High Graft CD8 Cell Dose Predicts Improved Survival and Enables Better Donor Selection in Allogeneic Stem-Cell Transplantation With Reduced-Intensity Conditioning

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ABSTRACT

Purpose

To characterize the impact of graft T-cell composition on outcomes of reduced-intensity conditioned (RIC) allogeneic hematopoietic stem-cell transplantation (alloHSCT) in adults with hematologic malignancies.

Patients and Methods

We evaluated associations between graft T-cell doses and outcomes in 200 patients who underwent RIC alloHSCT with a peripheral blood stem-cell graft. We then studied 21 alloHSCT donors to identify predictors of optimal graft T-cell content.

Results

Higher CD8 cell doses were associated with a lower risk for relapse (adjusted hazard ratio [aHR], 0.43; $P = .009$) and improved relapse-free survival (aHR, 0.50; $P = .006$) and overall survival (aHR, 0.57; $P = .04$) without a significant increase in graft-versus-host disease or nonrelapse mortality. A cutoff level of 0.72×10^8 CD8 cells per kilogram optimally segregated patients receiving CD8^{hi} and CD8^{lo} grafts with differing overall survival ($P = .007$). Donor age inversely correlated with graft CD8 dose. Consequently, older donors were unlikely to provide a CD8^{hi} graft, whereas approximately half of younger donors provided CD8^{hi} grafts. Compared with recipients of older sibling donor grafts (consistently containing CD8^{lo} doses), survival was significantly better for recipients of younger unrelated donor grafts with CD8^{hi} doses ($P = .03$), but not for recipients of younger unrelated donor CD8^{lo} grafts ($P = .28$). In addition, graft CD8 content could be predicted by measuring the proportion of CD8 cells in a screening blood sample from stem-cell donors.

Conclusion

Higher graft CD8 dose, which was restricted to young donors, predicted better survival in patients undergoing RIC alloHSCT.

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INTRODUCTION

Disease relapse occurs in 25% to 60% of patients after allogeneic hematopoietic stem-cell transplantation (alloHSCT) with reduced-intensity conditioning (RIC),¹⁻⁹ and is the primary barrier to long-term survival. Identification of modifiable factors that predict relapse and survival is fundamental to the design of better transplantation procedures.

In myeloablative peripheral blood stem-cell (PBSC) transplants, the doses of CD3, CD4, and CD8 cells did not correlate with outcomes.¹⁰⁻¹⁴

The majority of RIC transplantations use mobilized PBSC grafts that contain 10^{10} to 10^{11} T cells, the primary mediators of the immuno-

logic graft-versus-host and graft-versus-tumor (GVT) responses. Because the curative potential of RIC transplantation relies entirely on a potent GVT effect, T-cell doses and their subsets may be critical. The impact of T-cell doses on outcomes of commonly used RIC regimens is not well characterized.

Here we examine the impact of graft T-cell doses and subsets on disease relapse, graft-versus-host disease (GVHD), and survival. We also hypothesized that optimal graft T-cell content may be achieved by improved donor selection. To answer these questions, we studied a single-institution cohort of patients who underwent RIC alloHSCT with a uniform conditioning regimen.

PATIENTS AND METHODS

Patients and Treatment

We retrospectively studied 221 consecutive patients who underwent a first peripheral blood alloHSCT with fludarabine-busulfan conditioning for a hematologic malignancy between 2007 and 2014 at the University of Pennsylvania. Patients received fludarabine 120 mg/m² intravenously (IV) and busulfan 6.4 mg/kg IV, followed by the infusion of PBSCs from either a related or an unrelated donor without T-cell depletion. Participants received standard GVHD prophylaxis with tacrolimus or cyclosporine and IV methotrexate. Some patients (n = 51) also received maraviroc on clinical trials of GVHD prophylaxis.¹⁵ All participants received standard antimicrobial prophylaxis and daily granulocyte colony-stimulating factor until neutrophil engraftment.

PBSC collection, graft characterization, and study variables are described in the Data Supplement. The institutional review board approved the study, and patients provided informed consent for data collection before transplantation.

