Allogeneic Hematopoietic Cell Transplantation Outcomes in Acute Myeloid Leukemia: Similar Outcomes Regardless of Donor Type

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ABSTRACT
The use of alternative donor transplants is increasing as the transplantation-eligible population ages and sibling donors are less available. We evaluated the impact of donor source on transplantation outcomes for adults with acute myeloid leukemia undergoing myeloablative (MA) or reduced-intensity conditioning (RIC) transplantation. Between January 2000 and December 2010, 414 consecutive adult patients with acute myeloid leukemia in remission received MA or RIC allogeneic transplantation from either a matched related donor (n = 187), unrelated donor (n = 76), or umbilical cord blood donor (n = 151) at the University of Minnesota or Hôpital St. Louis in Paris. We noted similar 6-year overall survival across donor types: matched related donor, 47% (95% confidence interval [CI], 39% to 54%); unrelated donor, 54% (95% CI, 40% to 66%); and mismatched unrelated donor, 51% (95% CI, 28% to 70%) (P < .11). Survival differed based on conditioning intensity and age, with 6-year survival of 57% (95% CI, 47% to 65%), 39% (95% CI, 28% to 49%), 23% (95% CI, 6% to 47%), 47% (95% CI, 36% to 57%), and 28% (95% CI, 17% to 41%) for MA age 18 to 39, MA age 40+, or RIC ages 18 to 39, 39 to 40, and 57 to 74, respectively (P < .01). Relapse was increased with RIC and lowest in younger patients receiving MA conditioning (hazard ratio, 1.0 versus 2.5 or above for all RIC age cohorts), P < .01. Transplantation-related mortality was similar across donor types. In summary, our data support the use of alternative donors as a graft source with MA or RIC for patients with acute myeloid leukemia when a sibling donor is unavailable.

INTRODUCTION
Allogeneic hematopoietic cell transplantation (HCT) remains the only therapy that can provide extended disease-free survival (DFS) for the majority of patients with acute myeloid leukemia (AML) [1-3]. However, post-transplantation disease relapse remains a major therapeutic challenge. Efforts to identify patient, disease, and transplantation features playing a role in post-HCT relapse risk continue, with numerous reports documenting the role of cytogenetic risk, conditioning intensity, age, and disease status in transplantation outcomes for AML [4-11].

We analyzed the outcome of a large population of AML patients who underwent transplantation at 2 large centers, the University of Minnesota and Hôpital Saint Louis in Paris. We report the impact of specific patient, disease, and transplantation variables on clinical outcomes in cohorts receiving similar myeloablative (MA) and reduced-intensity conditioning (RIC) regimens. Our data highlight the interactions of age, conditioning intensity, and donor source on post-transplantation outcomes and support the use of alternative donors when a sibling donor is not available.
METHODS

Study Population

Between January 2000 and December 2010, 414 consecutive adult pa-
tients with AML in remission complete remission [CR 1, CR 2, or CR 3] received MA or RIC allogeneic HCT from either an HLA-identical matched related donor (MRD), unrelated donor (URD) (n = 76), or umbilical cord blood (UCB) donor (n = 151). Patients receiving more than 1 transplant for AML, those with French American British subtype M3, and those in relapse or with prior induction failure were excluded.

Risk Stratification

Patients were stratified based on disease status at transplantation (CR 1, CR 2, or CR 3) and by cytogenetic risk. Cytogenetic classification was limited by the differential availability of specific details between the 2 databases. The Paris data was available in ProMise (Project Manager Internet Server) and the European Group for Blood and Marrow Transplantation Web shared data base, in the following format: normal or abnormal chromosones, presence or absence of complex karyotype, presence or absence of molecular markers with partial reporting of which molecular marker (NPM-1 [nucleosomin 1], FLT-3 [FMS-like tyrosine kinase-3], BCR-ABL [breakpoint cluster region-ABL1 fusion], WT-1 [Wilms Tumor 1], ML [Mixed lineage leukemia with 11q23 abnormality, AML-ETO]) was present. The availability of this data was confounded by the time period of the study since 2000. Complete cytogenetic data were available for the majority of University of Minnesota cases and FLT-3 or NPM-1 molecular data was available in more recent years. Merging these 2 data sets, we classified using cytogenetic and molecular risk data as follows: standard risk included normal karyotype, favorable abnormalities including t(1;22) or inversion 16, CEBPA mutation, or NPM-1 mutation in the absence of FLT-3 ITD; poor risk included complex karyotype, monosomy 7, monosomy 5, monosomal karyotype, BCR-ABL, FLT-3 ITD, ML (11q23), or all other known high-risk abnormalities; and abnormal and uncertain significance included cases where an abnormality was documented without specifics or an abnormality of uncertain clinical significance was present (examples include CBF [core binding factor] + c-KIT [protooncogene encoding the tyrosine kinase KIT] + WT-1 or NPM-1 + WT-1).

