ASBMT American Society for Blood and Marrow Transplantation

# Favorable Outcomes in Patients with High Donor-Derived T Cell Count after In Vivo T Cell–Depleted Reduced-Intensity Allogeneic Stem Cell Transplantation

*Amir A. Toor,[1] Roy T. Sabo,[2] Harold M. Chung,[1] Catherine Roberts,[1] Rose H. Manjili,[1] Shiyu Song,[3] David C. Williams, Jr.,[4] Wendy Edmiston,[1] Mandy L. Gatesman,[1] Richard W. Edwards,[1] Andrea Ferreira-Gonzalez,[4] William B. Clark,[1] Michael C. Neale,[5,6] John M. McCarty,[1] Masoud H. Manjili[7]*

Patients with hematologic malignancies were conditioned using a rabbit antithymocyte globulin–based reduced-intensity conditioning regimen for allogeneic stem cell transplantation. Donor-derived $CD3^+$ cell count (ddCD3), a product of $CD3^+$ cell chimerism and absolute $CD3^+$ cell count, when $<110/\mu L$ at 8 weeks post-stem cell transplantation predicted a high risk of sustained mixed chimerism and relapse. Alternatively, patients with a higher ddCD3 developed graft-versus-host disease more frequently, and when partially chimeric, had higher rates of conversion to full donor chimerism after withdrawal of immunosuppression. Early data from our small cohort of patients indicate that ddCD3 at 8 weeks may be used to guide decisions regarding withdrawal of immunosuppression and administration of donor lymphocyte infusion in partially T cell–depleted reduced-intensity regimens.

*Biol Blood Marrow Transplant 18: 794-804 (2012) Published by Elsevier Inc. on behalf of American Society for Blood and Marrow Transplantation*

**KEY WORDS:** Absolute T cell count, Mixed chimerism, Antithymocyte globulin, Graft-versus-host disease, Relapse

## INTRODUCTION

Reduced-intensity conditioning regimens for allogeneic stem cell transplantation (SCT) are well tolerated but are characterized by variable immunologic recovery, particularly when T cell depletion (TCD) is performed to reduce graft-versus-host disease (GVHD) risk [1-5]. TCD may be performed either in vivo by administration of antithymocyte globulin (ATG) during conditioning or ex vivo by various allograft T cell–purging techniques. ATG reduces the risk of chronic GVHD and nonrelapse mortality in matched related donor (MRD) SCT recipients conditioned with a myeloablative regimen [6]. In addition, outcomes in unrelated donor (URD) SCT are improved when ATG is incorporated in the conditioning regimen [7,8] or when the allograft is ex vivo T cell–depleted with CD6 monoclonal antibodies [9].

However, when TCD is performed in SCT with a reduced-intensity conditioning regimen, post-SCT outcomes such as GVHD and relapse are influenced by the level of donor T cell chimerism achieved. Mixed donor–recipient chimerism in T cells often complicates such transplantations. A recent study found that the use of CD52 monoclonal antibody for TCD along with a reduced-intensity regimen resulted in a 50% incidence of mixed chimerism (MC) in T cells at day 100 post-SCT and that declining T cell chimerism was associated with increased risk of relapse [10]. Other studies have reported similarly poor outcomes with MC in the T cells in the first month after reduced-intensity SCT, particularly when T cell chimerism was <60% [11].

From the [1]Bone Marrow Transplant Program, Department of Internal Medicine; [2]Department of Biostatistics; [3]Department of Radiation Oncology; [4]Department of Pathology; [5]Department of Psychiatry; [6]Department of Human Genetics; and [7]Department of Microbiology and Immunology, Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia.

*Financial disclosure:* See Acknowledgments on page 803.

Correspondence and reprint requests: Amir A. Toor, MD, Associate Professor of Medicine, Massey Cancer Center, Virginia Commonwealth University, 1300 E Marshall St, Mail Stop 980157, Richmond, VA 23298-0157 (e-mail: atoor@mcvh-vcu.edu).

Received July 26, 2011; accepted October 10, 2011
Published by Elsevier Inc. on behalf of American Society for Blood and Marrow Transplantation
1083-8791/$36.00
doi:10.1016/j.bbmt.2011.10.011

The level of T cell chimerism after SCT also affects the response to donor lymphocyte infusion (DLI). In one study, patients conditioned with ATG and a reduced-intensity regimen before allografting had a high rate of graft loss despite prophylactic DLI if T cell chimerism was <20% donor and a high rate of conversion to full donor chimerism (FC) if T cell chimerism was >40% donor [12]. In addition to T cells, natural killer (NK) cell chimerism also has been reported to affect the risk for GVHD and graft loss in patients undergoing T cell–replete nonmyeloablative allogeneic SCT [13], highlighting the interactions among various effectors of cellular immunity. In general, studies incorporating T cell–replete allografts have reported frequent mixed donor–recipient chimerism in T cells early after reduced-intensity SCT, which over time converts to FC as immunosuppression is withdrawn. Often this shift in chimerism is accompanied by the development of GVHD, potentially compromising outcome. Conversely, in patients undergoing TCD allogeneic SCT, withdrawal of immunosuppression results in less precisely predictable outcomes in patients with mixed T cell chimerism, with either maintenance of stable MC or occasionally graft loss observed. MC is accompanied by an increased risk of relapse [14,15]. DLI may be used to convert patients with MC to FC and reduce the risk of relapse, but it is complicated by the development of acute or chronic GVHD in as many as 50% of patients [16,17], even when CD8-depleted DLI is used [18,19]. Alternative strategies in patients with MC, such as administration of low-dose prophylactic DLI, although less likely to cause GVHD, are ineffective [4].

Given the unfavorable outcomes associated with the mixed chimeric state, a reliable predictor for the expected evolution of mixed T cell chimerism is needed to help guide clinical decision making regarding withdrawal of immunosuppression and DLI. An alternative immune recovery parameter with prognostic value is T cell recovery posttransplantation [20,21]. We decided to combine this measure with T cell chimerism and to examine the predictive value of a calculated donor-derived T cell count for clinical outcomes after allogeneic SCT conditioned with rabbit ATG and reduced-intensity total body irradiation (TBI). This regimen is based on preclinical studies in murine transplantation demonstrating engraftment across the major histocompatibility complex barrier when T cell antibodies were combined with low-dose irradiation [22,23]. The feasibility of this approach in human transplantation has been demonstrated in clinical trials, which established a low risk of severe acute GVHD, albeit with high rates of mixed donor–recipient chimerism and occasional graft loss [1,3,12,24]. The present trial examined the effect of two doses of rabbit ATG in recipients of allogeneic SCT, with posttransplantation immune reconstitution as the trial's primary endpoint (Clinicaltrials.gov identifier: NCT00709592).

## PATIENTS AND METHODS

### Patients and Eligibility

Consecutive patients were enrolled on a prospective randomized phase II clinical trial, approved by the Institutional Review Board at Virginia Commonwealth University. To be eligible, patients had to be between 18 and 70 years of age, have a recurrent or high-risk hematologic malignancy, and have adequate end-organ function and performance status. Patients aged <50 years had to be ineligible for conventional myeloablative conditioning because of comorbidity. Each patient was required to have a 7/8 or 8/8 locus-matched related donor (MRD) or unrelated donor (URD), with high-resolution typing performed for HLA-A, -B, -C, and -DRB1.

