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Allogeneic Hematopoietic Cell Transplantation Following Minimal Intensity Conditioning: Predicting Acute Graft-vs.-Host Disease and Graft-vs.-Tumor Effects

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Abstract

Most patients with hematologic malignancies have received extensive chemotherapy before hematopoietic cell transplantation (HCT), resulting in neutropenia, lymphocytopenia, and use of antibiotics. Accordingly, patients have a wide range of neutrophil counts, lymphocyte counts, and prior antibiotic use. The minimal toxicity of the current conditioning regimen allowed asking whether peritransplant neutrophil or lymphocyte levels influenced the risks of acute graft-versus-host disease (GVHD) or relapse. We analyzed outcomes in 459 patients aged 7 to 75 (median 57) years who were conditioned with fludarabine and low-dose total body irradiation for HLA-matched HCT. We made two key findings. First, low neutrophil nadirs within the first 3 weeks after HCT had significant associations with increased risks of acute GVHD and 5-year non-relapse mortality (NRM), but showed no associations with the risk of relapse. Second, high lymphocyte counts immediately before transplantation had significant associations with reduced risks of relapse and overall mortality but showed no associations with the risks of GVHD or NRM. The findings suggested that the immunological mechanisms involved in acute GVHD might differ from those initiating graft-versus-tumor effects.

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AUTHORS' CONTRIBUTIONS

R.S.: Designed the research and wrote the paper.

B.G.: Assisted with data analysis and was principal investigator in one of the clinical trials.

B.E.S.: Carried out all statistical analyses.

D.G.M.: Principal investigator on clinical trials and contributed in writing the manuscript.

M.L.S.: Principal investigator on clinical trials and contributed in writing the manuscript.

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INTRODUCTION

Graft-vs.-host reactions occur after allogeneic hematopoietic cell transplantation (HCT) despite advances both in human leukocyte antigen (HLA) matching of the donor and the recipient and in immunosuppressive agents. Graft-vs.-host reactions can affect the recipient hematopoietic system, which may translate into graft-vs.-tumor (GVT) effects among patients with hematologic malignancies. These reactions can also affect the skin, gut and liver and the resulting graft-vs.-host disease (GVHD) can increase the risk of mortality. Among patients treated with conventional HCT, acute GVHD is initiated by the effects of high-intensity conditioning regimens, which cause tissue damage and subsequent production of inflammatory cytokines, translocation of bacterial products into the circulation, and heightened donor T-cell recognition of host antigens (reviewed in [1]). In a second phase donor T-cells are stimulated by minor histocompatibility antigens presented directly by host antigen-presenting cells [2,3] and indirectly by donor cells. In the third step, activated effector T-cells cause tissue injury that is recognized clinically as GVHD.

We developed a mild HCT-conditioning regimen that minimized tissue damage and could be tolerated by patients who were older or had serious comorbidities [4–7]. This regimen made it feasible to assess GVHD and GVT effects apart from the effects of the conditioning. In these patients, GVHD involvement of the liver has become very infrequent, especially after the introduction of prophylactic ursodiol [8,9]. Consequently, acute GVHD is now largely confined to skin and gut, both of which interface with the environment and possess characteristic microbiomes consisting of symbiotic, commensal bacteria [10,11]. The likely disturbance of microbiomes by neutropenia and antibiotics in patients undergoing chemotherapy before HCT, and the suspected roles of altered microbiomes in the development of autoimmune diseases (reviewed in [10,11]) raise the possibility that changes in commensal flora may modulate GVHD. In fact, a study in mice showed that pre-transplant treatment with the antibiotic ampicillin resulted in a loss of lactobacilli and aggravation of GVHD, whereas re-introduction of lactobacilli protected against GVHD [12]. Simultaneous studies in human patients identified increased microbial disturbances early after allogeneic HCT as a potential risk factor for GVHD [12].

Most patients with hematological malignancies have received extensive chemotherapy resulting in pancytopenia and use of broad-spectrum antibiotics. Accordingly, at arrival at our transplant center, patients have a wide range of neutrophil counts, lymphocyte counts, and prior antibiotic use. The widely ranging blood counts and the minimal toxicity of the conditioning regimen, allowed us to ask whether peri-transplant neutrophil or lymphocyte levels influenced the risks of acute GVHD or relapse (GVT effect) after HLA-matched, related and unrelated HCT.

METHODS

Patients

Between September 1, 2002 and December 31, 2010, 459 consecutive patients with hematologic malignancies were entered on prospective, minimal-intensity conditioning trials at the Fred Hutchinson Cancer Research Center (FHCRC) that were registered with ClinicalTrials.gov. All patients signed consent forms that were approved by the institutional review board of the FHCRC. Median follow-up of patients was 5 (range 1.5 to 9.9) years.

