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Mixed T Lymphocyte Chimerism after Allogeneic Hematopoietic Transplantation Is Predictive for Relapse of Acute Myeloid Leukemia and Myelodysplastic Syndromes

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Chimerism testing after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) represents a promising tool for predicting disease relapse, although its precise role in this setting remains unclear. We investigated the predictive value of T lymphocyte chimerism analysis at 90 to 120 days after allo-HSCT in 378 patients with AML/MDS who underwent busulfan/fludarabine-based myeloablative preparative regimens. Of 265 (70%) patients with available T lymphocyte chimerism data, 43% of patients in first or second complete remission (CR1/CR2) at the time of transplantation had complete (100%) donor T lymphocytes at day +90 to +120 compared with 60% of patients in the non-CR1/CR2 cohort ($P = .005$). In CR1/CR2 patients, donor T lymphocyte chimerism ≤ 85% at day +90 to +120 was associated with a higher frequency of 3-year disease progression (29%; 95% confidence interval [CI], 18% to 46% versus 15%; 95% CI, 9% to 23%; hazard ratio [HR], 2.1; $P = .04$). However, in the more advanced, non-CR1/CR2 cohort, mixed T lymphocyte chimerism was not associated with relapse (37%; 95% CI, 20% to 66% versus 34%; 95% CI, 25% to 47%; HR, 1.3; $P = .60$). These findings demonstrate that early T lymphocyte chimerism testing at day +90 to +120 is a useful approach for predicting AML/MDS disease recurrence in patients in CR1/CR2 at the time of transplantation.

INTRODUCTION

Although allogeneic hematopoietic transplantation (allo-HSCT) represents a potential curative therapy for patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), the major cause of treatment failure is disease recurrence [1]. The ability to predict relapse before detectable morphologic recurrence may allow for preemptive interventions, such as immune modulation, donor lymphocyte infusion (DLI), or initiation of hypomethylating agents, in high-risk patients to potentially augment graft-versus-leukemia (GVL) effects [2-5]. Post-transplantation peripheral blood chimerism analysis, which quantifies the relative levels of donor and recipient hematopoiesis, represents 1 potential tool to predict disease recurrence in the post-transplantation setting, especially in patients without specific cytogenetic, molecular, or immunophenotypic leukemic signatures that allow more direct monitoring of disease [6].

There have been a number of studies that have investigated the efficacy of chimerism analysis in predicting disease relapse, especially with the advent of more sensitive PCR-based methods and lineage-specific analyses to detect donor/recipient hematopoiesis [7]. However, the use of chimerism analysis to identify AML patients at high risk of relapse after allo-HSCT remains controversial. Several studies have suggested that mixed donor and recipient chimerism or chimerism kinetics (full donor chimerism to mixed chimerism) can predict disease recurrence [8-12], whereas other studies have suggested that such analyses cannot be used reliably [13-15]. Moreover, a substantial

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number of these studies have focused on pediatric patient populations or have been limited by small patient cohorts or disease heterogeneity inclusive of all leukemia subtypes with variable kinetics of relapse. Results may also vary depending on the preparative regimen. For example, the combination of busulfan and fludarabine, which has been widely used as a myeloablative preparative regimen for myeloid malignancies [16-23], is associated with a relatively high rate of mixed T lymphocyte chimerism [24]. We sought to examine the effect of mixed T lymphocyte chimerism on the risk of relapse after transplantation in patients with AML or MDS. In this single-institution retrospective analysis, we report the largest study to date to our knowledge investigating the potential utility of T lymphocyte chimerism analysis between post-transplantation day +90 to +120 in predicting relapse for AML/MDS patients after myeloablative allo-HSCT using the busulfan-fludarabine preparative regimen and tacrolimus/methotrexate graft-versus-host-disease (GVHD) prophylaxis.

