Published in final edited form as:

Leukemia. 2015 January ; 29(1): 137–144. doi:10.1038/leu.2014.173.

Comparison of Minimal Residual Disease as Outcome Predictor for AML Patients in First Complete Remission Undergoing Myeloablative or Nonmyeloablative Allogeneic Hematopoietic Cell Transplantation

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Abstract

Minimal residual disease (MRD) is associated with adverse outcome in AML after myeloablative (MA) hematopoietic cell transplantation (HCT). We compared this association with that seen after nonmyeloablative (NMA) conditioning in 241 adults receiving NMA (n=86) or MA (n=155) HCT for AML in first remission with pre-HCT bone marrow aspirates assessed by flow cytometry. NMA patients were older and had more comorbidities and secondary leukemias. Three-year relapse estimates were 28% and 57% for MRD^{neg} and MRD^{pos} NMA patients, and 22% and 63% for MA patients. Three-year overall survival (OS) estimates were 48% and 41% for MRD^{neg} and MRD^{pos} NMA patients and 76% and 25% for MA patients. This similar OS after NMA conditioning was largely accounted for by higher non-relapse mortality (NRM) in MRD^{neg} (30%) compared to MRD^{pos} (10%) patients, whereas the reverse was found for MRD^{neg} (7%) and MRD^{pos} (23%) MA patients. A statistically significant difference between MA and NMA patients in the association of MRD with OS (P<0.001) and NRM (P=0.002) but not relapse (P=0.17) was

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Presented in part at the 55th Annual Meeting of the American Society of Hematology; December 7–10, 2013; New Orleans, LA

CONFLICT OF INTEREST

The authors declare no competing financial interests.

Supplementary information is available at Leukemia's website.

confirmed. After adjustment, the risk of relapse was 4.51-times (P<0.001) higher for MRD^{pos} patients. These data indicate that the negative impact of MRD on relapse risk is similar after NMA and MA conditioning.

INTRODUCTION

For many patients with acute myeloid leukemia (AML), allogeneic hematopoietic cell transplantation (HCT) is considered definitive consolidation therapy once complete remission (CR) is achieved.[1, 2] Nevertheless, even without morphologically detectable disease at the time of transplantation, relapse remains a major cause of treatment failure post-HCT.[2] Increasing evidence from our center and others suggests that the presence of minimal residual disease (MRD) at the time of HCT, e.g. as detected by multiparameter flow cytometry (MFC), identifies a subset of patients that is at particularly high risk of relapse.[3] So far, however, studies have focused on the association between MRD and outcome after myeloablative (MA) conditioning.[4–9] On the other hand, very limited and mixed information is available on the role of MRD in AML patients undergoing nonmyeloablative (NMA) HCT, with a previous study from our institution on an earlier cohort of patients[10] indicating an adverse impact in univariate but not multivariate analyses, and a recent study from the University of Minnesota[11] indicating an independent adverse impact. Herein, we therefore conducted a comparative analysis to assess the relationship between MRD and outcome for AML patients undergoing NMA HCT relative to that seen in MA HCT, using a cohort of consecutive patients treated at our institution who underwent pre-HCT evaluation for MRD between 2006 and 2012.

PATIENTS AND METHODS

Study cohort

AML patients ≥18 years of age were included in this retrospective study if they were in first morphologic CR or CR with incomplete peripheral blood count recovery (CRi) irrespective of the presence of MRD, underwent allogeneic HCT with NMA or MA conditioning, and received peripheral blood or bone marrow as stem cell source. We included all patients meeting these criteria if they underwent pre-HCT work up from late April 2006, when a refined MFC-based MRD detection method was introduced at our institution and utilized routinely in all patients, until April 2012. Results on the first 136 MA patients have been reported previously.[8, 9] We used the 2008 WHO criteria to define AML[12] and the refined United Kingdom Medical Research Council (MRC) criteria to assign cytogenetic risk.[13] Secondary leukemia was defined as AML following a history of antecedent hematologic disorder (i.e. myelodysplastic syndrome or myeloproliferative neoplasm) or prior treatment with systemic chemotherapy and/or radiotherapy.

