

Kinetics of immune cell reconstitution predict survival in allogeneic bone marrow and G-CSF–mobilized stem cell transplantation

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Key Points

- Recipients of G-CSF–mobilized grafts from unrelated donors have faster immune reconstitution than BM transplant recipients.
- Better survival among recipients of G-CSF–mobilized grafts with more blood T cells and BM recipients with more blood DCs.

The clinical utility of monitoring immune reconstitution after allotransplant was evaluated using data from Blood and Marrow Transplant Clinical Trials Network BMT CTN 0201 (NCT00075816), a multicenter randomized study of unrelated donor bone marrow (BM) vs granulocyte colony-stimulating factor (G-CSF)–mobilized blood stem cell (G-PB) grafts. Among 410 patients with posttransplant flow cytometry measurements of immune cell subsets, recipients of G-PB grafts had faster T-cell reconstitution than BM recipients, including more naive CD4⁺ T cells and T-cell receptor excision circle–positive CD4⁺ and CD8⁺ T cells at 3 months, consistent with better thymic function. Faster reconstitution of CD4⁺ T cells and naive CD4⁺ T cells at 1 month and CD8⁺ T cells at 3 months predicted more chronic graft-versus-host disease (GVHD) but better survival in G-PB recipients, but consistent associations of T-cell amounts with GVHD or survival were not seen in BM recipients. In contrast, a higher number of classical dendritic cells (cDCs) in blood samples at 3 months predicted better survival in BM recipients. Functional T-cell immunity measured *in vitro* by cytokine secretion in response to stimulation with cytomegalovirus peptides was similar when comparing blood samples from BM and G-PB recipients, but the degree to which acute GVHD suppressed immune reconstitution varied according to graft source. BM, but not G-PB, recipients with a history of grades 2–4 acute GVHD had lower numbers of B cells, plasmacytoid dendritic cells, and cDCs at 3 months. Thus, early measurements of T-cell reconstitution are predictive cellular biomarkers for long-term survival and response to GVHD therapy in G-PB recipients, whereas more robust DC reconstitution predicted better survival in BM recipients.

Introduction

Reconstitution of functional immunity is a crucial indicator of success in allogeneic hematopoietic stem cell transplantation, because donor immune cells present in the graft mediate the anticancer graft-versus-leukemia activity of the allotransplant maneuver, confer protection against conventional and opportunistic infections, and limit graft-versus-host disease (GVHD).^{1,2} Following initial hematopoietic engraftment, *de novo* development and differentiation of functional donor-derived adaptive immunity takes a year or more to fully develop,^{3,4} and dysfunctional immune reconstitution includes failure of the graft-versus-leukemia effect or excess alloactivation of donor T cells and subsequent GVHD.

Submitted 13 December 2018; accepted 15 May 2019. DOI 10.1182/bloodadvances.2018029892.

Deidentified individual participant data are available indefinitely and can be accessed through Biological Specimen and Data Repository Information Coordinating Center by

contacting Alan Howard (ahoward@nmdp.org). The study protocol is available at the BMT CTN Web site (<https://web.emmes.com/study/bmt2/>).

The full-text version of this article contains a data supplement.

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Previous studies have noted differences in the kinetics of immune reconstitution between bone marrow (BM) and granulocyte colony-stimulating factor (G-CSF)-mobilized blood stem cell (G-PB) recipients, as well as an indication that the kinetics of T-cell and dendritic cell (DC) reconstitution may predict survival and GVHD after allogeneic transplantation. In randomized and nonrandomized series of BM vs G-PB transplants from related donors, G-PB recipients had faster T-cell recovery posttransplant, faster recovery of functional immunity, and fewer infections.^{5,6} Lower day-30 lymphocyte counts predicted worse survival and more GVHD in 381 G-PB allotransplant recipients receiving tacrolimus and mycophenolate mofetil immunoprophylaxis.⁷ Reddy et al studied 50 recipients of predominantly G-PB grafts and found that higher blood levels of total dendritic cell (DC) numbers (plasmacytoid DCs [pDCs] plus classical DCs [cDCs]) immediately after neutrophil engraftment predicted 2-year survival and freedom from GVHD.⁸ Gonçalves et al studied 111 allogeneic transplant recipients, half of whom received BM grafts, and found that greater pDC or cDC amounts at 3 weeks and 2 months posttransplant was associated with significantly improved overall survival (OS) and less acute GVHD (aGVHD) posttransplant.⁹ Elze et al found that early posttransplant pDC and cDC reconstitution in the blood of 45 children, half of whom received BM grafts, predicted less GVHD but more relapse.¹⁰ Taken together, these reports indicate that the kinetics of immune reconstitution are predictive for outcomes, but the relationships among immune reconstitution, graft source, and specific immune cell subsets are not clear.

To gain a better understanding of how posttransplant immune reconstitution is interrelated with transplant outcomes, particularly GVHD, we analyzed serial blood samples from a large series of 529 patients with myelodysplastic syndrome or leukemia enrolled in a multicenter national trial that randomly assigned them to allogeneic BM or G-PB grafts from unrelated donors.¹¹ We hypothesized that the amount of donor-derived immune cells measured in the blood at serial time points posttransplant would identify patients at higher subsequent risk for developing GVHD and relapse and that graft source and immune reconstitution, particularly DC subsets, may interact.¹² We report herein that the amount of donor CD4⁺ T cells in the graft was correlated with 1-month blood levels of CD4⁺ T cells that, in turn, predicted better survival in G-PB recipients, as well as that higher numbers of cDCs at 3 months were associated with better long-term survival in BM recipients, particularly among patients with a history of grade 2-4 aGVHD.

Methods

Study population

Blood and Marrow Transplant Clinical Trials Network BMT CTN 0201 was a national randomized clinical trial that transplanted 529 patients with acute or chronic leukemia or myelodysplastic syndrome using BM vs G-PB from unrelated donors.¹¹ In brief, donors and recipients were matched at 7/8 or 8/8 HLA loci based upon HLA typing at intermediate resolution for HLA-A, -B, and -C, and at high resolution for HLA-DRB1, consistent with National Marrow Donor Program standard procedures operative at the time of study enrollment. Patients were conditioned with 1 of a number of allowable myeloablative regimens, mostly busulfan cyclophosphamide and cyclophosphamide-total body irradiation, with a smaller fraction of patients receiving less-intensive regimens, including

fludarabine plus busulfan and fludarabine plus melphalan.¹¹ GVHD prophylaxis was predominantly a calcineurin inhibitor and methotrexate, with 10% of patients receiving anti-thymocyte globulin during conditioning (supplemental Table 1). Data on immune reconstitution were missing for 15% of transplant patients. There were only a few significant differences in the demographics of patients who had 1-month CD4⁺ T-cell counts vs those with missing data: more marrow grafts (55% vs 45%), more total body irradiation-based conditioning (51% vs 43%), and more tacrolimus-based immunoprophylaxis (72% vs 68%) (data not shown). Central data committees reviewed and adjudicated key clinical outcomes, including death, relapse, GVHD, and infections. In particular, GVHD deaths were defined as any death following a diagnosis of aGVHD or chronic GVHD (cGVHD) in a patient who had not relapsed and was still receiving immune-suppressive drug therapy. Because all patients in this trial received what was deemed to be a myeloablative conditioning regimen at the time (including fludarabine/busulfan and fludarabine/melphalan), centralized testing of donor chimerism was not mandatory. The study was approved by the Institutional Review Board at multiple participating BMT CTN sites.

