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ORIGINAL REPORT

Improved Survival After Transplantation of More Donor Plasmacytoid Dendritic or Naïve T Cells From Unrelated-Donor Marrow Grafts: Results From BMTCTN 0201

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ABSTRACT

Purpose
To characterize relationships between specific immune cell subsets in bone marrow (BM) or granulocyte colony-stimulating factor–mobilized peripheral blood (PB) stem cells collected from unrelated donors and clinical outcomes of patients undergoing transplantation in BMTCTN 0201.

Patients and Methods
Fresh aliquots of 161 BM and 147 PB stem-cell allografts from North American donors randomly assigned to donate BM or PB stem cells and numbers of transplanted cells were correlated with overall survival (OS), relapse, and graft-versus-host disease (GvHD).

Results
Patients with evaluable grafts were similar to all BMTCTN 0201 patients. The numbers of plasmacytoid dendritic cells (pDCs) and naïve T cells (Tns) in BM allografts were independently associated with OS in multivariable analyses including recipient and donor characteristics, such as human leukocyte antigen mismatch, age, and use of antithymocyte globulin. BM recipients of > median number of pDCs, naïve $CD8^+$ T cells (CD8Tns), or naïve $CD4^+$ T cells (CD4Tns) had better 3-year OS (pDCs, 56% *v* 35%; $P = .025$; CD8Tns, 56% *v* 37%; $P = .012$; CD4Tns, 55% *v* 37%; $P = .009$). Transplantation of more BM Tns was associated with less grade 3 to 4 acute GvHD but similar rates of relapse. Transplantation of more BM pDCs was associated with fewer deaths resulting from GvHD or from graft rejection. Analysis of PB grafts did not identify a donor cell subset significantly associated with OS, relapse, or GvHD.

Conclusion
Donor immune cells in BM but not PB stem-cell grafts were associated with survival after unrelated-donor allogeneic hematopoietic stem-cell transplantation. The biologic activity of donor immune cells in allogeneic transplantation varied between graft sources. Donor grafts with more BM-derived Tns and pDCs favorably regulated post-transplantation immunity in allogeneic hematopoietic stem-cell transplantation.

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INTRODUCTION

Much of the clinical utility of allogeneic hematopoietic stem-cell transplantation (alloHSCT) in treating patients with hematologic malignancies depends on the graft-versus-leukemia (GvL) activity of donor T cells.[1-3] Improving outcomes after alloHSCT requires understanding how the GvL or graft-versus-host disease (GvHD) functions of donor T cells are regulated, including interactions with donor or host dendritic cells (DCs) and homing to hematolymphoid or GvHD-target organs.[1,4] Previous reports have suggested the content of donor DCs is associated with incidence of chronic GvHD and relapse,[5] and the content of $CD34^+$ cells is associated with survival[6] and GvHD among peripheral blood (PB) stem-cell allograft recipients.[7]

To explore associations between cell subsets in the allograft with clinical outcomes in a prospective clinical trial, fresh aliquots of bone marrow (BM) and PB stem-cell grafts collected from unrelated volunteer donors recruited in BMTCTN (Blood and Marrow Transplant Clinical Trials Network) 0201 were analyzed for their content of $CD34^+$ and immune cells. BMTCTN 0201 randomly assigned patients with myelodysplastic syndrome or leukemia to receive either BM or PB stem cells and demonstrated equivalent overall survival (OS), acute

Table 1. Demographic Characteristics of BMTCTN 0201 Patients and Donors for Whom Cellular Constituents of Grafts Were Characterized

Variable	Bone Marrow (n = 161) No.	%	Peripheral Blood Stem Cells (n = 147) No.	%
Recipient sex				
Male	86	53.4	85	57.8
Female	75	46.6	62	42.2
Recipient age, years				
0-10	6	3.7	2	1.4
11-20	16	9.9	6	4.1
21-30	30	18.6	29	19.7
31-40	23	14.3	28	19.0
41-50	29	18.0	33	22.4
51-60	36	22.4	33	22.4
≥ 60	21	13.0	16	10.9
CMV status				
Positive	88	54.7	67	45.6
Negative	72	44.7	80	54.4
Missing	1	0.6	0	0
Karnofsky performance status, %				
90-100	107	66.5	88	59.9
< 90	41	25.5	43	29.3
Missing	13	8.1	16	10.9
Primary disease at enrollment				
Acute myelogenous leukemia	68	42.2	78	53.1
Acute lymphoblastic leukemia	40	24.8	30	20.4
Chronic myelogenous leukemia	16	9.9	21	14.3
Myelodysplastic syndrome	35	21.7	15	10.2
Chronic myelomonocytic leukemia	1	0.6	1	0.7
Agnogenic myeloid metaplasia with myelofibrosis	1	0.6	2	1.4
Disease risk				
Low	122	75.8	105	71.4
High	39	24.2	42	28.6
Interval from diagnosis to treatment, months				
≤ 6	58	36.0	55	37.4
6-12	41	25.5	41	27.9
> 12	61	37.9	51	34.7
Unknown	1	0.6	0	0.0
Conditioning regimen				
C-TBI	75	46.6	70	47.6
Bu-Cy	49	30.4	42	28.6
Flu-Bu-ATG	29	18.0	25	17.0
Flu-Mel	8	5.0	10	6.8
GvHD prophylaxis regimen				
Cyclosporine/methotrexate	39	24.2	26	17.7
Tacrolimus/methotrexate	108	67.1	112	76.2
Other	14	8.7	9	6.1
Donor sex				
Male	109	67.7	93	63.3
Female	52	32.3	54	36.7
Donor CMV status				
Positive	53	32.9	56	38.1
Negative	108	67.1	91	61.9
Donor age, years				
18-30	68	42.2	57	38.8
31-40	53	32.9	43	29.3
41-50	34	21.1	35	23.8
51-60	5	3.1	12	8.2
Missing	1	0.6	0	0
No. of HLA mismatches at HLA-A, -B, -C, -DRB1				
0	121	75.1	118	80.3
1	32	19.9	27	18.4
2	6	3.7	2	1.4
3	2	1.2	0	0

Abbreviations: BMTCT, Blood and Marrow Clinical Trials Network; Bu-Cy, busulfan and cyclophosphamide; CMV, cytomegalovirus; C-TBI, cyclophosphamide and total-body irradiation; Flu-Bu-ATG, fludarabine, busulfan, and antithymocyte globulin; Flu-Mel, fludarabine and melphalan; GvHD, graft-versus-host disease.

GvHD, and relapse rates in both arms, with significantly more chronic GvHD seen among recipients of PB stem-cell grafts.[8] Results of preplanned analyses of graft constituents with outcomes suggest a significant association of the content of donor plasmacytoid DCs (pDCs) and naïve T cells (Tns) in BM grafts with transplantation outcomes—associations that were not seen among recipients of PB stem-cell grafts.

PATIENTS AND METHODS

Study Population

BMTCTN 0201 enrolled 551 pairs of unrelated donors and corresponding patients age < 66 years with a diagnosis of leukemia, myelodysplasia, or myelofibrosis for whom allogeneic transplantation was planned. Details of randomization, eligibility, and the statistical design of the study have been published previously.[8] Our study included 308 of the 526 patients (59%) who underwent transplantation in BMTCTN 0201 and excluded transplantation recipients involving grafts acquired in Germany. Recipients of BM grafts had the entire graft infused (after RBC or plasma depletion, if required), whereas 84% of recipients of PB stem-cell grafts had a portion of the graft cryopreserved for possible donor leukocyte infusion. The final data set consisted of samples of 161 BM and 147 PB stem-cell allografts collected at North American donor centers and shipped at 4°C to a central laboratory for immediate analysis. The data set excluded samples without laboratory or clinical data necessary to calculate infused cell doses, samples that arrived too late at the central laboratory for analysis or failed quality control testing, and transplantation recipients without complete clinical data. Median follow-up among survivors was 36 months.

