Donor, recipient, and transplant characteristics as risk factors after unrelated donor PBSC transplantation: beneficial effects of higher CD34+ cell dose

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We report outcomes of 932 recipients of unrelated donor peripheral blood stem cell hematopoietic cell transplantation (URD-PBSC HCT) for acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and myelodysplastic syndrome enrolled on a prospective National Marrow Donor Program trial from 1999 through 2003. Preparative regimens included myeloablative (MA; N = 611), reduced-intensity (RI; N = 160), and nonmyeloablative (NMA; N = 161). For MA recipients, CD34+ counts greater than 3.8×10^6/kg improved neutrophil and platelet engraftment, whereas improved overall survival (OS) and reduced transplant-related mortality (TRM) were seen for all preparative regimens when CD34+ cell doses exceeded 4.5×10^6/kg. Higher infused doses of CD34+ cell dose did not result in increased rates of either acute or chronic graft-versus-host disease (GVHD). Three-year OS and disease-free survival (DFS) of recipients of MA, RI, and NMA approaches were similar (33%, 35%, and 32% OS; 33%, 30%, and 29% DFS, respec-tively). In summary, recipients of URD-PBSC HCT receiving preparative regimens differing in intensity experienced similar survival. Higher CD34+ cell doses resulted in more rapid engraftment, less TRM, and better 3-year OS (39% versus 25%, MA, P = .004; 38% versus 21% RI/NMA, P = .004) but did not increase the risk of GVHD. This trial was registered at www.clinicaltrials.gov as #NCT00785525. (Blood. 2009;114:2606-2616)

Introduction

In the early 1990s hematopoietic cell transplantation programs began using cytokine-mobilized peripheral blood stem cells (PBSCs) from sibling donors in lieu of bone marrow (BM) as a primary stem cell source.[1-4] Unrelated donor (URD) transplantation networks followed suit at the end of the 1990s,[5] and the use of URD-PBSC grafts has grown rapidly. In 2007, 59% of National Marrow Donor Program (NMDP)–facilitated URD transplantations involved PBSCs (versus bone marrow and cord blood) and adult recipients of non–cord blood donations received PBSC grafts 80% of the time. The marked increase in the use of URD PBSCs was fueled by early reports showing more rapid engraftment, good survival, and similar rates of graft-versus-host disease (GVHD) compared with URD BM.[6,7] The trend toward the use of URD PBSCs was further influenced by a report of lower rates of rejection and disease progression compared with the use of BM after a nonmyeloablative preparative regimen,[8] resulting in PBSCs being the preferred choice in many reduced toxicity regimen approaches. Finally, ease of acquisition (apheresis versus marrow harvest) and donor choice probably added to the increased use of URD PBSCs. This high rate of URD-PBSC usage continues despite recent studies raising concern about late chronic GVHD-related morbidity.[9-13]

Large studies have defined specific donor, graft, and transplant characteristics that lead to better outcome after URD BM transplantation.[14-17] Aside from a recent analysis of CD34+ cell dose,[18] the effect of other factors such as donor sex, HLA match, preparative regimen intensity, GVHD prophylactic regimen, and so forth, on survival and GVHD outcomes after URD-PBSC transplantation have not been studied in a large cohort.

Since 1999, all NMDP PBSC transplantations have been performed under a US Food and Drug Administration–accepted Investigational New Drug application protocol designed to assess URD-PBSC safety, collection efficacy, and recipient outcomes. To correlate transplant characteristics with URD-PBSC outcomes, we limited our cohort to recipients who received a transplant for the 4 most common hematologic malignancies (acute myeloid leukemia [AML], acute lymphoblastic leukemia [ALL], chronic myelogenous leukemia [CML], and myelodysplastic syndrome [MDS]) enrolled in the NMDP PBSC trial. We included key donor, product, and transplant-related variables.

Methods

Study cohort and data collection

The study cohort consisted of all recipients of primary PBSC transplants for AML, ALL, CML, or MDS facilitated by the NMDP from August 1999 through December 2003. Recipients of products that were manipulated for T-cell depletion or CD34+ cell selection were excluded from the analysis. This analysis was conducted on recipients who gave informed consent for submission of their outcome data to the NMDP for studies, in accordance with the Declaration of Helsinki. This study was approved by the NMDP central Institutional Review Board. This was done prospectively for all recipients since May 2002 but inconsistently for patients who received

transplants at some centers before then. In 2002, the NMDP asked surviving recipients who received a transplant before May 2002 to document their consent for study participation. To address bias introduced by the inclusion of only a proportion of surviving recipients (those documenting consent) but all deceased recipients of transplants before May 2002, random exclusion of recipients who died before initiation of the corrective action plan was performed to generate a "corrective action plan–corrected" dataset as previously described.[19] The final study population included 932 recipients from 99 transplantation centers. The analysis used the data collected on the NMDP Donor and Recipient Baseline and Follow-up Data Collection Forms and contract laboratory reports.

End points

Transplantation outcomes examined were neutrophil engraftment, platelet engraftment, overall survival, grades II-IV and grades III-IV acute GVHD, chronic GVHD, relapse, and transplant-related mortality (TRM). Neutrophil engraftment was defined as an achievement of an absolute neutrophil count of at least 500 neutrophils/mm^3 sustained for 3 consecutive laboratory measurements on different days. Platelet engraftment was defined as an achievement of a platelet count recovery of at least 50 000 platelets/mm^3 sustained for 3 consecutive laboratory measurements on different days with no platelet transfusions in the previous 7 days. A severity grade for acute GVHD was calculated according to the reported stages of skin, liver, and intestinal involvement with the use of the Glucksberg grading system.[20] Relapse was defined as hematologic recurrence; patients who failed to achieve remission after transplantation were considered to have had a recurrence at day 1. Treatment-related mortality was defined as death in continuous complete remission. Death from any cause was considered an event for overall survival.

Statistical methods

Patient-, disease-, transplant-, product-, and donor-related characteristics were compared for recipients of myeloablative (MA), reduced-intensity (RI), and nonmyeloablative (NMA) regimens with the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables. A conditioning regimen was considered MA if it included one of the following: total body irradiation (TBI) greater than 500 cGy as a single fraction; TBI greater than 800 cGy regardless of the number of fractions; busulfan 9.5 mg/kg or more; melphalan greater than 150 mg/m^2; any combination of busulfan and melphalan; or any combination of cyclophosphamide, etoposide (VP-16), and TBI. RI conditioning regimens included TBI between 200 and 500 cGy; TBI between 500 and 800 cGy as multiple fractions; busulfan less than 9.5 mg/kg; melphalan no greater than 150 mg/m^2; 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), etoposide, cytarabine, and melphalan (BEAM regimens) or cyclophosphamide, BCNU, and VP-16 (CBV regimens); or any combination of VP-16 and cyclophosphamide. NMA conditioning regimens included TBI dose of 200 cGy, fludarabine with 200 cGy TBI, any combination of fludarabine and cyclophosphamide, or any combination of fludarabine and cytarabine. Univariate probabilities of overall survival were calculated with the Kaplan-Meier estimator; the log-rank test was used for univariate comparisons of survival curves; the chi-square test was used for pointwise comparisons.[21] Probabilities of neutrophil and platelet recovery, acute and chronic GVHD, relapse, and TRM were calculated with the cumulative incidence function estimator with a subsequent transplantation as a censoring event.[22,23] For neutrophil and platelet engraftment and acute and chronic GVHD, death without an event is the competing risk. For TRM, relapse was the competing risk; for relapse, TRM was the competing risk. The analyses of neutrophil and platelet engraftment were restricted to patients receiving MA regimens.