PATIENTS AND METHODS

Patient Eligibility

Patients who underwent allo-HSCT for AML or MDS on 5 separate sequential clinical protocols involving myeloablative conditioning with busulfan and fludarabine at the University of Texas MD Anderson Cancer Center between 2001 and 2011 were included in this retrospective analysis. Informed consent was obtained from each patient per institutional guidelines before enrollment in each clinical trial. The institutional review board also reviewed and approved this retrospective analysis. All patients received myeloablative conditioning consisting of fludarabine 40 mg/m^2 with busulfan 130 mg/m^2 daily for 4 days or with busulfan given with pharmacokinetic dose adjustment, targeting a drug concentration area under the curve of 6000 mM × minute. Post-transplantation GVHD prophylaxis consisted of methotrexate 5 mg/m^2 on days 1, 3, 6, and 11 and tacrolimus, which was tapered after 4 to 6 months. Patients receiving unrelated donor transplants received antithymocyte globulin (Thymoglobulin; Genzyme Corporation, Cambridge, MA) 4.5 mg/kg pretransplantation in divided doses.

Chimerism Testing

Peripheral blood chimerism analysis was performed using 8 highly polymorphic microsatellite markers (Integrated DNA Technologies, Coralville, IA) in a multiplex PCR assay. Each marker was labeled with a fluorescent tag that allows size separation by using capillary electrophoresis in an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Data are presented as peaks, and the area under the curve represents the percentage of host-versus-donor hematopoiesis. In addition to looking at overall chimerism status, lineage-specific analysis was also performed by separating out myeloid and T lymphocyte cell populations using the RoboSep Magnetic Cell System and the EasySep whole blood CD3 positive or myeloid selection (anti-CD14 + CD66b) kits (Stem Cell Technologies, Vancouver, BC, Canada).

Endpoints and Statistical Analysis

The primary objective of the study was to assess the rate of disease progression according to peripheral blood T lymphocyte chimerism evaluated between day +90 to +120 after transplantation. *Leukemic disease progression* was defined by morphology as the appearance of >5% blasts on bone marrow aspirate smears. Only patients who were alive and free of disease progression at day +120 after transplantation were eligible for this assessment. The cumulative incidence of disease progression after day +120 (landmark analysis) was estimated considering death before disease progression as a competing risk. Predictors of disease progression were assessed using Cox's proportional regression analysis. Predictors included were chimerism status, age, gender, cytogenetic risk group, modified European Leukemia Net (ELN) risk stratification [25], donor type, cell type, and fixed or adjusted busulfan dose. Because of the limited number of patients who had both NPM1 and FLT3-ITD mutation status reported in this study, ELN classification was modified to categorize cytogenetically normal patients only on the basis of FLT3-ITD mutation status. Patient and transplantation characteristics were compared using Fisher's exact and chi-squared test for categorical variables and Mann-Whitney's test for continuous variables. Statistical significance was defined at the .05 level. Statistical analysis was performed using STATA 11.0 (StataCorp, College Station, TX).

RESULTS

Patient Characteristics

A total of 378 consecutive patients who were alive and without evidence of disease progression on day +120 after allo-HSCT met the eligibility criteria and were included in the study. Characteristics of these patients are delineated in Table 1 and characteristics of patients who were excluded from the study because of disease progression before day +120 are provided in Supplemental Table S1. In the study-eligible population, 305 (81%) patients had AML and 73 (19%) patients had MDS. The median age was 48 years, and 224 (59%) patients underwent transplantation in first or second complete remission (CR1/CR2). One hundred fifty-eight patients were not in remission or beyond CR2 (non-CR1/CR2). Peripheral blood stem cells were used in 242 (64%) patients and bone marrow was used in 36% patients. Over one half (52%) of all patients received a matched related donor transplant. Among the 378 eligible patients in the study, peripheral blood T lymphocyte and myeloid donor chimerism data between day +90 to +120 were available for 265 (70%) and 286 (76%) patients, respectively.