Analysis of Graft Constituents

Studies were conducted according to the BMTCTN manual of procedures on graft characterization. Bead-based quantitation of $CD34^+$ progenitor cells was performed by staining up to 10 million leukocytes for 10 minutes at room temperature with a stem-cell antibody panel (CD34 PE, CD45 FITC, 7AAD, and CD38 APC), followed by ammonium chloride–potassium buffer lysis of RBCs and addition of equal volume of phosphate-buffered saline without washing (lyse no-wash assay), and adding fluorescent counting beads (Perfect Count Beads; Life Technologies, Carlsbad, CA) just before evaluation by flow cytometry. Phenotyping of B cells, natural killer cells, T cells, and DCs[9] was performed by staining samples with antibody panels (Appendix Table A1, online only) at room temperature for 30 minutes followed by a 10-minute lysis of RBCs and washing and pelleting cells twice before resuspending in 500 μL of

staining media (Appendix Fig A1, online only). Subsets of lymphocytes and DCs are listed in Appendix Table A2 (online only).

Statistical Analyses

The primary objective of the study was to correlate cell subsets in the graft with OS. Because the appropriate cut point was not known for individual graft cell subsets, the sample of PB stem-cell and BM recipients was divided into those who survived ≥ 1 year and those who died in the first year, because nearly all deaths occurred within the first year. Median values of cells transplanted per kilogram for graft cell subsets of interest were then described separately for survivors and those who died and compared using a nonparametric Mann-Whitney Wilcoxon test. To reduce the number of comparisons, and because several of these measurements were strongly correlated with one another, preliminary analysis was restricted to 46 cell subsets that were not strongly correlated with one another (Pearson's or Spearman correlation > 0.8). We used the false discovery rate criterion (q) to screen for cell subsets and selected only those with a q value < 0.15 for further investigation.[10,11] Finally, for graft cell subsets of interest, Kaplan-Meier estimates of the survival curves and cumulative incidence estimates for competing risk end points (acute GvHD, chronic GvHD, treatment-related mortality [TRM], and relapse) based on dichotomizing the values above or below the median were constructed. Hazard ratios (HRs) and 95% CIs from univariable and multivariable Cox models were constructed.

RESULTS

Characteristics of Transplantation Recipients

The demographics and characteristics of the final study patients and their BM or PB stem-cell grafts are listed in Table 1. There were no significant differences between the recipients of BM and PB stem-cell grafts analyzed in this study, and both groups reflected the demographics of those randomly assigned in BMTCTN 0201 (data not shown). As was true for all patients enrolled onto BMTCTN 0201, OS was similar among the recipients of BM versus PB stem-cell grafts included in this study (Appendix Fig A2, online only).

Donor Characteristics

Donors from whom BM or PB stem-cell grafts were collected were similar to recipients with regard to sex, cytomegalovirus serostatus, age, and number of HLA mismatches (Table 1). Of note, 20% to 25% of donors were HLA mismatched, with most mismatches occurring at a single HLA locus.

Graft Characteristics

As expected, numbers of nucleated cells transplanted into recipients of the PB stem-cell grafts were approximately three-fold higher than cells infused into BM recipients (median, 780 *v* 260 × 10^6 cells/kg; Appendix Table A3, online only). In addition, PB stem-cell recipients received a median of 4.2 × 10^6 $CD34^+$ cells/kg versus a median of 2.8 × 10^6 $CD34^+$ cells/kg among recipients of BM grafts ($P = .003$). The T-cell content of the PB stem-cell grafts was approximately 10-fold higher than that of BM grafts; the content of pDCs was only two-fold higher among recipients of PB stem-cell grafts compared with BM grafts (0.6 *v* 0.3 × 10^6 cells/kg). Numbers of other transplanted cells were proportionally greater in PB stem-cell than BM grafts (Appendix Table A3, online only).

Content of pDCs and Tns in BM Grafts Associated With Improved OS

Analysis of the association of the dose of transplanted cells with 1-year survival among recipients of BM transplantations identified two cell subsets of interest (pDCs and naïve $CD8^+$ T cells [CD8Tns]) as being potentially associated with post-transplantation survival based on q values of 0.128 for each. We also included the results of an analysis of naïve $CD4^+$ T cells (CD4Tns) because of their strong correlation with CD8Tns and similar findings. BM transplantation recipients who survived 1 year received more pDCs (0.4 × 10^6 cells/kg) compared with deceased patients (0.2 × 10^6 cells/kg; $P = .006$). Surviving BM patients had also received more Tns (median, 1.7 × 10^6 CD8Tns/kg and 3.5 × 10^6 CD4Tns/kg) compared with deceased patients (median, 1.0 × 10^6 CD8Tns/kg and 1.8 × 10^6 CD4Tns/kg; $P = .004$ and .018, respectively). Of note, neither the content of transplanted $CD34^+$ cells (median, 2.5 *v* 2.8 × 10^6 cells/kg) nor the

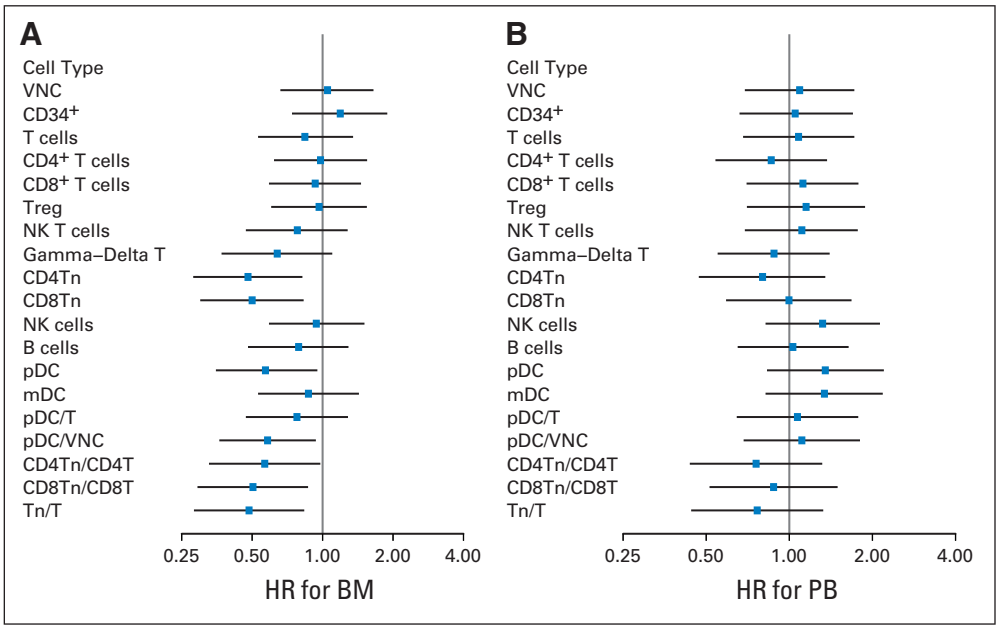

Fig 1. Significant association between content of cells in bone marrow (BM) and granulocyte colony-stimulating factor–mobilized peripheral blood (PB) stem-cell grafts with overall survival (OS) among those undergoing allogeneic transplantation with unrelated donor. Multivariable analysis showing hazard ratios (HRs) for OS based on content of viable nucleated cells (VNCs), $CD34^+$ cells, B cells, natural killer (NK) cells, T cells (T), and dendritic cells (DCs) as well as calculated ratios of cell subsets in graft. (A) Multivariable analysis of BM graft constituents. (B) Multivariable analysis of PB graft constituents. CD4Tn, naïve $CD4^+$ T cell; CD8Tn, naïve $CD8^+$ T cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; Tn, naïve T cell; Treg, T regulatory cell.

total number of T cells (median, 23.6 *v* 22.7 × 10^6/kg) was significantly different between deceased BM transplantation recipients and those who survived 1 year. No cell subset from PB stem-cell grafts was significantly associated with 1-year OS. In particular, patients who survived to 1 year after transplantation had received a median of 13.3 × 10^6 CD8Tns/kg and 0.5 × 10^6 pDCs/kg compared with transplanted cell doses of 13.5 × 10^6 CD8Tns/kg and 0.83 × 10^6 pDCs/kg among deceased PB stem-cell allograft recipients ($P > .05$). Multivariable analysis of individual cell subsets and ratios of Tns to total T cells, pDCs to total viable nucleated cells, and pDCs to T cells showed better OS among BM recipients of higher ratios of pDCs to viable nucleated cells Tns to T cells (Fig 1A). Analysis of PB stem-cell recipients showed a trend toward better OS among recipients of higher ratios of Tns to T cells that was not statistically significant; there was no indication that ratio of pDCs to nucleated cells or pDCs to T cells was associated with OS in recipients of PB grafts (Fig 1B). Multivariable analyses of other cell ratios listed in Appendix Table A3 (online only; eg, T regulatory cells [Tregs] to CD4 T cells, natural killer T cells to T cells, and memory T cells to T cells) did not reveal an association with relapse or GvHD in recipients of either graft type (data not shown). Subsequent analyses of associations of grafts constituents with clinical outcomes were therefore limited to BM graft recipients.