Measurements of posttransplant immune reconstitution

Standard procedures regarding the collection of clinical data and entry into a database were followed based on National Marrow Donor Program and National Heart, Lung, and Blood Institute guidelines. Analysis of immune reconstitution included 211 BM and 219 G-PB recipients who had ≥ 1 measurement of immune cells posttransplant. Blood samples were drawn at 1, 3, 6, 12, and 24 months posttransplant in heparinized tubes and shipped at 2°C to 8°C to a central reference laboratory and analyzed immediately by flow cytometry. Consistency and reproducibility of measurements of immune cell subsets from clinical samples shipped at 4°C over a 48-hour period have been established previously.¹³ Numbers of total T cells, B cells, natural killer (NK) cells, and DCs, as well as subsets of each population, were calculated as cells per microliter based upon their frequency in a lymphoid-mononuclear cell-gated population, and the absolute numbers of mononuclear cells were determined on an automated complete blood count, as previously described.¹² Plasma cytokine levels of interleukin-2 (IL-2) and IL-7 were determined by enzyme-linked immunosorbent assay. A mononuclear cell fraction was prepared by Ficoll-Hypaque centrifugation, and CD4⁺ and CD8⁺ T-cell subsets were isolated by fluorescence-activated cell sorting prior to determination of T-cell receptor excision circles (TRECs) by polymerase chain reaction amplification.¹⁴ Functional immunity was assessed by staining for expression of IL-2, interferon- γ (IFN- γ), or tumor necrosis factor- α (TNF- α) following 6 hours of activation with phorbol myristate acetate (PMA)/ionomycin or a pool of cytomegalovirus (CMV) peptides, as reported.¹⁵

Statistical analysis

Quantitative measures were summarized using median (range) and compared between groups using Wilcoxon rank-sum tests, whereas categorical variables were summarized using frequencies and compared between groups using χ^2 tests. Immune reconstitution measures were compared between treatment arms or by GVHD development within 100 days using Wilcoxon

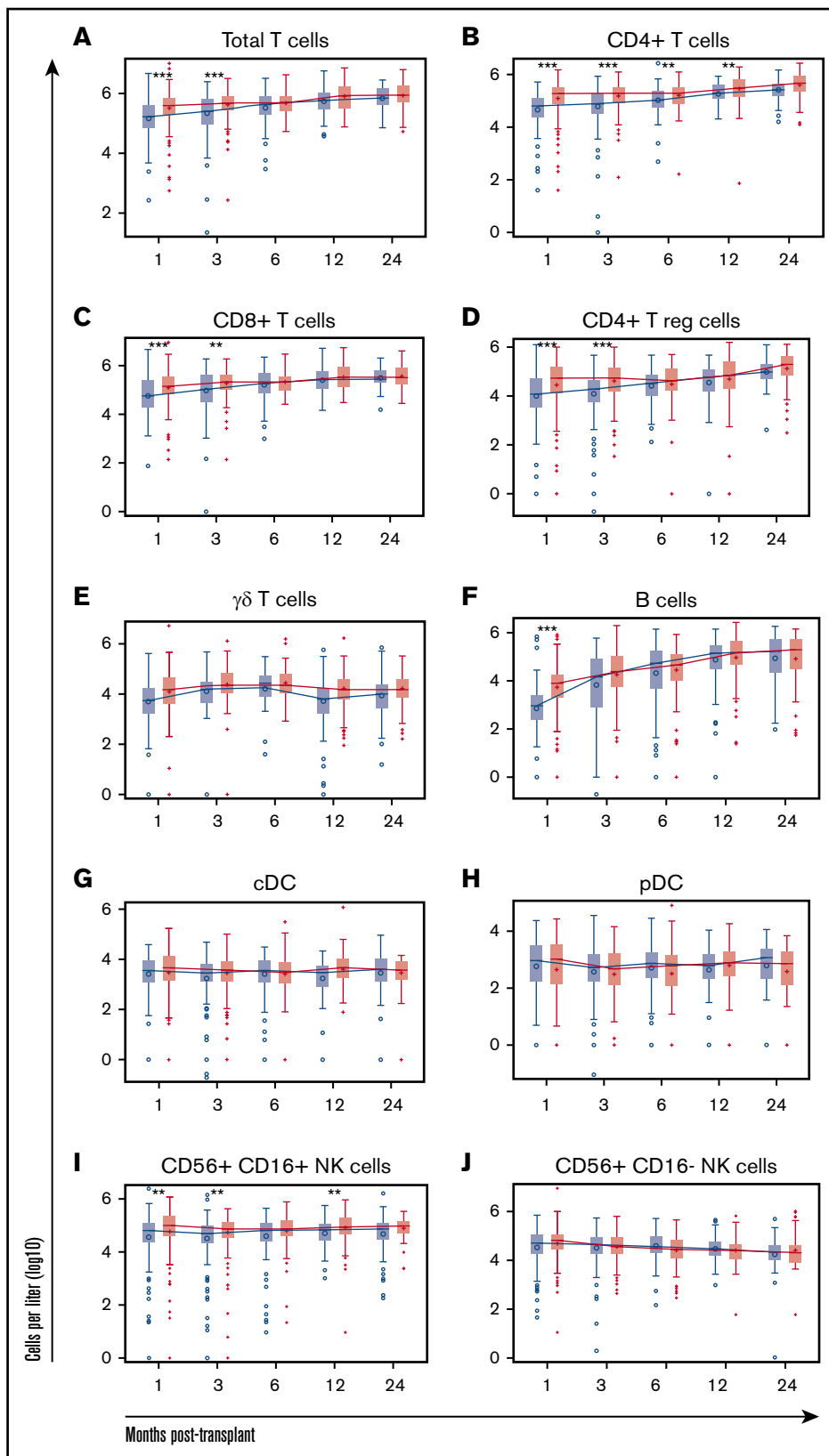


Figure 1. Recipients of G-PB grafts from unrelated donors have faster T-cell reconstitution than recipients of BM grafts. Mean numbers of immune cells in the blood are shown with the 25th and 75th percentiles at the upper and lower limits of the “box” and 95% confidence intervals (“whiskers”) at 1, 3, 6, 12, and 24 months posttransplant for recipients of BM (blue) or G-PB (red) grafts for total T cells (A), CD4⁺ T cells (B), CD8⁺ T cells (C), CD4⁺ Tregs (D), $\gamma\delta$ T cells (E), B cells (F), cDCs (G), pDCs (H), CD56⁺ CD16⁺ NK cells (I), and CD56⁺ CD16⁻ NK cells (J). *** $P < .001$, ** $P < .01$.