Assessment of potential risk factors for day 25 neutrophil engraftment and day 60 platelet engraftment was evaluated with the use of logistic regression. The estimated effects of each significant risk factor were given by odds ratios. Multivariate analyses of acute and chronic GVHD, relapse, TRM, and overall mortality were performed with the use of Cox proportional hazards regression.[24] The estimated effects of each significant risk factor are given as relative risks (RRs). The following risk factors were considered as candidate effects in the model building process of each regression analysis.

Recipient- and disease-related factors. These factors included recipient age, sex, race or ethnicity, body mass index (BMI), Karnofsky/Lansky performance score at conditioning, diagnosis and stage, disease risk, time from diagnosis to transplantation, coexisting disease, and CMV status. Disease risk was classified into 3 categories. Early disease included acute leukemia in first complete remission, chronic leukemia in first chronic phase, refractory anemia, or refractory anemia with ringed sideroblasts. Intermediate diseases included acute leukemia in second or higher complete remission or chronic leukemia in accelerated or second chronic phase. Advanced diseases included acute leukemia in relapse, chronic leukemia in blastic phase, refractory anemia with excess blasts, or refractory anemia with excess blasts in transformation.

Transplantation-related factors. These factors included donor/recipient HLA match, ABO match, sex match, race match, CMV status match, conditioning regimen type, use of TBI, GVHD prophylaxis, use of planned growth factors for engraftment defined as G-CSF or GM-CSF between day −3 and day 7, and year of transplantation. On the basis of the best available typing data at the time of analysis, HLA match was classified into 3 categories: well-matched, partially matched, and mismatched, according to a recently developed algorithm that considers level of typing resolution and matching at HLA-A, -B, -C, and -DRB1 loci as described by Weisdorf et al.[25] Well matched was defined as no known disparity between donor and recipient at HLA-A, -B, -C, and -DRB1, partially matched as one known or one likely disparity, and mismatched as 2 or more disparities.

Product-related factor. This included CD34+ cells per kilogram of recipient weight in infused product.

Donor-related factors. These factors included donor age, sex, race or ethnicity, BMI, CMV status, donor parity, 1-day versus 2-day collection, and the preapheresis day 5 values of donor white blood cell counts, platelet counts, and CD34+ cell counts.

A stepwise selection technique with a significance level of .05 was used in all regression analyses. Separate analyses were performed for MA transplants and RI/NMA transplants. For the Cox regression models, all possible risk factors were checked for proportional hazards with a time-dependent covariate approach, and there were no violations to the proportionality assumption. No significant first-order interactions were observed. For the cell dose variables, the optimal cutpoint was determined by examining the Martingale residual plots. *P* values are 2-sided. All analyses were done with the use of SAS Version 9.1 (SAS Institute Inc).

Results

Donor and recipient populations

No significant differences were noted between recipients of MA, RI, and NMA conditioning regimens in donor/recipient HLA, ABO, sex, and race matching, as well as CMV status and performance scores (Tables 1 and 2). Age of recipients varied significantly, with a median age of 38 years for patients receiving MA conditioning, compared with 56 and 57 years for recipients of RI and NMA regimens, respectively (*P* < .001). Fifty-two percent of recipients in the entire cohort had at least one coexisting medical comorbidity. The most common conditions included cardiac disease/hypertension (20%), followed by pulmonary, endocrine, and gastrointestinal disorders in 10%, 10%, and 8% of recipients, respectively (supplemental Table 1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). As would be expected in an older cohort, patients receiving RI and NMA regimens were more likely to have comorbid conditions (61% and 73% vs 45% of recipients of RI, NMA, and MA conditioning had comorbidities, respectively). The donor population in this study was predominantly younger than 40 years of age (69%). Donation for persons older than age 50 was rare (7%). Donor ages, sex, weight, parity, and other characteristics were similar among the 3 preparative regimen cohorts.

Table 1. Recipient and transplantation characteristics according to preparative regimen (N = 932 donor/recipient pairs undergoing harvest/transplantation)

Variable	Myeloablative	Reduced intensity	Nonmyeloablative	P
Recipient characteristics				
No. of patients	611	160	161	
No. of centers	79	52	36	
Median follow-up time among survivors, d (range)	1224 (228-2612)	1123 (364-2200)	1417 (593-2295)	
Male recipients, n (%)	345 (56)	92 (58)	90 (56)	.957
Recipient race/ethnicity				.011*
White, n (%)	525 (86)	142 (89)	153 (95)	
Hispanic, n (%)	39 (6)	6 (4)	4 (2)	
Asian/Pacific Islander, n (%)	19 (3)	6 (4)	1 (<1)	
Black/African American, n (%)	18 (3)	5 (3)	2 (1)	
Other/declines, n (%)	10 (2)	1 (<1)	1 (< 1)	
Recipient age, median (range)	38 (<1 to 65)	56 (1-75)	57 (17-73)	< .001
0-9 y, n (%)	28 (5)	2 (1)	0 (0)	< .001
10-19 y, n (%)	60 (10)	6 (4)	2 (1)	
20-29 y, n (%)	110 (18)	7 (4)	8 (5)	
30-39 y, n (%)	122 (20)	17 (11)	6 (4)	
40-49 y, n (%)	174 (28)	19 (12)	22 (14)	
50-59 y, n (%)	105 (17)	66 (41)	67 (42)	
60 y or older, n (%)	12 (2)	43 (27)	56 (35)	
Karnofsky performance score				.258
90-100, n (%)	371 (61)	91 (57)	87 (54)	
10-80, n (%)	184 (30)	54 (34)	63 (39)	
Unknown, n (%)	56 (9)	15 (9)	11 (7)	
Disease and stage				< .001†
Acute myelogenous leukemia, n (%)	249 (41)	99 (62)	71 (44)	
First CR, n (%)	87 (14)	33 (21)	34 (21)	
Second CR, n (%)	56 (9)	21 (13)	17 (11)	
Third CR, n (%)	4 (1)	2 (1)	2 (1)	
Not in remission, n (%)	102 (17)	43 (27)	18 (11)	
Acute lymphoblastic leukemia, n (%)	159 (26)	10 (6)	16 (10)	
First CR, n (%)	49 (8)	4 (3)	9 (6)	
Second CR, n (%)	46 (8)	3 (2)	4 (2)	
Third CR, n (%)	21 (3)	1 (< 1)	1 (< 1)	
Not in remission, n (%)	43 (7)	2 (1)	2 (1)	
Chronic myelogenous leukemia, n (%)	95 (16)	15 (9)	24 (15)	
First CP, n (%)	40 (7)	7 (4)	13 (8)	
Accelerated phase/second CP, n (%)	44 (7)	5 (3)	10 (6)	
Blast phase, n (%)	11 (2)	3 (2)	1 (< 1)	
Myelodysplastic syndromes (MDS), n (%)	108 (18)	36 (23)	50 (31)	
Refractory anemia, n (%)	31 (5)	9 (6)	6 (4)	
RAEB/RAEB-T, n (%)	35 (6)	14 (9)	13 (8)	
Other MDS, n (%)	42 (7)	13 (8)	31 (19)	
Disease risk				.013
Early, n (%)	207 (34)	53 (33)	62 (39)	
Intermediate, n (%)	213 (35)	45 (28)	65 (40)	
Advanced, n (%)	191 (31)	62 (39)	34 (21)	
Transplantation characteristics				
HLA match				.021
Well-matched, n (%)	359 (59)	88 (55)	108 (67)	
Partially matched, n (%)	173 (28)	53 (33)	46 (29)	
Mismatched, n (%)	79 (13)	19 (12)	7 (4)	
Donor/recipient sex match				.295
Male/male, n (%)	212 (35)	65 (41)	52 (32)	
Male/female, n (%)	143 (23)	42 (26)	46 (29)	
Female/male, n (%)	133 (22)	27 (17)	38 (24)	
Female/female, n (%)	123 (20)	26 (16)	25 (16)	