### ATG + TBI Conditioning Regimens

The patients were randomized between two different doses of rabbit ATG (Thymoglobulin; Genzyme, Cambridge, MA), 2.5 or 1.7 mg/kg adjusted ideal body weight/day, given i.v. on days -9 through -7, followed by TBI to a total dose of 4.5 Gy, delivered in three 1.5-Gy fractions, with two doses on day -1 and the final dose on day 0. Methylprednisolone 2 mg/kg was given as premedication for ATG. GVHD prophylaxis was tacrolimus given orally starting on day -2 with tapering starting at approximately 12 weeks posttransplantation. Mycophenolate mofetil was given orally at a dose of 15 mg/kg twice daily from day 0 to day 28. Granulocyte colony-stimulating factor (G-CSF) was given at a dose of 5 μg/kg/day from day 4 until myeloid engraftment occurred. Blood stem cells were collected using G-CSF 10 μg/kg/day s.c. on days 1-5. Escalating-dose DLI was permitted beyond 8 weeks post-SCT for the management of declining or persistent MC (initial dose, $1 \times 10^6$ $CD3^+$ cells/kg) and for disease progression (initial dose, $5 \times 10^6$ $CD3^+$ cells/kg).

### T Cell Engraftment Analysis

Donor engraftment was assessed by chimerism analyses performed at 4, 8, 12, and 24 weeks after SCT on whole-blood, granulocytes, and total T cells. Blood cell separation was done using immunomagnetic beads (Miltenyi Biotec, Auburn, CA) enriched for CD15- and CD3-expressing cells in a Miltenyi Biotech AutoMACS Pro Separator. DNA was isolated using a Qiagen EZ1 200-μL Whole-blood Isolation Kit (Qiagen, Valencia, CA) on a Qiagen EZ1 Biorobot. Polymerase chain reaction was performed with the

GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, CA) with the GenomeLab Human STR Primer Set (Beckman Coulter, Brea, CA), and capillary electrophoresis of the amplification products was performed on a Beckman Coulter Vidiera NsD Analyzer to determine the short tandem repeat (STR) alleles of the donor and patient. Donor chimerism was calculated for each STR from the areas of unique recipient and donor peaks using the manufacturer's software and averaged over all informative STR alleles. Immunophenotypic analysis of the blood for immune reconstitution was performed concomitantly with chimerism analysis, using a dual-platform technique on a Beckman Coulter Cytomics FC500 flow cytometer. Antibodies to CD3 and CD56 (Beckman Coulter) were used to enumerate T cells and NK cells.

Donor-derived $CD3^+$ T cell count (ddCD3) was calculated by multiplying the T cell chimerism (% recipient DNA, expressed as a fraction) with the absolute blood $CD3^+$ T cell count obtained simultaneously. The resulting absolute recipient-derived $CD3^+$ T cell count was then subtracted from the total absolute $CD3^+$ cell count to obtain the ddCD3 value.

### Study Design and Statistical Analysis

This randomized phase II study compared two different ATG doses used in conditioning and was designed with immune reconstitution as the primary endpoint, with engraftment, GVHD rate, and survival as secondary endpoints. Engraftment was defined as sustained hematopoietic recovery after SCT with <5% recipient chimerism in whole blood or a relevant cell fraction. Overall survival was calculated from the day after transplantation to the day of death. GVHD was classified according to consensus criteria. Acute GVHD was graded according to the Glucksberg criteria. Because of the small patient number and relatively low event rate, acute and chronic GVHD data were pooled to analyze GVHD risk. GVHD observations were censored if this complication developed after delivery of DLI. Disease-specific criteria were used to diagnose relapse or progression.

To determine the optimal cutoff for classifying our patients into high ddCD3 and low ddCD3 groups, we first measured the association between ddCD3 and clinical outcomes of interest to determine the logistic regression for each outcome, and then determined the area under the curve (AUC) of the receiver-operating characteristic (ROC) curve for logistic regression for each clinical outcome of interest against the ddCD3 value. The cutoff that maximized the sum of ROC-AUC across all measures was selected. The Kruskal-Wallis test was used to test for mean differences in continuous variables between the high ddCD3 and low ddCD3 groups. To further analyze these relationships, an informative normal prior was used for the mean measure in each ddCD3 group (based on the overall sample mean and variance) and was combined with a normal likelihood for the observed data. Fisher's exact test (FET) was used to analyze the relationships between ddCD3 groups and categorical measurements. As an additional analysis, noninformative beta priors were assumed on the proportions in both ddCD3 groups and were coupled with binomial likelihoods for the observed data. Kaplan-Meier curves were estimated to account for the timing of remission and overall survival, and differences between the high ddCD3 and low ddCD3 groups were tested using the log-rank test. Corroboratory Bayes methods were used as well. For both continuous and categorical cases, direct sampling (with 100,000 repetitions) from the conjugate posterior distributions for each ddCD3 group was used to estimate posterior probabilities (PPs) of differences between the two ddCD3 means or proportions [25]. For all frequentist analyses, a mean or rate difference between ddCD3 groups is considered significant for a $P$ value $<.05$, whereas for all Bayes analyses, a PP $> 0.95$ is considered sufficient evidence of a mean or proportional difference between the ddCD3 groups' data summaries. The Fisher's exact test and the Kruskal-Wallis test were applied using the FREQ and NPAR1-WAY procedures in SAS 9.2 (SAS Institute, Cary, NC), and Bayes analyses were conducted using R statistical software.

## RESULTS

### Patients

Between 2008 and 2011, a total of 25 patients were enrolled in this trial, 22 of whom were eligible for the present analysis. One patient was excluded because of disease progression and autologous reconstitution at 4 weeks after SCT, and two patients had not been enrolled sufficiently long to be evaluated for transplantation outcomes. The median age of the 22 evaluable patients was 58 years (range, 44-68 years). The diagnoses were multiple myeloma in seven patients, non-Hodgkin's lymphoma in six, chronic lymphocytic leukemia (or prolymphocytic leukemia) in seven, and myelodysplastic syndrome and acute myelogenous leukemia in one each. Most of the patients had experienced multiple relapses and had been heavily pretreated, having received a median of four previous rounds of chemotherapy (range, 2-10). Eleven patients had undergone previous autologous SCT. Fourteen patients were in complete remission at the time of SCT, whereas eight patients were in partial remission or had persistent, stable disease. Eleven patients (50%) were included in each ATG dose cohort. The conditioning regimen was well tolerated,

