HHS Public Access

Author manuscript

Bone Marrow Transplant. Author manuscript; available in PMC 2017 June 19.

Published in final edited form as:

Bone Marrow Transplant. 2017 April ; 52(4): 580–587. doi:10.1038/bmt.2016.322.

Fludarabine with pharmacokinetically-guided IV busulfan is superior to fixed-dose delivery in pretransplant conditioning of AML/MDS patients

Borje S. Andersson[1,9], **Peter F. Thall**[2], **Benigno C. Valdez**[1], **Denái R. Milton**[2], **Gheath Al-Atrash**[1], **Julianne Chen**[1], **Alison Gulbis**[3], **Diem Chu**[1], **Charles Martinez**[1], **Simrit Parmar**[1], **Uday Popat**[1], **Yago Nieto**[1], **Partow Kebriaei**[1], **Amin Alousi**[1], **Marcos de Lima**[1,4], **Gabriela Rondon**[1], **Qing H. Meng**[5], **Alan Myers**[6], **Jitesh Kawedia**[6], **Laura L. Worth**[7], **Marcelo Fernandez-Vina**[4,8], **Timothy Madden**[3], **Elizabeth J. Shpall**[1], **Roy B. Jones**[1], and **Richard E. Champlin**[1]

[1]Department of Stem Cell Transplantation & Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

[2]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

[3]Division of Pharmacy, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

[5]Division of Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

[6]Division of Pharmacy Research, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

[7]Division of Pediatrics, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

Abstract

We hypothesized that IV Busulfan (Bu) dosing could be safely intensified through pharmacokinetic (PK-) dose guidance to minimize the inter-patient variability in systemic exposure (SE) associated with body-sized dosing, and this should improve outcome of AML/MDS patients undergoing allogeneic stem cell transplantation (allo-HSCT). To test this hypothesis, we treated 218 patients (median age 50.7 years, male/female 50/50%) with fludarabine (Flu) 40 mg/m^2 once daily ×4, each dose followed by IV Bu, randomized to 130 mg/m^2 (N=107) or PK-guided to average daily SE, AUC of 6,000 μM-min (N=111), stratified for remission-status, and allo-grafting from HLA-matched donors. Toxicity and graft vs. host disease (GvHD) rates in the groups were similar; the risk of relapse or treatment-related mortality remained higher in the fixed-dose group throughout the 80-month observation period. Further, PK-guidance yielded safer

[9]**Correspondence:** Borje S. Andersson, M.D., Ph.D., Department of Stem Cell Transplantation & Cellular Therapy, Box 423, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, bandersson@mdanderson.org.

[4]Current Address: Department of Medicine-Hematology and Oncology, UH Case Medical Center, 11100 Euclid Ave., Cleveland, OH 44106

[8]Current Address: Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

disease-control, leading to improved overall and progression-free survival, most prominently in MDS-patients and in AML-patients not in remission at allo-HSCT. We conclude that AML/MDS patients receiving pretransplant conditioning treatment with our 4-day regimen may benefit significantly from PK-guided Bu-dosing. This could be considered an alternative to fixed dose delivery since it provides the benefit of precise dose delivery to a predetermined SE without increasing risk(s) of serious toxicity and/or GvHD.

Introduction

Allogeneic stem cell transplantation (allo-HSCT) is an established treatment with curative intent for patients with myeloid leukemias or MDS.[1,2] Recently, introduction of (a) nucleoside analog(s) (NAs), most commonly fludarabine (Flu), combined with IV busulfan (Bu) in a “reduced-toxicity” regimen, has gained popularity because of its high safety level when NAs are combined with Bu.[3–8] The antileukemic effects of such (a) combination(s) is/are very similar to those of the Bu-Cyclophosphamide (BuCy2) regimen when optimized for synergistic cytotoxicity of the two agents.[6,9,10]

Further, the clinical and pharmacological lessons from both oral and IV BuCy2 variant regimens suggested that a low Bu-systemic exposure (Bu-SE) was associated with higher risks for graft failure and leukemic relapse, while a high Bu-SE was associated with serious toxicity and graft vs host disease (GvHD). These results indicated the existence of an optimal therapeutic interval for Bu-SE. We proposed that this interval is a compromise between desirable antileukemic effect and complications arising with increasing dose intensity[11–16] (Suppl. Figure S1). The IV Bu data were used to define a therapeutic Bu-SE interval, represented by the area under the concentration vs time curve (AUC)[14], ranging from approximately 3,600 to 6,100 μM-min daily when translated into a once daily, 4-day schedule.[3,4,15–17] Inside this interval patients have an improved outcome, while at higher Bu-SE the risk for serious adverse events/treatment-related mortality (TRM) increases, and outweighs the anti-leukemic benefit of higher dose-intensity.[11–16]

We hypothesized that judicious use of pharmacokinetic (PK) information to guide/individualize Bu delivery would compensate for inter-individual variability in drug handling and metabolism. This PK-guided Bu dosing (a) would result in more precise, better standardized Bu-SE, (b) would yield better leukemia control without jeopardizing patient safety, and (c) could be safely escalated to improve disease control without increased TRM. Moreover, lethal complications are likely more common after fixed dosing in individuals with decreased drug clearance leading to a high Bu-SE. Our hypotheses also imply that PK-guided dose escalation would be most beneficial in patients with active disease at the start of conditioning therapy (Fig. S1).

Further support for PK-guidance was provided by the increased safety experienced with targeted Bu[7,8,14,16], and by Popat *et al* who demonstrated that standardized, PK-guided Bu dosing improved outcome in patients transplanted with Flu-IV Bu for myeloproliferative disorders compared with controls who received fixed-dose Bu in a similar, reduced intensity conditioning (RIC), regimen.[18]

To test our hypotheses, we designed a prospectively randomized trial of Flu with PK-guided vs. fixed-dose Bu in AML/MDS patients undergoing allo-HSCT. The end point of the study was to investigate if PK-guided Bu to an average daily AUC of 6,000-μM-min±10% is superior to a fixed dose of 130 mg/m^2 (daily AUC ~5,000 μM-min, range ~3,000–8,000), in terms of time to treatment failure (relapse or death from any cause) in patients with AML or MDS. Data regarding engraftment, toxicity, relapse over time, and long-term overall (OS) and progression-free survival (PFS) were collected. Patients were stratified only based on disease status, i.e. whether they had a cytological (bone marrow; BM) complete remission (CR) or active disease. The trial was limited to AML/MDS patients, to avoid possibly confounding effects of differential drug sensitivity of different diseases. Our results demonstrate for the first time in a randomized, prospectively controlled trial, that PK-guided Bu delivery confers significant advantages over the more traditional prescription of (pretransplant conditioning) chemotherapy based only on body weight/body surface area.

Patients and Methods

Eligibility Criteria

Patient Eligibility—AML patients in first CR should have failed initial induction chemotherapy, i.e. to have needed a salvage regimen to attain CR1, or have high-risk disease, characterized by cytogenetics other than translocation (8;21 or 15;17), inversion (16), or by the need for more than one cycle of chemotherapy to achieve CR.[4] All patients beyond CR1 or having chemotherapy-refractory disease were eligible. Subjects with MDS were eligible if they had an International Prognostic Scoring System (IPSS) score ≥ 2 (ref. 19), or if they failed to achieve CR with chemotherapy.

The eligibility criteria further 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, -C, and HIV, LVEF ≥45%, FEV1, FVC and DLCO ≥50% of predicted, no active infection, and no chemotherapy within 30 days prior to study entry. A human leukocyte antigen (HLA-) compatible related (fully matched or one antigen mismatched) or matched unrelated donor (MUD) was required. The study was approved by the institutional review board prior to its initiation, and all patients signed informed consent prior to randomization per institutional guidelines.