CR indicates complete remission; CP, chronic phase; RAEB, refractory anemia with excess of blasts; RAEB-T, RAEB in transformation; N/A, not applicable; and MTX, methotrexate.

*White compared with others.

†Comparing broad diseases.

‡CsA compared with FK506

§Other GVHD prophylaxis included MTX, mycophenolate mofetil, corticosteroids, and G-CSF.

Table 1. Recipient and transplantation characteristics according to preparative regimen (N = 932 donor/recipient pairs undergoing harvest/transplantation)—Continued

Variable	Myeloablative	Reduced intensity	Nonmyeloablative	P
Donor/recipient CMV status				.200
Negative/negative, n (%)	187 (31)	35 (22)	52 (33)	
Negative/positive, n (%)	199 (33)	63 (39)	57 (36)	
Positive/negative, n (%)	88 (15)	20 (13)	21 (13)	
Positive/positive, n (%)	131 (22)	42 (26)	29 (18)	
Unknown, n (%)	6 (N/A)	0 (N/A)	2 (N/A)	
TBI, n (%)	399 (65)	25 (16)	129 (80)	< .001
GVHD prophylaxis				< .001‡
CsA + MTX ± other, n (%)	327 (54)	25 (16)	4 (2)	
CsA ± other (no MTX), n (%)	18 (3)	55 (34)	132 (82)	
FK506 + MTX ± other, n (%)	220 (36)	33 (21)	12 (7)	
FK506 ± other (no MTX), n (%)	40 (7)	46 (29)	8 (5)	
Other, n (%)§	6 (<1)	1 (<1)	5 (3)	
Use of planned growth factors, n (%)	187 (31)	66 (41)	27 (17)	< .001

CR indicates complete remission; CP, chronic phase; RAEB, refractory anemia with excess of blasts; RAEB-T, RAEB in transformation; N/A, not applicable; and MTX, methotrexate.

*White compared with others.
†Comparing broad diseases.
‡CsA compared with FK506.
§Other GVHD prophylaxis included MTX, mycophenolate mofetil, corticosteroids, and G-CSF.

Transplant characteristics, engraftment, and overall survival

Table 1 reviews characteristics of the transplants included in the analysis. Sixty percent of the transplants were from well-matched donors. Most recipients received MA conditioning procedures (66%). Most recipients received cyclosporine-based GVHD prophylaxis, but nearly 40% of the recipients received FK506. Forty-six percent of recipients underwent sex-mismatched procedures, and 56% of recipients were CMV positive.

The median time to neutrophil engraftment for patients receiving MA regimens was 14 days with a 92% and 95% cumulative incidence of engraftment at 25 and 100 days, respectively. The median time for platelets to reach 50 000 mm³ was 21 days with a cumulative incidence of 70% at 60 days and 77% at 1 year. Data were not available to assess the timing or cumulative incidence of

lymphocyte recovery. The probability of overall survival of the entire cohort at 100 days, 1 year, 2 years, and 3 years was 75%, 47%, 39%, and 33%, respectively.

Multivariate analysis of transplantation outcomes in patients receiving MA conditioning

Because the time course of some transplantation outcomes differs after MA versus RI/NMA regimens, multivariate analyses attempting to define key factors contributing to outcomes was performed separately for MA versus RI/NMA approaches. The risk factors considered as candidate effects for the model building process of each regression analysis are described in "Statistical methods."

Neutrophil and platelet engraftment. Table 3 shows logistic regression results for neutrophil engraftment at day 25 and platelet recovery to 50 000 mm³ at day 60 in the MA cohort. Recipients

Table 2. Donor and product characteristics according to preparative regimen

Variable	Myeloablative	Reduced intensity	Nonmyeloablative	P
Product characteristics				
Median CD34+ cell dose, × 10⁶/kg (range)*	6.2 (0.4-56.0)	5.4 (0.7-55.4)	6.3 (0.3-29.0)	.415
Donor characteristics				
Male donors, n (%)	355 (58)	107 (67)	98 (61)	.128†
Donor race/ethnicity				.004†
White, n (%)	484 (79)	130 (81)	146 (91)	
Hispanic, n (%)	47 (8)	13 (8)	5 (3)	
Multiple, n (%)	24 (4)	7 (4)	4 (2)	
Asian/Pacific Islander, n (%)	26 (4)	5 (3)	1 (< 1)	
Black/African American, n (%)	16 (3)	3 (2)	1 (< 1)	
Other/declines/unknown, n (%)	14 (2)	2 (1)	4 (2)	
Median donor age at donation, y (range)	35 (19-60)	37 (19-58)	36 (18-60)	.286
18-30 y, n (%)	213 (35)	42 (26)	51 (32)	.237
31-40 y, n (%)	217 (36)	70 (44)	54 (34)	
41-50 y, n (%)	135 (22)	39 (24)	44 (27)	
51-60 y, n (%)	46 (8)	9 (6)	12 (7)	
Donor parity (female only)				.252
0, n (%)	100 (39)	11 (21)	27 (43)	
1-2, n (%)	77 (30)	21 (40)	17 (27)	
3 or more, n (%)	60 (23)	15 (28)	15 (24)	
Unknown, n (%)	19 (7)	6 (11)	4 (6)	

*CD34+ cell dose is missing in 176 myeloablative cases, 46 reduced-intensity cases, and 39 nonmyeloablative cases.
†White compared with others.