Pretransplantation comorbidities were assessed retrospectively using the HCT-specific comorbidity index (HCT-CI).[14, 15] Treatment responses were categorized as proposed by the European LeukemiaNet.[16] Criteria for diagnosis and grading of acute and chronic GVHD have been reported previously.[17, 18] Information on post-transplant outcomes was captured via the Long-Term Follow-Up Program through medical records from our outpatient clinic

and local clinics that provided primary care for patients in addition to records obtained on patients on research studies. All patients were treated on Institutional Review Board-approved protocols or standard treatment protocols and gave consent in accordance with the Declaration of Helsinki. Follow-up was current as of October 1, 2013.

MFC detection of MRD

Ten-color MFC was performed as a routine clinical test on bone marrow aspirates obtained as routine baseline assessment before HCT with a panel consisting of three antibody combinations, as described previously.[8, 9] Up to 1 million events per tube were acquired on a custom-built LSRII and data compensation and analysis performed using software developed in our laboratory (WoodList). MRD was identified by visual inspection as a population showing deviation from the normal patterns of antigen expression seen on specific cell lineages at specific stages of maturation as compared with either normal or regenerating marrow.[9] This approach is required due to the predominance of referred patients in this population for whom the abnormal immunophenotype is not available or useful. In this cohort, 89 of 241 patients (36.9%) had one or more positive flow cytometric studies in our laboratory prior to the pre-transplant sample evaluation. This approach is estimated to be applicable to roughly 90% of AML patients as assessed by concordance of concurrent FISH and flow cytometric assay results.[19] The routine sensitivity of this assay was estimated at 0.1% although a higher level of sensitivity was possible for a subset of leukemias featuring more frankly aberrant immunophenotypes (see Table 1 for median and ranges seen in these cohorts). When identified, the abnormal population was quantified as a percentage of the total $CD45^+$ white cell events. Any level of residual disease was considered MRD^{pos}.[8, 9] The results from MFC assessment of MRD were available to the transplant teams.

Statistical analysis

Similar to our previous analysis,[9] there was no statistically significant evidence that increasing levels of MRD were associated with increasing risk of relapse or death, we focused our analysis of a comparison of MRD^{pos} and MRD^{neg} patients. Unadjusted probabilities of overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method, and probabilities of NRM, relapse, and acute as well as chronic graft-versus-host disease (GVHD) were summarized using cumulative incidence estimates. NRM was defined as death without prior relapse and was considered a competing risk for relapse, while relapse was a competing risk for NRM; death was considered a competing risk for acute and chronic GVHD. All outcomes were treated as time-to-event endpoints. Outcomes between MRD^{pos} and MRD^{neg} groups were compared using Cox regression. Multivariate models included the following additional factors: conditioning regimen (NMA *vs.* MA), age at the time of HCT, HCT comorbidity index (HCT-CI) scores, cytogenetic risk group at time of AML diagnosis (unfavorable *vs.* favorable/intermediate), type of AML at diagnosis (secondary *vs.* de novo), number of chemotherapy cycles, karyotype at time of HCT (normalized *vs.* not normalized for patients presenting with abnormal karyotypes), and peripheral blood counts at the time of HCT (CR *vs.* CRi). Missing cytogenetic risk and karyotype were accounted for as separate categories. Categorical patient characteristics were compared between MRD^{pos} and MRD^{neg} groups using Pearson's Chi Square tests, and

continuous characteristics were compared with two-sample t-tests. No adjustments were made for multiple comparisons, and all two-sided *P*-values from the regression models were derived from the Wald test. Statistical analyses were performed using STATA (StataCorp LP, College Station, TX).

RESULTS

Patient characteristics

We identified 241 patients undergoing NMA (n=86) or MA (n=155) HCT from a matched-related or an unrelated donor for AML in first remission who had pre-HCT MFC studies available for retrospective analysis. All patients had <5% bone marrow blasts and thus met the morphological criteria for leukemia-free state and CR.[16] Among these, 21 (24.4%) of the NMA and 30 (19.4%) of the MA HCT patients had MRD by MFC (i.e. were MRD^{pos}), whereas the others failed to have flow cytometric evidence of MRD (i.e. were MRD^{neg}). The characteristics of the study population, induction and consolidation chemotherapies, donors, and transplants are summarized in Table 1. Of note, NMA patients differed from MA patients in that they were older (median age [range]: 62.5 [20.0–75.0] *vs*. 48.6 [18.2–69.7] years; $P<0.0001$), had more comorbid illnesses (median HCT-CI: 3 [0–9] *vs*. 2 [0–7], $P<0.001$), and more often had secondary AML (48.8% *vs*. 35.5%; $P=0.04$) and incomplete peripheral blood count recovery pre-HCT (31.4% *vs*. 10.3%; $P<0.0001$). NMA patients also received a higher number of consolidation chemotherapy cycles than MA patients before HCT ($P=0.03$). In contrast, the median time between achievement of CR and transplantation was similar for NMA and MA patients (108 [26–788] *vs*. 120 [16–465] days: $P=0.84$).