HLA Typing, Matching, and Donor Selection

HLA-identical MRD were primarily siblings based on family testing. URDs were defined as matched (8/8) if HLA-A, -C, -B, and -DRB1 were iden-
tical at the allele level [12]. Stem cells were harvested for sibling or URDs via marrow harvest (n = 74) or flgristim-mobilized peripheral blood (n = 189). UCB unit nucleated cell dose and matching have been described elsewhere [13]; however, in brief they were required to have a minimum of 4/6 antigen match between each cord and the recipient. In the absence of a sibling donor, UCB was the graft choice of preference for the University of Minne-
sota based on research priorities, whereas Hôpital Saint-Louis utilized URDs in this situation. Preparative regimens were classified as either MA or RIC by established Center for International Blood and Marrow Transplant Research functional definitions [14-16].

Treatment

 Patients received either MA or RIC conditioning. MA conditioning from Paris included 120 mg/kg cyclophosphamide (60 mg/kg, on each of 2 days) and busulfan (3.2 mg/kg i.v. daily on 4 consecutive days), or 12 Gy total body irradiation (TBI) in a fractionated regimen. For the University of Minnesota, the MA regimen for MRD and UCB was cyclo-
phosphamide (60 mg/kg × day –6 and –5) plus TBI (165 GyG) twice daily for 8 fractions on days –4 through –1. UCB MA conditioning consisted of fludarabine (25 mg/m² daily on days –8 through –6), cyclophosphamide (60 mg/kg i.v. daily on days –7 and –6), and TBI (165 GyG twice daily for 8 fractions on days –4 through –1). MA at the Hôpital Saint-Louis consisted predominantly of fludarabine (30 mg/m² i.v. daily from days –5 through –1), busulfan (3.2 mg/kg i.v. twice daily on days –4 and –3) plus rabbit antithymocyte globulin (ATG; 5 mg/kg for siblings and 10 mg/kg for URDs on days –2 and –1). The University of Minnesota RIC regimen con-
sisted of cyclophosphamide (50 mg/kg on day –6), fludarabine (30 to 40 mg/ m² i.v. daily on days –6 through –2), and TBI (200 cGy/cGy on day –1) for all donor sources. Equine ATG (15 mg/kg twice daily for 6 doses from day –6 through day –4) in the setting of RIC was used for those URDs who had only 1 cycle of multigenet chemotherapy within 3 months or for related donors with only 1 cycle of multigenet chemotherapy within 6 months before HCT. Graft-versus-host disease (GVHD) prophylaxis included cyclosporine (day –3 through +100 to 180) plus mycophenolate mofetil (days –3 to +30) (565) or cyclosporine plus methotrexate (40%).

Supportive care was similar in both institutions. Patients were hospi-
talized in single rooms utilizing high efficiency air filtration systems. Pa-
tients received prophylactic acyclovir for herpes simplex virus or cytomegalovirus prophylaxis plus antibacterial prophylaxis until day +21 or longer if on prednisone for GVHD; fungal prophylaxis with either flucona-
zole or voriconazole for 100 days; and pneumocystis jirovecii prophylaxis typically with trimethoprism sulfamethoxazole for 1 year.

Data Collection

All patients were treated on protocols approved by the institutional review board of each hospital with prior informed consent for treatment and data analysis. Data were prospectively collected. Data from Hôpital Saint-Louis in Paris were retrieved through the European Group for Blood and Marrow Transplantation and data from the University of Minnesota were prospectively collected in the institutional blood and marrow transplantation database. Data were merged for the combined analysis.

Statistical Analysis

The primary endpoint was overall survival (OS). Secondary endpoints included hematopoietic recovery, occurrence of acute GVHD and chronic GVHD, transplantation-related mortality (TRM), incidence of relapse, and DFS. OS was defined as time to death from any cause and a 6-year time point was used because of the availability of extended follow-up. Hematopoietic recovery was defined as time to absolute neutrophil count (ANC) ≥ 500 neutrophils/μL for 3 consecutive days. Incidence and grade of acute GVHD (aGVHD) at day +100 and absence or presence of chronic GVHD (cGVHD) at day +24 were recorded based on consensus criteria [17,18]. TRM was defined as death followed by any cause death after day +28 without evidence of relapsed leukemia. Relapse was reported at 2 years as most post-transplant relapses are evident within that time period. DFS was defined as survival without death or relapse censoring at the date of last contact.

