible for the front-line program (BuCy2 and Bu-Flu, respectively).

Supportive Care

All supportive care measures were utilized according to extant institutional protocols. All patients received Filgrastim (Neupogen®, Amgen, Inc., Thousand Oaks, CA) 5 μg/kg subcutaneously daily from day +7 until achieving an absolute neutrophil count (ANC) ≥1.5 × 10^9/L for 3 days. Phenytoin was used during and 1 day after i.v. Bu-based therapy.

The cytotoxic drugs were infused via a controlled-rate infusion pump through a central venous catheter. Flu dosing was according to actual body weight. The alkylating agents were dosed per patients' actual weight up to 120% of ideal body weight, above which the doses were based on adjusted ideal body weight (ideal weight plus 50% of the difference between ideal and actual weight). All groups received graft-versus-host disease (GVHD) prophylaxis with tacrolimus (Prograf®, Fujisawa Healthcare, Inc., Deerfield, IL) and minidose methotrexate (MTX) 5 mg/m^2 on days 1, 3, 6, and 11 following transplant [35]. Tacrolimus was to be continued for 6-8 months. Patients with a 1-antigen mismatched related or an unrelated donor received equine ATG (ATGAM®, Pharmacia & Upjohn Company, Kalamazoo, MI) 20 mg/kg daily (days –3 to –1 (in the BuCy2 group), or rabbit-ATG (Thymoglobulin®, Genzyme Inc., Cambridge, MA), 0.5 mg/kg on day –3, 1.5 mg/kg on day –2, and 2.0 mg/kg on day –1) (Bu-Flu group). In addition, Pentostatin (Nipent®, Supergen, Dublin, CA) was added in 10 cases receiving unrelated (n = 8) or 1-antigen mismatched related donor grafts (n = 2), under an investigational protocol (i.v. Bu-Flu arm only). The dose of pentostatin was 0.5 mg/m^2 (n = 2), 1 mg/m^2 (n = 4), and 1.5 mg/m^2 (n = 4) given on days 8, 15, 22, and 30 following HSCT.

Hematopoietic Stem Cell Grafts

Procurement of donor peripheral blood progenitor cells (PBPC) has been described [36]. Donors were treated with Filgrastim 10-12 μg/kg every 12 hours over 3 days and in the morning of day 4 prior to PBPC collection. The donor's total blood volume was processed 3 times per apheresis procedure. In case a second apheresis procedure was performed, Filgrastim treatment was continued through prior to the second procedure. The PBPC dose was targeted to approximately 5 × 10^6 CD34$^+$ cells/kg patient body weight, in keeping with the observation of a correlation between higher cell doses and incidence of GVHD [37]. Bone marrow or PBPC from unrelated donors were obtained through the National Marrow Donor Program.

HLA Typing

HLA typing for class I antigens was performed using standard serologic techniques. Class II alleles (HLA-DRB1) were resolved with low-resolution molecular typing using sequence specific oligonucleotide primers for hybridization of amplified DNA, followed by high-resolution typing in all patients and donors. Donor-recipient pairs were considered fully matched by compatibility for HLA-A, -B, and -DRB1.

Analysis of Chimerism

Peripheral blood or bone marrow donor-recipient chimerism was evaluated by analysis of DNA microsatellite polymorphisms by polymerase chain reaction (PCR) with D6S264, D3S1282, D18S62, and D3S1300 fluorescence-labeled primers, and analyzed using GeneScan software (Applied Biosystems, Foster

City, CA). In addition, we used conventional cytogenetic analysis with G-banding or fluorescein in situ hybridization studies for the Y-chromosome in sex-mismatched cases. Mixed chimerism was defined as the presence of any detectable (≥1%) recipient DNA or cells in addition to donor-derived DNA or cells.

Clinical Outcome Variables

Time of engraftment was defined as the first of 3 consecutive days with ANC $\geq 0.5 \times 10^9$/L. Failure to engraft in the absence of malignancy by day +30 was considered primary engraftment failure. Secondary graft failure was initial engraftment with documented donor-derived hematopoiesis followed by loss of graft function without recurrent malignancy. Time of platelet engraftment was defined as the first of 7 consecutive days with a platelet count $\geq 20 \times 10^9$/L without transfusion support. Criteria for CR prior to transplant included absence of circulating blasts, <5% marrow blasts, lack of chromosomal abnormalities, and platelet count $\geq 100 \times 10^9$/L. CR posttransplant was defined using the same criteria except for platelet count, with documented donor cell engraftment.

Cytogenetics were considered prognostically favorable for patients with t(15,17), inv 16, or t(8,21); poor risk ("bad") for patients with deletions of chromosome 5 and/or 7, multiple chromosomal abnormalities or trisomy of chromosome 8; and intermediate risk in all others [31]. Standard morphologic criteria, conventional cytogenetics, or both were used to diagnose recurrent disease. Cytogenetic relapse was documented by the presence of a clonal chromosomal abnormality in >2 consecutive tests, taken at least 4 weeks apart. Time to relapse/progressive disease was calculated from transplant to the day of documented event. Patients who did not achieve a CR after transplant were scored as failures at the date of the documented persistent disease. Toxicity was scored using the modified National Cancer Institute criteria (CTC 2.0).

