TITLE

Impact of T-cell dose on the outcome of T-cell replete HLA matched allogeneic peripheral blood stem cell transplantation.

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ABSTRACT:

Background: Data on whether T-cell dose of allogeneic peripheral blood stem cell (PBSC) product influences transplant outcome are conflicting.
Methods: Using CIBMTR database, we identified 2,736 adult patients who underwent first allogeneic peripheral blood stem cell (PBSC) transplant for acute leukemia (AML, ALL) or myelodysplastic syndrome (MDS) between 2008-2014 using an HLA-matched sibling donor (MSD) or 8/8-matched unrelated donor (MUD). We excluded *ex-vivo* and *in-vivo* T-cell depleted transplants. Correlative analysis was performed between CD3+ T-cell dose and risk of graft-versus-host-disease (GVHD), relapse, non-relapse mortality (NRM), disease free survival (DFS) and overall survival (OS).
Results: Using maximum likelihood estimation method, we identified CD3+ T-cell cell dose cutoff that separated risk of acute GVHD (aGVHD) grade II-IV in both MSD and MUD groups. A CD3+ T-cell dose cutoff of 14 $x10^7$ cells/kg identified MSD/low CD3+ (n=223) and MSD/high CD3+ (n=1214), and a dose of 15 $x10^7$ cells/kg identified MUD/low CD3+ (n=197) and MUD/high CD3+ (n=1102). With univariate analysis, MSD/high CD3+ group had higher cumulative incidence of day 100 aGVHD grade II-IV of 33% vs 25% when compared to MSD/low CD3+ group (P value =0.009). There was no other difference between both groups in engraftment rate, risk of aGVHD grade III-IV or chronic GVHD (cGVHD), NRM, relapse, DFS, or OS. MUD/high CD3+ group had higher cumulative incidence of day 100 aGVHD grade II-IV of 49% vs 41% when compared to MUD/low CD3+ group (P value =0.04). There was no other difference between both groups in engraftment rate, risk of severe aGVHD or cGVHD, NRM, relapse, DFS, or OS. Multivariate analysis of MSD and MUD groups failed to show an association between CD3+ T-cell dose and risk of either aGVHD grade II-IV (p value =0.1 and 0.07 respectively) or cGVHD (p value=0.8 and 0.3 respectively). Sub-analysis of CD4, CD8 and CD4/CD8 ratio failed to identify cutoff values predictive of transplant outcome. Using log-rank test, the sample size was, however, suboptimal to identify difference at these cutoff cell dose.
Conclusion: In this registry study, CD3+ T-cell dose of PBSCT product did not influence risk of aGVHD or cGVHD or other transplant outcomes when using HLA-matched sibling or 8/8 unrelated donors. Subset analysis of CD4+ and CD8+ T-cell dose was not possible for small sample size.

INTRODUCTION

Allogeneic hematopoietic cell transplant (HCT) when performed for hematologic malignancies, relies on both conditioning regimen as well as the immunotherapy exploiting the graft versus tumor (GVT) effect, which is primarily derived from donor immune effector cells.[1, 2] A complex interplay between the immune effector cells including antigen presenting cells, CD3 + cells (CD4+ T cells, CD8+ T cells, regulatory T cells (T regs)), and natural killer (NK) cells is responsible for both the GVT and the graft-versus-host-disease (GVHD)[3], among which the most well-studied cells are the CD3+ T-cells.

Though the CD3+ T-cells can exert a strong GVT[4], the risk of aGVHD also rises with a higher dose as demonstrated by both observational and prospective studies.[5, 6] T-cell depleted (TCD) allogeneic HCT have led to a decreased risk of GVHD but at an expense of increasing the risk of relapse, as demonstrated by some trials in both *ex-vivo*[7] and *in-vivo* depletion[8]. The higher risk of GVHD in peripheral blood stem cell (PBSC) graft compared to the bone marrow (BM) source is apparent from both observational studies[9] and clinical trials[10] as the PBSCs are known to carry 10-15 times the quantity of CD3+ T-cells comparatively.[11] Thus many attempts have been made to separate out the GVT from GVHD which include utilizing CD34+ selection[12], naïve T-cell depletion[13], post-transplant cyclophosphamide[14], microtransplantation[15] and NK-cell graft engineering. Few single center studies have evaluated the role of CD3+ T-cell dose with respect to both relapse and GVHD outcomes post-HCT, however, these studies varied significantly in the selection criteria with no consensus on an optimal CD3+ T-cell dose cutoff value.[16-19] A recent large registry study indicated that in HCTs utilizing unrelated donors, the CD3+ and CD34+ doses were significantly associated with an increased risk for grade III-IV aGVHD (hazard ratio [HR] = 3.6; 95% CI: 1.45-9.96, P = .006 and 2.65 (95% CI: 1.07-6.57), P = .04,

respectively).[20] Since the studies mentioned above have used different types of donors, different diseases, and different conditioning regimens, optimum cut-offs for the CD3+ T-cell dose which can potentially avoid GVHD while still promote GVT, are unknown.

