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Impact of T Cell Dose on Outcome of T Cell-Replete HLA-Matched Allogeneic Peripheral Blood Stem Cell Transplantation

Ayman Saad[1], Lawrence Lamb[2], Tao Wang[3,4], Michael T. Hemmer[4], Stephen Spellman[5], Daniel Couriel[6], Amin Alousi[7], Joseph Pidala[8], Hisham Abdel-Azim[9], Vaibhav Agrawal[10], Mahmoud Aljurf[11], Amer M. Beitinjaneh[12], Vijaya Raj Bhatt[13], David Buchbinder[14], Michael Byrne[15], Jean-Yves Cahn[16], Mitchell Cairo[17], Paul Castillo[18], Saurabh Chhabra[19], Miguel Angel Diaz[20], Shatha Farhan[21], Yngvar Floisand[22], Hadar A. Frangoul[23], Shahinaz M. Gadalla[24], James Gajewski[25], Robert Peter Gale[26], Manish Gandhi[27], Usama Gergis[28], Betty Ky Hamilton[29], Peiman Hematti[30], Gerhard C. Hildebrandt[31], Rammurti T. Kamble[32], Abraham S. Kanate[33], Pooja Khandelwal[34], Aleksandr Lazaryan[8], Margaret MacMillan[35], David I. Marks[36], Rodrigo Martino[37], Parinda A. Mehta[34], Taiga Nishihori[8], Richard F. Olsson[38,39], Sagar S. Patel[40], Muna Qayed[41], Hemalatha G. Rangarajan[42], Ran Reshef[43], Olle Ringden[44], Bipin N. Savani[15], Harry C. Schouten[45], Kirk R. Schultz[46], Sachiko Seo[47], Brian C. Shaffer[48], Melhem Solh[49], Takanori Teshima[50], Alvaro Urbano-Ispizua[51], Leo F. Verdonck[52], Ravi Vij[53], Edmund K. Waller[54], Basem William[1], Baldeep Wirk[55], Jean A. Yared[56], Lolie C. Yu[57], Mukta Arora[58,*], Shahrukh Hashmi[11,59]

[1] *Division of Hematology, The Ohio State University, Columbus, Ohio*
[2] *University of Alabama at Birmingham, Birmingham, Alabama*
[3] *Division of Biostatistics, Institute for Health and Equity, Medical College of Wisconsin, Milwaukee, Wisconsin*
[4] *Center for International Blood and Marrow Transplant Research, Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin*
[5] *Center for International Blood and Marrow Transplant Research, National Marrow Donor Program/Be the Match, Minneapolis, Minnesota*
[6] *Utah Blood and Marrow Transplant Program, Salt Lake City, Utah*
[7] *Department of Stem Cell Transplantation, Division of Cancer Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, Texas*
[8] *Department of Blood and Marrow Transplantation, H. Lee Moffitt Cancer Center, Tampa, Florida*
[9] *Division of Hematology, Oncology and Blood and Marrow Transplantation, Children's Hospital of Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, California*
[10] *Division of Hematology-Oncology, Indiana University School of Medicine, Indianapolis, Indiana*
[11] *Oncology Center, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia*
[12] *University of Miami, Miami, Florida*
[13] *The Fred and Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, Nebraska*
[14] *Division of Pediatric Hematology, Children's Hospital of Orange County, Orange, California*
[15] *Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, Tennessee*
[16] *Department of Hematology, CHU Grenoble Alpes, Grenoble, France*
[17] *Division of Pediatric Hematology, Oncology, and Stem Cell Transplantation, Department of Pediatrics, New York Medical College, New York, New York*
[18] *UF Health Shands Children's Hospital, Gainesville, Florida*
[19] *Division of Hematology/Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin*
[20] *Department of Hematology/Oncology, Hospital Infantil Universitario Nino Jesus, Madrid, Spain*
[21] *Henry Ford Hospital Bone Marrow Transplant Program, Detroit, Michigan*
[22] *The National Hospital, Oslo, Denmark*
[23] *Children's Hospital at TriStar Centennial and Sarah Cannon Research Institute, Nashville, Tennessee*
[24] *Division of Cancer Epidemiology & Genetics, Clinical Genetics Branch, National Cancer Institute, Rockville, Maryland*
[25] *Consultant at Lu Daopei Hospital, Beijing, China*
[26] *Hematology Research Center, Division of Experimental Medicine, Department of Medicine, Imperial College London, London, United Kingdom*
[27] *Division of Transfusion Medicine, Mayo Clinic, Rochester, Minnesota*
[28] *Hematologic Malignancies & Bone Marrow Transplant, Department of Medical Oncology, New York Presbyterian Hospital/Weill Cornell Medical Center, New York, New York*
[29] *Blood & Marrow Transplant Program, Cleveland Clinic Taussig Cancer Institute, Cleveland, Ohio*
[30] *Division of Hematology/Oncology/Bone Marrow Transplantation, Department of Medicine, University of Wisconsin Hospital and Clinics, Madison, Wisconsin*

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* Correspondence and reprint requests: Mukta Arora, MD, University of Minnesota Medical Center, Division of Hematology, Oncology and Transplantation, Minneapolis, 420 Delaware St, SE Minneapolis, MN 55455.
E-mail address: arora005@umn.edu (M. Arora).

