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The Effect of the Composition of Unrelated Donor Bone Marrow and Peripheral Blood Progenitor Cell Grafts on Transplantation Outcomes

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Abstract

To test the hypothesis that the outcome of hematopoietic stem cell grafts is at least partially determined by the cellular composition of the graft, the National Marrow Donor Program analyzed the correlation of cellular phenotypes of unrelated grafts with graft outcome. Samples from 94 bone marrow (BM) and 181 peripheral blood progenitor cell (PBPC) grafts for transplantations at 40 U.S. transplant centers between 2003 and 2005 were analyzed at a single immunophenotyping reference laboratory. Samples were shipped from transplant centers upon receipt of graft. Graft cellular composition included analysis of leukocyte total cell numbers, and subsets of myeloid [CD34+, CD34 + CD38−], lymphoid [CD3+, CD3+ CD4+, CD3+ CD8+], and activated lymphoid cells [CD3+ CD25 +, CD3+ CD69+, CD3+ HLA-DR+] coexpressing CD3+. There was substantial variability in the cellular composition of BM and PBPC grafts before and after graft processing by red blood cell (RBC) removal or plasma depletion in preparation for transplant. With BM grafts, cellular composition was not associated with hematopoietic recovery, graft-versus-host disease (GVHD), or survival. With PBPC grafts, survival rates were higher with CD34 + $>5 \times 10^6$/kg, 59% compared to 34% with CD34+ $\leq 5 \times 10^6$/kg at 1-year. Platelet recovery was higher with PBPC containing CD3+ CD8+ $>8 \times 10^7$/kg. Neutrophil recovery or GVHD could not be predicted by any cellular subsets of

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PBPC grafts. Though survival was superior with PBPC grafts containing >5 × 10^6 CD34+/ kg an optimal graft mix of myeloid, lymphoid and activated lymphoid subsets was not identified.

Keywords

Graft composition; unrelated donor transplant; hematopoietic recovery; overall survival

INTRODUCTION

The clinical outcome of hematopoietic cell transplantation (HCT) is determined by a variety of patient, disease and graft factors. While donor-recipient HLA matching is the major determinant of transplant-outcome after unrelated donor transplantation, among graft factors, product cellular composition, cell dose, and the effects of post harvest, pre-infusion processing are considered sufficiently important to influence transplant-outcome. However, after almost three decades of allogeneic HCT using volunteer unrelated bone marrow (BM) or peripheral blood progenitor cells (PBPC), the optimal cellular composition of these grafts has not been defined. Prior trials using diverse patient populations have reported conflicting results in correlating the composition of BM and PBPC grafts (1-8) with transplant-outcome. Therefore, this study was designed to examine the composition of volunteer unrelated donor grafts and the effect on graft outcomes.

The various methods of graft processing may affect the cellular composition of the final product. The most common graft manipulations are red blood cell (RBC) removal, plasma depletion, and mononuclear cell concentration. The aim of these simple manipulations is to volume reduce or remove erythrocyte antigens or plasma antibodies (isohemagglutinins) from the graft without significant loss of hematopoietic progenitor cells or lymphocyte subsets. In contrast, the more complex procedures of T-cell depletion or CD34+ cell selection profoundly affect graft composition with the specific aim of modifying numbers of certain cell types to improve engraftment or decrease graft-versus-host disease (GVHD) (6,7). Flow cytometric analysis of the graft may provide a rapid predictive measure of graft outcome (2-5,7,8). However, inter-laboratory standardization in analyzing grafts is difficult, as gating and subset enumeration still remains an art rather than a precise science, particularly for complex cellular samples such as BM or mobilized PBPC. The use of a single reference laboratory offers the advantages of consistency and efficiency, but the logistics and costs of sample collection and shipment to the reference laboratory can limit the feasibility of standardized data collection. Furthermore, the quality of the data obtained may be compromised by the effects of transport time and temperature on overall sample integrity and cellular viability (9).

MATERIALS AND METHODS

Study population: patient, disease and transplant characteristics

This study was developed to prospectively determine the relationship between graft myeloid and lymphoid subset cell dose and clinical outcome for BM and PBPC grafts, using data from a single immuno-phenotyping reference laboratory. The study was developed and coordinated by the National Marrow Donor Program, (Minneapolis, MN), following approval of the protocol from the NMDP Institutional Review Board. Forty-five participating transplant centers were recruited through the NMDP, however, only 40 transplant centers provided patient consent, samples and complete phenotypic and clinical data (see list of participating transplant centers, Appendix 1). Consequently, the study population (94 BM and 181 PBPC recipients) were transplanted at 40 centers in the U.S between July 2003 and March 2005. Recipients of T-cell depleted (BM; n=29) and CD34 selected (PBPC; n=17) transplants from these centers

were excluded as the most centers did not send a sample to the reference laboratory post-processing.

All grafts were characterized for cellular quantity and quality and studied to determine the effects of laboratory processing on graft composition. Specifically, we examined the impact of graft composition measured by total WBC numbers, and the number of cells belonging to the myeloid subset [CD34+, CD34+ CD38−], the lymphoid subset [CD3+, CD3+ CD4+, CD3 + CD8+], and activated lymphoid cells [CD3+ CD25+, CD3+ CD69+, CD3+ HLA-DR+] coexpressing CD3+. Constraints of cell numbers dictated that other subpopulations of lymphocytes, notably Tregs, were not analyzed. The outcome parameters of hematological recovery, acute and chronic GVHD, and survival were analyzed, with a median follow-up of 2 years. Data were adjusted for patient, disease and transplant characteristics.