Clinical Outcomes

Time to disease relapse, grade 2 to 4 acute GVHD (aGVHD), moderate to severe chronic GVHD (cGVHD), nonrelapse mortality (NRM), relapse-free survival (RFS), and overall survival (OS) were defined as the time from transplantation to the event. Patients were censored at the time of last contact or a second transplantation for all outcomes, and at the time of donor lymphocyte infusion for GVHD outcomes. Disease relapse was defined as morphologic, cytogenetic, or radiologic evidence of disease demonstrating pretransplantation characteristics. Restaging evaluation, including bone marrow biopsies and appropriate imaging studies, was routinely performed at day 100 or earlier in patients with signs indicating early relapse. The Consensus Conference criteria and National Institutes of Health criteria were used for aGVHD and cGVHD grading, respectively.^{16,17} Donor T-cell chimerism levels were measured after immunomagnetic positive selection of CD3⁺ cells from peripheral blood samples (STEMCELL Technologies, Vancouver, BC, Canada).

Stem-Cell Donors

We studied 21 randomly selected PBSC donors to identify clinical and immunologic factors that predict graft T-cell content. These donors underwent apheresis with similar blood volumes (12 to 15 L). Blood samples were collected during donor screening by using an institutional review board–approved protocol. The proportions of CD4 and CD8 T-cell subsets were determined by flow cytometry, conducted on FACSCanto flow cytometer (BD Biosciences, San Jose, CA) and were analyzed by using FlowJo software (Tree-Star, Ashland, OR).

Statistical Analysis

Correlations between cell doses and clinical variables were assessed by using Pearson and *t* tests. Logarithmic transformation was used to normalize cell doses only in these analyses. Competing risks regression analyses were conducted to identify predictors of time to relapse and GVHD and NRM outcomes, allowing for death without the event as a competing risk. Cox regression was used to identify predictors of OS and RFS. Univariable and multivariable analyses were performed to identify significant independent predictors. Variables that exhibited univariable significance of *P* < .10 were considered for multivariable modeling, and a step-wise elimination method was then used. Separate models were constructed for the primary variables of interest, CD8, CD4, and CD3 cell doses. CD34 cell doses were analyzed by using similar methodology. Because the GVHD prophylaxis regimen was not randomly assigned, it was entered into the models as a fixed covariate for adjustment only. For each model, the statistical significance of predictors was assessed by the Wald test, interactions were examined, and the assumption of proportional hazards was tested. No adjustment for multiple testing was performed. A univariable classification and regression tree procedure was used to dichotomize groups with differing RFS and OS. Univariable comparisons of OS between various donor subsets were conducted with the log-rank test and

confirmed by multivariable Cox regression (only log-rank results are reported). Crude 1-year incidence rates were compared with the χ^2 test. Analyses were conducted in STATA v13.1 (STATA, College Station, TX).

RESULTS

Between April 2007 and May 2014, 221 consecutive patients underwent a first alloHSCT for a hematologic malignancy with fludarabine-busulfan conditioning and a PBSC graft. Of these, 200 patients (90%) had complete graft T-cell data and were included in this analysis. Patient, disease, and transplantation characteristics are summarized in Table 1. Twenty-one excluded patients are described in the Data Supplement. The median follow-up was 29.4 months (range, 0.4 to 85.5 months).

We observed significant heterogeneity in CD3, CD4, and CD8 doses among grafts, with a more than 20-fold difference between the smallest and the largest grafts (Table 1). T-cell doses correlated with age and donor type. Donor age inversely correlated with CD3 (Pearson *r*, −0.33; *P* < .001) and CD8 (*r*, −0.45; *P* < .001) doses but not with CD4 (*r*, −0.11; *P* = .10) doses. Unrelated donors were younger than sibling donors (mean age, 32 v 56 years; *P* < .001) and had grafts with significantly higher mean T-cell doses (CD3: 2.76 v 1.96 × 10⁸ cells per kg; *P* < .001; CD8: 0.71 v 0.38 × 10⁸ cells per kg; *P* < .001; CD4: 1.57 v 1.28 × 10⁸ cells per kg; *P* = .008). In addition, apheresis total blood volume greater than the median (15 L) was associated with higher mean T-cell doses compared with a lower volume (CD3: 2.93 v 1.97 × 10⁸ cells per kg, *P* < .001; CD8: 0.73 v 0.38 × 10⁸ cells per kg, *P* < .001; and CD4: 1.76 v 1.28 × 10⁸ cells per kg, *P* < .001).