Overall survival (OS) was calculated from the day of transplant, with patients alive at the time of last follow-up administratively censored. TRM was defined as death because of any cause other than relapse, whereas nonrelapse-related survival (NRRS) was defined as the time from HSCT to death for reasons other than relapse, with relapse being a censoring event. Event-free survival (EFS) time was counted from day zero to relapse or death. Relapse-free survival (RFS) was defined as time from HSCT to relapse with death or time of last follow-up in CR counting as censoring events.

Adverse events and hematologic parameters were monitored daily, clinical chemistry parameters at least twice weekly during the initial hospitalization and then at least once weekly up to HSCT day +100. Subsequently, patients were followed at least quarterly during the first year, then at gradually increasing intervals.

Statistical Methods

General methods. Patient characteristics were summarized using the median (range) for numeric variables or frequencies (percentages) for categoric variables. Differences in the distributions of patient characteristics between groups were assessed using Kruskal-Wallis or generalized Fisher exact tests [38]. Unadjusted probabilities of event times were estimated using the method of Kaplan and Meier (KM) [39]. The log-rank test [40] was used to compare unadjusted OS, NRRS, EFS, and RFS between subgroups. Bayesian log-normal regression models were used to assess the joint effects of patient covariates and treatments on OS, and similarly on each of the other outcomes NRRS, EFS, and RFS. The covariates included cytogenetics (bad versus other), disease status at BMT (in CR versus not in CR), donor type (sibling or other-related versus unrelated donor), age, whether any blasts were present in the patient's peripheral blood (PB), and PB platelet count. The log-normal model was selected after assessing goodness of fit for several parametric models, including the exponential, Weibull, and log-logistic, using the Bayesian Information Criterion (BIC) and the Bayesian chi-squared method [41]. The log-normal model assumes a normal distribution for the log-transformed event time, denoted $\log(T) \sim N(\mu, r)$, where μ is a linear combination of covariate effects and treatment effects, and r is the precision parameter, equal to the inverse of the variance, σ^2, of $\log(T)$.

For each model fit, we assumed that each parameter in μ followed a noninformative normal prior with mean 0 and variance 1000, and a noninformative inverse-Gamma prior for σ^2, with mean 1 and variance 1000. All statistical analyses were carried out in Splus 6.1 [42] or, for the Bayesian model fits, in WinBugs 1.4 [43].

Bayesian method for comparing Bu-Cy versus Bu-Flu. Patients were not randomized between Bu-Flu and BuCy2 with all BuCy2 patients enrolled prior to t^* = 4/18/2001 and all Bu-Flu patients enrolled after this date. Consequently, the Bu-Flu versus BuCy2 ("treatment") effect was confounded with the post-t^* versus pre-t^* ("period") effect in the data from the 215 patients, and this treatment effect cannot be estimated from these data. To address this problem, we first fit a Bayesian log normal regression model to the data from the 215 BuCy2 and Bu-Flu patients, including patient prognostic covariates and a parameter θ accounting for the confounded treatment-period effect by including an indicator [Bu-Flu] in the linear term μ. We assumed that this parameter was the sum of the actual Bu-Flu-versus-BuCy2 (treatment) effect, θ_{TRT}, and a period effect, θ_{PERIOD}, formally, $\theta = \theta_{TRT} + \theta_{PERIOD}$. We also fit a similar Bayesian regression model, including the same covariates and an indicator [PERIOD] = 1 if the patient was enrolled after t^* and

0 if before t^*, to the data on the 78 MF patients treated over a period of time spanning the 2 periods both before and after t^*. Because this provided a posterior estimate of θ_{PERIOD}, we obtained the treatment effect of interest as $\theta_{TRT} = \theta - \theta_{PERIOD}$. That is, under the above additivity assumption that the confounded effect of Bu-Flu after t^* versus BuCy2 before t^* was equal to the sum of the Bu-Flu versus BuCy2 treatment effect plus the post-t^* versus pre-t^* period effect, the period effect was estimated separately, and the i.v. Bu-Flu versus i.v. BuCy2 effect was obtained by subtraction [2,30]. All treatment-covariate interactions were included initially in the model, and interaction terms for which the posterior probability of a beneficial effect was not either >0.90 or <0.10 were dropped.

RESULTS

Patients Treated with BuCy2 versus Bu-Flu (N = 215)

Table 1a summarizes characteristics of the 215 BuCy2 and Bu-Flu patients. The only significant covariate imbalances were that the Bu-Flu patients were on average 7 years older and had a lower percentage of Sib/related donors (52.7% versus 79.1%). In terms of age and donor type, therefore, because patients were not randomized between the 2 regimens, the Bu-Flu group would be at a disadvantage in any statistical comparison that does not account for these covariates. Only a minority of patients were transplanted while in any CR (47.8% of BuCy2 patients and 46.6% of the Bu-Flu patients).

Patients Treated with MF (N = 78)

Table 1b summarizes patient characteristics of the 78 MF patients. The only significant imbalance within the group was that the MF patients treated after April 18, 2001, had a higher fraction of patients transplanted in CR, compared with those treated before April 18, 2001 (35.7% versus 12.0%).