Definition of outcomes

The primary outcome of this analysis was overall survival defined as time from infusion of graft to death from any cause. Surviving patients were censored at last follow-up. Secondary endpoints evaluated include: 1) neutrophil recovery defined as achieving neutrophil count $\geq 0.5 \times 10^9$/L for three consecutive measurements, 2) platelets recovery to $\geq 20 \times 10^9$/L for seven days, unsupported, 3) grade 2-4 acute GVHD as defined by the Glucksberg scale (10) and 4) chronic GVHD (11).

Sample collection and analysis

Unrelated donor BM or filgrastim-mobilized PBPC grafts were couriered by standard NMDP shipment protocols from the collection center to the transplant center's processing laboratory. Upon arrival at the transplant center processing laboratory, a sample of the original product was shipped to the reference laboratory (sample 1). If the graft underwent further processing (RBC or plasma depletion) at the transplant center, the processing laboratory sent a sample of the graft after processing (sample 2). Both samples were packaged and shipped (in insulated cool packs) to the central reference laboratory, Baylor College of Medicine Center for Cell and Gene Therapy (CAGT). The samples were shipped inside secondary containers with frozen gel packs in Class 6.2 shipping containers and transported by overnight delivery service to the reference laboratory.

The transplant center processing laboratory reported data on type of processing (no processing, mononuclear cell concentration, plasma depletion, red blood cell removal), time of collection and time of processing. Upon receipt of the samples, the reference laboratory recorded the condition of the sample, the packaging condition (orientation, temperature and number of gel packs), and the time from collection and processing. Each sample was assessed for overall sample integrity and filtered using a 40μm Cell Strainer (Falcon #352340) if cellular aggregates were present. Only samples that were free of visible clumps and were at least 70% viable (by 7 AAD staining) were included in the analysis. Leukocyte count of the sample was enumerated using a Coulter AcT 8/10 Analyzer (Beckman-Coulter, Miami FL). In preparation for immunophenotyping each sample was adjusted to 1×10^7 cells per mL. Saturation concentrations of conjugated monoclonal antibodies (BDIS : CD45, CD34+, CD38, CD3+, CD3+ CD4+, CD3+ CD8+, CD3+ CD69+, CD3+ CD25+ & CD3+ HLA-DR+) were added to 12×75mm polystyrene tubes (Falcon 352008), followed by 100μL (10^6) cells and incubated 15 minutes at ambient temperature (18°C-22°C) in the dark. The antibodies and reagents are ASR reagents purchased from Becton-Dickinson Biosciences and include CD45 clone 2D1, CD34 class III clone 8G12, CD3 clone SK7, CD4 clone SK3, CD8 clone SK1, CD69 clone L78, CD25 clone 2A3, and HLA-DR clone L243. Also included, but not requested in the study design, CD19 clone 4G7 and CD16 clone B73.1 + CD56 clone MY31, to serve as internal Lympho-Sum control. All antibodies were used a saturated concentrations against the antigen

concentration and determined by in-house checkerboard titration and/or used at the manufacturers suggested concentration. Commercial reference controls (CD-Chex Plus from Streck Laboratories, Inc) were run each day of sample evaluation as quality control of the staining-acquisition-and analysis for CD34, CD3, CD4, CD8 and HLA markers, in addition to the B-cell and NK-cell markers. There were no commercial controls available for the activation markers CD69 and CD25. Additionally, a combination of CD45 and CD14 was included to provide a 3-part differential which was also used as an internal control to ensure that the sum of T+B+NK cells was equal to the lymphocytes. The samples were processed using a programmable Lyse-Wash Assistant (BDIS), lysing the erythrocytes with ammonium chloride-based solution (PharM Lyse, BDIS). Viability after phenotyping was assessed using 7AAD (ViaProbe, BDIS). Data were acquired using single laser (488nm) flow cytometer with Cell-Quest Pro™ software. For progenitor cells, a total of 100,000 events were stored in list-mode; and 20,000 total events for lymphocyte and viability data. The acquisition software was also used for the analysis of the list-mode data. The CD34+ enumeration was performed using ISHAGE gating strategy (12). The lymphocytes were enumerated by defining a light scatter region around the lymphocyte population. The region was confirmed by CD3-backgating and the subsets co-expressing CD3 were enumerated (CD3+ CD4+, CD3+ CD8, CD3+ CD69+, CD3+ CD25+, and CD3+ HLA-DR+).

Statistical analysis

Median value and ranges are reported for continuous variables and percentages for categorical variables. The probability of overall survival was calculated using the Kaplan-Meier estimator (13). The probabilities of neutrophil and platelet recovery and acute and chronic GVHD were calculated with the cumulative-incidence estimator (14). Death was considered a competing risk for hematopoietic recovery and GVHD. Patients were censored if they underwent a second transplant, or at last follow-up. 95% confidence intervals (CI) were calculated with log transformation.