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[31] Markey Cancer Center, University of Kentucky, Lexington, Kentucky
[32] Division of Hematology and Oncology, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas
[33] Osborn Hematopoietic Malignancy and Transplantation Program, West Virginia University, Morgantown, West Virginia
[34] Division of Bone Marrow Transplant and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[35] University of Minnesota Blood and Marrow Transplant Program, Pediatrics, Minneapolis, Minnesota
[36] Adult Bone Marrow Transplant, University Hospitals Bristol NHS Trust, Bristol, United Kingdom
[37] Division of Clinical Hematology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
[38] Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden
[39] Centre for Clinical Research Sormland, Uppsala University, Uppsala, Sweden
[40] Blood and Marrow Transplant Program, Cleveland Clinic Foundation, Cleveland, Ohio
[41] Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia
[42] Department of Pediatric Hematology, Oncology, Blood and Marrow Transplantation, Nationwide Children's Hospital, Columbus, Ohio
[43] Blood and Marrow Transplantation Program and Columbia Center for Translational Immunobiology, Columbia University Medical Center, New York, New York
[44] Translational Cell Therapy Research, Karolinska Institute, Stockholm, Sweden
[45] Department of Hematology, Academische Ziekenhuis, Maastricht, Netherlands
[46] Department of Pediatric Hematology, Oncology and Bone Marrow Transplant, British Columbia's Children's Hospital, The University of British Columbia, Vancouver, Britich Columbia, Canada
[47] Department of Hematology and Oncology, Dokkyo Medical University, Tochigi, Japan
[48] Memorial Sloan Kettering Cancer Center, New York, New York
[49] The Blood and Marrow Transplant Group of Georgia, Northside Hospital, Atlanta, Georgia
[50] Hokkaido University Hospital, Sapporo, Japan
[51] Department of Hematology, Hospital Clinic, University of Barcelona, IDIBAPS, and Josep Carreras Institute of Research, Barcelona, Spain
[52] Department of Hematology/Oncology, Isala Clinic, Zwolle, The Netherlands
[53] Division of Hematology and Oncology, Washington University School of Medicine, St Louis, Missouri
[54] Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, Georgia
[55] Division of Bone Marrow Transplant, Seattle Cancer Care Alliance, Seattle, Washington
[56] Blood & Marrow Transplantation Program, Division of Hematology/Oncology, Department of Medicine, Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, Maryland
[57] Division of Hematology/Oncology and HSCT, Center for Cancer and Blood Disorders, Children's Hospital/Louisiana State University Medical Center, New Orleans, Louisiana
[58] Division of Hematology, Oncology and Transplantation, Department of Medicine, University of Minnesota Medical Center, Minneapolis, Minnesota
[59] Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota

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A B S T R A C T

Data on whether the T cell dose of allogeneic peripheral blood stem cell (PBSC) products influences transplantation outcomes are conflicting. Using the Center for International Blood and Marrow Transplant Research database, we identified 2736 adult patients who underwent first allogeneic PBSC transplantation for acute leukemia or myelodysplastic syndrome between 2008 and 2014 using an HLA-matched sibling donor (MSD) or an 8/8-matched unrelated donor (MUD). We excluded ex vivo and in vivo T cell-depleted transplantations. Correlative analysis was performed between $CD3^+$ T cell dose and the risk of graft-versus-host-disease (GVHD), relapse, nonrelapse mortality (NRM), disease-free survival (DFS), and overall survival (OS). Using maximum likelihood estimation, we identified $CD3^+$ T cell dose cutoff that separated the risk of acute GVHD (aGVHD) grade II-IV in both the MSD and MUD groups. A $CD3^+$ T cell dose cutoff of 14×10^7 cells/kg identified MSD/low $CD3^+$ (n = 223) and MSD/high $CD3^+$ (n = 1214), and a dose of 15×10^7 cells/kg identified MUD/low $CD3^+$ (n = 197) and MUD/high $CD3^+$ (n = 1102). On univariate analysis, the MSD/high $CD3^+$ group had a higher cumulative incidence of day +100 aGVHD grade II-IV compared with the MSD/low $CD3^+$ group (33% versus 25%; $P = .009$). There were no differences between the 2 groups in engraftment rate, risk of aGVHD grade III-IV or chronic GVHD (cGVHD), NRM, relapse, DFS, or OS. The MUD/high $CD3^+$ group had a higher cumulative incidence of day +100 aGVHD grade II-IV compared with the MUD/low $CD3^+$ group (49% versus 41%; $P = .04$). There were no differences between the 2 groups in engraftment rate, risk of severe aGVHD or cGVHD, NRM, relapse, DFS, or OS. Multivariate analysis of the MSD and MUD groups failed to show an association between $CD3^+$ T cell dose and the risk of either aGVHD grade II-IV ($P = .10$ and .07, respectively) or cGVHD (P = .80 and .30, respectively). Subanalysis of $CD4^+$ T cells, $CD8^+$ T cells, and CD4+/CD8+ ratio failed to identify cutoff values predictive of transplantation outcomes; however, using the log-rank test, the sample size was suboptimal for identifying a difference at this cutoff cell dose. In this registry study, the $CD3^+$ T cell dose of PBSC products did not influence the risk of aGVHD or cGVHD or other transplantation outcomes when using an MSD or an 8/8-matched MUD. Subset analyses of $CD4^+$ and $CD8^+$ T cell doses were not possible given our small sample size.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) performed for hematologic malignancies relies on both the conditioning regimen and immunotherapy exploiting the graft-versus-tumor (GVT) effect, which is derived primarily 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 (Tregs), and natural killer (NK) cells, is responsible for both the GVT effect and the graft-versus-host disease (GVHD) [3]. Among these, the most well-studied cells are $CD3^+$T cells.

Although $CD3^+$T cells can exert a strong GVT effect [4], the risk of acute GVHD (aGVHD) also rises with increasing dose, as demonstrated in both observational and prospective studies [5,6]. T cell-depleted (TCD) allogeneic HCT has led to a decreased risk of GVHD but at the expense of an increased risk of relapse, as demonstrated by trials of both ex vivo [7] and in vivo depletion [8]. The higher risk of GVHD in peripheral blood stem cell (PBSC) grafts compared with bone marrow (BM)

grafts is apparent from both observational studies [9] and clinical trials [10], as PBSCs are known to carry 10 to 15 times the quantity of $CD3^+$T cells as BM [11]. Thus, numerous attempts have been made to separate out the GVT effect from GVHD, including the use of $CD34^+$T cell selection [12], naïve T cell depletion [13], post-transplantation 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 terms of selection criteria, with no consensus on an optimal $CD3^+$T cell dose cutoff value [16-19]. In a recent large registry study, in HCTs using unrelated donors, higher $CD3^+$ and $CD34^+$T cell doses were significantly associated with an increased risk of grade III-IV aGVHD (hazard ratio [HR], 3.6; 95% confidence interval [CI], 1.45 to 9.96; $P = .006$ and 2.65 (95% CI, 1.07 to 6.57); $P = .04$, respectively) [20]. Because the aforementioned studies used different types of donors, different diseases, and different conditioning regimens, the optimum cutoff $CD3^+$T cell dose that can potentially avoid GVHD while still promoting the GVT effect are unknown.

We hypothesized that there exists a T cell dose range that promotes GVT, whereas levels above this range carry a higher risk of both severe aGVHD and cGVHD with subsequent increased nonrelapse mortality (NRM).

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 onsite audits. Computerized checks for discrepancies, physician reviews of submitted data, and onsite 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 the 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 2 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 (BM and/or PBSCs), conditioning regimen, post-transplantation disease progression and survival, development of new malignancy, and cause of death. All CIBMTR centers contribute TED data. More detailed disease and pretransplantation and post-transplantation clinical information are collected on a subset of registered patients selected for CRF data via a weighted randomization scheme. TED- and CRF-level data are collected pre-transplantation, at 100 days and 6 months post-HCT. and annually thereafter or until death. Data for the present analysis were retrieved from CIBMTR (TED and CRF) report forms.

Patients

We analyzed data of adult patients (age ≥18 years) who underwent first allogeneic HCT for acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), or myelodysplastic syndrome (MDS) between 2008 and 2014 with PBSC grafts using an HLA-identical sibling donor (MSD) or an 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 a comparable risk of relapse and susceptibility to the GVT effect. We excluded ex vivo (TCD and $CD34^+$-selected grafts) and in vivo TCD (antithymocyte globulin or alemtuzumab) HCT. All patients had available data on $CD3^+$T cell dose; however, some patients were missing data on $CD4^+$and/or $CD8^+$ T cell dose.