Conditioning regimen

Fludarabine-IV Busulfan (Flu-Bu)—When eligibility had been confirmed, and patients had signed informed consent, they were prospectively randomized between the two arms of the protocol, stratifying by disease status (CR vs active disease). After randomization, each patient was admitted to start conditioning therapy, or to receive the Bu test dose that preceded conditioning in the PK-guided arm. The treatment consisted of Flu, 40 mg/m^2, (Fludara®, Berlex Labs., Inc., Montville, NJ), dosed according to actual body weight and given IV over 60 minutes daily × four (days −6 to −3), each dose was followed by Bu over 3 hours (IV Busulfex® (busulfan) Injection, Otsuka America Pharmaceuticals Inc., Princeton, NJ), at 130 mg/m^2 body surface area (“fixed-dose”), or calculated to target an average daily AUC of 6,000 μM-min±10% (total course AUC 24,000 μM-min±10%). The drugs were

infused via controlled-rate infusion pump through a central venous catheter (CVC). Busulfan in the fixed-dose arm was given per actual weight to 120% of ideal weight, above which it was based on adjusted ideal body weight, calculated as ideal weight plus 50% of the difference between ideal and actual weight. The Bu dose in the PK-guided cohort was calculated based on PK-parameters derived from a Bu test dose of 32 mg/m^2 administered IV over 45 min 2–5 days before the therapeutic conditioning program. All Bu analyses and PK-parameters were performed as previously described.[17,20–25] Briefly, blood samples for Bu analysis were collected from a peripheral IV line to avoid possible cross-contamination caused by the proximity between different ports of the CVC used for drug administration. Samples were collected on wet ice, separated at 4°C, and the plasma cryopreserved at −70°C until analysis using high-pressure liquid chromatography with mass-spectrometric detection.[17,20,21] The PK-modeling was performed using the ADAPT II Software program, Version 4.0 (BMRS, University of Southern California, Los Angeles, CA).[26]

Supportive Care—Supportive care was given according to extant institutional protocols. All patients received filgrastim (Neupogen®, Amgen, Inc., Thousand Oaks, CA) 5 μg/kg s.c. daily from day +7 until achieving an absolute neutrophil count (ANC) ≥1.5 × 10^9/L for three days. Phenytoin was given as seizure prophylaxis, started in the evening before and then in the morning of the Bu test dose and then restarted the evening before therapeutic Bu administration, and again given before each daily therapeutic Bu dose with the last dose administered on day −2.

All patients received GvHD prophylaxis with tacrolimus (Prograf®, Fujisawa Healthcare, Inc., Deerfield, IL) and methotrexate, 5 mg/m^2 IV on days +1, +3, +6 and +11.[27] Tacrolimus was continued for 6–8 months. Patients with a one-antigen mismatched related or unrelated donor received rabbit-ATG (Thymoglobulin®, Sanofi-Genzyme Inc. Cambridge, MA), 0.5 mg/kg on days HSCT − 3, 1.5 mg/kg on day −2, and 2 mg/kg on day −1.

Stem Cell Grafts—Procurement of donor peripheral blood progenitor cells (PBPC) after stimulation with filgrastim has been described.[4] Bone marrow was obtained under general anesthesia and targeted to 3 × 10^8 nucleated cells per kg patient body weight (BW). The PBPC dose was targeted to approximately 5 × 10^6 CD34+ cells/kg patient BW.[4] Bone marrow and PBPC from MUD donors were obtained through the National Marrow Donor Program.

Human Leukocyte Antigen Typing—HLA-typing for class I and class II antigens was performed with low resolution molecular typing using sequence-specific oligonucleotide primers for hybridization of amplified DNA, followed by high resolution typing for all patients and donors. Patient-donor pairs were considered fully matched by compatibility for HLA-A, -B, -C and -DRB1 and -DQ.[4]

Clinical Outcome Variables—The endpoint of the study was to investigate if Bu PK-guided to an average daily AUC of 6,000 μM-min (±10%) is superior to a fixed dose of 130 mg/m^2 in terms of time to treatment failure, i.e. relapse or death from any cause, after allo-SCT in patients with AML or MDS. Data on engraftment, toxicity, relapse over time, as well as long-term overall and progression-free survival were collected. Time of engraftment was

defined as the first of three days with an absolute neutrophil count ≥0.5 × 10^9/L. Failure to engraft in the absence of malignancy in the bone marrow by day +30 was considered primary graft failure. Secondary graft failure was initial engraftment with donor-derived hematopoiesis followed by loss of graft function without recurrent leukemia. Time of platelet engraftment was defined as the first of seven days with a platelet count ≥20 × 10^9/L without transfusions. The criteria for CR prior to transplant followed conventional cytological criteria, without circulating blasts and <5% marrow blasts and normal maturation. Minimal residual disease was assayed by PCR- or FISH- or flow cytometry-based technique, but it was not consistently employed prior to study entry. Peripheral blood CR criteria, including platelets ≥100 × 10^9/L and granulocytes ≥1.5 × 10^9/L, were waived because of commonly ongoing maintenance chemotherapy. Post-transplantation, CR was defined by the same criteria and with documented donor cell engraftment by PCR.[28]

Cytogenetics were considered favorable for patients with translocation (15;17 or 8;21), or inversion (16); unfavorable ("poor risk/bad") for deletion of chromosome 5 and/or 7, multiple chromosomal abnormalities or trisomy of chromosome 8; and intermediate risk in all others.[29] Morphological criteria, conventional cytogenetics, and/or flow-cytometry or PCR-based molecular criteria were used to diagnose recurrent/progressive disease. Cytogenetic relapse was documented by the presence of a clonal abnormality in two consecutive tests, obtained at least four weeks apart. Time to relapse/progressive disease was calculated from the transplant to the documented event. Patients with active disease who did not achieve CR after transplantation were scored as failures at the time of documented persistent/progressive disease. Toxicity was scored using the modified NCI criteria (CTC v3.0).[30]

Overall survival (OS) was calculated from day of transplant, with patients alive at the time of last follow-up administratively censored. Treatment-related mortality (TRM) was defined as death due to any cause other than recurrent disease. Patients alive at last follow-up or who experienced disease progression were censored for TRM. Progression-free survival (PFS) time was counted from allo-HSCT to relapse or death, censored for patients alive in CR at last follow-up.

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

Statistical Methods

Demographics, clinical measurements, and toxicities (including grade III–IV acute GvHD in the first 100 days) were summarized for all patients and by Bu dose group. Categorical variables were summarized by frequencies and percentages, and their associations were assessed using either Fisher's exact test, or generalized Fisher's exact test.[31,32] Age at allo-HSCT date was summarized by median and range (minimum, maximum) and compared between Bu dose groups using the Wilcoxon rank sum test.[33]

Unadjusted Event Time Analyses—The Kaplan-Meier (KM) method[34] was used to estimate unadjusted distributions of OS, PFS, and TRM and the log-rank test[35] was used to assess unadjusted differences between treatment groups.

Time to Event Regression Analyses—Bayesian piecewise exponential survival time regression[36] was used to assess relationships between each of OS, PFS, and TRM times and patient covariates and treatment arm. Because cytogenetic risk category information was missing for two patients, these patients were not included in the regression model fits. Non-informative N(0,100) prior distributions were used for the regression model coefficients and a gamma (0.001, 0.001) prior for the variance was assumed. A chain size of 10,000 was used in the Monte Carlo Markov chains to compute posteriors.

Interpretation of Fitted Bayesian Event Time Regression Models—To interpret each fitted Bayesian regression model, for a model parameter β that is the coefficient of a covariate or treatment indicator in the model, the numerical values labeled “Probability of a beneficial effect (pbe)” are posterior probabilities of the form $Pr(\beta > 0 \mid Data)$, which indicates that survival increases with the covariate. For example, since the PK-guided Bu dose group had a pbe = 0.92 of OS, this says that the posterior probability of PK-guided Bu being superior to fixed-dose Bu was 0.92. Values $0.90 < pbe < 0.94$ or $0.06 < pbe < 0.10$ may be considered moderately significant, $0.95 < pbe < 0.99$ or $0.01 < pbe < 0.05$ may be considered significant, and pbe > 0.99 or < 0.01 highly significant.