Unadjusted Analyses

OS. For this analysis, the event was defined as death from any cause. Among the 215 i.v. Bu patients, 120 (55.8%) died (47 [70%] in the BuCy2 group and 73 [49%] in the Bu-Flu group). The median OS time was 24.6 months (95% confidence interval [CI] 16.6-51.1 months). The median follow-up time was 74.6 months (95% CI 69.8-83.6 months) for the BuCy2 group and 39.1 months (95% CI 36.7-45.4 months) for the Bu-Flu group. Figure 1a shows the Kaplan-Meier estimates for OS in these 2 groups, indicating that the Bu-Flu patients survived significantly longer, but had a shorter follow up time.

EFS. For this analysis, the event was defined as progression or death from any cause. Among the 215 i.v. Bu patients, 133 (61.9%) progressed or died (50 BuCy2 patients and 83 Bu-Flu patients). The median EFS time was 11.8 months for the whole group (95% CI, 7.6-20 months). Figure 1b shows the Kaplan-Meier estimates for the EFS in these 2 groups, indicating that the Bu-Flu patients had a longer EFS, compared with the BuCy2 group (19.1 months versus 8.4 months, respectively).

NRRS. For this analysis, the event was defined as death without disease recurrence. Among the 215 Bu-treated patients, 36 (16.7%) died of treatment-related causes and without recurrent disease (18, 26.9%, of the BuCy2 and 18, 12%, of the Bu-Flu patients). The median NRRS time has not been reached

Table 1a. *Patient Characteristics by Treatment Group in Patients Treated with BuCy2 or Bu-Flu*

Variable	BuCy2 (N = 67)*	Bu-Flu (N = 148)	*P*-Value
Cytogenetics			1.00
Others	47 (70.1)	104 (70.3)	
Bad	20 (29.9)	44 (29.7)	
Disease Status			.88
Others	35 (52.2)	79 (53.4)	
CR	32 (47.8)	69 (46.6)	
CR1	12 (18)	36 (24)	
Allo type			.0003
Others	14 (20.9)	70 (47.3)	
Sib/related	53 (79.1)	78 (52.7)	
PB blast			.11
>0	24 (35.8)	37 (25.0)	
0	43 (64.2)	111 (75.0)	
Age	39 (13-64)	46 (19-66)	.01
PB PLT	86 (2-330)	89 (3-463)	.41

Cyto indicates cytogenetics; PB, peripheral blood, Allo, allogeneic; Sib, sibling; Rel, related; PLT, platelet; CR, complete remission.
*Number in each cell is N (%) for categoric variable and median (range) for continuous variable.

Table 1b. *Patient Characteristics by Treatment Group in Patients Treated with MF*

Variable	Before 4/18/2001 (N = 50)*	After 4/18/2001 (N = 28)	*P*-Value
Cytogenetics			.16
Others	23 (46.0)	18 (64.3)	
Bad	27 (54.0)	10 (35.7)	
Disease Status			.02
Others	44 (88.0)	18 (64.3)	
CR	6 (12.0)	10 (35.7)	
Allo type			.24
Others	32 (64.0)	14 (50.0)	
Sib/related	18 (36.0)	14 (50.0)	
PB blast			.08
>0	19 (38.0)	5 (17.9)	
0	31 (62.0)	23 (82.1)	
Age	54 (23-66)	54 (22-74)	.69
PB PLT	46 (2-284)	54 (9-377)	.16

Cyto indicates cytogenetics; PB, peripheral blood, Allo, allogeneic; Sib, sibling; Rel, related; PLT, platelet; CR, complete remission.
*Number in each cell is N (%) for categoric variable and median (range) for continuous variable.

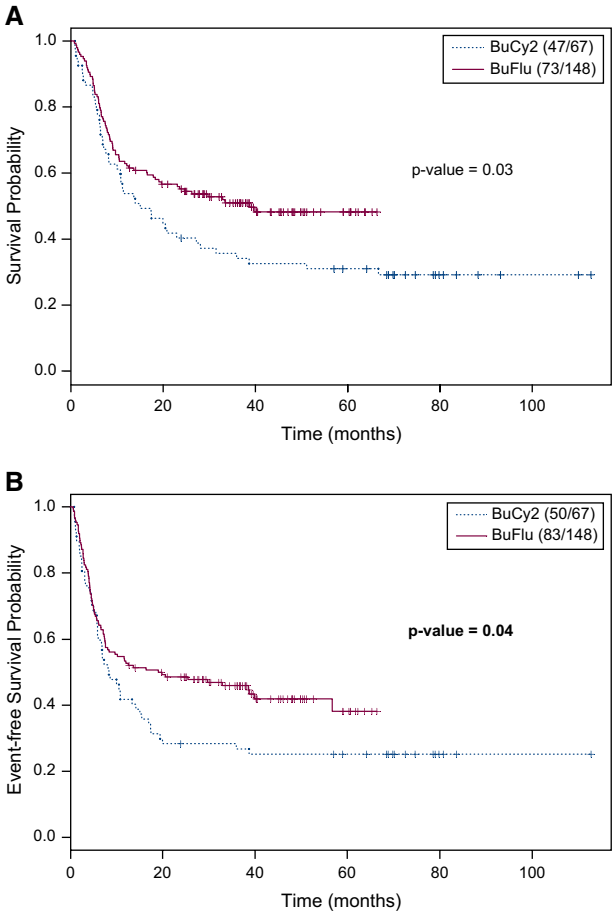

Figure 1. Kaplan-Meier estimates for the probabilities of (**a**) OS, and (**b**) EFS by treatment group in 215 patients treated with BuCy2 (- - -) and Bu-Flu (—) (the numbers within parenthesis indicate number of events and cohort size).