Consistent with our previous findings,[8, 9] MRD^{pos} and MRD^{neg} patients differed in several baseline characteristics from each other. Specifically, MRD^{pos} patients more likely had AML with unfavorable cytogenetics ($P=0.001$) and had a higher prevalence of secondary AML ($P=0.002$). Furthermore, the time between CR achievement and HCT was shorter for MRD^{pos} patients ($P=0.008$), and they received fewer consolidation chemotherapy courses ($P=0.04$). In contrast, MRD^{neg} and MRD^{pos} patients were of comparable age ($P=0.16$) and were similarly likely to have incomplete blood count recovery and thus be classified as having CRi rather than CR at the time of HCT ($P=0.23$).

Acute and chronic GVHD

The 100-day cumulative incidences of grade 3 or 4 acute GVHD differed considerably between patient strata, with estimates of 10% and 27% for MRD^{neg} and MRD^{pos} MA patients, and 9% *vs*. 0% for MRD^{neg} and MRD^{pos} NMA patients, respectively (Figure 1A and Table 2). In contrast, the 180-day cumulative incidences of chronic GVHD were relatively similar for MA and NMA patients, with slightly lower incidences noted in MRD^{pos} patients (58% and 38% for MRD^{neg} and MRD^{pos} MA patients, and 51% *vs*. 30% for MRD^{neg} and MRD^{pos} NMA patients, respectively; Figure 1B and Table 2).

Association between MRD status and post-HCT outcome

There were a total of 101 deaths, 78 relapses, and 39 NRM events contributing to the probability estimates for OS, DFS, relapse, and NRM stratified by MRD status for NMA

and MA patients. The median follow-up after HCT among survivors was 50.6 (13.7–85.3) months for NMA patients and 38.8 (12.2–84.8) months for MA patients, respectively. As summarized in Table 2, among NMA patients, the 3-year estimates of OS were 48% and 41% for MRD^{neg} and MRD^{pos} patients, respectively; among MA patients, 3-year OS was estimated to be 76% and 25%, respectively (Figure 2A). For DFS, similar estimates were 42% and 33% for MRD^{neg} and MRD^{pos} NMA patients and 71% and 13% for MRD^{neg} and MRD^{pos} MA patients (Figure 2B). Three-year estimates of relapse for NMA patients were 28% and 57%, respectively, and 22% and 63%, respectively, for those who received MA conditioning (Figure 2C). Finally, following NMA conditioning, the 3-year estimates of NRM where 30% and 10% for MRD^{neg} and MRD^{pos} patients, respectively, whereas similar estimates were 7% and 23% following MA conditioning (Figure 2D).

Relationship between MRD status and pre-HCT conditioning

Regression models for OS, DFS, relapse, and NRM were fit to assess the relationship between MRD and pre-HCT conditioning as well as post-HCT outcome. As summarized in Table 3, being MRD^{pos} at the time of HCT was significantly associated with increased risk of relapse (P=0.005) in patients undergoing NMA conditioning, whereas there was no statistically significant association between MRD status and OS, DFS, or NRM in these patients. In contrast, being MRD^{pos} was significantly associated with shorter OS (P<0.001) and DFS (P<0.001) as well as an increased risk of relapse (P<0.001) and NRM (P<0.001) in patients undergoing MA conditioning. Consistent with these findings, tests for interaction indicated that the association of MRD with relapse among NMA patients was similar to that among MA patients (e.g., P=0.17) whereas the associations of MRD with OS, DFS, and NRM differed between NMA and MA patients (P<0.001, P<0.001, and P<0.002, respectively).