2-to-1 Matched-Pair Analysis—To further evaluate the impact of PK-guided Bu dosing, we compared the outcomes of patients in the fixed-dose Bu group whose natural metabolic capacity for busulfan yielded an average daily Bu AUC of 6,000 μM-min (±10%) when they received 130 mg/m^2 with patients in the PK-guided Bu dose group using a matched-pair analysis. Thus, we performed a 2-to-1 matched pair analysis on the fixed-dose Bu patients who achieved AUC levels between 5,400 and 6,600 μM-min, using nearest neighbor matching, which selects the best control match(es) (in this case PK-guided Bu dose) for each individual in the treatment group (in this case fixed-dose Bu).[37,38] Matching was done in a 2:1 ratio, such that two PK-guided Bu patients were matched to each fixed-dose Bu patient. Matching was done using a distance measure which was estimated using a logistic regression model to compute propensity scores, which are estimated probabilities of a patient receiving the treatment they actually received. Matches were chosen for each fixed-dose Bu patient one at a time from largest to smallest distance measure value. Variables used in the propensity score-based matching were diagnosis (MDS vs. other), age, and disease status (CR vs. no CR) at allo-HSCT. The matched groups were compared using KM survival curves for OS and PFS, and the log rank test.

All statistical analyses were performed using SAS 9.3 for Windows (Copyright © 2011 by SAS Institute Inc., Cary, NC). KM survival curves for the matched groups and the OS posterior distribution were produced in R version 3.0.1. All statistical tests used a significance level of 5%. No adjustments for multiple testing were made.

Results

Two-hundred-eighteen patients were prospectively randomized to receive PK-guided Bu (N=111, 51%) or fixed-dose Bu (N=107, 49%), stratified for CR vs. active disease. Table 1 summarizes the covariate distributions for all patients and by Bu dose group. Half of the patients were male and 82% were white with a median age at SCT of 50.7 years. Twenty-four percent of the patients had MDS and 31% were FLT-3 positive. About 70% of the patients received PBPC, and 50% had MUD donors. Eighty-three patients (38%) were in a poor cytogenetic risk category at diagnosis, while 62% were in CR. The only differences between the two sub-groups were observed for FLT-3 status (p=0.008) and cytogenetic risk status (p=0.050) (Table 1), both of which have been associated with a poor outcome.[29,39–41] The median follow-up time for all patients was 37 months (range: 0.1 – 112.2), and the minimum follow-up time was 10 months in surviving patients.

There was no statistically significant difference in serious toxicities or serious acute GvHD between the two treatment groups in any of the patient cohorts. The detailed incidences of grade III–IV acute GvHD within 100 days for all patients, as well as severe treatment-related events for all patients are summarized in Suppl. Table S1.

Table 2, Figures 1, 2, and Supplemental Figures S2–S5 present summary statistics and KM-graphs for OS, PFS, and TRM for all patients and for non-CR, CR, and the MDS sub-groups. A significant difference was seen for OS in all patients and FLT-3 negative patients and for PFS in all, non-CR, MDS, and FLT-3 negative patients; longer survival was experienced by patients in the PK-guided Bu group compared with their fixed-dose Bu counterparts. There was a trend for less TRM in the PK-guided Bu group for all patients in spite of the similarity of toxicity(-ies) and GvHD between the arms. This was primarily due to a higher incidence of late infections in the fixed-dose arm (Fig. S4). Interestingly, the hazard function for overall survival/risk of dying (all patients) was higher for the fixed-dose Bu group, this difference was highest in the first year after transplant, but it did not reach a plateau. It persisted over the entire course of observation, up to and beyond 80 months post-transplantation (Fig. S6).

Patients with FLT-3 mutations have a worse prognosis and there was, by chance, a major overrepresentation of FLT-3 mutated (FLT-3 positive) patients in the PK-guided treatment arm which had not been accounted for in the up-front randomization. Therefore, we attempted to retrospectively address the issue of PK-guided versus fixed-dose Bu effects in sub-groups determined by FLT-3 status and whether the patient was in CR at transplant. It was first noted that there was a highly significant association between FLT-3 status and CR, with 75% of FLT-3 positive patients who received PK-guided Bu dosing in CR whereas only 42% of the FLT-3 positive patients in the fixed-dose group were in CR (p-value = 0.008). Any treatment comparisons of OS, PFS, or TRM within any of the five subgroups CR, Non CR, MDS, FLT-3 (+), and FLT-3(−) given in Table 2 should be viewed with caution. This is due the facts that (i) these within-subgroup treatment comparisons were not planned in the trial design, (ii) subsample sizes within many of the subgroups are small, hence comparisons would have limited reliability, and (iii) the tabled p-values of treatment comparisons within

individual subgroups were not adjusted upward to control the overall false positive rate due to multiple testing.

To assess the PK-guided versus fixed-dose Bu effect while accounting for FLT-3 status, a Bayesian piecewise exponential model was fit including three-way treatment-FLT3-CR interaction terms. This was done because, due to the play of chance, FLT-3 status (+/−) and disease status (CR, non-CR) were highly associated (p = 0.009), hence a fitted model including only a two-way interaction for treatment-FLT3 could misleadingly show an actual CR-treatment effect as a treatment-FLT3 effect. The fitted model indicated that there was no significant beneficial effect of PK-guided Bu dosing among FLT-3 positive patients. In contrast, a large, significant beneficial effect of PK-guided Bu dosing among FLT-3 negative patients (pbe=0.978) was observed, regardless of whether they were in CR. Accounting for PK-guided-versus fixed dose treatment effects within the four FLT-3-CR sub-groups in this way, the overall PK-guided versus fixed-dose Bu posterior mean hazard ratio was 0.64, with 95% ci 0.35 – 0.94. However, we caution that these model-based inferences must be qualified by the facts that (i) they were not planned in the trial design, (ii) the eight treatment-FLT3-CR subgroup sample sizes were small, and (iii) the CR-FLT3 subgroup sample sizes were very imbalanced between the two treatment arms.

Associations between OS, PFS, and TRM and patient covariates for all patients and for the non-CR subgroup and for those with MDS are presented in Table 3, and Suppl. Tables S2 – S9. The posterior probability that PK-guided dosing is superior to fixed-dose delivery in all patients is 0.922 (Figure 3). Similarly, the posterior probability that PK-guided Bu dosing is superior to fixed-dose Bu delivery was 0.938 and 0.958 for PFS and TRM, respectively (Suppl. Tables S2, S3). The difference between the two treatment groups for both OS and PFS for all patients at 5 years post-transplantation was also significant (p=0.042; Fig. 1a). Moreover, the posterior probability that non-CR patients have a superior PFS if receiving PK-guided Bu dosing was 0.931 (Figure S7). The difference in relapse rate and TRM between the two treatment groups did not reach statistical significance, neither for the whole patient population nor for the non-CR patients (Supplemental Figs. S8a, S8b and S9, respectively).

The matched-pair analysis identified 34 patients in the fixed-dose Bu group who achieved average daily AUC levels of 6,000 μM-min ±10%, and 68 patients from the PK-guided group with similar characteristics. After matching, the fixed-dose Bu group and the PK-guided group had very similar OS distributions (Fig. 4a; p=0.84), and PFS distributions (Fig. 4b; p=0.61). These comparisons confirm that the observed differences in outcome between the two treatment arms likely were caused by the different modes of Bu-administration, namely PK-guided dosing vs. fixed-dose delivery. The difference in dose between the PK-guided and fixed-dose groups is illustrated by the range in dose delivered by PK-guidance, with a median of 157.5 mg/m^2 (range 87 – 234.4 mg/m^2, Table 1).