Study 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, and patients alive without evidence of disease relapse/progression were censored at last follow-up. 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 ≥500/μL after a post-transplantation nadir. Platelet recovery was defined as the first of 3 successive days with a platelet count ≥20,000/μL without transfusion support for at least 7 days. Data were 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 severity of aGVHD and cGVHD, OS, DFS, relapse, and NRM following PBSC HCT with MSD and 8/8-matched MRD HCT. In a subset analysis of subjects with available $CD4^+$ and $CD8^+$T cell dose data, we also tested for an association between graft T cell subset dose and ratio of $CD4^+$/$CD8^+$T cell doses and these transplantation outcomes in univariate analysis due only to smaller sample size. T cell dose cutoff values were determined using a maximum likelihood method based on a Cox proportional hazards model for the aGVHD grade II-IV endpoint.

Categorical data are summarized as frequency, and continuous data are summarized as median and range. Probabilities of DFS and OS were calculated as described previously [23]. Cumulative incidences of aGVHD grade II-IV, aGVHD grade III-IV, cGVHD, NRM, relapse/progression, platelet recovery, and hematopoietic cell 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, and then a stepwise forward model selection procedure was used to select adjusted clinical variables for each outcome, with a threshold of .05 to be entered into and be retained 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 .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 2736 adult patients who met the selection criteria described above. Regimen intensity, classified as myeloablative (MAC), reduced-intensity (RIC), or nonmyeloablative (NMA), was defined as described previously [26]. Using a Cox proportional hazards model, we determined the cutoff value for $CD3^+$T cell dose and separated each group (MSD and MUD) into low risk and high risk of grade II-IV aGVHD: 14×10^7 cells/kg for MSD and 15×10^7 cells/kg for MUD. Then the patients were divided into 4 groups based on the donor type (MSD or MUD) and T cell dose cutoff value: MSD/low $CD3^+$ (n = 223), MSD/high $CD3^+$ (n = 1214), MUD/low $CD3^+$ (n = 197), and MUD/high $CD3^+$ (n = 1102). The median $CD3^+$T cell dose was 11×10^7 in the MSD/low group, 29×10^7in the MSD/high group, 10×10^7in the MUD/low group, and 28×10^7in the MUD/high group. The MSD and MUD groups were analyzed separately. Baseline patient-, disease- and transplantation-related characteristics are summarized in Tables 1 and 2.

MSD Groups

Univariate analysis showed a day +100 cumulative incidence of aGVHD grade II-IV of 25% (95% CI [confidence interval], 19% to 31%) in the MSD/low $CD3^+$group and 33% (95% CI, 30% to 36%) in the MSD/high $CD3^+$group ($P = .009$). However, there was no significant between-group difference in the risk of aGVHD grade III-IV ($P = .40$) or in the risk of cGVHD at 2 years, NRM, relapse, DFS, and OS. There was also no difference in the day +100 engraftment rate between the 2 groups.

In multivariate analysis, $CD3^+$T cell dose did not influence aGVHD (grades II-IV and III-IV) (Table 3), cGVHD, relapse, NRM, DFS, or OS (Supplemental Table 1). However, the risk of aGVHD grade II-IV was higher with any donor-recipient sex

Table 1
Characteristics of Adult Patients Undergoing First Allogeneic HCT for AML, ALL, and MDS Between 2008 and 2014 with PBSCs from an HLA-Identical Sibling Donor with Valid $CD3^+$ Cell Dose Data, as Reported to the CIBMTR