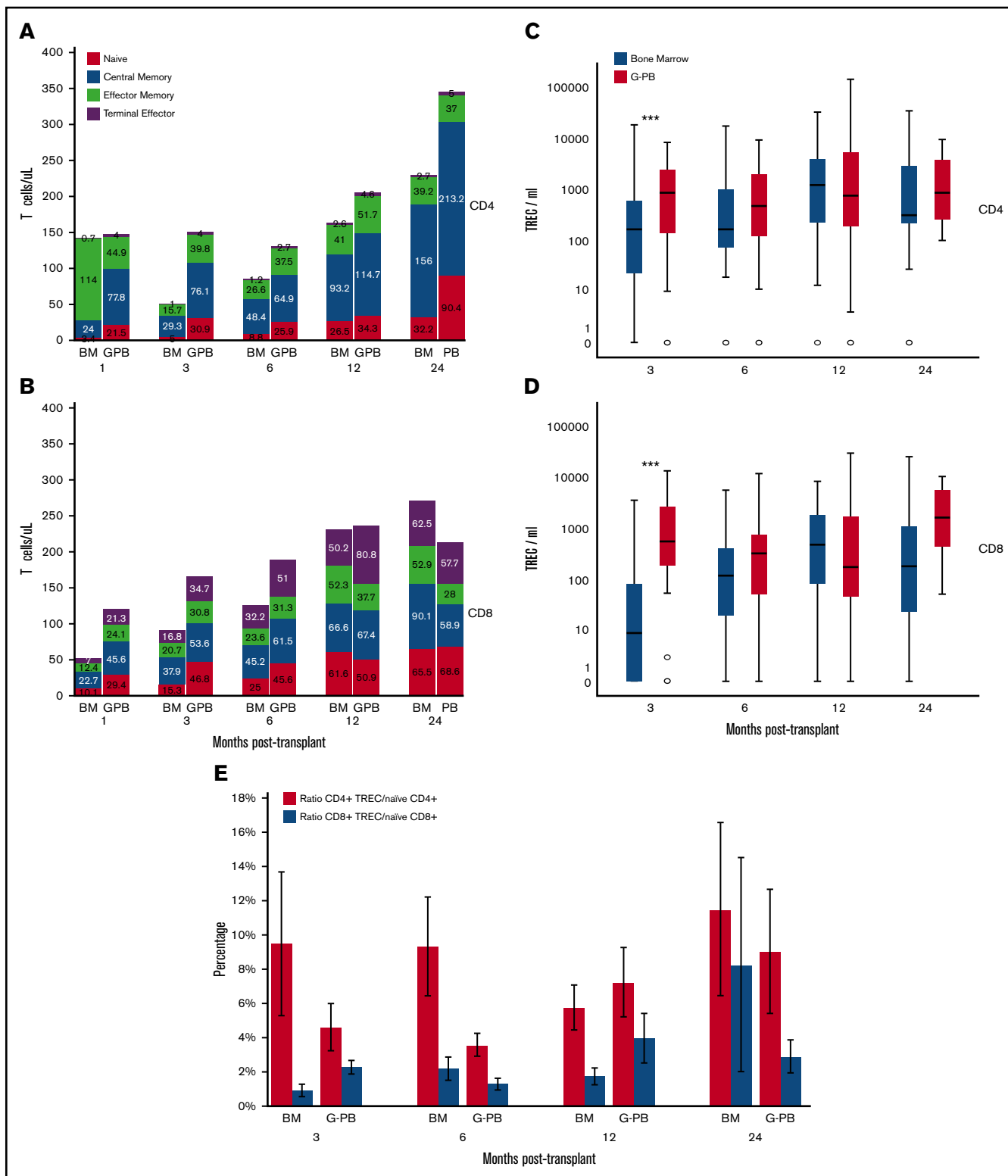


Figure 2. Recipients of G-PB grafts have faster T-cell reconstitution and thymopoiesis than BM graft recipients with more naive and TREC⁺ T cells. The proportions of CD4⁺ (A) and CD8⁺ (B) T cells with naive, central memory, effector memory, and terminal effector memory phenotype were determined by flow cytometry, as previously described,¹² in blood samples obtained at 1, 3, 6, 12, and 24 months posttransplant from recipients of BM or G-PB grafts. Numbers of TREC⁺ CD4⁺ T cells (C) and TREC⁺ CD8⁺ T cells (D) per milliliter. (E) Percentages of TREC⁺ CD4⁺ naive T cells and TREC⁺ CD8⁺ naive T cells among recipients of BM or G-PB grafts.

*** $P < .001$.

Table 1. Clinical outcomes for transplant recipients stratified by median numbers of CD4⁺ T cells and CD4⁺ T-cell subsets

	Greater than or equal to the median	Less than the median	P
Total CD4⁺ T cells	≥109/μL	<109/μL	
OS in all patients			
No. evaluated	138	138	.05
At 3 mo	96 (92-98)	88 (83-93)	.02
At 6 mo	85 (78-90)	75 (67-82)	.03
At 12 mo	76 (69-83)	62 (53-70)	.009
At 24 mo	62 (54-70)	47 (39-55)	.01
OS in BM recipients			
No. evaluated	40	84	.30
At 3 mo	95 (86-100)	92 (85-97)	.47
At 6 mo	85 (72-94)	77 (68-86)	.29
At 12 mo	72 (58-85)	67 (56-76)	.52
At 24 mo	59 (44-74)	50 (39-61)	.32
OS in G-PB recipients			
No. evaluated	98	54	.05
At 3 mo	96 (91-99)	83 (72-92)	.02
At 6 mo	85 (77-91)	70 (58-82)	.05
At 12 mo	78 (69-85)	54 (40-67)	.003
At 24 mo	63 (54-72)	43 (30-56)	.01
Grade 2-4 aGVHD in all patients			
No. evaluated	138	138	.40
At 3 mo	43 (35-52)	41 (33-50)	.71
At 6 mo	51 (42-59)	46 (37-54)	.40
Grade 3-4 aGVHD in all patients			
No. evaluated	138	138	.10
At 3 mo	10 (4-18)	3 (0-10)	.13
At 6 mo	10 (4-18)	3 (0-10)	.13
cGVHD in all patients			
No. evaluated	138	138	.005
At 3 mo	4 (2-8)	4 (1-7)	.76
At 6 mo	30 (22-38)	23 (17-31)	.22
At 12 mo	58 (50-66)	41 (33-49)	.003
At 24 mo	67 (59-74)	48 (40-56)	.001
Relapse in all patients			
No. evaluated	138	138	.58
At 3 mo	10 (6-16)	10 (6-16)	1.00
At 6 mo	18 (12-25)	20 (14-27)	.65
At 12 mo	23 (17-31)	27 (20-34)	.49
At 24 mo	28 (21-36)	30 (23-38)	.70
TRM			
No. evaluated	138	138	.11
At 3 mo	3 (1-6)	10 (6-16)	.01
At 6 mo	8 (4-13)	15 (10-22)	.06
At 12 mo	12 (7-17)	21 (15-28)	.03
At 24 mo	17 (11-23)	27 (20-34)	.04

Transplant patients were stratified into 2 groups based upon blood levels of CD4⁺ T cells above or below the median at 1 month posttransplant, and the frequency (%) of clinical events of interest (survival, grade 2-4 aGVHD, cGVHD, relapse, and TRM) were calculated at 3, 6, 12, and 24 months with 95% confidence intervals in parentheses. Numbers of evaluable samples analyzed for each immune cell subset are shown. Comparisons with $P \leq .01$ were considered significant.