Univariate probabilities of DFS and OS were calculated using the Kaplan-
Meier estimator with variance estimated by Greenwood’s formula [19]. Probabilities of aGVHD, cGVHD, TRM, and relapse were calculated using cumulative incidence curves to accommodate competing risks [20]. Ninety-five percent confidence intervals (CI) for all probabilities and P values of pair-wise comparisons were derived from point-wise estimates and calcu-
lated. Single variable comparisons were made using log-rank tests with standard weights.

Multivariable regression models were fit for each outcome: Cox regression [21] for OS and DFS, and Fine and Gray [22] competing risks regression for all other outcomes, reported as hazard ratios (HR). TRM was analyzed with a competing risk of relapse, and relapse, GVHD, and he-
matopoietic recovery were analyzed with a competing risk of mortality. All models were prespecified and included categorical factors for cytogenetic (standard, poor, abnormal but unknown significance), donor type (MRD, UCB, matched URB, mismatched URD), disease status (CR1, CR2, CR3), and age at conditioning. Subcombinations (MA 18 to 39, MA 40 to 56, RIC 18 to 39, RIC 40 to 56, and RIC 57 to 74) because of their association. Subgroup analysis investigation showed no significant association between donor source and conditioning and, thus, was not included in final modeling. Treatment center had minimal influence; thus, was not included in the final models. SAS software (SAS Institute, Cary, NC) was used to perform statis-
tical analyses.

RESULTS

Patient Characteristics

Patient characteristics (Table 1) were similar across donor types (MRD, UCB, URD) with respect to gender, Karnofsky performance status, and age. MRD had fewer cases with poor-risk cytogenetic/molecular profile compared with UCB or to matched and mismatched URD (38% versus 53%, 51%, and 52%, respectively). There were many MRD treated in CR1 (78% versus 58% in UCB, 73% in matched URD, and 48% in mismatched URD). UCB (64%) transplant recipients were more likely to receive RIC compared with MRD (40%) and compared with matched (25%) or mismatched URDs (10%). Those receiving URD stem cell sources were more likely to be exposed to ATG in their conditioning compared with MRD and UCB (45% to 48% matched and mismatched URD versus 11% MRD and 15% UCB). GVHD prophylaxis associated with conditioning intensity, with a higher percentage of cyclo-
sporine/methotrexate in the MRD and URD cohorts.

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The median time to engraftment of ANC was 16 days with 96% recovering by day +50. Twelve events of primary graft failure were noted; 10 in the UCB group. In multivariate analysis, the only factors predictive of time to neutrophil recovery were conditioning intensity and donor type, with quicker ANC recovery in the RIC groups (age 18 to 39: HR, .5; 95% CI, .3 to .9; age 40 to 56: HR, .5; 95% CI, .3 to .6; and age 57 to 74: HR, .6; 95% CI, .4 to .8; \( P < .01 \)) and slower, less frequent recovery in UCB donors (HR, 2.8; 95% CI, 2.1 to 3.6; \( P < .01 \)).

Survival

After a median follow-up of 5.2 years, 184 patients survived. The 6-year probability of survival was 44% (95% CI, 38% to 49%).

We observed similar 6-year OS across donor types: MRD, 47% (95% CI, 39% to 54%); UCB, 36% (95% CI, 28% to 44%); matched URD, 54% (95% CI, 40% to 66%); and mismatched URD, 51% (95% CI, 28% to 70%) (\( P < .11 \)) (Table 2, Figure 1). We observed a nonsignificant finding of somewhat poorer survival in patients undergoing RIC UCB at 28% (95% CI, 19% to 38%) compared with RIC MRD of 46% (95% CI, 33% to 58%) or matched URD at 52% (95% CI, 29% to 72%) (\( P < .23 \)). However, the patients undergoing RIC UCB were more often in CR2 or CR3 (39% versus 28% in MRD and 21% in matched URD) and a greater proportion had poor-risk cytogenetics (55% versus 44% in MRD).