**Table 1. Patient Outcomes According to ddCD3 Cell Count at 8 Weeks Posttransplantation**

| Patient | Donor | ATG Dose, mg/kg | CD3 Cell Count, Cells/μL | T Cell Chimerism; % Recipient DNA in T Cells | ddCD3 Cell Count, Cells/μL | GVHD (Grade) | Disease Status | Blood Engraftment Weeks 12-54 | DLI |
|---|---|---|---|---|---|---|---|---|---|
| 16 | MRD | 5.1 | 5 | 4.7 | 3 | No | Relapse | MC | |
| 5 | URD | 5.1 | 5 | 1 | 5 | Acute (II)>prog | CR | FC | |
| 18 | URD | 5.1 | 39 | 23 | 30 | No | Relapse/PD | MC | Yes |
| 7 | URD | 7.5 | 60 | NE | 60[b] | No | CR | MC | |
| 23 | MRD | 7.5 | 90 | 26 | 67 | No | CR | FC | |
| 20 | MRD | 7.5 | 85 | 11 | 76 | No | Relapse | MC | Yes |
| 17 | URD[a] | 7.5 | 84 | 1 | 76 | No | Relapse/PD | MC | Yes |
| 2 | MRD[a] | 7.5 | 2,646 | 96 | 106 | No | Relapse | MC | Yes |
| 6 | MRD | 7.5 | 128 | 0 | 128 | DOA (III)[c] | CR | FC | |
| 8 | URD | 7.5 | 336 | 0 | 336 | OS | CR | FC | |
| 24 | URD | 5.1 | 413 | 0 | 413 | Acute (II) | CR | FC | |
| 4 | MRD | 5.1 | 1,054 | 57 | 453 | No | CR | FC | |
| 14 | URD[a] | 5.1 | 736 | 0 | 736 | DOA (IV) | CR | FC | |
| 11 | MRD[a] | 7.5 | 770 | 0 | 770 | Chronic | CR | FC | |
| 13 | URD | 5.1 | 828 | 0 | 828 | Acute (II)>prog | CR | FC | |
| 12 | URD | 5.1 | 882 | 0 | 882 | No | CR | FC | |
| 15 | URD | 7.5 | 957 | 0 | 957 | Chronic | CR | FC | |
| 10 | URD | 7.5 | 1,170 | 0 | 1,170 | Chronic | Relapse | FC | Yes |
| 19 | MRD | 5.1 | 1,530 | 4.7 | 1,458 | DOA (III) | CR | FC | |
| 9 | URD | 7.5 | 1,550 | 0.7 | 1,539 | Chronic | CR | FC | |
| 3 | URD | 5.1 | 2,050 | 6 | 1,923 | No | CR | FC | |
| 21 | MRD | 5.1 | 2,464 | 0 | 2,464 | No | Relapse | FC | |

CR indicates complete response; PD, persistent disease; NE, not evaluable; prog, progression to persistent grade III acute GVHD; DOA, delayed-onset acute GVHD; OS, overlap syndrome.

[a]Single-locus HLA class I mismatch (7/8 matched donor); blood chimerism: last value <5% recipient FC, >5% MC.

[b]There was inadequate sample size for T cell chimerism determination at 8 weeks; thus, total $CD3^+$ cell count was assumed to equal ddCD3 cell count; MC at 12 weeks (51% recipient DNA).

[c]Progressed to extensive chronic GVHD.

with no day +100 transplantation-related mortality observed.

## Engraftment and Chimerism

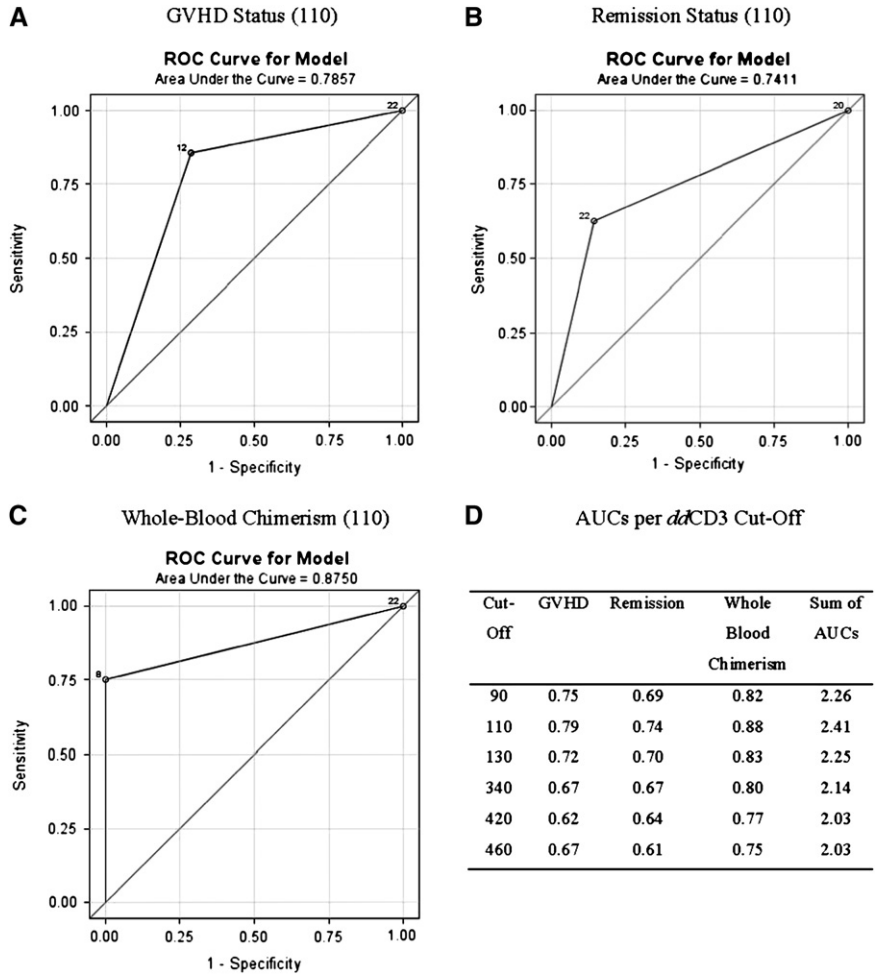
Predominantly donor-derived hematopoiesis was established promptly after SCT. The median whole-blood hematopoietic and granulocyte chimerism was 0% recipient-derived at all time points evaluated after transplantation in the 22 evaluable patients. Median T cell chimerism measured simultaneously was 5% at 4 weeks posttransplantation, 1.5% at 8 weeks, 5% at 12 weeks, and 0% at 24 weeks. T cell chimerism could not be evaluated in seven of the 22 patients at 4 weeks posttransplantation because of inadequate DNA yield related to low T cell recovery at that time. Nine of the 22 patients had mixed T cell chimerism (≥5% recipient DNA) at 8 weeks posttransplantation, and three of these nine (33%) developed FC after withdrawal of immunosuppression over the ensuing weeks. One of the nine patients demonstrated improved donor chimerism after DLI, and one of the 13 patients with FC reverted to MC.

## Clinical Outcomes with ATG + TBI Conditioning

Classical-onset acute cutaneous GVHD developed in three patients (grade I-II in all), two of whom evolved to progressive delayed-onset acute grade III-IV gastrointestinal GVHD. Two other patients developed delayed-onset acute gastrointestinal GVHD (grade III-IV) after withdrawal of immunosuppression (Table 1). Chronic GVHD has developed in five other patients (four with classical onset and one with overlap syndrome). All patients who developed GVHD demonstrated FC at the time of GVHD diagnosis. Relapse or persistent disease was observed in seven of 22 patients (32%), five of whom had MC or declining T cell chimerism after initial engraftment. Five patients received DLI at a median of 204 days after SCT (range, 99-362 days) for the management of persistent or relapsing disease with MC (n = 4) or without MC (n = 1). Two of these patients died from progressive disease (one patient despite developing GVHD), whereas the other three remained progression free.