Table 1 shows patient and disease characteristics. The median patient age was 57 (range 7–75) years. Unmodified grafts consisted of granulocyte colony stimulating factor (G-CSF)-mobilized blood mononuclear cells (G-PBMC) and contained medians of 8.6 (range 0.2–29.9) $\times 10^6$ CD34⁺ cells/kg and 3.0 (range 0.1–10.8) $\times 10^8$ CD3⁺ cells/kg. Patients had

received a median of 3 (range 0–23) chemotherapy regimens before HCT. HCT Comorbidity Index (HCT-CI) scores were assigned as described [13]. Fifty-one percent of patients had serious comorbidities with scores of 3 and higher. The designation of low, standard or high risk of relapse or progression for each disease and disease stage followed a previously described algorithm [14].

Conditioning and postgrafting immunosuppression

Four hundred thirty patients received 2 Gy TBI with ($n=343$) or without ($n=87$) fludarabine, 30 mg/m²/day, on days 4, 3, and 2 before HCT (Table 1). Twenty-four patients received 3 Gy and five patients received 4 Gy TBI. Immunosuppression after HCT included MMF (28 days for related [4] and at least 96 days for unrelated [5,6] recipients) and a calcineurin inhibitor (up to 180 days; either cyclosporine [CSP; $n=270$] or tacrolimus [TAC; $n=189$]). Thirty-seven unrelated recipients also received sirolimus as part of a randomized trial [15]. All patients received prophylactic ursodiol from approximately 14 days before transplantation until at least day 180 after transplantation. Infection prophylaxis and treatment followed FHCRC standard practice guidelines including acyclovir for herpes simplex and varicella zoster virus prophylaxis, fluconazole for yeast prophylaxis, trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis, and monitoring for cytomegalovirus reactivation. Antibacterial prophylaxis with levofloxacin was started when neutrophil counts declined below 500 cells/ μ L and was continued until neutrophil counts recovered to above 500 cells/ μ L for 2 consecutive days in the absence of infection. Transplantations were planned as outpatient procedures.

Monitoring after transplantation

As a rule, patients had marrow aspirations to assess disease status on days 28, 84, 180, 365, and then as indicated. Donor chimerism was evaluated on days 28, 84, and 365 after HCT. Acute and chronic GVHD were graded as described [16–20]. The diagnoses of relapse, defined as recurrence of malignancy, or of progression, were based on previously published criteria [14].

Causes of death

Relapse mortality included deaths after relapse or progression of pre-transplant disease, regardless of other events. Non-relapse mortality (NRM) included all deaths without relapse or progression. GVHD mortality included all deaths in patients with a history of GVHD who died during immunosuppressive therapy. Infection was listed as a cause of death in patients without relapse, progression, or a history of GVHD.

Statistical analysis

Overall survival was estimated by the Kaplan-Meier method [21]. Cumulative incidence rates of acute and chronic GVHD, NRM, and relapse mortality were estimated by methods previously described [22]. Death was a competing risk for all other endpoints. Relapse was a competing risk for NRM, and NRM was a competing risk for relapse and relapse-related mortality. Since some time-to-event endpoints also occurred during this time period, progressive neutrophil nadir was treated as a time-dependent covariate when analyzed as a risk factor. Cox regression was used for risk factor analysis for all time-to-event endpoints. Logistic regression was used to analyze risk factors for neutropenia nadir <500 cells/ μ L. For this analysis pre-transplant neutropenia was defined as the lowest value from day –15 through day –1 before starting the conditioning regimen. For purposes of display and characterization, neutrophil nadirs during days 1 through 20 after transplant were divided in four ranges.

All statistical analyses were adjusted to account for possible uneven distribution of the following variables: related/unrelated donor; HLA allele-level mismatch; patient age in years (<50; ≥50); donor/patient gender (F→M, other); fludarabine use (no, yes); calcineurin inhibitor (CSP, TAC); diagnosis (multiple myeloma, lymphoma, other); preceding high-intensity transplant (yes, no). Statistical analyses involving neutrophil nadir as a risk factor for acute and chronic GVHD, relapse, NRM, and overall mortality were also adjusted for pre-transplant lymphocyte counts. In separate analyses pre-transplant lymphocyte counts were analyzed as a risk factor for the same outcomes with adjustments both for post-transplant neutrophil counts and the other variables. Pre-transplant lymphocyte levels were determined by the minimum value between day -15 to day -1 before start of the conditioning regimen.

RESULTS

Post-transplantation neutrophil nadir

Neutrophil counts declined exponentially after HCT reaching nadirs between days 10–15, followed by exponential recoveries to the normal range by day 30. This is shown in Figure 1A, where the neutrophil nadirs were divided in four ranges for purposes of illustration.