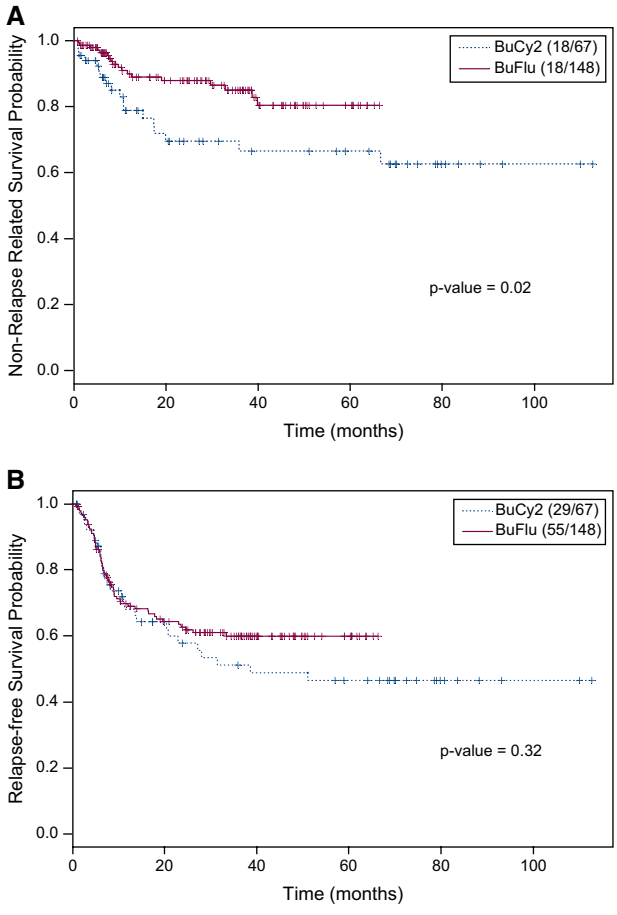

Figure 2. Kaplan-Meier Estimates for the probabilities of (**a**) NRRS, and (**b**) RFS, by treatment group in 215 patients treated with BuCy2 (- - - -) and Bu-Flu (—) (the numbers within parenthesis indicate number of events and cohort size).

in either group. Figure 2a shows the Kaplan-Meier estimates for the NRRS in these 2 groups, indicating that the Bu-Flu patients had a longer NRRS time, compared to the BuCy2 group; the estimated NRRS at 3 years was 86% in the Bu-Flu group and 66% in the BuCy2 group.

RFS. For this analysis, the event was defined as death because of persistent or recurrent disease, with all other terminating events considered to be right (administrative) censoring. Among the 215 I.V. Bu patients 84 (39.1%) died of persistent or recurrent disease, 55 (37%) of the Bu-Flu patients, and 29 (43%) of the BuCy2 patients. The median RFS time has not been reached (95% CI 38.6-NA). Figure 2b shows the Kaplan-Meier estimates for the RFS in these 2 groups, indicating that there was no significant difference between the i.v. Bu-Flu and i.v. BuCy2 patients in terms of RFS. Bearing in mind, again, that patients were not randomized, and moreover, that KM curves ignore covariate effects, it is notable that the plateau for the Bu-Flu RFS curve is about 0.10 higher than that of the BuCy2 RFS curve.

Early Disease, CR1 Patients

Patients transplanted in CR1 constituted only a small subgroup (Table 1a), 12 (18%) in the BuCy2 group and 36 (24%) of the Bu-Flu patients. However, the most striking differences in outcome were encountered when comparing these subgroups; the 3-year OS was 78% after Bu-Flu and 42% after BuCy2, and the 3-year EFS percentages were 74% and 42%, respectively (Figure 3a and b). Further, there were no differences in outcome related to the use of matched related versus unrelated donors (data not shown). Young patients (up to and including age 40), fared even better with Bu-Flu; their 3-year OS and EFS were 94% and 83%, respectively (Figure 3c and d). Finally, the 1-year TRM for patients transplanted in CR using the Bu-Flu program was 6%, significantly better than that achieved with the BuCy2 regimen (21%).

GVHD

The overall incidence of aGVHD grades II-IV was 33.3% after BuCy2, 26.1% after Bu-Flu, and 42.1%