DISCUSSION

Previous trials demonstrated that both oral BuCy2 and Cy-TBI are efficacious pretransplant conditioning treatments for patients with AML/MDS.[1,2,42,43] Recently published analyses

indicate that IV Bu-based conditioning therapy yields both improved safety and better disease control/event-free survival.[44–46] The subsequent introduction of nucleoside analog (Flu) - IV Bu-combinations increased the safety of the conditioning treatment, and recent reports suggest that such (a) regimen(s) is/are at least equivalent to IV BuCy2. (Refs. 6,9,10, provided that drug-sequencing and -timing in the Flu-IV Bu combination is optimized.[47,48] We hypothesized that PK-guided Bu-dosing to a predetermined Bu-SE would safely allow intensified therapy and improve outcome, i.e. optimized patient benefit from the dose-response relationship observed in AML,[49,50,51] without increased regimen-related serious toxicity. Such PK-guidance might be of particular interest when the other drug(s) in the conditioning program is/are independent of GSH-conjugation and CYP 450-isoenzyme mediated metabolism, which introduce(s) further uncertainty as to how conditioning is standardized.[52]

In this study, Flu with fixed-dose Bu represents standard of care, beyond which the PK-guided group was intensified by about 20%, targeted towards the upper end of the previously suggested therapeutic interval, or about 4 × 1,500 μM-min for an average daily SE of 6,000 μM-min in the 4-day regimen.[7,8,14–17] This was possible because the IV Bu-formulation consistently allows targeting a Bu-SE within a narrow range for the total treatment course or average daily Bu-SE.

Our results demonstrated a lower relapse rate (38% vs. 56%) and lower TRM (24% vs. 39%), respectively, at three years in the PK-guided group compared with the standard fixed-dose arm in non-CR patients (p=non-significant). The rates of significant acute GvHD were similar, while GvHD-related deaths during the first four years after allo-HSCT were fewer in the PK-guided arm. These results confirm the hypotheses underlying the study design; they further suggest that precise Bu- (alkylating agent-) delivery may be more important than previously thought, giving lower regimen-related toxicity, greater antileukemic efficacy, and lower rate of GvHD, all affecting survival beyond the first (few) year(s). When outcomes were compared between a subgroup of fixed-dose patients who, because of their inherent metabolic capacity achieved an average daily Bu-SE of 6,000 μM-min±10%, and matched patients from the PK-guided group, the results provide additional evidence that the difference in outcome between the two treatment arms was indeed due to the mode of Bu-administration, since both OS and PFS were very similar between the two matched groups (Fig. 4). It appears that it would not matter if patients achieve the optimized Bu-SE from their biological make-up or whether it is provided through active PK-guidance as part of the treatment program’s design.

The PK-targeted systemic exposure of an average daily AUC of 6,000 M-min was extrapolated from our previous investigation utilizing the IV BuCy2-regimen.[14] It is conceivable that a detailed investigation of an optimal Bu therapeutic interval, when given with (a) nucleoside analog(s), may yield a slightly revised estimate. The present investigation suggests that disease characteristics, and -volume ought to be considered in addition to patient-unique features/comorbidities if/when computing a desirable individualized Bu-SE interval.[53,54] We based our study design partly on the assumption that an optimal therapeutic interval does exist for IV Bu in the Flu-Bu combination used here. Once we analyze a larger data set regarding PK/Bu-SE relative to clinical outcome for

patients treated on newer Flu-IV Bu protocols, we anticipate that the optimal Bu-SE interval will be similar to what we used here, and that the upper recommendable average limit for once daily Bu dosing in a 4-day regimen will be in the range from approximately 5,500 to about 7,000 μM-min per day.[7,8,14–16], representing Bu in a once daily 4-dose regimen, where each dose is preceded by a dose of Flu, at 40–50 mg/m^2. (Refs. 47,48)

Finally, while PK information correlated with outcome, at first glance clinical disease status did not; PK-guided dosing was overall beneficial for all patients, and it conferred patients with active AML and MDS a major benefit. For AML-patients in CR, the difference in outcomes between the dosing groups favored PK-guided dosing without added toxicity, similar to that of the cohort(s) with active disease and to the whole patient population, but it did not reach statistical significance. One can argue that PK-guided Bu-dosing may benefit patients with a cytological CR and measurable minimal residual disease, who behave more like patients in relapse than those with a true CR.[55] In contrast, it may not be possible to conclusively demonstrate any difference in OS or PFS in a group of patients in a true CR without evidence of MRD.[55] However, the latter group of patients would still benefit from the enhanced safety level obtained with PK-guidance to a Bu-SE in an optimized exposure interval, while also retaining an optimal anti-leukemic effect, since there was no significant "price to pay" when it comes to serious adverse events and GvHD.

The finding of a smaller difference in outcome between these CR-groups may also partly explain why, for patients in cytological CR1, the use of Flu-IV Bu in reduced-intensity- vs. myeloablative-conditioning settings with fixed-dosing yield similar outcomes, as was recently reported from the EBMT.[56] Thus, in CR1, patient heterogeneity outweighs the difference in outcome that could be attributed to different dose intensities. In contrast, another recent publication from the EBMT demonstrated improved outcome after more intensive Flu-Bu conditioning in patients with AML in CR2, where the relapse risk is significantly higher than in CR1.[51] Again, this supports that safely intensified conditioning therapy benefits our patients, and that not all CRs are created equal.

In summary, our results strongly suggest that outcome of patients with AML/MDS transplanted after conditioning with our variant Flu-Bu 4-day regimen can be further improved by individualized PK-guided dosing, based on PK-results from a preliminary subtherapeutic "test" dose to target an optimized therapeutic Bu-SE interval. For such optimization cytotoxic drug dose delivery must be considered in terms of systemic exposure rather than administered dose; Bu doses in excess of 200 mg/m^2 can be delivered safely in selected patients when utilizing PK-guidance. This is an area of future investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by the National Cancer Institute grants P01-CA49639 and 2P30-CA16672, and by support from Various Donors' Fund and the Stephen and Lavinia Boyd Fund for Leukemia Research. The authors would like to acknowledge the excellent work of the clinical staff, research nurses, and nursing and pharmacy staff of the

blood and marrow transplantation unit, without whom this project would not have been possible. The assistance of Mr. Matthew Hernandez in preparation of this manuscript is gratefully acknowledged.

DISCLOSURES/CONFLICT OF INTEREST

BSA was previously a consultant for Otsuka America Pharmaceuticals, Inc., and REC has received research funding from Otsuka America Pharmaceuticals, Inc.

REFERENCES

1. Thomas, ED. A history of allogeneic hematopoietic cell transplantation. In: Appelbaum, FR.Forman, SJ.Negrin, RS., Blume, KG., editors. Thomas' Hematopoietic Cell Transplantation. 4th. Oxford, UK: Wiley-Blackwell; 2009. p. 3-7.
2. Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Elurnoy N, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. Blood. 1977; 49:511–533. [PubMed: 14751]
3. Russell JA, Tran HT, Quinlan D, Chaudhry A, Duggan P, Brown C, et al. Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. Biol Blood Marrow Transplant. 2002; 8:468–476. [PubMed: 12374451]
4. de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R, et al. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood. 2004; 104:857–864. [PubMed: 15073038]
5. Russell JA, Savoie ML, Balogh A, Turner AR, Larratt L, Chaudhry MA, et al. Allogeneic transplantation for adult acute leukemia in first and second remission with a novel regimen incorporating daily intravenous busulfan, fludarabine, 400 cGy total-body irradiation, and thymoglobulin. Biol Blood Marrow Transplant. 2007; 13:814–821. [PubMed: 17580259]
6. Andersson BS, de Lima M, Thall PF, Wang X, Couriel D, Korbling M, et al. Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclosphosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. Biol Blood Marrow Transplant. 2008; 14:672–684. [PubMed: 18489993]
7. Pidala J, Kim J, Anasetti C, Kharfan-Dabaja MA, Field T, Perkins J, et al. Targeted i.v. BU and fludarabine (t-i.v. BU/Flu) provides effective control of AML in adults with reduced toxicity. Bone Marrow Transplant. 2011; 46:641–649. [PubMed: 20711242]
8. Perkins JB, Kim J, Anasetti C, Fernandez HF, Perez LE, Ayala E, et al. Maximally tolerated busulfan systemic exposure in combination with fludarabine as conditioning before allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2012; 18:1099–1107. [PubMed: 22198540]
9. Liu H, Zhai X, Song Z, Sun J, Xiao Y, Nie D, et al. Busulfan plus fludarabine as a myeloablative conditioning regimen compared with busulfan plus cyclophosphamide for acute myeloid leukemia in first complete remission undergoing allogeneic hematopoietic stem cell transplantation: a prospective and multicenter study. J Hematol Oncol. 2013; 6:15. [PubMed: 23394705]
10. Rambaldi A, Grassi A, Masciulli A, Boschini C, Micò MC, Busca A, et al. Busulfan plus cyclophosphamide versus busulfan plus fludarabine as a preparative regimen for allogeneic haemopoietic stem-cell transplantation in patients with acute myeloid leukaemia: an open-label, multicentre, randomised, phase 3 trial. Lancet Oncol. 2015; 16:1525–1536. [PubMed: 26429297]
11. Slattery JT, Sanders JE, Buckner CD, Schaffer RL, Lambert KW, Langer FP, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. Bone Marrow Transplant. 1995; 16:31–42. [PubMed: 7581127]
12. Slattery JT, Clift RA, Buckner CD, Radich J, Storer B, Bensinger WI, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. Blood. 1997; 89:3055–3060. [PubMed: 9108427]
13. Dix SP, Wingard JR, Mullins RE, Jerkunica I, Davidson TG, Gilmore CE, et al. Association of busulfan area under the curve with veno-occlusive disease following BMT. Bone Marrow Transplant. 1996; 17:225–230. [PubMed: 8640171]