To analyze the association of cellular composition (white blood cell count, CD34+, CD34+ CD38−, CD3+, CD3+ CD4+, CD3+ CD8+, CD3+ CD25+, CD3+ CD69+ and CD3+ HLA-DR+) and clinical outcomes, multivariate logistic regression models were constructed for each cellular component for hematopoietic recovery, and Cox proportional hazards regression models (15) were constructed for acute and chronic GVHD, and overall survival. All the cell dose effects were analyzed as a binary variable. To dichotomize the continuous variables, Martingale residuals were plotted for each cell dose in order to determine the optimal cut point. We concluded that the medians were good cut points for all cell doses and thus a binary variable with median as cut point was used for all subsets analyzed. Other variables tested included age, performance score, disease (malignant vs. non-malignant), disease status at transplantation (early vs. intermediate vs. advanced disease vs. non-malignant disease), conditioning regimen (myeloablative vs. reduced intensity conditioning vs. conditioning for non-malignant diseases), donor-recipient HLA match (well matched vs. partially matched vs. mismatched) (16), donor-recipient CMV serostatus, ABO mismatch, donor age and graft processing (mononuclear cell concentration vs. plasma depletion vs. red blood cell removal vs. none). Due to the multiple comparisons included in the statistical analysis, a $p<.01$ was defined as statistically significant. All p-values were two-sided. All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC).

RESULTS

The demographic, disease and transplant characteristics of the 94 BM and 181 PBPC recipients are outlined on Table 1. The median age was 20 years (range <1-66) for BM recipients and 45 years (<1-70) for PBPC recipients. Most patients had high performance scores; performance

scores were ≥90 for 81% of BM and 71% of PBPC recipients. Nearly all transplants (85% BM and 94% PBPC) were for treatment of malignancy. Transplant conditioning regimens for hematological malignancies included both myeloablative (70% of BM and 54% of PBPC transplants) and reduced intensity regimens (15% of BM and 40% of PBPC transplants). Only 14 (15%) BM and 10 (6%) PBPC transplants were for non-malignant diseases. Donor and recipient were HLA well-matched for 73% of BM and 66% of PBPC transplants.

Cellular composition of grafts and effect of processing

Samples were assessed for temperature of gel packs, sample integrity (absence of visible clumps after gentle agitation or clots requiring filtration), viability (>70%) and immunophenotype (CD34+, CD3+, and CD3+ T-cell subsets, CD3+ CD25+, CD3+ CD69+, CD3+ HLA-DR+). Samples from BM and PBPC products included herein met sample integrity and viability requirements. Compared to BM grafts, PBPC grafts contained more nucleated cells (7.23×10^8/kg vs. 2.34×10^8/kg), CD3+ cells (2.37×10^8/kg vs. 2.54×10^7/kg) and CD34 + cells (5.41×10^6/kg vs. 3.62×10^6/kg). Data on differences in cellular composition before and after processing (BM or PBPC) and type of processing are shown in Figures 1-2. Fifteen BM grafts were subject to plasma depletion and 32 to RBC removal. Plasma depletion of BM was not associated with changes in CD34+ and CD34+ CD38−, CD3+ and CD3+ HLA-DR+ recovery (Figure 1A). In contrast, RBC removal of BM grafts resulted in lower CD34+, CD34 + CD38− and CD3+ doses (Figure 1B). Thirty-seven PBPC grafts were subject to plasma depletion. CD3+ dose was lower but there were no differences in CD34+, CD34+ CD38− or CD3+ HLA-DR+ doses before and after plasma depletion (Figure 2).

Effect of graft composition on post-HCT outcomes

Hematopoietic recovery—Ninety two of 94 (98%) recipients of BM grafts and 171 of 181 (94%) recipients of PBPC grafts achieved neutrophil recovery. The median time to neutrophil recovery was 18 days and 13 days after transplantation of BM and PBPC grafts, respectively. Corresponding day-28 probabilities of neutrophil recovery were 94% (95% CI 87-98%) and 93% (95% CI 88-96%). In multivariate analysis, neutrophil recovery after BM or PBPC transplantation was not associated with white blood cell count, myeloid or lymphoid subsets or activated lymphoid cells (Table 2). 69 of 94 (73%) recipients of BM grafts and 149 of 181 (82%) recipients of PBPC grafts achieved platelet recovery. The median time to platelet recovery was 27 days and 20 days after transplantation of BM and PBPC grafts, respectively. Corresponding day-60 probabilities of platelet recovery were 67% (95% CI 57-76%) and 79% (95% CI 73-85%). In multivariate analysis, platelet recovery after BM transplantation was not associated with white blood cell count, myeloid or lymphoid subsets or activated lymphoid cells. Platelet recovery was more likely with PBPC grafts containing $>8 \times 10^7$/kg CD3+ CD8 + cells (OR 2.80, 95% CI 1.25 – 6.28, p=0.01) (Table 2).

Acute and chronic GVHD—The day-100 probabilities of grade 2-4 acute GVHD were 43% and 54% after transplantation of BM and PBPC, respectively. The 1-year probabilities of chronic GVHD were 31% and 44% after transplantation of BM and PBPC, respectively. In multivariate analysis, acute and chronic GVHD were not associated with white blood cell count, myeloid or lymphoid subsets or activated lymphoid cells (Table 3).

Overall survival—Forty six of 94 (49%) recipients of BM and 67 of 181 (37%) recipients of PBPC transplants were alive at the last contact. In multivariate analysis of BM transplants, overall mortality was not associated with white blood cell count, myeloid or lymphoid subsets or activated lymphoid cells (Table 4). In contrast, higher CD34+ cell dose ($>5 \times 10^6$/kg) was associated with lower risks of mortality (RR 0.58, 95% CI 0.39 – 0.84, p<0.01) after PBPC transplants. No other myeloid or lymphoid subset or activated lymphoid cells were associated

with overall survival (Table 4). The 1-year probabilities of overall survival were 59% and 34% when PBPC grafts contained >5 × 10^6/kg and ≤5 × 10^6/kg CD34+ cells, respectively.