## Donor-Derived $CD3^+$ Cell Count

T cell chimerism and simultaneously measured absolute $CD3^+$ cell counts were used to calculate ddCD3 at 8 weeks after SCT, because reliable data were available for all patients for this interval. The median ddCD3 value at 8 weeks was 433/μL (range, 3-2,464/μL). After calculating the sum of ROC-AUCs for each of the potential ddCD3 cutoff values (Figure 1), a ddCD3 of 110/μL was found to be optimal, with both the highest AUC sum and the highest AUC for each measure (cumulative GVHD present or absent, 0.79; remission present or absent, 0.74; whole blood chimerism, FC vs MC, 0.88). Thus, we used 110/μL




| Cut-Off | GVHD | Remission | Whole Blood Chimerism | Sum of AUCs |
|---|---|---|---|---|
| 90 | 0.75 | 0.69 | 0.82 | 2.26 |
| 110 | 0.79 | 0.74 | 0.88 | 2.41 |
| 130 | 0.72 | 0.70 | 0.83 | 2.25 |
| 340 | 0.67 | 0.67 | 0.80 | 2.14 |
| 420 | 0.62 | 0.64 | 0.77 | 2.03 |
| 460 | 0.67 | 0.61 | 0.75 | 2.03 |

**Figure 1.** AUC for ROC curves for GVHD status, remission status, and whole blood chimerism against the ddCD3 group. The table lists ddCD3 cutoff values calculated from the sum of AUCs for different ROC curves.

as the cutoff value to distinguish between low (<110) and high (>110) ddCD3 values. Based on this criterion, there were eight patients in the low ddCD3 group and 14 patients in the high ddCD3 group. Notably, based on the cutoff of 110/ μL, the median ddCD3 in the corresponding groups was 60/μL in the low ddCD3 group and 855/μL in the high ddCD3 group, suggesting a marked difference in T cell reconstitution between the two groups.

## Clinical Impact of Donor-Derived CD3$^+$ Cell Count

Significant relationships were found between ddCD3 value and cumulative GVHD status (present or absent), remission status (present or absent), and whole blood chimerism status (FC or MC), reflecting engraftment at and beyond 12 weeks after SCT (Table 2). Using frequentist methods, the proportion

**Table 2. Relative Clinical Outcomes in Patients with Low and High ddCD3 Recovery**

| | ddCD3 <110 | | | ddCD3 >110 | | |
|---|---|---|---|---|---|---|
| Measurement | (n) | Frequency | Proportion | (n) | Frequency | Proportion |
| GVHD (yes) | 7 | 1 | 0.14 | 14 | 10 | 0.71 |
| Remission (yes) | 8 | 3 | 0.38 | 14 | 12 | 0.86 |
| Whole blood chimerism (MC) | 8 | 6 | 0.75 | 14 | 0 | 0.00 |

**Table 3. Summary of Continuous (Sample Size [n], Median, and Interquartile Range [IQR]) and Categorical (Sample Size [n], Frequency, and Proportion) Measurements**

| | ddCD3 <110 | | | ddCD3 >110 | | |
|---|---|---|---|---|---|---|
| Measurement | (n) | Median | IQR | (n) | Median | IQR |
| Donor age | 8 | 52 | 41-61 | 14 | 45 | 32-63 |
| CD34+ cell dose | 8 | 5.4 | 3.3-6.4 | 14 | 5.7 | 3.9-6.8 |
| CD3+ cell dose | 8 | 2.83 | 1.85-5.12 | 14 | 2.94 | 1.66-3.54 |
| NK cell count at 4 weeks | 8 | 183 | 53-201 | 14 | 185 | 144-264 |
| NK cell count at 8 weeks | 8 | 129 | 49-210 | 14 | 221 | 85-300 |
| ddCD3 | 8 | 63 | 18-76 | 14 | 855 | 453-1458 |

| Measurement | (n) | Frequency | Proportion | (n) | Frequency | Proportion |
|---|---|---|---|---|---|---|
| Donor type (MRD) | 8 | 4 | 0.50 | 14 | 5 | 0.36 |
| ATG dose (5.1) | 8 | 3 | 0.38 | 14 | 8 | 0.57 |

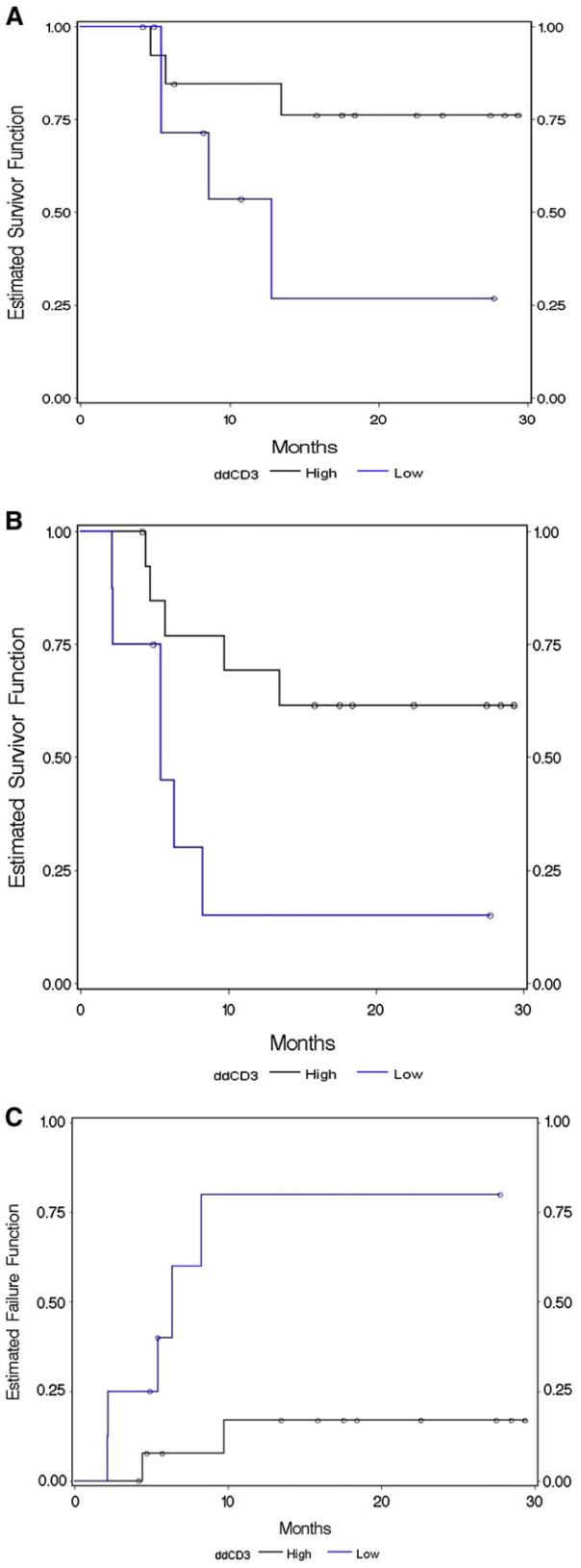



**Figure 2.** (A) Kaplan-Meier survival curve for overall survival time, comparing the high ddCD3 (>110) and low ddCD3 (<110) groups. (B) Kaplan-Meier survival curve for progression-free survival time, comparing the high ddCD3 and low ddCD3 groups. (C) Kaplan- Meier failure curve for time to relapse, comparing the high ddCD3 and low ddCD3 groups.

