Efficacy of CD34+ Stem Cell Dose in Patients Undergoing Allogeneic Peripheral Blood Stem Cell Transplantation after Total Body Irradiation

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ABSTRACT

We estimated the effect of CD34+ stem cell dose during peripheral blood stem cell transplantation (PBSCT) in predicting mortality after total body irradiation (TBI). Between 1997 and 2004, 146 consecutive patients with hematologic malignancies received fractionated TBI (12-13.6 Gy) in 8 fractions over 4 days before undergoing PBSCT; 61 patients received TBI with reduced radiation dose to the lung (6-9 Gy). The number of CD34+ cells transplanted was recorded for all patients. A cubic spline representation for CD34+ dose within a Cox proportional hazards model was used to model the relationship between the CD34+ dose and mortality. Median follow-up was 44 months (range, 12–90 months). The CD34+ cell dose ranged from 2.45 to 15.90 × 10^6 cells/kg (median, 5.15 × 10^6 cells/kg). Risk of mortality decreased with CD34+ doses between 4–8 × 10^6 cells/kg and then began to increase. For all patients, CD34+ doses of 5.1–12.9 × 10^6/kg resulted in at least a doubling of median survival associated with the lowest CD34+ value. In patients treated with lung dose reduction, a similar range of CD34+ dose (4.3–10.2 × 10^6 cells/kg) produced at least a 5-fold improvement from the survival associated with the lowest CD34+ dose; however, the relationship between CD34+ dose and mortality was not statistically different when analyzed by lung dose reduction. A method for assessing risk of mortality by CD34+ dose as a continuous variable is presented. Risk of mortality decreased with CD34+ doses between 4–8 × 10^6 cells/kg and then began to increase.

INTRODUCTION

Peripheral blood stem cell transplantation (PBSCT) is a key component in the treatment of various malignancies [1-5]. Preparatory regimens for PBSCT often include total body irradiation (TBI) [5-7]. Increasing numbers of CD34+ stem cells given during transplantation are reported to correlate with decreasing time to engraftment [8-10]. This effect is retained until a given threshold dose, beyond which no extra benefit occurs [10]. Relatively higher CD34+ doses are reported to be associated with increased incidence of chronic graft-versus-host disease (GVHD) [11-13]; however, the effect of relatively higher CD34+ stem cell dose on survival remains unclear [12-15].

A preliminary analysis of this cohort, using the median dose as a cutpoint, showed that CD34+ cell dose was a risk factor for pulmonary transplantation-related mortality (relative risk [RR] = 9.4 for a CD34+ dose < 5 × 10^6 cells/kg [16]. Lung dose-reduced TBI has also been shown to improve survival in certain patients with poor pretreatment pulmonary function test values [17].

The present analysis was performed to estimate the effect of CD34+ stem cell dose (as a continuous variable) during PBSCT in predicting all-cause mortality after TBI performed with or without dose reduction to the lung.

METHODS

Study Group

Between July 1997 and August 2004, 146 consecutive patients with hematologic malignancies under-

### Conditioning Regimens

Three conditioning regimens were used in consecutive time periods. Regimen A (April 1997–December 2001) consisted of 13.6 Gy of TBI and cyclophosphamide 120 mg/kg, with no lung dose reduction (n = 85). Regimen B (February 2002–May 2003) consisted of 12.0 Gy of TBI, with 9.0 Gy to the lungs, cyclophosphamide 120 mg/kg, and fludarabine 125 mg/m² (n = 35). Regimen C (June 2003–August 2004) consisted of 12.0 Gy of TBI, with 6.0 Gy to the lungs, cyclophosphamide 120 mg/kg, and fludarabine 125 mg/m² (n = 26).

### Radiation Techniques

The radiation techniques used in this cohort have been described previously [17].

### Transplantation Approach

In the first protocol, patients received a T-cell–depleted granulocyte colony-stimulating factor (G-CSF)-mobilized PBSCT using the Ceptrate selection system (CellPro, Bothell, WA). Subsequent protocols used an Isolex 300 cell separator (Baxter, Deerfield, IL), as described previously [18]. CD34⁺ cells were positively selected using anti-CD34⁺ beads, and residual T cells were removed with a cocktail of anti-CD2, -CD6, and -CD7 antibody–coated beads. The CD34⁺ cell dose ranged from 2.45 to 15.90 × 10⁹/kg (median, 5.15 × 10⁹/kg); the T-cell dose was 0.2-1.0 × 10⁹ CD3⁻ cells/kg recipient weight. In the absence of GVHD or unless molecular remission was documented in chronic myeloid leukemia, T cells were added back on days 45 and 100 (n = 140) or on day 60 (n = 6). The cyclosporine (CSA) dose varied according to protocol; 36 patients received standard-dose CSA (target plasma level, 200-400 ng/mL), 20 received low-dose CSA (target plasma level, 100-200 ng/mL) starting on day −4 and continuing until an oral dose was tolerated, and 90 received no CSA during the first 6 weeks after transplantation. All patients started CSA either on day 44 (if T cells were added back on day 45) or on day 59 (if T cells were added back on day 60), and it was continued until at least day 130 (or longer, if chronic GVHD [cGVHD] occurred). Standard prophylaxis against infection included fluconazole to day 100, co-trimoxazole for 6 months, and weekly surveillance for cytomegalovirus antigenemia, as described previously [18,19]. Acute GVHD (aGVHD) was managed with high-dose steroids. Steroid-refractory patients (ie, no response to 7 days of treatment) received combined treatment with anti–tumor necrosis factor (infliximab) and anti-CD25 (daclizumab) monoclonal antibodies, as described previously [20].

