Early recovery of T cell function predicts improved survival after T-cell depleted allogeneic transplant

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Abstract

Infection, relapse and GVHD can complicate allogeneic hematopoietic stem cell transplantation (allo-HSCT). While the effect of poor immune recovery on infection risk is well-established, there are limited data on the effect on relapse and survival, especially following T cell-depletion (TCD). To characterize the pattern of immune reconstitution in the first year after transplant and its effects on survival and relapse, we performed a retrospective study in 375 recipients of a myeloablative TCD allo-HSCT for hematologic malignancies. We noted that different subsets recover sequentially, CD8+ T cells first, followed by total CD4+ and naïve CD4+ T cells, indicating thymic recovery during the first year after HSCT. In the multivariate model, a fully HLA-matched donor and recovery of T cell function, assessed by PHA response at 6 months, were the only factor independently associated with OS and EFS. In conclusion, T cell recovery is a predictor of outcome after TCD allo-HSCT.

Keywords

immune reconstitution; allogeneic hematopoietic stem cell transplant; T cell depletion

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established treatment for hematologic malignancies. However, it is associated with significant adverse effects including infection, relapse, and graft versus host disease (GVHD). One variable that may affect these outcomes is the recovery of the immune system after transplantation.[1-7] Deficiencies in post-transplant T-cell reconstitution, and in particular of CD4+ T cells, correlate with an increased risk of infections.[1,2] Several groups have shown that early recovery of absolute lymphocyte count (ALC) after unmodified or partially T-cell depleted (TCD) allo-HSCT is associated with improved overall survival (OS), decreased relapse and lower transplant-related mortality (TRM).[4-7] There are, however, incomplete data regarding the effect of the quantitative and functional recovery of T cells on relapse and survival.[8-10] Data on immune reconstitution following TCD allo-HSCT are significantly more limited than in the unmodified setting.[1,11-14]

TCD allo-HSCT lowers the incidence and severity of both acute and chronic GVHD without increased relapse or decreased survival when used in selected patient populations.[13,15-22] This approach is now being investigated in a multicenter randomized phase 3 trial (BMT CTN 1301, NCT NCT02345850). However, immune recovery after TCD-HSCT is delayed because of the paucity of T cells administered in the graft. Thus, understanding the delayed immune reconstitution in recipients of TCD grafts is potentially critical to improving outcomes in these patients. To that end, we characterized the pattern of immune recovery in the first year after TCD allo-HSCT and its effects on post-transplant morbidity and mortality, in a retrospective study in 375 patients transplanted for hematologic malignancies.

Materials and Methods

Patients

Three hundred and seventy-five patients with hematologic malignancies underwent an allogeneic TCD-HSCT at Memorial Sloan Kettering Cancer Center (MSKCC) from January 1997 through December 2005. Patients transplanted after 2005 were excluded because they received palifermin, for which pre-clinical data suggest a positive effect on immune recovery.[23-26] One additional patient was excluded because he received post-transplant interleukin-7, which enhances immune reconstitution.[27] All patients received grafts from an HLA-identical or single HLA-mismatched (A, B, C, DR) related or unrelated donor. HLA matching was established for all patients by DNA sequence-specific oligonucleotide typing for HLA-A, -B, and DR-B1 loci, and additionally for C and DQ-B1 loci for unrelated donors. Written informed consent for treatment was obtained from all patients and donors. Approval for this retrospective review was obtained under a waiver of authorization from the Institutional Review and Privacy Board.

Transplant procedure and supportive care

Patients received myeloablative cytoreduction with either total body irradiation (TBI) or chemotherapy-based regimens. Either of two TBI-based regimens were used: TBI 1375 or 1500 cGy, followed by 2 daily doses of thiotepa (5 mg/kg/day) and, either 2 daily doses of

cyclophosphamide (60 mg/kg/day, n=218) starting after thiotepa (TBI/THIO/CY), or 5 daily doses of fludarabine (25 mg/m^2/day, n=109) beginning the first day of thiotepa (TBI/THIO/FLU).[13,15] The chemotherapy-based regimen (n=48) consisted of busulfan (0.8 mg/kg/dose) every 6 hours for 10 doses, melphalan (70 mg/m^2/day) for 2 doses and fludarabine (25 mg/m^2/day) for 5 doses (BU/MEL/FLU).[28] The three conditioning regimens did not change over the time period of the study and continue in use at our center. Both the TBI/THIO/CY and BU/MEL/FLU regimens are included in the ongoing BMT CTN 1301 randomized trial (NCT NCT02345850). T cells were removed from bone marrow grafts (n=190) or granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC, n=153) as previously described.[13,15,29] T cells were removed from bone marrow grafts by sequential soybean lectin agglutination and sheep red blood cell (sRBC)-rosette depletion.[15,29] Positive selection of CD34+ cells was performed by using Isolex 300i Magnetic Cell Separator (Baxter, Deerfield, IL) and subsequent sheep RBC rosette depletion,[13] or using the CliniMACS CD34 Reagent System (Miltenyi Biotech, Gladbach, Germany).[21,30] In addition, 32 patients received combined T cell depleted BM and PBSC allografts, on a protocol in which both products were being administered. Equine anti-thymocyte globulin (ATG, 15 mg/kg/dose × 2 equine) or rabbit ATG (2.5 mg/kg/dose × 2) provided graft rejection prophylaxis.[13,15,31] Patients who received mismatched grafts received 3 doses of ATG. Four patients received alemtuzumab instead of ATG prior to transplant. Recipients of HLA-matched related donors treated with TBI, thiotepa, and fludarabine (n=53) and 21 patients under the age of 24 treated with TBI, thiotepa, and cyclophosphamide did not receive ATG rejection prophylaxis. All patients received ex vivo T cell depleted grafts and no post transplant GVHD prophylaxis was given to any patient. All patients received supportive care and prophylaxis against opportunistic infections according to standard guidelines. Briefly, patients were monitored weekly using the pp65 antigenemia assay from engraftment until day 100,[32] and on a similar schedule by EBV PCR. Pre-emptive therapy was used for CMV (antivirals) and EBV (rituximab). There was no monitoring for fungal infections and all patients received mold active anti-fungal prophylaxis.