DISCUSSION

The composition of a hematopoietic progenitor cell graft is a major determinant of clinical outcome. It has been seen in grafts from both related (17,18) and unrelated (19,20) donors that when grafts are analyzed for cell dose by various phenotypic markers (e.g., CD34, DR, CD3) associations are seen with clinical outcomes such as survival, rate and type of hematologic recovery, or GVHD. The bone marrow or PBPC source for the graft has a profound effect on cellular composition and the cell dose, since mobilized peripheral blood contains a larger number but lesser percentage of primitive myeloid cells and a much greater numbers and higher percentage of mature T cells than bone marrow. Not surprisingly, this reciprocal relationship of cell types and doses between bone marrow and peripheral blood progenitor cell grafts can lead to vastly different clinical outcomes (21,22,23,24). However, though several comparative analyses have been published, including the Blood and Marrow Transplant Clinical Trials Network protocol 0201 which recently completed enrollment of 550 patients randomized to unrelated donor marrow or mobilized blood grafts. In earlier trials, prospective testing has not been done using a single reference laboratory to determine the relationship between graft composition and transplantation outcome.

Laboratory processing of bone marrow or PBPC also has a major effect on the cellular composition of the final graft. The common laboratory procedures of plasma depletion and RBC reduction are done to reduce risks of transfusion reactions in the recipient with minimal stem cell loss. The effect of processing on graft composition is necessary to plan the optimal volume of marrow cells to be collected or number of apheresis procedures.

As expected, in this study substantial variability was seen in the cellular graft composition of BM and PBPC grafts both before and after common manipulations of RBC removal or plasma depletion. In BM grafts the cellular composition was not associated with hematopoietic recovery, GVHD, or survival while in PBPC grafts, superior survival was seen when grafts contained > 5 × 10^6 CD34+/kg, and superior platelet recovery was seen when grafts contained > 8 × 107 CD3+ CD8+/kg. However, neither survival nor GVHD were altered by any other cellular subsets in PBPC grafts.

In this report, a central reference laboratory was used to ensure that data on the composition of the grafts examined were consistent and reliable. Importantly the study demonstrated that the data integrity is dependent on the products (samples analyzed) being an accurate representation of the transplanted graft and that the sample analyzed is relatively unaffected by storage or transportation prior to analysis. Improper storage and transport of products could differentially affect the viability of myeloid and lymphoid elements, leading to misleading interpretations. Multicenter studies using central reference laboratories must utilize uniform shipping protocols that ensure graft viability and integrity including temperature control and prompt delivery to the analyzing laboratory.

The clinical decision determining the optimal cell source and total cell number in the graft is dependent on the choice of cell source, the expected yield, how the graft is processed, and the reliability of phenotyping data obtained from samples. Determining the optimal graft composition of BM and PBPC could better serve the recipients and minimize demands on volunteer unrelated donors.

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Appendix 1: Participating transplant centers

Barbara Ann Karmanos Cancer Inst.- Wayne State University

Baylor University Medical Center

Children's Hospital Medical Center

Children's Hospital of Orange County

Children's Hospital of Philadelphia

Children's Hospital of Wisconsin

Children's Memorial Hospital

City of Hope National Medical Center

Cleveland Clinic Foundation

Duke University Medical Center

Emory University Egleston Children's Hospital

Georgetown University Hospital

H. Lee Moffitt Cancer Center

Henry Ford Health Systems

Loyola University Medical Center

Medical College of Wisconsin/Froedtert Memorial Lutheran Hospital Cancer Center

Memorial Sloan-Kettering Cancer Center

North Shore University Hospital

Rainbow Babies & Children's Hospital

Rocky Mountain Cancer Center/Presbyterian St. Luke's

Roswell Park Cancer Institute

Schneider Children's Hospital

St Louis Children's Hospital

St. Luke's Hospital of Kansas City

Stanford University Medical Center

The Jewish Hospital

The Ohio State University/Arthur G. James Cancer Hospital and Richard J. Solove Research Institute

University of California San Francisco Children's Hospital

University of Chicago Children's Hospital

University of Iowa Hospital

University of Michigan Medical Center

University of Minnesota Hospitals & Clinics

University of Mississippi Medical Center

University of Nebraska Medical Center

University of North Carolina Hospitals

University of Oklahoma Health Sciences Center/HCA Health Services of Oklahoma, Inc.

University of Pittsburgh Medical Center Cancer Pavilion

University of Rochester Medical Center/Strong Memorial Hospital

University of Utah Medical Center

West Virginia University Hospitals, Inc.