of patients with MC in whole blood was significantly higher in the low ddCD3 group compared with the high ddCD3 group ($P < .0001$, FET). However, the proportion of patients diagnosed with GVHD was significantly lower in the low ddCD3 group ($P = .024$, FET). The proportion of patients in sustained remission was marginally lower in the low ddCD3 group ($P = .0524$, FET). These results were corroborated by Bayes analysis, which found higher whole blood MC rates (PP = 0.99), lower GVHD rates (PP = 0.99), and lower remission rates (PP = 0.99) in the low ddCD3 group. Consequently, with a median follow-up of 18 months (range, 4-29 months) in the surviving patients, both overall survival and progression-free survival (Figure 2A and B), as well as time to relapse (Figure 2C), were more favorable in the high ddCD3 group compared with the low ddCD3 group. But although the relationship was significant for relapse ($\chi^2_1 = 0.79$; $P = .005$) and progression-free survival ($\chi^2 = 5.3$; $P = .02$), it was only marginally significant for overall survival ($\chi^2_1 = 3.2$; $P = .07$). Correspondingly, 1-year Kaplan-Meier estimates for overall survival (84% vs 54%) and progression-free survival (69% vs 15%) were superior in the high ddCD3 group, with all relapses occurring in the first year after SCT. Notably, treatment-related mortality was similar in the two groups (16% in the high ddCD3 group and 15% in the low ddCD3 group).

To confirm the utility of ddCD3 value in predicting clinical outcomes, we performed additional analyses to assess the impact of its factors in isolation. We found no significant associations between absolute T cell count and T cell chimerism at 8 weeks after SCT with mortality (T cell recovery, $P = .65$; % T cell chimerism, $P = .57$), relapse (T cell recovery, $P = .51$; % T cell chimerism, $P = .47$), or GVHD (T cell recovery, $P = .35$; % T cell chimerism, $P = .21$).

## Kinetics of Donor-Derived T Cell Reconstitution

When examined over time, patients with a ddCD3 value <110/μL and MC had either declining donor chimerism or persistent MC as immunosuppression was withdrawn (Figure 3A), whereas those with ddCD3 >110/μL had increasing donor T cell chimerism over time as immunosuppression was tapered (Figure 3B). This resulted in a progressive rise in the absolute ddCD3 value over time in the patients with higher ddCD3 values (Figure 3C). Patients in the high ddCD3 group demonstrated predominantly donor chimerism in T cells at 6 months posttransplantation (Figure 3D). A modulating effect of ddCD3 value on immunosuppression withdrawal was shown most clearly in the rare patient with mixed granulocyte chimerism (Figure 4). Early DLI arrested the decline of

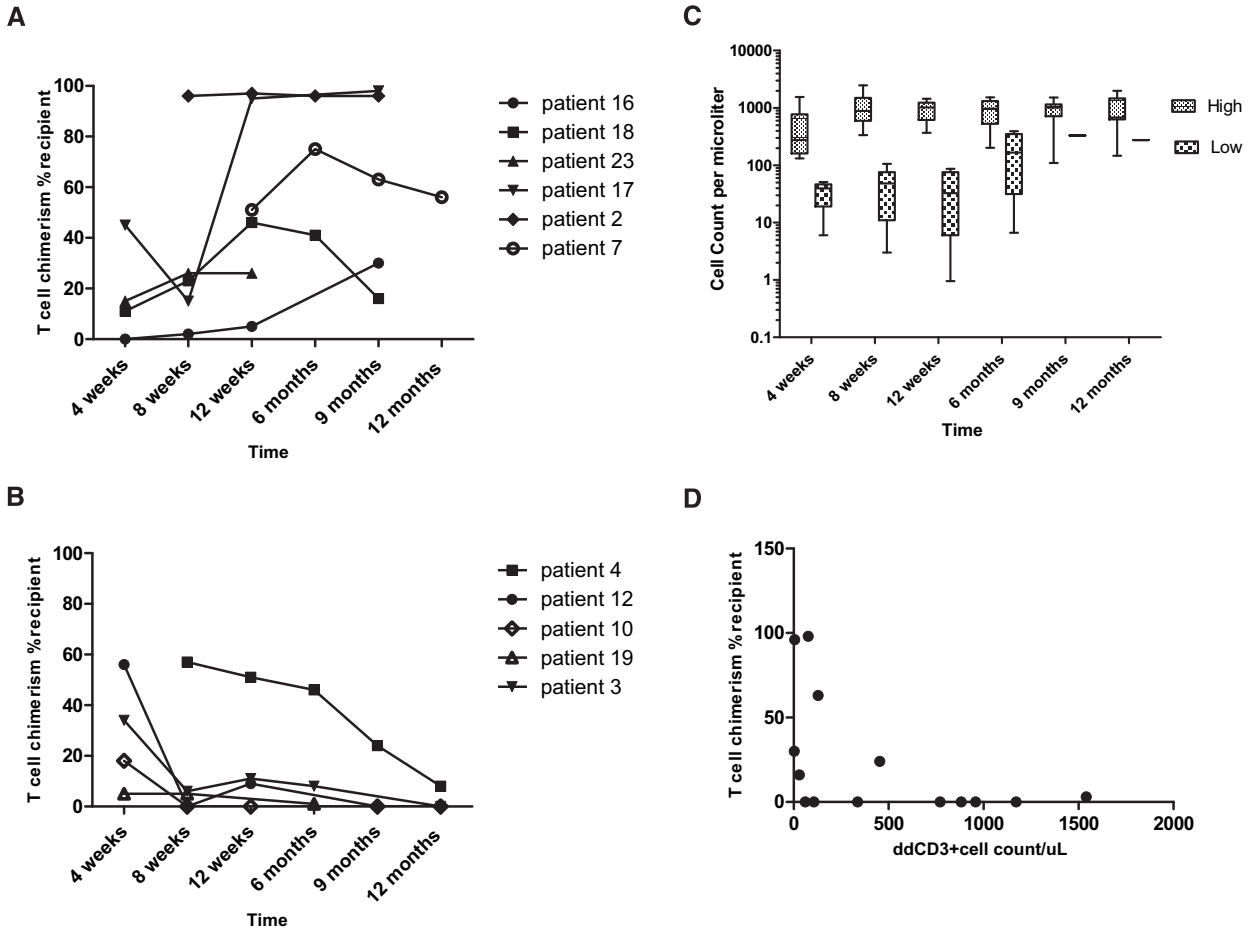



**Figure 3.** (A) T cell chimerism trend in patients with MC and ddCD3 <110/μL. (B) T cell chimerism trend in patients with MC and ddCD3 >110/μL. (C) Trends in ddCD3 cell count over time in the high and low recovery groups. (D) Effect of ddCD3 count at 8 weeks on T cell chimerism at 24 weeks.

donor hematopoiesis in the patients with low ddCD3 recovery.