Acute GVHD (aGVHD) was diagnosed clinically, confirmed pathologically by biopsy whenever possible, and classified according to standard criteria.[33] Patients with late aGVHD were scored as having aGVHD, per consensus guidelines.[34] Chronic GVHD (cGVHD) was classified as limited or extensive by the criteria of Sullivan,[35] because the retrospective nature of the study precluded accurate scoring of chronic GVHD severity per consensus criteria in all patients. Patients who engrafted were evaluable for aGVHD, and patients surviving at least 100 days were evaluable for cGVHD. Cause of death was determined using a standard algorithm .[36]

Immune recovery monitoring

Data were collected for ALC at days +30, +60 and +90 after transplant. If the patient was not available on those specific days, the ALC within one day of those time points was used. Circulating lymphocyte subsets (total CD4+, CD4+45RA+ and CD8+ T cells, NK cells) and T cell proliferative responses (measured by ^{3}HTdR incorporation) to the mitogen phytohemagglutinin (PHA) were measured at varying time points for 12 months after

transplant, as previously published.[1] Immune recovery data were censored at time of donor lymphocyte infusion (DLI) or second transplant.

Statistical analysis

Immunologic data were collected longitudinally, and early measurements were used as predictors of survival time. Other baseline factors included age, diagnosis (ALL vs. others), conditioning regimen (TBI-based or chemotherapy only), HLA matching status (identical or not), ATG (yes vs. no), remission status (first vs. others), transplant type (PBSC vs. others), donor sex match status, and year of transplant (1997-2001 vs. 2002-2005). Four outcomes were evaluated: OS was defined from the date of HSCT to the date of death or last follow-up; event-free survival (EFS) was defined from the date of HSCT to the earliest date of relapse/progression, death, or last follow-up; time to relapse/progression was the time interval between the date of HSCT and the date of relapse/progression, and the patients without relapse/progression were censored at the last available follow-up; time to non-relapse mortality (NRM) was defined as the same time interval as EFS, and patients who relapsed were censored at the date of relapse.

The association between baseline factors and each immune recovery variable measured over time was investigated using a mixed effects model. The mixed effects model contained subject-specific and time-random effects, with linear and quadratic functions of time, and baseline factor fixed effects. Baseline factors included here are all baseline factors used in outcome analyses plus patient sex. The slopes of the baseline factors within the model were estimated along with the 95% confidence intervals (CI) and tested in the mixed effects models.

Landmark survival analyses at 3 months and 6 months were performed to examine the association between each survival outcome and immune reconstitution factors (total CD4, CD8, NK, naïve CD4 and PHA) plus baseline factors. Landmark survival analyses at 1 month were performed for OS, EFS, relapse and NRM associations with total CD4, CD8 and NK. The effect of ALC recovery on each survival outcome was examined at landmark times of 30, 60 and 90 days. The immune reconstitution value was recorded at the closest time point prior to the landmark. The values measured after relapse/progression were not used in EFS, relapse or NRM outcome analyses. Univariate analysis was first performed on each immune reconstitution factor or baseline factor using the Cox regression. Multivariable Cox regression started with all baseline and immune reconstitution factors in the model, and used stepwise selection with significance level < 0.01. For the landmark analyses of OS and EFS at 6 months, we plotted a smooth nonparametric curve showing the 75th quantile of the survival distribution as a function of the immune recovery [37]. A test with p value < 0.01 was considered statistically significant. Statistical analyses were performed in software packages SAS 9.2 (SAS Institute Inc., Cary, NC, USA), and R version 2.13 (The R Foundation for Statistical Computing).

Results

Patient characteristics and transplant outcomes

Pre-transplant characteristics of the 375 patients are detailed in Table 1. The median age was 40 years (range 2-68). Diseases transplanted included ALL (n=85), AML (n=150), CML (n=55), MDS (n=36) and NHL (n=49). Immune recovery data was available for 354 patients. The other 21 patients were not evaluable because of primary (n=5) or early secondary (n=3) graft failure or early death (n=13). Sixty-seven patients were censored at the time of unselected DLI (n=64) or EBV-specific cytotoxic lymphocytes infusion (n=3). Reasons for DLI included disease (morphologic relapse or minimal residual disease, n=36), infection including EBV lymphoproliferative disorder (n=22), mixed chimerism (n=5), and other (n=4). The median time to first DLI was 293 days (range 47-1617 days). Thirty-four patients were censored at the time of second transplant (n=21) or stem cell "boost" (n=13). Reasons for second transplant included disease (n=14) or graft failure (n=7); while reasons for a stem cell boost included graft failure (n= 10), disease (n=2), or low cell dose (n=1). The median time to second transplant or boost was 217 days (range 5-4345 days).

At one year, the cumulative incidence of grade II-IV and III-IV aGVHD in 363 evaluable patients was 0.177 (95%CI: 0.13, 0.216) and 0.100 (95%CI: 0.069, 0.132), respectively. The cumulative incidence of cGVHD and extensive cGVHD at 1 year in 304 evaluable patients was 0.093 (95%CI: 0.060, 0.127) and 0.047 (95%CI: 0.027, 0.071), respectively. The non-relapse mortality (NRM) at 1 year was 0.296 (95%CI: 0.250, 0.342). Non-relapse causes of death included infection (n=64), GVHD (n=35), organ failure (n=17), non-engraftment (n=9), secondary malignancy (n=6), and other causes (n=11).