High Graft CD8 T-Cell Dose Protects Against Relapse and Predicts Improved Survival

The cumulative incidence of relapse was 42% (95% CI, 35% to 49%) at 1 year and 47% (95% CI, 40% to 55%) at 5 years. There were no significant differences between the 1-year relapse rates in patients with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and non-Hodgkin lymphoma (NHL), the most common diseases in our cohort (42%, 46%, and 41%, respectively; *P* = .44).

We assessed the impact of CD3, CD4, and CD8 T-cell doses and other covariates on time to relapse, RFS, and OS in univariable (Data Supplement) and multivariable (Table 2) analyses. Cell doses were assessed as continuous variables and therefore the hazard ratios (HRs) reflect the increased or decreased risk for the outcome for each unit (1 × 10⁸ cells per kilogram) of T cells within the graft.

In multivariable analysis, the CD8 cell dose was an independent predictor of relapse (adjusted hazard ratio [aHR], 0.43; *P* = .009), RFS (aHR, 0.50; *P* = .006), and OS (aHR, 0.57; *P* = .04). Total CD3, CD4, and CD34 cell doses had no significant associations with these outcomes.

In addition to demonstrating a linear association between CD8 cell dose and outcomes, we used classification and regression tree analysis to identify a cutoff for CD8 cell dose that optimally segregated patients with differing RFS and OS. For both outcomes, a cutoff level of 0.72 × 10⁸ CD8 cells per kilogram was identified, with 60 patients (30%) having received CD8 cell doses above this cutoff level. Compared with patients with CD8 cell doses below the cutoff (CD8^{lo}), patients who received CD8 cell doses above this cutoff (CD8^{hi}) had significantly better RFS (*P* = .005) and OS (*P* = .007), as shown in Figure 1A and 1B. The crude 1-year survival rates were 77% (95% CI,

Table 1. Characteristics of Patients With Available T-Cell Doses (N = 200)

Variable	No. (%)
Median recipient age, years (range)	62 (21-76)
Median donor age, years (range)	43 (18-73)
Recipient sex: male	58%
Donor sex: male	54%
Female into male	21%
Diagnosis	
Myeloid disease	141 (71)
Acute myeloid leukemia	86 (43)
CR1	50 (25)
CR2	21 (11)
Not in CR	15 (8)
Favorable cytogenetics*	3 (2)
Intermediate/unknown cytogenetics*	55 (28)
Adverse cytogenetics*	28 (14)
Myelodysplastic syndrome	44 (22)
Low risk*	17 (9)
High risk*	27 (14)
Intermediate/unknown cytogenetics*	22 (11)
Adverse cytogenetics*	21 (11)
Myeloproliferative neoplasms	8 (4)
Chronic myeloid leukemia (chronic phase)	3 (2)
Lymphoid disease	59 (30)
Non-Hodgkin lymphoma	30 (15)
Indolent B-cell non-Hodgkin lymphoma	4 (2)
Mantle cell lymphoma	3 (2)
Aggressive B-cell non-Hodgkin lymphoma	4 (2)
Peripheral T-cell lymphoma	8 (4)
Cutaneous T-cell lymphoma	11 (6)
Chronic lymphocytic leukemia	8 (4)
Prolymphocytic leukemia	2 (1)
Multiple myeloma	2 (1)
Hodgkin lymphoma	6 (3)
Acute lymphoblastic leukemia	11 (6)
CR1	10 (5)
CR2	1 (1)
Disease Risk Index*	
Low	11 (6)
Intermediate	138 (69)
High	45 (23)
Very high	6 (3)
Hematopoietic cell transplantation comorbidity index	
Low	74 (37)
Intermediate	73 (37)
High	51 (26)
Unknown	2
Karnofsky performance status, %	
> 80	81 (41)
≤ 80	116 (59)
Unknown	3
Donor	
Sibling	85 (43)
Unrelated	115 (58)
HLA compatibility	
8/8 match	171 (86)
Single-antigen mismatch	29 (15)
Recipient cytomegalovirus serostatus	
Negative	110 (55)
Positive	89 (45)
Unknown	1

