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Higher infused CD34+ cell dose and overall survival in patients undergoing in vivo T-cell depleted, but not t-cell repleted, allogeneic peripheral blood hematopoietic cell transplantation

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BACKGROUND AND OBJECTIVES: Understanding the effect of cellular graft composition on allogeneic hematopoietic cell transplantation (AHCT) outcomes is an area of great interest. The objective of the study was to analyze the correlation between transplant-related outcomes and administered CD34+, CD3+, CD4+ and CD8+ cell doses in patients who had undergone peripheral blood, AHCT and received either in vivo T-cell depleted or T-cell replete allografts.

DESIGN AND SETTING: Comparison of consecutive patients who underwent peripheral blood AHCT in our institution between January 2003 and December 2009.

PATIENTS AND METHODS: The cohort of 149 patients was divided into two groups; non T-cell depleted (NTCD) (n=54) and T-cell depleted (TCD) (n=95). Study endpoints were overall survival (OS), progression free survival (PFS), engraftment kinetics (neutrophil and platelet recovery), incidence of acute graft versus host disease (acute GVHD), chronic GVHD, non relapse mortality (NRM) and disease relapse.

RESULTS: Multivariate analysis showed that higher infused CD34+ cell dose improved OS (relative risk 0.58, 95% CI 0.34-0.98, *P*=.04), PFS (relative risk 0.59, 95% CI 0.35-1.00, *P*=.05) and NRM (relative risk 0.49, 95% CI 0.24-0.99, *P*=.048) in the TCD group. By multivariate analysis, there was no difference in engraftment, grades II-IV acute GVHD, extensive chronic GVHD and relapse in the two groups relative to the infused cell doses. There was a trend towards improved OS (relative risk 0.54, 95% CI 0.29-1.01, *P*=.05) with higher CD3+ cell dose in the TCD group.

CONCLUSION: Our findings suggest that higher CD34+ cell dose imparts survival benefit only to in vivo TCD peripheral blood AHCT recipients.

Allogeneic hematopoietic cell transplantation (AHCT) remains a potentially curative treatment modality for a variety of malignant and benign hematological disorders. The cellular composition of the donor grafts can affect clinical outcomes following AHCT. In myeloablative AHCT using bone marrow as a graft source, a clear association between higher CD34+ cell dose and improved survival has been reported.[1,2] Similarly, in the setting of peripheral blood AHCT, higher infused CD34+ cell doses have been shown to be associated with early neutrophil and platelet engraftment[3-5] and improved survival[5-9] in some but not all studies.[10] The correlation of higher infused CD34+ doses with graft-versus-host disease (GVHD) in patients undergoing peripheral blood AHCT is even more controversial with some reports

showing increased incidence of grades II-IV acute GVHD[11,12] and extensive chronic GVHD,[4,9] while others report no such correlation.[5,10] Similarly the correlation between CD3+, CD4+ and CD8+ cell doses and GVHD has been discrepant with many reports suggesting no association.[4,10,13,14]

Even though the influence of cellular composition of the infused allografts on transplantation outcomes has been the subject of many previous studies, there is paucity of data on the relative importance of cellular composition of the infused allograft on the transplantation outcomes of patients undergoing peripheral blood AHCT with in vivo T-cell depletion compared to patients receiving T-cell replete allografts. A recent report has suggested a survival benefit with higher infused CD34+ and CD3+ cell doses in pediatric patients undergoing AHCT with in vivo TCD.[15] We report the impact of cellular graft composition on transplantation outcomes of adult patients who underwent T-cell depleted peripheral blood AHCT compared to patients who underwent T-cell replete peripheral blood AHCT.

METHODS

The patient cohort included 149 consecutive patients who underwent granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood AHCT between January 2003 through December 2009 at our transplant center, who received either myeloablative (MA) (n=114) or non-myeloablative/reduced intensity conditioning (NMA/RIC) (n=35) regimens. The patient population was divided in two groups based on whether they received in vivo T-cell depletion; non T-cell depleted (NTCD group) and T-cell depleted (TCD group). Sixty-three percent (n=95) received in vivo T-cell depletion with alemtuzumab (n=39) (Campath, Genzyme, Massachusetts, USA) or rabbit antithymocyte globulin (ATG) (Thymoglobulin, Genzyme) (n=52). Four patients with aplastic anemia received equine ATG 30 mg/kg (Atgam, Pfizer). High-resolution human leukocyte antigen (HLA) typing was done at the allele level for class-I (HLA-A, -B, -C) and class II (HLA-DRB1) molecules as described before.[16] Patients with 8/8 allele level match for HLA -A, -B, -C and -DRB1 with their respective donors were considered a full match.[17] The study was approved by the Institutional Review Board and Scientific Review Committee at our institution.