Recipients of More pDCs in BM Grafts Had Less TRM and Improved Long-Term OS

Three-year OS for BM recipients receiving > the median number of donor pDCs (0.3 × 10^6 cells/kg) was 56% versus 35% for those patients who received < the median number ($P = .025$; Fig 2A). Incidence curves for TRM (Fig 2B) show increased early mortality among recipients of a smaller number of pDCs in the graft but no significant effect on relapse (Fig 2C), acute GvHD (Fig 2D), or chronic GvHD (Fig 2E). Multivariable analyses of the association of donor pDCs with OS, disease-free survival (DFS), relapse, TRM, and chronic and acute GvHD are shown graphically as HRs in Figure 2F. Patients who received > the median number of pDCs had improved OS and DFS and less TRM compared with patients who received fewer pDCs. Of note, there were significantly fewer deaths resulting from graft rejection and from GvHD among patients who received > the median number of pDCs in BM grafts compared with recipients of fewer pDCs (Table 2).

Recipients of More Tns in BM Grafts Had Less TRM and Acute GvHD and Improved Long-Term OS

OS was significantly associated with a higher content of CD8Tns and CD4Tns (HR, 0.51 and 0.48; $P = .01$ and .007, respectively), as was a reduction in TRM (HR for death, 0.3 and 0.4; $P = .01$ and .035,

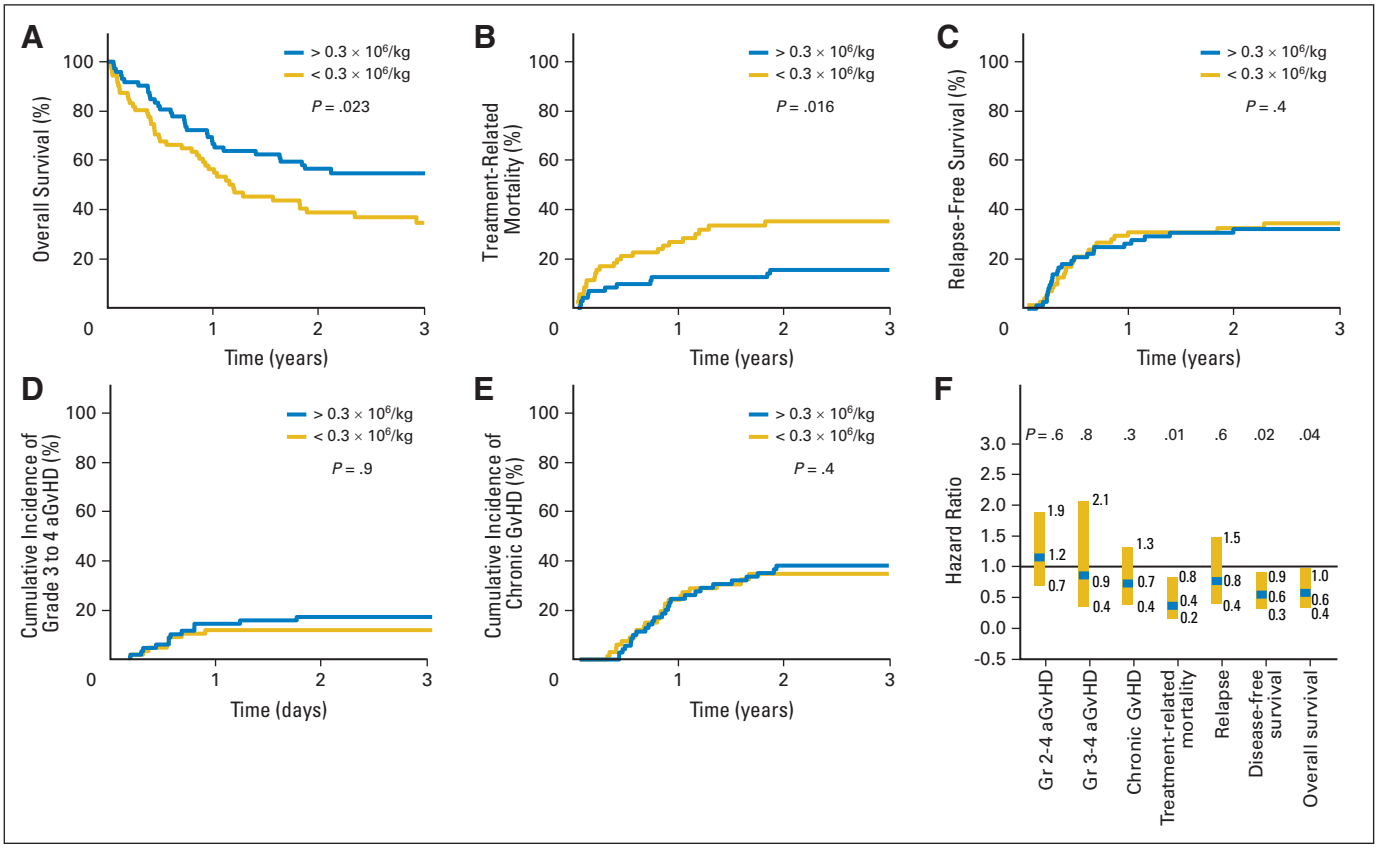

Fig 2. Larger numbers of donor plasmacytoid dendritic cells (pDCs) in bone marrow (BM) graft are associated with improved survival and decreased treatment-related mortality (TRM) after allogeneic transplantation with unrelated donor. (A) Estimated probability of 3-year overall survival (OS), stratifying 143 evaluable recipients of BM grafts by median number of transplanted donor pDCs; (B) incidence of TRM; (C) incidence of relapse; (D) incidence of grade 3 to 4 acute graft-versus-host disease (aGvHD); (E) incidence of chronic GvHD; and (F) multivariable analysis showing hazard ratio of content of pDCs in BM graft with aGvHD, chronic GvHD, TRM, relapse, disease-free survival, and OS. *P* values in (A) to (E) represent log-rank tests for patients undergoing transplantation with number of pDCs < or > median value of 0.3 × 10^6 cells/kg. *P* values in (F) represent results of multivariable analysis, as described in Patients and Methods.

Table 2. Causes of Death Among Deceased BM Transplantation Recipients Stratified by Content of pDCs, CD4Tns, and CD8Tns in Graft

Cause of Death	Content of pDCs in BM Allograft*			
	$< 0.3 \times 10^6$/kg (n = 71)		$> 0.3 \times 10^6$/kg (n = 72)	
	No.	% of 46 Deaths	No.	% of 33 Deaths
Relapse	21	46	22	67
Graft rejection	5	11	0	0
Acute GvHD	6	13	2	6
Chronic GvHD	5	11	3	9
Infection	3	7	1	3
Other	6	13	5	15
Total	46	100	33	100
	Content of CD4Tns in BM Allograft†			
	$< 2.6 \times 10^6$/kg (n = 66)		$> 2.6 \times 10^6$/kg (n = 63)	
	No.	% of 44 Deaths	No.	% of 26 Deaths
Relapse	22	50	17	65
Graft rejection	1	2	1	4
Acute GvHD	6	14	1	4
Chronic GvHD	4	9	4	15
Infection	5	11	0	0
Other	6	14	3	12
Total	44	100	26	100
	Content of CD8Tns in BM Allograft‡			
	$< 1.3 \times 10^6$/kg (n = 67)		$> 1.3 \times 10^6$/kg (n = 62)	
	No.	% of 44 Deaths	No.	% of 26 Deaths
Relapse	20	46	19	73
Graft rejection	2	5	0	0
Acute GvHD	7	16	0	0
Chronic GvHD	5	11	3	12
Infection	5	11	0	0
Other	5	11	4	15
Total	44	100	26	100

Abbreviations: BM, bone marrow; CD4Tn, naïve $CD4^+$ T cell; CD8Tn, naïve $CD8^+$ T cell; GvHD, graft-versus-host disease; pDC, plasmacytoid dendritic cell.