(continued in next column)

Table 1. Characteristics of Patients With Available T-Cell Doses (N = 200) (continued)

Variable	No. (%)
Donor cytomegalovirus serostatus	
Negative	122 (61)
Positive	78 (39)
GVHD prophylaxis	
Cyclosporine + methotrexate or mycophenolate mofetil	19 (10)
Tacrolimus + methotrexate	130 (65)
Tacrolimus + methotrexate + maraviroc	51 (26)
Era	
2006-2010	77 (39)
2011-2014	123 (62)
Median time to neutrophil engraftment, days (range)	14 (7-32)
Never dropped below 500/ μ L	6 (3)
Never engrafted	2 (1)
Median time to platelet engraftment, days (range)	19 (9-141)
Never dropped below 20,000/ μ L	21 (11)
Never engrafted	4 (2)
Median apheresis total blood volume, L (range)	15 (9-30)
Unknown	44
Apheresis collection sessions	
One	176 (89)
Two	21 (11)
Unknown	3
Median nucleated cell dose, cells per kilogram $\times 10^8$ (range)	8.1 (1.3-22.4)
Median CD34 ⁺ dose, cells per kilogram $\times 10^6$ (range)	6.0 (1.4-21.4)
Median CD3 ⁺ dose, cells per kilogram $\times 10^8$ (range)	2.2 (0.4-8.1)
Median CD4 ⁺ dose, cells per kilogram $\times 10^8$ (range)	1.3 (0.2-5.4)
Median CD8 ⁺ dose, cells per kilogram $\times 10^8$ (range)	0.5 (0.1-2.2)
CD4:CD8 ratio (range)	2.6 (0.3-18.9)

NOTE. Percentages do not always add to 100% because of rounding.

Abbreviations: CR, complete remission; CR1, first complete remission; CR2, second complete remission; GVHD, graft-versus-host disease.

*Disease categories and Disease Risk Index summarized in Armand et al.¹⁸

66% to 88%) for CD8^{hi} grafts and 50% (95% CI, 46% to 55%) for CD8^{lo} grafts ($P < .001$), and survival rates remained significantly different 4 years after transplantation. CD8^{hi} grafts also exhibited a significant survival benefit in subsets of common disease groups (AML/MDS, $P = .048$; NHL, $P = .01$).

CD8^{hi} Grafts Predict Better Outcomes Independent of Disease Risk

Our analysis demonstrated a significant association between the Disease Risk Index and relapse, RFS, and OS (Data Supplement), in agreement with previous reports.¹⁸ High CD8 cell doses resulted in better RFS and OS in patients with high-risk or very-high-risk disease ($n = 51$) and in patients with low-risk or intermediate-risk disease ($n = 149$; Figs 1C and 1D). In particular, patients with high-risk disease who received a CD8^{lo} graft had a poor outcome, with OS of 26.2% at 1 year from their transplantation. Moreover, patients with high-risk disease who received a CD8^{hi} graft had outcomes similar to those of patients with low-risk disease who received a CD8^{lo} graft, demonstrating the ability of high graft CD8 content to overcome the poor prognosis associated with high-risk disease features.