Determination of CD34+, CD3+, CD4+ and CD8+ cell doses was performed at the West Virginia University Flow Cytometry Laboratory (Morgantown, West Virginia). The BD FACSCanto II flow cytometer, Becton Dickinson (San Jose, California) was used for all analyses. Red blood cell (RBC) lysed and washed samples were used for CD34+ enumeration with phycoerythrin (PE) labeled, 8G12 clone, immunoglobulin (Ig) G1 (Becton Dickinson, San Jose, California) based on International Society of Hematotherapy and Graft Engineering guidelines.[18] An unwashed but RBC lysed sample was used to count CD3+, CD4+ and CD8+ cell doses. Four-color direct immunofluorescence reagent was used; BD MultiTEST™ fluoresein isothiocyanate (FITC)-labeled CD3, clone SK7; allophycocyanin (APC)-labeled CD4, clone SK3; PE-labeled CD8, clone SK1; peridinin chlorophyll protein (PerCP)-labeled CD45, clone 2D1 (Becton Dickinson, San Jose, California). Used in conjunction with Trucount tubes, the absolute numbers of CD3+, CD4+ and CD8 + was calculated using the BD MultiSET software (Becton Dickinson, San Jose, California).

The study endpoints included engraftment, acute and chronic GVHD, relapse rate, nonrelapse mortality (NRM), overall survival (OS) and progression-free survival (PFS). Neutrophil engraftment was defined as days to an absolute neutrophil count (ANC) $\geq 0.5\times10^9$/L for 3 consecutive days post-transplantation nadir and platelet engraftment as days to platelet count $\geq 20\times10^9$/L for 7 consecutive days without platelet transfusion.[19] Acute and chronic GVHD were graded using the standard criteria.[20-23] All patients who successfully engrafted neutrophils were considered evaluable for acute GVHD. Patients surviving at least 100 days post transplantation were considered evaluable for chronic GVHD. OS was defined as the time to death due to any cause from the date of transplant. Surviving patients were censored at time of last follow up. Death, disease relapse and/or progression were considered events for PFS. NRM was defined as death from any cause other than disease progression or relapse.

The patient population was divided into NTCD or TCD groups. The cell doses of CD34+, CD3+, CD4+ and CD8+ in the NTCD and TCD groups were dichotomized as ≥ and <50th percentile based on median values.[8,15] Baseline categorical variables for NTCD and TCD groups were compared by using the chi-square test or Fisher exact test, as appropriate; while continuous variables were compared using the *t*-test. All *P* values are two sided. OS and PFS were estimated using the Kaplan-Meier method and differences between the patient groups analyzed with the log-rank test. Cumulative incidence was estimated for NRM and relapse rate, with relapse as a competing risk for the former and death in remission for the latter. The Gray test was used to assess the difference between various subgroups for NRM and relapse rate.[24] A propor-

tional hazards model was created to assess the impact of infused cell doses on engraftment, acute GVHD, chronic GVHD, NRM, relapse, PFS and OS initially measured by univariate analysis. Multivariate logistic regression analysis was constructed for variables showing significance on univariate analysis ($P<0.1$). All statistical analyses were performed with JMP-version 9, SAS Institute Inc., Cary, NC. P-values based on the Gray test were calculated using R-project version 2.8.1 (The R Foundation for Statistical Computing, 2008).