We hypothesized that there exists a T-cell dose range that promotes GVT while levels above this range result in higher risk of both severe acute and chronic GVHD with subsequent increased non-relaspe mortality (NRM).

MATERIALS and METHODS

Data sources

The Center for International Blood and Marrow Transplant Research (CIBMTR) is a working group of more than 420 transplantation centers worldwide that contribute detailed data on HCT to a statistical center at the Medical College of Wisconsin. Participating centers are required to report all transplantations consecutively; patients are followed longitudinally and compliance is monitored by on-site audits. Computerized checks for discrepancies, physicians' review of submitted data, and on-site audits of participating centers ensure data quality. Observational studies conducted by the CIBMTR are performed in compliance with all applicable federal regulations pertaining to the protection of human research participants. Protected Health Information used in the performance of such research is collected and maintained in CIBMTR's capacity as a Public Health Authority under the HIPAA Privacy Rule. The Institutional Review Boards of the Medical College of Wisconsin and the National Marrow Donor Program approved this study. The CIBMTR collects data at two levels: Transplant Essential Data (TED) and Comprehensive Report Form (CRF) data. TED level data include disease type, age, gender, pre-HCT disease stage and chemotherapy-responsiveness, date of diagnosis, graft type (bone

marrow- and/or blood-derived stem cells), conditioning regimen, post-transplant disease progression and survival, development of a new malignancy, and cause of death. All CIBMTR centers contribute TED data. More detailed disease and pre- and post-transplant clinical information are collected on a subset of registered patients selected for CRF data by a weighted randomization scheme. TED and CRF level data are collected pre-transplant, 100 days, and six months post-HCT and annually thereafter or until death. Data for the current analysis were retrieved from CIBMTR (TED and CRF) report forms.

Patients

We analyzed data of adult (≥18 years) patients who underwent first allogeneic HCT for acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or myelodysplastic syndrome (MDS) between 2008 and 2014 with PBSC using HLA-identical sibling donor (MSD) or 8/8-matched unrelated donor (MUD) matched at the allele-level at HLA-A, -B, -C and -DRB1. We limited the disease types to AML, ALL, and MDS hypothesizing that these patients have comparable risk of relapse and susceptibility to GVT effect. We excluded *ex-vivo* (TCD and CD34 selected grafts) and *in-vivo* TCD (antithymoglobulin or alemtuzumab) HCT. All patients had available CD3+ T-cell dose, however, some patients were missing CD4+ T-cell and/or CD8+ T cell dose.

Definitions of endpoints

For overall survival (OS), death from any cause was considered an event and surviving patients were censored at last contact. For disease-free survival (DFS), either progression/relapse or death from any cause was considered an event while patients alive without evidence of disease relapse/progression were censored at last follow-up. Non-relapse mortality (NRM) was defined as death without evidence of primary disease progression/relapse with the latter event considered

a competing risk. AGVHD and cGVHD were graded using standard criteria[21, 22] Neutrophil recovery was defined as the first of 3 successive days with absolute neutrophil count (ANC) ≥500/μL after post-transplantation nadir. Platelet recovery was defined as the first of 3 successive days with platelet counts ≥20,000/μL without transfusion support for at least 7 days. Data are censored for mortality events before neutrophil recovery.

Statistical analysis

The primary objective of the study was to correlate the graft T-cell dose with the incidence and grade of aGVHD and cGVHD, OS, DFS, relapse and NRM following PBSC HCT in matched sibling and 8/8 matched URD HCT. In a subset analysis for subjects with available CD4+, CD8+ T-cell doses, we also tested for association of the graft T-cell subset dose and the ratio of CD4+/CD8+ T-cell and these transplant outcomes in univariate analysis only due to smaller sample size. T-cell dose cutoff values were determined using maximum likelihood method based on Cox proportional hazards model for aGVHD grade II-IV endpoint.

Categorical data were summarized using frequencies while continuous data were summarized using medians and ranges. Probabilities of DFS and OS were calculated as described previously.[23] Cumulative incidence of aGVHD grade II-IV, aGVHD grade III-IV, cGVHD, NRM, relapse/progression, platelet recovery and hematopoietic recovery were calculated to accommodate for competing risks.[24] Associations among patient-, disease-, and transplantation-related variables and outcomes of interest were evaluated using Cox proportional hazards regression. All the clinical variables were tested for the affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted through stratification. Then a stepwise forward model selection procedure was used to select adjusted clinical variables for each outcome with a threshold of 0.05 for both entry and stay in the model.