The pace of recovery after allo-HSCT differs for different T cell subsets

The pattern of immune recovery is depicted in Figure 1. There was a rapid normalization of the ALC with engraftment and, subsequently, a small increase during the first 3 months post transplant. The pattern of recovery of NK cells mirrored the recovery of ALC. These findings are consistent with other studies in the unmodified setting that show early ALC values represent mostly NK cells.[4,38,39]

While we observed increases in CD4+ T cells, CD8+ T cells, naïve CD4+ T cells, and PHA responses over time, we noted differences between subsets. This included more rapid recovery to a normal range in CD8+ T cells compared with CD4+ T cells, resulting in pertubation of the expected CD4+: CD8+ ratio. At 6 months, median CD8+ T cell count was 132/mm^3 (range 0-3643/mm^3), which is above the lower limit of normal (LLN, 98/mm^3). In contrast, median CD4+ T cell count was 135/mm^3 (range 1-1771/mm^3) at 6 months and 252/mm^3 (range 10-1151/mm^3) at 12 months, both below the LLN (359/mm^3). In patients who had immune reconstitution parameters measured at 12 months, 28% (33/119) achieved CD4+ T cell counts in the normal range, while 79% (94/119) had normal CD8+ T cell counts (median 269/mm^3). Furthermore, the recovery of naïve CD4+ T cells trailed that of total CD4+ T cells, consistent with delayed thymic recovery. The median naïve CD4+ T cell count was 3/mm^3 (range 0-177/mm^3) at 6 months and 40/mm^3 (range 0-748/mm^3, LLN 67/mm^3) at 12 months. However, by 12 months the proportion of patients with normal naïve

CD4+T cell counts (30%, 13/43) and normal total CD4+ T cell counts were nearly identical. There was a modest correlation between these patients (Spearman correlation coefficient 0.586, P<.001). Finally, functional T cell responses, assessed by response to PHA, paralleled CD4+ T cell recovery. At 12 months, median response to PHA was 55,512 cpm (range 503-253691 cpm, limits of normal differed over time), with 38% (44/117) of patients achieving a PHA response in the normal range. Although median CD4+ and CD8+ counts appeared to decrease near the end of the observation period, this likely reflects continued immune recovery monitoring of patients with delayed recovery at 12 months post transplant and beyond.

Effect of transplant factors on immune recovery and clinical outcomes

The effect of transplant factors on immune recovery is presented in Table 2. In multivariate analysis, receiving an HLA identical graft was associated with improved recovery of ALC, CD8+ T cells, and NK cells. Increased age was associated with decreased recovery of T cell function as measured by PHA response (slope on log (PHA) -0.17, P<.001). Surprisingly, age did not otherwise strongly affect immune reconstitution, including recovery of naïve CD4+ T cells. Diagnosis and remission status did not have an effect on immune reconstitution. ATG had an adverse effect on recovery of ALC, CD4+ T cells, naïve T cells, and PHA response (Figure 1, Table 2).

The effect of transplant factors on outcomes is presented in Table 3. The following factors were significantly associated with improved OS, NRM and EFS for patients alive at 1 month: not receiving ATG (p=0.003, p=0.001, and p=0.001) and having an HLA identical match (p<0.001 for all). At 3 months, not receiving ATG was associated with improved EFS, (P=.007) and having an HLA identical match was associated with improved OS as well as EFS (P<.001 in both). In addition, a TBI-based preparative regimen was associated with improved EFS at 3 months, although P=.046. At 6 months, HLA match (P<.001, P<.001) still predicted OS and EFS. In the multivariate model, a fully HLA-matched donor was the only factor independently associated with OS and EFS in landmark analyses at 1, 3 and 6 months and NRM in a landmark analysis at 1 month.

Baseline transplant factors including HLA matching status and preparative regimen type also influenced relapse rate, but did not reach statistical significance level (p 0.01 for all factors). For example, at 3 and 6 months post allo-HSCT, having an HLA match (P=.020, P=.013) and receiving a TBI- based preparative regimen (P=.016, P=.027) was associated with a decreased relapse rate. In the multivariate model, having an HLA-matched donor was associated with a decreased relapse rate at 6 months (P=.013). Receipt of a TBI-based regimen was independently predictive of decreased relapse at 3 months (P<.001).

Immune Recovery predicts increased EFS and OS

We also examined the impact of early recovery of immune parameters on survival outcomes and relapse. Higher NK cell counts at 1 month was associated with improved NRM after 1 month (p=0.008). The effects of CD4+ and CD8+ T cell counts on EFS and OS at 1, 3 and 6 months by landmark analysis are depicted in Table 3 and Figure 2. Total CD4+ T cells at 6 months were significantly associated in a univariate analysis with improved EFS (p=0.001)

and OS (p=0.002) after 6 months. However, the effect of these parameters did not appear to be independent as they were not predictive of EFS or OS in a multivariate model. There was no effect of either CD4+ or CD8+ T cell recovery on relapse at 3 or 6 months. Because there were a small number of immune reconstitution data points at 12 months, biased towards patients with poorer outcomes, we did not examine the effect of immune recovery on survival outcomes at this time point.

Furthermore, in univariate analysis, T cell response to the mitogen PHA at 6 months predicted improved EFS (P<.001) and OS (P<.001) (Table 3). The PHA response was independent of other factors, predicting improved EFS (P=.002) and OS (P<.001) at 6 months in a multivariate model. In contrast, the earlier PHA response measured at 3 months did not predict OS or EFS.

Discussion

Our results represent the most extensive analysis to date of immune recovery following TCD allo-HSCT. We focused on TCD allo-HSCT, because delayed immune reconstitution is one of the potential limitations of this approach and a source of morbidity and mortality. Recognizing the limitations of a retrospective study, we illustrate the pattern and timing of reconstitution of T cell subsets and function over the first year after TCD-HSCT in a cohort of 375 patients. Additionally, we provide evidence T cell function is associated with improved OS and EFS.

T cell subsets recover to different degrees and with different kinetics. Notably, CD8+ T cells reach the normal range within the 1st year in a majority of patients, while this only occurs in 28% of patients for CD4+ T cells. It is particularly interesting that, while naïve CD4+ T cell recovery is not robust in the first 6 months, there is clear generation of naïve CD4+ T cells at later time points in many patients. In fact, the percentage of patients who reach normal naïve CD4+ T cell counts at 12 months is nearly identical to the percentage of those who reach normal total CD4+ T cell counts. Thus, early reconstitution of T cells likely results from peripheral expansion either of residual host CD4+ T cells or of the limited CD4+ T cells in the graft. At later time points, reconstitution of T cells using thymic pathways becomes more prominent as the production of naïve CD4+ T cells increases. However, the modest correlation between patients with normal naïve CD4+ T cell counts and those with normal total CD4+ T cell counts suggests that peripheral expansion remains an important contributor to immune reconstitution at 1 year post allo-HSCT. While we do not have data on CD4 or CD8 subsets, beyond naïve CD4 T cells, in this cohort, we did examine T cell subsets in our phase 1 study of recombinant human IL-7, and showed that the main subset present early after TCD-HCT was effector memory T cells.[27] In that study, the percent of regulatory T cells was within the normal range and was not affected by treatment with IL-7, which is not surprising as they don't express CD127. While that represents a very small group of patients (n=12) that requires prospective validation in a larger dataset, it is consistent with the notion that early recovery is driven by peripheral expansion. In addition, we were not able to examine B cell recovery or T cell chimerism, both parameters that would enhance the overall analysis.

