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Increasing Chimerism after Allogeneic Stem Cell Transplantation Is Associated with Longer Survival Time

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Donor chimerism after allogeneic stem cell transplantation (allo-SCT) is commonly used to predict overall survival (OS) and disease-free survival (DFS). Because chimerism is observed at 1 or more times after allo-SCT and not at baseline, if chimerism is in fact associated with OS or DFS, then the occurrence of either disease progression or death informatively censors (terminates) the observed chimerism process. This violates the assumptions underlying standard statistical regression methods for survival analysis, which may lead to biased conclusions. To assess the association between the longitudinal post–allo-SCT donor chimerism process and OS or DFS, we analyzed data from 195 patients with acute myelogenous leukemia (n = 157) or myelodysplastic syndrome (n = 38) who achieved complete remission after allo-SCT following a reduced-toxicity conditioning regimen of fludarabine/intravenous busulfan. Median follow-up was 31 months (range, 1.1 to 105 months). Fitted joint longitudinal-survival time models showed that a binary indicator of complete (100%) donor chimerism and increasing percent of donor T cells were significantly associated with longer OS, whereas decreasing percent of donor T cells was highly significantly associated with shorter OS. Our analyses illustrate the usefulness of modeling repeated post–allo-SCT chimerism measurements as individual longitudinal processes jointly with OS and DFS to estimate their relationships.

INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) is an effective and potentially curative treatment modality for patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). The 2 primary goals of allo-SCT are to reestablish hematopoiesis in the patient after receiving a myeloablative preparative regimen and to mount a graft-versus-leukemia immune response to eliminate residual leukemia. Numerous prognostic factors currently are followed post–allo-SCT to detect disease relapse in patients who achieve complete remission (CR), including hematologic parameters and cytogenetic and molecular mutations in the bone marrow. Early detection and treatment of minimal residual disease before overt hematologic relapse after allo-SCT are associated with better outcomes, because it often leads to implementation of adaptive therapeutic decisions, such as decreasing immunosuppression, donor lymphocyte infusions, or administration of a chemotherapeutic agent, such as azacitidine, to consolidate and maintain remission [1-3].

Chimerism (percent of donor-derived blood cells) after allo-SCT also has been used prognostically for disease relapse [2,4-8]. This requires measuring chimerism at 1 or more time points after allo-SCT. The presence of cells of host origin after allo-SCT in the absence of an overt diagnosis of residual AML may indicate inadequate myeloablation or persistence of host-derived malignant cell clones, which ultimately can lead to clinical disease recurrence. Consequently, a high percentage of patient cells after allo-SCT may predict impending disease relapse.

Assessing a possible association between chimerism and disease-free survival (DFS) or overall survival (OS) is not entirely straightforward because chimerism is measured longitudinally at 1 or more times after allo-SCT; consequently, chimerism is a treatment outcome process and not simply a baseline covariate. If the chimerism process is associated with DFS or OS, then the direction and rate of change of the chimerism process, specifically the slope of the chimerism timeline, may be the key aspect that is predictive of OS or DFS. Standard statistical survival time regression methods, such as a Cox model analysis, cannot reveal such relationships because they require covariates to be measured only at baseline (allo-SCT) or, alternatively, require a landmark analysis [9] wherein time is measured from a single chimerism measurement. Another problem is that relapse or death may be an informative censoring variable for the longitudinal chimerism process, which leads to biased

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estimates. A valid approach accounts for the joint distribution of the longitudinal process of successive chimerism measurements and the time of the terminating event (relapse or death) [10-12]. The chimerism process, DFS, or OS also may be influenced by other factors recorded at the time of allo-SCT, including the intensity of the preparative regimen, T cell composition of the graft [13,14], chimerism lineage (lymphoid versus myeloid), sample source (bone marrow versus peripheral blood), timing of the chimerism evaluations after allo-SCT, and method used to measure chimerism.

Studies examining the predictive ability of percent chimerism have produced conflicting results. Although some have shown that early chimerism detection can be used to predict relapse in pediatric patients with acute lymphoblastic leukemia (ALL) and AML [6,7,15], other studies have concluded that the prognostic value of chimerism is limited or not associated with disease relapse in patients, specifically in patients with ALL whose chimerism status was evaluated 80 days after allo-SCT [16,17]. Despite these conflicting conclusions, there remains a need for a reliable prognostic variable for disease relapse after allo-SCT. A report by the National Cancer Institute on the prevention and treatment of relapse after allo-SCT highlighted the need for surrogate markers and proposed several specific objectives concerning the predictive value of early detection methods such as percent chimerism [18]. The workshop highlights the critical role of determining the frequency for monitoring minimal residual disease and chimerism after allo-SCT and assessment of the efficacy of interventional strategies based on changes in minimal residual disease and/or chimerism to prevent overt clinical relapse [18].