Pre-HCT MRD status as independent prognostic factor

Finally, we fit multivariate models for OS, DFS, relapse, and NRM using MRD status (MRD^{pos} *vs.* MRD^{neg}), type of conditioning (NMA vs. MA) age at HCT, HCT-CI, cytogenetic disease risk at diagnosis (adverse *vs.* intermediate/favorable), type of AML (secondary *vs.* primary), number of chemotherapy cycles before HCT, pre-HCT karyotype (not normalized *vs.* normalized for patients initially presenting with abnormal karyotype), and pre-HCT peripheral blood count recovery (CRi *vs.* CR) as covariates. After adjustment for these factors, being MRD^{pos} remained statistically significantly associated with shortened OS (hazard ratio [HR]=2.16 [95% confidence interval: 1.34–3.49], P=0.002) and DFS (HR=3.24 [2.03–5.17], P<0.001) as well as increased risk of relapse (HR=4.51 [2.57–7.90], P<0.001), whereas there was no association with risk of NRM (HR=1.46 [0.59–3.60], P=0.41; Table 4). In these analyses, there was a trend toward shorter OS (HR=1.58 [0.95–2.62], P=0.08), shorter DFS (HR=1.48 [0.92–2.38], P=0.11), and increased risk of NRM (HR=2.06 [0.87–4.87], P=0.10) for patients undergoing NMA conditioning. A slightly different multivariate model, in which we used the number of induction chemotherapy cycles and type of consolidation therapy (none *vs.* high-dose cytarabine [HIDAC]-containing *vs.* non-HIDAC containing) as covariates revealed very similar finding, We then performed additional multivariate models restricting the study cohort to those 199 patients who received peripheral blood as stem cell source and, overall, found very comparable

results as those obtained in the entire study cohort (Supplemental Table 1). Finally, we fit multivariate models for relapse separately for NMA and MA patients. Because of the smaller sample sizes, a smaller number of covariates (age, HCT-CI, cytogenetic disease risk at diagnosis, type of AML, and number of chemotherapy cycles) were included. In these analyses, MRD was associated with increased risk of relapse in both NMA (HR=2.79 [1.23–6.36], P=0.01) as well as MA (HR=7.68 [3.65–16.2], P<0.001) patients, respectively.

DISCUSSION

The use of MRD as a biomarker for the intrinsic resistance of the leukemia to therapy has come of age in AML: both during and after conventional induction and consolidation chemotherapy, its presence identifies a subset of patients with an increased risk of disease recurrence and worse outcome among those who meet the standard morphologic criteria for CR.[20–22] Similarly, for AML patients undergoing HCT while in CR, several studies congruently demonstrated the value of MRD as a marker of increased disease recurrence and shorter survival. Yet, although several studies have included small numbers of patients undergoing NMA conditioning,[23, 24] most studies have focused on patients undergoing MA conditioning,[4–9] and the role of MRD as marker of increase risk, if any, after NMA conditioning has not been well defined. In fact, in a previous study on 274 patients with AML undergoing NMA HCT at 17 centers, including 231 in first or second CR, we found that an adverse impact of MRD – as determined by a combination of MFC, karyotype analysis, and fluorescence in situ hybridization at the time of HCT – on relapse in an univariate analysis, but this association was lost after multivariable adjustment.[10] A limitation of that study was that MRD assessments were not standardized and that patients received NMA HCT between 1998 and 2008, a decade over which MFC assessments for MRD have changed significantly. On the other hand, in a very recent report on 85 patients undergoing NMA HCT for AML in CR between 2000 and 2012 at the University of Minnesota, the presence of MRD by MFC at the time of HCT was found to be associated with a significantly increased relapse rate even after multivariate adjustment, a finding reminiscent of that seen with MA HCT, although the number of patients with detectable MRD was small (n=8).[11]

In the current study, we aimed to further clarify the potential role of MRD at the time of HCT as predictor of adverse outcome by assessing adults undergoing NMA HCT for AML in morphologic first remission at our institution since April 2006. At that time, a refined MRD assay was introduced that fundamentally remained unchanged since then. As a bone marrow assessment with flow cytometric analysis of MRD is considered standard of care during the pretransplant work-up, our study includes essentially all patients with CR1 AML undergoing NMA HCT over a 6-year period. As a non-randomized comparison, we contrasted our findings with those obtained in the cohort of CR1 AML patients undergoing MA HCT during the same time period. Together, our results support three main conclusions: first, the presence of MRD at the time of NMA HCT is associated with an increased risk of post-HCT relapse, a finding similar to that found in MA HCT; second, despite a high rate of NRM among MRD^{neg} NMA patients, approximately half of these patients are alive 3 years after transplantation, indicating that this treatment modality is associated with favorable outcomes and, perhaps, cure in many; and third, due to a relatively low NRM among

MRD^{pos} NMA patients, approximately one third of these patients are alive without evidence of disease recurrence, suggesting that long-term disease control is possible even in a subset of patients with MRD at the time of NMA HCT.

