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Early Donor Chimerism Levels Predict Relapse and Survival after Allogeneic Stem Cell Transplantation with Reduced-Intensity Conditioning

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The success of hematopoietic stem cell transplantation (HSCT) with reduced-intensity conditioning (RIC) is limited by a high rate of disease relapse. Early risk assessment could potentially improve outcomes by identifying appropriate patients for preemptive strategies that may ameliorate this high risk. Using a series of landmark analyses, we investigated the predictive value of early (day-30) donor chimerism measurements on disease relapse, graft-versus-host disease, and survival in a cohort of 121 patients allografted with a uniform RIC regimen. Chimerism levels were analyzed as continuous variables. In multivariate analysis, day-30 whole blood chimerism levels were significantly associated with relapse (hazard ratio [HR] = .90, $P < .001$), relapse-free survival (HR = .89, $P < .001$), and overall survival (HR = .94, $P = .01$). Day-30 T cell chimerism levels were also significantly associated with relapse (HR = .97, $P = .002$), relapse-free survival (HR = .97, $P < .001$), and overall survival (HR = .99, $P = .05$). Multivariate models that included T cell chimerism provided a better prediction for these outcomes compared with whole blood chimerism. Day-30 chimerism levels were not associated with acute or chronic graft-versus-host disease. We found that high donor chimerism levels were significantly associated with a low lymphocyte count in the recipient before transplant, highlighting the impact of pretransplant lymphopenia on the kinetics of engraftment after RIC HSCT. In summary, low donor chimerism levels are associated with relapse and mortality and can potentially be used as an early predictive and prognostic marker. These findings can be used to design novel approaches to prevent relapse and to improve survival after RIC HSCT.

INTRODUCTION

Reduced-intensity conditioning (RIC) regimens are associated with decreased treatment-related mortality and make allogeneic hematopoietic stem cell transplantation (HSCT) feasible in older patients and those with comorbidities. The primary barrier to the success of RIC HSCT is disease relapse [1]. The risk of relapse after RIC is 25% to 60% [2-7], and the median time to disease relapse is 3 to 7 months [8-11], implying that identification of patients at high risk for relapse should be done very early, optimally within the first few weeks after transplant. The ability to detect relapse early in the post-transplant period is fundamental to the design of interventions that can potentially prevent disease recurrence and improve survival, such as maintenance regimens or preemptive donor lymphocyte infusions (DLIs).

The level of donor–recipient chimerism is an established method to document donor engraftment [12] and can be conducted in whole blood (WB) and bone marrow and in cellular subsets such as T cells, myeloid cells, and $CD34^+$ cells [13,14]. The kinetics of donor chimerism after myeloablative transplants have been characterized, but associations between attainment of complete donor chimerism and disease relapse or survival have not been consistently demonstrated [15-18].

In contrast to myeloablative transplants, RIC HSCT frequently results in varying degrees of mixed chimerism that may persist for months [19,20], but the underlying biological features that determine this heterogeneity among

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patients are not well characterized. In addition, previous studies of RIC HSCT have shown conflicting results regarding the correlation between early chimerism levels and disease relapse [19-22]. As a result, interpreting chimerism measurements in this setting remains uncertain, therefore limiting their clinical utility.

Our goal was to examine the utility of early chimerism measurement for prediction of disease relapse, graft-versus-host disease (GVHD), and survival. We therefore used a landmark analysis to investigate the predictive power of day-30 WB and T cell chimerism levels for subsequent outcomes of patients undergoing RIC HSCT with a uniform and commonly used conditioning regimen.

METHODS

Patients and Treatment

We reviewed data on adult recipients of a first allogeneic peripheral blood HSCT who were allografted with a uniform RIC regimen (fludarabine + busulfan) for a malignant hematological disorder between August 2006 and April 2013 at the University of Pennsylvania. We excluded patients who were transplanted for primary myelofibrosis where it is difficult to accurately define relapse and patients who did not have available results of day-30 chimerism levels. Because graft rejection was rare in this cohort (n = 3), we excluded these patients. Our study population included 121 patients. To account for the heterogeneity of the cohort in disease type and disease burden, we reviewed relevant disease characteristics (ie, cytogenetics in acute myeloid leukemia and myelodysplastic syndrome, disease subtype in myelodysplastic syndrome, disease stage and status in all diseases) and calculated the Disease Risk Index (DRI), a stratification system that predicts overall survival (OS) based on disease parameters. We used the 3-group version of the DRI that was recently validated using a large dataset from the Center for International Blood and Marrow Transplant Research [23]. Additional variables collected were the Karnofsky performance status and the hematopoietic cell transplantation–specific comorbidity index [24]. The Institutional Review Board of the University of Pennsylvania approved the study. All participants provided written informed consent for data collection at the time of their transplant.

All participants received a uniform conditioning regimen of fludarabine i.v. 120 mg/m^2 and busulfan i.v. 6.4 mg/kg, followed by the infusion of granulocyte colony-stimulating factor–mobilized peripheral blood stem cells from either a related or an unrelated donor. T cell depletion was not used. Participants received standard GVHD prophylaxis with oral tacrolimus .06 mg/kg/d or cyclosporine 5 mg/kg/d in 2 divided doses starting on day −3 and intravenous methotrexate 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11. Tacrolimus and cyclosporine doses were adjusted to attain trough levels between 5 and 15 ng/mL and 200 and 400 ng/mL, respectively. Some patients (n = 29) received maraviroc, a CCR5 antagonist, as part of a clinical trial in GVHD prophylaxis at doses of 150 or 300 mg twice daily between day −2 and day +30 [25]. All participants received standard antimicrobial prophylaxis. Patients did not receive prophylactic DLIs.