Characteristic	$CD3^+$ T Cell Dose	
	$<14 \times 10^7$	$\geq 14 \times 10^7$
Number of patients	223	1214
Number of centers	58	95
Recipient age, yr		
Median (range)	51 (18-71)	54 (18-78)
18-29, n (%)	20 (9)	110 (9)
30-39, n (%)	28 (13)	130 (11)
40-49, n (%)	55 (25)	232 (19)
50-59, n (%)	74 (33)	419 (35)
60+, n (%)	46 (21)	323 (27)
Recipient sex, n (%)		
Male	123 (55)	694 (57)
Female	100 (45)	520 (43)
Recipient race, n (%)		
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), n (%)	2 (<1)	25 (2)
Normal (18.5-<25), n (%)	52 (23)	366 (30)
Overweight (25-<30), n (%)	67 (30)	433 (36)
Obese (≥30), n (%)	101 (45)	390 (32)
Missing, n (%)	1 (<1)	0
KPS score, n (%)		
<90	92 (41)	478 (39)
90-100	125 (56)	718 (59)
Missing	6 (3)	18 (1)
Sorror comorbidity index, n (%)		
0-1	101 (45)	560 (46)
2-3	68 (30)	382 (31)
4+	51 (23)	261 (21)
Missing	3 (1)	11 (<1)
Disease, n (%)		
AML	137 (61)	640 (53)
ALL	33 (15)	224 (18)
MDS	53 (24)	350 (29)
Disease status, n (%)		
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 DRI, n (%)		
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 HCT, mo		
Median (range)	6 (1-156)	5 (<1-279)
<6, n (%)	121 (54)	695 (57)
6-<12, n (%)	52 (23)	257 (21)
≥12, n (%)	50 (22)	262 (22)
$CD3^+$ cell dose, $\times 10^7$/kg, median (range)	11 (3-14)	29 (14-113)
$CD4^+$ cell dose, $\times 10^7$/kg, quartiles		
Median (range)	8 (3-169)	19 (<1-180)
<10.6, n (%)	73 (33)	30 (2)
10.6-16.79, n (%)	4 (2)	99 (8)
16.8-28.79, n (%)	0	103 (8)
≥28.8, n (%)	12 (5)	90 (7)
Missing, n (%)	134 (60)	892 (73)
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, $\times 10^6$/kg		
Median (range)	5 (<1-22)	6 (<1-28)
<2, n (%)	36 (16)	53 (4)
2-<4, n (%)	54 (24)	211 (17)
4-<8, n (%)	101 (45)	624 (51)
≥8, n (%)	26 (12)	314 (26)
Missing, n (%)	6 (3)	12 (<1)
$CD4^+/CD8^+$ cell dose ratio, quartiles		
Median (range)	2 (<1-9)	2 (<1-13)
<1.53, n (%)	26 (12)	78 (6)
1.53-2.189, n (%)	20 (9)	82 (7)
2.19-3.149, n (%)	23 (10)	79 (7)
≥3.15, n (%)	19 (9)	84 (7)
Missing, n (%)	135 (61)	891 (73)
Donor/recipient sex match, n (%)		
Female/female	48 (22)	255 (21)
Female/male	55 (25)	312 (26)
Male/female	52 (23)	265 (22)
Male/male	68 (30)	382 (31)
Donor/recipient CMV serostatus match, n (%)		
-/-	56 (25)	276 (23)
-/+	52 (23)	304 (25)
+/-	26 (12)	141 (12)
+/+	84 (38)	477 (39)
Missing	5 (2)	16 (1)
Donor/recipient ABO match, n (%)		
Matched	105 (47)	565 (47)
Minor mismatch	26 (12)	136 (11)

Table 1 (*Continued*)

Characteristic	$CD3^+$ T Cell Dose	
	$<14 \times 10^7$	$\geq 14 \times 10^7$
Major mismatch	22 (10)	149 (12)
Bidirectional mismatch	7 (3)	36 (3)
Missing	63 (28)	328 (27)
Conditioning regimen intensity, n (%)		
MAC	167 (75)	840 (69)
RIC/NMA	56 (25)	374 (31)
Conditioning regimen, MAC, n (%)		
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, n (%)		
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, n (%)		
Yes	81 (36)	461 (38)
No	142 (64)	753 (62)
GVHD prophylaxis, n (%)		
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 transplantation, n (%)		
2008-2010	110 (49)	555 (46)
2011-2014	113 (51)	659 (54)
Follow-up of survivors, mo, median (range)	47 (3-101)	49 (3-107)

CMV, cytomegalovirus, Bu, busulfan; Cy, cyclophosphamide; TBI, total body irradiation; Flu, fludarabine; ETOP, etoposide; CsA, cyclophosphamide; MTX, methotrexate; MMF, mycophenolate mofetil; Tac, tacrolimus.

mismatch (P= .02 and .009 for female to male and male to female, respectively). The risk of severe aGVHD grade III-IV was worse in patients with a Karnofsky Performance Status (KPS) score <90 relative to those with KPS score of 90 to 100 (P = .005). The risk of cGVHD was increased in patients age >29 years (overall P = .006), in transplants from a female donor (P< .002), and in transplantations performed before 2011 (P = .01). DFS was worse in patients age ≥60 years (P = .01), patients with a high/very high Disease Risk Index (DRI) (P < .0001), and patients with a lower KPS score (P < .0001). OS was worse in patients with a high/very high DRI (P = .0001), those with a lower KPS score (P < .0001), and those with a Hematopoietic Stem Cell Transplantation Comorbidity Index (HCT-CI) >3 (P = .003). NRM was worse in patients with MDS (P = .002), a lower KPS (P = .007), and HCT-CI >3 (P = .0006). The relapse risk was higher in patients with advanced disease before HCT (P= .0007) and lower KPS score (P= .002).

Data from subset analyses of $CD4^+$ T cells, $CD8^+$ T cells, and $CD4^+/CD8^+$ ratio were available for only a limited number of patients. No significant associations of these variables were detected for aGVHD, cGVHD, NRM, relapse, DFS, or OS.