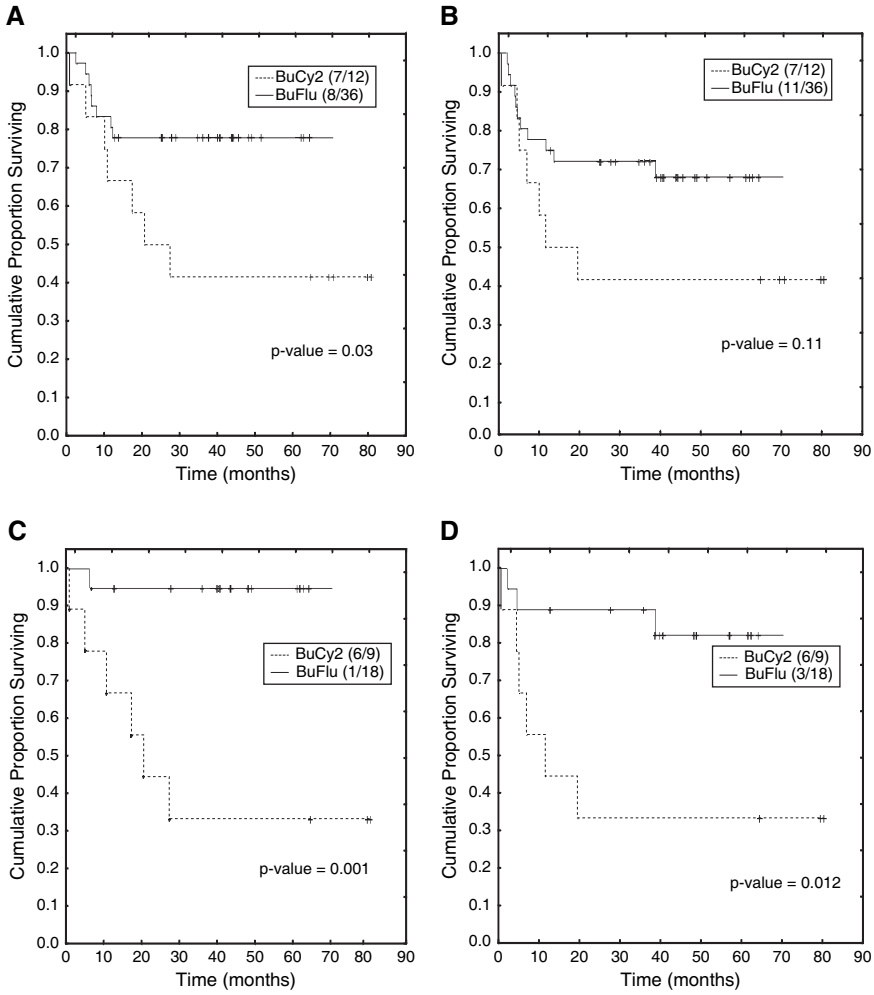

Figure 3. Survival of patients transplanted in CR1, "early disease" with BuCy2 (- - - -) and Bu-Flu (—); (**a**) OS, and (**b**) EFS in all CR1 patients. In graphs (**c**) OS, and (**d**) EFS is depicted for patients up to and including 40 years of age (the numbers within parenthesis indicate number of events and cohort size).

after the MF regimen. Among patients who had a fully HLA-matched related donor the incidence of aGVHD grades II-IV was 32.7% after BuCy2, 15.8% after Bu-Flu, and 25% after MF. The corresponding incidence of extensive chronic GVHD (cGVHD) was 36.1% after BuCy2, 34.1% after Bu-Flu, and 39.4% after MF.

Covariate Adjusted Analyses

Table 2a summarizes a fitted Bayesian log-normal survival model for OS for the 215 i.v. Bu patients, including 67 treated with BuCy2 and 148 who received Bu-Flu. Table 2b summarizes a similar model fit to the data from the 78 MF patients, with a period effect in place of the confounded Bu-Flu-versus-BuCy2 treatment effect. Assuming, as described above, that this time period effect was distributed in a similar fashion in the FM and BuCy2-Bu-Flu data sets, the posterior distribution of the treatment effect obtained by subtracting the period effect is summarized in Figure 4a, which indicates that, after accounting for the period effect, i.v. Bu-Flu was greatly superior to i.v. BuCy2 in terms of OS. Remarkably, even the uncorrected Bu-Flu-versus-BuCy2 effect favored Bu-Flu over BuCy2 (Table 2a), despite the fact that the MF data showed a detrimental effect of the later period 2001-2004 when most of the Bu-Flu trial was conducted. Similar analyses are given in Table 3 and Figure 4b for EFS, and Tables 4 and Figure 5a for NRRS. Table 5 and Figure 5b examine the relationship between treatment arm (BuCy2 versus Bu-Flu) and RFS. After accounting for covariates and subtracting the period effect in the Bayesian analyses, the posterior probability that Bu-Flu is superior to BuCy2 in terms of NRRS is >0.99 and in terms of RFS is only 0.17. It may be argued that, because nondisease-related and disease-related deaths are competing risks, Bu-Flu has a much lower overall death rate but may result in a slightly higher death rate because of recurrent disease.

DISCUSSION

Several investigators have reported a dose-response relationship between the pretransplant

Table 2a. *Fitted Bayesian Log-Normal Survival Model for Overall Survival of 215 Patients, Including 67 Patients Treated with BuCy2 and 148 Treated with Bu-Flu*

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect
			2.50%	97.50%	
Intercept	2.372	0.563	1.263	3.474	—
Cyto = bad (versus other)	−0.114	0.277	−0.657	0.433	0.339
Disease status = CR (versus not in CR)	0.241	0.339	−0.431	0.903	0.764
Allo type = sib/other Rel (versus unrelated)	0.647	0.273	0.109	1.195	0.991
Age	−0.023	0.011	−0.045	−0.001	0.020
PB Blast = 0 (versus >0)	1.129	0.319	0.494	1.778	1.000
PB PLT	0.003	0.001	0.000	0.006	0.990
Confounded Bu-Flu (versus BuCy2) effect	0.591	0.292	0.015	1.164	0.977
R	0.363	0.054	0.268	0.478	