14. Andersson BS, Thall PF, Madden T, Couriel D, Wang X, Tran HT, et al. Busulfan systemic exposure relative to regimen-related toxicity and acute graft-versus-host disease: defining a therapeutic window for i.v. BuCy2 in chronic myelogenous leukemia. Biol Blood Marrow Transplant. 2002; 8:477–485. [PubMed: 12374452]
15. Geddes M, Kangarloo SB, Naveed F, Quinlan D, Chaudhry MA, Stewart D, et al. High busulfan exposure is associated with worse outcomes in a daily i.v. busulfan and fludarabine allogeneic transplant regimen. Biol Blood Marrow Transplant. 2008; 14:220–228. [PubMed: 18215782]
16. Russell JA, Kangarloo SB, Williamson T, Chaudhry MA, Savoie ML, Turner AR, et al. Establishing a target exposure for once-daily intravenous busulfan given with fludarabine and thymoglobulin before allogeneic transplantation. Biol Blood Marrow Transplant. 2013; 19:1381–1386. [PubMed: 23871781]
17. Madden T, de Lima M, Thapar N, Nguyen J, Roberson S, Couriel D, et al. Pharmacokinetics of once daily IV busulfan as part of pretransplant preparative regimens; a comparison with an every 6 hour dosing schedule. Biol Blood Marrow Transplant. 2007; 13:56–64. [PubMed: 17222753]
18. Popat U, Bassett R, Chen J, et al. Allogeneic transplantation for myelofibrosis: benefit of dose intensity. J Clin Oncol. 2013; 31(suppl) abstr #7011.
19. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International Scoring System for evaluating prognosis in myelodysplastic syndromes. Blood. 1997; 89:2079–2088. [PubMed: 9058730]
20. Andersson BS, Madden T, Tran HT, Hu WW, Blume KG, Chow DS, et al. Acute safety and pharmacokinetics of intravenous busulfan when used with oral busulfan and cyclophosphamide as pretransplantation conditioning therapy: a phase I study. Biol Blood Marrow Transplant. 2000; 6:548–554. [PubMed: 11071260]
21. Andersson BS, Kashyap A, Gian V, Wingard JR, Fernandez H, Cagnoni PJ, et al. Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: a phase II study. Biol Blood Marrow Transplant. 2002; 8:145–154. [PubMed: 11939604]
22. Andersson BS, Valdez BC, de Lima M, Wang X, Thall PF, Worth LL, et al. Clofarabine ±Fludarabine with Once Daily IV Busulfan as Pretransplant Conditioning Therapy for Advanced Myeloid Leukemia and MDS. Biol Blood Marrow Transplant. 2011; 17:893–900. [PubMed: 20946966]
23. Kebriaei P, Madden T, Wang X, Thall PF, Ledesma C, de Lima M, et al. Intravenous BU plus Mel: an effective, chemotherapy-only transplant conditioning regimen in patients with ALL. Bone Marrow Transplant. 2013; 48:26–31. [PubMed: 22732703]
24. Kebriaei P, Madden T, Kazerooni R, Wang X, Thall PF, Ledesma C, et al. Intravenous Busulfan Plus Melphalan Is a Highly Effective, Well-Tolerated Preparative Regimen for Autologous Stem Cell Transplantation in Patients with Advanced Lymphoid Malignancies. Biol Blood Marrow Transplant. 2011; 17:412–420. [PubMed: 20674757]
25. Nieto Y, Thall P, Valdez B, Andersson B, Popat U, Anderlini P, et al. High-Dose Infusional Gemcitabine Combined with Busulfan and Melphalan with Autologous Stem-Cell Transplant in Patients with Refractory Lymphoid Malignancies. Biol Blood Marrow Transplant. 2012; 18:1677–1686. [PubMed: 22643322]
26. D'Argenio, DZ., Schumitzky, A. ADAPT II User's Guide: Pharmacokinetic / Pharmacodynamic Systems Analysis Software. Biomedical Simulations Resource. Los Angeles: 1997.
27. Przepiorka D, Khouri I, Ippoliti C, Ueno NT, Mehra R, Körbling M, et al. Tacrolimus and minidose methotrexate for prevention of acute graft-versus-host disease after HLA-mismatched marrow or blood stem cell transplantation. Bone Marrow Transplant. 1999; 24:763–768. [PubMed: 10516680]
28. Tang X, Alatrash G, Ning J, Jakher H, Stafford P, Zope M, et al. Increasing Chimerism after Allogeneic Stem Cell Transplantation Is Associated with Longer Survival Time. Biol Blood Marrow Transplant. 2014; 20:1139–1144. [PubMed: 24727332]
29. Keating MJ, Smith TL, Gehan EA, McCredie KB, Bodey GP, Spitzer G, et al. Factors related to length of complete remission in adult acute leukemia. Cancer. 1980; 45:2017–2029. [PubMed: 6989483]