*Recipients of BM allografts analyzed for content of pDCs (n = 143) were stratified according to median number of pDCs transplanted per kilogram.

†Recipients of BM allografts analyzed for content of CD4Tns (n = 129) were stratified according to median number of CD4Tns transplanted per kilogram.

‡Recipients of BM allografts analyzed for content of CD8Tns (n = 129) were stratified according to median number of CD8Tns transplanted per kilogram.

respectively) and grade 3 to 4 acute GvHD (HR, 0.3 and 0.27; $P = .04$ and .012, respectively). Representing these associations graphically, patients who received > the median number of 1.3×10^6 CD8Tns/kg had a probability of 3-year OS of 56% compared with 37% among patients who received fewer CD8Tns/kg (Fig 3A). TRM was significantly higher among patients who received < the median number of CD8Tns (Appendix Fig A3, online only); there was no association with relapse (Appendix Fig A3, online only). Incidence of grade 3 to 4 acute GvHD was significantly higher among patients who received < the median number of CD8Tns compared with patients who received more CD8Tns (Fig 3B). Of note, the incidence of chronic GvHD at 2 years was not significantly higher (55%) among patients who received more CD8Tns, compared with 25% incidence among a larger number of surviving patients who received transplantations with fewer CD8Tns (Appendix Fig A3, online only). Results of a multivariable analysis for the association of the content of CD8Tns with clinical outcomes are shown in Figure 3C. Analysis of the same clinical outcomes with respect to CD4Tns in BM grafts yielded similar survival and incidence curves for OS, TRM, relapse, and acute and chronic GvHD in univariable and multivariable analyses (Fig 3D to 3F; Appendix Fig A3, online only). There were significantly fewer deaths resulting from acute GvHD ($P = .03$) among patients receiving > the median number of CD4Tns or CD8Tns in BM grafts compared with recipients of fewer Tns (Table 2). Additional phenotypic analysis suggested that transplantation of more BM $CD4^+$ and $CD8^+$ T cells expressing CD45RA, CCR7, and CD62L (Tns), CD127 (interleukin-7 receptor), and Ki-67 (proliferating T cells) was associated with better survival. Patients who survived > 1 year after transplantation received a median number of 2.6×10^6 $CD45RA^+/CCR7^+/CD62L^+$ CD4Tns/kg and 0.9×10^6 $CD45RA^+/CCR7^+/CD62L^+$ CD8Tns/kg compared with corresponding median values of 1.2 and 0.4×10^6/kg for CD4Tn and CD8Tn subsets among patients who died within the first year after transplantation ($P = .006$ and .003, respectively). These data suggest that transplanting activated Tns expressing interleukin-7 receptor and receptors targeting high endothelial venules (CD62L) and lymph nodes (CCR7) is associated with improved survival among recipients of BM grafts from unrelated donors.

Independent Effect of Donor-Naïve T Cells and pDCs on Transplantation Outcomes After Unrelated-Donor BM Transplantation

OS for the BM transplantation recipients who received > the median numbers of both CD8Tns and pDCs was superior to that of groups receiving more of one and less of the other cell subsets or < the median number of both cell subsets (Fig 4). This exploratory analysis suggested that there was no interaction between the numbers of either cell subsets in the graft ($P = .9$), but the small numbers in each subgroup precluded formal analysis of interactions between the groups.

DISCUSSION

Analysis of the association of cell subsets among > 300 patients undergoing transplantation in BMTCTN 0201 suggests significant associations of the content of pDCs, CD4Tns, and CD8Tns with OS among patients who received BM grafts. The study population was representative of recent transplantation practices (2003 to 2009) for patients receiving myeloablative conditioning regimens, and the demographics and clinical outcomes of patients whose grafts were available for analysis were similar to all patients enrolled onto BMTCTN 0201. Strengths of our study include enrollment of a large number of patients across multiple sites and prospective analysis of fresh aliquots of donor grafts. Findings from our study are relevant to the immunology of hematopoietic cell transplantation; more BM transplantations are performed because of lower rates of chronic GvHD.[8] However, conclusions from this study may not be applicable to patients with more advanced malignancies or those undergoing nonmyeloablative conditioning regimens.

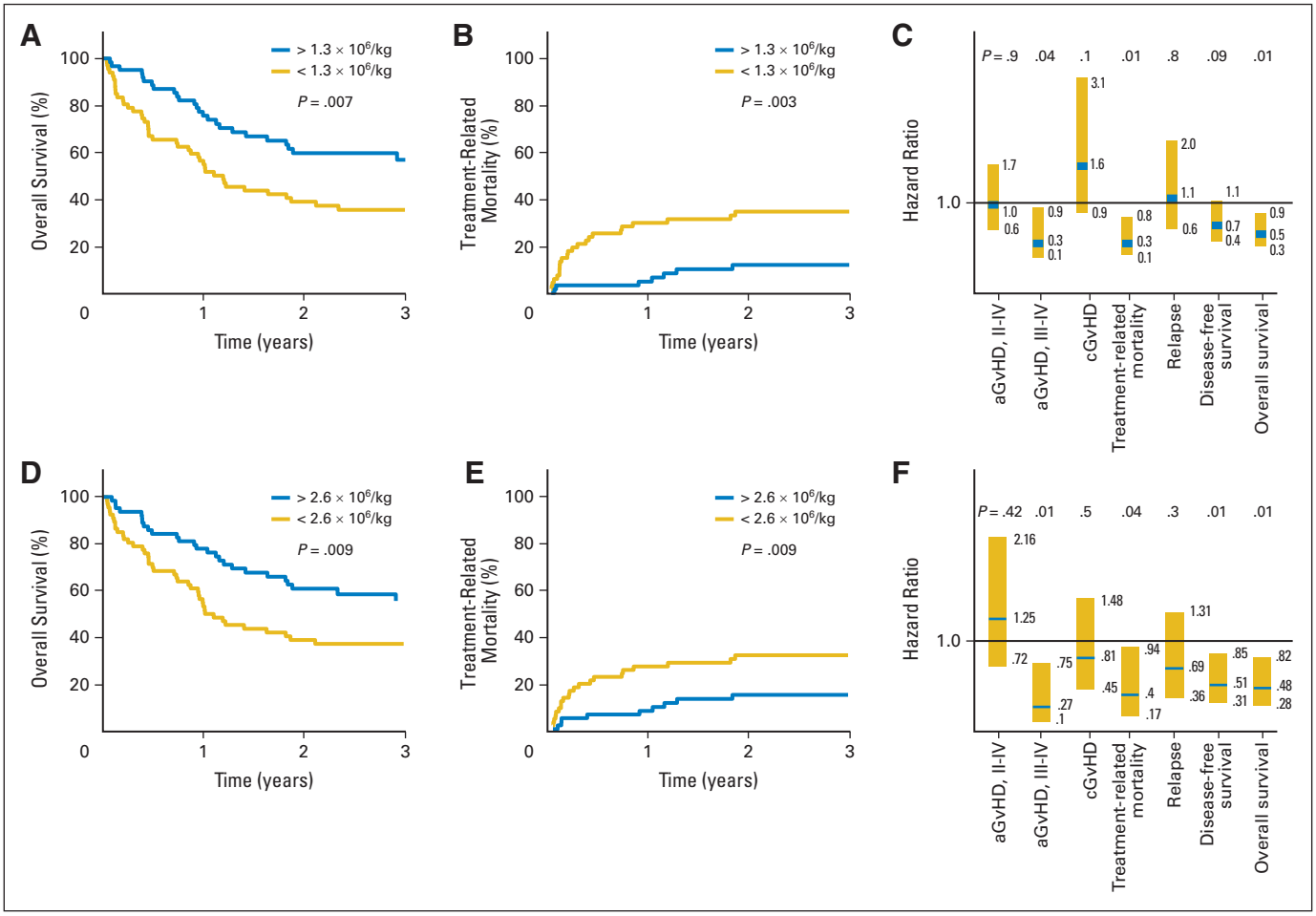

Fig 3. Larger numbers of donor naïve T cells (Tns) in bone marrow (BM) graft are associated with improved survival, decreased treatment-related mortality (TRM), and decreased grade 3 to 4 acute graft-versus-host disease (aGvHD) after allogeneic transplantation with unrelated donor. Estimated probability of 3-year overall survival (OS), stratifying 129 evaluable recipients of BM grafts by median number of transplanted donor (A) naïve $CD8^+$ T cells (CD8Tns) or (B) naïve $CD4^+$ T cells (CD4Tns). Incidence of TRM stratified by content of donor (B) CD8Tns or (E) CD4Tns. Multivariable analysis showing hazard ratio of content of (C) CD8Tns or (F) CD4Tns in BM graft with aGvHD, chronic GvHD (cGvHD), TRM, relapse, disease-free survival (DFS), and OS. *P* values in (A), (B), (D), and (E) represent log-rank tests for survival of patients undergoing transplantation with number of CD8Tns or CD4Tns < or > median value of 1.3×10^6 cells/kg or 2.6×10^6 cells/kg, respectively. *P* values in (C) and (F) represent results of multivariable analysis, as described in Patients and Methods.