Table 2
Characteristics of Adult Patients Undergoing First Allogeneic HCT for AML, ALL, and MDS Between 2008 and 2014 with PBSCs from an 8/8-matched MUD with Valid $CD3^+$ Cell Dose Data, as Reported to the CIBMTR

Characteristic	$CD3^+$ T Cell Dose	
	$<15 \times 10^7$	$\geq 15 \times 10^7$
Number of patients	197	1102
Number of centers	55	80
Age, yr		
Median (range)	55 (19-76)	56 (18-78)
18-29, n (%)	18 (9)	111 (10)
30-39, n (%)	25 (13)	120 (11)
40-49, n (%)	32 (16)	182 (17)
50-59, n (%)	61 (31)	264 (24)
60+, n (%)	61 (31)	425 (39)
Recipient sex, n (%)		
Male	123 (62)	629 (57)
Female	74 (38)	473 (43)
Recipient race, n (%)		
Caucasian	190 (96)	1022 (93)
Non-Caucasian	7 (4)	61 (6)
Missing	0	19 (2)
Body mass index		
Median (range)	29 (19-52)	28 (8-62)
Underweight (<18.5), n (%)	0	21 (2)
Normal (18.5-<25), n (%)	45 (23)	309 (28)
Overweight (25-<30), n (%)	68 (35)	418 (38)
Obese (≥30), n (%)	84 (43)	354 (32)
KPS score, n (%)		
<90	81 (41)	428 (39)
90-100	113 (57)	662 (60)
Missing	3 (2)	12 (1)
Sorror comorbidity index, n (%)		
0-1	64 (32)	473 (43)
2-3	61 (31)	357 (32)
4+	70 (36)	264 (24)
Missing	2 (1)	8 (<1)
Disease, n (%)		
AML	116 (59)	619 (56)
ALL	22 (11)	142 (13)
MDS	59 (30)	341 (31)
Disease status, n (%)		
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 DRI, n (%)		
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)

(*continued*)

Table 2 (*Continued*)

Characteristic	$CD3^+$ T Cell Dose	
	$<15 \times 10^7$	$\geq 15 \times 10^7$
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 HCT, mo		
Median (range)	6 (2-156)	6 (<1-297)
<6, n (%)	94 (48)	505 (46)
6-<12, n (%)	55 (28)	292 (26)
≥12, n (%)	47 (24)	305 (28)
Missing, n (%)	1 (<1)	0
$CD3^+$ cell dose, $\times 10^7$/kg, median (range)	10 (3-14)	28 (14-113)
$CD4^+$ cell dose, $\times 10^7$/kg, quartiles		
Median (range)	6 (2-57)	18 (<1-190)
<9.6, n (%)	63 (32)	20 (2)
9.6-14.89, n (%)	3 (2)	80 (7)
14.9-23.39, n (%)	0	81 (7)
≥23.4, n (%)	6 (3)	77 (7)
Missing, n (%)	125 (63)	844 (77)
$CD8^+$ cell dose, $\times 10^7$/kg		
Median (range)	4 (<1-30)	10 (<1-145)
<5.19, n (%)	58 (29)	24 (2)
5.19-8.519, n (%)	8 (4)	76 (7)
8.52-14.439, n (%)	1 (<1)	81 (7)
≥14.44, n (%)	5 (3)	78 (7)
Missing, n (%)	125 (63)	843 (76)
$CD34^+$ cell dose, $\times 10^6$/kg		
Median (range)	5 (<1-24)	7 (1-30)
<2, n (%)	10 (5)	11 (<1)
2-<4, n (%)	40 (20)	98 (9)
4-<8, n (%)	117 (59)	511 (46)
≥8, n (%)	27 (14)	455 (41)
Missing, n (%)	3 (2)	27 (2)
$CD4^+/CD8^+$ cell dose ratio		
Median (range)	2 (<1-6)	2 (<1-19)
<1.31, n (%)	19 (10)	62 (6)
1.31-1.649, n (%)	14 (7)	68 (6)
1.65-2.259, n (%)	19 (10)	65 (6)
≥2.26, n (%)	20 (10)	62 (6)
Missing, n (%)	125 (63)	845 (77)
MUD age, yr		
Median (range)	30 (18-60)	28 (18-61)
18-32, n (%)	116 (59)	692 (63)
33-49, n (%)	59 (30)	296 (27)
50+, n (%)	14 (7)	63 (6)
Missing, n (%)	8 (4)	51 (5)
Donor/recipient sex match, n (%)		
Female/female	15 (8)	163 (15)
Female/male	19 (10)	174 (16)
Male/female	59 (30)	310 (28)
Male/male	104 (53)	455 (41)
Donor/recipient CMV serostatus match, n (%)		
-/-	65 (33)	300 (27)
-/+	71 (36)	409 (37)
+/-	15 (8)	116 (11)
+/+	42 (21)	265 (24)
Missing	4 (2)	12 (1)
Donor/recipient ABO match, n (%)		
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, n (%)		
MAC	134 (68)	668 (61)
RIC/NMA	63 (32)	434 (39)
Conditioning regimen, MAC, n (%)		
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, n (%)		
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, n (%)		
Yes	57 (29)	391 (35)
No	140 (71)	711 (65)
GVHD prophylaxis, n (%)		
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 transplantation, n (%)		
2008-2010	69 (35)	482 (44)
2011-2014	128 (65)	620 (56)
Follow-up of survivors, mo, median (range)	37 (21-96)	48 (3-102)