Mixed T Lymphocyte Chimerism and Disease Status at Transplantation

There was a significant association between disease status at transplant and day +90 to +120 donor T lymphocyte chimerism. Of 164 patients in the CR1/CR2 cohort, 43% of patients had complete donor T lymphocyte chimerism compared with 60% of patients in the non-CR1/CR2 cohort ($P = .005$). Most (90%) patients in the CR1/CR2 and non-CR1/CR2 cohorts achieved complete (100%) or near complete (99%) donor myeloid chimerism by day +90 to +120 (Table 2). Because of the high rates of full donor myeloid chimerism by day +90 to +120 in all patients, signifying the low value of myeloid chimerism at this time point, subsequent analyses focused solely on the impact of T lymphocyte chimerism and post-transplantation disease progression. Moreover, given the differences in donor T lymphocyte chimerism based on disease status at transplantation, predictors of disease progression were analyzed separately for CR1/CR2 and non-CR1/CR2 patients at the time of transplantation.

Age, donor type, and a history of grade ≥ 2 acute GVHD, and cytogenetics risk group were comparable ($P > .10$) between the CR1/CR2 and non-CR1/CR2 groups (Table 1). Among CR1/CR2 patients with T lymphocyte chimerism data available, 40% were greater than 50 years of age, 56% had a related donor transplantation, 26% had a history of grade ≥ 2 acute GVHD, and 30% had poor risk, 57% intermediate risk, 10% good risk, and 2% unknown risk cytogenetics. The corresponding proportions in the non-CR1/CR2 group were 44%, 44%, 30%, 36%, 43%, 19%, and 3%, respectively.

Predictors of Disease Progression

CR1/CR2 cohort

Based on the distribution of the percentages of donor T lymphocyte chimerism in the CR1/CR2 cohort, T lymphocyte chimerism was further subdivided by the following intervals: 100%, 96% to 99%, 86% to 95%, 76% to 85%, 51% to 75%, and ≤50%. The rate of disease progression was comparably low in patients with 100%, 96% to 99% or 86% to 95%, and comparably high in patients with 76% to 85%, 51% to 75%, or ≤50% (Table S2). Based on these findings, 85% was chosen as the cut-off for subsequent analyses of T lymphocyte donor chimerism. In landmark analysis, the

Table 1
Patient Characteristics

Characteristic	Overall	T Lymphocyte Chimerism Available	T Lymphocyte Chimerism Available CR1/CR2	T Lymphocyte Chimerism Available Non-CR1/CR2
Total patients	378	265	164	101
Sex				
Female	185 (49)	125 (47)	75 (46)	50 (50)
Male	193 (51)	140 (53)	89 (54)	51 (50)
Age				
≤50	231 (61)	156 (59)	99 (60)	57 (56)
>50	147 (39)	109 (41)	65 (40)	44 (44)
Diagnosis				
AML	305 (81)	186 (70)	152 (93)	61 (60)
MDS	73 (19)	79 (30)	12 (7)	40 (40)
Disease status at transplantation				
CR1	158 (42)	119 (45)		
CR2	66 (17)	45 (17)		
Not CR	154 (41)	101 (38)		
Cytogenetics				
Good risk	50 (13)	36 (14)	17 (10)	19 (19)
Intermediate risk	196 (52)	136 (51)	93 (57)	43 (43)
Poor risk	121 (32)	86 (32)	50 (30)	36 (36)
Unknown	11 (3)	7 (3)	4 (2)	3 (3)
FLT3 ITD mutation				
Yes	45 (12)	36 (14)	31 (19)	5 (5)
No	224 (59)	161 (61)	95 (58)	66 (65)
N/A	109 (29)	68 (26)	38 (23)	30 (30)
Modified ELN classification				
Favorable	114 (30)	82 (31)	50 (30)	32 (32)
Intermediate 1*	32 (8)	27 (10)	24 (15)	5 (5)
Intermediate 2	75 (20)	53 (20)	38 (23)	15 (15)
Adverse	85 (22)	60 (23)	29 (18)	31 (31)
Cg normal, FLT3 ITD N/A	56 (15)	30 (11)	16 (10)	14 (14)
Cg N/A	16 (4)	11 (4)	7 (4)	4 (4)
Stem cell source				
Bone marrow	135 (36)	92 (35)	54 (33)	38 (38)
Peripheral blood	242 (64)	173 (65)	110 (67)	63 (63)
Cord blood	1 (0)	0 (0)	0 (0)	0 (0)
Donor type				
Matched related donor	195 (52)	137 (52)	92 (56)	45 (44)
Other	183 (48)	128 (48)	72 (44)	56 (55)
GVHD prophylaxis				
Tacrolimus/methotrexate	335 (89)	237 (89)	149 (91)	88 (87)
Tacrolimus/methotrexate/pentostatin	42 (11)	28 (11)	15 (9)	13 (13)
Other	1 (0)	0 (0)	0 (0)	0 (0)