30. Common Terminology Criteria v3.0. 2003 Jun. NIH Publication No. 03-5410.
31. Fisher RA. On the interpretation of χ^2 from contingency tables, and the calculation of P. J Royal Stat Soc. 1922; 85:87–94.
32. Randles, R., Wolfe, D. Introduction to the Theory of Nonparametric Statistics. John Wiley; 1979.
33. Freeman GH, Halton JH. Note on exact treatment of contingency, goodness of fit and other problems of significance. Biometrika. 1951; 38:141–149. [PubMed: 14848119]
34. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Amer Statist Assoc. 1958; 53:457–481.
35. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep. 1966; 50:163–170. [PubMed: 5910392]
36. Ibrahim, JG., Chen, M-Hui, Sinha, D. Bayesian Survival Analysis. New York: Springer; 2004.
37. Rosenbaum PR, Rubin DB. Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. Amer Stat. 1985; 39:33–38.
38. Ho D, Imai K, King G, Stuart E. Matching as Nonparametric Preprocessing for Reducing Model Dependence in Parametric Causal Inference. Pol Anal. 2007; 15:199–236.
39. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood. 2001; 98(6):1752–1759. [PubMed: 11535508]
40. Abu-Dahier FM, Goodeve AC, Wilson GA, Gari MA, Peake IR, Rees DC, et al. FLT3 internal tandem duplication mutations in adult acute myeloid leukaemia define a high-risk group. Br J Haematol. 2000; 111:190–195. [PubMed: 11091200]
41. Kurosawa S, Yamaguchi T, Miyawaki S, Uchida N, Sakura T, Kanamori H, et al. Prognostic factors and outcomes of adult patients with acute myeloid leukemia after first relapse. Haematologica. 2010; 95:1857–1864. [PubMed: 20634493]
42. Santos GW, Tutschka PJ, Brookmayer R, Saral R, Beschorner WE, Bias WB, et al. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. N Engl J Med. 1983; 309:1347–1353. [PubMed: 6355849]
43. Tutschka PJ, Copelan EA, Klein JP. Bone marrow transplantation for leukemia following a new busulfan and cyclophosphamide regimen. Blood. 1987; 70:1382–1388. [PubMed: 3311203]
44. Nagler A, Rocha V, Labopin M, Unal A, Ben Othman T, Campos A, et al. Allogeneic hematopoietic stem-cell transplantation for acute myeloid leukemia in remission: comparison of intravenous busulfan plus cyclophosphamide (Cy) versus total-body irradiation plus Cy as conditioning regimen--a report from the acute leukemia working party of the European group for blood and marrow transplantation. J Clin Oncol. 2013; 31:3549–3556. [PubMed: 23980086]
45. Copelan EA, Hamilton BK, Avalos B, Ahn KW, Bolwell BJ, Zhu X, et al. Better leukemia-free and overall survival in AML in first remission following cyclophosphamide in combination with busulfan compared with TBI. Blood. 2013; 122:3863–7380. [PubMed: 24065243]
46. Bredeson C, LeRademacher J, Kato K, Dipersio JF, Agura E, Devine SM, et al. Prospective cohort study comparing intravenous busulfan to total body irradiation in hematopoietic cell transplantation. Blood. 2013; 122:3871–3878. [PubMed: 24081656]
47. Yamauchi T, Nowak BJ, Keating MJ, Plunkett W. DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. Clin Cancer Res. 2001; 7:3580–3589. [PubMed: 11705880]
48. Valdez BC, Andersson BS. Interstrand crosslink inducing agents in pretransplant conditioning therapy for hematologic malignancies. Environ Mol Mutagen. 2010; 51:659–668. [PubMed: 20577993]
49. Shimoni A, Hardan I, Shem-Tov N, Yerushalmi R, Avigdor A, Ben-Bassat I, et al. Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. Leukemia. 2006; 20:322–328. [PubMed: 16307018]
50. De Lima M, Anagnostopoulos A, Munsell M, Shahjahan M, Ueno N, Ippoliti C, et al. Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and

high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. Blood. 2004; 104:865–872. [PubMed: 15090449]

51. Kharfan-Dabaja MA, Labopin M, Bazarbachi A, Socie G, Kroeger N, Blaise D, et al. Higher busulfan dose intensity appears to improve leukemia-free and overall survival in AML allografted in CR2: An analysis from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Leuk Res. 2015; 39:933–937. [PubMed: 26003666]
52. Hassan M, Andersson BS. Role of pharmacogenetics in busulfan/ cyclophosphamide conditioning therapy prior to hematopoietic stem cell transplantation. Pharmacogenomics. 2013; 14:75–87. [PubMed: 23252950]
53. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, Storer B. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005; 106:2912–2919. [PubMed: 15994282]
54. Sorror ML, Storb RF, Sandmaier BM, Maziarz RT, Pulsipher MA, Maris MB, et al. Comorbidity-Age Index: A clinical measure of biologic age before allogeneic hematopoietic cell transplantation. J Clin Oncol. 2014; 32:3249–3257. [PubMed: 25154831]
55. Kanakry CG, O'Donnell PV, Furlong T, de Lima MJ, Wei W, Medeot M, et al. Multi-institutional study of post-transplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. J Clin Oncol. 2014; 32:3497–3505. [PubMed: 25267759]
56. Kharfan-Dabaja MA, Labopin M, Bazarbachi A, Hamladji RM, Blaise D, Socie G, et al. Comparing i.v. BU dose intensity between two regimens (FB2 vs FB4) for allogeneic HCT for AML in CR1: a report from the Acute Leukemia Working Party of EBMT. Bone Marrow Transplant. 2014; 49:1170–1175. [PubMed: 24978140]

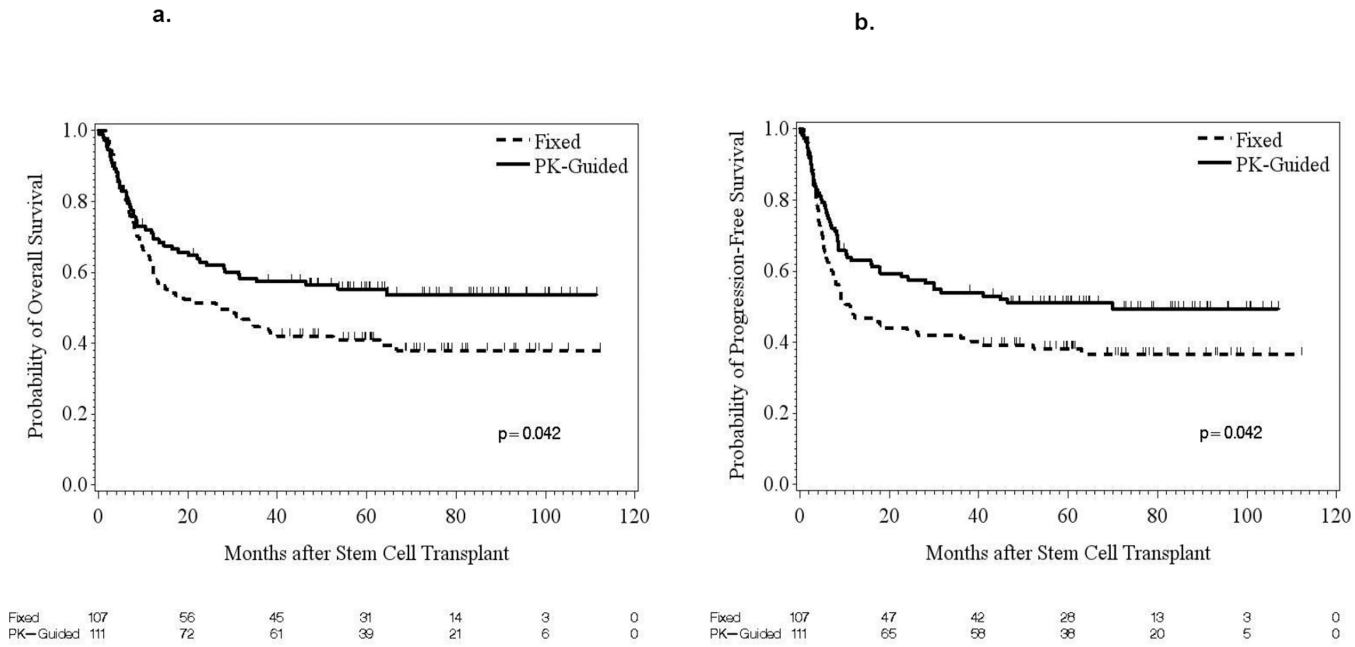

Figure 1.

Survival and mortality of all patients. (a) Overall survival (Fixed dose N=107, number of deaths = 65); PK-guided dose N = 111, number of deaths = 50). (b) Progression-free survival (Fixed dose N = 107, number of events = 67); PK-guided dose N = 111, number of events = 65.