Interactions between T-cell dose and the adjusted clinical variables were examined and no significant interactions were detected. Center effect was adjusted as a random factor for all outcomes.[25] The significance level of 0.01 was used for the overall effects of factors followed by Bonferroni adjustment for pairwise comparisons to account for multiple testing. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC)

RESULTS:

Baseline Characteristics

We identified 2,736 adult patients who met the selection criteria described above. Regimen intensity as myeloablative (MAC), reduced intensity (RIC), or non-myeloablative (NMA)was defined as previously described.[26] Based on Cox proportional hazards model, we detertmined the cutoff value for CD3+ T-cell dose and separated each group (MSD and MUD) into low and high risk of grade II-IV aGVHD. These were 14 $x10^7$ cells/kg and 15 $x10^7$ cells/kg for MSD and MUD groups respectively. Then, patients were divided into 4 groups based on the donor type (MSD or MUD) and T-cell dose cutoff values. The 4 groups were MSD/low CD3+ (n= 223), MSD/high CD3+ (n= 1214), MUD/low CD3+ (n= 197), MUD/high CD3+ (n= 1102). Median CD3+ T-cell dose were 11 and 29 (x 10^7) in the MSD/Low and MSD/High groups, respectively, and 10 and 28 ($x10^7$) in the MUD/Low and MUD/High groups respectively. MSD and MUD groups were analyzed separately. The baseline patient-, disease- and transplantation-related characteristics are shown in **Tables 1 and 2.**

Matched sibling donor (MSD) groups

Univariate analysis showed cumulative incidence of aGVHD grade II-IV at day +100 of 25% (95% CI [confidence interval]: 19-31) and 33% (95% CI: 30-36) in MSD/low CD3+ and MSD/high CD3+ respectively (p = 0.009). However, there was no difference in the risk of

aGVHD grade III-IV (p= 0.4). Likewise, risk of cGVHD at 2 years, NRM, relapse, DFS, and OS were not shown to be statistically different. There was also no difference in the day 100 engraftment rate between both groups.

In multivariate analysis, CD3+ T-cell dose did not influence aGVHD (II-IV, and III-IV) **(Table 3),** cGVHD, relapse, NRM, DFS, or OS **(Supplemental Table 1)**. However, aGVHD grade II-IV risk was higher with any gender mismatch (p = 0.02 and 0.009 for female-male and male-female, respectively). Risk of severe aGVHD grade III-IV was worse among patients with lower Karnofsky Performance Status (KPS) (<90) relative to KPS 90-100 (p = 0.005). Risk of cGVHD was worse in patients older than 29 years old (overall p = 0.006), with female donors (p = <0.002), and in transplant done before 2011 (p = 0.01). DFS was worse among older patients (≥60 years old) (p = 0.01), high/very high Disease Risk Index (DRI) (p <0.0001), and lower KPS (p = <0.0001)]. OS was worse among high/very high DRI (p = 0.0001), lower KPS (p = <0.0001), and HCT-CI >3 (p = 0.003). Non-relapse mortality was worse among MDS (p = 0.002), lower KPS (p = 0.007), and HCT-CI >3 (p = 0.0006). Relapse risk was worse among patients with advanced disease prior to transplant (p = 0.0007), and lower KPS (p = 0.002).

Subset analysis of CD4, CD8 and CD4/CD8 ratio was available only in limited number of patients. No significant association of these variables were detected for aGVHD, cGVHD, NRM, relapse, DFS, or OS. Likewise, CD34+ cell dose was also not significantly associated with any of the transplant outcomes.

Matched unrelated donor (MUD) groups

Univariate analysis showed cumulative incidence of aGVHD grade II-IV at day +100 of 41% (95% CI: 35-48) and 49% (95% CI: 46-52) in MUD/low CD3+ and MUD/high CD3+ respectively (p = 0.04). However, there was no difference in the risk of aGVHD grade III-IV (p=

0.9). Likewise, risk of cGVHD at 2 years, NRM, relapse, DFS, and OS were not statistically different. There was also no difference in the day 100 engraftment rate between both groups.