N/A indicates not available; Cg, cytogenetics.
Data presented are n (%), unless otherwise indicated.
* Intermediate-I included only FLT3-ITD mutant patients because of limited number of patients with both NPM1 and FLT3-ITD mutation reported.

cumulative incidence of disease progression at 3 years after transplantation was significantly higher (29%; 95% confidence interval [CI], 18% to 46%) in the ≤85% group compared with the >85% group (15%; 95% CI, 9% to 23%; hazard ratio [HR], 2.1; $P = .04$) (Table S4, Figure 1A). Patients classified as adverse according to the modified ELN risk stratification system had a significantly higher rate of disease progression (HR, 3.0; $P = .004$). Nevertheless, on bivariate analysis, ≤85% donor T lymphocyte chimerism at day +90 to +120 remained an independent predictor of disease progression regardless of adverse modified ELN risk (HR, 2.4; $P = .02$). None of the remaining patient or transplantation characteristics assessed (age, gender, cytogenetic risk group, donor type, stem cell source, and fixed or adjusted busulfan dose) were significantly associated with the rate of disease progression in the CR1/CR2 cohort (data not shown).

Table 2
Myeloid and T Lymphocyte Chimerism at Day +90 to +120 according to Disease Status at Transplantation

	CR1/CR2	Non-CR1/CR2	*P* Value
% Donor T lymphocyte chimerism	n = 164 (%)	n = 101 (%)	
100	70 (43%)	61 (60%)	.005
86-99	49 (30%)	21 (21%)	Ref.
≤85	45 (27%)	19 (19%)	Ref.
% Donor myeloid chimerism	n = 179 (%)	n = 107 (%)	
100	120 (67%)	80 (75%)	.20
99	44 (25%)	13 (12%)	Ref.
≤98	15 (8%)	14 (13%)	Ref.

Ref. indicates reference.

Non-CR1/CR2 cohort

Using the same categorization of chimerism as described for the CR1/CR2 cohort, there was no significant association between mixed T lymphocyte donor chimerism and the rate of disease progression by 3 years after transplantation in the non-CR1/CR2 cohort (Tables S3 and S4). In a landmark analysis, the cumulative incidence of disease progression at 3 years after transplantation was 37% (95% CI, 20% to 66%) in the ≤85% and 34% (95% CI, 25% to 47%) in the >85% group (HR, 1.3; $P = .60$) (Figure 1B). When examined at earlier time points before the 3-year landmark analysis, there was a trend towards increased rate of disease progression in patients with ≤85% T lymphocyte chimerism at 6 months and 1 year, although this did not reach statistical significance (Table S5).