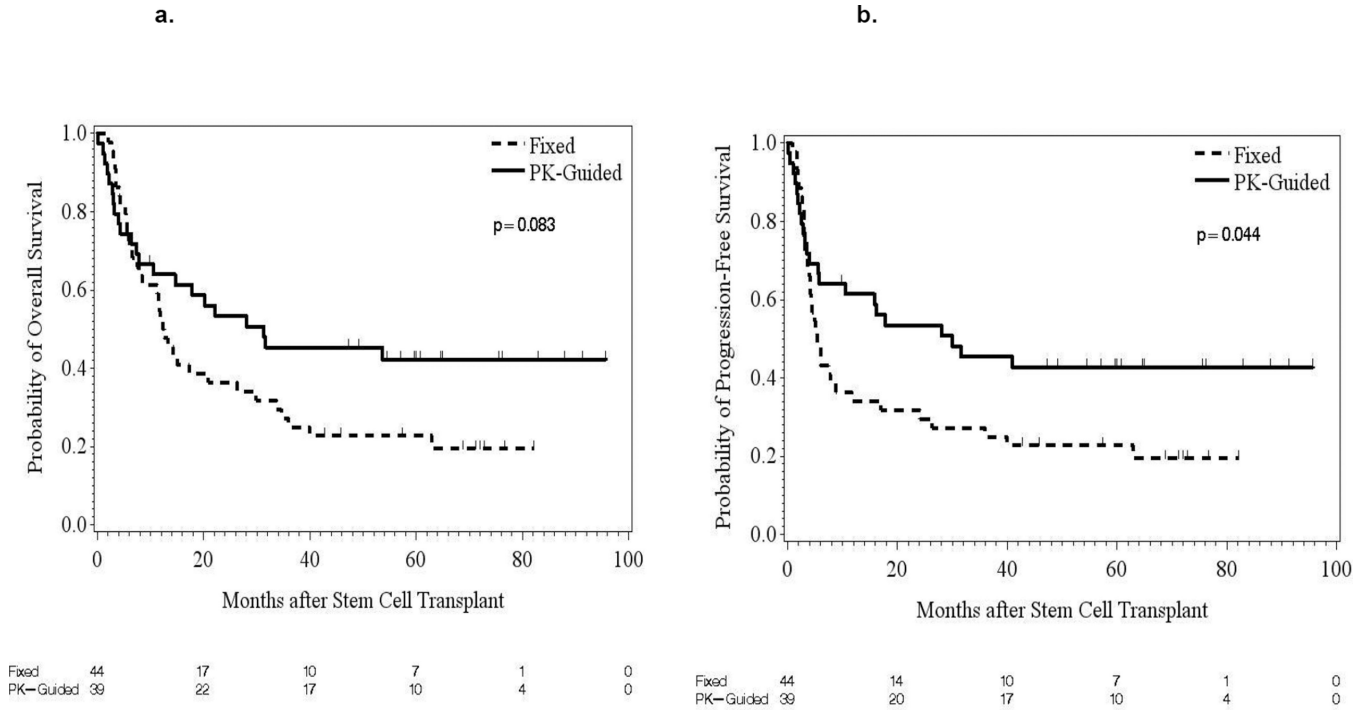

Figure 2.

Survival and mortality of non-CR patients. (a) Overall survival (Fixed dose N=44, number or deaths=35; PK-guided dose N=39, number of deaths=22). (b) Progression-free survival (Fixed dose N=44, number of events=35; PK-guided dose N=39, number of events=22).

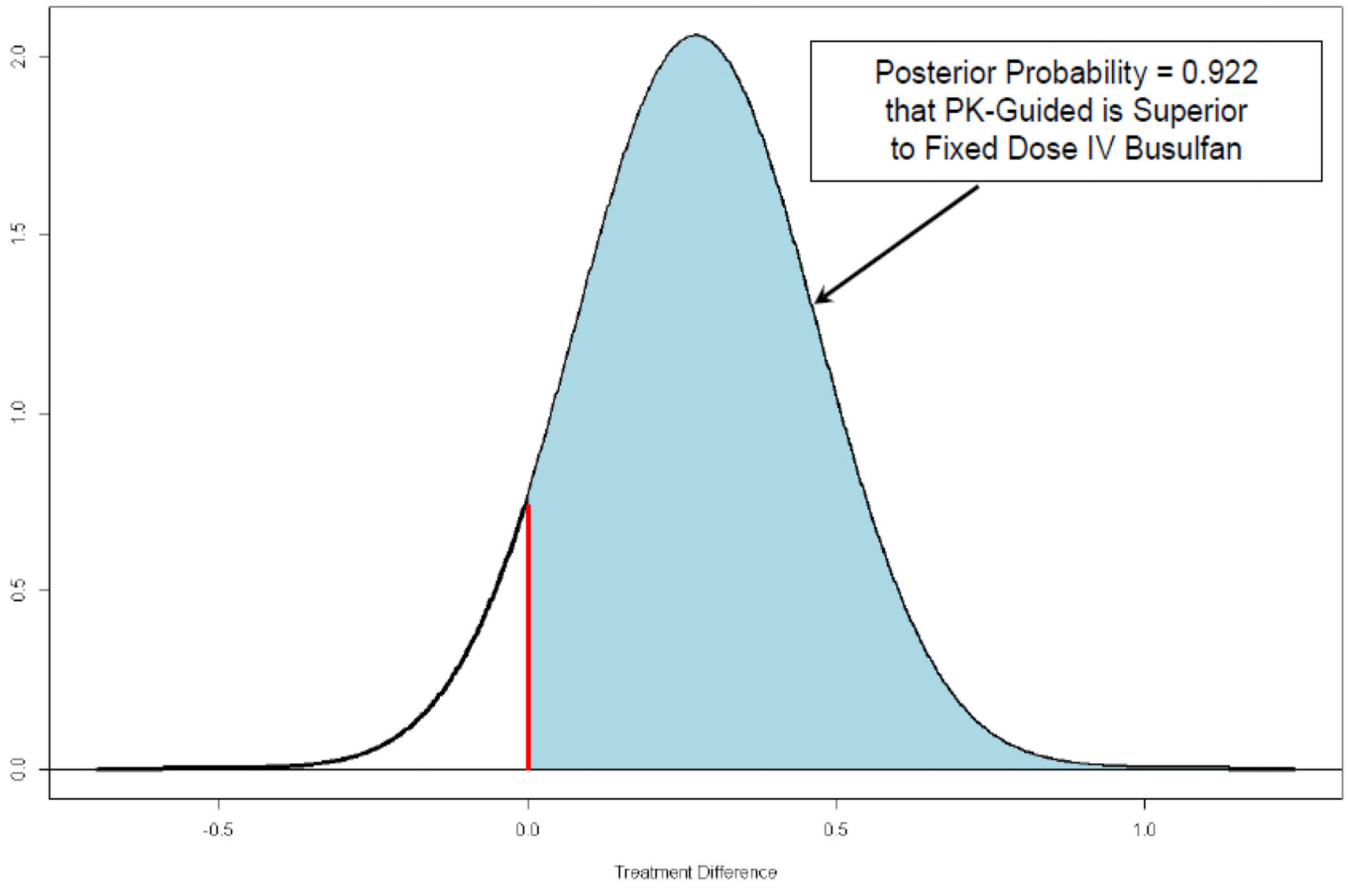

Figure 3.
Posterior distribution of the Fixed-vs-PK-guided busulfan dose effect on overall survival of all patients.