High Graft CD8 Cell Doses Are Associated With Rapid T-Cell Engraftment

We hypothesized that the reduction in relapse risk and improvement in survival observed with high CD8 cell doses were associated

Table 2. Multivariable Associations of Graft T-Cell Doses With Transplantation Outcomes

Dose $\times 10^8/\text{kg}$	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
		Relapse*			RFS†			OS‡	
CD8	0.43	0.23 to 0.81	.009	0.50	0.30 to 0.82	.006	0.57	0.33 to 0.97	.04
CD4	0.92	0.69 to 1.24	.60	0.91	0.72 to 1.16	.45	1.04	0.82 to 1.33	.75
CD3	0.85	0.68 to 1.06	.14	0.87	0.73 to 1.03	.10	0.93	0.77 to 1.11	.40
		aGVHD (grade 2 to 4)‡			cGVHD (moderate to severe)§			NRM§	
CD8	0.96	0.55 to 1.65	.87	1.84	0.89 to 3.78	.10	0.80	0.36 to 1.76	.58
CD4	1.27	0.96 to 1.68	.10	1.04	0.74 to 1.46	.84	0.93	0.67 to 1.29	.66
CD3	1.08	0.87 to 1.34	.51	1.16	0.91 to 1.48	.24	0.97	0.77 to 1.23	.82

NOTE. P values < .05 are presented in **bold** font.

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic GVHD; HR, hazard ratio; NRM, nonrelapse mortality; OS, overall survival; RFS, relapse-free survival.

*Adjusted for donor sex, Disease Risk Index, and GVHD prophylaxis regimen.

†Adjusted for Disease Risk Index and GVHD prophylaxis regimen.

‡Adjusted for HLA mismatching and GVHD prophylaxis regimen.

§Adjusted for GVHD prophylaxis regimen.

with more rapid engraftment of donor T cells. Donor T-cell chimerism levels, measured on days 30, 60, and 100, were significantly higher in patients who received higher CD8 cell doses (Data Supplement). Similar associations were observed on day 180 and at 1 year but did not reach statistical significance, possibly as a result of a smaller sample size.

Survival Advantage Associated With Younger Unrelated Donors Is Limited to CD8^{hi} Grafts

Outcomes of RIC transplantations may be better when using younger unrelated donors compared with older sibling donors, but previous studies have shown conflicting results.^{19,20} We sought to

Fig 1. Higher CD8 cell doses are associated with better relapse-free survival (RFS) and overall survival (OS). Kaplan-Meier plots of (A) RFS and (B) OS are shown for patients who received CD8^{hi} and CD8^{lo} grafts (cutoff, 0.72×10^8 CD8 cells per kilogram). P values were generated by the log-rank test. Similar plots are presented for (C) RFS and (D) OS for four groups of patients, categorized by the CD8 graft content and the Disease Risk Index.

Fig 2. CD8 cell doses decrease with donor age. A scatter plot of donor age and graft CD8 cell dose is shown. Donor age inversely correlated with CD8 doses (Pearson r , -0.45 ; $P < .001$). The horizontal reference line at 0.72×10^8 cells per kilogram shows the optimal cutoff of CD8 cell dose for prediction of relapse-free survival and overall survival. None of the 22 donors older than age 60 years had graft CD8 doses above the optimal cutoff.

determine whether differences in outcomes related to donor age were driven by differences in graft CD8 content. We found that donor age inversely correlated with CD8 cell dose (Fig 2); only 13% (10 of 75) of donors older than age 50 years provided a CD8^{hi} graft, and all 22 donors older than age 60 years provided a CD8^{lo} graft. Even among younger donors, the proportions of CD8^{hi} grafts were only 40% (50 of 125) for donors younger than age 50 years and 53% (31 of 58) for donors younger than age 30 years.

We then compared the OS of older recipients (age ≥ 50 years; $n = 185$) who received a graft from either HLA-matched sibling donors (age ≥ 50 years) or younger unrelated donors (age younger than 50 years) stratified by CD8^{hi} or CD8^{lo} grafts (Fig 3). Unrelated donor groups were inclusive of HLA-mismatched donors. Compared with older sibling donors, OS was significantly better for younger unrelated donors with a CD8^{hi} graft ($P = .03$), but not for younger unrelated donors with a CD8^{lo} graft ($P = .28$). The 4-year OS rates were 59% (95% CI, 39% to 74%) for younger unrelated donors with CD8^{hi} grafts, 18% (95% CI, 7% to 33%) for younger unrelated donors with CD8^{lo} grafts, and 33% (95% CI, 20% to 47%) for older sibling donors. These results demonstrate that the superiority of younger unrelated donors is dependent on the graft CD8 cell dose.