Another important finding is the wide variability in immune recovery, implying that factors aside from TCD affect T cell recovery. For example, CD4+ T cell counts ranged between 0-1771/mm^3 at 6 months and 0-2960/mm^3 at 12 months. While studies in the non-TCD setting have suggested that patient age, CD4+ transplant dose, donor type, graft source, administration of ATG, CMV reactivation, and the development of GVHD are important, [8,9,40,41] we examined factors influencing immune recovery after fully TCD allo-HSCT. We identified ATG as a factor that impaired immune recovery, suggesting that we could potentially improve outcomes if we focus on strategies to perform TCD allo-HSCT without ATG.[13] However, we cannot exclude potential bias in this finding because patients who did not require ATG were either young or received an HLA-matched graft. In addition, receiving an HLA-matched graft predicted improved recovery of all immune reconstitution parameters, while lower patient age predicted improved T cell function post TCD allo-HSCT. Although GVHD impacts immune recovery in non-TCD transplants, there were only a small number of GVHD events in the present study.

In this large patient population, we demonstrate that recovery of T cell function as measured by response to the mitogen PHA is independently predictive of OS and EFS at 6 months post transplant. Our data confirm and significantly expand on a prior report of 69 patients receiving conventional allo-HSCT, which showed an improvement in OS with CD4+ T cell recovery at 3 months.[8] This is the first description of the effect of immune recovery on OS and EFS after TCD allo-HSCT. In this population of patients who receive a fully TCD allo-HSCT, we did not confirm the impact of ALC at day 30 on OS and EFS that has been seen in conventional and partially TCD studies.[4-7] This finding may result from a lower NK cell content administered in the graft with TCD. We also did not demonstrate an independent effect of T cell count recovery in the multivariate analysis.

Our study also seeks to assess the effect of immune recovery on disease relapse over the first year after TCD allo-HSCT. While no effect on relapse was seen in our study, our ability to detect such a difference was limited by the small number of these events observed. Our results are in agreement with a study in which neither CD4+ T cell nor CD8+ T cell recovery affected relapse in recipients of conventional grafts.[42] Interestingly, this study demonstrated an NK cell count >150 cells/uL at 12 months (but not earlier) is associated with a decreased risk of relapse. The discrepancy between this finding and our study may be related to the use of GVHD prophylaxis in the Bühlmann paper.

There have been other studies that demonstrate the impact of immune reconstitution after allo-HSCT. A previous report in 71 patients from MSKCC showed that CD4+ T cell recovery can mitigate opportunistic infections.[1] Berger et al [9] also reported that early CD4+ T cell recovery after conventional BMT leads to decreased infections, resulting in reduced TRM. No difference in TRM was noted for CD8+ and CD56+ cell counts. Pao et al[43] also reported that higher levels of circulating naïve CD4+ T cells correlated with an improved response to pneumococcal vaccination.

There are limitations to our study. First, it is retrospective and our findings would be strengthened if confirmed in a prospective study. There also several additional factors that are known to impact post-transplant outcomes that we were not able to include in the

analysis. These include the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), which we recently reported is also predictive in TCD all-HSCT,[44] and the disease risk index (DRI). Other factors, such as ABO have been shown to have at best a modest effect in a recent CIBMTR analysis of over 10,000 patients.[45] The dose of ATG has been shown in some series to affect outcomes. Those reports have all been with unmodified grafts. In our series, the dose of ATG was standard and the main effect was seen in the presence or absence of ATG.

Despite these limitations, the study provides a clear picture of the pattern of immune recovery after TCD allo-HSCT. Important findings include a description of the kinetics of naïve CD4+ T cell recovery and the demonstration that recovery of T cell function, assessed by PHA response at 6 months, independently predicts improved OS and EFS and may therefore provide a marker for patients whose outcomes could improve with investigational agents for the promotion of immune reconstitution. While the main immune parameters measured, CD4 and CD8 T cells, were not independently associated with outcomes in the multivariable analysis, their analysis and correlations with risk of infection and response to vaccines for example could still indicate their clinical utility.