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Table 1. (continued)

	Greater than or equal to the median	Less than the median	P
OS based upon CD4⁺ T-cell subsets			
Naive CD4 ⁺ T cells in all patients	≥9/μL	<9/μL	
No. evaluated	135	134	.23
At 3 mo	96 (92-99)	87 (81-93)	.007
At 6 mo	84 (78-90)	76 (69-83)	.08
At 12 mo	75 (67-82)	63 (55-71)	.04
At 24 mo	61 (52-69)	52 (44-60)	.15
Central memory CD4 ⁺ T cells in all patients	≥21/μL	<21/μL	
No. evaluated	135	134	.86
At 3 mo	93 (89-97)	90 (85-95)	.36
At 6 mo	81 (75-88)	79 (72-86)	.62
At 12 mo	70 (62-77)	69 (61-76)	.87
At 24 mo	58 (50-67)	54 (46-63)	.52
Effector memory CD4 ⁺ T cells in all patients	≥39/μL	<39/μL	
No. evaluated	135	134	.05
At 3 mo	96 (88-97)	88 (86-95)	.02
At 6 mo	85 (75-88)	75 (72-86)	.04
At 12 mo	75 (62-77)	63 (59-75)	.04
At 24 mo	63 (50-66)	50 (46-62)	.03
Terminal effector CD4 ⁺ T cells in all patients	≥1/μL	<1/μL	
No. evaluated	135	134	1.00
At 3 mo	93 (89-97)	90 (85-95)	.36
At 6 mo	81 (74-87)	80 (73-86)	.85
At 12 mo	67 (59-75)	71 (63-78)	.53
At 24 mo	58 (50-67)	55 (46-63)	.52
CD4 ⁺ Tregs in all patients	≥28/μL	<28/μL	
No. evaluated	140	138	.62
At 3 mo	93 (88-97)	90 (84-94)	.37
At 6 mo	81 (75-87)	77 (69-83)	.34
At 12 mo	72 (64-79)	64 (56-72)	.17
At 24 mo	56 (48-65)	52 (43-60)	.46

Transplant patients were stratified into 2 groups based upon blood levels of CD4⁺ T cells above or below the median at 1 month posttransplant, and the frequency (%) of clinical events of interest (survival, grade 2-4 aGVHD, cGVHD, relapse, and TRM) were calculated at 3, 6, 12, and 24 months with 95% confidence intervals in parentheses. Numbers of evaluable samples analyzed for each immune cell subset are shown. Comparisons with $P \leq .01$ were considered significant.

rank-sum tests at each time point. Landmark analyses of survival and disease-free survival based on high (greater than or equal to the median) vs low (less than the median) immune reconstitution measures at the landmark time were conducted using Kaplan-Meier estimates and compared using the log-rank test. Landmark analyses of cGVHD, treatment-related mortality (TRM), or relapse were summarized using cumulative incidence. Multivariate modeling of OS, starting at a landmark time of 100 days, was done using Cox regression, adjusted for the same covariates as identified in the primary trial.¹¹ Forest plots were constructed by adding select immune reconstitution measurements at the landmark time (above median vs below median) to the multivariate model and estimating the hazard ratio and associated confidence interval for mortality. Spearman correlations between donor graft cell doses and the corresponding immune reconstitution measurement

were estimated at each time point. $P \leq .01$ was considered significant in all analyses as an ad hoc adjustment for multiple comparisons.

Results

Recipients of G-PB grafts have faster T-cell reconstitution than recipients of BM grafts

First, we compared the kinetics of immune cell reconstitution between BM and G-PB recipients. Of note, recipients of G-PB received a median of 246×10^6 donor T cells per kilogram vs 23×10^6 donor T cells per kilogram among recipients of BM grafts.¹² Total T cells, CD4⁺ T cells, CD8⁺ T cells, and regulatory T cell (Treg) subsets recovered more quickly among recipients of G-PB grants than BM grafts, consistent with previous reports¹⁶ (Figure 1A-D).

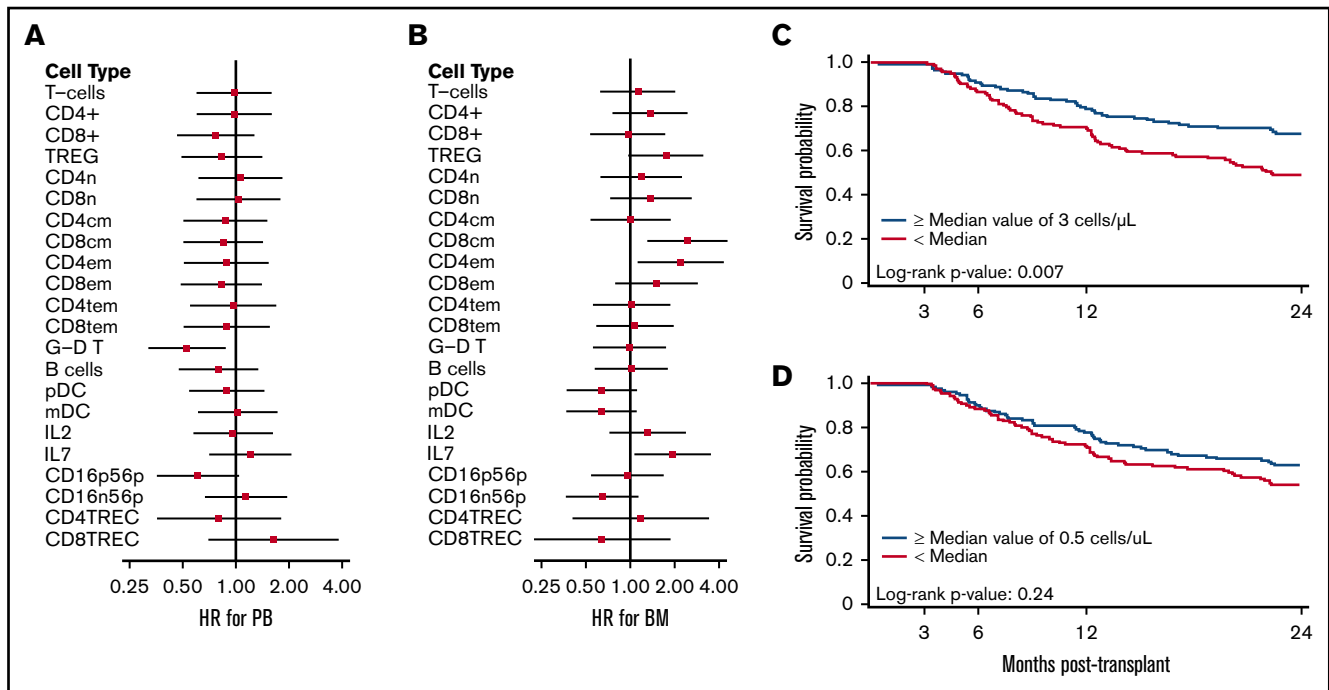


Figure 3. The amount of cDCs in the blood at 3 months posttransplant predicts survival after allogeneic HSCT from unrelated donors. (A) Multivariate analysis showing the hazard ratios (HRs) for the numbers of viable T cells, T-cell subsets, B cells, NK cells, DC subsets (cDCs/myeloid DCs and pDCs), CD4⁺ and CD8⁺ TREC, and plasma IL-2 and IL-7 levels with OS for recipients of G-PB grafts. (B) Multivariate analysis showing HRs for the numbers of immune cells and plasma cytokine levels with OS for recipients of BM grafts. (C) Estimated probability of 3-year OS, stratifying 291 evaluable recipients of BM and G-PB grafts by the median number of cDCs in blood samples at 3 months posttransplant. (D) Estimated probability of 3-year OS by the median number of pDCs in blood samples at 3 months posttransplant.