RESULTS

The baseline patient characteristics are detailed in **Table 1**. All patients received G-CSF mobilized peripheral blood allografts. The TCD and NTCD groups were matched for median patient age (50 years and 48 years respectively; $P=.32$) and sex ($P=1.0$). As expected, notable differences were present at baseline among patients receiving a T-cell depleted vs a T-cell replete allograft. All patients with chronic myeloid leukemia in first chronic phase, acute myeloid leukemia and acute lymphoblastic leukemia in first complete remission and myelodysplastic syndrome with refractory anemia and refractory anemia with ringed sideroblasts were considered standard-risk, while all remaining patients were considered high-risk for relapse as defined previously.[25,26] The intensity of the conditioning regimens was defined using the working group recommendations[27] and the two groups were matched for MA versus NMA/RIC regimens ($P=.17$). There was no difference between the NTCD and TCD groups with regards to HLA-mismatched transplants ($P=.29$). Among those receiving HLA identical transplants more patients in the TCD group received allografts from unrelated donors (URD) ($P<.01$).

The median infused CD34+ cell dose was 5.8×10^6/kg patient weight (PW); range 1.2-16. Median cell doses for CD3+, CD4+ and CD8+ cells were 30.8×10^7/kg PW (4.5-100.8), 18.7×10^7/kg PW (1.9-63) and 11.3×10^7/kg PW (0.8-52.4), respectively. Patients in the TCD group received a higher CD34+ cell dose (median; 6.2×10^6/Kg PW versus 5.4×10^6/Kg PW, $P=.008$). There was no difference in the CD3+, CD4+ and CD8+ cell doses between the groups (**Table 2**).

One hundred and thirty-eight patients had successful neutrophil engraftment after posttransplant nadir. Seven patients never achieved ANC $\geq0.5\times10^9$/L, considered primary engraftment failure 28 and 4 patients who received NMA conditioning had a nadir above the threshold. Median time to neutrophil engraftment in the NTCD and TCD groups was 16 and 15 days, respectively (mean NTCD 15.8 days, 95%CI 14.7-16.8 and mean TCD 15.9 days, 95%CI 15.2-16.7); $P=.78$). In the TCD group, univariate analysis showed a trend toward early neutrophil engraftment with higher CD34+ cell dose (15.4 vs. 16.8 days, $P=.08$), however there was no correlation between CD3+, CD4+ and

Table 1. Baseline patient characteristics.

Patient Characteristics	NTCD n=54	TCD n=95	P
Median age (years/range)	**48 (20-63)**	**50 (17-69)**	**.32**
Sex (%)			
Male	63 (34)	63.2 (60)	1.0
Female	37 (20)	36.8 (35)	
Disease (%)			
Acute leukemia/MDS	64.8 (35)	55.7 (53)	.30
Non-Hodgkin lymphoma	7.4 (4)	23.2 (22)	.01
Hodgkin lymphoma	3.7 (2)	3.2 (3)	1.0
Chronic myeloid leukemia	22.2 (12)	6.3 (6)	.007
Chronic lymphoid leukemia	1.8 (1)	3.1 (3)	1.0
Multiple myeloma	0	3.2 (3)	.55
Others[a]	0	5.2 (5)	.16
Risk group[b] (%)			
Standard risk	38.9 (21)	13.7 (13)	<.01
High risk	61.1 (33)	86.3 (82)	
Conditioning regimen (%)			
Myeloablative	83.3 (45)	72.6 (69)	.17
Reduced intensity / non-myeloablative	16.7 (9)	27.4 (26)	
Donor/match[c] (%)			
Sibling HLA-identical	68.5 (37)	37.9 (36)	<.01
URD HLA-identical	24.1 (13)	48.4 (46)	<.01
HLA-mismatched	7.4 (4)	13.7 (13)	.29
GVHD prophylaxis (%)			
Tacrolimus-based	31.5 (17)	66.3 (63)	<.01
Cyclosporine-based	68.5 (37)	33.7 (32)	

NTCD: non T-cell depleted, TCD: T-cell depleted, MDS: myelodysplastic syndrome, URD: unrelated donor, HLA: human leukocyte antigen, GVHD: graft versus host disease. P values were calculated using the Fisher exact test for categorical variables and using the t test for continuous variables. [a]Aplastic anemia (n=4) and myelofibrosis (n=1). [b]Standard risk includes refractory anemia, refractory anemia with ringed sideroblasts, acute leukemia in 1st complete remission and CML in chronic phase (all others high risk). [c]High-resolution allele level matching for HLA -A, -B, -C, and -DRB1.

Table 2. Median cell doses in NTCD and TCD groups.