Likewise, $CD34^+$ cell dose was not significantly associated with any of the transplantation outcomes.

MUD Groups

Univariate analysis showed a cumulative incidence of aGVHD grade II-IV at day +100 of 41% (95% CI, 35% to 48%) in the MUD/low $CD3^+$group and 49% (95% CI, 46% to 52%) in the MUD/high $CD3^+$group (P= .04). However, there was no between-group difference in the risk of aGVHD grade III-IV (P= .90). Likewise, the risks of cGVHD at 2 years, NRM, relapse, DFS, and OS were not statistically different between the 2 groups. There also was no difference in the day +100 engraftment rate.

In multivariate analysis, $CD3^+$T cell dose did not influence the risk of aGVHD grade II-IV and III-IV (Table 4), cGVHD, relapse, NRM, DFS, or OS (Supplemental Table 2). However, the risk of aGVHD grade II-IV was higher in patients who received

Table 3
Multivariate Analysis of the MSD Group Showing the Influence of $CD3^+$ T Cell Dose

$CD3^+$ Cell Dose, $\times 10^7$/kg	aGVHD II-IV		aGVHD III-IV		cGVHD		Relapse		NRM		DFS		OS	
	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
>14	1		1		1		1		1		1		1	
≤14	.79 (.60-1.04)	.10	.78 (.51-1.18)	.25	.97 (.79-1.21)	.81	1.02 (.81-1.29)	.85	.97 (.70-1.36)	.87	.99 (.82-1.20)	.96	.94 (.77-1.15)	.55

Table 4
Multivariate Analysis of the MUD Group Showing the Influence of $CD3^+$ T Cell Dose

$CD3^+$ Cell Dose, $\times 10^7$/kg	aGVHD II-IV		aGVHD III-IV		cGVHD		Relapse		NRM		DFS		OS	
	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
>15	1		1		1		1		1		1		1	
≤15	.81 (.65-1.02)	.07	.85 (.61-1.19)	.34	.89 (.73-1.10)	.29	1.01 (.78-1.29)	.96	.95 (.71-1.27)	.73	.97 (.80-1.18)	.77	.96 (.78-1.17)	.66

an MAC regimen ($P = .02$). The risk of severe aGVHD grade III-IV was elevated in underweight patients ($P = .01$), and with older donors (age >32 years) ($P = .01$). The risk of cGVHD was lower in patients with ALL ($P = .003$), and those who underwent HCT after 2010 ($P = .0003$). DFS was worse with older donors (age >50 years) ($P = .0001$) and those with a high/very high DRI ($P < .0003$). Worse OS was associated with older patients (age ≥50 years) ($P = .008$), older donors (age ≥50 years) ($P = .0001$), high/very high DRI ($P = .0005$), and lower KPS ($P < .009$). NRM was worse with older donors (age >50 years) ($P = .0006$). The risk of relapse was worse in patients with a high/very high DRI ($P = .0002$). Subset analyses of $CD4^+$ T cells, $CD8^+$ T cells, and $CD4^+/CD8^+$ ratio was available in only a limited number of patients. No significant associations were detected between these variables for aGVHD, cGVHD, NRM, relapse, DFS, or OS. Likewise, $CD34^+$ cell dose was also not significantly associated with any of the transplantation outcomes.

DISCUSSION

Our data show no associations between the $CD3^+$T cell dose of PBSC grafts and the risk of aGVHD, cGVHD, or relapse in our study cohort. Nonetheless, the subgroup analyses suggest certain associations that merit further exploration prospectively. Although the univariate analysis showed a correlation between $CD3^+$T cell dose and the risk of aGVHD in both the MSD and 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), 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 for aGVHD (eg, inadequate information on $CD4^+$, $CD8^+$, and $CD56^+$T cells and dendritic cells in PBSC grafts). The only group with an increased risk of aGVHD grade II-IV on multivariate analysis was patients who underwent MUD HCT using an MAC regimen. This finding is consistent with a CIBMTR study showing that RIC regimens were associated with decreased risk of aGVHD in MUD HCT recipients [27].