Graft T-Cell Doses Have No Significant Impact on GVHD or NRM

Although high CD4 cell doses were weakly associated with a higher risk for aGVHD, and high CD8 doses correlated with a higher risk for cGVHD (Table 2 and Data Supplement), these associations did not reach statistical significance, and the risk for NRM was unaffected by cell doses. Subset analyses of T-cell doses and GVHD outcomes by GVHD prophylaxis regimens also revealed no significant associations (data not shown). High CD34 cell doses were weakly associated with a higher risk for aGVHD (Data Supplement), but did not reach statistical significance in multivariable analysis (aHR, 1.05; 95% CI, 0.99 to 1.11; $P = .11$).

Fig 3. Overall survival (OS) of patients age ≥ 50 years depends on donor type and graft CD8 dose. Kaplan-Meier OS plots are shown for transplant recipients age 50 years or older ($n = 185$) who received peripheral blood stem-cell grafts from three different sources: matched sibling donors (age ≥ 50 years), unrelated donors (age younger than 50 years) with a CD8 graft above 0.72×10^8 cells per kilogram (CD8^{hi}), and unrelated donors (age younger than 50 years) with a lower CD8 cell dose (CD8^{lo}). Younger unrelated donors with a CD8^{hi} graft resulted in better OS compared with older sibling donors ($P = .03$) or young unrelated donors with a CD8^{lo} graft ($P = .001$). Younger unrelated donors with a CD8^{lo} graft and older sibling donors had no significant differences in OS ($P = .28$), suggesting that donor age had no independent effect on transplantation outcome.

Screening for Donors Who Can Provide Grafts With Higher CD8 Cell Doses

A potential strategy for optimizing graft T-cell content would be to predict the graft composition during donor screening. We therefore studied 21 randomly selected alloHSCT donors with similar apheresis blood volumes and known graft T-cell content. We explored whether immunophenotypic or clinical characteristics correlated with graft CD8 T-cell dose. Donors with a higher proportion of CD8 cells subsequently donated grafts with a higher CD8 cell dose (Pearson r , 0.67 ; $P < .001$; Fig 4), although donors with a higher proportion of CD4 T

Fig 4. Prediction of graft CD8 T-cell dose using screening blood samples from peripheral blood stem-cell donors. Baseline donor CD8 proportions in a screening blood sample from 21 stem-cell donors successfully predicted the subsequent graft CD8 content. Scatter plots are shown for the correlation between the CD8 graft content and the baseline proportion of CD8 T cells in a screening blood sample from each donor. Pearson correlation coefficients and P values are presented.

cells and higher CD4:CD8 ratio donated grafts with lower CD8 cell content (r , -0.53 ; $P = .01$ and r , -0.53 ; $P = .01$, respectively). There were no significant correlations between the CD8 graft content and donor clinical variables such as weight, sex, viral serologies, or apheresis parameters.

DISCUSSION

We show that high CD8 cell doses in PBSC grafts predicted a significantly reduced relapse risk and improved survival in patients with hematologic malignancies undergoing RIC alloHSCT, independent of disease risk. In addition, our data suggest that OS after RIC transplantation can be improved by preferentially choosing young unrelated donors with a CD8^{hi} graft over older sibling donors; no benefit was associated with young unrelated donors with a CD8^{lo} graft. Finally, we show that the proportion of CD8 cells or the CD4:CD8 ratio in a baseline donor blood sample predict a CD8^{hi} graft.

These findings indicate that improved survival after RIC transplantations could be achieved by optimizing donor selection and PBSC collection to increase the likelihood of mobilizing grafts containing high CD8 cell doses. We show that selecting a younger donor increases the chance for a CD8^{hi} graft. This is increasingly practical because donor registries are enriched for younger donors and most patients are likely to have at least one acceptable donor²¹ and often more than one, so that the likelihood of identifying an appropriate young donor is high.