The ratio of CD4⁺/CD8⁺ T cells was similar between the 2 groups of patients; the median value in most cases was between 0.5 and 2 (supplemental Figure 1). In contrast, kinetics of $\gamma\delta$ T cells, B cells, cDCs, pDCs, and NK cells was similar comparing recipients of BM vs G-PB grafts (Figure 1E-J). Examining naive, central memory, effector memory, and terminal effector T-cell subsets defined by expression patterns of CD45RA and CD62L,¹² we found that reconstitution of CD4⁺ and CD8⁺ naive and central memory T-cell subsets was faster for G-PB than BM graft recipients (Figure 2A-B). Analysis of the amount of TRECs within sorted CD4⁺ and CD8⁺ T cells showed that TREC⁺ CD4⁺ T cells were more frequent than TREC⁺ CD8⁺ T cells and that recipients of G-PB grafts had significantly more TREC⁺ CD4⁺ T cells at 3 months posttransplant than BM recipients, consistent with enhanced thymopoiesis (Figure 2C-D). The percentages of naive CD4⁺ T cells that were TREC⁺ were similar between 3 and 24 months posttransplant (~6%) in recipients of BM and G-PB grafts (Figure 2E). The percentage of TREC⁺ naive CD8⁺ T cells was more variable, with most mean values ~2% (Figure 2E). We tested whether the kinetics of immune reconstitution reflects the numbers of corresponding immune cells in the graft. Only numbers of pDCs and CD4⁺ T cells in the graft were correlated with blood levels of the corresponding cells posttransplant; the numbers of CD4⁺ T cells in the graft correlated with blood levels of CD4⁺ T cells 1 and 3 months posttransplant (supplemental Table 2). In contrast, the amount of donor pDCs in the graft was significantly associated with pDC levels at 1 month posttransplant but not at subsequent time points (supplemental Table 2). These data are consistent with the biology of T cells, which are long lived,

whereas marrow-derived pDCs have a posttransplant survival that is measured in weeks.¹⁷

Measurements of T-cell subsets in blood at 1 and 3 months posttransplant predicted survival in G-PB recipients

Next, we addressed the hypothesis that measurements of specific immune cells at defined times posttransplant predict clinical outcomes, including GVHD, relapse, and death. Given the central role of T cells in GVHD and graft-versus-leukemia, we first compared posttransplant survival for subgroups of patients defined by the median number of T cells in blood at 1, 3, and 6 months posttransplant. Overall survival at 1 and 2 years ($P = .009$ and 0.01 , respectively) was greater among patients with more than the median numbers of CD4⁺ T cells at 1 month posttransplant (Table 1). Separate analyses of BM and G-PB graft recipients showed significantly better survival among G-PB patients with higher 1-month CD4⁺ T cell counts but not among BM recipients (Table 1). Higher numbers of naive and central memory CD4⁺ T cell subsets at 1 month were also significantly associated with better survival among G-PB recipients but not BM recipients (supplemental Table 3). There was no difference in relapse-related death at 4 years of follow-up when comparing BM recipients with greater than the median vs less than the median number of CD4⁺ T cells (29% vs 30%) or in G-PB recipients (28% vs 31%). The major contributor to increased death among patients with fewer CD4⁺ T cells was TRM, with a trend toward higher 3-month TRM in patients with fewer vs more CD4⁺ T cells at

Table 2. Clinical outcomes among patients stratified by the median numbers of cDCs or pDCs in the blood at 3 months posttransplant

	Greater than or equal to the median	Less than the median	P
cDCs	≥3/μL	<3/μL	
No. evaluated	134	133	
Incidence of posttransplant events			
Cumulative grade 2-4 aGVHD at 6 mo	45 (36-53)	60 (52-68)	.01
Cumulative grade 3-4 aGVHD at 6 mo	12 (6-20)	11 (4-20)	.89
Cumulative cGVHD at 2 y	60 (51-68)	63 (55-71)	.7
TRM at 2 y	13 (8-19)	23 (17-31)	.02
Relapse at 2 y	31 (23-39)	31 (23-39)	.97
Disease-free survival at 2 y	57 (48-65)	46 (37-54)	.07
OS at 2 y for all patients	68 (60-76)	49 (40-57)	.001
OS for BM recipients (N = 124 across strata)	65 (52-76)	45 (33-58)	.03
OS for G-PB recipients (N = 143 across strata)	65 (54-76)	58 (46-69)	.35
No. of deaths at 2 y	n = 69	n = 90	
Cause of death, n (%)			
Primary disease	46 (67)	46 (51)	
Graft failure	1 (1)	2 (2)	
aGVHD	6 (9)	7 (8)	
cGVHD	10 (14)	29 (32)	
Infection	2 (3)	4 (4)	
Organ failure	0	1 (1)	
New malignancy	1 (1)	0	
TTP	0	1 (1)	
IPN	1 (1)	0	
GI bleed	1 (1)	0	
Unknown	1 (1)	0	
pDCs	≥0.5/μL	<0.5/μL	
No. evaluated	134	133	
Cumulative grade 2-4 aGVHD at 6 mo	42 (34-50)	63 (55-71)	<.001
Cumulative grade 3-4 aGVHD at 6 mo	9 (3-16)	14 (7-24)	.31
Cumulative cGVHD at 2 y	57 (49-66)	65 (57-73)	.18
TRM @ 2 y	13 (8-19)	23 (17-31)	.02
Relapse at 2 y	34 (26-42)	28 (21-36)	.31
Disease-free survival at 2 y	54 (45-62)	49 (40-57)	.43
OS at 2 y	63 (54-71)	54 (46-63)	.15
OS for BM patients (n = 124 across strata)	63 (51-74)	47 (35-59)	.03
OS for G-PB patients (n = 143 across strata)	61 (50-72)	62 (50-73)	.92
No. of deaths at 2 y	n = 77	n = 82	
Cause of death, n (%)			
Primary disease	51 (66)	41 (50)	.57
Graft failure	1 (1)	2 (2)	
aGVHD	4 (5)	9 (11)	
cGVHD	15 (19)	24 (29)	
Infection	3 (4)	3 (4)	
Organ failure	0	1 (1)	
New malignancy	1 (1)	0	

The cumulative incidence of posttransplant complications, including death, relapse, TRM, aGVHD, and cGVHD, were determined among patients with greater than or fewer than the median number of cDCs or pDCs in the blood at 3 months posttransplant. Causes of death were assigned by a central data adjudication committee. Unless otherwise indicated, data are % (95% confidence interval).

GI, gastrointestinal; IPN, interstitial pneumonia; TTP, thrombotic thrombocytopenic purpura.

Table 2. (continued)

	Greater than or equal to the median	Less than the median	P
TTP	0	1 (1)	
IPN	1 (1)	0	
GI bleed	1 (1)	0	
Unknown	0	1 (1)	

The cumulative incidence of posttransplant complications, including death, relapse, TRM, aGVHD, and cGVHD, were determined among patients with greater than or fewer than the median number of cDCs or pDCs in the blood at 3 months posttransplant. Causes of death were assigned by a central data adjudication committee. Unless otherwise indicated, data are % (95% confidence interval).

GI, gastrointestinal; IPN, interstitial pneumonia; TTP, thrombotic thrombocytopenic purpura.