Given recent increased interest in TCD in the setting of the ongoing randomized trial, [17,18,30,46] and the risk of poor immune recovery associated with TCD, it will become increasingly important to identify patients at risk for delayed immune recovery and develop agents for the promotion of the immune system after TCD. Our demonstration of naïve T cell recovery post TCD allo-HSCT implies that patients can have thymic recovery following myeloablative allo-HSCT. Thus, preserving the thymus, perhaps with KGF or androgen ablation, or directly effecting T cells with agents such as IL-7,[27] are worthwhile strategies to pursue for promoting immune reconstitution. Future plans include further assessing the close relationship between immune recovery and the development of infections including how the development of infection affects the immune system. In addition, it will be critical to further dissect the relationship between T cell numbers, function and T cell receptor diversity.[14]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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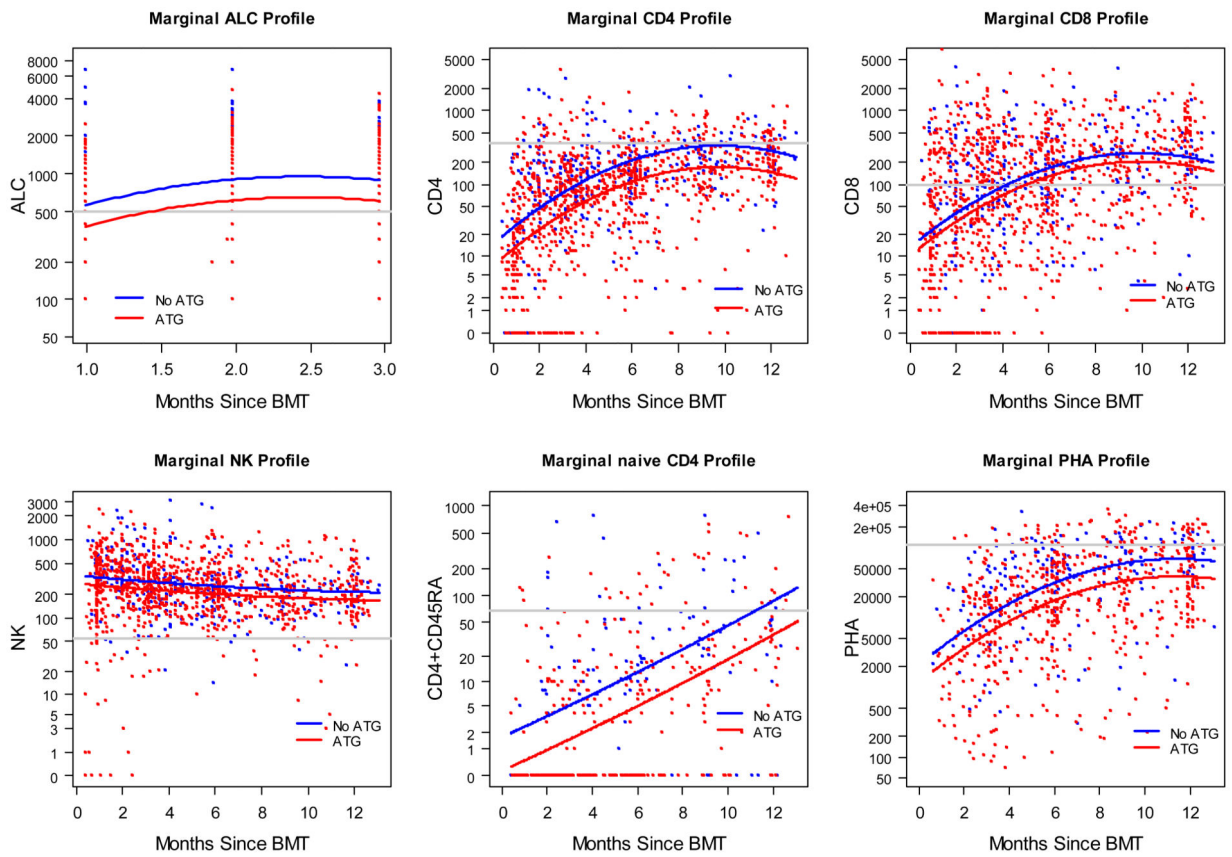
**Figure 1. Immune recovery for the first 12 months after allo-HSCT with and without ATG**
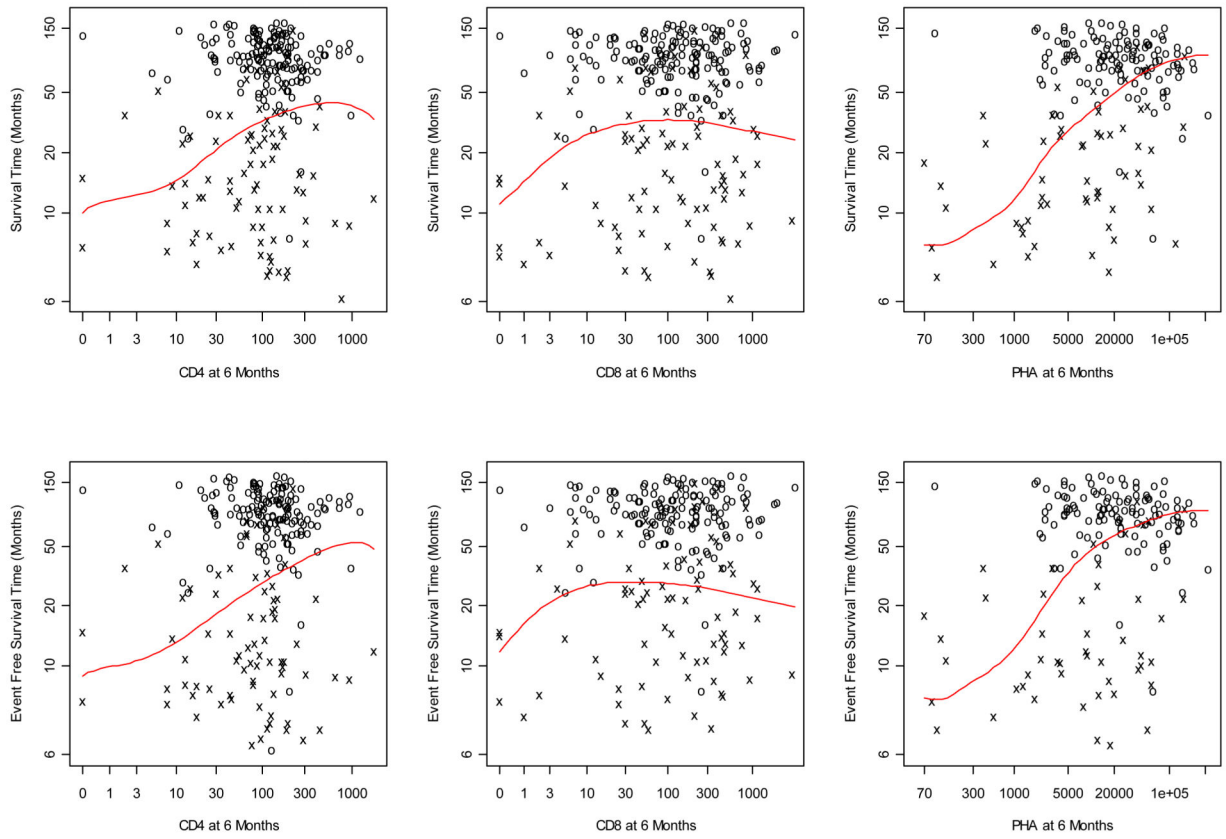
Figure 2. Landmark analysis of immune reconstitution at 6 months on OS and EFS

Table 1

Patient Characteristics

Patient Characteristics	
Age (years)	
Median [Range]	40 [2-68]
<18	69
<18-30	64
30-49	150
>50	92
Disease	
AML	150
ALL	85
CML	55
NHL	49
MDS	36
Donor	
Matched Related	202
Mismatched Related	36
Matched Unrelated	77
Mismatched unrelated	60
Status at HSCT	
1st complete remission, 1st chronic phase, untreated MDS without increased blasts	178
active relapse, refractory disease, progressive disease	22
Other	175
Conditioning Regimen	
TBI/Thiotepa/Cyclophosphamide	218
TBI/Thiotepa/Fludarabine	109
Busulfan/Melphalan/Fludarabine	48
Stem Cell Source	
Bone Marrow	190
Peripheral Blood Stem Cells	153
Bone Marrow & Peripheral Blood Stem Cells	32

AML: acute myelogenous leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myelegenous leukemia; NHL: non-Hodgkin lymphoma; MDS: Myelodysplastic syndrome; HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation.