Cell doses (per kg patient weight)	NTCD median (range)	TCD median (range)	*P*
CD34+×10^6	5.4 (1.2-12.9)	6.2 (1.9-16)	.008
CD3+×10^7	29.6 (5.7-44.7)	31.1 (4.5-100.8)	.91
CD4+×10^7	18.7 (4.6-44.7)	18.6 (1.9-63)	.80
CD8+×10^7	10.6 (0.8-52.4)	11.6 (2.1-44.7)	.9

NTCD: non T-cell depleted, TCD: T-cell depleted. *P* value estimated using t test.

Table 3. Univariate analysis showing effects of higher and lower cell doses in NTCD and TCD groups on neutrophil and platelet engraftment.

Days to neutrophil engraftment		NTCD group (95% CI) [*P*]	TCD group (95% CI) [*P*]
CD34+	<50th centile	16.3 (15.0-17.7) [.18]	16.8 (15.6-18.0) [.08]
	>50th centile	14.9 (13.2-16.6)	15.4 (14.4-16.4)
CD3+	<50th centile	15.9 (14.1-17.4) [.95]	16.1 (14.9-17.3) [.73]
	>50th centile	15.8 (14.4-17.3)	15.8 (14.8-16.9)
CD4+	<50th centile	15.3 (13.8-16.9) [.37]	16.0 (14.9-17.0) [1.0]
	>50th centile	16.3 (14.8-17.8)	16.0 (14.9-17.0)
CD8+	<50th centile	15.7 (14.4-17.6) [.82]	16.2 (15.1-17.3) [.5]
	>50th centile	16.0 (14.2-17.2)	15.7 (14.7-16.8)
Days to platelet engraftment			
CD34+	<50th centile	23.2 (18.9-27.5) [.35]	23.1 (17.7-28.5) [.55]
	>50th centile	19.8 (14.1-25.6)	20.9 (16.5-25.4)
CD3+	<50th centile	22.0 (17.5-26.5) [.96]	26.5 (21.6-31.5) [.013]
	>50th centile	21.9 (16.4-27.3)	18.0 (13.6-22.5)
CD4+	<50th centile	21.6 (16.9-26.3) [.82]	26.2 (21.5-30.8) [.01]
	>50th centile	22.4 (17.3-27.6)	17.5 (12.9-22.2)
CD8+	<50th centile	22.6 (17.9-27.3) [.71]	25.2 (20.4-30.0) [.05]
	>50th centile	21.3 (16.1-26.4)	18.6 (13.9-23.3)

NTCD: non T-cell depleted, TCD: T-cell depleted. *P* values estimated using *t* test.

CD8+ cell doses and neutrophil recovery (**Table 3**). In the NTCD group, univariate analysis did not show any association between cell doses and neutrophil engraftment.

For the evaluation of platelet recovery, 14 patients did not recover their counts and 19 patients had a nadir above 20×10^9/L. Median time to platelet recovery was 18 days in both groups, (mean NTCD group 22 days, 95%CI, 18.5-25.4 and mean TCD group 21.8 days, 95%CI 18.4-25.3, *P*=.95). Univariate analysis was suggestive of earlier platelet engraftment in the TCD group with higher (>50th centile) doses of CD3+, CD4+ and CD8+ cell doses (**Table 3**). However, multivariate analysis showed no association between platelet recovery and higher infused cell doses for CD3+ (*P*=.62), CD4+ (*P*=.29) and CD8+ (*P*=.68) in the TCD group.

Seven patients who were deemed to have primary graft failure were excluded in the acute GVHD analysis. The incidence of grade II-IV acute GVHD in the NTCD and TCD groups was 51.9% (n=27) and 44.4% (n=40) respectively (RR=0.87, 95% CI=0.62-1.21; *P*=.49). The incidence of grade III-IV acute GVHD in the NTCD and TCD groups was 19.2% (n=10) and 18.9% (n=17) respectively (RR = 0.99, 95% CI = 0.84-1.18; *P*=1.0). Univariate analysis did not show any significant association between CD34+, CD3+, CD4+ and CD8+ cell doses and rates of grade II-IV and grade III-IV acute GVHD, precluding a multivariate analysis (**Table 4**).