Because only 53% of donors younger than age 30 years mobilized CD8^{hi} grafts, other strategies to increase the CD8 cell content can be considered. A higher apheresis total blood volume increases T-cell doses, as shown by our study and by previous reports,^{22,23} although this strategy increases both CD8 and CD4 doses. Importantly, screening peripheral blood from potential donors for the relative proportions of CD8 and CD4 cells identifies donors most likely to mobilize CD8^{hi} grafts. This is also a practical consideration because this assay is rapid, is routinely performed in clinical laboratories, and can easily be done at the time of confirmatory HLA typing. It is important to note that in our analysis, there was a linear association between CD8 dose and survival, suggesting that even when a CD8^{hi} graft is not achievable, we should aim for the highest CD8 cell dose among available donors rather than aim for a specific target number. How well these strategies translate into meaningful improvement in outcome can now be examined prospectively.

The importance of T-cell doses in PBSC grafts has been previously examined. Several studies did not show a correlation between T-cell doses and outcomes of myeloablative PBSC transplantations,^{10,14,24} and CD8 cell depletion failed to reduce GVHD.²⁵ In RIC transplantations, a previous report has shown the prognostic importance of the CD8 dose in 63 patients who received PBSC grafts.²⁶ That study evaluated predominantly non-AML patients who received low-dose total-body irradiation with or without fludarabine, whereas our study included patients allografted with fludarabine-busulfan, the most commonly used RIC regimen according to Center for International Blood and Marrow Transplant Research data,²⁷ and showed significant advantage for CD8^{hi} grafts in both myeloid and lymphoid diseases. Interestingly, although our study focused on RIC PBSC transplantations, a recent study identified a role for CD8 cell doses in myeloablative umbilical cord blood transplants, suggesting relevance in other settings.²⁸

The use of alloHSCT in elderly patients has increased in recent years,²⁷ and identifying the optimal donor for older patients is critical. A Center for International Blood and Marrow Transplant Research analysis showed worse OS after alloHSCT from younger matched unrelated donors compared with older sibling donors in patients with a good performance status.¹⁹ Another retrospective registry study of patients with MDS showed a survival advantage for unrelated donors younger than age 30 years compared with matched related siblings, but the survival difference was not driven by a lower relapse rate.²⁰

Both analyses included conditioning regimens with various intensities, multiple graft sources, and T-cell depletion in some patients, making conclusions difficult to apply clinically. Another analysis of RIC transplantations showed no difference in NRM between older sibling and younger unrelated donors, but relapse and survival outcomes were not reported.²⁹ Most importantly, graft T-cell doses and their subsets are not reported by most transplantation centers, limiting the ability to include them in analyses of registry data.

The effect of donor age on the GVT response has not been clearly identified. Worse outcomes for transplantations using older donors have been reported, but the biologic reason is not clear.³⁰⁻³² Aging stem cells are characterized by ineffective lymphopoiesis,³³ and aging individuals have a higher proportion of regulatory cell populations that may weaken antitumor responses.³⁴⁻³⁶ Our results suggest that the critical predictor of a potent GVT response is not age but the number of CD8 cells in the graft, with donor age being a surrogate marker for this number.

Our study has certain limitations. We retrospectively examined a single common RIC regimen (fludarabine-busulfan) in a single center. Whether the role of graft T-cell content remains important with other regimens is unknown. In addition, we analyzed a heterogeneous patient population in terms of disease characteristics (eg, disease type, cytogenetics). The validated Disease Risk Index was used to adjust our analyses to overcome this barrier.¹⁸ In addition, a subset analysis of disease groups (ie, AML/MDS and NHL) maintained a survival advantage for high CD8 doses, but detailed analyses of specific diseases were underpowered. Finally, detailed analyses of CD4 and CD8 subsets, other cell types, and activation markers were not part of this analysis and may provide mechanistic insight.

RIC alloHSCT remains the only potentially curative option for the increasing number of older patients who may not tolerate myeloablative alloHSCT. Modifiable factors such as graft content and donor optimization may improve the outcome of RIC transplantations for patients with hematologic malignancies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

High Graft CD8 Cell Dose Predicts Improved Survival and Enables Better Donor Selection in Allogeneic Stem-Cell Transplantation With Reduced-Intensity Conditioning

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