Several previous studies have indicated that older or medically infirm AML patients can experience long-term remissions after NMA allogeneic HCT.[10, 11, 25–30] The survival estimates from our current study are very similar to those obtained in our previous study and many of the other reports, and further support this notion. Our finding that pre-transplant MRD is associated with an increased risk of relapse after NMA HCT is consistent with that by Ustun *et al*.[11] In fact, in their analysis, the presence of MRD was associated with a 3.7-fold increased relapse risk after multivariate adjustment, a point estimate similar to ours (2.8-fold increase among NMA patients). Together, these studies validate MRD as marker for increased risk of disease recurrence, and thus extend the data from the MA to the NMA setting.

At our institution, patients with AML in CR are routinely assigned to MA conditioning unless significant comorbidities are present, patients are otherwise ineligible to undergo fully ablative HCT, or they were entered in a randomized study comparing conditioning intensity; conversely, the presence of MRD, although perceived as indicator of increased disease recurrence after transplantation, typically played no role in the decision of MA *vs*. NMA conditioning. As the assignment to NMA or MA conditioning was non-random in our study cohort and driven largely by these factors (i.e. age, comorbidities and protocol availability) – factors that we may only have been partially able to adjust for in multivariate analyses – our findings should not be used to directly compare the outcomes of MA and NMA conditioning for adults with AML in CR. Nonetheless, it is interesting to note that we observed a 7.7-fold increased risk of disease recurrence in MRD^{pos} relative to MRD^{neg} patients after multivariate adjustment in the contemporary patients undergoing MA HCT. In assessing a possible interaction between MRD and the type of conditioning, we were unable to detect a statistically significant difference for the association between MRD and relapse risk for patients undergoing NMA and those undergoing MA HCT. However, such interaction analyses are limited by low power, and future studies will be required to further test the possibility that the adverse effect of MRD on post-transplant relapse risk is less marked in patients undergoing NMA relative to MA HCT, and whether such a difference, if noted, is due to a higher risk of relapse in MRD^{neg} patients or a relatively smaller risk increase when MRD is detectable.

In our NMA cohort, a relatively high rate of NRM was noted in the subset of MRD^{neg} relative to that in the MRD^{pos} NMA or MRD^{neg} MA patients. We were unable to discern an obvious explanation for this difference. While a higher proportion of MRD^{neg} than MRD^{pos} NMA patients were diagnosed with grade 3 or 4 acute GVHD, only small imbalances were noted between these two patient subsets with regard to other characteristics (e.g. fewer males and slightly higher proportions of patients receiving consolidation therapy, having a HCT-CI ≥3, or receiving an unrelated donor transplant), and adjusting for these imbalances (e.g. amount of consolidation therapy) had little impact on NRM (data not shown). Moreover, MRD^{neg} NMA patients experiencing NRM did not differ in age (P=0.78), HCT-CI (P=0.90), gender (P=0.24), cytogenetic risk (P=0.53), type of AML (P=0.23), number of

induction or consolidation courses given (P=0.62 and P=0.43), or blood count recovery before HCT, relative to MRD^{pos} NMA patients. As the cumulative incidence estimates for relapse were relatively similar for MRD^{neg} NMA and MA patients, these NRM differences largely explained the inferior OS and DFS estimates for the NMA cohort. In contrast, the OS and DFS estimates for MRD^{pos} NMA patients compared favorably to those for MRD^{pos} MA patients, primarily because of a lower NRM rate in the former.

As one potential limitation of our study, the majority of patients were referred to our institution for transplantation after having received induction and consolidation chemotherapy elsewhere. Thus, abnormal immunophenotypes were only available in 89 of the 241 patients. The frequency of MRD detection for those patients having an available positive sample prior to the pre-transplant evaluation was 29.2% in comparison with 16.5% for those lacking a prior sample (P=0.02). While we do not fully understand the reasons for this difference, it is conceivable that having access to a prior study that established the individual's abnormal immunophenotype improves the ability to identify MRD in pre-transplant bone marrow specimens. However, we suspect that other confounders are likely present, as the level of MRD was higher in patients in whom prior abnormal immunophenotypes were established (median of 0.4% *vs*. 0.2% in patients without availability of prior flow cytometric studies) – we would have expected that knowledge of prior abnormal immunophenotype might facilitate the detection and not quantification of very low level MRD. Nonetheless, the negative impact of MRD appeared relatively similar in the subsets with/without established abnormal immunophenotype (adjusted HR for OS: 2.15 [0.86–5.39] *vs*. 2.51 [1.36–4.65]; adjusted HR for DFS: 4.12 [1.74–9.77] *vs*. 3.72 [2.08–6.65]; and adjusted HR for relapse: 7.04 [2.37–20.9] *vs*. 4.47 [2.17–9.18], respectively).