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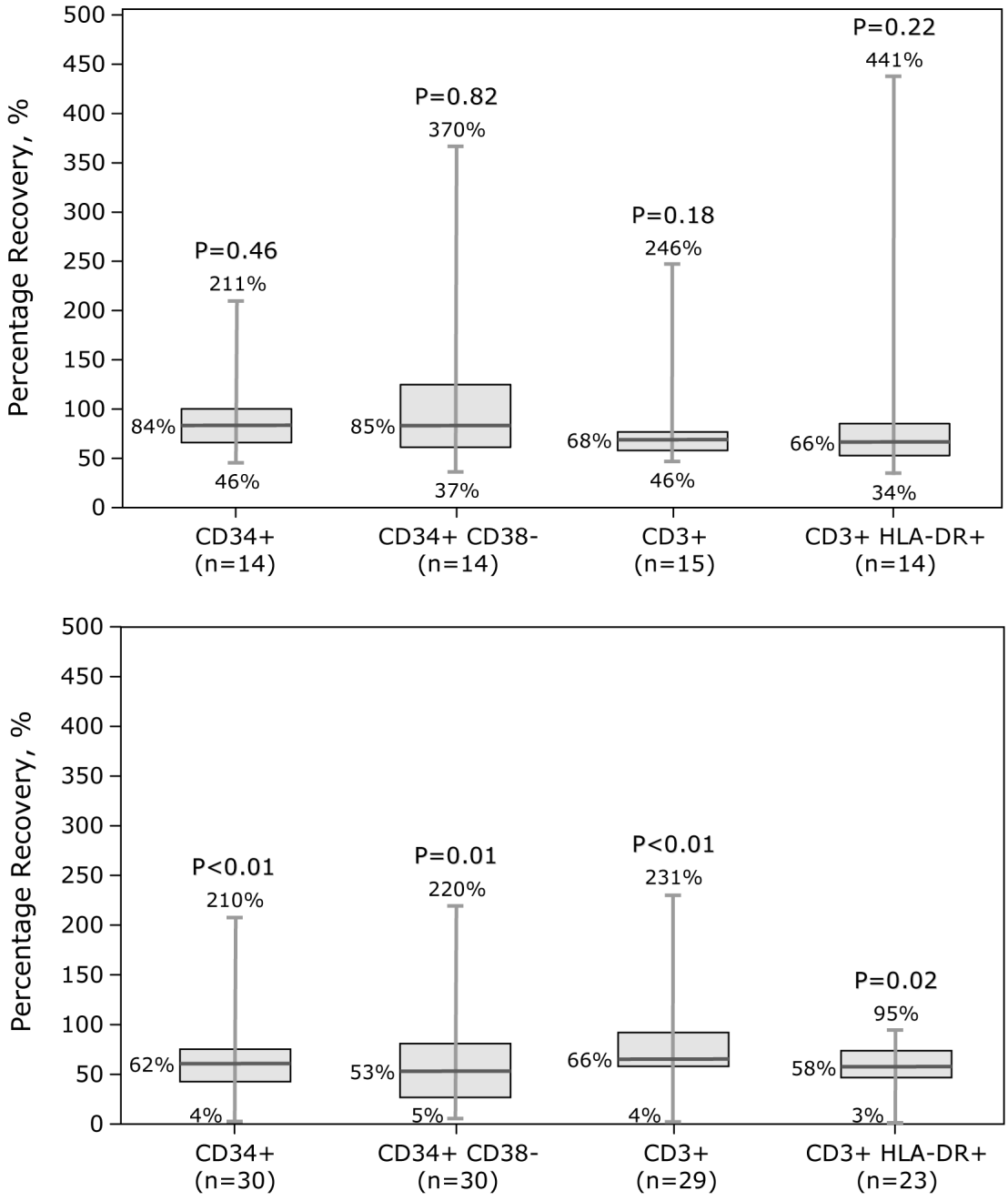

Figure 1.
Effect of processing on BM product graft composition.
1A: Plasma depletion. 1B: RBC removal.

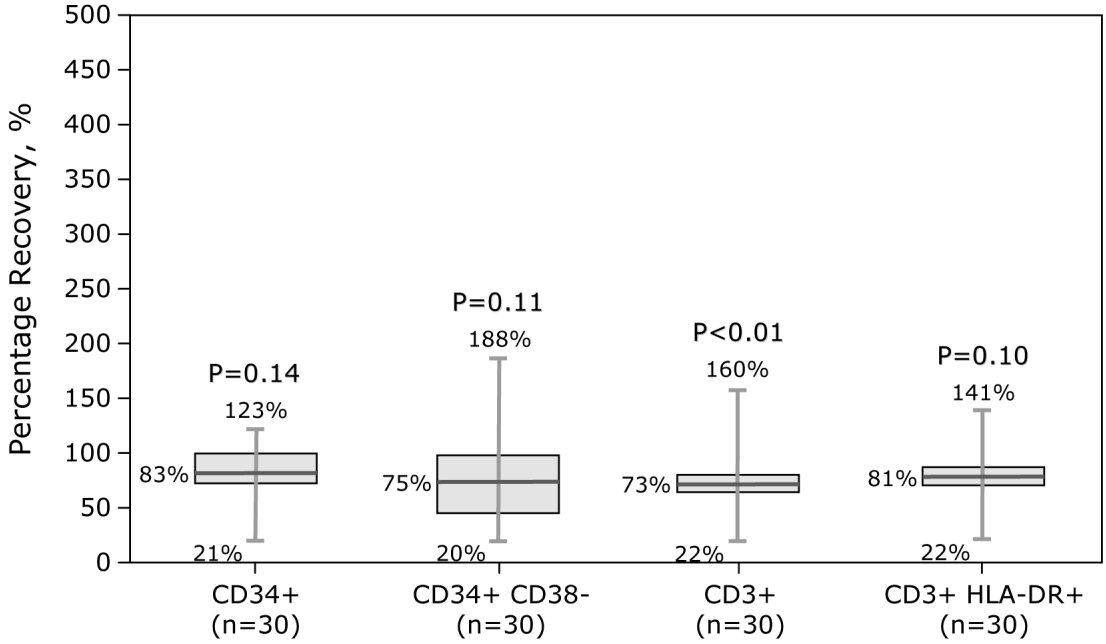

Figure 2.
Effect of processing on PBPC product graft composition – plasma depletion.
Numbers given on the figures were minimum, median and maximum of percentage recovery, and p-value from Kruskal-Wallis test to compare cell doses between before and after processing.