### Statistical Methods

We sought to estimate the relationship between CD34⁺ dose and mortality in a flexible way. Standard approaches, such as Cox proportional hazards models with linear effects, make strong assumptions about the form of the relationship between CD34⁺ dose and mortality (ie, that a change of 1 unit in CD34⁺ dose will have the same relative effect on mortality for any value of CD34⁺ dose). Therefore, the relationship of CD34⁺ dose as a continuous variable and mortality was modeled with a cubic spline representation for CD34⁺ dose within a Cox proportional hazards model [21]. This approach allows for a smoothed representation of a continuous relationship between CD34⁺ dose and mortality. The cubic spline terms were fit with 2 knot points, which allowed for sufficient flexibility with this relatively small sample size. We sought to estimate the relationship between risk of mortality and CD34⁺ dose. Models were fit on (1) the whole patient group and (2) by whether or not patients received lung dose–reduced TBI.

Formal statistical inference was done using likelihood ratio tests. We tested for a relationship between CD34⁺ dose and mortality using a χ² test with 3 degrees of freedom, both with and without adjustment for other potential prognostic variables. We formally tested for whether the relationship between CD34⁺ dose and mortality varied depending on other factors, such as lung dose–reduced TBI, combined ventilation/diffusion capacity (CVDC) deficit (defined as having both a forced expiratory volume in 1 minute [FEV₁] and diffusing capacity of the lung for CO [DLCO] of <100% predicted), and age at transplantation by testing for a statistical interaction between the spline terms of CD34⁺ and these other factors.

Curves of the log hazard ratio (HR), relative to the CD34⁺ dose corresponding to the worst survival in that group, were estimated. Efficacious CD34⁺ ranges were defined as those corresponding to an HR of <0.5 (−0.06 on the log scale) relative to the lowest CD34⁺ dose in that group (ie, worst survival). This range corresponds to a doubling of the median survival relative to the lowest CD34⁺ dose. For the lung dose reduction group, we also present an efficacious CD34⁺ dose region corresponding to a 5-fold increase in median survival, because the efficacious region based on a 2-fold increase was too wide to be useful.

Summary statistics, such as sample proportions, medians, standard deviations, and 95% confidence intervals, were used to describe the patient characteristics and the 1-year survival probabilities for patients in...
different subgroups. Kaplan-Meier curves were used to display the distributions of survival among subgroups of patients. Log-rank tests were used to compare survival curves. Data analysis was performed using S-PLUS version 7.0 statistical software (Insightful Corp, Seattle, WA).

RESULTS

Full demographic and outcomes data for this cohort have been reported previously [17]. Briefly, median follow-up was 44 months (range, 12–90 months). The 1-year overall survival was 70% in those treated with lung dose reduction and 64% in those treated without lung dose reduction ($P = .61$). Survival of patients treated with regimens A (85 patients), B (35 patients), and C (26 patients) did not differ significantly.

A cubic spline Cox model, with 2 knot points throughout, was used to examine the relationship of CD34 dose and survival in more detail. Figure 1A plots the log HR (relative to the lowest value of CD34, which was $2.45 \times 10^6$ cells/kg) as a function of log-transformed CD34 dose for all patients. Risk of mortality decreased with CD34 doses between $4-8 \times 10^6$ cells/kg and then began to increase. On the original scale, the dashed lines correspond to CD34 dose values of 5.1 and $12.9 \times 10^6$ cells/kg. Median survival over this range was at least double the survival associated with the lowest CD34 dose. A statistical test of whether CD34 dose was associated with survival produced a statistically significant result ($P = .002$). Figure 1B shows an estimate of the relationship between CD34 and survival, adjusting for CVDC deficit, lung shielding, and age at transplantation. CD34 dose remained significantly associated with survival even after these adjustments ($P = .004$). Furthermore, the relationship as represented by the cubic spline was nearly identical with and without adjustments.