Cyto indicates cytogenetics; PB, peripheral blood, Allo, allogeneic; Sib, sibling; Rel, related; PLT, platelet; CR, complete remission.

conditioning regimen and long-term outcome after allogeneic HSCT in acute leukemia [3,4,10,12,28]. In this context, i.v. Bu provides a valuable tool for safe and reproducible delivery of intensive conditioning treatment; the intra- and interindividual variability in systemic Bu exposure is considerably lower than what is typically obtained with oral Bu [17,20,21,44]. Nonrandomized comparisons between patients conditioned with i.v. Bu-based [7,19] and those receiving oral BuCy2 [6], or TBI-based therapy [27,28], appeared favorable for the i.v. Bu-based combinations relative to a lower TRM/increased safety. The main benefit was seen in terms of increased safety/lower TRM at 100 days and at 1 year posttransplantation [6,7,17,19,27]. The experience of both our group [17,45], and that of Russell et al. [18,19], suggested that (minor) variants of this Bu-Flu ± ATG combination would be well-tolerated and safe, reduced-toxicity myeloablative conditioning treatments. There was some concern, however, that the "favorable" comparison between i.v. Bu-Flu and i.v. BuCy2 constituted a nonrandomized assessment of sequential conditioning programs during a time when supportive care had improved in a way that greatly favored the more recent program. A similar quandary was highlighted by Chae et al. [46], who reported on a favorable outcome when comparing Bu-flu to BuCy2 in a mixed patient population transplanted for a variety of hematologic malignancies. Although these authors reported a greatly improved outcome after Bu-Flu, their analysis was complicated by reporting observations made in a mixed-patient population with varying ages, performed as 2 sequential programs, and further by changing from oral to i.v. Bu and then changing Cy to Flu, all of which may unpredictably contribute to the final clinical observations. The classical approach to comparison of different treatment programs is to perform a prospective randomized trial to obtain an unbiased estimate of the difference in treatment effects. Although this ideal route is frequently used, a large body of data results from single arm trials, as was the present case. Any comparison of such single-arm, consecutive trials will suffer from the confounding effect(s) of unknown factors, here including possible changes in referral patterns, improved supportive care routines with introduction of new antifungal, antiviral, and antibacterial agents as well as use of rabbit-ATG, and increasing experience of the nursing and medical staff. We have argued that a Bayesian sensitivity analysis can provide a basis for a comparative evaluation of different treatment programs in the presence of such confounding effects [2,29]. In the present

Table 2b. *Fitted Bayesian Log-Normal Survival Model for Overall Survival of 78 Patients Treated with MF*

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect
			2.50%	97.50%	
Intercept	3.370	1.437	0.523	6.237	—
Cyto = bad (versus other)	−0.878	0.500	−1.875	0.087	0.038
Disease status = CR (versus not in CR)	1.377	0.708	0.012	2.798	0.976
Allo type = sib/other rel (versus urelated)	0.550	0.506	−0.451	1.558	0.865
Age	−0.023	0.025	−0.072	0.026	0.169
PB Blast = 0 (versus >0)	0.770	0.559	−0.323	1.875	0.919
PB PLT	0.001	0.002	−0.003	0.004	0.679
After 04/01 (versus before 04/01)	−0.811	0.573	−1.945	0.313	0.078
R	0.250	0.056	0.151	0.371	—

Cyto indicates cytogenetics; PB, peripheral blood, Allo, allogeneic; Sib, sibling; Rel, related; PLT, platelet; CR, complete remission.

Table 3. *Fitted Bayesian Log-Normal Survival Model for Event-Free Survival in 215 Patients, Including 67 Patients Treated with BuCy2 and 148 Treated with Bu-Flu*

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect
			2.50%	97.50%	
Intercept	1.622	0.583	0.491	2.752	—
Cyto = bad (versus other)	−0.090	0.287	−0.660	0.473	0.386
Disease status = CR (versus not in CR)	0.501	0.341	−0.158	1.162	0.932
Allo type = sib/other rel (versus unrelated)	0.230	0.279	−0.320	0.781	0.799
Age	−0.011	0.011	−0.033	0.012	0.171
PB Blast = 0 (versus >0)	1.307	0.336	0.650	1.965	1.000
PB PLT	0.002	0.001	0.000	0.004	0.961
Confounded Bu-Flu (versus BuCy2) effect	0.416	0.295	−0.141	1.011	0.922
R	0.327	0.045	0.248	0.424	—

Cyto indicates cytogenetics; PB, peripheral blood, Allo, allogeneic; Sib, sibling; Rel, related; PLT, platelet; CR, complete remission.

investigation, we exploited the data from the separate MF trial, a program that remained unaltered from 1997-2004 [26], by estimating the combined confounding factors as a "period" effect using the MF trial data. We assumed that the "period"-related changes that influenced treatment outcome for the MF patients would similarly influence outcome for patients allocated to the BuCy2 and Bu-Flu regimens over time, because there was no systematic bias in allocating patients to the MF regimen versus that of the Bu-based regimens during this time period. Moreover, all 3 trials were conducted in the same institution. It may be argued on fundamental grounds that patients treated more recently should have benefited from more advanced supportive care routines, more highly skilled medical staff, etc. After accounting for known patient characteristics in the analysis of the MF data, however, both OS and the chance for RFS worsened over time, indicating that the remaining period effect favored the earlier period (1997-2001) over the later time period. Thus, improvements in supportive care were apparently more than balanced out by a changing referral pattern, an increasing median patient age, increasing use of alternative donors and possibly other, unknown factors. Given that the estimated period effect greatly favored the earlier period, when the BuCy2 trial was conducted, it is quite remarkable that even the uncorrected, confounded Bu-Flu-versus-BuCy2 effect (Table 2 and Figures 1a, 2b, and 3a and b) showed Bu-Flu to be a superior regimen.