Table 1

Patient, disease and transplant characteristics

	Bone marrow	Peripheral blood progenitor cells
Variables	**Number (%)**	**Number (%)**
Number of cases	94	181
Sex, male	52 (55)	111 (61)
Age, in years, median (range)	20 (<1-66)	45 (<1-70)
10 or under 10	22 (23)	13 (7)
11-20	22 (23)	16 (9)
21-30	6 (6)	23 (13)
31-40	14 (15)	22 (12)
41-50	13 (14)	41 (23)
51-60	13 (14)	44 (24)
over 60	4 (4)	22 (12)
Karnofsky performance score 90-100	65 (81)	118 (71)
Diseases		
Acute myeloid leukemia	29 (31)	62 (34)
Acute lymphoblastic leukemia	15 (16)	39 (22)
Other leukemia	4 (4)	15 (8)
Chronic myeloid leukemia	13 (14)	13 (7)
Myelodysplastic syndrome	10 (11)	24 (13)
Non-Hodgkin lymphoma	8 (9)	16 (9)
Plasma cell disorder, multiple myeloma	2 (2)	3 (2)
Other malignancy	0	2 (1)
Severe aplastic anemia	7 (7)	5 (3)
SCID and other immune system disorders	1 (1)	0
Inherited disorder of metabolism	2 (2)	1 (1)
Histiocytic disorders	3 (3)	1 (1)
Disease status prior to transplant*		
Early	22 (23)	45 (25)
Intermediate	24 (26)	42 (23)
Advanced	34 (36)	84 (46)
Non-malignant	14 (15)	10 (6)
Conditioning regimen		
Malignant diseases, myeloablative	66 (70)	98 (54)
Malignant diseases, reduced intensity	14 (15)	73 (40)
Non-malignant diseases, other	14 (15)	10 (6)
HLA disparity†		
Well-matched	69 (73)	119 (66)
Partially matched	20 (21)	41 (23)
Mismatched	5 (5)	21 (12)
Cytomegalovirus serostatus		

	Bone marrow	Peripheral blood progenitor cells
Variables	**Number (%)**	**Number (%)**
Donor negative /recipient negative	27 (29)	52 (29)
Donor negative / recipient positive	35 (37)	63 (35)
Donor positive / recipient negative	11 (12)	25 (14)
Donor positive / recipient positive	20 (21)	38 (21)
Unknown	1 (1)	3 (2)
ABO match		
Matched	45 (48)	67 (37)
Minor mismatch	15 (16)	55 (30)
Major mismatch	28 (30)	41 (23)
Bi-directional	5 (5)	18 (10)
Unknown	1 (1)	0
Donor-recipient sex match		
Male donor - male recipient	31 (33)	85 (47)
Male donor - female recipient	34 (36)	42 (23)
Female donor – male recipient	21 (22)	26 (14)
Female donor – female recipient	8 (9)	28 (15)
Donor age, years		
18-30	27 (29)	56 (31)
31-40	37 (39)	68 (38)
41-50	25 (27)	46 (25)
51-60	5 (5)	11 (6)
Year of Transplant		
2003	22 (23)	46 (25)
2004	56 (60)	100 (55)
2005	16 (17)	35 (19)
Graft processing type		
Mononuclear cell concentration	6 (6)	0
Plasma depletion	15 (16)	37 (20)
Red blood cell removal	32 (34)	1 (1)
None	41 (44)	143 (79)
Median follow-up survival, months (range)	24 (6-30)	24 (3-37)
Cell dose per kg, median (range)		
WBC	2.34×10^{8} (4.33×10^{6}-9.56×10^{8})	7.23×10^{8} (4.06×10^{7}-4.69×109)
CD34+	3.62×10^{6} (1.02×10^{5}-1.84×10^{7})	5.41×10^{6} (3.08×10^{5}-3.23×10^{7})
CD34+ CD38−	9.44×10^{4} (8.76×10^{2}-7.39×10^{5})	1.54×10^{5} (8.12×10^{3}-1.07×10^{6})
CD3+	2.54×10^{7} (6.43×10^{5}-1.21×10^{8})	2.37×10^{8} (2.19×10^{7}-2.05×10^{9})
CD3+ CD4+	1.33×10^{7} (2.87×10^{5}-6.06×10^{7})	1.45×10^{8} (1.43×10^{7}-1.29×10^{9})
CD3+ CD8+	1.09×10^{7} (2.94×10^{5}-7.52×10^{7})	8.37×10^{7} (7.07×10^{6}-6.69×10^{8})
CD3+ CD25+	3.05×10^{6} (1.08×10^{5}-1.72×10^{7})	2.46×10^{7} (2.34×10^{6}-2.40×10^{8})
CD3+ CD69+	3.38×10^{6} (1.43×10^{5}-2.64×10^{7})	3.55×10^{6} (1.51×10^{5}-3.89×10^{7})

	Bone marrow	Peripheral blood progenitor cells
Variables	**Number (%)**	**Number (%)**
CD3+ HLA-DR+	3.92×10^{6} (8.59×10^{4}-3.50×10^{7})	2.18×10^{7} (1.62×10^{6}-2.06×10^{8})

* Early is defined as CR1, CP1, RA, and RARS; intermediate is defined as ≥ CR2+, AP, and ≥CP2; and advanced is defined as PIF, BP, relapse, RAEB, RAEBT, CMMoL, Durie-Salmon Stage III, and Waldenstrom macroglobulinemia.