Table 2

Effect of baseline factors on immune reconstitution

	Univariate Analysis			Multivariable Analysis		
	Slope	95%CI	P-value	Slope	95%CI	P-value
log(ALC) n =324						
Age (10-year increment)	0.04	-0.02 , 0.09	.195			
Sex (Male)	0.11	-0.09 , 0.31	.275			
ATG use	-0.38	-0.61 , -0.16	.001			
HLA Identical	0.43	0.22 , 0.65	<.001	0.43	0.22 , 0.65	<.001
Regimen (chemo only)	0.14	-0.14 , 0.43	.332			
1st Remission	-0.02	-0.22 , 0.19	.865			
Diagnosis (ALL)	-0.19	-0.41 , 0.04	.103			
PBSC only	0.16	-0.04 , 0.36	.111			
Donor Sex (Male)	0.17	-0.02 , 0.37	.079			
log(CD4), n = 315						
Age (10-year increment)	-0.01	-0.08 , 0.06	.698			
Sex (Male)	0.28	0.03 , 0.52	.027			
ATG use	-0.66	-0.94 , -0.39	<.001	-0.66	-0.94 , -0.39	<.001
HLA Identical	0.43	0.15 , 0.71	.003			
Regimen (chemo only)	0.07	-0.30 , 0.44	.698			
1st Remission	0.25	0 , 0.51	.052			
Diagnosis (ALL)	-0.07	-0.35 , 0.21	.622			
PBSC only	0.14	-0.11 , 0.38	.275			
Donor Sex (Male)	-0.05	-0.30 , 0.19	.666			
log(CD8), n = 314						
Age (10-year increment)	-0.01	-0.10 , 0.07	.746			
Sex (Male)	0.27	-0.04 , 0.58	.085			
ATG use	-0.26	-0.62 , 0.09	.149			

	Univariate Analysis			Multivariable Analysis		
	Slope	95%CI	P-value	Slope	95%CI	P-value
HLA Identical	0.50	0.15 , 0.85	.006	0.50	0.15 , 0.85	.006
Regimen (chemo only)	-0.08	-0.54 , 0.39	.745			
1st Remission	-0.10	-0.43 , 0.22	.527			
Diagnosis (ALL)	-0.03	-0.38 , 0.32	.854			
PBSC only	-0.40	-0.70 , -0.09	.011			
Donor Sex (Male)	-0.24	-0.54 , 0.07	.132			
log(NK), n = 314						
Age (10-year increment)	0.05	0 , 0.09	.059			
Sex (Male)	-0.06	-0.22 , 0.11	.508			
ATG use	-0.23	-0.42 , -0.04	.019			
HLA Identical	0.28	0.08 , 0.47	.005	0.28	0.08 , 0.47	.005
Regimen (chemo only)	0.17	-0.08 , 0.42	.190			
1st Remission	0.04	-0.13 , 0.22	.640			
Diagnosis (ALL)	-0.15	-0.34 , 0.04	.118			
PBSC only	0.16	0 , 0.33	.055			
Donor Sex (Male)	0.14	-0.02 , 0.3	.097			
log(CD45), n= 187						
Age (10-year increment)	-0.10	-0.20 , 0.01	.066			
Sex (Male)	0.23	-0.17 , 0.63	.266			
ATG use	-0.86	-1.30 , -0.42	<.001	-0.86	-1.30 , -0.42	<.001
HLA Identical	0.38	-0.03 , 0.80	.072			
Regimen (chemo only)	-0.19	-0.72 , 0.35	.488			
1st Remission	0.12	-0.30 , 0.54	.585			
Diagnosis (ALL)	0.18	-0.25 , 0.60	.419			
PBSC only	0.08	-0.32 , 0.48	.699			
Donor Sex (Male)	0.08	-0.32 , 0.47	.711			
log(PHA), n =258						

	Univariate Analysis			Multivariable Analysis		
	Slope	95%CI	P-value	Slope	95%CI	P-value
Age (10-year increment)	-0.17	-0.26 , -0.08	<.001	-0.16	-0.25 , -0.07	.001
Sex (Male)	0.00	-0.34 , 0.33	.980			
ATG use	-0.58	-0.95 , -0.21	.002	-0.53	-0.89 , -0.16	.005
HLA Identical	0.32	-0.06 , 0.71	.100			
Regimen (chemo only)	0.04	-0.45 , 0.52	.883			
1st Remission	0.26	-0.09 , 0.60	.144			
Diagnosis (ALL)	0.20	-0.17 , 0.57	.285			
PBSC only	0.02	-0.31 , 0.35	.917			
Donor Sex (Male)	0.07	-0.26 , 0.40	.678			

[a]The factors without estimates and p value were not significant in multivariable analysis.

ALC: absolute lymphocyte count; ATG: antithymocyte globulin; ALL: acute lymphoblastic leukemia; PBSC: peripheral blood stem cells; NK: natural killer; PHA: phytohemagglutinin.