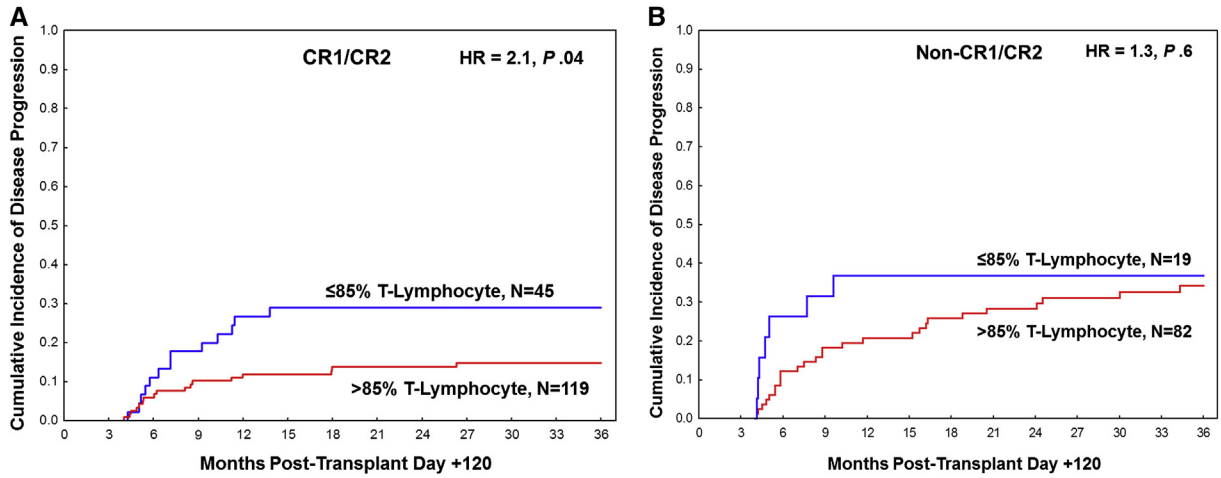

Figure 1. Cumulative incidence of disease progression at 3 years after transplantation based on T lymphocyte chimerism in (A) CR1/CR2 patients at transplantation and (B) non-CR1/CR2 patients at transplantation.

Patients classified as having adverse risk under the modified ELN stratification system had higher rates of disease progression, although this did not reach statistical significance (HR, 2.1; $P = .06$). On bivariate analysis, neither modified ELN risk stratification nor day +90 to +120 T lymphocyte chimerism were significant predictors of disease progression. Moreover, age, gender, cytogenetic risk group, donor type, cell type, and fixed or adjusted busulfan dose were not associated with the rate of disease progression in the non-CR1/CR2 cohort. The overall survival related to T-cell chimerism is shown in (Figure 2).

T Lymphocyte Chimerism, GVHD, and Disease Progression

There was no significant difference in the history of acute GVHD based on donor T lymphocyte chimerism levels at day +90 to +120 when stratified at the 85% cut-off point (Table 3). Grade II to IV acute GVHD occurred in 15 of 64 patients (23%) with ≤85% T lymphocyte chimerism compared with in 58 of 201 patients (29%) with >85% T lymphocyte chimerism ($P = .40$). A history of grade II to IV or III and IV acute GVHD was not associated with the rate of disease progression (Table 4). Likewise, the frequency of chronic GVHD did not differ significantly based on T lymphocyte chimerism levels at day +90 to +120. The prevalence of chronic GVHD by day +90 to +120 was 9% in ≤85% group and 11% in the >85% group ($P = .70$) (Table 3); and the incidence of chronic GVHD after day +90 to +120 was 52% and 59% ($P = .90$) (Table 5) in the 2 groups, respectively. Because of the known associations between complete donor T lymphocyte chimerism and GVHD [26-28], which may influence relapse risk, we evaluated the impact of chronic GVHD on disease progression as time-dependent covariates. Indeed, chronic GVHD was associated with lower rate of disease progression regardless of disease status at transplantation. However, this association was only statistically significant in CR1/CR2 patients ($P = .02$) and not in the non-CR1/CR2 cohort ($P = .20$). On bivariate analysis taking into consideration both chronic GVHD as a time-dependent variable and mixed T lymphocyte chimerism ≤ 85% (Table 4), both factors remained significant predictors of disease progression for the CR1/CR2 group ($P = .02$, $P = .03$, respectively) but not in the non-CR1/CR2 cohort ($P = .20$, $P = .60$, respectively).