† Well-matched includes: 8/8 allele-level matched (BM n=46, PBPC n=91); allele-level matched A, B, DRB1 and low res matched at HLA-C (BM n=7, PBPC n=8); low res matched at A, B and C and allele-level DRB1 (BM n=16, PBPC n=19), allele-level matched at A, B, DRB1, and HLA-C unknown (PBPC n=1)

Partially matched includes: single locus antigen or allele-level MM at A, B, C or DRB1(BM n=19, PBPC n=39); matched at low-res A, B and high-res DRB1 and HLA-C unknown (BM n=1, PBPC n=1); matched at low-res A, B, C and DRB1 (PBPC n=1).

Mismatched includes: >1 allele or antigen MM at A, B, C, DRB1 (BM=4, PBPC n=20); 1 antigen mismatch at high-res A, B, DRB1, and HLA-C unknown (BM n=1); 1 mismatch at lowres A, B, high-res at DRB1 and HLA-C unknown (PBPC n=1)

Table 2

Multivariate analysis of hematopoietic recovery

Bone marrow	Neutrophil recovery OR (95%CI), *p*-value	Platelet recovery OR (95%CI), *p*-value
WBC >2.34 vs. ≤2.34, 10^8 /kg	5.48 (0.61-48.80), 0.13	1.98 (0.82-4.76), 0.13
CD34+ >3.62 vs. ≤3.62, 10^6 /kg	1.00 (0.19-5.23), 1.00	0.75 (0.32-1.78), 0.51
CD34+ CD38− >9.44 vs. ≤9.44, 10^4 /kg	2.09 (0.36-0.41), 0.41	1.34 (0.57-3.17), 0.51
CD3+ >2.54 vs. ≤2.54, 10^7 /kg	1.00 (0.19-5.23), 1.00	0.75 (0.32-1.78), 0.51
CD3+ CD4+ >1.33 vs. ≤1.33, 10^7 /kg	1.03 (0.14-7.66), 0.98	0.47 (0.18-1.21), 0.12
CD3+ CD8+ >1.09 vs. ≤1.09, 10^7 /kg	0.32 (0.03-3.18), 0.33	0.80 (0.31-2.03), 0.63
CD3+ CD25+ >3.05 vs. ≤3.05, 10^6 /kg	1.50 (0.24-9.50), 0.67	1.70 (0.66-4.35), 0.27
CD3+ CD69+ >3.38 vs. ≤3.38, 10^6 /kg	0.65 (0.10-4.11), 0.65	1.55 (0.62-3.88), 0.35
CD3+ HLA-DR+ >3.92 vs. ≤3.92, 10^6 /kg	0.48 (0.08-2.75), 0.41	0.82 (0.34-1.97), 0.66

Peripheral blood progenitor cells	Neutrophil recovery OR (95%CI), *p*-value	Platelet recovery OR(95%CI), *p*-value
WBC >7.23 vs. ≤7.23, 10^8 /kg	1.17 (0.38-3.62), 0.79	2.04 (0.90-4.60), 0.09
CD34+ >5.41 vs. ≤5.41, 10^6 /kg	1.17 (0.38-3.62), 0.79	2.32 (1.05-5.16), 0.04
CD34+ CD38− >1.54 vs. ≤1.54, 10^5 /kg	0.61 (0.19-1.94), 0.40	1.00 (0.46-2.17), 0.99
CD3+ >2.37 vs. ≤2.37, 10^8 /kg	1.64 (0.52-5.21), 0.40	1.92 (0.88-4.17), 0.10
CD3+ CD4+ >1.45 vs. ≤1.45, 10^8 /kg	0.60 (0.19-1.90), 0.38	1.22 (0.56-2.66), 0.62
CD3+ CD8+ >8.37 vs. ≤8.37, 10^7 /kg	2.36 (0.70-7.96), 0.17	2.80 (1.25-6.28), 0.01
CD3+ CD25+ >2.46 vs. ≤2.46, 10^7 /kg	6.13 (1.32-28.49), 0.02	2.29 (1.02-5.13), 0.04
CD3+ CD69+ >3.55 vs. ≤3.55, 10^6 /kg	3.62 (0.96-13.64), 0.06	1.18 (0.55-2.55), 0.67
CD3+ HLA-DR+ >2.18 vs. ≤2.18, 10^7 /kg	2.36 (0.70-7.96), 0.17	1.69 (0.78-3.67), 0.19

Abbreviations: OR=Odds Ratio; CI=Confidence Interval.