The strongest predictors for a neutrophil nadir of <500/μL after HCT were low neutrophil counts before HCT (Table 2). Compared to patients with pre-transplant neutrophil counts >2000/μL, those with counts between 500 and 1999/μL had an overall risk (O.R.) of 3.55 (95% C.I. 2.0–6.2; p<0.0001) and those with counts <500/μL an O.R. of 5.94 (95% C.I. 2.1–17; p=0.0009). Another risk factor for low neutrophil nadirs after HCT was having transplantation from an unrelated donor (O.R. 3.62; 95% C.I. 1.9–6.8; p<0.0001). Patients with MM (O.R. 0.24; 95% C.I. 0.1–0.6; p=0.002) and those with prior high-intensity transplantation (O.R. 0.46; 95% C.I. 0.2–0.9; p=0.03) were significantly less likely to have a nadir of <500 neutrophils/μL after HCT. No significant associations with neutrophil nadir were seen with donor age, patient age, CD34 cell dose, number of preceding chemotherapy regimens and HCT-CI scores. In order to estimate the effect size associated with these risk factors, the post-transplantation neutrophil nadir was analyzed as a continuous outcome on a log scale and the results were expressed as nadir multipliers (Table 2). For example, a pre-transplant neutrophil count <500/μL reduced the post-transplantation neutrophil nadir by 82%, and a diagnosis of multiple myeloma increased the nadir by 54%.

GVHD—Two hundred sixty-four patients developed grade II-IV acute GVHD, of whom 101 had isolated gut GVHD, 60 had isolated skin GVHD, 102 had both skin and gut GVHD, and one had isolated, stage 1 liver GVHD. Eight patients had liver GVHD in addition to gut involvement. Among the 203 patients with gut GVHD, 159 (78%) had upper gut involvement. The distributions of organ involvement in patients were comparable over the range of neutrophil nadirs. The overall cumulative incidence rates of grades II–IV and III–IV acute GVHD were 56% and 8%, respectively.

Table 3A shows post-transplantation neutrophil nadirs, per log decrease in neutrophil counts, as risk factors for GVHD. Significantly increased hazard ratios were seen for the association of neutrophil nadirs with grades II-IV and III-IV acute GVHD and de novo chronic GVHD.

In Table 4, cumulative incidence estimates of grades II-IV and III-IV acute GVHD were analyzed within four subgroups of patients according to their neutrophil nadir. The results suggested that the neutrophil nadir represented a continuous predictor of the risk for acute GVHD. For example, the overall risk of grade II-IV acute GVHD rose from 36% in the

highest nadir group to 69% in the lowest nadir group, and the risk of grade III-IV acute GVHD tripled from 3.5% to 11%.

Relapse or progression, NRM and overall survival at 5 years—Table 3A also examined the neutrophil nadir as a time-dependent risk factor for relapse or progression, NRM and overall mortality. No statistically significant association between neutrophil nadir and relapse or progression was seen (HR 1.11; $p=0.39$) while associations with increased NRM and overall mortality were statistically significant (HRs 1.54 and 1.46, respectively; $p=0.001$ and <0.0001 , respectively). Table 4 shows comparable 5-year relapse mortality in the four nadir groups ranging from 32–39% (see also Figure 2A). In contrast, 5-year NRM steadily increased from 3% in the highest nadir range to 25% in the lowest nadir range (see also Figure 2B). Five-year overall survival, affected both by relapse mortality and NRM, ranged from 58% in the highest nadir range to 40% in the lowest nadir range.

Causes of NRM

Eighty-seven patients died of nonrelapse causes. Of these, 78 (90%) died from complications related to GVHD and its treatment. Among the 78, 59 died from bacterial, fungal, or viral infections and 19 died from organ failure including bronchiolitis obliterans organizing pneumonia. Among the nine patients without GVHD, two experienced late graft failure and died of infections after receiving pentostatin and donor lymphocyte infusions (days 158 and 344, respectively). Four patients died of solid tumors (in two cases recurrent) 2.9, 5.0, 5.1, and 5.5 years after HCT, and two died in car accidents 1.3 and 2.5 years after HCT. One patient with a history of CNS radiation, intrathecal chemotherapy, and two preceding high-intensity conditioning transplantations died on day 97 from leukoencephalopathy.

Pre-transplantation lymphocyte counts

In the preceding multivariate analyses, we used lymphocyte counts immediately before transplantation as a continuous adjustment factor. Here, we analyzed pre-transplant lymphocyte counts as continuous variable and transplantation outcomes (Table 3B) with the post-transplantation neutrophil nadir used as an additional adjustment factor. While no statistically significant associations were seen with acute GVHD, chronic GVHD or NRM, low lymphocyte counts predicted significantly higher relapse risk (HR 1.48; $p=0.003$) and increased overall mortality (HR 1.41; $p=0.007$).