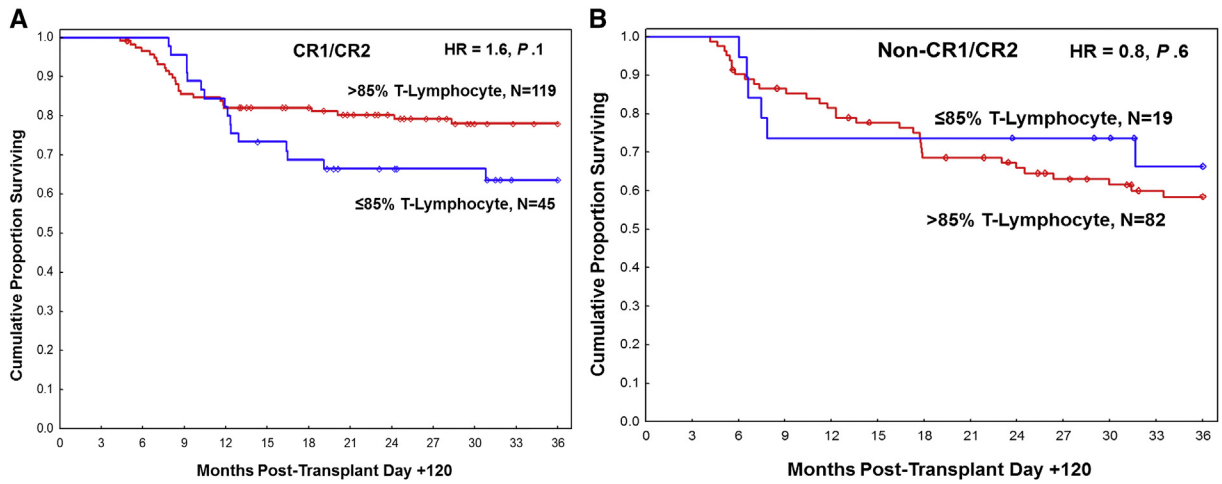

Figure 2. Overall survival at 3 years after transplantation based on T lymphocyte chimerism in (A) CR1/CR2 patients at transplantation and (B) non-CR1/CR2 patients at transplantation.

Table 3
Correlation of Day +90 to +120 T Lymphocyte Chimerism with GVHD

	T Lymphocyte Chimerism ≤ 85% n = 64 (%)	T Lymphocyte Chimerism > 85% n = 201 (%)	*P*
History of aGVHD			
Grade 1	14 (22)	55 (27)	
Grades 2-4	15 (23)	58 (29)	.40
Grade 2	13 (20)	44 (22)	
Grades 3-4	2 (3)	14 (7)	.20
cGVHD before day +120	6 (9)	22 (11)	.70

aGVHD indicates acute graft-versus-host-disease; cGVHD, chronic graft-versus-host-disease.

DISCUSSION

In this analysis, we report the largest study to date to our knowledge investigating the role of chimerism testing between after transplantation day +90 to +120 in predicting disease progression in AML/MDS patients. Several studies have evaluated the role of chimerism analysis in predicting disease recurrence in AML/MDS after transplantation, yet these reports have often been limited by small patient cohorts [29,30], heterogeneous disease populations with variable kinetics of relapse [10,31], or lack of lineage-specific analyses [32,33]. This study focused on patients receiving a uniform, widely used, busulfan-fludarabine myeloablative preparative regimen, which is known to have a relatively high rate of mixed lymphocyte chimerism [24]. All patients received tacrolimus/methotrexate post-transplantation immunosuppressive therapy. We hypothesized that a higher degree of mixed lymphocyte peripheral blood chimerism may be associated with an increased risk of leukemia relapse after transplantation.

We found a significant association between disease status at transplantation and the kinetics of T lymphocyte engraftment, as a lower proportion of patients in CR1/CR2 at transplantation had complete T lymphocyte engraftment at day +90 to +120 compared with in the non-CR1/CR2 cohort. This is likely explained by the greater pretransplantation chemotherapy exposure in the non-CR1/CR2 patients, resulting in greater cumulative immunosuppression, which would facilitate donor engraftment. This is consistent with previous reports that demonstrated a significant association between the number of pretransplantation chemotherapy regimens and T lymphocyte engraftment kinetics [34-37].