One significant difference between BM and PB stem-cell grafts is that PB stem-cell grafts contain 10-fold more T cells than BM grafts, despite having similar numbers of $CD34^+$ cells and DC subsets. To explore whether the relative number of cell subsets might show an association with OS, we examined ratios of different graft cell subsets and OS in recipients of BM and PB stem cells. We found significantly less mortality in BM recipients who received grafts with a higher ratio of Tns to T cells and a nonsignificant trend favoring a higher ratio of Tns to T cells in PB stem-cell grafts. Although Tns per kilogram were higher in BM grafts from younger donors, multivariable models that showed an effect of Tns per kilogram on OS, relapse, and GvHD were adjusted for donor age. Although pDC content and ratio of pDCs to T cells were significantly associated with survival among BM recipients, there was no suggestion of an association between ratio of pDCs to T cells in PB stem-cell grafts and survival. Notably, BM grafts had a higher ratio of pDCs to T cells than PB stem-cell grafts (Appendix Table A3, online only). The salutatory effect of pDCs in BM may thus reflect the relatively higher proportions of pDCs compared with total T cells in the graft.

Another explanation for the lack of a significant association between the content of donor immune cells with transplantation outcomes in recipients of PB stem-cell grafts is that phenotypically defined cell subsets in BM and PB stem-cell grafts have distinct immune functions. The finding that the content of pDCs from BM but not PB stem-cell grafts is associated with less GvHD and graft rejection is supported by murine studies in which immature $CCR9^+$ pDCs are tolerogenic[12] and immunosupressive[13] compared with more mature pDCs from granulocyte colony-stimulating factor–mobilized blood.[14] Analysis of chemokine expression on murine pDCs suggests CCR9 expression is necessary for pDCs to home to sites of inflammation,[15] and recent studies have suggested chemokine receptor expression varies between pDCs from BM versus PB stem-cell grafts (unpublished data, S. Hosoba and E. Waller, 2014). Although an earlier report on BM transplantation from HLA-matched siblings found that more donor pDCs were associated with more relapse and less chronic GvHD,[5] our study found that transplantation of more donor BM–derived pDCs in BMTCTN 0201 was associated with decreased TRM and graft rejection, with a trend toward fewer deaths

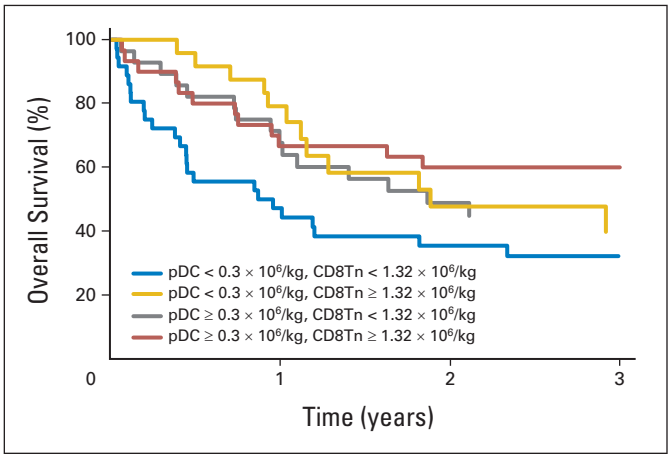

Fig 4. Overall survival (OS) of bone marrow (BM) transplantation recipients stratified by content of both naïve $CD8^+$ T cells (CD8Tns) and plasmacytoid dendritic cells (pDCs) in BM graft. Estimated probability of 3-year OS, stratifying 129 evaluable recipients of BM grafts by median number of transplanted donor CD8Tns and pDCs into four groups: patients who received > median number of both cell subsets (red line), patients who received > median number of one cell subset and < median number of other cell subset (gold and gray lines), and patients who received < median number of both cell subsets (blue line).

resulting from GvHD. A consistent finding from both studies is that donor pDCs reduced alloimmunity (GvL and/or GvHD) of donor T cells. BMTCTN differed from the earlier study from our institution in the use of HLA-mismatched unrelated rather than HLA-matched donors; also, fewer patients with chronic myelogenous leukemia underwent transplantation (who were sensitive to the GvL activity of donor T cells) in BMTCTN 0201.[16] The finding that pDCs modulate the alloreactivity of host cells is corroborated by preclinical observations from murine BM transplantation studies and human kidney allogeneic transplantations, in which donor pDCs have homed to and expanded in the lymph nodes of transplantation recipients,[17,18] facilitating donor allograft survival[19,20] and stem-cell engraftment.[19] Murine studies have shown that interferon gamma–induced expression of indoleamine 2,3-dioxygenase in donor pDCs regulates post-transplantation immunity,[21,22] limiting GvHD by generating Tregs[23] without inhibiting GvL activity of donor T cells.[18] Thus, donor pDCs may modulate both donor and host T-cell alloreactivity and attenuate both donor-versus-graft and GvHD reactions. Of note, pDCs are the primary source of interferon alfa in response to viral infections,[24] and a study correlating donor pDCs with post-transplantation infections is under way.

A weakness of our study is that associations of particular cell subsets in the grafts with survival or GvHD could represent chance or the effect of another highly correlated cell subset. The independent association of both CD4Tns and CD8Tns with transplantation outcomes makes a chance error less likely, but the high correlation between the content of both Tn subsets obscures the distinction between effects of CD4Tns versus CD8Tns on transplantation outcomes. Findings that the content of CD4Tns and CD8Tns is associated with OS and severe acute GvHD are in contrast with a body of work from murine models of BM transplantation that suggests donor splenic Tns are responsible for acute GvHD.[25-27] Explanations for the difference between our clinical data and that from murine studies include species differences for Tns in post-transplantation immunology, as has been observed in other systems,[28-30] and the fact that source of T cells can affect their alloreactivity, such that that BM T cells cause less GvHD than the peripheral T cells typically used in murine studies.[31] In our study, among recipients of more CD4Tns and CD8Tns, there were fewer deaths resulting from infection, graft rejection, or GvHD, suggesting donor Tns contributed to reconstitution of normal immune function. Although transplantation of allogeneic Tregs has been associated with protection from severe acute GvHD,[18] direct measurement of Treg content in the BM grafts in this study did not suggest a significant association with OS. In conclusion, on the basis of these results, additional clinical studies to evaluate BM-derived pDCs and Tns in alloHSCT are warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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Manuscript writing: All authors
Final approval of manuscript: All authors

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Appendix

Table A1. Antibody Panels Used to Characterize Cellular Subsets in Aliquots of BM and G-CSF–Mobilized PB Stem-Cell Allografts From Donors Enrolled Onto BMTCTN 0201