DISCUSSION

The study yielded two major, new results. First, the depth of the neutrophil nadirs within the first 3 weeks after transplantation was highly associated with the risks of acute GVHD and associated NRM but not with GVT effects. Second, peripheral blood lymphocyte counts immediately before transplantation were associated with GVT effects but not with acute GVHD or NRM. These findings suggested that the immunological mechanisms involved in acute GVHD differed from those initiating GVT effects.

The first result showed that patients with the lowest neutrophil nadirs had significantly increased risks of both acute GVHD and 5-year NRM compared to patients with higher nadirs. For example, the risk of grades III-IV acute GVHD rose steadily from 3.5% in the highest neutrophil nadir range to 11% in the lowest nadir range, and the risk of 5-year NRM rose from 3% to 25%. The data suggested that the neutrophil nadir might serve as a continuous predictor of risks for GVHD and NRM. While the risks of acute GVHD and NRM increased significantly with decreasing nadir, both the cumulative incidence rates and the hazard ratios of relapse were unchanged across the range of neutrophil nadirs. It could be

argued that some diseases, which are especially susceptible to GVT effects such as B-cell malignancies, were unevenly distributed among the nadir ranges, but the multivariate analyses were adjusted for the type of malignancy.

Unsurprisingly, the most significant factor predicting low post-transplantation neutrophil nadirs was pre-transplant neutropenia. Other factors included underlying malignancies, having received a prior transplant with high-intensity conditioning, and unrelated grafts, but these were included as adjustments in all analyses. Low pre-transplant neutrophil counts likely resulted from extended chemotherapy and impaired marrow function before referral to HCT. Typically, neutropenic patients are treated with prophylactic, broad-spectrum antibiotics. Antibiotics can impart unintended collateral damage to symbiotic microorganisms that live in the human gastrointestinal tract [23]. Specifically, repeated exposure to broad-spectrum antibiotics can dramatically alter the composition of human gut microbial communities, and these changes can persist for long periods of time [24,25]. It is possible that both the neutropenia and the altered microbiota emerging after antibiotic treatments can lead to a pro-inflammatory state which induces production of cytokines, such as interferon- γ , TNF- α , IL-1, and IL-6, and increased expression of HLA-DR and costimulatory molecules, not only on antigen-presenting cells, but also on skin and gut epithelia. In this hypothetical sequence of events, skin and gut epithelia might conceivably present minor histocompatibility antigens to donor T-cells, thereby amplifying GVHD manifestations specifically in these two organs at the exclusion of GVT effects or immune reactions against other organs, such as those typically involved in autoimmune diseases. Alternatively, indirect antigen-presentation from these cells might be enhanced by low-grade inflammation associated with neutropenia and altered microbial flora.

Results from previous studies support the hypothesis that gut and skin microbiomes can influence the development of GVHD. “Germ-free” murine recipients of H-2 incompatible HCT had less clinically detectable GVHD and survived significantly better than conventionally raised mice, and their histologic GVHD lesions were less pronounced [26–28]. Human aplastic anemia patients given marrow grafts from HLA-identical siblings experienced both significantly less acute GVHD and better survival when the transplantation was carried out in a protective, “bacteria-free” environment compared to a conventional environment [29]. Similarly, a pediatric study showed improved prevention of GVHD with suppression of microbial flora [30]. In another study, human HCT recipients randomly assigned to antibiotic prophylaxis with ciprofloxacin and metronidazole had less acute GVHD than those given ciprofloxacin alone [31]. The authors suggested that anaerobic flora, favored by ciprofloxacin monotherapy, played a role in the pathogenesis of acute GVHD.

In mice, outgrowth of *Escherichia (E.) coli* bacteria was observed during gut GVHD and GVHD was ameliorated when *E. coli* growth was curtailed by oral polymyxin B, consistent with the hypothesis that microorganisms could modulate GVHD [32]. Another murine study confirmed outgrowth of *E. coli* during GVHD, a finding that was interpreted to represent a shift toward pro-inflammatory bacterial species [33]. Probiotics appeared to reduce GVHD in experimental models [34], as did oral administration of *Lactobacillus rhamnosus*, while administration of ciprofloxacin led to worse survival, possibly as a result of a disturbed microbiome [35]. These results were consistent with the adverse effect on GVHD caused by pretreatment of mice with ampicillin and the reversal of this effect by introduction of lactobacilli [12]. Findings in human allogeneic HCT recipients did not identify special bacterial populations as potential risk factors for subsequent GVHD but found greater microbial disturbance early after transplantation in patients who developed GVHD [12]. Sequential high-resolution 16s rRNA studies of skin and gut microbiomes, especially among patients with high vs. low post-transplantation neutrophil nadirs, might provide insight into

whether microbiome changes could be implicated in the causal pathway leading to development of acute GVHD.