Table 3. Multivariate analysis of factors associated with engraftment in patients undergoing myeloablative unrelated donor PBSC transplantation

Variable	n	Engrafted, n	OR (95% CI)	P
Day 25 neutrophil engraftment				
Karnofsky score				.002
90-100	364	344	1.00	
10-80	176	151	0.37 (0.18-0.64)	< .001
Missing	56	53	1.24 (0.34-4.49)	.738
CD34$^+$ cells dose, $\times 10^6$/kg				.001
3.8 or less	107	94	1.00	
More than 3.8	319	305	3.70 (1.61-8.47)	.002
Missing	170	149	0.97 (0.45-2.11)	.941
Recipient BMI, kg/m^2				.034
Less than 18.5	47	41	0.8 (0.32-2.41)	.798
18.5-24.9	220	196	1.00	
25-29.9	185	175	2.36 (1.07-5.22)	.033
30 or greater	144	136	2.72 (1.14-6.46)	.024
Use of planned growth factors				
No	412	373	1.00	
Yes	184	175	2.37 (1.08-5.17)	.031
Day 60 platelet 50 000 engraftment				
Karnofsky score				.007
90-100	362	272	1.00	
10-80	180	110	0.54 (0.36-0.81)	.003
Missing	52	35	0.61 (0.32-1.17)	.135
CD34$^+$ cell dose, $\times 10^6$/kg				< .001
3.8 or less	102	55	1.00	
More than 3.8	321	238	2.69 (1.68-4.33)	< .001
Missing	171	125	2.48 (1.46-4.21)	< .001
HLA matching status				.016
Well-matched	352	261	1.00	
Partially matched	166	111	0.74 (0.48-1.12)	.150
Mismatched	76	45	0.47 (0.27-0.80)	.005
Recipient CMV status				
Negative	268	202	1.00	
Positive	326	215	0.68 (0.47-0.99)	.042

were more likely to engraft neutrophils at day 25 and platelets at day 60 if the Karnofsky score at transplantation was at least 90, if they received planned doses of growth factors (filgrastim or sargramostim), or if the CD34$^+$ cell dose exceeded 3.8×10^6/kg recipient weight. Recipients whose BMI was below 25 kg/m^2 were less likely to engraft neutrophils. Recipients who were CMV positive and those who received HLA-mismatched grafts were less likely to achieve platelet engraftment.

Grades II-IV and grades III-IV acute and chronic GVHD. Table 4 shows Cox proportional hazards regression results for grades II-IV and grades III-IV acute GVHD and chronic GVHD in the MA cohort. As anticipated, HLA mismatching increased the risk of grades III-IV acute GVHD. Of note, higher CD34$^+$ dose was not associated with an increase in acute or chronic GVHD. The risk of grades II-IV acute GVHD was noted to be less in based prophylaxis regimens compared with cyclosporine-based regimens (RR = 0.68, P < .001). The risk of chronic GVHD was also less with based GVHD prophylaxis (RR = 0.59, P < .001) or when TBI was used (RR = 0.72, P = .007).

Relapse and TRM. Recipients in the MA cohort with AML or ALL were more likely to relapse, with markedly lower rates in patients who received transplants for MDS or CML (Table 4). Disease risk was a significant determinant of relapse outcomes in the MA group, with a RR of 3.72 and 2.17 for patients with advanced disease and intermediate disease, respectively, compared with those with early disease (P < .001 and P = .002, respectively). Of note, TRM in the MA cohort was not associated with

advanced disease as it has been in previous URD BM studies; instead, TRM was associated with HLA mismatching, CD34$^+$ dose 4.5×10^6/kg or less, lower Karnofsky scores, and the use of FK506–based GVHD prophylaxis regimens.

Multivariate analysis of transplantation outcomes in patients receiving RI/NMA conditioning

Acute GVHD, relapse, and TRM. Because many recipients of RI/NMA conditioning did not become neutropenic or require platelet transfusions, engraftment was not assessed by multivariate analysis. In addition, insufficient data were available for chimerism analysis in this cohort.

FK506-based prophylaxis was associated with lower risk of acute GVHD in the RI/NMA group (grades II-IV: RR = 0.62, P = 0.040; grades III-IV: RR = 0.52, P = .033; Table 5). The risk of acute GVHD tended to decrease through the years. The risk of significant acute GVHD (grades III-IV) was also lower in patients receiving NMA versus RI conditioning (RR = 0.37, P < .001). The only factor associated with relapse in the RI/NMA cohort was disease risk: patients who received a transplant for advanced disease were twice as likely to recur compared with those who received a transplant for early disease (RR = 2.00, P = .003). Advanced disease was also associated with TRM of patients receiving RI/NMA conditioning, with a RR of 1.91 for patients with advanced disease compared with those with early disease (P = .008). Finally, TRM was decreased in the RI/NMA group

Table 4. Multivariate analysis of factors associated with GVHD, relapse, and TRM in patients undergoing myeloablative unrelated donor PBSC transplantation

Variable	n	RR (95% CI)	P
Grades II-IV acute GVHD			
GVHD prophylaxis			
CsA-based	337	1.00	
FK506-based	260	0.68 (0.54-0.85)	< .001
Grades III-IV acute GVHD			
HLA matching status			.001
Well-matched	345	1.00	
Partially matched	173	1.57 (1.13-2.19)	.007
Mismatched	79	1.93 (1.29-2.88)	.001
Chronic GVHD			
GVHD prophylaxis			
CsA-based	342	1.00	
FK506-based	260	0.59 (0.46-0.75)	< .001
Conditioning regimen			
Non-TBI	209	1.00	
TBI	393	0.72 (0.57-0.91)	.007
Year of transplantation			
1999-2000	95	1.00	
2001	126	1.28 (0.88-1.87)	.193
2002	158	1.55 (1.08-2.23)	.018
2003	223	1.70 (1.21-2.40)	.002
Relapse			
Disease			< .001
AML	238	1.00	
ALL	157	0.83 (0.56-1.23)	.348
CML	93	0.18 (0.07-0.46)	< .001
MDS	107	0.43 (0.25-0.73)	.002
Disease risk			< .001
Early	203	1.00	
Intermediate	210	2.17 (1.33-3.54)	.002
Advanced	182	3.72 (2.33-5.93)	< .001
Transplant-related mortality			
HLA matching status			< .001
Well-matched	348	1.00	
Partially matched	169	1.47 (1.12-1.92)	.005
Mismatched	76	2.30 (1.63-3.26)	< .001
CD34⁺ cells dose, ×10⁶/kg			.031
4.5 or less	138	1.00	
More than 4.5	287	0.68 (0.50-0.92)	.013
Missing	170	0.89 (0.64-1.23)	.472
Karnofsky score			< .001
90-100	362	1.00	
10-80	177	1.83 (1.41-2.37)	< .001
Missing	56	0.94 (0.57-1.54)	.799
GVHD prophylaxis			
CsA-based	339	1.00	
FK506-based	256	1.51 (1.18-1.93)	.001