1 month (median, 10% [range, 6-16] vs 3% [range, 1-6], $P = .01$; 2-year TRM: median, 27% [range, 20-34] vs 17% [range, 11-23], $P = .04$, respectively). Notably, the association of lower 1-month CD4⁺ T-cell numbers with higher TRM at 3 months trended toward significance among G-PB recipients, with a median of 13% (range, 5-23) TRM vs 2% (range, 0-6) TRM ($P = .02$) after stratification by low vs high CD4⁺ T-cell counts, respectively, compared with 8% (range, 3-15) TRM vs 5% (range, 0-14) TRM ($P = .47$) among BM recipients stratified by median CD4⁺ T-cell counts at 1 month. Deaths from GVHD and infection contributed to the differences in TRM, with aGVHD-related death occurring in 10% of patients with less than the median number of CD4⁺ T cells and 5% of patients with more than the median number and death from infection and idiopathic pneumonia (without GVHD) occurring in 4% vs <1% of patients stratified by low vs high CD4⁺ T-cell counts, respectively. cGVHD-related death occurred in 8% vs 12% of patients stratified by low vs high CD4⁺ T cells, respectively ($P =$ not significant). Of note, deaths from infection among patients receiving active therapy for GVHD were scored by the data adjudication committee for this study as GVHD-related deaths. There was no significant difference in the distribution of causes of death when comparing BM vs G-PB recipients stratified by 1-month CD4⁺ T-cells counts, although death from aGVHD or cGVHD was higher among G-PB recipients than BM recipients (22% vs 13%), as has been reported previously. Survival based upon numbers of total T cells, CD8⁺ T cells, NK cells, B cells, and DCs at 1 month posttransplant did not meet the prespecified threshold of significance (supplemental Table 4).

Next, we examined the association of T-cell counts at 3 months with survival. In a univariate analysis, 2-year survival was not different when all subjects (pooling BM and G-PB recipients) were divided according to their median numbers of total T cells or CD4⁺, CD8⁺, $\gamma\delta$ TCR⁺, or Treg T-cell subsets at 3 months (supplemental Table 5; supplemental Figure 2). Separate multivariable analyses in BM and G-PB recipients showed that higher numbers of $\gamma\delta$ TCR⁺ T cells were associated with a decreased risk for death among G-PB graft recipients (Figure 3A), particularly those patients with a history of grade 2-4 aGVHD. Patients with more than the median number of $\gamma\delta$ TCR⁺ T cells (10 cells per microliter) had a median 75% (range, 65-84) 2-year survival vs 51% (range, 40-62) 2-year survival ($P = .002$) for those with less than the median number. Notably, higher levels of $\gamma\delta$ TCR⁺ T cells were not associated with a decreased risk for relapse.¹⁸ Although not significant at the threshold of $P \leq .01$, death from relapse was more frequent in patients with greater than the median number vs less than the median number of $\gamma\delta$ TCR⁺ T cells at 3 months (45 deaths vs 33 deaths, respectively), whereas

death from aGVHD or cGVHD was more common in patients with fewer vs more $\gamma\delta$ TCR⁺ T cells: 25 deaths vs 19 deaths, respectively. Analysis of subgroups of BM vs G-PB recipients showed a similar trend (data not shown). In contrast, BM recipients with greater than the median number of CD4⁺ effector memory and CD8⁺ central memory T-cell numbers at 3 months had worse survival (Figure 3B).

Higher numbers of blood DCs at 3 months posttransplant predicted better long-term survival in BM recipients

In a multivariable analysis of survival performed separately on BM and G-PB recipients, there was a trend toward an association between the amounts of pDC and cDC (also called myeloid DCs) in blood at 3 months and 2-year survival (Figure 3A-B). To address the relevance of immune monitoring to the posttransplant clinical management of patients in whom the graft source has already been selected, we pooled data on recipients of BM and G-PB grafts for univariate analyses. Median 2-year survival among patients with more than the median number (of 3 cDCs per microliter) at 3 months was 68% (range, 60-76) vs 49% (range, 40-57) among patients with <3 cells per microliter ($P = .001$) (Table 2; Figure 3C). In contrast, there was no significant association between blood pDC numbers at 3 months posttransplant and 2-year survival in the pooled population of patients (Figure 3D). Analyzing the association between pDC and cDC counts and survival by graft type, the predictive significance of higher DC subsets tended to be greater in BM recipients than in G-PB recipients, with a relative excess of death from GVHD among patients who had fewer cDCs or fewer pDCs (Table 2). The numbers of cDCs and pDCs (above or below the median at 6 months posttransplant) showed a nonsignificant trend with subsequent survival, with a median of 78% (range, 70-86) vs 66% (range, 57-74) 2-year survival for pDCs ($P = .04$) and 78% (range, 70-85) vs 67% (range, 58-75) 2-year survival for cDCs ($P = .07$), respectively.

Lower blood DC counts reflect prior aGVHD and increased risk for GVHD-related death

Because GVHD can cause “immune paralysis,”¹⁹ we examined whether lower blood levels of CD4⁺ T cells at 1 month and DCs at 3 months are surrogate markers for a history of aGVHD and whether their association with survival is a consequence of GVHD. Notably, the incidence of grade 1-4 aGVHD that had occurred by the time of blood sampling at 1 month was not significantly different

Table 3. Relationship between history of aGVHD and immune cell subsets in blood at 3 months posttransplant.

	No grade 2-4 aGVHD by day 100	Grade 2-4 aGVHD by day 100	P
No. evaluated	164*	144*	
Decreased among patients with a history of grade 2-4 aGVHD, n/μL (95% CI)			
$\gamma\delta$ TCR ⁺ T cells	14 (2-1682)	7 (0-316)	<.001
BM	11 (0-578)	3 (0-315)	<.001
G-PB	107 (0.1-715)	9 (0-5120)	.22
B cells	34 (0-932)	8 (0-2014)	<.001
BM	36 (0-571)	2 (0-606)	<.001
G-PB	33 (0-932)	16 (0-2014)	.17
cDCs	4 (0-101)	2 (0-32)	.001
BM	4 (0-39)	3 (0-34)	<.001
G-PB	5 (0-101)	16 (0-2014)	.15
pDCs	0.7 (0-36)	0.4 (0-9)	<.001
BM	0.8 (0-36)	0.3 (0-6)	<.001
G-PB	0.6 (0-15)	0.4 (0-9)	.11
Not affected by a history of grade 2-4 aGVHD, n/μL (95% CI)			
Total T cells	447 (0-3247)	346 (0-2629)	.06
Total CD4 ⁺ T cells	135 (0-994)	114 (0-1210)	.52
Naive CD4 ⁺ T cells	11 (0-282)	9 (0-423)	.88
Effector memory CD4 ⁺ T cells	32 (0-420)	24 (0-583)	.53
Central memory CD4 ⁺ T cells	59 (0-678)	53 (0-584)	.33
Terminal effector CD4 ⁺ T cells	2 (0-279)	2 (0-86)	.67
CD4 ⁺ Tregs	29 (0-693)	41 (0-979)	.3
Total CD8 ⁺ T cells	197 (0-1872)	151 (0-1872)	.09
Naive CD8 ⁺ T cells	35 (0-539)	21 (0-1360)	.21
Effector memory CD8 ⁺ T cells	28 (0-580)	24 (0-580)	.09
Central memory CD8 ⁺ T cells	53 (0-1197)	38 (0-1168)	.06
Terminal effector CD8 ⁺ T cells	32 (0-907)	21 (0-1368)	.16
NK cells			
16 ⁺ 56 ⁺ double positive	70 (0-1376)	50 (0-395)	.3
16 ⁻ 56 ⁺ single positive	46 (0-450)	36 (0-610)	.54

Blood samples drawn 3 months posttransplant from patients with a history of grade 2-4 aGVHD had lower numbers of B cells, $\gamma\delta$ TCR⁺ T cells, and DC subsets compared with patients without a history of grade 2-4 aGVHD.