In multivariate analysis, CD3+ T-cell dose did not influence risk of aGVHD (II-IV, and III-IV) **(Table 4),** cGVHD, relapse, NRM, DFS, or OS **(Supplemental Table 2).** However, aGVHD grade II-IV risk was higher among patients who received myeloablative regimens (P = 0.02). Risk of severe aGVHD grade III-IV was worse among underweight patients (p = 0.01), and with older donors (>32 years old) (p = 0.01). Risk of cGVHD was less in patients with ALL (p = 0.003), and in transplant done after 2010 (p = 0.0003). DFS was worse with older donors (>50 years old) (p = 0.0001), and high/very high DRI (p < 0.0003). OS had a worse outcome among older patients (≥50 years old) (p = 0.008), older donor (≥50 years old) (p = 0.0001), high/very high DRI (p = 0.0005), and lower KPS (p = <0.009). Non-relapse mortality was worse with older donor (>50 years old) (p = 0.0006). Relapse risk was worse among patients with high/very high DRI (p = 0.0002). Subset analysis of CD4, CD8 and CD4/CD8 ratio was available only in limited number of patients. No significant association of these variables were detected for aGVHD, cGVHD, NRM, relapse, DFS, or OS. Likewise, CD34+ cell dose was also not significantly associated with any of the transplant outcomes.

DISCUSSION

This study demonstrated no association of the CD3+ T-cell dose of PBSC graft and risk of acute or chronic GVHD, nor did it influence the risk of relapse in the cohort. Nonetheless, the subgroup analyses project certain associations worth exploring further prospectively. Although the univariate analysis showed a correlation between the CD3+ T-cell dose and the risk of aGVHD in both the MSD and the MUD groups, the multivariable analysis failed to prove such an association. It is possible that the subgroups selected for multivariate analysis were not large

enough to power a detection in difference in the binary outcome (presence or absence of grade II-IV aGVHD) thus leading to the possibility of type II error. It is also possible that the variables chosen in the univariate analysis did not include some potential risk factors of aGVHD (e.g. inadequate information on CD4+, CD8+, CD56+ cells, and dendritic cells in the PBSC graft). The only group with increased risk of aGVHD grade II-IV (on multivariate analysis) was patients who underwent MUD HCT using MAC regimens. This is consistent with the previous CIBMTR study that showed that among MUD HCT, RIC regimens were associated with decreased risk of aGVHD.[27]

Our data contrasts with the European Society of Blood and Marrow Transplant (EBMT) study of MUD HCT that showed that CD3+ T-cell dose >35 × 10^7/kg to be associated with higher risk of aGVHD.[20] This discrepancy may be attributed to difference in median CD3+ T-cell doses in PBSC grafts in both studies, and the statistical methodology used for categorization of the primary outcome variable (CD3+ T-cell dose was categorized by interquartile range in the EBMT study, whereas in the current study we used a cutoff values of CD3 T-cell dose based on the differential risk of aGVHD grade II-IV). Moreover, EBMT study included TCD allogeneic HCT, whereas the current study excluded it. Additionally, some of the conditioning regimens used in the EBMT study were not evaluated in the current study. It is worthy noting that the BMT CTN0201 trial has also failed to show an association of the T-cell dose of the PBSC graft with survival or GVHD in patients with AML or MDS.[28] A single instiurion study using bone marrow (rather than PBSC) graft has demonstrated a paradoxical increase of risk of cGVHD with lower CD3+ T-cell dose in a subset of patients who received myeloablative busulfan/cyclophosphamide regimen (p=0.006).[29]

Due to the limited sample size of our cohort, further analysis was not possible in order to detect outcome differences based on T cell phenotypic subsets; CD4+, CD8+, or their ratio. However, transplant outcome may depend on functional T cell subsets; naïve T cells, effector T cells, and/or central memory T cells. In particular, depletion of naïve T cells (either CD4+ or CD8+) was associated with less risk of cGVHD and more likelihood of steroid-responsive aGVHD in small phase II study.[13] T_{reg} (regulatory T cells: CD4+/CD25+/FOXP3+) another small subset of CD4 has been shown to ameleriorate cGVHD.[30] Unbalanced recovery of T_{reg} and effector T cells after transplant has been also correlated with risk of cGVHD.[31]

Though, a PBSC graft includes a co-infusion of both CD34+ and CD3+ T-cells in HCTs, the dose of CD3+ is not evaluated routinely in most transplant centers since it continues to be controversial. Farhan *et al.* retrospectively evaluated the CD3+ T-cell dose in both MUD and MSD HCTs, and found no significant correlation with aGVHD, however, they observed that the OS was significantly affected by a higher dose of CD3+ (mean dose 12 x 10^7/kg) in their cohort.[32] This CD3+ T-cell dose differs from the dose in our cohort and the EBMT cohort, thus perhaps contributing to different outcome.