Table 3a

Landmark analysis of effect of baseline factors and immune recovery at 1 month on survival outcomes, NRM and relapse

1 Month	Total N	Univariate Analysis		Multivariable Analysis*	
		OS			
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
Baseline Factor					
Age (10-year increment)	355	1.07 (0.98, 1.17)	.129		
ATG (yes)	355	1.86 (1.23, 2.80)	.003		
HLA identical (yes)	355	0.51 (0.37, 0.69)	<.001	0.51 (0.37, 0.69)	<.001
Regimen (chemo only)	355	1.22 (0.80, 1.85)	.354		
1st Remission (yes)	355	0.74 (0.54, 1.03)	.073		
ALL	355	1.24 (0.89, 1.72)	.210		
PBSC only	355	0.81 (0.60, 1.10)	.183		
Donor Sex match (yes)	355	0.96 (0.72, 1.29)	.806		
Transplant Year (2002–2005)	355	0.77 (0.57, 1.04)	.087		
Immune recovery parameter					
ALC	323	0.88 (0.66, 1.17)	.364		
log(CD4)	193	0.94 (0.84, 1.05)	.253		
log(CD8)	193	0.95 (0.87, 1.04)	.267		
log(NK)	192	0.85 (0.74, 0.98)	.025		
		NRM			
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
Baseline Factor					
Age (10-year increment)	353	1.12 (1.00, 1.25)	.042		
ATG (yes)	353	2.77 (1.56, 4.93)	.001		
HLA identical (yes)	353	0.45 (0.31, 0.65)	<.001	0.45 (0.31, 0.65)	<.001
Regimen (chemo only)	353	1.06 (0.62, 1.82)	.838		
1st Remission (yes)	353	0.71 (0.48, 1.06)	.095		
ALL	353	1.09 (0.72, 1.65)	.676		

1 Month	Total N	Univariate Analysis		Multivariable Analysis*	
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
PBSC only	353	0.81 (0.56, 1.17)	.257		
Donor Sex match (yes)	353	0.99 (0.69, 1.41)	.942		
Transplant Year (2002–2005)	353	0.64 (0.44, 0.93)	.018		
Immune recovery parameter					
ALC	322	0.74 (0.49, 1.12)	.158		
log(CD4)	193	0.92 (0.81, 1.05)	.216		
log(CD8)	193	0.93 (0.83, 1.04)	.183		
log(NK)	192	0.81 (0.7, 0.95)	.008		
EFS					
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
Baseline Factor					
Age (10-year increment)	353	1.04 (0.96, 1.13)	.353		
ATG (yes)	353	1.94 (1.29, 2.91)	.001		
HLA identical (yes)	353	0.50 (0.37, 0.68)	<.001	0.50 (0.37, 0.68)	<.001
Regimen (chemo only)	353	1.34 (0.90, 2.01)	.149		
1st Remission (yes)	353	0.74 (0.54, 1.01)	.059		
ALL	353	1.21 (0.87, 1.67)	.258		
PBSC only	353	0.80 (0.59, 1.08)	.147		
Donor Sex match (yes)	353	0.95 (0.71, 1.27)	.735		
Transplant Year (2002–2005)	353	0.80 (0.59, 1.07)	.132		
Immune recovery parameter					
ALC	322	0.79 (0.58, 1.08)	.144		
log(CD4)	193	0.97 (0.87, 1.07)	.505		
log(CD8)	193	0.99 (0.90, 1.08)	.755		
log(NK)	192	0.84 (0.74, 0.96)	.011		
Relapse					
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
Baseline Factor					

1 Month	Total N	Univariate Analysis		Multivariable Analysis*
Age (10-year increment)	353	0.90 (0.78, 1.04)	.169	
ATG (yes)	353	1.11 (0.61, 2.01)	.742	
HLA identical (yes)	353	0.63 (0.37, 1.09)	.101	
Regimen (chemo only)	353	2.04 (1.10, 3.77)	.023	
1st Remission (yes)	353	0.78 (0.45, 1.34)	.364	
ALL	353	1.42 (0.82, 2.47)	.210	
PBSC only	353	0.83 (0.49, 1.40)	.486	
Donor Sex match (yes)	353	0.80 (0.48, 1.32)	.385	
Transplant Year (2002-2005)	353	1.29 (0.78, 2.15)	.319	
Immune recovery parameter				
ALC	322	0.92 (0.58, 1.44)	.708	
log(CD4)	193	1.07 (0.90, 1.27)	.457	
log(CD8)	193	1.11 (0.96, 1.29)	.168	
log(NK)	192	0.93 (0.71, 1.21)	.583	

[a] The factors without estimates and p value were not significant in multivariable analysis.

ALC: absolute lymphocyte count; ATG: antithymocyte globulin; ALL: acute lymphoblastic leukemia; PBSC: peripheral blood stem cells; NK: natural killer; PHA: phytohemagglutinin.

Table 3b

Landmark analysis of effect of baseline factors and immune recovery at 3 months on survival outcomes and relapse

3 Months	Total N	Univariate Analysis		Multivariable Analysis[a]	
		OS			
		HR of Parameter (95%CI)	**P-value**	**HR of Parameter (95%CI)**	**P-value**
Baseline Factor					
Age (10-year increment)	314	1.08 (0.98, 1.19)	.132		
ATG (yes)	314	1.71 (1.09, 2.68)	.019		
HLA identical (yes)	314	0.44 (0.31, 0.62)	<.001	0.44 (0.31, 0.62)	<.001
Regimen (chemo only)	314	1.44 (0.92, 2.26)	.110		
1st Remission (yes)	314	0.79 (0.55, 1.14)	.214		
ALL	314	1.40 (0.97, 2.02)	.075		
PBSC only	314	0.85 (0.60, 1.20)	.347		
Donor Sex match (yes)	314	0.93 (0.67, 1.30)	.678		
Transplant Year (2002–2005)	314	0.83 (0.59, 1.16)	.271		
Immune recovery parameter					
log(CD4)	252	0.89 (0.82, 0.98)	.012		
log(CD8)	252	0.91 (0.84, 0.98)	.014		
log(NK)	251	0.93 (0.68, 1.26)	.637		
log(CD45)	114		Na		
log(PHA)	89	0.94 (0.79, 1.12)	.473		
		EFS			
		HR of Parameter (95%CI)	**P value**	**HR of Parameter (95%CI)**	**P value**
Baseline Factor					
Age (10-year increment)	304	1.06 (0.96, 1.18)	.232		
ATG (yes)	304	1.90 (1.19, 3.02)	.007		
HLA identical	304	0.41 (0.29, 0.58)	<.001	0.41 (0.29, 0.58)	<.001
Regimen (chemo only)	304	1.58 (1.01, 2.47)	.046		