One hundred and twenty patients who survived until 100 days post-transplant were considered eligible for the analysis. The incidence of chronic GVHD in the NTCD and TCD groups was 55.6% (n=25) and 52% (n=39) respectively (RR 1.07, 95%CI 0.76-1.50, *P*=.85). Rates of extensive chronic GVHD in the NTCD and TCD group was 46.7% (n=21) and 36% (n=27) respectively (RR 1.29, 95%CI 0.84-2.0, *P*=.26). On univariate analysis (**Table 4**) no correlation was seen in the incidence of chronic GVHD and cell doses in the TCD group. In the NTCD group no association existed between CD3+, CD4+ and CD8+ cell doses and chronic GVHD and extensive chronic GVHD, but an increased incidence of chronic GVHD was noted with lower CD34+ cell doses; 70.4% (n=19) vs. 33.3% (n=6), (RR 0.47, 95% CI 0.24-0.95, *P*=.03). Univariate analysis also showed a trend towards increased extensive chronic GVHD with lower CD34+ cell dose in the NTCD group; 59.2% (n=16) vs. 27.8% (n=5) (RR 0.47, 95%CI 0.21-1.10, *P*=.06). A multivariate model was not constructed because no significance was noted with any other cell subtype.

Median follow-up for surviving patients was 3 years (1071 days, range: 146-2394 days). The OS in the NTCD and TCD groups at 1 year was 51.8% and 54.7%, respectively, and at 3 years was 38.4% and

Table 4. Univariate analysis comparing the incidence of grades II-IV and III-IV acute graft versus host disease (GVHD) and chronic and extensive chronic GVHD by cell doses (< and > 50th percentile) in the non T-cell depleted (NTCD) and T-cell depleted (TCD) groups.

Grade II-IV acute GVHD	NTCD group RR (95% CI); [*P*]	TCD group RR (95% CI); [*P*]
CD34+	1.44 (0.74-2.82); [.40]	0.93 (0.66-1.33); [.83]
CD3+	0.67 (0.35-1.26); [.26]	0.97 (0.68-1.40); [1.00]
CD4+	0.72 (0.44-1.32); [.41]	1.08 (0.72-1.61); [.83]
CD8+	0.74 (0.41-1.35); [.40]	1.16 (0.79-1.71); [.52]
Grade III-IV acute GVHD		
CD34+	0.60 (0.31-1.14); [.28]	1.28 (0.75-2.19); [.42]
CD3+	1.43 (0.54-3.79); [.49]	1.25 (0.73-2.15); [.42]
CD4+	1.18 (0.54-2.58); [.73]	1.14 (0.66-1.96); [.79]
CD8+	1.07 (0.48-2.38); [1.00]	1.67 (0.85-3.27); [.11]
Chronic GVHD		
CD34+	0.47 (0.24-0.95) [.03]	1.13 (0.72-1.77) [.64]
CD3+	1.02 (0.59-1.74) [1.0]	1.41 (0.87-2.28) [.17]
CD4+	0.91 (0.53-1.56) [.77]	0.97 (0.63-1.50) [1.0]
CD8+	1.17 (0.67-2.03) [.76]	0.97 (0.62-1.49) [1.0]
Extensive chronic GVHD		
CD34+	0.47 (0.21-1.10) [.06]	0.88 (0.48-1.61) [.81]
CD3+	1.47 (0.76-2.81) [.36]	1.67 (0.84-3.32) [.15]
CD4+	1.37 (0.70-2.68) [.38]	1.15 (0.63-2.12) [.81]
CD8+	0.98 (0.51-1.88) [1.0]	1.21 (0.65-2.24) [.63]

RR: relative risk, CI: confidence interval. *P* values estimated using the Fisher exact test.

Table 5. Univariate analysis showing overall survival, progression free survival and non-relapse mortality in non T-cell depleted (NTCD) and T-cell depleted (TCD) group, illustrating the impact of higher cell doses (> 50th percentile).