Table 5. Multivariate analysis of factors associated with GVHD, relapse, and TRM in patients undergoing reduced-intensity or nonmyeloablative unrelated donor PBSC transplantation

Variable	n	RR (95% CI)	P
Grades II-IV acute GVHD			
GVHD prophylaxis			
CsA-based	215	1.00	
FK506-based	99	0.62 (0.40-0.98)	.040
Year of transplantation			.028
1999-2000	43	1.00	
2001	53	0.74 (0.42-1.31)	.307
2002	93	0.56 (0.33-0.95)	.033
2003	125	0.47 (0.28-0.79)	.004
Grades III-IV acute GVHD			
GVHD prophylaxis			
CsA-based	215	1.00	
FK506-based	99	0.52 (0.28-0.95)	.033
Year of transplantation			.016
1999-2000	43	1.00	
2001	53	1.06 (0.51-2.18)	.877
2002	93	0.56 (0.27-1.15)	.114
2003	125	0.41 (0.20-0.85)	.016
Conditioning intensity			
Reduced intensity	159	1.00	
Nonmyeloablative	155	0.37 (0.22-0.64)	< .001
Relapse			
Disease risk			.005
Early	114	1.00	
Intermediate	107	1.15 (0.71-1.84)	.572
Advanced	95	2.00 (1.27-3.14)	.003
Transplant-related mortality			
CD34⁺ cells dose, × 10⁶/kg)			.039
No more than 4.5	79	1.00	
More than 4.5	154	0.56 (0.36-0.88)	.013
Missing	83	0.63 (0.38-1.04)	.073
Disease risk			.029
Early	114	1.00	
Intermediate	107	1.38 (0.87-2.18)	.175
Advanced	95	1.91 (1.18-3.09)	.008

HLA mismatching. Other important factors increasing risk of mortality included the use of FK506-based regimens (RR = 1.37, $P = .002$) and CD34⁺ doses less than 4.5×10^6/kg (RR = 0.75, $P = .021$). Finally, there was a statistically significant increase in mortality when both donor and recipient were CMV positive compared with when both were negative (RR = 1.61, $P < .001$).

Only 2 variables reached significance in the regression analysis of mortality in the RI/NMA cohort. As expected, patients with advanced disease did poorly compared with those with early disease (RR = 1.85, $P < .001$). As with recipients of MA conditioning, cell dose was important. CD34⁺ cell doses exceeding 4.5×10^6/kg recipient weight were associated with a decrease in overall mortality (RR = 0.66, $P = .010$).

Effect of higher CD34⁺ cell doses on engraftment, GVHD, and survival

We further analyzed the effect of infused CD34⁺ dose on key outcomes (Figures 1-3). Figure 1 shows the cumulative incidence of neutrophil and platelet engraftment after MA transplantation in recipients who received 3.8×10^6 CD34⁺ cells/kg recipient weight or less (Low) compared with those who received more than the cutoff value (High; $P = .025$ for neutrophil engraftment at 25 days; $P < .001$ for platelet engraftment at 60 days). The difference in both the rapidity and eventual ability to achieve platelet engraftment with higher doses of CD34⁺ cells is marked.

when CD34⁺ cell doses exceeded 4.5×10^6/kg recipient weight (RR = 0.58, $P = .017$).

Multivariate analysis of mortality of patients receiving MA and RI/NMA conditioning

We analyzed both cohorts for overall mortality and for treatment failure (defined as TRM or relapse). Because extended survival after relapse was rare, outcomes from both analyses were interchangeable, and we present only the mortality analysis. Table 6 outlines key transplant characteristics associated with increased risk of mortality in the study cohorts. For recipients of an MA transplant, intermediate and advanced disease significantly increased a patient's risk of death, as did low Karnofsky score and

Table 6. Multivariate analysis of factors associated with overall mortality in patients undergoing myeloablative or reduced intensity/nonmyeloablative unrelated donor PBSC transplantation

Variable	n	RR (95% CI)	P
Myeloablative			
HLA matching status			< .001
Well-matched	352	1.00	
Partially matched	169	1.11 (0.89-1.40)	.351
Mismatched	78	1.84 (1.38-2.44)	< .001
CD34$^+$ cells dose, ×10^6/kg			.024
4.5 or less	138	1.00	
More than 4.5	289	0.75 (0.58-0.96)	.021
Missing	172	0.97 (0.74-1.27)	.808
Karnofsky score			< .001
90-100	363	1.00	
10-80	180	1.79 (1.43-2.23)	< .001
Missing	56	0.94 (0.64-1.39)	.775
GVHD prophylaxis			
CsA-based	342	1.00	
FK506-based	257	1.37 (1.12-1.67)	.002
Donor/recipient CMV status			.007
Negative/negative	186	1.00	
Negatuve/positive	196	1.19 (0.93-1.53)	.173
Positive/negative	87	1.17 (0.84-1.62)	.357
Positive/positive	130	1.61 (1.23-2.12)	< .001
Disease risk			< .001
Early	203	1.00	
Intermediate	210	1.32 (1.03-1.69)	.029
Advanced	186	1.74 (1.35-2.24)	< .001
Reduced intensity/nonmyeloablative			
CD34$^+$ cells dose, ×10^6/kg			.024
4.5 or less	80	1.00	
More than 4.5	156	0.66 (0.48-0.91)	.010
Missing	85	0.67 (0.47-0.96)	.031
Disease risk			< .001
Early	115	1.00	
Intermediate	110	1.17 (0.84-1.63)	.355
Advanced	96	1.85 (1.33-2.56)	< .001

We explored in more depth whether higher CD34$^+$ cell doses were associated with increased rates of acute or chronic GVHD or both. Figure 2A and B shows the cumulative incidence of grades
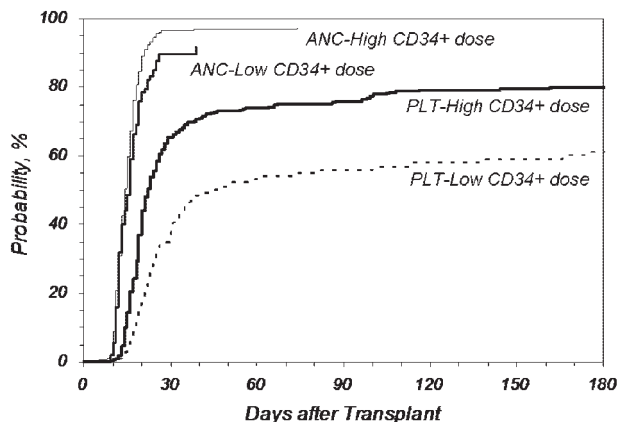
Figure 1. Cumulative incidence of neutrophil and platelet engraftment after MA URD-PBSC transplantation by CD34$^+$ dose. CD34$^+$ cell doses higher than 3.8 × 10^6/kg recipient weight improved neutrophil and platelet engraftment compared with lower doses (P = .025 for neutrophil engraftment at 25 days; P < .001 for platelet engraftment > 50 000/μL at 60 days). ANC indicates neutrophil engraftment; PLT, platelet engraftment; Low, no more than 3.8 × 10^6 CD34$^+$/kg (n = 107, ANC; n = 106, PLT); High, greater than 3.8 × 10^6 CD34$^+$/kg (n = 327, ANC; n = 324, PLT).