In summary, the negative impact of MRD on post-transplant relapse risk among AML patients in CR1 undergoing NMA HCT is similar to the negative impact seen in patients undergoing MA HCT. Because of the high rate of NRM among MRD^{neg} NMA patients the survival difference between MRD^{neg} and MRD^{pos} patients was much larger in MA than NMA patients in this analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research reported in this publication was supported by grants P01-CA078902, P01-CA018029, and R00-HL088021 from the National Cancer Institute/National Institutes of Health, Bethesda, MD, USA. R.B.W. is a Leukemia & Lymphoma Society Scholar in Clinical Research. S.A.B. is the recipient of a Trainee Research Award and a "Hematology Opportunity for the Next-Generation of Research Scientists" (HONORS) Award from the American Society of Hematology.

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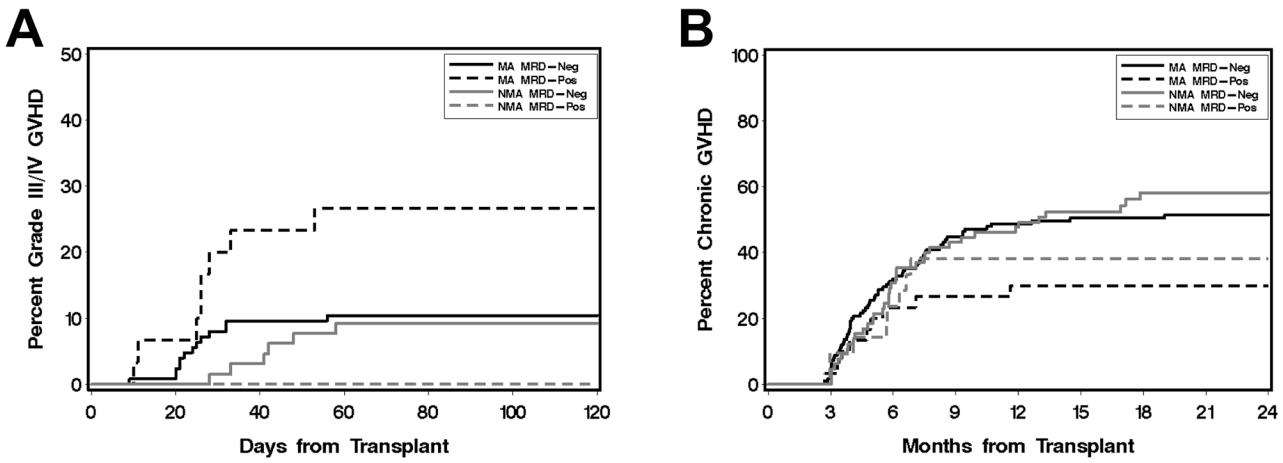

Figure 1. Cumulative incidences of acute GVHD and chronic GVHD stratified by conditioning type and MRD status

Estimates of **(A)** grade 3 or 4 acute GVHD and **(B)** chronic extensive GVHD following myeloablative allogeneic HCT for AML in complete first morphologic remission, shown individually for MRD^{neg} (n=65) and MRD^{pos} (n=21) NMA patients as well as MRD^{neg} (n=125) and MRD^{pos} (n=30) MA patients, respectively.