The second major finding of the current study was that lymphocyte counts immediately before transplantation had significant associations with the risk of relapse and, consequently, with overall mortality but showed no associations with the risks of acute GVHD, chronic GVHD or NRM. Specifically, patients with higher lymphocyte counts had significantly less risk of relapse or progression and better overall survival than those with lower lymphocyte counts. Because pretransplant flow cytometry examinations of the peripheral blood were focused on detecting malignant cells, we have no detailed information on lymphocyte or mononuclear subsets in these patients. In the absence of that knowledge, we would hypothesize that lymphocyte levels might serve as a proxy for the numbers of host antigen-presenting cells with the result that larger numbers of these cells are associated with stronger GVT effects. Even though our findings are currently unexplained, they are consistent with the possibility that GVT effects can be separated from acute GVHD, and that they are initiated by different mechanisms. Alternatively, it could be argued that pre-transplant lymphocyte counts were a reflection of the intensity of the preceding chemotherapy used to reduce the tumor burden. Thus, low lymphocyte counts might be a surrogate marker for “resilience” of the malignancy to respond to therapy. In order to offset this confounder, the analysis was adjusted for underlying diagnoses.

Four studies in patients treated with either myeloablative or reduced-intensity conditioning regimens showed that the risk of relapse was not reduced by acute GVHD [7,36–38]. In contrast, a recent large study in patients with leukemias and myelodysplastic syndromes treated with high-intensity regimens did find significant incremental effects of acute GVHD on risk of relapse, although this effect was observed only beyond the first 18 months [39]. Others showed GVT effects associated with acute GVHD in patients with ALL and more advanced AML but not AML in first remission [16,40]. Ishiyama also reported GVT effects associated with acute GVHD [41]. A large, retrospective CIMBTR study [42] analyzed results in patients given allogeneic HCT for AML and MDS. Acute GVHD after myeloablative conditioning did not affect relapse, but both chronic GVHD alone and acute plus chronic GVHD were associated with reduced relapse risks. In contrast, all three manifestations of GVHD were associated with highly significant reductions in risk of relapse in patients given a variety of reduced-intensity conditioning regimens. The discordance between current results after minimal-intensity conditioning and some of the previous results after high-intensity conditioning might, in part, reflect differences in the diseases treated, which might have different susceptibilities to GVT effects. Previous studies included mainly patients with acute leukemias and chronic myeloid leukemia, while more than half of the current patients had B-cell malignancies, multiple myeloma, or Hodgkin lymphoma, and only two patients had CML.

In conclusion, low posttransplant neutrophil counts predicted increased risks of acute GVHD, de novo chronic GVHD and NRM, while high pretransplant lymphocyte counts were associated with a lower risk of relapse and improved survival. These results suggested that different mechanisms were involved in the two types of localized, immunologic graft-vs.-host reactions.