Immune Subset Panels	Tube No.	FL1	FL2	FL3	FL4	FL5	FL6	FL7
T-cell subsets	1	CD8 FITC	CD27 PE	7AAD	CD69 Cy7-PE	CD3 PE-Alx610	CD25 APC	CD4 Cy7-APC
NK and γ/δ T cells	2	CD8 FITC	CD56 PE	7AAD	CD16 Cy7-PE	CD3 PE-Alx610	TCR γ/δ APC	CD8 Cy7-APC
CD4/CD8 T cells (Ki67)	3	CD45RA FITC	Ki-67 PE	CD8 PerCP	CCR7 Cy7-PE	CD3 PE-Alx610	CD62L APC	CD4 Cy7-APC
CD4/CD8 T cells (CD127)	4	CD45RA FITC	CD127 PE	CD8 PerCP	CCR7 Cy7-PE	CD3 PE-Alx610	CD62L APC	CD4 Cy7-APC
B cells	5	CD5 FITC	CD27 PE	7AAD	CD19 Cy7-PE	CD3 PE-Alx610	CD14 APC	HLD-DR CY7-APC
Activated T cells and Tregs	7	CD8 FITC	foxp3 PE	—	CD69 Cy7-PE	CD3 PE-Alx610	CD25 APC	CD4 Cy7-APC
Isotype controls	8	IgG FITC	IgG PE	7AAD	IgG Cy7-PE	IgG PE-Alx610	IgG APC	IgG Cy7-APC

NOTE. Eight seven-color flow cytometry analysis tubes were prepared from fresh (nonfrozen) aliquot of each graft product shipped overnight at 4°C from collection center. Analysis of plasmacytoid and myeloid dendritic cell subsets used commercial cocktail of lineage antibodies (Becton Dickson, Mountain View, CA), as previously described.[9]

Abbreviations: APC, allophycocyanin; BM, bone marrow; BMTCTN, Blood and Marrow Clinical Trials Network; FITC, fluorescein; G-CSF, granulocyte colony-stimulating factor; IgG, immunoglobulin G; NK, natural killer; PB, peripheral blood; PE, phycoerythrin; PerCP, peridinin chlorophyll; Treg, T regulatory cell.

Table A2. Hematopoietic Cell Subsets Enumerated in Aliquots of BM and G-CSF–Mobilized PB Stem-Cell Allografts From Donors Enrolled Onto BMTCTN 0201

Selected Cell Subsets	Tube No.	Phenotype	Subset Definition
T-cell subsets	1	$CD3^+$	T cells
		$CD3^+$ $CD4^+$	CD4 T cells
		$CD3^+$ $CD8^+$	CD8 T cells
		$CD3^+$ $CD25^+$	$CD25^+$ T cells
		$CD3^+$ $CD25^+$ $CD69^+$	Activated T cells
		$CD3^+$ $CD4^+$ CD8- $CD69^+$	Activated CD4 T cells
		$CD3^+$ $CD4^-$ $CD8^+$ $CD69^+$	Activated CD8 T cells
		$CD3^+$ $CD4^+$ $CD8^-$ $CD25^+$ $CD69^+$	IL-$2R^{++}$ activated CD4 T cells
		$CD3^+$ $CD4^-$ $CD8^+$ $CD25^+$ $CD69^+$	IL-$2R^{++}$ activated CD8 T cells
		$CD3^+$ $CD4^+$ $CD8^-$ $CD25^+$ $CD27^+$ $CD69^-$	Regulatory T-cell compartment
NK and γ/δ T cells	2	$CD3^-$ $CD4^-$ $CD8^+$	$CD8^+$ NK cells
		$CD3^-$ $CD16^+$ $CD56^+$	NK cells
		$CD3^+$ $CD56^+$	NK-T cells
		$CD3^+$ $\gamma\delta TCR^+$	γ/δ T cells
		$CD3^+$ $\gamma\delta TCR^+$ $CD8^+$	$CD8^+$ γ/δ T cells
		$CD3^+$ $\gamma\delta TCR^+$ $CD56^+$	$CD56^+$ γ/δ T cells
		$CD3^-$ $CD16^-$ $CD56^+$	CD16− NK cells
T-cell subsets	3 and 4	$CD3^+$	T cells
		$CD3^+$ $CD127^+$	IL-$7R^+$ T cells
		$CD3^+$ $Ki67^+$	Proliferating T cells
		$CD3^+$ $CD4^+$	CD4 T cells
		$CD3^+$ $CD8^+$	CD8 T cells
		$CD3^+$ $CD4^+$ $CD127^+$	IL-$7R^+$ $CD4^+$ T cells
		$CD3^+$ CD4 $Ki67^+$	Proliferating $CD4^+$ T cells
		$CD3^+$ $CD8^+$ $CD127^+$	IL-$7R^+$ $CD8^+$ T cells
		$CD3^+$ $CD8^+$ $Ki67^+$	Proliferating $CD4^+$ T cells
		$CD3^+$ $CD4^+$ $CD45RA^+$ $CD62L^+$	Naïve CD4 T cells
		$CD3^+$ $CD4^+$ $CD45RA^-$ $CD62L^+$	Central memory CD4 T cells
		$CD3^+$ $CD4^+$ $CD45RA^-$ $CD62L^-$	Effector memory CD4 T cells
		$CD3^+$ $CD4^+$ $CD45RA^+$ $CD62L^-$	Terminal effector memory CD4 T cells
		$CD3^+$ $CD8^+$ $CD45RA^+$ $CD62L^+$	Naïve CD8 T cells
		$CD3^+$ $CD8^+$ $CD45RA^-$ $CD62L^+$	Central memory CD8 T cells
		$CD3^+$ $CD8^+$ $CD45RA^-$ $CD62L^-$	Effector memory CD8 T cells
		$CD3^+$ $CD8^+$ $CD45RA^+$ $CD62L^-$	Terminal effector memory CD8 T cells
		$CD3^+$ $CD4^+$ $CD45RA^+$ $CD62L^+$ $CCR7^+$ $CD127\pm$	Triple-positive naïve CD4 T cells
		$CD3^+$ $CD8^+$ $CD45RA^+$ $CD62L^+$ $CCR7^+$ $CD127\pm$	Triple-positive naïve CD8 T cells
B cells	5	$CD3^-$ $CD19^+$ $CD14^-$	B cells
		$CD3^-$ $CD19^+$ $CD14^-$ $CD27^+$	Activated B cells
		$CD3^-$ $CD5^+$ $CD14^-$ $CD19^+$	B1 B cells
		$CD3^-$ $CD5^+$ $CD14^-$ $CD19^+$ $CD27^+$	Activated B1 B cells
DCs	6	$CD3^-$ Lin^- $HLADR^+$ $CD16^-$ CD123lo $CD11c^+$	mDC precursors
		$CD3^-$ Lin^- $HLADR^+$ $CD16^-$ $CD123^+$ $CD11c^-$	pDC precursors
		$CD3^-$ $CD16^+$ $HLADR^+$ Lin^-	$CD16^+$ DC
Activated T cells and Tregs	7	$CD3^+$	T cells
		$CD3^+$ $CD4^+$	CD4 T cells
		$CD3^+$ $CD8^+$	CD8 T cells
		$CD3^+$ $CD25^+$	$CD25^+$ T cells
		$CD3^+$ $CD25^+$ $CD69^+$	Activated T cells
		$CD3^+$ $CD4^+$ $CD8^-$ $CD69^+$	Activated CD4 T cells
		$CD3^+$ $CD4^-$ $CD8^+$ $CD69^+$	Activated CD8 T cells
		$CD3^+$ $CD4^+$ $CD8^-$ $CD25^+$ $CD69^+$	IL-$2R^{++}$ activated CD4 T cells
		$CD3^+$ $CD4^-$ $CD8^+$ $CD25^+$ $CD69^+$	IL-$2R^{++}$ activated CD8 T cells
		$CD3^+$ $CD4^+$ $CD8^-$ $CD25^+$ foxp3$^+$ $CD69^-$	Regulatory T cells

NOTE. Phenotype of NK cell, T-cell, B-cell, and DC subsets (analyzed according to markers in flow cytometry panels in Appendix Table A1) are shown with description of subset to right.

Abbreviations: BM, bone marrow; BMTCTN, Blood and Marrow Clinical Trials Network; DC, dendritic cell; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; PB, peripheral blood; mDC, myeloid dendritic cell; NK, natural killer; pDC, plasmacytoid dendritic cell; Treg, T regulatory cell.