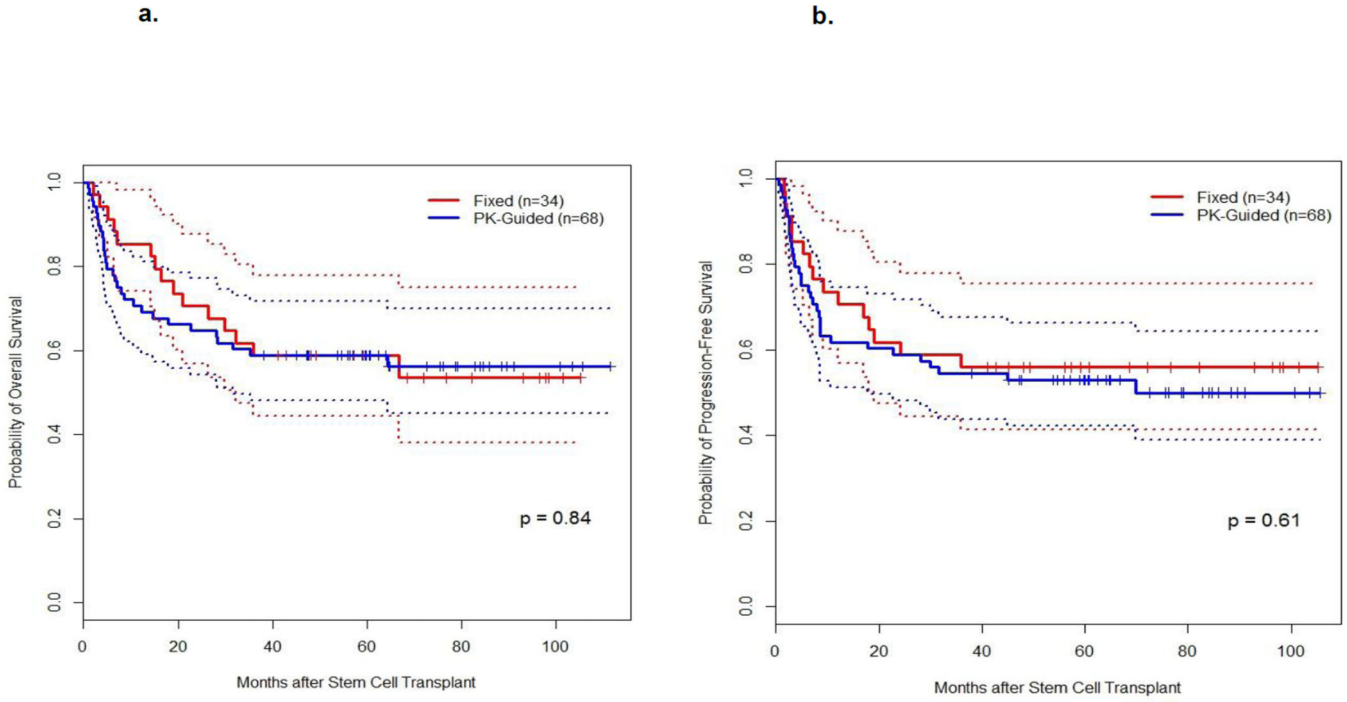

Figure 4.

Survival of matched pairs of patients, Kaplan-Meier estimates with 95% confidence bands. (a) Overall survival (Fixed dose N=34, number of deaths=15; PK-guided dose N=68, number of deaths=29). (b) Progression-free survival (Fixed dose N=34, number of deaths=15; PK-guided dose N=68, number of deaths=33).

Table 1

Summary of Patient Characteristics and Clinical Characteristics, Overall and by Treatment Group

Measure	All Patients (N=218)	Busulfan Dose Group		
		PK-Guided (N=111)	Fixed (N=107)	p-value[a]
Age at SCT (years)				
Mean (SD)	47.5 (12.3)	46.9 (13.0)	48.2 (11.5)	0.66[b]
Median	50.7	50.3	51.5	
Minimum, Maximum	13.2, 65.8	14.4, 65.3	13.2, 65.8	
Gender, n (%)				
Male	110 (50)	55 (50)	55 (51)	0.79
Race, n (%)				
White	179 (82)	91 (82)	88 (82)	1.00
MDS, n (%)				
Yes	52 (24)	26 (23)	26 (24)	1.00
No	166 (76)	85 (77)	81 (76)	
Donor type, n (%)				
Related	110 (50)	55 (50)	55 (51)	0.79
Unrelated	108 (50)	56 (50)	52 (49)	
Cell source, n (%)				
HPC-A	155 (71)	79 (71)	76 (71)	1.00
HPC-M	63 (29)	32 (29)	31 (29)	
Cytogenetic risk, n (%)				
Poor	83 (38)	35 (32)	48 (45)	0.050
Not poor	133 (62)	75 (68)	58 (55)	
Missing	2	1	1	
Complete response, n (%)				
Yes	135 (62)	72 (65)	63 (59)	0.40
No	83 (38)	39 (35)	44 (41)	
FLT-3 (+), n (%)				
Yes	68 (31)	44 (40)	24 (22)	0.008
No	150 (69)	67 (60)	83 (78)	
Total dose[c]				
Median		157.5		
Minimum, Maximum		87.0, 234.4		
Zubrod Score				

Measure	All Patients (N=218)	Busulfan Dose Group		
		PK-Guided (N=111)	Fixed (N=107)	p-value[a]
n	194	97	97	0.83
Median	0	0	0	
Minimum, Maximum	0, 2	0,1	0,2	
Karnofsky score				
n	202	104	98	0.94
Median	90	90	90	
Minimum, Maximum	60, 100	70,100	60, 100	
HCT-CI score				
n	217	111	106	0.15
Median	2	2	2	
Minimum, Maximum	0, 7	0, 7	0, 7	
< 4, n (%)	174 (80)	85 (77)	89 (84)	0.18[b]
≥ 4, n (%)	43 (20)	26 (23)	17 (16)	
HLA-				
matched	198	98	100	
1-Ag mismatched	20	13	7	
-A	7	4	3	
-B	3	2	1	
-C	2	1	1	
DRB1	2	-	2	
DQB1	6	6	-	

[a] Fisher's exact test

[b] Wilcoxon rank sum test

[c] busulfan administered by PK-guidance, dose normalized to body surface area [mg/m^2/day]

Table 2

Summary of Clinical Outcomes

Patients	Outcome Median (95% CI)	Busulfan Dose Group		p-value
		PK-Guided (N=111)	Fixed (N=107)	
All	**OS**	NE (31.3, NE)	26.4 (12.3, 63.0)	0.042
	PFS	69.9 (17.9, NE)	11.2 (7.1, 38.2)	0.042
	TRM	NE (NE, NE)	NE (63.0, NE)	0.07
CR	**OS**	NE (46.4, NE)	NE (19.1, NE)	0.35
	PFS	NE (22.7, NE)	52.2 (9.2, NE)	0.51
	TRM	NE (NE, NE)	NE (NE, NE)	0.37
Non CR	**OS**	31.3 (7.8, NE)	12.5 (7.5, 26.3)	0.08
	PFS	30.0 (5.6, NE)	5.4 (3.9, 11.7)	0.044
	TRM	NE (NE, NE)	63.0 (7.9, NE)	0.10
MDS	**OS**	53.6 (20.2, NE)	12.7 (7.9, 40.0)	0.09
	PFS	46.4 (17.8, NE)	6.6 (4.1, 36.0)	0.034
	TRM	NE (31.6, NE)	40.0 (7.9, NE)	0.15
FLT-3 (+)	**OS**	64.4 (16.4, NE)	31.2 (7.5, NE)	0.52
	PFS	23.4 (8.5, NE)	21.7 (3.7, NE)	0.65
FLT-3 (−)	**OS**	NE (28.1, NE)	26.3 (12.2, 63.0)	0.042
	PFS	NE (28.1, NE)	11.2 (7.9, 40.0)	0.025

CI = confidence interval;
CR = complete response;
NE = not estimated/not reached;
OS = overall survival;
PFS = progression-free survival;
TRM = treatment-related mortality

Table 3

Bayesian piecewise exponential survival time regression - Overall Survival All Patients (N=216[#], number of deaths=114)

	Posterior Quantities				
Variable	**Mean of β**	**SD of β**	**Posterior 95% Credible Interval**		**Probability of a beneficial effect**
PK-Guided IV-BU Dose	−0.271	0.192	−0.645	0.104	0.922[†]
Centered Age	0.016	0.008	−0.000	0.033	0.028[*]
MDS	−0.416	0.249	−0.880	0.088	0.952[*]
Poor Cytogenetic Risk	0.390	0.192	−0.012	0.750	0.024[*]
CR	−0.906	0.222	−1.324	−0.468	1.000[**]
FLT-3 (+)	−0.113	0.202	−0.279	0.507	0.289

[**] Highly significant;

[*] Significant;

[†] Moderately significant.

Notes:

SD = standard deviation

Number of intervals used: 4

PK-Guided vs. Fixed Mean HR (95% HPD Interval) = 0.78 (0.50, 1.08)

[#] Two patients were lacking cytogentetic information and are therefore excluded from this modeling.