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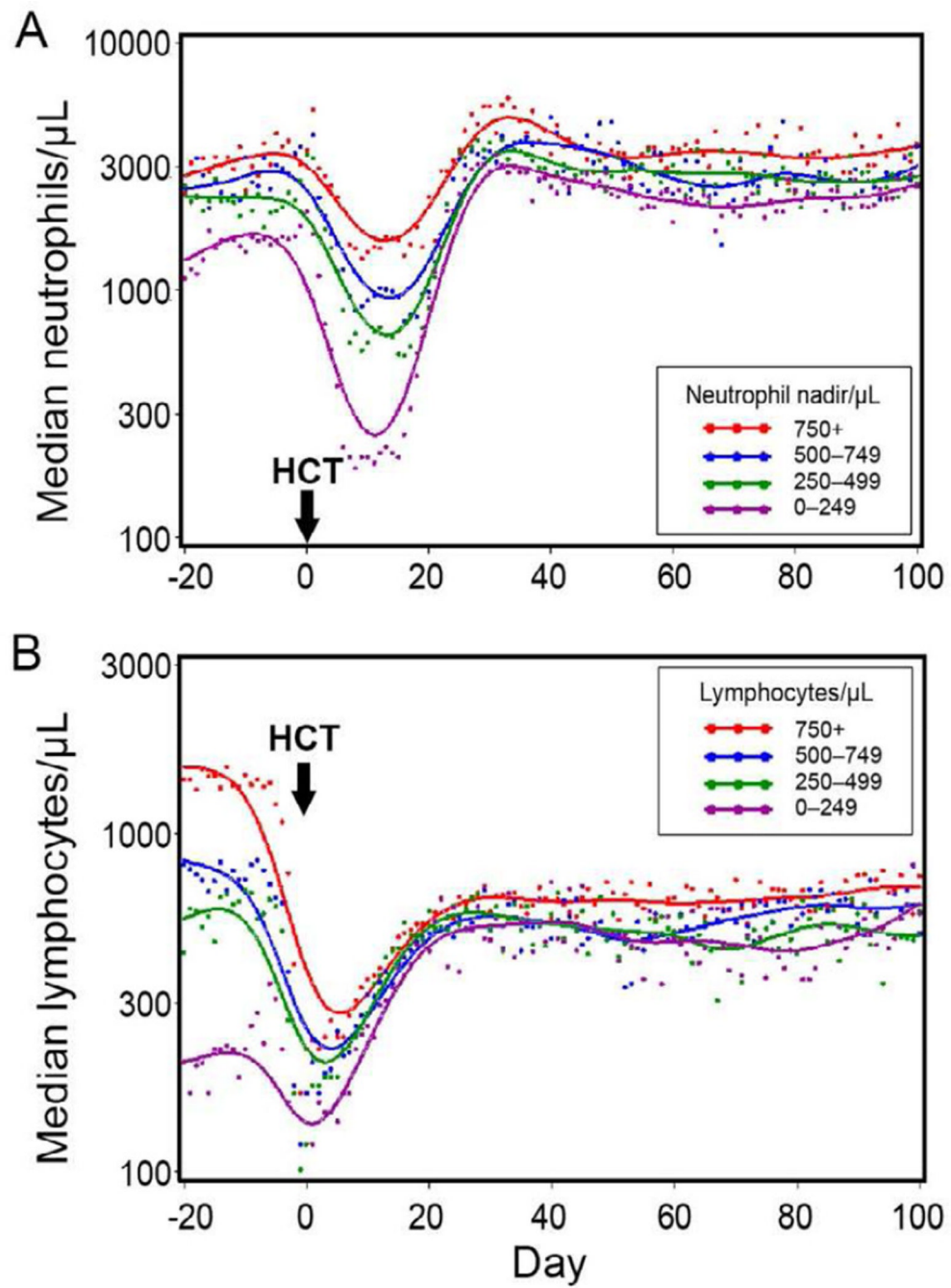


Figure 1. Pre- and post-transplant neutrophil changes among patients in four post-transplant neutrophil nadir ranges (**A**). Pre- and post-transplant lymphocyte changes among patients in four pre-transplant lymphocyte count ranges (**B**).