Table A3. Median and Interquartile Range of No. of Hematopoietic Progenitors and Immune Cells Transplanted in Recipients of BM and PB Stem-Cell Grafts

	BM			PB Stem Cells			
Cell Subset	No.	Median	IQR	No.	Median	IQR	*P*
Nucleated cells	161	259	205-331	136	778	486-1,144	< .001
$CD34^+$ progenitors	157	2.8	1.9-4.5	131	4.2	1.9-7.8	.003
$CD38^-$	148	0.11	0.06-0.26	130	0.07	0.03-0.14	< .001
$BAAA^+$	98	1.7	0.67-2.1	100	3	0.82-6.2	< .001
T cells	148	23	14-32	132	246	131-409	< .001
$CD4^+$ T cells	148	12	6.7-17	132	148	83-274	< .001
Naïve	129	2.6	0.89-4.7	107	31	3.9-80	< .001
Central memory	127	3.2	1.3-6.2	107	27	7-56	< .001
Effector memory	128	2.3	0.96-4.6	107	32	3.9-73	< .001
TEMRA	128	1.7	0.6-3.4	108	14	2.4-51	< .001
Tregs	146	2.2	0.68-6.3	132	30	8-92	< .001
$CD69^+$ (activated)	147	0.73	0.42-1.2	132	2	0.67-5.1	< .001
$CD25^+$	148	2.7	0.87-7.6	132	31	8.7-102	< .001
$Ki67^+$ (proliferating)	104	0.28	0.14-0.48	62	0.75	0.24-2	.001
IL-7 receptor positive	130	7.5	3.3-13	109	117	45-185	< .001
$CD8^+$ T cells	130	9.2	5.3-13	112	67	30-117	< .001
Naïve	129	1.3	0.55-2.1	107	13	1.9-39	< .001
Central memory	129	0.28	0.13-0.61	109	3.2	0.45-7.9	< .001
Effector memory	129	3.3	1.6-5	108	13	3.7-23	< .001
TEMRA	129	3.3	2-5.5	108	24	6-47	< .001
$CD69^+$ (activated)	144	2	1.2-3.4	132	2.3	0.80-4.9	.442
$Ki67^+$ (proliferating)	103	0.16	0.05-0.36	61	0.36	0.11-1.2	.004
IL-7 receptor positive	130	3.8	1.9-7.2	111	40	10-72	< .001
Gamma delta T cells	145	0.63	0.29-1.28	127	5.2	2-10	< .001
$CD8^+$	144	0.16	0.06-0.28	127	0.65	0.23-1.7	< .001
$CD56^+$	142	0.04	0.02-0.13	126	0.53	0.13-1.3	< .001
NK T cells	145	2.5	0.71-8.4	127	14	4.4-102	< .001
B cells	146	8.3	5.5-14	131	48	21-97	< .001
$CD69^+$ (activated)	146	1.2	0.64-2.1	130	8.5	3.6-17	< .001
$CD5^+$ $CD69^+$ (activated)	136	0.15	0.06-0.25	124	0.77	0.19-1.7	< .001
$CD16^+/CD56^{++}$	144	1.2	0.71-2.5	127	16	6.5-27	< .001
$CD16^-/CD56^+$	145	0.66	0.36-1.2	127	2.4	1.2-4.6	< .001
$CD8^+$	145	1.1	0.61-2.1	127	11	4.5-17	< .001
DCs							
mDCs	144	0.41	0.12-1.16	122	0.58	0.18-1.3	.175
pDCs	143	0.31	0.17-0.66	122	0.56	0.19-2.1	< .001
$CD16^+$	56	0.007	0.002-0.061	46	0.054	0.013-0.28	< .001
Relative concentrations							
Naïve CD4/CD4 T cells	120	24%	10%-38%	105	28%	12%-45%	.478
Naïve CD8/CD8 T cells	118	14%	8%-25%	105	28%	9.5%-56%	< .001
Naïve T/T cells	120	18%	8.9%-29%	103	27%	13%-42%	.015
pDCs/T cells	132	1.7%	0.85%-2.6%	119	0.39%	0.12%-0.86%	< .001
pDCs/NCs	143	0.15%	0.06%-0.24%	121	0.09%	0.04%-0.22%	.031
Central memory CD4/CD4 T cells	119	19%	11%-32%	105	25%	11%-41%	.254
Central memory CD8/CD8 T cells	118	3.6%	1.6%-6.7%	107	6.2%	1.4%-14%	.046
Gamma delta T cell/T cells	133	3.1%	1.6%-5.3%	124	2.1%	1.3%-4%	.005
Treg/CD4 T cells	146	26%	7.3%-64%	132	32%	7.5%-65%	.519
NK T cells/T cells	133	11%	3.9%-34%	124	9.4%	2.5%-50%	.801

NOTE. Nos. of cells transplanted represent 10^6 cells/kg for each specified subset. *P* values comparing No. of cells transplanted in BM versus PB stem-cell grafts are based on Mann-Whitney Wilcoxon test. Relative concentrations of cells in graft are shown as percentages in bottom portion of table. Quality control procedures compared content of total T cells analyzed in separate tubes using different antibody panels and excluded samples in which frequency of T cells was > 10% more or less than mean frequency of T cells based on all panels/tubes analyzed for that sample (Appendix Tables A1 and A2). No. columns represent Nos. of samples evaluable for each cell subset or relative concentration of cells in BM or PB stem-cell grafts. Analysis of Tregs and ratio of Tregs to CD4 T cells used larger No. of evaluable samples from panel 1, in which Tregs were defined as $CD3^+$ $CD4^+$ $CD69^-$ $CD25^+$ $CD27^+$. BAAA is fluorescent substrate for ALDH, which diffuses into intact and viable cells. In presence of ALDH, BAAA is converted into fluorescent reaction product BODIPY-aminoacate, which is retained inside cells.

Abbreviations: ALDH, Aldehyde dehydrogenase; BAAA+, BODIPY-amino acetaldehyde; BM, bone marrow; DC, dendritic cell; IQR, interquartile range; mDC, myeloid dendritic cell; NC, nucleated cell; NK, natural killer; PB, peripheral blood; pDC, plasmacytoid dendritic cell; TEMRA, T-cell effector memory, CD45RA positive; Treg, T regulatory cell.