Although, our analysis did not show an impact of CD34+ cell dose on transplant outcome, it is worth noting that most of patients in our cohorts (more than 50%) received CD34+ cell dose of 4-8 x10*6 cells/kg and minority (5-10%) received a dose <2 x10*6 cells/kg (tables 1 and 2). In our opinion, this precludes an accurate conclusion on the impact of CD34+ cell dose on transplant outcome. Prior studies have evaluated this question with favorable outcome with higher CD34+ cell dose[33-35] albeit observing higher risk of cGVHD with CD34+ cell dose >8 x10*6 cells/kg,[35, 36] or >10 x10*6 cells/kg.[37]

Donor age group was found to be a risk factor for the development of severe aGVHD and for a worse DFS (donor age > 50 years) in MUD HCT. The effect of donor age on the clinical outcomes is similar to another study,[16] where there was a correlation of donor's age and the CD8+ content of the PBSC graft. Given the limited availability of CD8+ dose in the PBSC grafts in our cohorts, we could not assess this association with age. This study was congruent with other large studies for results pertaining to well known risk factors for GVHD, e.g. older recipient age[38], and a lower KPS[39]. Expectedly, a higher DRI predicted greater risk of relapse in both MUD and MSD groups.[40]

Strength of our study lies in a large sample size in both the MUD and MSD groups, which allowed us to categorize the entire cohort into 4 groups based on the donor and the CD3+ T-cell dose in the PBSC graft. Another strength of the study was the availability of comprehensive data on both the transplant (including both MAC and RIC/NMA regimens) and disease associated risk factors (in the 3 disease types selected for the study), and a long median follow-up of 4 years (49 months for MSD, 47 months for MUD).

To our knowledge, this is the largest study addressing the question of impact of T-cell content of PBSC grafts on transplant outcomes. In this registry study, the CD3+ T-cell dose in the PBSCT product did not influence the risk of aGVHD or cGVHD or other transplant outcomes when using HLA- matched sibling or 8/8 unrelated donors. Prospective studies are needed to determine whether T-cell subsets; CD4+, CD8+, T_{reg}, or naïve T-cell content of the allografts have meaningful influence on transplant outcome. Results of the ongoing phase II clinical trial using standardized CD3+ T cell dose with HLA-matched related PBSC transplant is awaited (NCT00959140). Additionally, in the current era of post-transplant cyclophosphamide (PTCy) for prevention of GVHD, it may be imperative to assess the impact of these T-cell subsets in

haploidentical and HLA-matched HCT. Interestingly, a multicenter study has indeed indicated an increased risk of all grade cGVHD with an elevated CD3+ T-cell dose with haploidentical PBSC HCT using post-transplant cyclophosphamide.[41] CD3 T-cell dose has also been shown to be predictive of graft failure with TCD allogeneic HCT.[42]

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Table 1. Characteristics of adult patients undergoing first allogeneic HCT for AML, ALL, and MDS between 2008-2014 with PBSC from an HLA-identical sibling donor with valid CD3+ cell dose data, as reported to the CIBMTR.

CD3+ T-cell dose	**< 14 x10^7**	**≥ 14 x10^7**
Number of patients	**223**	**1214**
Number of centers	58	95
Recipient Age		
Median (range)	51 (18-71)	54 (18-78)
18-29	20 (9)	110 (9)
30-39	28 (13)	130 (11)
40-49	55 (25)	232 (19)
50-59	74 (33)	419 (35)
60+	46 (21)	323 (27)
Recipient gender		
Male	123 (55)	694 (57)
Female	100 (45)	520 (43)
Recipient race		
Caucasian	176 (79)	1057 (87)
Non-Caucasian	37 (17)	117 (10)
Missing	10 (4)	40 (3)
Body mass index		
Median (range)	29 (18-62)	27 (15-56)
Underweight (<18.5)	2 (<1)	25 (2)
Normal (18.5-<25)	52 (23)	366 (30)
Overweight (25-<30)	67 (30)	433 (36)
Obese (≥30)	101 (45)	390 (32)
Missing	1 (<1)	0
Karnofsky performance status		
< 90	92 (41)	478 (39)
90-100	125 (56)	718 (59)
Missing	6 (3)	18 (1)
Sorror co-morbidity index		
0-1	101 (45)	560 (46)
2-3	68 (30)	382 (31)
4+	51 (23)	261 (21)
Missing	3 (1)	11 (<1)
Disease		
AML	137 (61)	640 (53)
ALL	33 (15)	224 (18)
MDS	53 (24)	350 (29)