We tested for interactions between CD34 dose and other important factors, such as lung dose-reduced TBI, CVDC deficit, and age at transplantation. The interactions of CD34 dose with lung dose-reduced TBI, CVDC deficit, or age at transplantation were all statistically insignificant ($P = .35$, .20, and .39, respectively), demonstrating a lack of strong evidence for a differential relationship between CD34 dose and mortality by these 3 factors.

However, because an examination of CD34 dose and mortality by type of TBI was of particular interest, we estimated the relationship separately by lung dose-reduced TBI status. Figure 2 plots the log HR (relative to the lowest value of CD34 dose for no lung dose-reduced TBI) as a function of log-transformed CD34 dose for patients with and without lung dose-reduced TBI. The dose range between the vertical dashed lines correspond to a 2-fold increase in median survival for those without lung dose reduction and a 5-fold increase in median survival for those with lung dose reduction. On an original scale, the dashed lines correspond to values of $5.8-13.6 \times 10^6$ cells/kg (without lung dose reduction) and $4.3-10.2 \times 10^6$ cells/kg (with lung dose reduction). Relative to the lowest CD34 dose in the lung dose reduction group, the range corresponding to a 2-fold increase in median survival for this group was $3.3-14.3 \times 10^6$ cells/kg.

DISCUSSION

This article describes risk of mortality as a function of CD34 dose, treated as a continuous variable,
during PBSCT. Risk of mortality decreased with CD34+ doses between $4 \times 8 \times 10^6$ cells/kg and reached a minimum, with a subsequent rise at higher doses.

Other investigators have found similar ranges of “optimal” CD34+ dose. Mohty et al. [12] suggested that to minimize the increased mortality from chronic GVHD but yet allow acceptable engraftment kinetics, the optimum CD34+ dose was $4 \times 8 \times 10^6$ cells/kg. Zaucha et al. [11] also suggested that increasing CD34+ doses were significantly associated with accelerated neutrophil ($P = .03$) and platelet ($P = .01$) engraftment, but higher CD34+ doses ($> 8.0 \times 10^6$ cells/kg) were also associated with a significantly increased hazard of extensive GVHD ($P = .001$). Based on similar considerations, however, Kamel et al. [10] suggested a higher but very narrow optimum CD34+ dose range of 8.95–10 $\times 10^6$ cells/kg.

Our data are very consistent with the findings of Mohty et al. [12], Zaucha et al. [11], and Kamel et al. [10]. Figure 3 shows our data with the dose ranges suggested by these other investigators as dashed lines. These lines correspond exactly with the portion of the curve with decreasing risk of mortality.

Why the risk of mortality increases from a minimum point with increasing CD34+ doses ($> 8 \times 10^6$ cells/kg in this analysis) is unclear. CD34+ cells are known to be a heterogeneous population in which the true “stem cell” subset has yet to be defined [10]. Thus, it remains possible that with higher CD34+ doses, some population of cells that are deleterious to survival may be increased. Alternatively, because there are only a few patients with doses $> 10 \times 10^6$ cells/kg, this increase may be an artifact of the smoothing procedure.

Treatment-related factors also may affect survival as a function of CD34+ dose. With T-cell–replete regimens, for example, T cell numbers may increase with increasing CD34+ dose, resulting in increased risk of GVHD [22]. However, the effect of T-cell dose may be less important in regimens that use G-CSF [11,23].

Further complicating matters, patient factors also interact with treatment-related factors. A recent analysis of this cohort’s pretreatment findings revealed that presence of a CVDC deficit (defined as both FEV$_1$ and DLCO $< 100\%$ predicted) identi-
fied a subgroup of patients who gained a significant (20%) 1-year survival benefit from the use of lung dose–reduced TBI (70% vs 50%; $P = .04$, log-rank test) [17].

Because no optimum conditioning regimen before PBSCT has yet been defined, it is particularly important that treatment-related factors are analyzed in the context of each conditioning regimen. For all patients in our cohort, CD34 doses of 5.1–12.9 × 10^6 cells/kg resulted in at least a doubling of median survival associated with the lowest CD34 value. For patients treated without lung dose reduction, this doubling of median survival occurred at a range of CD34 doses of 5.8–13.6 × 10^6 cells/kg. For patients treated with lung dose reduction, a similar range of CD34 doses (4.3–10.2 × 10^6 cells/kg) produced at least a 5-fold improvement from the survival associated with the lowest CD34 value. The magnitude of this change in risk of mortality over a range of CD34 doses by type of TBI used was not statistically significant. However, it is possible that the interaction test had low power, and a statistically significant difference may be observed in a larger study.

The effect of treatment-related factors (eg, TBI, T-cell dose, and use of G-CSF) on mortality as a function of CD34 dose suggests that the stem cell dose associated with minimum risk of mortality may depend on the conditioning regimen used. Consequently, analyses such as ours that treat CD34 doses as a continuous variable might allow investigators to determine an optimal range of CD34 doses for their particular conditioning regimen.

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