Six-year OS differed in significance based on age and conditioning intensity, as follows: MA age 18 to 39, 57% (95% CI, 47% to 65%); MA age 40 to 56, 39% (95% CI, 28% to 49%); RIC age 18 to 39, 23% (95% CI, 6% to 47%); RIC age 40 to 56, 47% (95% CI, 36% to 57%); and RIC age 57 to 74, 28% (95% CI, 17% to 41%) (\( P < .01 \)) (Table 2, Figure 2). The interaction of age and conditioning remained important in multivariate analysis as younger patients (age 18 to 39) undergoing MA had superior survival compared with a small cohort of similar aged patients undergoing RIC HCT (HR, 2.3; 95% CI, 1.1 to 4.6; \( P < .01 \)). This small cohort (RIC, age 18 to 39; \( n = 13 \)), in comparison to the MA (age 18 to 39) cohort, had more with Karnofsky performance status \( \leq 80 \) (46% versus 20%), advanced remission status CR2 or CR3 (46% versus 34%), and poor-risk cytogenetics (54% versus 43%). Notably, TRM and relapse risks were higher in this group and account for their poor survival.

The majority of deaths were due to disease recurrence (\( n = 119, 52\% \)) with GVHD (\( n = 32, 14\% \)), infection (\( n = 28, 12\% \)), other miscellaneous HCT-related complications (\( n = 46, 20\% \)) (septic shock, organ failure, graft failure,
hemorrhage, acute respiratory distress syndrome, secondary malignancy, graft failure), and unknown (n = 5, 2%) accounting for the remainder.

DFS was 41% (95% CI, 36% to 46%); however, as the majority of relapsing patients died, DFS was similar to OS.

Relapse and TRM

The overall incidence of relapse at 2 years was 29% (95% CI, 24% to 34%). Relapse was more frequent in those with higher risk disease as defined by cytogenetics or remission status at transplantation. Two-year relapse for those with poor-risk cytogenetic/molecular profiles was 34% (95% CI, 27% to 41%) versus 26% for those with standard risk (95% CI, 18% to 33%) or 24% for those with abnormal/unknown significance (95% CI, 14% to 34%) (P < .04). For those who underwent transplantation in CR2 or CR3, relapse was increased at 36% (95% CI, 27% to 45%) versus 26% (95% CI, 21% to 31%) for those in CR1 (P < .04). Lastly, conditioning intensity influenced relapse, with those receiving RIC showing an incidence of 38% to 39% across all age cohorts compared with 18% and 25% in the MA age 18 to 39 and 40 to 56 age groups, respectively (P < .01).

In univariate analysis, relapse was slightly increased in those receiving UCB transplants at 36% (95% CI, 28% to 45%) and mismatched URD at 33% (95% CI, 13% to 53%) compared with matched URD at 20% (95% CI, 9% to 31%) and MRD at 26% (95% CI, 19% to 32%) (P < .05) (Figure 3A). However, UCB recipients were more likely to receive RIC, and in multivariate analysis, conditioning/age remained significantly associated with relapse (P < .01) but donor type did not (P < .34) (Table 3). Subgroup analysis showed that the combination of UCB and RIC had particularly high relapse of 46% (95% CI, 35% to 57%) compared with 20% (95% CI, 9% to 31%) in those receiving MA UCB (P < .01). As noted, these RIC UCB recipients had more advanced disease and more poor-risk cytogenetics, and in multivariate analysis of relapse, significantly higher relapse rates were observed across all age cohorts of RIC HCT (P < .001) (Table 3).

TRM at 1 year was 20% (95% CI, 16% to 24%). We observed no significant differences based on remission status, donor type (Figure 3B), cytogenetic/molecular risk group, or gender. Compared with the younger MA group, TRM was highest in older MA patients, ages 40 to 56, compared with the younger MA group (HR, 1.8; 95% CI, 1 to 3) and was also higher in the youngest RIC cohort, ages 18 to 39, as explained...
by their higher risk disease and poorer performance status (HR, 1.5; 95% CI, 5 to 4.3), \( P < .04 \).

**aGHVD and cGVHD**

The incidence of day +100 aGVHD grade II to IV for the entire cohort was 62% (95% CI, 56% to 67%) with only 16% (95% CI, 12% to 19%) severe grade III and IV aGVHD. Severe aGVHD was lowest in MRD at 9% (95% CI, 5% to 13%) compared with URD at 15% (95% CI, 5% to 24%), UCB at 24% (95% CI, 17 to 31%), and mismatched URD at 24% (95% CI, 6 to 42%) \( P < .01 \). In multivariate analysis, a higher risk of severe aGHVD was observed in those receiving an UCB graft source (HR, 3.2; 95% CI, 1.6 to 6.2) or mismatched URD (HR, 2.6; 95% CI, 1.3 to 5.0) \( P < .01 \) and in males (HR, 1.7; 95% CI, 1.3 to 2.1, \( P < .05 \)).