Overall survival	NTCD group RR (95% CI); [P]	TCD group RR (95% CI); [P]
CD34+	0.96 (0.48-1.85) [.91]	0.63 (0.38-1.04) [.07]
CD3+	0.98 (0.50-1.90) [.95]	0.52 (0.31-0.87) [.01]
CD4+	1.38 (0.71-2.72) [.34]	0.66 (0.39-1.1) [.12]
CD8+	0.84 (0.43-1.62) [.61]	0.54 (0.32-0.9) [.02]
Progression free survival		
CD34+	1.07 (0.56-2.00) [.83]	0.64 (0.39-1.06) [.08]
CD3+	0.97 (0.51-1.81) [.91]	0.56 (0.34-0.92) [.02]
CD4+	1.27 (0.68-2.43) [.45]	0.63 (0.36-1.04) [.07]
CD8+	0.91 (0.48-1.69) [.76]	0.60 (0.36-0.98) [.04]
Non relapse mortality		
CD34+	0.73 (0.28-1.73) [.49]	0.49 (0.24-0.99) [.04]
CD3+	1.44 (0.61-3.53) [.41]	0.51 (0.25-1.01) [.05]
CD4+	1.56 (0.66 – 3.94) [.33]	0.66 (0.33-1.31) [.24]
CD8+	0.93 (0.39-2.23) [.96]	0.42 (0.20-0.85) [.04]

RR: relative risk, CI: confidence interval. *P* values calculated using log-rank test.

39.7%, respectively (log-rank *P*=.86). Univariate analysis showed a significant association between CD34+, CD3+ and CD8+ cell doses and OS in the TCD group (**Table 5**).

On multivariate regression analysis, only CD34+ cell dose >5.8×10^6/kg PW (median) was associated with improved OS (RR=0.58, 95% CI=0.34-0.98, *P*=.04) in the TCD group (**Figure 1**). Higher CD3+ cell dose, >30.8×10^7/kg PW, showed a trend towards improved OS (RR= 0.54, 95% CI=0.29-1.01, *P*=.053).

The PFS in the NTCD and TCD groups at 1 year was 46.2% and 45.1% respectively and at 3 years was 28.9% and 34.2% respectively (log-rank *P*=.7). Univariate analysis showed a significant association with higher CD34+, CD3+, CD4+ and CD8+ cell doses and PFS in the TCD group; *P*<.1 (**Table 5**). However, multivariate analysis suggested improved PFS with higher CD34+ cell doses only (RR 0.59, 95% CI=0.35-1.00, *P*=.05). The 1-year cumulative incidence of NRM in the NTCD and TCD groups was 30% and 25%, respectively, and at 3 years it was 35% and 32%, respectively (Gray test *P*=.64). By univariate analysis, in the TCD group higher CD34+, CD3+ and CD8+ cell doses was associated with improved NRM (**Table 5**). In multivariate analysis statistical significance was seen only with CD34+ cell dose >5.8×10^6/kg PW, (RR 0.49, 95%CI 0.24-0.99, *P*=.048). Relapse in the NTCD and TCD groups at 1 year was 26% and 31% respectively, and at 3 years was 35% for both groups, *P*=.95. By univariate analysis there was no difference in relapse rates between the NTCD and TCD groups stratified by cell doses (*P*>.1).

DISCUSSION

In this study, we analyzed the effects of cellular graft composition on transplantation outcomes of patients receiving either T-cell replete or T-cell depleted peripheral blood AHCT. Interesting observations were made in patients undergoing AHCT with in vivo