### Effect of Pretransplantation and Posttransplantation Variables on ddCD3

No significant relationships were found between ddCD3 level and donor age, donor type (MRD vs URD), total ATG dose (5.1 mg/kg vs 7.5 mg/kg), or infused $CD34^+$ or $CD3^+$ cell dose (Table 3). Using frequentist methods, mean values for donor age ($\chi^2_1 = 0.5$; $P = .49$), infused $CD3^+$ ($\chi^2_1 = 0$; $P = .84$), and $CD34^+$ cell dose ($\chi^2_1 = 0.5$; $P = .49$), as well as proportions for donor type ($P = .66$, FET) and ATG dose ($P = .66$, FET), were not significantly different between the low ddCD3 and high ddCD3 groups. These results were corroborated by the Bayes statistical analysis, which showed low PPs for mean differences between the ddCD3 groups for donor age (PP = 0.83) and $CD34^+$ cell dose (PP = 0.72), as well as proportional differences between the ddCD3 groups for ATG dose (PP = 0.80) and donor type (PP = 0.26).

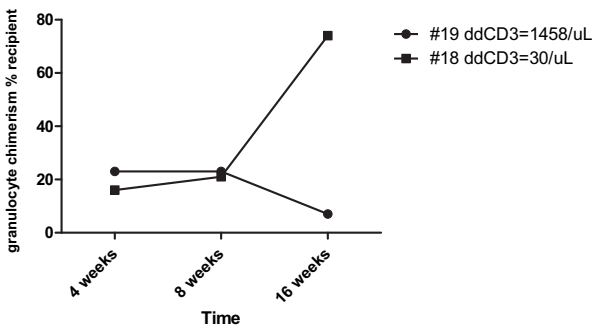



**Figure 4.** Effect of immunosuppression withdrawal on granulocyte chimerism in the high ddCD3 and low ddCD3 groups.

NK cell reconstitution after SCT was evaluated to examine its relationship with ddCD3 value. NK cell counts were higher in the high ddCD3 group than in the low ddCD3 group at 8 weeks ($\chi^2_1 = 4.1$; $P = .044$; PP = 0.99). However, posttransplantation NK cell recovery at 4 and 8 weeks did not influence the ddCD3 count ($\chi^2_1 = 0.8$; $P = .37$; PP = 0.88). We analyzed the effect of NK cell recovery on clinical outcomes of interest, including GVHD ($\chi^2_1 = 0.4$;

$P = .55$), remission status ($\chi^2_1 = 2.4$; $P = .12$), and whole blood engraftment ($\chi^2_1 = 3.3$; $P = .07$), as well as on time-to-event measurements of mortality ($P = .56$), relapse ($P = .10$), and GVHD ($P = .40$), and unlike with ddCD3 value, found no measurable independent effect in our patient cohort.

## DISCUSSION

Cellular immune reconstitution after SCT is known to be of prognostic relevance, particularly with respect to relapse. Patients undergoing T cell–depleted SCT have a higher rate of MC and thus a higher rate of delayed immune reconstitution with slower recovery of donor T cells. Neither chimerism analysis nor T cell subset recovery consistently predicts the effect of withdrawing immunosuppression in these patients. Patterns of T cell chimerism trends generally fall into four broad categories: (1) early establishment of stable FC; (2) early MC evolving to FC with withdrawal of immunosuppression; (3) stable MC despite withdrawal of immunosuppression; and (4) MC (or early FC) with late autologous reconstitution and graft loss [26-28]. Generally, patients with MC have inferior outcomes; in particular, those with high recipient chimerism in T cells are at a greater risk for eventual graft loss when receiving a T cell–depleted allograft. Clearly, early identification of the chimerism pattern that a patient is likely to develop will allow modification of therapy and reduce the risk of relapse and graft loss in that patient.

To get a more accurate picture of the magnitude of alloimmune reconstitution and its downstream effect, we examined the impact of donor-derived T cell recovery (ie, ddCD3 value) on posttransplantation outcomes in patients conditioned with an ATG-based preparative regimen. This parameter was calculated from the simultaneously measured $CD3^+$ T cell chimerism and absolute $CD3^+$ cell count in circulation. The earliest time point at which the ddCD3 value could be determined in all of our patients was 8 weeks after SCT. This was likely secondary to the effect of ATG and the extended course of mycophenolate therapy that our patients received for GVHD prophylaxis, with the testing schedule an obvious determining factor. Nevertheless, even at this relatively early time point, ddCD3 had predictive value for clinical outcomes developing later in the course of transplantation. This effect was particularly striking for donor engraftment when the ROC curves for the effect of ddCD3 value on common posttransplantation parameters were determined and when hematopoietic and T cell chimerism were plotted over time for the high ddCD3 and low ddCD3 groups at 8 weeks after SCT. The absolute magnitude of donor T cell recovery and its effect in our patients reflects the conditioning regimen used and the underlying disease biology and thus is unique to this patient population and most likely subject to revision with a larger sample size. This is particularly true because of the relatively low ddCD3 cutoff value (110/μL) in our patients, likely a manifestation of the TCD regimen. Thus, the timing and magnitude of a prognostically important ddCD3 value likely will differ based on the conditioning regimen and particularly with different TCD strategies.

Timing and magnitude aside, the most clinically relevant aspect of the ddCD3 value is its association with hematopoietic and T cell chimerism after withdrawal of immunosuppression. Generally, mixed T cell chimerism after reduced-intensity SCT is managed by cessation of immunosuppression in T cell–replete allografts; however, this may result in progressive graft loss in T cell–depleted allografts, especially in patients with MC. That patients with a higher ddCD3 have a more readily predictable trajectory in both chimerism and T cell reconstitution over time is intuitive and provides a discriminator for decision making regarding DLI in recipients of T cell–depleted reduced-intensity allografts. We did not address whether this adds to the general value of T cell chimerism measurement alone; however, it appears to be informative in our small dataset, particularly when the downstream effects of T cell engraftment (ie, relapse and GVHD) are examined. Because this measure accounts for both quantitative and qualitative donor T cell recovery posttransplantation through a simple calculation, the observed association with downstream outcome is likely to be confirmed in larger cohorts.

Similar findings have been reported in patients undergoing transplantation after T cell–depleted allogeneic SCT and scheduled DLI. In a cohort of patients with breast cancer, a relative preponderance of host-derived T cells was observed at 28 and 100 days after transplantation in patients with MC [29]. Conversely, patients who developed grade II-IV GVHD despite T cell depletion had a significantly higher number of donor-derived T cells in circulation at day 100 [29]. These findings are consistent with our finding of a higher rate of GVHD in patients with a higher ddCD3 value at 8 weeks. Another study found that in recipients of T cell–replete allografts conditioned with fludarabine and 2 Gy TBI and with early discontinuation of immunosuppression, a day 14 donor-derived $CD8^+$ cell count $>0.043*10^6$/mL ($>43$/μL) was strongly associated with grade II-IV acute GVHD [30]. Similar to our cohort, no predictors for donor-derived $CD8^+$ cell recovery were revealed by an analysis of donor age and graft composition [30]. Taken together, these findings and our results suggest that the magnitude of donor-derived T cell recovery plays a critical role in alloimmune responses after SCT.