The overall incidence of cGVHD at 2 years was 34% (95% CI, 29% to 39%). In multivariate analysis, risk of cGVHD was higher in males (HR, 1.8; 95% CI, 1.3 to 2.6) \( P < .01 \) and HCTs during CR2/CR3 (HR, 1.5; 95% CI, 1.0 to 2.1; \( P < .03 \)). There was no significant influence of donor type, age, or conditioning intensity on the incidence of cGVHD.

**DISCUSSION**

We investigated patient, disease, and transplantation factors affecting survival, relapse, and TRM risk in a well-characterized population of 414 adult AML patients receiving consistent MA and RIC HCT regimens. Our analyses revealed similar outcomes for transplantations across donor sources and with MA or RIC regimens. These findings build upon earlier reports in the older RIC setting [23] and provide solid support for use of alternative donors when a sibling donor is unavailable. Our data also highlight the interaction of conditioning intensity and age, the impact of remission status at transplantation and cytogenetic risk, and the increased relapse risks in those receiving RIC conditioning. Thus, our data support the use of MA conditioning to limit relapse risk when feasible.

Of notable importance, our study evaluates a heterogeneous cohort of patients treated with 3 different donor sources across both MA and RIC conditioning approaches. As may occur in any retrospective review, the groups are not evenly balanced with respect to disease or transplantation characteristics, which could affect outcome. Extensive subset analysis when patient numbers within subsets are small can potentially lead to incorrect conclusions. Although we...
analyze important subsets in our manuscript, we attempted to minimize overgeneralizations based upon statistically insignificant analyses. Overall, we demonstrated that OS was similar across donor types and that high-risk disease features, including poor-risk cytogenetics, advanced disease stage (CR2 or CR3), and RIC contributed more to risk of relapse than did donor source. Although we observed a trend to inferior OS in those patients undergoing RIC, paired interactions of donor source and conditioning intensity were not statistically significant, supporting our main conclusions.

Prior comparisons of HCT using alternative donor sources have had mixed results. Some reports have suggested increased TRM using mismatched URDs and UCB donor sources and, consequently, lower survival [24,25]. Others have reported improved outcomes for older patients with sibling donors versus URDs [26] yet comparable results with URDs and UCB [27]. We recently reported a collaborative analysis investigating donor source in an older (age 50-+) cohort of AML patients, all receiving RIC, and revealed similar outcomes using MRDs, URDs, and UCB donors [23]. The current study, a larger group of adults with AML receiving RIC and MA conditioning at our 2 centers, highlights similar survival, DFS, relapse, and TRM across donor sources with either conditioning intensity. Our study did not include haploidentical donor sources. However, as this alternative donor options is studied further and is currently the basis of an ongoing Blood and Marrow Transplant Clinical Trials Network randomized trial using RIC (protocol 1101), forthcoming data will allow for comparisons including this alternative donor source.

Clear data on the value of greater conditioning regimen intensity for AML and MDS are still lacking, though the use of RIC has increased to offer allogeneic HCT to older patients. Many retrospective studies have highlighted lower risks of TRM offset by increased rates of relapse in RIC with similar OS [28-31]. More recent studies note similar outcomes for those in complete remission [32,33]. This ongoing conditioning intensity debate prompted a national randomized trial within the Blood and Marrow Transplant Clinical Trials Network 0901 to prospectively answer this question. Although closed to accrual, future reports of their data will be helpful in answering this question. In the interim, our data strongly suggest lower relapse in patients receiving MA conditioning.

Rates of advanced aGVHD were highest in those receiving UCB or mismatched URD stem cell sources, but they were in line with other published reports [27]. Our UCB and mismatched URD cohorts included a higher proportion of those with advanced disease status (CR2 and CR3) and poor-risk cytogenetics and, thus, they may have been more heavily pretreated entering transplantation, thereby increasing their risk of severe aGVHD. cGVHD rates were associated with more advanced disease.

Our data highlight long follow-up of a sizeable population treated uniformly in 2 experienced centers and support the use of alternative donors as a graft source for patients with nonfavorable risk AML when a sibling donor is unavailable. Clinically suitable patients should be considered for MA conditioning to reduce relapse risk.

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