Because the value of chimerism at a single time point after allo-SCT may be misleading as it ignores the path (direction and slope) of the chimerism process over time, we sought to determine whether longitudinal chimerism measurements can be used to more accurately predict relapse in patients after allo-SCT. Here, we describe a retrospective analysis investigating the significance of chimerism measurements over time as a prognostic factor for DFS and/or OS. To account for the association between chimerism and event time, we performed analyses based on "shared random effects" models [10-12], wherein patient-specific random effects are included in both the mean of the longitudinal chimerism process and the linear predictor in the hazard function of the event time model.

METHODS

Patient Selection

We studied 206 patients with AML (n = 165) or MDS (n = 41) transplanted at The University of Texas M.D. Anderson Cancer Center between April 2001 and October 2007. All protocols, including this retrospective analysis, were approved by the institutional review board of the University of Texas M.D. Anderson Cancer Center Patients provided written informed consent for their treatment and were treated in accordance with the Declaration of Helsinki.

All preparative regimens included i.v. busulfan and fludarabine at a myeloablative dose intensity [19,20]. Of the 206 patients, 51% of patients (n = 105) had received matched related donor transplants, 40% (n = 82) received matched unrelated donor transplants, and 9% (n = 19) received a 1-antigen-mismatched transplant. Antithymocyte globulin was administered to patients who received grafts from matched unrelated donor or mismatched grafts [19,20]. All patients received tacrolimus and minidose methotrexate for graft-versus-host disease (GVHD) prophylaxis.

Cytogenetic risk categories were defined as follows: favorable-risk cytogenetics included patients with translocation (t)(8;21); inversion (inv)(16) or t(16;16); or t(15;17). Adverse-risk cytogenetics included patients with a complex karyotype (≥4 abnormalities); inv(3) or t(3;3); t(6;11); del (5q); del 7q; 11q23 abnormalities excluding t(9;11) and t(11;19). Intermediate-risk cytogenetics were defined as patients with a normal karyotype, as well as those who did not fit the criteria for favorable- or adverse-risk cytogenetics [21,22].

DNA chimerism in blood and bone marrow was measured using PCR-based technology, as previously described [20]. Briefly, DNA microsatellite polymorphisms were analyzed by PCR using standard primers followed by analysis using GeneScan software (Applied Biosystems, Foster City, CA). Percent chimerism was calculated using the fraction of donor to total DNA in the analyzed sample.

Statistical Methods

Frequencies and percentages were used to summarize patient characteristics. OS and DFS were recorded from the time of allo-SCT. Unadjusted OS and DFS distributions were estimated using the method of Kaplan and Meier [23]. To assess the association between chimerism measured at 1 or more of days 30, 60, and 90 after allo-SCT and OS or DFS, a joint model for the longitudinal chimerism process and the event time distribution [10-12] was fit. This was done (1) for the longitudinal process of complete (100%) T cell chimerism and event time (OS or DFS) and (2) for the longitudinal process of percent T cell chimerism and event time. In each joint model, to account for the association between chimerism and event time, 1 or more random patient-specific parameters were included in both the linear term of the mean chimerism process and the hazard function of the event time. Details are given in Supplemental Methods. Computations for all statistical analyses were conducted in R (version 2.14.1; R Development Core Team) and SAS (version 9.2; SAS Institute Inc., Cary, NC).

RESULTS

Patient Characteristics

Two hundred six patients with either AML (n = 165) or MDS (n = 41) received allo-SCT between April 2001 and October 2007. The median age at time of transplant was 47 years; 83 patients (40%) were older than 50 years. Ninety-eight patients (48%) were women, and 108 (52%) were men. One hundred ninety-five patients achieved CR after allo-SCT and were used in the chimerism analysis. We focused our chimerism analysis on this subgroup of patients because patients who fail to achieve CR by definition have a mixed chimera and worse outcomes and therefore intrinsically bias the effect of chimerism on OS and DFS. Pretransplant patient characteristics for this subgroup of patients are presented in Table 1.

CR was defined as the achievement of a normalized marrow maturation profile and less than 5% blasts (cytologic CR). Specialized data regarding the assessment of minimal residual disease such as flow cytometry, fluorescence in situ hybridization, or cytogenetics were not included; also, strict peripheral blood criteria for CR, such as platelets > 100,000 or absolute neutrophil count ≥ 1500, were not applied [24].

Table 1
Characteristics of Patients Who Achieved CR after Allo-SCT (n = 195)

Variable	Value	Number (%)
Gender	Male	104 (53.3)
	Female	91 (46.7)
Age, yr	>50	72 (36.9)
	≤50	123 (63.1)
Disease	AML	157 (80.5)
	MDS	38 (19.5)
Disease status at transplantation	CR	112 (57.4)
	Active disease	83 (43.6)
Cytogenetics	Good	18 (9.2)
	Intermediate	102 (52.3)
	Poor	74 (37.9)
	Unknown	1 (.5)
Donor type	MRD	98 (50.2)
	MUD	78 (40.0)
	Mismatched	19 (9.7)
Stem cell source	Bone Marrow	87 (44.6)
	Peripheral Blood	108 (55.4)

MRD indicates matched related donor; MUD, matched unrelated donor.