CD3+ T-cell dose	**< 14 x10^7**	**≥ 14 x10^7**
Disease status		
AML	137	640
Early	87 (64)	386 (60)
Intermediate	21 (15)	107 (17)
Advanced	29 (21)	147 (23)
ALL	33	224
Early	17 (52)	167 (75)
Intermediate	7 (21)	37 (17)
Advanced	9 (27)	20 (9)
MDS	53	350
Early	35 (66)	228 (65)
Intermediate	17 (32)	104 (30)
Advanced	1 (2)	18 (5)
Revised Disease Risk Index (DRI)		
AML	137	640
Low	8 (6)	41 (6)
Intermediate	90 (66)	355 (55)
High/Very high	25 (18)	135 (21)
Missing	14 (10)	109 (17)
ALL	33	224
Intermediate	17 (52)	167 (75)
High/Very high	16 (48)	57 (25)
MDS	53	350
Intermediate	29 (55)	194 (55)
High/Very high	12 (23)	81 (23)
Missing	12 (23)	75 (21)
Time from diagnosis to transplant, months		
Median (range)	6 (1-156)	5 (<1-279)
< 6	121 (54)	695 (57)
6 - < 12	52 (23)	257 (21)
≥ 12	50 (22)	262 (22)
CD3+ cell dose, x 10^7/kg, median (range)	11 (3-14)	29 (14-113)
CD4+ cell dose, x 10^7/kg, quartiles		
Median (range)	8 (3-169)	19 (<1-180)
< 10.6	73 (33)	30 (2)
10.6 - 16.79	4 (2)	99 (8)
16.8 - 28.79	0	103 (8)
≥ 28.8	12 (5)	90 (7)
Missing	134 (60)	892 (73)

CD3+ T-cell dose	**< 14 x10^7**	**≥ 14 x10^7**
CD8+ cell dose, x 10^7/kg, quartiles		
Median (range)	4 (<1-59)	8 (<1-253)
< 4.52	61 (27)	43 (4)
4.52 - 7.179	16 (7)	87 (7)
7.18 - 12.769	2 (<1)	102 (8)
≥ 12.77	11 (5)	92 (8)
Missing	133 (60)	890 (73)
CD34+ cell dose, x 10^6/kg		
Median (range)	5 (<1-22)	6 (<1-28)
< 2	36 (16)	53 (4)
2-<4	54 (24)	211 (17)
4-<8	101 (45)	624 (51)
≥ 8	26 (12)	314 (26)
Missing	6 (3)	12 (<1)
CD4+/CD8+ cell dose ratio, quartiles		
Median (range)	2 (<1-9)	2 (<1-13)
< 1.53	26 (12)	78 (6)
1.53 - 2.189	20 (9)	82 (7)
2.19 - 3.149	23 (10)	79 (7)
≥ 3.15	19 (9)	84 (7)
Missing	135 (61)	891 (73)
D/R gender match		
F/F	48 (22)	255 (21)
F/M	55 (25)	312 (26)
M/F	52 (23)	265 (22)
M/M	68 (30)	382 (31)
D/R CMV status match		
-/-	56 (25)	276 (23)
-/+	52 (23)	304 (25)
+/-	26 (12)	141 (12)
+/+	84 (38)	477 (39)
Missing	5 (2)	16 (1)
D/R ABO match		
Matched	105 (47)	565 (47)
Minor mismatch	26 (12)	136 (11)
Major mismatch	22 (10)	149 (12)
Bidirectional mismatch	7 (3)	36 (3)
Missing	63 (28)	328 (27)
Conditioning regimen intensity		

CD3+ T-cell dose	**< 14 x10^7**	**≥ 14 x10^7**
MA	167 (75)	840 (69)
RIC/NMA	56 (25)	374 (31)
Conditioning regimen, MA		
BU+CY±others	52 (31)	242 (29)
TBI+CY	48 (29)	275 (33)
BU+FLU	40 (24)	206 (25)
TBI+ETOP	10 (6)	71 (8)
Others	17 (10)	46 (5)
Conditioning regimen, RIC/NMA		
BU+FLU	20 (36)	156 (42)
FLU+MEL	23 (41)	104 (28)
TBI+FLU	2 (4)	64 (17)
FLU+others	10 (18)	41 (11)
Others	1 (2)	9 (2)
TBI used in conditioning regimen		
Yes	81 (36)	461 (38)
No	142 (64)	753 (62)
GVHD prophylaxis		
CsA + MTX ± others	9 (4)	123 (10)
Tac + MTX ± others	161 (72)	716 (59)
CsA + MMF ± others	13 (6)	92 (8)
Tac + MMF ± others	18 (8)	145 (12)
Others	22 (10)	138 (11)
Year of transplant		
2008-2010	110 (49)	555 (46)
2011-2014	113 (51)	659 (54)
Follow-up of survivors, months, median (range)	47 (3-101)	49 (3-107)

Abbreviations: HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen; CIBMTR, Center for Blood and Marrow Transplant Research; D, donor; R, recipient; F, female; M, male; CMV, cytomegalovirus, MA, myeloablative; RIC/NMA, reduced intensity conditioning/non-myeloablative, BU, busulfan; CY, cyclophosphamide; TBI, total body irradiation; FLU, fludarabine; ETOP, etoposide; GVHD, graft-versus-host disease; CsA, cyclosphamide; MTX, methotrexate; MMF, mycophenolate mofetil; Tac, tacrolimus.