Our Bayesian analyses led to the conclusion that the observed differences in 100-day and 1-year mortality rates between the BuCy2 and Bu-Flu regimens are likely attributable to a superior safety and tolerance profile of the latter program in patients with AML/MDS. In reference to a low TRM, this conclusion is further supported by Russell et al. [18], who reported similar findings both in patients with advanced hematologic malignancies, and more recently, in better prognosis patients with AML and ALL [19]. The

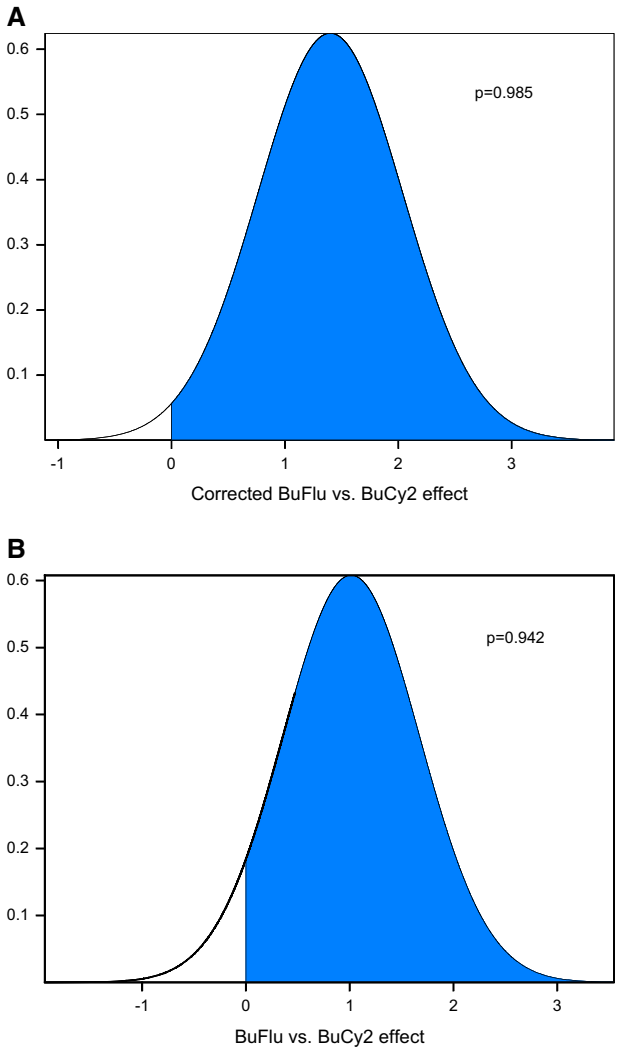

Figure 4. Posterior distribution under the lognormal regression model for (**a**) OS, and (**b**) EFS of the corrected Bu-Flu versus BuCy2 treatment effect. In these plots, p denotes the probability of a beneficial effect of Bu-Flu versus BuCy2, and is represented by the area of the shaded region in the respective figure.

Table 4. *Fitted Bayesian Log-Normal Survival Model for Nonrelapse-Related Survival (NRRS) in 215 Patients, Including 67 Patients Treated with BuCy2 and 148 Treated with Bu-Flu*

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect
			2.50%	97.50%	
Intercept	5.131	1.343	2.671	7.944	—
Cyto = bad (versus other)	0.996	0.681	−0.294	2.371	0.934
Disease status = CR (versus not in CR)	−0.092	0.712	−1.488	1.313	0.448
Allo type = sib/other rel (versus unrelated)	1.396	0.602	0.277	2.684	0.994
Age	−0.055	0.025	−0.109	−0.009	0.009
PB Blast = 0 (versus >0)	0.922	0.709	−0.466	2.334	0.909
PB PLT	0.002	0.002	−0.002	0.007	0.755
Confounded Bu-Flu (versus BuCy2) effect	1.880	0.651	0.664	3.258	0.999
R	0.166	0.049	0.087	0.277	—

Cyto indicates cytogenetics; PB, peripheral blood, Allo, allogeneic; Sib, sibling; Rel, related; PLT, platelet; CR, complete remission.