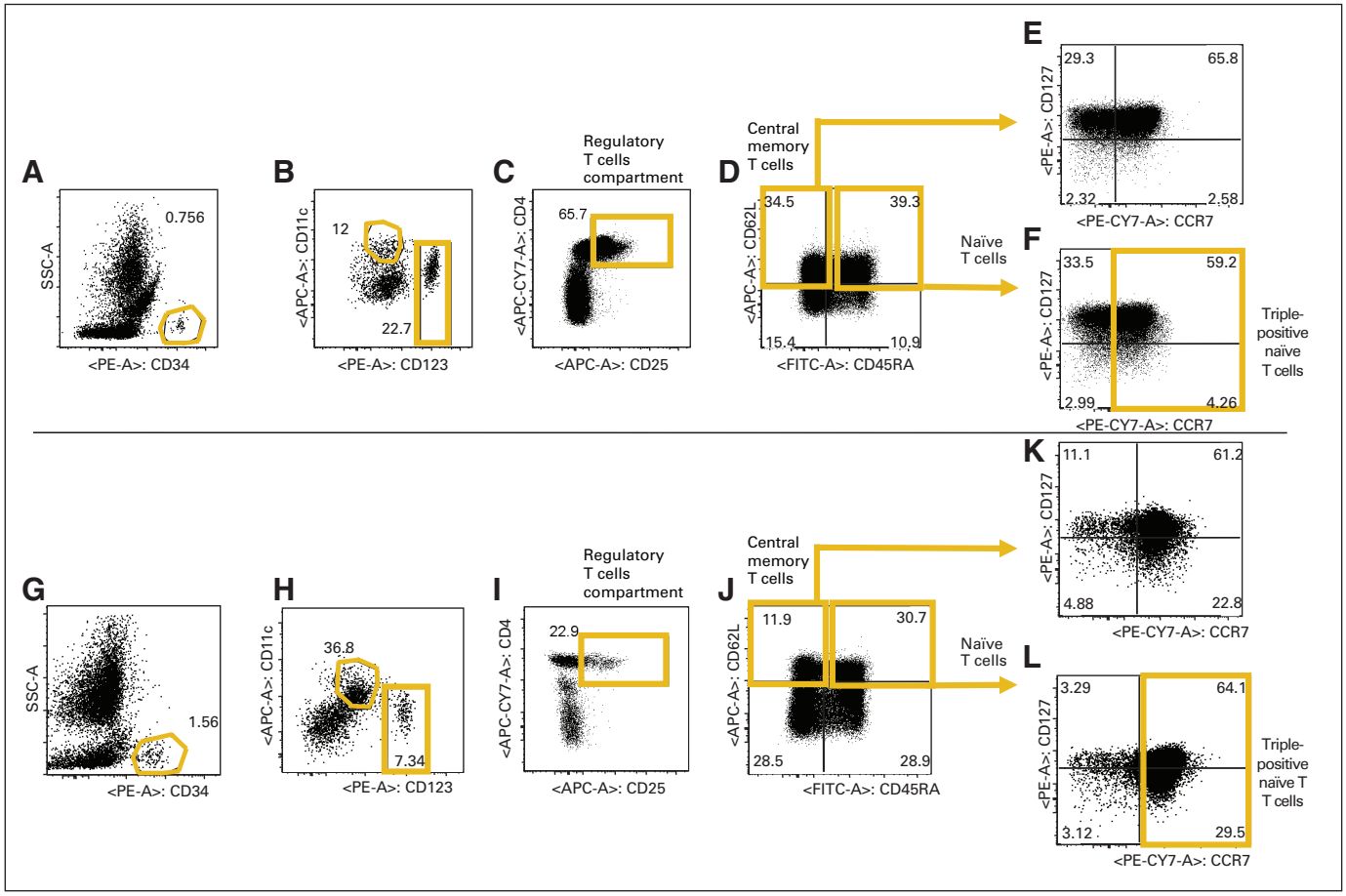

Fig A1. Flow cytometry dot plots of representative data showing phenotype of $CD34^+$ cells, dendritic cells, T regulatory cells (Tregs), and memory and naïve T-cell (Tn) subsets for single patient in donor peripheral blood (PB) apheresis sample (A to F) and donor bone marrow (BM) from participants in BMTCTN (Blood and Marrow Transplant Clinical Trials Network) 0201 (G to L). Cell populations of interest are shown gated with gold polygons. (A, G) Samples are gated on forward scatter and side scatter (SSC), with percentage of $CD34^+$ stem cells shown as fraction of $CD45lo/^+$ $7AAD^-$ population. (B, H) Percentages of $CD11c^+$ myeloid and $CD123^+$ plasmacytoid dendritic cells as fraction of lineage-negative (CD3, CD14, CD16, CD19, CD20) HLA DR^+ population are shown. (C, I) Data shown are gated on $CD27^+$, $CD69^-$ PB and BM T cells and show percentage of $CD4^+$ T cells with Treg phenotype $CD3^+$ $CD4^+$ $CD27^+$ $CD69^-$ $CD25^+$. (D, J) Percentages of central memory and Tn subsets within total population of $CD3^+$ T cells are shown. (E, F, K, L) Expression of CCR7 and CD127 (interleukin-7 receptor) on central memory and Tn subsets from the previously gated subsets in (D) and (J). (F, L) $CD45RA^+$, $CD62L^+$, and $CCR7^+$ triple-positive Tn populations are shown.

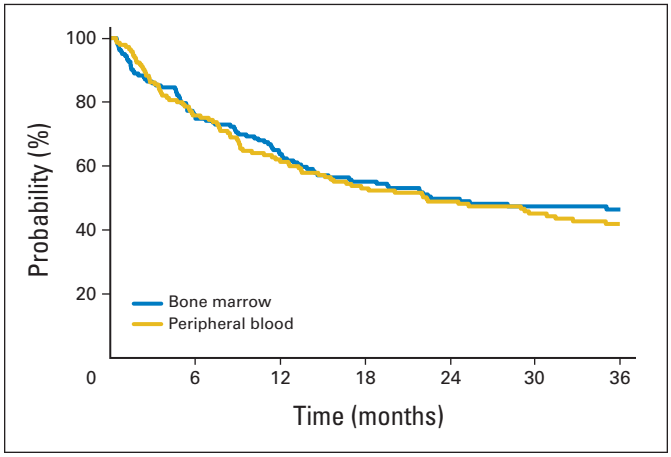

Fig A2. Overall survival among recipients enrolled onto BMTCTN (Blood and Marrow Transplant Clinical Trials Network) 0201 for whom graft samples were analyzed.

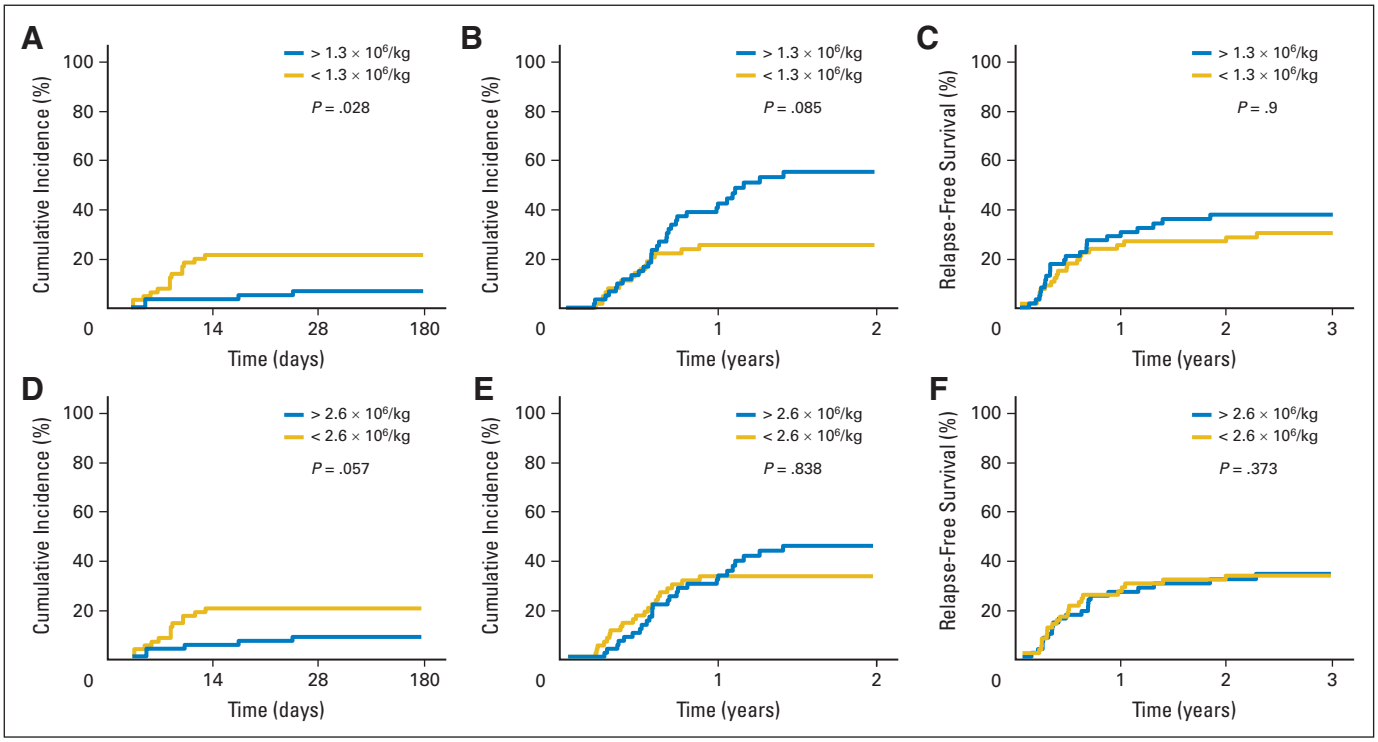

Fig A3. Larger numbers of donor naïve T cells (Tns) in bone marrow (BM) grafts are associated with decreased incidence of severe acute graft-versus-host disease (GvHD) after allogeneic transplantation with unrelated donor without significant effect on relapse or chronic GvHD. Incidence of grade 3 to 4 acute GvHD stratifying 129 evaluable recipients of BM grafts by median number of transplanted donor (A) naïve $CD8^+$ T cells (CD8Tns) or (B) naïve $CD4^+$ T cells (CD4Tns). Incidence of chronic GvHD stratified by content of donor (B) CD8Tns or (E) CD4Tns. Incidence of relapse stratified by content of donor (C) CD8Tns or (F) CD4Tns.