Table 2: Characteristics of adult patients undergoing first allogeneic HCT for AML, ALL, and MDS between 2008-2014 with PBSC from an 8/8-matched unrelated donor with valid CD3+ cell dose data, as reported to the CIBMTR.

Variable	**< 15 x10^7**	**≥ 15 x10^7**
Number of patients	**197**	**1102**
Number of centers	55	80
Age		
Median (range)	55 (19-76)	56 (18-78)
18-29	18 (9)	111 (10)
30-39	25 (13)	120 (11)
40-49	32 (16)	182 (17)
50-59	61 (31)	264 (24)
60+	61 (31)	425 (39)
Recipient gender		
Male	123 (62)	629 (57)
Female	74 (38)	473 (43)
Recipient race		
Caucasian	190 (96)	1022 (93)
Non-Caucasian	7 (4)	61 (6)
Missing	0	19 (2)
Body mass index, median (range)		
Body mass index		
Median (range)	29 (19-52)	28 (8-62)
Underweight (<18.5)	0	21 (2)
Normal (18.5-<25)	45 (23)	309 (28)
Overweight (25-<30)	68 (35)	418 (38)
Obese (≥30)	84 (43)	354 (32)
Karnofsky performance status		
< 90	81 (41)	428 (39)
90-100	113 (57)	662 (60)
Missing	3 (2)	12 (1)
Sorror co-morbidity index		
0-1	64 (32)	473 (43)
2-3	61 (31)	357 (32)
4+	70 (36)	264 (24)
Missing	2 (1)	8 (<1)
Disease		
AML	116 (59)	619 (56)
ALL	22 (11)	142 (13)
MDS	59 (30)	341 (31)

Variable	**< 15 x10^7**	**≥ 15 x10^7**
Disease status		
AML	116	619
Early	70 (60)	351 (57)
Intermediate	20 (17)	124 (20)
Advanced	26 (22)	141 (23)
Missing	0	3 (<1)
ALL	22	142
Early	12 (55)	91 (64)
Intermediate	5 (23)	31 (22)
Advanced	5 (23)	20 (14)
MDS	59	341
Early	41 (69)	233 (68)
Advanced	16 (27)	92 (27)
Missing	2 (3)	16 (5)
Revised Disease Risk Index (DRI)		
AML	116	619
Low	9 (8)	39 (6)
Intermediate	64 (55)	352 (57)
High/Very high	26 (22)	129 (21)
Missing	17 (15)	99 (16)
ALL	22	142
Intermediate	12 (55)	91 (64)
High/Very high	10 (45)	51 (36)
MDS	59	341
Intermediate	33 (56)	210 (62)
High/Very high	11 (19)	73 (21)
Missing	15 (25)	58 (17)
Time from diagnosis to transplant, months		
Median (range)	6 (2-156)	6 (<1-297)
< 6	94 (48)	505 (46)
6 - < 12	55 (28)	292 (26)
≥ 12	47 (24)	305 (28)
Missing	1 (<1)	0
CD3+ cell dose, x 10^7/kg, median (range)	10 (3-14)	28 (14-113)
CD4+ cell dose, x 10^7/kg, quartiles		
Median (range)	6 (2-57)	18 (<1-190)
< 9.6	63 (32)	20 (2)
9.6 - 14.89	3 (2)	80 (7)
14.9 - 23.39	0	81 (7)