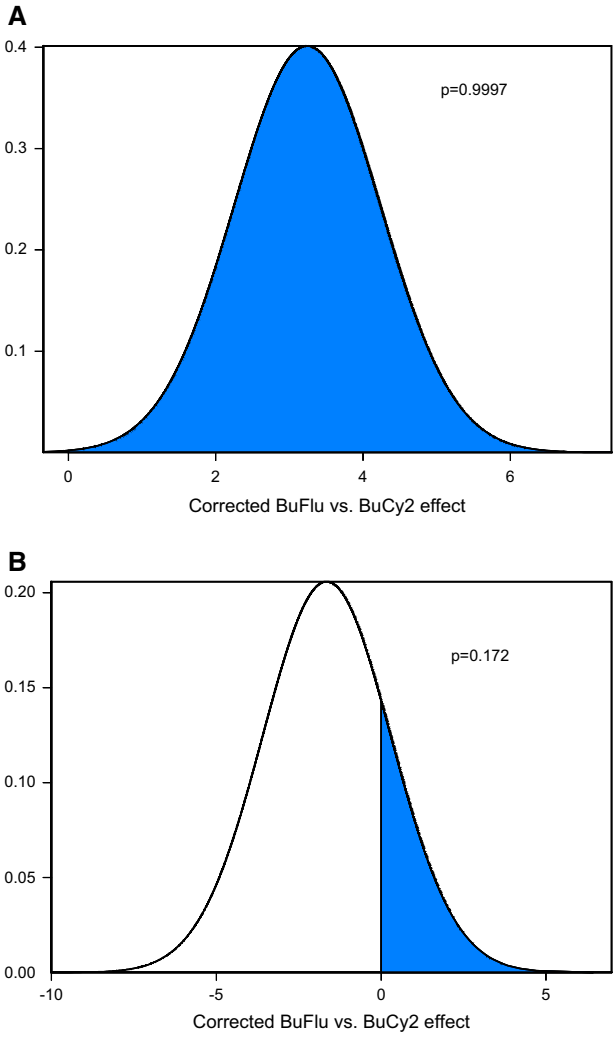

Figure 5. Posterior distributions under the lognormal regression model for (**a**) NRRS, and (**b**) RFS of the corrected Bu-Flu versus BuCy2 treatment effect within the 215 Bu-treated patients. In the plot, p denotes the probability of beneficial effect of Bu-Flu versus BuCy2, and is represented by the area of the shaded region in the respective figure.

lingering concern that a replacement of Cy with Flu would represent an overall decreased treatment intensity that translated into less side effects at the price of inferior antileukemic effect, especially in subjects with active leukemia at time of transplant [4], also appears largely unfounded, and our results indicate that patients receiving Bu-Flu would not be at any disadvantage in this respect as depicted in Figure 5b. It is important to remember that both Cy and Flu were primarily used because of their immunosuppressive rather than antileukemic properties, that is, to enhance engraftment. It is true, however, that although a high relapse rate exists in patients transplanted with chemotherapy-refractory leukemia and a clinically high tumor load, the search should continue for ways to further improve the Bu-Flu regimen. This program should be considered primarily as a therapeutic platform, to which other, both pre- and postgrafting components may be added safely to improve tumor control. Overall, it is tempting to conclude that Bu-Flu ± ATG represents a significant improvement for patient safety, at least in the first (few) year(s) after allogeneic HSCT, because the outcome of patients treated with Bu-Flu was significantly better than what would be expected based on our past experience with the BuCy2 regimen and from comparisons with data using other conditioning programs in patients with AML/MDS [26,47–50]. Although it was not a primary objective to compare GVHD rates after the different patient populations because of their disparity in age, proportion of donors other than fully matched related donors, etc., it was noteworthy that the incidence of GVHD among patients transplanted after Bu-Flu (~16%) with a fully matched related donor was only half of that observed after BuCy2 (~33%).

The favorable outcome of the Bu-Flu patients, in view of a relative long follow-up time (a median of about 40 months) and a comparatively large number of patients with a high median age (46 years), appears to challenge the concept that an age above 50 or 55 years necessitates a reduced-intensity (RIC) regimen for

Table 5. *Fitted Bayesian Log-Normal Survival Model for Relapse-Free Survival (RFS) in 215 Patients, Including 67 Patients Treated with BuCy2 and 148 Treated with Bu-Flu*

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect
			2.50%	97.50%	
Intercept	2.856	0.659	1.575	4.145	—
Cyto = bad (versus other)	−0.549	0.325	−1.189	0.088	0.046
Disease status = CR (versus not in CR)	0.219	0.450	−0.663	1.096	0.688
Allo type = sib/other rel (versus unrelated)	0.398	0.331	−0.248	1.046	0.889
Age	−0.012	0.014	−0.038	0.014	0.181
PB blast = 0 (versus >0)	1.322	0.394	0.560	2.109	1.000
PB PLT	0.005	0.002	0.001	0.009	0.998
Confounded Bu-Flu (versus BuCy2) effect	0.042	0.356	−0.652	0.760	0.547
R	0.309	0.056	0.208	0.429	—

Cyto indicates cytogenetics; PB, peripheral blood, Allo, allogeneic; Sib, sibling; Rel, related; PLT, platelet; CR, complete remission.

allogeneic HSCT in AML/MDS. Finally, the Bu-Flu data suggest that it may be time for a prospective evaluation of allogeneic HSCT versus conventional induction and consolidation chemotherapy for AML/MDS for all patients who do not have APL or core binding factor leukemia, regardless of cytogenetic risk pattern. Such a study should, as a minimum, cover the population up to age 40. Previous comparisons of allogeneic HSCT and conventional maintenance chemotherapy mostly have relied on total body irradiation (TBI)-based conditioning therapy, which yielded excessive TRM without corresponding patient benefit [51,52].

In summary, the consistent and reproducible systemic Bu exposure that was achieved with a parenteral Bu formulation, when paired with Flu ± rabbit-ATG, is likely to continue having a significant impact on (early) posttransplant safety and survival in the studied patient population(s).

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