III-IV acute GVHD based on CD34$^+$ doses by quartiles for recipients of MA and RI/NMA conditioning, respectively. No difference was noted between the quartiles (P = .599 and .305 at 180 days for MA and RI/NMA, respectively). For recipients of MA conditioning, the incidence of grades III-IV acute GVHD in the top quartile of CD34$^+$ cells doses (> 9.5 × 10^6/kg) compared with doses in the second quartile (between 3.8 and 6.2 × 10^6/kg) had a RR of 0.81 (P = .393); doses above the 90th percentile (> 14.9 × 10^6/kg) had a similarly nonsignificant RR of 1.13 (P = .696). For recipients of RI/NMA conditioning, the RR of grades III-IV acute GVHD in the top quartile (> 9.4 × 10^6/kg) and the top 10% (> 14.6 × 10^6/kg) compared with the second quartile (between 3.6 and 5.9 × 10^6/kg) were 0.62 (P = .301) and 0.64 (P = .488), respectively. An analysis of grades II-IV acute GVHD similarly showed no increase in incidence with higher cell doses (data not shown).

Figure 2C and D shows the incidence of chronic GVHD by quartiles, demonstrating no increase in incidence with higher cell doses for recipients of MA and RI/NMA conditioning, respectively (P = .068 and .189 at 2 years, respectively). Further analysis of patients receiving cell doses above the top quartile and the 90th percentile compared with the second quartile similarly shows no evidence of an increase in chronic GHVD for recipients of MA and RI/NMA conditioning (MA: RR = 1.17, P = .405 for top quartile vs second quartile; RR = 1.24, P = .389 for top 10% vs second quartile; RI/NMA: RR = 1.35, P = .262 for top quartile vs second quartile; RR = 1.24, P = .508 for top 10% vs second quartile).

Higher cell doses were independent predictors of better survival regardless of preparative regimen approaches. CD34$^+$ doses between 4.5 and 9.5 × 10^6/kg recipient weight resulted in a 12% improvement in 3-year survival in recipients of MA conditioning compared with lower doses (37% vs 25%; P = .020, Medium vs Low; Figure 3A). However, doses greater than 9.5 × 10^6/kg did not further improve the survival rate (P = .489 at 3 years, Medium vs High). Three-year survival after RI/NMA preparative regimens also significantly improved in patients with CD34$^+$ doses between 4.5 and 9.5 × 10^6/kg recipient weight compared with patients with lower doses (34% vs 21%; P = .045, Medium vs Low; Figure 3B). Similar to the MA cohort, CD34$^+$ cell doses greater than 9.5 × 10^6/kg did not further improve outcome in this cohort (P = .157 at 3 years; Medium vs High).

We also analyzed CD34 doses based on ideal body weight as opposed to actual body weight. This analysis was restricted to adults only because of the differences in computing ideal body weight for children. Compared with CD34$^+$ cells/kg based on actual body weight, CD34$^+$ cells/kg based on ideal body weight was similarly associated with neutrophil and platelet engraftment, but it was not significantly associated with TRM or survival in MA transplantations. Among NMA/RI transplants, CD34 dose based on ideal body weight was only significantly associated with survival but not TRM (data not shown). This indicates that cell dose based on ideal body weight may be a less sensitive predictor of transplantation outcomes in URD-PBSC transplantations, compared with cell dose based on actual body weight.

Comparison of outcomes between preparative regimen cohorts

Although the preparative regimen cohorts differed by age and the presence of coexisting conditions, these variables did not come out as significant in multivariate outcome analysis; therefore, comparisons of the cohorts may be instructive. NMA regimens resulted in significantly less severe (grades III-IV)

acute GVHD compared with RI or MA regimens (cumulative incidence at 180 days: 16% vs 26% vs 30%, NMA vs RI vs MA, $P < .001$; Figure 4A), but chronic GVHD was statistically identical between the regimens, with an incidence just above
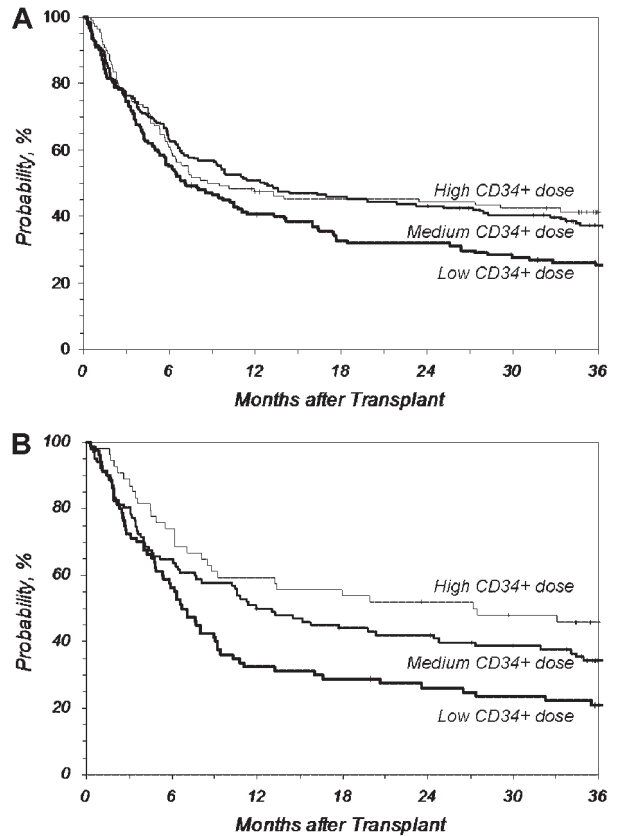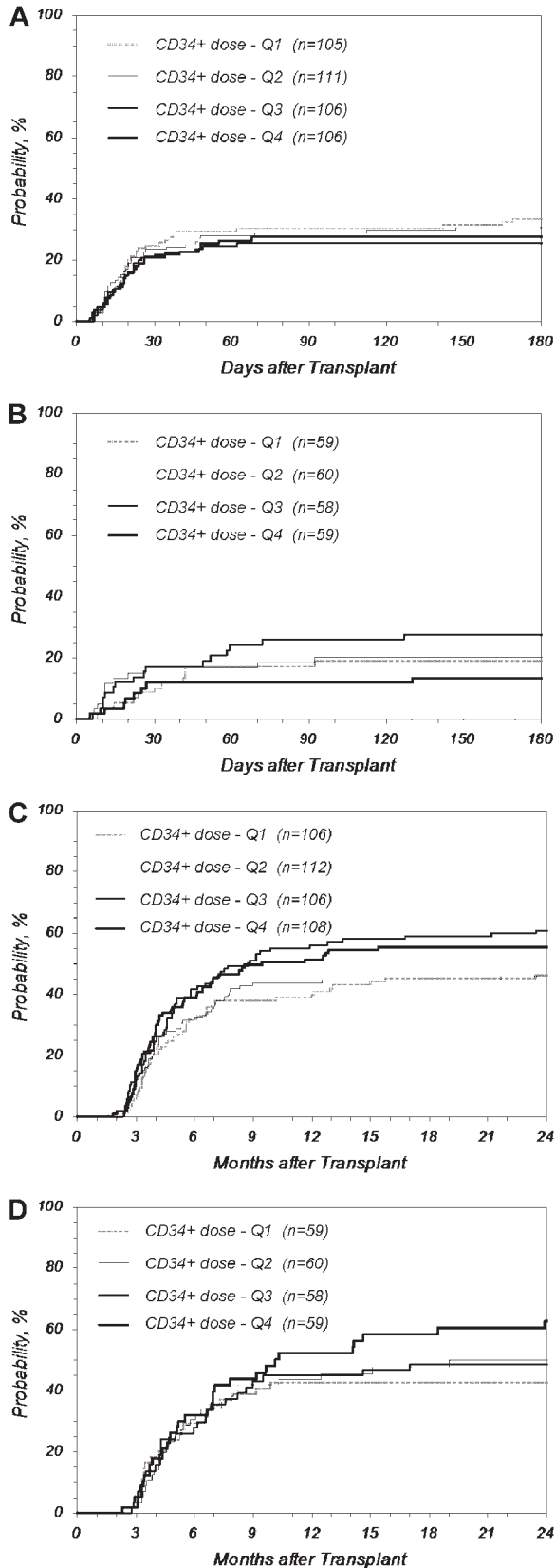