Table 3

Multivariate analysis of Grade 2-4 acute and chronic graft-versus-host disease

Bone marrow	Acute GVHD 2-4 RR (95% CI), *p*-value	Chronic GVHD RR (95% CI), *p*-value
WBC >2.34 vs. ≤2.34, 10^8 /kg	0.67 (0.33-1.12), 0.11	1.05 (0.51-2.14), 0.90
CD34+ >3.62 vs. ≤3.62, 10^6 /kg	0.79 (0.43-1.44), 0.43	0.94 (0.47-1.90), 0.87
CD34+ CD38− >9.44 vs. ≤9.44, 10^4 /kg	0.85 (0.47-1.55), 0.60	1.24 (0.61-2.53), 0.55
CD3+ >2.54 vs. ≤2.54, 10^7 /kg	0.57 (0.31-1.06), 0.07	0.95 (0.47-1.91), 0.88
CD3+ CD4+ >1.33 vs. ≤1.33, 10^7 /kg	0.93 (0.49-1.76), 0.83	0.90 (0.43-1.91), 0.79
CD3+ CD8+ >1.09 vs. ≤1.09, 10^7 /kg	0.80 (0.42-1.53), 0.49	1.05 (0.50-2.21), 0.90
CD3+ CD25+ >3.05 vs. ≤3.05, 10^6 /kg	0.94 (0.50-1.91), 0.85	0.76 (0.36-1.61), 0.47
CD3+ CD69+ >3.38 vs. ≤3.38, 10^6 /kg	0.71 (0.37-1.36), 0.30	0.81 (0.37-1.79), 0.61
CD3+ HLA-DR+ >3.92 vs. ≤3.92, 10^6 /kg	0.88 (0.47-1.62), 0.67	0.58 (0.28-1.19), 0.14

Peripheral blood progenitor cells	Acute GVHD 2-4 RR (95% CI), *p*-value	Chronic GVHD RR (95% CI), *p*-value
WBC >7.23 vs. ≤7.23, 10^8 /kg	1.19 (0.79-1.78), 0.41	1.07 (0.69-1.66), 0.77
CD34+ >5.41 vs. ≤5.41, 10^6 /kg	1.17 (0.78-1.76), 0.44	0.79 (0.51-1.22), 0.29
CD34+ CD38− >1.54 vs. ≤1.54, 10^5 /kg	1.63 (1.08-2.46), 0.02	0.98 (0.63-1.52), 0.93
CD3+ >2.37 vs. ≤2.37, 10^8 /kg	1.13 (0.75-1.69), 0.57	1.21 (0.77-1.89), 0.41
CD3+ CD4+ >1.45 vs. ≤1.45, 10^8 /kg	1.19 (0.79-1.78), 0.41	1.41 (0.90-2.21), 0.13
CD3+ CD8+ >8.37 vs. ≤8.37, 10^7 /kg	1.08 (0.72-1.62), 0.72	1.48 (0.95-2.32), 0.09
CD3+ CD25+ >2.46 vs. ≤2.46, 10^7 /kg	1.20 (0.80-1.82), 0.38	1.42 (0.91-2.23), 0.13
CD3+ CD69+ >3.55 vs. ≤3.55, 10^6 /kg	1.05 (0.70-1.57), 0.83	1.10 (0.71-1.71), 0.68
CD3+ HLA-DR+ >2.18 vs. ≤2.18, 10^7 /kg	0.79 (0.53-1.18), 0.25	0.89 (0.57-1.37), 0.58

Abbreviations: RR = Relative Risk; CI=Confidence Interval

Table 4

Multivariate analysis of overall mortality

Bone marrow		**Peripheral blood progenitor cells**	
Graft composition	**RR (95% CI), *p*-value**	**Graft composition**	**RR (95% CI), *p*-value**
WBC >2.34 vs. ≤2.34, 10^8 /kg	0.64 (0.36-1.16), 0.14	WBC >7.23 vs. ≤7.23, 10^8 /kg	0.68 (0.46-0.98), 0.04
CD34+ >3.62 vs. ≤3.62, 10^6 /kg	0.71 (0.40-1.27), 0.25	CD34+ >5.41 vs. ≤5.41, 10^6 /kg	0.58 (0.39-0.84), <0.01
CD34+ CD38− >9.44 vs. ≤9.44, 10^4 /kg	0.67 (0.37-1.21), 0.18	CD34+ CD38− >1.54 vs. ≤1.54, 10^5 /kg	1.04 (0.71-1.51), 0.85
CD3+ >2.54 vs. ≤2.54, 10^7 /kg	0.72 (0.41-1.29), 0.27	CD3+ >2.37 vs. ≤2.37, 10^8 /kg	0.75 (0.52-1.09), 0.13
CD3+ CD4+ >1.33 vs. ≤1.33, 10^7 /kg	0.98 (0.52-1.86), 0.96	CD3+ CD4+ >1.45 vs. ≤1.45, 10^8 /kg	0.80 (0.55-1.16), 0.25
CD3+ CD8+ >1.09 vs. ≤1.09, 10^7 /kg	0.86 (0.45-1.65), 0.66	CD3+ CD8+ >8.37 vs. ≤8.37, 10^7 /kg	0.66 (0.45-0.96), 0.03
CD3+ CD25+ >3.05 vs. ≤3.05, 10^6 /kg	0.83 (0.41-1.68), 0.60	CD3+ CD25+ >2.46 vs. ≤2.46, 10^7 /kg	0.89 (0.62-1.30), 0.55
CD3+ CD69+ >3.38 vs. ≤3.38, 10^6 /kg	0.55 (0.29-1.04), 0.07	CD3+ CD69+ >3.55 vs. ≤3.55, 10^6 /kg	0.77 (0.53-1.12), 0.18
CD3+ HLA-DR+ >3.92 vs. ≤3.92, 10^6 /kg	0.83 (0.46-1.52), 0.55	CD3+ HLA-DR+ >2.18 vs. ≤2.18, 10^7 /kg	0.67 (0.46-0.98), 0.04

Abbreviation: RR=Relative Risk; CI=Confidence Interval.