Although ddCD3 value was largely predictive of relapse (and thus the need for DLI) in our patients, two patients with a high ddCD3 value relapsed. Both of these patients had heavily pretreated myeloma with an adverse cytogenetic profile, suggesting the primacy of disease biology in prognostic determination. The adverse impact of disease status and cytogenetics in patients undergoing reduced-intensity in vivo T cell–depleted allografts is well known [31,32]. Thus, ddCD3 values should be interpreted in light of disease biology when used in decision making regarding the need for DLI versus ongoing immunosuppression in patients who have undergone T cell–depleted reduced-intensity allogeneic SCT.

Our dataset is not large enough to establish whether ddCD3 cell count can augment isolated T cell chimerism and absolute T cell count in most patients undergoing SCT. Nonetheless, in selected patients with poor immune reconstitution, this more precise metric has additional predictive value for post-SCT outcomes beyond the factors that determine it. In our estimation, ddCD3 cell count appears to be of the greatest value in patients with low T lymphocyte recovery or high levels of recipient chimerism. To better understand this value, consider the general nature of biological dose-response relationships, which often tend to follow a sigmoid or logistic relationship with threshold effects observed. Although this may not be entirely true for T cell–mediated responses, which are antigen specific, or binary, increasing donor-derived T cell counts would increase the likelihood of the presence of relevant recipient or tumor antigen-specific T cell clones, thus the association of higher ddCD3 counts with GVHD and freedom from relapse. We speculate that our data best define outcomes of the patients on the steep part of the aforementioned sigmoid relationships between T cell reconstitution and posttransplantation outcome likelihood. This is reflected by the ROC-AUC curves for GVHD, relapse, and achievement of FC. Additional studies examining the impact of ddCD3 in larger cohorts of patients are needed before this value can be established as a qualitative and quantitative measure to predict the onset and magnitude of GVHD and graft-versus-tumor effects, which are largely determined by minor histoincompatibilities between HLA-identical donors and recipients [33-35]. In addition, the likelihood of eventually developing GVHD may be predicted more accurately by assays such as T cell clonality.

The interaction between hematopoietic lineages and T cells is likely important in determining the rate and durability of T cell engraftment, as well as donor-derived T cell recovery. In an investigation of patterns in T cells and dendritic cells, MC in dendritic cells with FC in T cells predicted the development of an alloimmune response [36]. NK cell reconstitution after allogeneic SCT has been studied as a predictor of posttransplantation outcomes and found to have variable effects on such outcomes as graft rejection and relapse [13,37,38]. Although we were not able to identify an effect of posttransplantation NK cell reconstitution on ddCD3 cell count recovery, patients with a high ddCD3 cell count also had a higher NK cell count at the 8-week mark, suggesting interactions among factors that determine cellular immune reconstitution. None of the graft and patient characteristics that we studied, including $CD3^+$ cell dose infused and ATG dose used in conditioning, was predictive of cellular immune recovery. However, such parameters as ATG level at the time of transplantation, tacrolimus and mycophenolate levels after transplantation, and minor histocompatibility differences are not accounted for in our analysis and may well have a deterministic effect on ddCD3 cell recovery and subsequent outcomes. Thus, it is reasonable to conclude that clinicians caring for SCT recipients should closely monitor cellular immune reconstitution after transplantation and, depending on disease biology, intervene with such measures as DLI if this process is delayed.

Our findings must be considered only preliminary, keeping in mind the small sample size of our cohort and the heterogeneity of disorders for which the SCTs were performed. Thus, to increase the confidence in the validity of our findings, we evaluated Bayes methods along with our proposed classical methods, because they account for small sample sizes and yield interpretable inference on the parameters of interest, rate or mean differences, that is often similar to inference from frequentist methods [39]. In analyses such as ours, a particular concern is that a substantial sample size may be required to deliver adequate statistical power and yield appropriate asymptotic behavior of the statistics being used [25]. However, the posterior probabilities obtained using Bayes methods are valid even for small sample sizes, given that those probabilities are conditioned on the observed data and, by necessity, on the sample size. Thus, a posterior probability reflects the data at hand and requires more statistical evidence to produce a high probability with smaller sample sizes than with larger sample sizes.

In conclusion, here we report the effect of donor T cell reconstitution, easily calculated from routinely measured posttransplantation variables, on clinical outcomes, particularly the stability of engraftment and disease relapse. The ddCD3 cell count may aid in the decision making regarding withdrawal of immunosuppression and timing of DLI in recipients of in vivo T cell–depleted allografts. The optimal ddCD3 cutoff value needs to be refined by examining this parameter in larger patient cohorts with comparable disease biology over a longer period of time and with a wider range of conditioning regimens before this parameter can be widely applied in the clinical setting.

## ACKNOWLEDGMENTS

We thank the nurses, nurse practitioners, and coordinators in the Bone Marrow Transplant Program at Virginia Commonwealth University. Special thanks are extended to Cheryl Jacocks-Terrell for help with preparing the manuscript and Carol Cole and Laura Couch for protocol management.

*Financial Disclosure:* Genzyme Corporation has provided a research grant to Drs. Toor and Manjili.