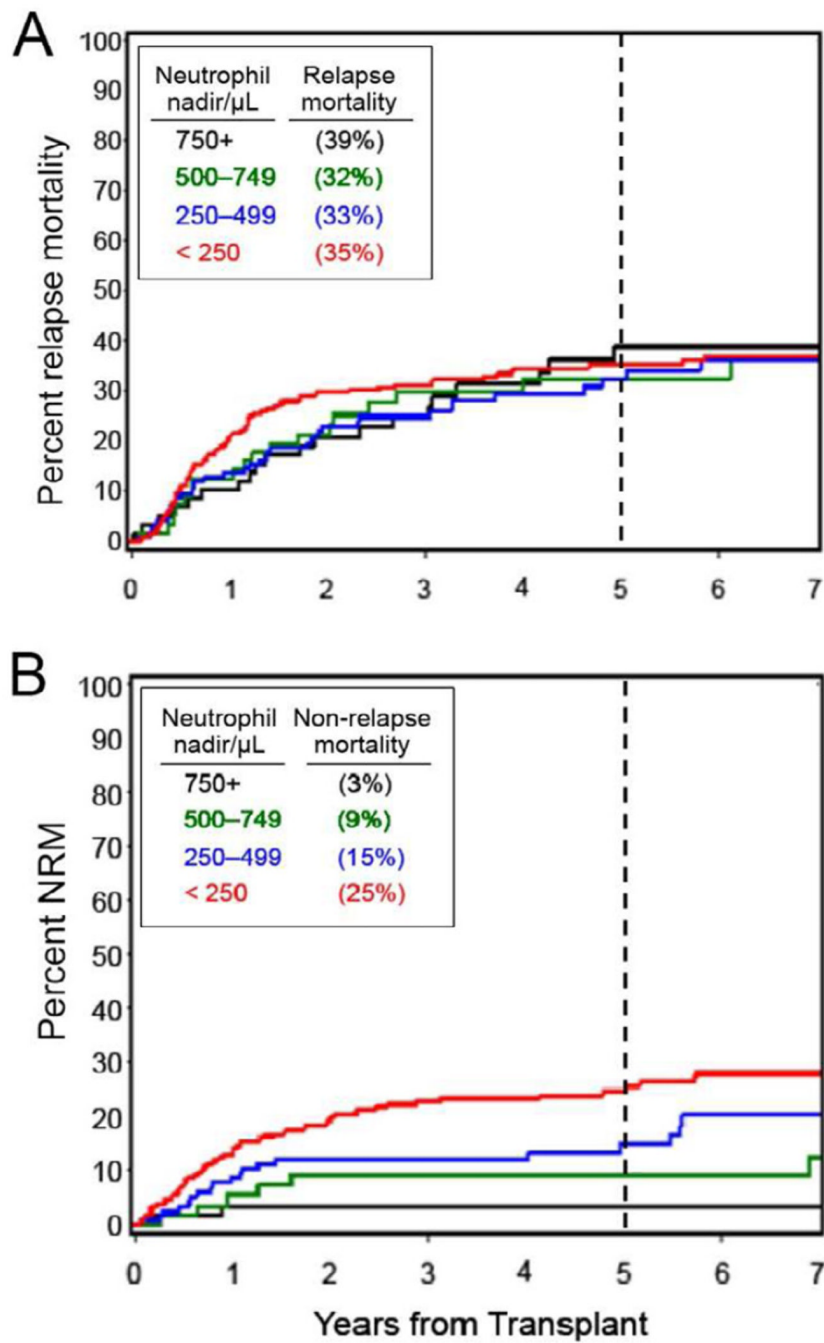


Figure 2. Cumulative incidences of relapse mortality (**A**) and non-relapse mortality (**B**) in relation to four neutrophil nadir ranges. The percentages shown within the boxes are 5-year data.

Table 1

Patient and disease characteristics (n=459) *

Characteristic	n (%)
Sex	
Female	183 (40)
Male	276 (60)
Patient age, years	
0–19	7 (2)
20–29	17 (4)
30–39	28 (6)
40–49	68 (15)
50–59	152 (33)
60–69	169 (37)
70–79	18 (4)
Median (range)	57 (7–75)
Diagnosis	
ALL	27 (6)
AML	111 (24)
CLL	50 (11)
CML	2 (<1)
HL	29 (6)
MDS	53 (12)
MPD	5 (1)
MM	86 (19)
NHL	91 (20)
Waldenstrom's	5 (1)
Relapse risk group [14]	
Low	111 (24)
Standard	220 (48)
High	128 (28)
Conditioning regimen	
2Gy TBI	87 (19)
2Gy TBI + Flu	343 (75)
3Gy TBI + Flu	24 (5)
4Gy TBI + Flu	5 (1)
Prior high-intensity transplant	
No	243 (53)

Characteristic	n (%)
Yes [†]	216 (47)
Donor	
HLA-id sibling	217 (47)
Other HLA-matched related	4 (1)
HLA-matched unrelated	216 (47)
HLA-mismatched unrelated [‡]	22 (5)
HCT-CI	
0	83 (18)
1	45 (10)
2	96 (21)
3	113 (25)
4	45 (10)
5	38 (8)
6	25 (5)
7+	12 (3)
CMV seropositive	
Patient	250 (54)
Donor	184 (40)

* All patients reported here were entered on prospective clinical trials that were registered with ClinicalTrials.gov. Data on other patients, for example those whose insurance denied participation in clinical trials and who were therefore placed on treatment plans, are not included in this analysis.

[†]Autologous HCT as part of tandem autologous/allogeneic HCT: n=114; failed autologous HCT: n=87; preceding autologous HCT for other disease: n=9; failed allogeneic HCT: n=15.

[‡]One HLA-allele mismatched.

Abbreviations, e.g., ALL=acute lymphocytic leukemia, AML=acute myeloid leukemia, CLL=chronic lymphocytic leukemia, CML=chronic myeloid leukemia, HL=Hodgkin lymphoma, MDS=myelodysplastic syndrome, MPD=myeloproliferative disorder, MM=multiple myeloma, NHL=non-Hodgkin lymphoma, TBI=total body irradiation, Flu=fludarabine, HLA=human leukocyte antigen, HCT-CI=Hematopoietic Cell Transplantation-Comorbidity Index, CMV=cytomegalovirus.