Our data contrast with the European Society of Blood and Marrow Transplant (EBMT) study of MUD HCT showing an association between $CD3^+$T cell dose $>35 \times 10^7$/kg and increased risk of aGVHD [20]. This discrepancy may be attributed to differences in median $CD3^+$T cell doses in PBSC grafts in the 2 studies, as well as in the statistical methodology used to categorize the primary outcome variable. In the EBMT study, $CD3^+$T cell dose was categorized by interquartile range, whereas in the present study, we used a cutoff values of $CD3^+$ T cell dose based on the differential risk of aGVHD grade II-IV. Moreover, the EBMT study included TCD allogeneic HCT, whereas the present study excluded it. In addition, some of the conditioning regimens used in the EBMT study were not evaluated in the present study. Of note, the BMT CTN 0201 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-institution study using BM rather than PBSC grafts demonstrated a paradoxical increase in the risk of cGVHD with lower $CD3^+$T cell dose in a subset of patients who received an MAC regimen with busulfan and cyclophosphamide ($P = .006$) [29].

Owing to our limited sample size, further analysis was not possible in order to detect outcome differences based on T cell phenotypic subsets: $CD4^+$, $CD8^+$, or $CD4^+/CD8^+$ ratio. However, transplantation outcomes 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 a lower risk of cGVHD and a greater risk of steroid-responsive aGVHD in small phase II study [13]. Tregs ($CD4^+/CD25^+/FOXP3^+$), another small subset of $CD4^+$ T cells, have been shown to ameliorate cGVHD [30]. Unbalanced recovery of Tregs and effector T cells after transplantation also has been correlated with an increased risk of cGVHD [31].

Even though PBSC grafts include coinfusions of both $CD34^+$ and $CD3^+$T cells in HCTs, the dose of CD3+ is not evaluated routinely in most transplantation centers, because it continues to be controversial. Farhan et al [32] retrospectively evaluated $CD3^+$T cell doses in both MUD and MSD HCTs and found no significant correlation with aGVHD; however, they observed that OS was significantly affected by a higher $CD3^+$cell dose (mean dose 12×10^7/kg) in their cohort. This $CD3^+$T cell dose differs from the doses in our cohort and the EBMT cohort, perhaps contributing to different outcomes.

Although our analysis did not show an impact of $CD34^+$T cell dose on transplant outcome, it is worth noting that >50% of the patients in our cohort received a $CD34^+$ cell dose of 4 to 8×10^6 cells/kg, and a minority (5% to 10%) received a dose $<2 \times 10^6$ cells/kg (Tables 1 and 2). In our opinion, this precludes an accurate conclusion as to the impact of $CD34^+$ cell dose on transplantation outcomes. Previous studies have evaluated this question with favorable outcomes with higher $CD34^+$T cell doses [33-35], although observing a higher risk of cGVHD with $CD34^+$ cell doses $>8 \times 10^6$ cells/kg [35,36] or $>10 \times 10^6$ cells/kg [37].

Donor age group was identified as a risk factor for the development of severe aGVHD and for worse DFS (donor age >50 years) in MUD HCT. The effect of donor age on the clinical outcomes is similar that seen in another study [16], which found a correlation between donor age and the $CD8^+$ T cell dose of the PBSC graft. Given the limited availability of $CD8^+$T cell dose in the PBSC grafts in our cohort, we could not assess this association with age. This study was congruent with other large studies in terms of results pertaining to well-known risk factors for GVHD, such as older recipient age [38] and a lower KPS score [39]. As expected, a higher DRI was predictive of a greater risk of relapse in both the MUD and MSD groups [40].

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

To our knowledge, this is the largest study reported to date addressing the impact of the T cell content of PBSC grafts on transplantation outcomes. In this registry study, the $CD3^+$T cell dose in the PBSC products did not influence the risk of aGVHD or cGVHD or other transplantation outcomes when using MSDs or 8/8 MUDs. Prospective studies are needed to determine whether the T cell subset ($CD4^+$, $CD8^+$, Tregs, and naïve T cells) contents of the allografts have a meaningful influence on transplantation outcomes. The results of the ongoing phase II clinical trial using a standardized $CD3^+$ T cell dose with HLA-matched related PBSC transplantations are awaited (NCT00959140). In addition, in the current era of post-transplantation 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 cGVHD of all severity with an elevated $CD3^+$ T cell dose with haploidentical PBSC HCT using PTCy [41]. $CD3^+$ T cell dose has also been shown to be predictive of graft failure with TCD allogeneic HCT [42].

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SUPPLEMENTARY DATA

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