Figure 3. Overall survival after URD-PBSC transplantation by CD34+ dose. CD34+ cell doses higher than 4.5×10^6/kg recipient weight improved overall survival compared with lower doses. However, doses much higher than 4.5×10^6/kg did not further improve the survival rate compared with doses just above 4.5×10^6/kg. (A) Overall survival after MA transplantation ($P = .020$ at 3 years for Medium vs Low; $P = .489$ at 3 years for Medium vs High). (B) Overall survival after RI/NMA transplantation ($P = .045$ at 3 years for Medium vs Low; $P = .157$ at 3 years for Medium vs High). Low indicates no greater than 4.5 (n = 142, MA; n = 80, RI/NMA); Medium, 4.5 to 9.5 (n = 183, MA; n = 102, RI/NMA); High, greater than 9.5 (n = 110, MA; n = 54, RI/NMA) ($\times 10^6$ CD34+/kg).

50% at 2 years (Figure 4B). TRM was higher after MA procedures (cumulative incidence at 3 years: 34% vs 34% vs 43%, NMA vs RI vs MA, $P = .027$; Figure 4C), but any gains in survival from decreased TRM in NMA and RI conditioning were offset by increased relapse (cumulative incidence at 3 years: 37% vs 35% vs 24%, NMA vs RI vs MA, $P < .001$; Figure 4D). This resulted in overall survival that was indistinguishable among the 3 cohorts, ranging from 32% to 35% at 3 years (Figure 4E). Three subanalyses were performed: (1) excluding ALL, (2) AML first complete remission alone, and (3) patients aged 40 to 60 years with AML/MDS. Survival in the 3 subanalyses was the same in each of the 3 preparative regimen cohorts (data not shown).

The most common single cause of death was relapse (28%-38%) followed by infection, organ failure, and acute and chronic

Figure 2. Cumulative incidence of GVHD after URD-PBSC transplantation by quartile (Q) of CD34+ dose. Higher CD34+ cell doses did not increase the incidence of GVHD. (A) Grades III-IV acute GVHD after MA transplantation ($P = .599$ at 180 days); (B) grades III-IV acute GVHD after RI/NMA transplantation ($P = .305$ at 180 days); (C) chronic GVHD after MA transplantation ($P = .068$ at 2 years); (D) chronic GVHD after RI/NMA transplantation ($P = .189$ at 2 years). MA: Q1 indicates no greater than 3.8; Q2, 3.8 to 6.2; Q3,6.2 to 9.5; Q4, greater than 9.5; RI/NMA: Q1, no greater than 3.6; Q, 3.6 to 5.9; Q3, 5.9 to 9.4; Q4, greater than 9.4 ($\times 10^6$ CD34+/kg).
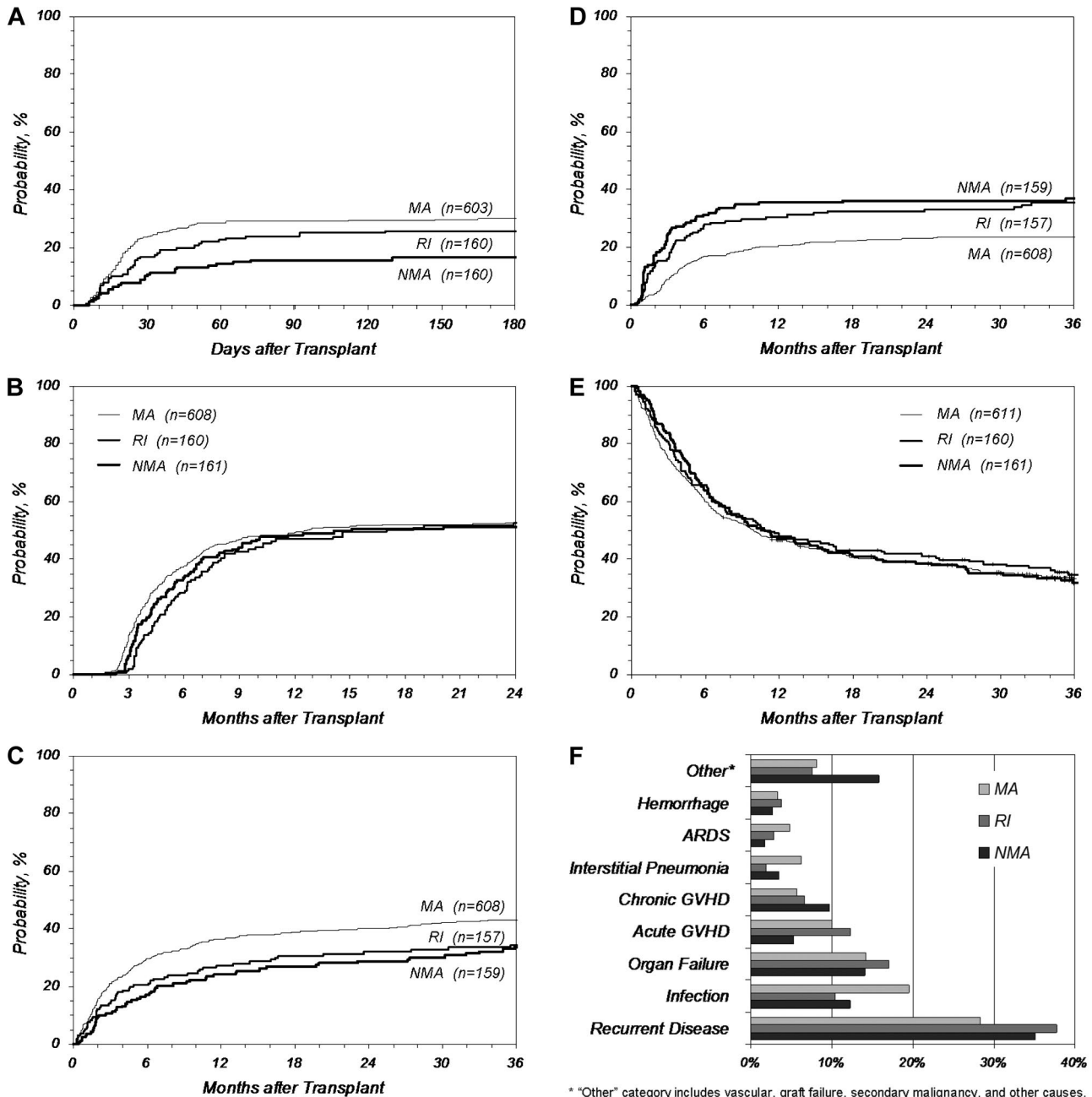
Figure 4. Key outcomes after URD-PBSC transplantation by preparative regimen. (A) Grades III-IV acute GVHD, (B) chronic GVHD, (C) TRM, (D) relapse, (E) overall survival, and (F) primary cause of death. ARDS indicates acute respiratory distress syndrome.