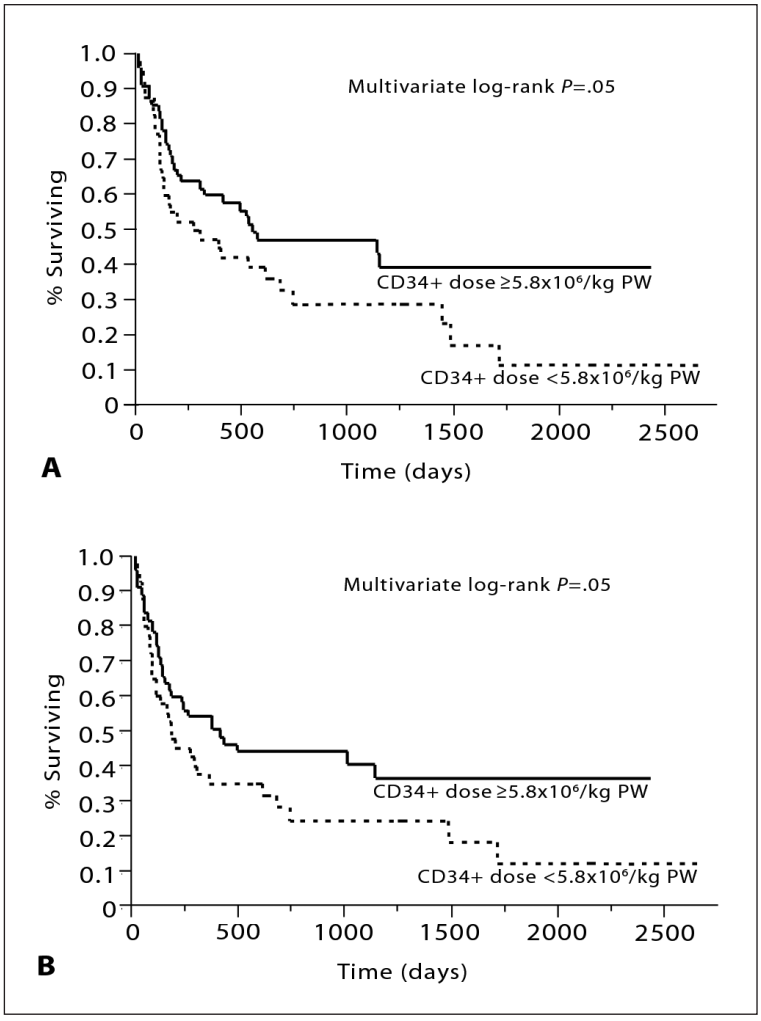

Figure 1. Kaplan-Meir estimate of overall survival and progression free survival by CD34+ cell doses in the T-cell depleted (TCD) group. The bold line represents CD34+ cell dose >50th centile and the broken line represents <50th centile; median cell dose = 5.8×10^6/kg patient weight (PW). A. OS: risk ratio 0.58, 95% confidence interval 0.34-0.98; log-rank *P*=.04. B. PFS: risk ratio 0.59, 95% confidence interval 0.35-1.00; *P*=.05.

T-cell depletion (TCD group). Multivariate analysis showed that higher CD34+ cell dose (>5.8×10^6/kg PW) improved OS, PFS and NRM, but had no impact on disease relapse. Although there was no association between infused cell doses and engraftment kinetics in multivariate analysis, univariate analysis showed early neutrophil engraftment with higher CD34+ cell dose. Univariate analysis was suggestive of better platelet engraftment with higher CD3+, CD4+ and CD8+ cell doses in the TCD group without any impact from the CD34+ cell dose.

Great interest exists in understanding the effects of cellular allograft composition on clinical outcomes of patients undergoing AHCT. The ideal CD34+ cell dose infused to optimize survival outcomes of peripheral blood AHCT remains a matter of controversy. While some studies have not reported any impact on survival with higher infused CD34+ cell doses,[10,29] others have shown improved OS with higher CD34+ cell doses in URD peripheral blood AHCT.[5,8] Collins et al have suggested improved OS following peripheral blood URD transplantation in patients receiving CD34+ cell doses >5×10^6/kg.[8] Similarly, Pulsifer et al have shown improved OS with URD peripheral blood transplantation with CD34+ cell doses between 4.5-9.5×10^6/kg.[5] Interestingly this beneficial effect was lost in patients receiving doses greater than 9.5×10^6/kg. These findings underscore the need to better define the optimal CD34+ dose for patients receiving peripheral blood allografts.

The optimal infused CD34+ cell dose for patient receiving in vivo TCD allografts is even more controversial, with no data (to our knowledge) assessing the relative importance of infused doses in T-cell replete versus T-cell depleted setting. Kalwak et al reported improved OS and disease-free survival in pediatric patient population with CD34+ cell dose >10×10^6/kg, specifically with in vivo TCD as GVHD prophylaxis.[15] In contrast Tsirigotis et al, in a study which included both adult and pediatric patients receiving ATG as part of transplant conditioning reported no effect on OS of CD34+ doses >10×10^6/kg. This observation is in line with the finding from Pulsifer et al where CD34+ cell doses greater than 9.5×10^6/kg had no beneficial effect on survival outcomes.[5] Interestingly, in our study the benefit of higher CD 34+ cells doses (doses >5.8×10^6/kg PW) in terms of improved OS, PFS and NRM was restricted to patients receiving TCD allografts. The survival benefit in the TCD group with higher CD34+ cell dose may be due to a more robust immune reconstitution leading to decreased NRM rates.