In contrast, nearly all patients who were alive and free of disease at the time of chimerism testing achieved complete donor myeloid chimerism by day +90 to +120, regardless of disease status at transplantation. Overall, T lymphocyte chimerism lagged behind the myeloid compartment, which is consistent with a prior study evaluating lineage-specific chimerism kinetics using a busulfan-fludarabine conditioning regimen [24]. Some reports have also suggested that conditioning regimens with busulfan, such as those used in this study, may result in less lymphodepletion compared with melphalan-based regimens [37], but a recent study reported no difference in T lymphocyte engraftment kinetics between the 2 agents [38]. Taken together, these studies imply that the rates of lymphocyte chimerism vary for different preparative regimens, and that the predictive value of mixed lymphocyte chimerism may also vary depending on the type and intensity of conditioning, use of anti–T cell antibodies, or T cell depletion of the graft.

As most patients achieved complete or near complete donor myeloid chimerism by post-transplantation day +90 to +120, our analysis focused on the impact of mixed T lymphocyte chimerism on disease progression. T lymphocyte donor chimerism ≤ 85% was a significant predictor of disease progression in CR1/CR2 patients. In contrast, mixed T lymphocyte chimerism did not significantly affect disease progression rates in the non-CR1/CR2 cohort at 3 years, although there was a trend towards increased risk of disease progression in this group when examined at earlier time points within the first year after transplantation. This may suggest that achieving complete donor T lymphocyte chimerism and maximizing the GVL effect has a larger impact in maintaining remission in CR1/CR2 patients. This is in contrast to non-CR1/CR2 patients, where the diminished predictive value of T lymphocyte chimerism suggests that the presence of active disease at the time of transplantation poses a greater risk for relapse than delayed immune reconstitution with donor T lymphocytes. The fact that chronic GVHD, when analyzed as a time-dependent variable, was a significant predictor of disease progression only in the CR1/CR2 patients lends further support to this idea.

The role of mixed T lymphocyte donor chimerism in predicting AML relapse after transplantation has been controversial, although no previous studies have stratified patients based on disease status at transplantation as in the current analysis. Four studies have reported no association of mixed T lymphocyte chimerism and disease recurrence, although 3 of these studies contained different preparative regimens and very heterogeneous disease populations, including acute and chronic leukemias of both myeloid and lymphoid lineages [27,35,38]. However, the potential importance of T lymphocyte chimerism in predicting disease

Table 4
Impact of aGVHD and cGVHD on Disease Progression

	CR1/CR2 n = 164			Non-CR1/CR2 n = 101		
	HR	95% CI	*P*	HR	95% CI	*P*
History of aGVHD II-IV	1.7	.8-3.6	.2	.5	.2-1.2	.10
History of aGVHD III-IV	No events*		.30	1.2	.3-5.0	.80
cGVHD (as time-dependent variable)	.3	.1-.8	.02	.6	.3-1.3	.20
Bivariate analysis cGVHD and T lymphocyte chimerism						
cGVHD (as time-dependent variable)	.3	.1-.8	.02	.6	.3-1.3	.20
Chimerism ≤ 85%	2.2	1.1-4.6	.03	1.2	.5-2.9	.60

* No events in 2 patients with grade III-IV aGVHD.