## REFERENCES

1. Dey BR, McAfee S, Sackstein R, et al. Successful allogeneic stem cell transplantation with nonmyeloablative conditioning in patients with relapsed hematologic malignancy following autologous stem cell transplantation. *Biol. Blood Marrow Transplant*. 2001;7:604-612.
2. Remberger M, Storer B, Ringden O, et al. Association between pretransplant thymoglobulin and reduced nonrelapse mortality rate after marrow transplantation from unrelated donors. *Bone Marrow Transplant*. 2002;29:391-397.
3. Toor A, Rodriguez T, Bauml M, et al. Feasibility of conditioning with thymoglobulin and reduced-intensity TBI to reduce acute GVHD in recipients of allogeneic SCT. *Bone Marrow Transplant*. 2008;42:723-731.
4. Rodriguez-Luaces M, Ferra C, Martin-Henao G, et al. Mixed chimerism is frequent after allogeneic peripheral blood stem cell transplantation with positive CD34 selection, and is not reverted by low doses of donor T-cell add-back. *Eur J Haematol*. 2004;73:162-168.
5. Juliusson G, Theorin N, Karlsson K, et al. Subcutaneous alemtuzumab vs ATG in adjusted conditioning for allogeneic transplantation: influence of Campath dose on lymphoid recovery, mixed chimerism and survival. *Bone Marrow Transplant*. 2006; 37:503-510.
6. Russell JA, Turner AR, Larratt L, et al. Adult recipients of matched related donor blood cell transplants given myeloablative regimens including pretransplant antithymocyte globulin have lower mortality related to graft-versus-host disease: a matched-pair analysis. *Biol Blood Marrow Transplant*. 2007;13: 299-306.
7. Duggan P, Booth K, Chaudhry A, et al. Unrelated donor BMT recipients given pretransplant low-dose antithymocyte globulin have outcomes equivalent to matched sibling BMT: a matched-pair analysis. *Bone Marrow Transplant*. 2002;30:681-686.
8. Portier DA, Roberts CH, Fletcher DR, et al. Use of rabibit antithymocyte globulin for conditioning in matched unrelated donor hematopoietic cell transplantation provided comparable dose dependant outcomes to matched related donor recipients. *(Abstract) Biol Blood Marrow Transplant*. 2011;17:S303.
9. Alyea EP, Weller E, Fisher DC, et al. Comparable outcome with T-cell–depleted unrelated-donor versus related-donor allogeneic bone marrow transplantation. *Biol. Blood Marrow Transplant*. 2002;8:601-607.
10. van Besien K, Dew A, Lin S, et al. Patterns and kinetics of T-cell chimerism after allo transplant with alemtuzumab-based conditioning: mixed chimerism protects from GVHD, but does not portend disease recurrence. *Leuk Lymphoma*. 2009;50: 1809-1817.
11. Saito B, Fukuda T, Yokoyama H, et al. Impact of T cell chimerism on clinical outcome in 117 patients who underwent allogeneic stem cell transplantation with a busulfan-containing reduced-intensity conditioning regimen. *Biol. Blood Marrow Transplant*. 2008;14:1148-1155.
12. Dey BR, McAfee S, Colby C, et al. Impact of prophylactic donor leukocyte infusions on mixed chimerism, graft-versus-host disease, and antitumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow transplantation. *Biol Blood Marrow Transplant*. 2003;9:320-329.
13. Baron F, Baker JE, Storb R, et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *Blood*. 2004;104:2254-2262.
14. Bader P, Kreyenberg H, Hoelle W, et al. Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem cell transplantation: possible role for preemptive immunotherapy? *J Clin Oncol*. 2004;22:1696-1705.
15. Bader P, Kreyenberg H, Hoelle W, et al. Increasing mixed chimerism defines a high-risk group of childhood acute myelogenous leukemia patients after allogeneic stem cell transplantation where preemptive immunotherapy may be effective. *Bone Marrow Transplant*. 2004;33:815-821.
16. Tomblyn M, Lazarus HM. Donor lymphocyte infusions: the long and winding road. How should it be traveled? *Bone Marrow Transplant*. 2008;42:569-579.
17. Norlin AC, Remberger M. A comparison of Campath and Thymoglobulin as part of the conditioning before allogeneic hematopoietic stem cell transplantation. *Eur J Haematol*. 2011;86: 57-66.
18. Meyer RG, Britten CM, Wehler D, et al. Prophylactic transfer of CD8-depleted donor lymphocytes after T-cell–depleted reduced-intensity transplantation. *Blood*. 2007;109:374-382.
19. Meyer RG, Wagner EM, Konur A, et al. Donor CD4 T cells convert mixed to full donor T-cell chimerism and replenish the CD52-positive T-cell pool after alemtuzumab-based T-cell–depleted allo-transplantation. *Bone Marrow Transplant*. 2010;45:668-674.
20. Koehl U, Bochennek K, Zimmermann SY, et al. Immune recovery in children undergoing allogeneic stem cell transplantation: absolute $CD8^+$ $CD3^+$ count reconstitution is associated with survival. *Bone Marrow Transplant*. 2007;39:269-278.
21. Parkman R, Cohen G, Carter SL, et al. Successful immune reconstitution decreases leukemic relapse and improves survival in recipients of unrelated cord blood transplantation. *Biol Blood Marrow Transplant*. 2006;12:919-927.
22. Tomita Y, Sachs DH, Khan A, et al. Additional monoclonal antibody (mAb) injections can replace thymic irradiation to allow induction of mixed chimerism and tolerance in mice receiving bone marrow transplantation after conditioning with anti-T cell mAbs and 3-Gy whole body irradiation. *Transplantation*. 1996;61:469-477.
23. Nikolic B, Zhao G, Swenson K, et al. A novel application of cyclosporine A in nonmyeloablative pretransplant host conditioning for allogeneic BMT. *Blood*. 2000;96:1166-1172.
24. Dey BR, McAfee S, Colby C, et al. Anti-tumour response despite loss of donor chimaerism in patients treated with nonmyeloablative conditioning and allogeneic stem cell transplantation. *Br J Haematol*. 2005;128:351-359.
25. Spiegelhalter DJ, Abrams KR, Myles JP. *Bayesian Approaches to Clinical Trials and Health Care Evaluation*. West Sussex, UK: Wiley; 2004.
26. Lawler M, McCann SR, Marsh JC, et al. Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe aplastic anaemia (SAA): indication for routine assessment of chimerism post SCT for SAA. *Br J Haematol*. 2009;144:933-945.
27. Shaw BE, Byrne JL, Das-Gupta E, et al. The impact of chimerism patterns and predonor leukocyte infusion lymphopenia on survival following T cell–depleted reduced intensity conditioned transplants. *Biol Blood Marrow Transplant*. 2007;13: 550-559.
28. Saito AM, Kami M, Mori S, et al. Prospective phase II trial to evaluate the complications and kinetics of chimerism induction following allogeneic hematopoietic stem cell transplantation with fludarabine and busulfan. *Am J Hematol*. 2007;82:873-880.

29. Hardy NM, Hakim F, Steinberg SM, et al. Host T cells affect donor T cell engraftment and graft-versus-host disease after reduced-intensity hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:1022-1030.
30. Petersen SL, Madsen HO, Ryder LP, et al. Chimerism studies in HLA-identical nonmyeloablative hematopoietic stem cell transplantation point to the donor CD8(+) T-cell count on day +14 as a predictor of acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2004;10:337-346.
31. Craddock C, Nagra S, Peniket A, et al. Factors predicting long-term survival after T-cell–depleted reduced-intensity allogeneic stem cell transplantation for acute myeloid leukemia. *Haematologica*. 2010;95:989-995.
32. Zeiser R, Spyridonidis A, Wasch R, et al. Evaluation of immunomodulatory treatment based on conventional and lineage-specific chimerism analysis in patients with myeloid malignancies after myeloablative allogeneic hematopoietic cell transplantation. *Leukemia*. 2005;19:814-821.
33. Shlomchik WD. Graft-versus-host disease. *Nat Rev Immunol*. 2007;7:340-352.
34. Goulmy E, Schipper R, Pool J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med*. 1996;334: 281-285.
35. Spellman S, Warden MB, Haagenson M, et al. Effects of mismatching for minor histocompatibility antigens on clinical outcomes in HLA-matched, unrelated hematopoietic stem cell transplants. *Biol Blood Marrow Transplant*. 2009;15:856-863.
36. Levenga H, Woestenenk R, Schattenberg AV, et al. Dynamics in chimerism of T cells and dendritic cells in relapsed CML patients and the influence on the induction of alloreactivity following donor lymphocyte infusion. *Bone Marrow Transplant*. 2007; 40:585-592.
37. Koenecke C, Shaffer J, Alexander SI, et al. NK cell recovery, chimerism, function, and recognition in recipients of haploidentical hematopoietic cell transplantation following nonmyeloablative conditioning using a humanized anti-CD2 mAb, Medi-507. *Exp Hematol*. 2003;31:911-923.
38. Sobecks RM, Ball EJ, Askar M, et al. Influence of killer immunoglobulin-like receptor/HLA ligand matching on achievement of T-cell complete donor chimerism in related donor nonmyeloablative allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2008;41:709-714.
39. Carlin BP, Louis TA. *Bayes and Empirical Bayes Methods for Data Analysis*, 2nd ed. New York: Chapman & Hall/CRC; 2000.