CI, confidence interval.

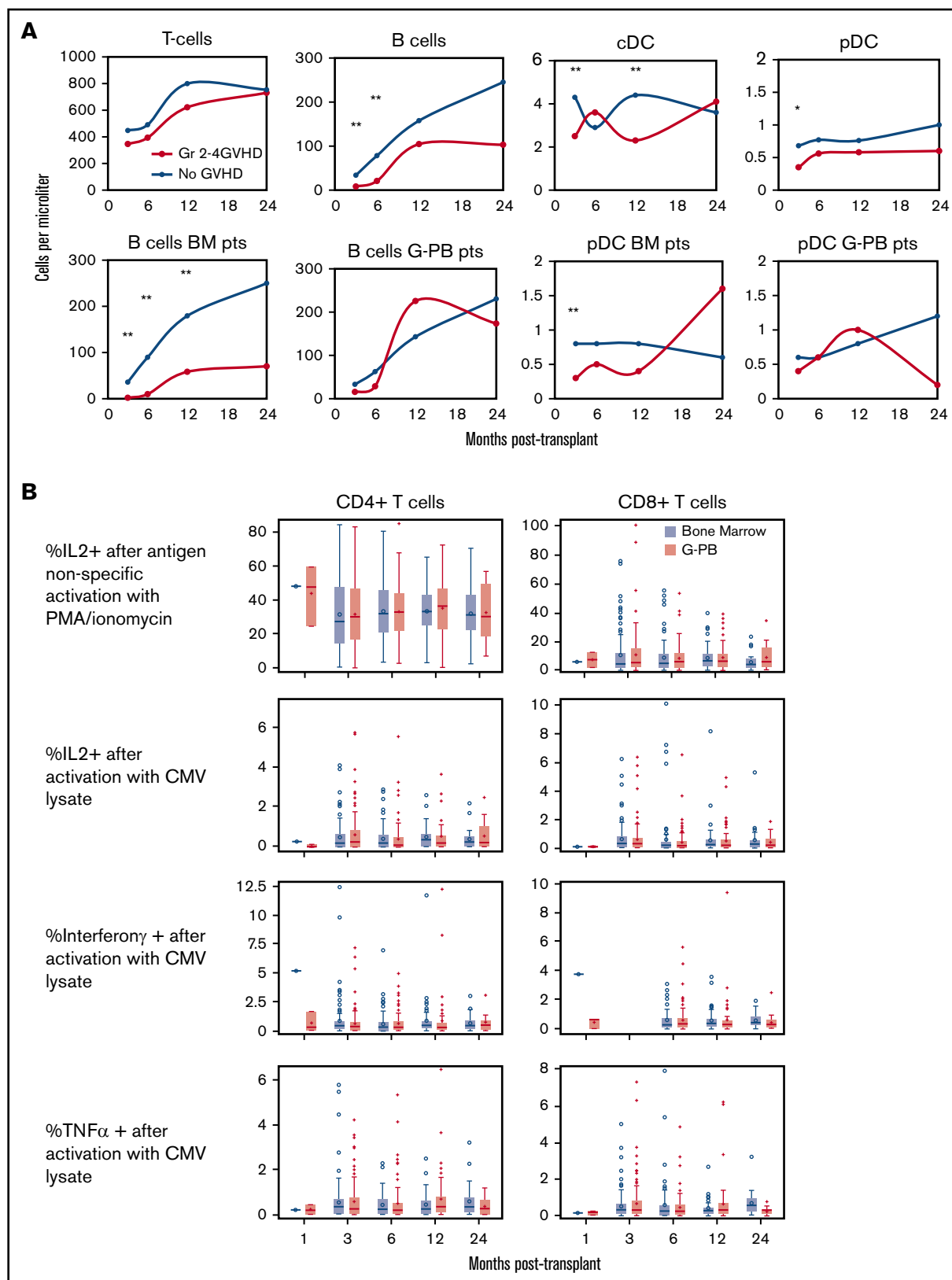
*Listed numbers of samples are for analysis of total T cells. Numbers of samples for analysis of other immune cell subsets were slightly less in some cases.

when comparing patients with higher 1-month CD4⁺ T-cell counts (44%) vs those with number below the median (39%). In contrast, a history of grade 2-4 aGVHD was associated with lower numbers of $\gamma\delta$ TCR⁺ T cells, B cells, cDCs, and pDCs in 3-month blood samples, especially among BM recipients (Table 3). Although patients with greater than median blood levels of cDCs had lower cumulative incidences of grade 2-4 aGVHD, the rates of grade 3-4 aGVHD, cGVHD, TRM, or relapse did not vary in association with cDC numbers (Table 2). Notably, the numbers of cDCs in the blood at 3 months was associated with a trend toward better 2-year survival among patients with a history of grade 2-4 aGVHD, with 63% (range, 51-74) survival among patients with more than the median number of cDCs vs 43% (range, 32-55) survival for patients with fewer cDCs ($P = .02$), with a similar trend toward higher rates of death from GVHD

among patients with fewer cDCs or pDCs ($P = .025$ and $.036$, respectively).

Functional immunity was similar comparing recipients of BM vs G-PB grafts

Because GVHD triggers a series of inflammatory cytokines that suppress lymphoid development, as well as pDC maturation,¹⁹ we measured the kinetics of immune reconstitution following a diagnosis of grade 2-4 aGVHD. Median numbers of B cells, cDCs, and pDCs were significantly higher at 3 months posttransplant among patients with no history of grade 2-4 aGVHD than among patients without a history of GVHD (Figure 4A, upper panels). The kinetics of B-cell recovery among BM recipients was slower among those who had developed grade 2-4 aGVHD,



whereas recipients of G-PB had equivalent kinetics of B-cell recovery, irrespective of their GVHD history (Figure 4A, lower panels). Of note, B-cell recovery was not predictive of OS (supplemental Table 4). With respect to whether more robust thymopoiesis protects from GVHD, the amount of CD4⁺ and CD8⁺ TRECs at 3 months was not associated with the subsequent incidence of cGVHD (data not shown). Finally, we measured functional immunity in T cells by incubating mononuclear cells with a CMV lysate overnight and then measuring the frequency of CD4⁺ and CD8⁺ T cells that expressed IL-2, IFN- γ , or TNF- α . Comparing blood samples from recipients of BM vs G-PB, there was no significant differences in the median percentage of CD4⁺ or CD8⁺ T cells that synthesized IL-2, IFN- γ , or TNF- α in response to nonspecific activation with PMA/ionomycin or antigen-specific activation following short-term incubation with CMV peptides (Figure 4B).