Table 2Multivariate analysis of risk factors for neutrophil nadir < 500/ μ L

	OR (95% Ci)	P	Nadir multiplier [†]	P
Pre-transplant neutrophil level (μ L)				
>2000	1.0			
500–1999	3.55 (2.0–6.2)	<0.0001	0.56 (0.42–0.75)	<0.0001
<500	5.94 (2.1–17)	0.0009	0.18 (0.12–0.27)	<0.0001
Disease group				
ALL,AML,MDS,CML	1.0			
HL,NHL,CLL	0.57 (0.3–1.1)	0.11	0.91 (0.66–1.27)	0.58
MM	0.24 (0.1–0.6)	0.002	1.54 (0.96–2.47)	0.08
Donor				
Related	1.0			
Unrelated	3.62 (1.9–6.8)	<0.0001	0.56 (0.39–0.80)	0.001
Patient age, years				
<50	1.0			
50–64	1.51 (0.8–2.8)	0.18	0.74 (0.53–1.05)	0.09
65+	2.40 (0.8–7.2)	0.12	0.72 (0.45–1.16)	0.18
Donor age, years				
<50	1.0			
50+	0.87 (0.5–1.7)	0.68	0.87 (0.59–1.27)	0.47
CD34 cell dose $\times 10^6$ /kg				
5.9+	1.0			
<5.9 (lowest quartile)	1.29 (0.7–2.4)	0.41	0.91 (0.67–1.23)	0.55
Prior high-intensity transplant				
No	1.0			
Yes*	0.46 (0.2–0.9)	0.03	1.68 (1.18–2.39)	0.004
No. of prior chemotherapy regimens				
0–3	1.0			
4+	0.95 (0.5–1.7)	0.87	0.92 (0.69–1.23)	0.59
HCT-CI score				
0–2	1.0			
3+	1.12 (0.7–1.9)	0.66	0.89 (0.68–1.15)	0.37

* Autologous HCT as part of tandem autologous/allogeneic HCT: n=114; failed autologous HCT: n=87; preceding autologous HCT for other disease: n=9; failed allogeneic HCT: n=15.

† The nadir is a continuous outcome on a log scale – the effects of risk factors are expressed as multipliers. For example, a pre-transplant count <500 reduces the nadir by 82%; a diagnosis of MM increases the nadir by 54%.

Table 3

Post-transplantation neutrophil nadir (per log decrease in absolute neutrophil count as continuous variable) and pre-transplantation absolute lymphocyte counts (per log decrease in absolute lymphocyte levels as continuous variable) as risk factors for various outcomes*

Outcome	No. Events	HR (95% CI)	P value	
A. Post-transplant neutrophil nadirs				
GVHD				
Acute [†]	Grade II-IV	263	1.45 (1.2-1.7)	<0.0001
	Grade III-IV	39	1.63 (1.1-2.4)	0.02
Chronic	Any	270	1.08 (0.9-1.3)	0.42
	De Novo	103	1.81 (1.2-2.8)	0.007
Relapse or progression		207	1.11 (0.9-1.4)	0.39
NRM		87	1.54 (1.2-2.0)	0.001
Overall mortality		241	1.46 (1.21-1.8)	<0.0001
B. Pre-transplant absolute lymphocyte levels				
GVHD				
Acute [†]	Grade II-IV	263	1.17 (0.9-1.5)	0.23
	Grade III-IV	39	0.94 (0.5-1.7)	0.84
Chronic	Any	270	0.94 (0.7-1.2)	0.62
	De Novo	103	0.82 (0.5-1.3)	0.40
Relapse or progression		207	1.48 (1.1-1.9)	0.003
NRM		87	1.00 (0.6-1.6)	0.99
Overall mortality		241	1.41 (1.1-1.8)	0.007

* Adjusted for: donor (related, unrelated); HLA allele-level mismatch (no, yes); patient age in years (<50, 50); donor/patient gender (F→M, other); HCT-CI (0-2, 3); fludarabine use (no, yes); GVHD prophylaxis (CSP, TAC), diagnosis (multiple myeloma, lymphoma, other); post-transplant neutrophil nadir (continuous); prior high-intensity transplant (no, yes).

[†]The analysis of post-transplantation neutrophil nadir was also adjusted for pre-transplantation lymphocyte counts (continuous). The analysis of pre-transplantation lymphocyte levels was also adjusted for post-transplantation neutrophil nadir (continuous).

[‡]In one patient, GVHD grade was not available. This case was excluded from multivariate analysis.

Cumulative incidences of acute GVHD, relapse mortality, and NRM, and Kaplan-Meier estimates of overall survival in relation to four ranges of post-transplantation neutrophil nadir

Table 4

Post-transplant neutrophil nadir, cells/ μ L	No. of patients	% Acute GVHD, grades		% 5-year	
		II-IV	III-IV	Relapse mortality	NRM
>750	58	36	3.5	39	3
500-749	56	46	5.4	33	9
250-499	118	54	9.3	32	15
0-249	227	69	11.0	35	25
					40