Table 5
Correlation of Day +90 to +120 T Lymphocyte Chimerism with 3-Year Cumulative Incidence of cGVHD

	T Lymphocyte Chimerism ≤ 85%	T Lymphocyte Chimerism > 85%			
	% 3-Year Cumulative Incidence* (95% CI)	% 3-Year Cumulative Incidence* (95% CI)	HR	95% CI	*P*
cGVHD overall	52% (41-63)	59% (53-66)	.8	.6-1.2	.40
cGVHD in CR1/CR2	58% (45-74)	61% (53-71)	.94	.6-1.5	.80
cGVHD in non-CR1/CR2	37% (20-66)	56% (46-68)	.6	.2-1.4	.20

* Accounting for disease progression or death before cGVHD as competing risks.

recurrence has been highlighted by several recent studies. In a large analysis by Koreth et al., mixed total donor cell and T lymphocyte chimerism < 90% at day +30 and day +100 in patients who underwent allo-HSCT was predictive of increased risk of relapse, with day +100 total donor cell chimerism most predictive [39]. Two additional studies have also described the significance of day +30 mixed T lymphocyte chimerism in predicting disease relapse. Saito et al. reported that T lymphocyte chimerism < 90% at day +30 portended poor outcomes in a cohort of 117 patients with both myeloid and lymphoid malignancies who underwent busulfan-based reduced-intensity conditioning allo-HSCT [36]. Finally, day +30 mixed whole blood and T lymphocyte chimerism were predictive of early relapse and shorter overall survival in a cohort of 121 patients with myeloid and lymphoid malignancies when chimerism was assessed as a continuous variable [40]. In this particular model, T lymphocyte chimerism was a stronger predictor of relapse and overall survival compared with whole blood chimerism testing. Likewise, the current study also supports the value of T lymphocyte chimerism in predicting relapse in AML/MDS after allo-HSCT, although its applicability is limited to patients who are alive and without disease progression at day +120. This represented 78% of all AML/MDS patients who initially underwent allo-HSCT in our study. However, assessing the predictive impact of day +90 to +120 chimerism analysis may have particular therapeutic relevance if prophylactic DLIs are considered, as the timing usually corresponds to the tapering of immunosuppression after allo-HSCT. As a result, prophylactic DLIs given during this time period may be more tolerable with lower rates of GVHD, compared with earlier administration [41].

Given the emergence of multiparameter flow cytometry (MFC) and reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) as highly sensitive tools in assessing minimal residual disease (MRD), 1 limitation of this study is the lack of MRD data that was incorporated in this analysis [42,43]. The value of MRD testing to predict relapse after transplantation is being actively studied. Relapse can occur if residual clonogenic leukemia cells are present in the absence of an effective GVL immune effect. Indeed, integration of chimerism data with MFC or RT-qPCR MRD measurements to develop relapse risk models may represent the most practical use of such data [44]. Chimerism data remain a potential useful tool in assessing relapse risk in subsets of patients who may not have specific molecular markers to monitor by RT-qPCR [42]. Moreover, although almost all AML patients will have an identifiable leukemia-associated immunophenotype at the time of diagnosis with MFC [45], changes in leukemia-associated immunophenotype expression patterns at the time of relapse can occur in up to 80% to 90% of patients [46,47] which represents 1 limitation of such testing.

Identifying AML/MDS patients at high risk for impending disease relapse in the post-transplantation setting remains a challenge. In this large analysis, we demonstrate that mixed T lymphocyte chimerism at day +90 to +120 after transplantation is a promising approach of detecting patients at higher risk for disease recurrence, and its interpretation depends on disease status at transplantation. Chimerism assessment should be considered with the disease risk index, MRD assessment, and potentially other factors in identifying patients at high risk of relapse. Strategies to enhance early full donor T cell chimerism after transplantation and early preemptive therapies need assessment to prevent relapse in high-risk patients, such as immune modulation, DLI, or initiation of hypomethylating agents or tyrosine kinase inhibitors. These approaches warrant study in prospective clinical trials.

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Authorship statement: R.C. designed the research. J.C. provided database support. R.M.S. performed statistical analyses. L.J.M. performed the chimerism analysis. H.C.L., R.M.S., G.A., U.P., and R.C. analyzed and interpreted data. H.C.L., R.M.S., and R.C. wrote the manuscript. All authors reviewed and edited the manuscript and approved the final version.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found at 10.1016/j.bbmt.2015.07.005.

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