Donor–recipient chimerism levels were measured in the peripheral blood on day 30 using short tandem repeat analysis [26,27]. Chimerism levels were measured in WB samples and in the T cell subset after immunomagnetic positive selection of CD3$^+$ cells (StemCell Technologies, Vancouver, Canada). The graft composition, including the nucleated cell dose and the CD34$^+$, CD3$^+$, CD4$^+$, and CD8$^+$ cell doses, were determined using standard procedures [28]. Absolute lymphocyte counts (ALCs) were measured on routine complete blood counts on day −6 before starting the conditioning regimen and again on day 0, before the stem cell infusion.

Clinical Outcomes

The clinical outcomes of interest were time to disease relapse, grades II to IV acute GVHD, moderate to severe chronic GVHD, relapse-free survival (RFS), and OS. Disease relapse was defined as morphological, cytogenetic, or radiological evidence of disease demonstrating pretransplant characteristics. Bone marrow biopsies and appropriate imaging studies were routinely performed at day 100 or earlier in patients with signs indicating early relapse. The Consensus Conference criteria and National Institutes of Health criteria were used for acute and chronic GVHD grading, respectively [29,30].

Statistical Analysis

Descriptive statistics were used to characterize distributions of variables. Linear correlations between WB and T cell donor chimerism at day 30 and other continuous variables were assessed by Pearson's correlation coefficient, and differences between groups defined by categorical variables were assessed by either Wilcoxon rank sum or Kruskal-Wallis tests. No adjustment for multiple testing was performed in the analysis of predictors of chimerism levels. A landmark approach was used for time-to-event outcomes by measuring the time from chimerism measurement (approximately day 30) to the event, which allowed us to evaluate day-30 WB and T cell donor chimerism as predictors. Time to relapse was defined as the time from day-30 chimerism measurement to first documented relapse or last patient contact without relapse. Other outcomes were similarly defined. Patients were censored at the time of a second transplant in all analyses and at the time of DLI for GVHD analyses.

Competing risks regression analyses were conducted to identify predictors of time to relapse and time to GVHD outcomes, allowing for death without the event as a competing risk. Cox regression was used to identify predictors of survival and RFS. Univariate and multivariate analyses were performed to identify significant independent predictors and the primary variables of interest, day-30 WB and T cell chimerism, were entered into all models separately. The GVHD prophylaxis regimen was entered as a fixed covariate in the models for adjustment only because patients were not randomized to these treatments. Additional variables considered for model building exhibited univariate significance of $P \leq .10$, and a step-wise elimination method was then used.

Statistical significance of predictors in the models was assessed by the Wald test. The Akaike Information Criterion (AIC) was used to assess the relative goodness of fit of the models built for WB and T cell chimerism. Analyses were conducted in STATA v13.1 (STATA Corp, College Station, TX) and R using the cmprsk package (The R Project for Statistical Computing, http://www.rproject.org).

RESULTS

Patient and transplant characteristics are summarized in Table 1. The median follow-up was 22.5 months (range, 1.4 to 57.9 months). The median day-30 WB chimerism level was 96% (range, 77% to 100%). T cell chimerism levels were available in 103 of 121 patients; the median day-30 T cell chimerism was 65% (range, 18% to 100%).

Predictors of Day-30 Chimerism Levels

Our goal was to assess the associations between day-30 chimerism levels and RIC HSCT outcomes. We first examined whether day-30 chimerism levels were associated with various patient, disease, and transplant characteristics (Table 2).

The primary variable associated with day-30 chimerism levels was the recipient's ALC before transplant. Low ALC, both preconditioning (day −6) and on day 0, was strongly associated with higher levels of WB and T cell chimerism levels (Figure 1, $P \leq .0001$ for all associations). The DRI showed a significant association with day-30 WB chimerism and a trend ($P = .07$) with day-30 T cell chimerism with higher disease risk correlating with lower chimerism levels. In addition, the total nucleated cell dose demonstrated a positive association with WB and T cell chimerism, a slightly higher WB chimerism was observed in female recipients, and higher T cell chimerism was observed in HLA-mismatched transplants. The use of tacrolimus versus cyclosporine was associated with lower T cell chimerism, and maraviroc did not seem to affect chimerism levels.

We wanted to check whether the association between pretransplant ALC and day-30 chimerism was driven primarily by patients with lymphoid malignancies who are more likely to receive lymphodepleting therapies before transplant. Surprisingly, we found that recipients' ALC was associated with day-30 chimerism levels regardless of disease type (Table 3). Both preconditioning and day 0 ALCs were highly correlated with WB and T cell chimerism in both lymphoid and myeloid diseases ($P < .005$). The only association that was strong but did not reach statistical significance was between preconditioning ALC and WB chimerism in myeloid disease ($P = .08$). These results demonstrate that