Our data did not show any difference in relapse rates between the NTCD and TCD group and no statistically significant association with CD34+, CD3+, CD4+ and CD8+ cell doses, which is contrast with previous reports showing decreased relapse rates with higher infused CD34+ cell dose.[9,29] Lower NRM seen in the TCD group with higher CD34+ cell doses seen in our study has been reported in patients undergoing NTCD transplants.[5]

Our analysis did not show any difference between the TCD and NTCD groups with regards to neutrophil engraftment and platelet recovery in the multivariate model. It must be noted however that univariate analysis suggested faster neutrophil engraftment with higher CD34+ cell doses. Interestingly, in the TCD

group univariate analysis was also suggestive of faster platelet recovery with a higher T-cell dose. A prior study has also shown early platelet engraftment with higher CD8+ (>8×10^7/kg) cell doses without affecting neutrophil recovery.[8]

The association between cell doses, CD34+ in particular and incidence of GVHD has been controversial. Several studies have shown increased incidence of acute and chronic GVHD with higher CD34+ cell doses.[4,9,11,12] An explanation for this interesting finding remains a mystery. In an elegant study, Reisner et al showed that donor CD34+ cells can act as 'veto' cells and may suppress host T-cells capable of donor alloreactivity. Thus, theoretically, infusion of large numbers of donor CD34+ cells can eliminate 'host-versus-graft' reaction, turning the balance in favor of the 'graft-versus-host' reaction, leading to increased incidence of GVHD.[30,31] In our study, we did not see any association between CD34+ cell dose and acute GVHD in the NTCD and TCD groups. These results are in concordance with several recent reports. There was no association between incidence of chronic GVHD to CD34+, CD3+, CD4+ and CD8+ cell doses in the TCD group, while in the NTCD group; lower infused CD34+ cell dose was associated with increased chronic extensive GVHD, contrary of previous reports.[4,9] This finding is indeed perplexing and we have no satisfactory explanation for it. It is likely due to the nature of the analysis (retrospective), and the low number of patients.

The rates of acute GVHD in the TCD group in our study appear to be higher than the recent report by Socie et al.[32] However it is important to point out that majority of patients in our study received TCD with Thymoglobulin, unlike Socie et al's study were ATG-Fresenius was employed. Our acute GVHD rates in fact are very similar to a recent CIBMTR study, where rates of grade II-IV acute GVHD in the Thymoglobulin group was 38%.[33] Moreover a significant proportion of TCD group patients were also HLA mismatched with their donors, further explaining the incidence of acute GVHD in our study. In a prospective study Bacigalupo et al, patients receiving ATG doses of 7.5 mg/kg had grade III-IV acute GVHD rates of 36%. The ATG dose used in our study was lower (6 mg/kg), which is another likely explanation of slightly higher acute GVHD rates in our experience.[34]

Similarly the disparities noted between our study and the report by Socie et al in terms of rates of chronic GVHD are likely reflective of various ATG preparations used in these studies (Thymoglobulin vs. ATG-Fresenius) with differing biological activities, divergent biologically active doses, different administration schedules, differences ntensity of conditioning regimens (as indicated in CIBMTR data) and the degree of patient/donor HLA match in the two studies studies.[32,33] The relevance of such biological differences between various ATG types has been recently reviewed.[35]

Our retrospective analysis is limited by the heterogeneity of the patient population, with the TCD group having more high-risk patients and URD transplants. Acknowledging these limitations, our report is suggestive of improved survival and NRM with higher CD34+ cell doses without increasing the risk of GVHD and relapse, in patients undergoing in vivo T-cell depleted AHCT. The improved OS seen with higher CD34+ cell dose (>5.8×10^6/kg PW) in TCD transplants is likely due to improved rates of NRM. We hypothesize that the improved NRM and OS in TCD transplant might be due to a more robust immune reconstitution with higher cell doses thereby reducing infectious complication otherwise associated with TCD.

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Conflict of interest

None

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