Discussion

The current randomized study represents the largest prospective multicenter study of immune reconstitution in allogeneic transplant recipients described in the literature. Although G-PB grafts have, on average, ~10-fold more T cells than allogeneic BM grafts,¹² the incidence of aGVHD and relapse is comparable between recipients of these 2 graft sources in randomized clinical trials,¹¹ as well as registry studies.²⁰ Our primary focus was to compare reconstitution kinetics of lymphocytes and DCs between BM and G-PB recipients and to determine whether posttransplant measurements of engrafted immune cells predict clinical outcomes. Results confirm that G-PB recipients have faster immune reconstitution than BM recipients and show that the CD4⁺ T-cell numbers in the graft were correlated with the initial kinetics of CD4⁺ T-cell reconstitution. Higher numbers of donor-derived CD4⁺ T cells and naive CD4⁺ T cells at 1 month and higher numbers of cDCs at 3 months posttransplant predicted long-term survival and informed subsequent risks for death, particularly among patients with a history of grade 2-4 aGVHD. Surprisingly, the predictive significance of specific immune cells posttransplant varied by the specific graft type received. Faster reconstitution of CD4⁺ T cells at 1 month posttransplant was associated with improved survival in G-PB recipients, with decreased TRM and increased cGVHD, consistent with reports that physical depletion of donor T cells from the graft²¹ or partial *in vivo* depletion of donor T cells by anti-thymocyte globulin leads to a decreased incidence of aGVHD and cGVHD posttransplant^{22,23} but often to increased death from opportunistic infections.²³⁻²⁵ In contrast, higher 3-month levels of DCs were associated with better survival in BM recipients. Of note, there was no association between the numbers of CD34⁺ T cells in the graft and the 1-month or 3-month CD4⁺ T cell count in BM or G-PB recipients (data not shown).

An interesting aspect of this study is the relationship between GVHD and immune reconstitution. The 3-month blood samples reflect the effects of prior aGVHD, because they were drawn on day 90 \pm 14 days, with aGVHD occurring at a median onset of 35 days, and 90% of aGVHD occurring by day 90 posttransplant. Blood levels of pDCs and B cells were lower in BM transplant

recipients who developed aGVHD, consistent with previous studies, using murine BM transplantation models, reporting that GVHD limits pDC differentiation²⁰ and cGVHD impairs B-cell maturation² and suppresses B-cell quantity and diversity,²⁶ leading to increased autoreactive B cells²⁷ and decreased numbers of naive and transitional B cells.²⁸ Lower pDC and B-cell counts may reflect the effects of inflammation associated with GVHD (IFN- γ and TNF- α) that compromise stem cell differentiation into pDCs and B cells,¹² as well as the effects of steroid therapy to treat GVHD. The basis for differences in pDC and B-cell recovery after GVHD between BM and G-PB recipients in this setting is unknown. Current findings support previous reports that immune cell monitoring posttransplant may predict response to GVHD treatment, including extracorporeal photopheresis,²⁹ although a recent prospective study showed no association between Tregs and response to extracorporeal photopheresis.³⁰

A limitation of this study is the range of measured values for immune cells in blood, shown as outliers in Figure 1. We used median values as the cut point, as well as nonparametric analyses for comparisons of clinical end points, to limit the effect of outliers. Further analyses are needed to optimally define a cut point or functional relationship between a cell subset and clinical outcome. A second issue is that immune reconstitution data were missing for 15% of patients. The demographics of patients for whom blood samples were evaluable for immune reconstitution were not substantially different from those without evaluable samples (see "Methods"). A comparison of patients with and without evaluable CD4⁺ T-cell counts at 1 month did not translate into differences in the incidence of GVHD or, most importantly, survival, with a median 2-year survival of 55% (range, 49-60) in patients with 1-month samples vs 50% (range, 44-57) in patients without evaluable 1-month samples ($P = .33$). Although these data show that early lymphoid reconstitution is correlated with the numbers of the corresponding cells in the graft, other clinical events, such as CMV infection, that were not analyzed as part of the current study have been shown to influence T-cell reconstitution kinetics.³¹ Finally, because of the large number of immune reconstitution analyses performed, results should still be considered exploratory and subject to confirmation in future studies.

In conclusion, blood levels of immune cells at 1 and 3 months posttransplant may be predictive for survival and response to GVHD treatment. Higher CD4⁺ T-cell counts were associated with better survival among G-PB recipients, and higher DC counts were associated with better survival among BM recipients. These data suggest that qualitative differences in immune cell function that are linked to the graft source may persist well beyond the initial period of engraftment. Current results provide indirect support for the role of donor-derived DCs in limiting donor T-cell alloreactivity and GVHD and functional differences between BM and G-CSF-mobilized donor pDCs.^{17,19,32} Consistent with this view, we have recently shown that G-CSF-mobilized donor pDCs have a lower cell-intrinsic ability to regulate T-cell activation than BM pDCs¹⁶ and lead to more cGVHD (Mojibade Hassan, Alina Ulezko Antonova, Jingru Zhu, Christopher T. Petersen, Yiwen Li, Sakura Hosoba, Jian Ming Li, C.R.G., Jeanne Kowalski, Adam J. Perricone,

Figure 4. (continued) Intracellular expression of IL-2, IFN- γ , and TNF- α in CD4⁺ and CD8⁺ T cells from recipients of BM and G-PB grafts. Percentages of CD4⁺ and CD8⁺ T cells that synthesized IL-2 in response to antigenic nonspecific activation with PMA/ionomycin (top row). Percentages of CD4⁺ and CD8⁺ T cells that expressed IL-2 (second row), IFN- γ (third row), or TNF- α (bottom row) following overnight coculture with CMV lysate. pts, patients.

David L. Jaye, E.K.W., manuscript submitted February 2019). Furthermore, a large retrospective study of Center for International Blood and Marrow Transplant Research data has shown that BM allografts from unrelated donors are associated with significantly better GVHD-free relapse-free survival compared with PB grafts.³³ However, because current clinical practice remains frustratingly agnostic with respect to the best graft source from an unrelated donor, analysis of how immune reconstitution predicts survival pooled across graft sources may be more clinically relevant (Table 2). Future clinical trials should focus on novel strategies to enhance the quality of donor stem cells and DCs that may help to restore immune homeostasis posttransplant.

Acknowledgments

This work was supported by grants 1R01CA188523-01A1 (E.K.W.) and U10HL069294 from the National Cancer Institute and National Heart, Lung, and Blood Institute, National Institutes of Health, which support the Blood and Marrow Transplant Clinical Trials Network that sponsored the clinical trial. The Department of the Navy, Office of Naval Research, and the National Marrow Donor Program also provided support for the BMT CTN 0201 study. Enrollment support was provided by DKMS Germany.

Any views, opinions, findings, conclusions or recommendations expressed in this material are those of the authors and do not reflect the views or the official policy or position of the above-mentioned parties.

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Authorship

Contribution: E.K.W. analyzed data and wrote the manuscript; B.R.L. and M.F. analyzed data and performed statistical analyses; S.J.L., S.C., S.M.F., and C.A. analyzed data and edited the manuscript; D.C. edited the manuscript; A.H. collected data and edited the manuscript; and C.R.G. analyzed data and wrote and edited the manuscript.

Conflict-of-interest disclosure: E.K.W. has received payments and other support from Cambium Medical Technologies, grants from Celldex Therapeutics, payments from Kalytera Therapeutics, grants and payments from Novartis, grants and nonfinancial support from Pharmacyclics, has equity ownership in Cerus Corporation and Chimerix that is outside the scope of this study, and has intellectual property related to the immune-modulatory effects of vasoactive intestinal polypeptide that have been licensed to Cambium Oncology LLC, a biotechnology company in which he holds equity. The remaining authors declare no competing financial interests.

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