Variable	**< 15 x10^7**	**≥ 15 x10^7**
≥ 23.4	6 (3)	77 (7)
Missing	125 (63)	844 (77)
CD8+ cell dose, x 10^7/kg		
Median (range)	4 (<1-30)	10 (<1-145)
< 5.19	58 (29)	24 (2)
5.19 - 8.519	8 (4)	76 (7)
8.52 - 14.439	1 (<1)	81 (7)
≥ 14.44	5 (3)	78 (7)
Missing	125 (63)	843 (76)
CD34+ cell dose, x 10^6/kg		
Median (range)	5 (<1-24)	7 (1-30)
< 2	10 (5)	11 (<1)
2-<4	40 (20)	98 (9)
4-<8	117 (59)	511 (46)
≥ 8	27 (14)	455 (41)
Missing	3 (2)	27 (2)
CD4+/CD8+ cell dose ratio		
Median (range)	2 (<1-6)	2 (<1-19)
< 1.31	19 (10)	62 (6)
1.31 - 1.649	14 (7)	68 (6)
1.65 - 2.259	19 (10)	65 (6)
≥ 2.26	20 (10)	62 (6)
Missing	125 (63)	845 (77)
Unrelated donor age, years		
Median (range)	30 (18-60)	28 (18-61)
18-32	116 (59)	692 (63)
33-49	59 (30)	296 (27)
50+	14 (7)	63 (6)
Missing	8 (4)	51 (5)
D/R gender match		
F/F	15 (8)	163 (15)
F/M	19 (10)	174 (16)
M/F	59 (30)	310 (28)
M/M	104 (53)	455 (41)
D/R CMV status match		
-/-	65 (33)	300 (27)
-/+	71 (36)	409 (37)
+/-	15 (8)	116 (11)
+/+	42 (21)	265 (24)

Variable	< 15 x10^7	≥ 15 x10^7
Missing	4 (2)	12 (1)
D/R ABO match		
Matched	60 (30)	397 (36)
Minor mismatch	42 (21)	216 (20)
Major mismatch	34 (17)	168 (15)
Bidirectional mismatch	5 (3)	66 (6)
Missing	56 (28)	255 (23)
Conditioning regimen intensity		
MA	134 (68)	668 (61)
RIC/NMA	63 (32)	434 (39)
Conditioning regimen, MA		
BU+CY±others	50 (37)	211 (32)
TBI+CY	33 (25)	157 (24)
BU+FLU	29 (22)	203 (30)
TBI+ETOP	6 (4)	29 (4)
Others	16 (12)	68 (10)
Conditioning regimen, RIC/NMA		
BU+FLU	27 (43)	117 (27)
FLU+MEL	20 (32)	145 (33)
TBI+FLU	8 (13)	100 (23)
FLU+others	6 (10)	43 (10)
Others	2 (3)	29 (7)
TBI used in conditioning regimen		
Yes	57 (29)	391 (35)
No	140 (71)	711 (65)
GVHD prophylaxis		
CsA + MTX ± others	6 (3)	41 (4)
Tac + MTX ± others	130 (66)	611 (55)
CsA + MMF ± others	11 (6)	97 (9)
Tac + MMF ± others	30 (15)	192 (17)
Others	20 (10)	161 (15)
Year of transplant		
2008-2010	69 (35)	482 (44)
2011-2014	128 (65)	620 (56)
Follow-up of survivors, months, median (range)	37 (21-96)	48 (3-102)

Abbreviations: HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen; CIBMTR, Center for Blood and Marrow Transplant Research; D, donor; R, recipient; F, female; M, male; CMV, cytomegalovirus, MA, myeloablative; RIC/NMA, reduced intensity conditioning/non-myeloablative, BU, busulfan; CY, cyclophosphamide; TBI, total body irradiation; FLU, fludarabine; ETOP, etoposide; GVHD, graft-versus-host disease; CsA, cyclosphamide; MTX, methotrexate; MMF, mycophenolate mofetil; Tac, tacrolimus.

Table 3: Multivariate analysis of MSD showing influence of CD3+ T-cell dose.

	aGVHD II-IV *		aGVHD III-IV*		cGVHD*		Relapse **		NRM		DFS		OS	
Factor	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
CD3 cell dose, x 10^7/kg														
> 14	1		1		1		1		1		1		1	
≤ 14	0.79 (0.60-1.04)	0.10	0.78 (0.51-1.18)	0.25	0.97 (0.79-1.21)	0.81	1.02 (0.81-1.29)	0.85	0.97 (0.70-1.36)	0.87	0.99 (0.82-1.20)	0.96	0.94 (0.77-1.15)	0.55

Abbreviations: MSD, matched sibling donor; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

Table 4: Multivariate analysis of MUD groups showing influence of CD3+ T-cell dose.

	aGVHD II-IV *		aGVHD III-IV**		cGVHD		Relapse		NRM		DFS		OS	
Factor	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
CD3 cell dose, x 10^7/kg														
> 15	1		1		1		1		1		1		1	
≤ 15	0.81 (0.65-1.02)	0.07	0.85 (0.61-1.19)	0.34	0.89 (0.73-1.10)	0.29	1.01 (0.78-1.29)	0.96	0.95 (0.71-1.27)	0.73	0.97 (0.80-1.18)	0.77	0.96 (0.78-1.17)	0.66

Abbreviations: MUD, matched unrelated donor; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.