3 Months	Total N	Univariate Analysis		Multivariable Analysis[a]	
		HR of Parameter (95%CI)	P value	HR of Parameter (95%CI)	P value
1st Remission	304	0.79 (0.55, 1.14)	.205		
ALL	304	1.28 (0.88, 1.86)	.205		
PBSC only	304	0.80 (0.57, 1.14)	.214		
Donor Sex match (yes)	304	0.84 (0.60, 1.17)	.294		
Transplant Year (2002–2005)	304	0.84 (0.60, 1.19)	.330		
Immune recovery parameter					
log(CD4)	243	0.93 (0.85, 1.02)	.128		
log(CD8)	243	0.93 (0.86, 1.01)	.083		
log(NK)	242	0.87 (0.64, 1.19)	.399		
log(CD45)	111		Na		
log(PHA)	85	0.92 (0.78, 1.09)	.350		

Relapse		HR of Parameter (95%CI)	P value	HR of Parameter (95%CI)	P value
Baseline Factor					
Age (10-year increment)	304	0.93 (0.79, 1.10)	.385		
ATG (yes)	304	1.14 (0.58, 2.24)	.700		
HLA identical	304	0.49 (0.27, 0.90)	.020		
Regimen (chemo only)	304	2.29 (1.17, 4.94)	.016		
1st Remission	304	0.82 (0.45, 1.52)	.536		
ALL	304	1.43 (0.76, 2.66)	.264		
PBSC only	304	0.81 (0.45, 1.45)	.472		
Donor Sex match (yes)	304	0.51 (0.28, 0.93)	.028		
Transplant Year (2002–2005)	304	1.49 (0.83, 2.65)	.180		
Immune recovery parameter					
log(CD4)	243	1.06 (0.91, 1.23)	.447		
log(CD8)	243	1.02 (0.89, 1.16)	.777		
log(NK)	242	0.85 (0.66, 1.10)	.226		
log(CD45)	111		Na		
log(PHA)	85	0.94 (0.72, 1.23)	.662		

[a]The factors without estimates and p value were not significant in multivariable analysis.

ALC: absolute lymphocyte count; ATG: antithymocyte globulin; ALL: acute lymphoblastic leukemia; PBSC: peripheral blood stem cells; NK: natural killer; PHA: phytohemagglutinin.

Table 3c

Landmark analysis of effect of baseline factors and immune recovery at 6 months on survival outcomes and relapse

6 Months	Total N	Univariate Analysis		Multivariable Analysis[a]	
		OS			
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
Baseline Factor					
Age (10-year increment)	272	1.07 (0.95, 1.21)	.255		
ATG (yes)	272	1.52 (0.91, 2.53)	.111		
HLA identical (yes)	272	0.45 (0.29, 0.68)	<.001	0.48 (0.27, 0.82)	.008
Regimen (chemo only)	272	1.37 (0.79, 2.37)	.267		
1st Remission (yes)	272	0.95 (0.62, 1.44)	.805		
ALL	272	1.43 (0.92, 2.22)	.110		
PBSC only	272	0.96 (0.64, 1.44)	.843		
Donor Sex match (yes)	272	0.88 (0.59, 1.31)	.528		
Transplant Year (2002–2005)	272	1.10 (0.74, 1.65)	.640		
Immune recovery parameter					
log(CD4)	238	0.76 (0.65, 0.90)	.002		
log(CD8)	238	0.91 (0.79, 1.04)	.149		
log(NK)	238	1.10 (0.83, 1.46)	.505		
log(CD45)	121	0.86 (0.71, 1.04)	.118		
log(PHA)	179	0.70 (0.61, 0.80)	<.001	0.74 (0.65, 0.86)	<.001
		EFS			
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
Baseline Factor					
Age (10-year increment)	254	1.03 (0.91, 1.17)	.647		
ATG (yes)	254	1.56 (0.91, 2.69)	.106		
HLA identical	254	0.37 (0.24, 0.56)	<.001	0.37 (0.21, 0.67)	.001
Regimen (chemo only)	254	1.54 (0.87, 2.74)	.137		

6 Months	Total N	Univariate Analysis		Multivariable Analysis[a]	
		HR of Parameter (95%CI)	P-value	HR of Parameter (95%CI)	P-value
1st Remission	254	0.85 (0.54, 1.32)	.464		
ALL	254	1.41 (0.88, 2.24)	.150		
PBSC only	254	0.99 (0.64, 1.52)	.957		
Donor Sex match (yes)	254	0.97 (0.64, 1.47)	.869		
Transplant Year (2002–2005)	254	1.13 (0.74, 1.73)	.567		
Immune recovery parameter					
log(CD4)	222	0.76 (0.64, 0.90)	.001		
log(CD8)	222	0.94 (0.81, 1.08)	.374		
log(NK)	222	1.02 (0.76, 1.37)	.897		
log(CD45)	115	0.81 (0.65, 1.00)	.046		
log(PHA)	164	0.73 (0.63, 0.85)	<.001	0.80 (0.69, 0.92)	.002

Relapse		HR of Parameter (95%CI)	P-value		
Baseline Factor					
Age (10-year increment)	254	0.91 (0.73, 1.13)	.380		
ATG (yes)	254	0.87 (0.38, 1.97)	.731		
HLA identical	254	0.38 (0.18, 0.82)	.013		
Regimen (chemo only)	254	2.63 (1.12, 6.22)	.027		
1st Remission	254	0.86 (0.39, 1.91)	.717		
ALL	254	1.41 (0.62, 3.21)	.411		
PBSC only	254	0.99 (0.47, 2.11)	.982		
Donor Sex match (yes)	254	0.51 (0.24, 1.10)	.087		
Transplant Year (2002–2005)	254	1.82 (0.83, 3.98)	.132		
Immune recovery parameter					
log(CD4)	222	0.91 (0.65, 1.28)	.603		
log(CD8)	222	1.06 (0.81, 1.39)	.646		
log(NK)	222	1.14 (0.67, 1.92)	.632		
log(CD45)	115	0.82 (0.55, 1.23)	.339		
log(PHA)	164	1.02 (0.75, 1.38)	.903		

[a]The factors without estimates and p value were not significant in multivariable analysis.

ALC: absolute lymphocyte count; ATG: antithymocyte globulin; ALL: acute lymphoblastic leukemia; PBSC: peripheral blood stem cells; NK: natural killer; PHA: phytohemagglutinin.