GVHD (Figure 4F). Recipients receiving MA procedures died more frequently of infection, acute respiratory distress syndrome, and interstitial pneumonia compared with patients who received RI/NMA conditioning, whereas patients who received RI/NMA conditioning died more frequently of recurrent disease, chronic GVHD (recipients of NMA conditioning), and other causes (recipients of NMA conditioning). Causes of death did not vary by CD34+ cell dose.

Discussion

We have shown in this large, multi-institutional prospective study that transplantation with NMDP-facilitated URD PBSCs results in

rapid engraftment and survival comparable to published transplantation experiences with URD BM.[17,26] Because PBSCs from URDs has become the most commonly used URD stem cell source, the multivariate risk factor analysis presented here is useful in defining prognosis and identifying populations at risk to design strategies aimed at improving outcome.

One of the key findings of this study is the independent predictive value of higher CD34+ dose for improvements in major transplantation outcomes. Cell dose has long been recognized to be important in allogeneic transplantation. Early studies showed less rejection and better survival in patients undergoing transplantation for severe aplastic anemia who received higher mononuclear cell doses in their BM grafts.[27,28] More recent studies involving MA approaches have shown

faster count recovery, less TRM, and better survival in recipients of matched sibling BM or PBSCs containing higher numbers of CD34+ cells.[29-35] One study of matched sibling donor MA transplantation correlated clinical chronic extensive GVHD with high CD34+ doses, but survival was not affected.[36] Studies of RI regimens have correlated higher cell doses with better survival, but at a cost of more chronic GVHD.[37-40] Two of these studies associated better outcomes with higher CD8+ cell doses, whereas the other 2 studies correlated higher CD34+ doses with chronic GVHD. High CD4+, CD8+, total T-cell, monocyte, natural killer cell, and CD34+ counts have been associated with more rapid achievement of full-donor chimerism and a trend toward decreased rejection in NMA approaches.[41,42]

Studies correlating cell dose with outcomes in URD-PBSC transplantation are few and limited. Nakamura et al[18] reviewed a single center experience of URD-PBSC transplantation with the use of either MA or RI regimens for a variety of hematologic malignancies and myeloproliferative disorders. They showed by multivariate analysis that higher CD34+ doses were associated with faster recovery of absolute lymphocyte counts on day 30 and a reduced rate of relapse. The group also noted a greater reduction in relapse associated with RI regimens when a high dose of CD34+ cells was given compared with MA regimens. Small numbers of URD-PBSC products have been included in a few of the analyses described in the previous paragraph,[38,41] but the study we present is the only large, multicenter trial describing the effect of URD-PBSC CD34+ cell dose on patients undergoing a variety of transplantation regimens.

The lack of an association between higher cell doses and increased rates of acute and/or chronic GVHD in our study may seem to be surprising; however, although a handful of studies has suggested an association of CD34+ cell dose with increased chronic GVHD after sibling donor transplantation,[36,39,40,43] other studies of sibling donors and URDs find either no association of cell dose and acute or chronic GVHD[29,35,38,41,44] or a decrease in grades III-IV acute[34] or chronic GVHD[18] in recipients of higher cell doses. We were unable to define any specific adverse outcome associated with high URD-PBSC cell doses. That said, as long as patients achieved the cell dose cutoff associated with better outcomes (4.5×10^6 CD34+ cells/kg), we were not able to discern specific advantages to receiving doses significantly higher than that threshold.

In summary, cell dose is a key factor after URD-PBSC transplantation. Collection practices leading to acquisition and infusion of at least 4.5×10^6 CD34+ cells/kg may improve survival and decrease morbidity in patients receiving this stem cell source for transplantation, regardless of regimen intensity. Other factors identified in this study may help individual patients understand risk and may assist investigators in targeting high-risk populations for studies aimed at improving outcome.

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Authorship

Contribution: M.A.P. had primary responsibility for study design, data analysis, data interpretation, and manuscript writing and had primary responsibility for the entire paper as an accurate and verifiable report; P.C. had primary responsibility for study design, data file preparation, data analysis, interpretation of data, and manuscript writing; B.R.L. participated in study design, data analysis, interpretation of data, and manuscript writing; S.F.L. and P.A. participated in interpretation of data and manuscript writing; J.P.K. participated in study design, data analysis, and interpretation of data; M.M.H. participated in study design, interpretation of data, and manuscript writing; J.P.M. and R.J.K. participated in data file preparation, interpretation of data, and manuscript writing; and D.L.C. had responsibility for study design, data file preparation, data analysis, data interpretation, and manuscript writing and had responsibility for the entire paper as an accurate and verifiable report.

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