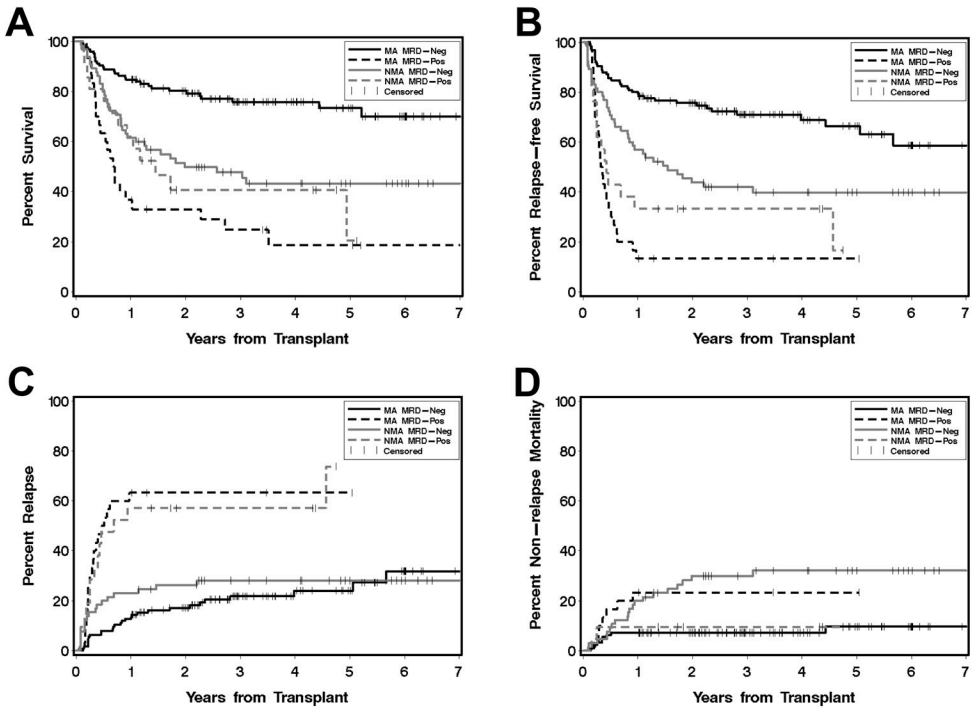

Figure 2. Association between pre-HCT MRD, as determined by multiparameter flow cytometry, and outcome for AML patients following nonmyeloablative (NMA) or myeloablative (MA) HCT

Estimates of **(A)** overall survival, **(B)** disease-free survival, **(C)** cumulative incidence of relapse, and **(D)** cumulative incidence of non-relapse mortality following myeloablative allogeneic HCT for AML in complete first morphologic remission, shown individually for MRD^{neg} (n=65) and MRD^{pos} (n=21) NMA patients as well as MRD^{neg} (n=125) and MRD^{pos} (n=30) MA patients, respectively.

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TABLE 1

Pre-transplantation Demographic and Clinical Characteristics of Study Cohort, Stratified by HCT Type and MRD Status

	Nonmyeloablative HCT (n=86)		Myeloablative HCT (n=155)	
	MRD^{neg} (n=65)	**MRD^{pos} (n=21)**	**MRD^{neg} (n=125)**	**MRD^{pos} (n=30)**
Median Age at HCT (range), years	62.1 (20.0–75.0)	63.8 (33.1–74.9)	47.5 (19.1–69.7)	50.8 (18.2–66.8)
Male Gender	64.6%	90.5%	51.2%	60.0%
Median WBC at Diagnosis, x10^3/μL	4.2 (0.8–238)	1.7 (0.7–105)	9.1 (0.2–280)	2.4 (0.3–145)
Cytogenetics				
Favorable	6.2%	0%	4.8%	0%
Intermediate	67.7%	47.6%	73.6%	46.7%
Adverse	21.5%	52.4%	19.2%	50.0%
Missing	4.6%	0%	2.4%	3.3%
Secondary AML	46.2%	57.1%	29.6%	60.0%
No. of Induction Courses				
1	58.5%	57.1%	64.0%	50.0%
2	24.6%	19.1%	31.2%	40.0%
≥3	15.4%	23.8%	4.8%	10.0%
Missing	1.5%	0%	0%	0%
No. of Consolidation Courses				
0	12.3%	28.6%	12.8%	36.7%
1	33.9%	38.1%	53.6%	40.0%
≥2	53.9%	33.3%	33.6%	23.3%
Median CR Duration (range), days	110 (26–788)	77 (35–356)	128 (20–465)	83 (16–210)
Recovered Peripheral Blood Counts before	69.2%	66.7%	91.2%	83.3%
HCT*				
Routine Cytogenetics before HCT				
Normal karyotype	44.6%	33.3%	48.8%	50.0%
Abnormal karyotype	9.2%	38.1%	7.2%	26.7%
Missing/inadequate data	46.2%	28.6%	44.0%	23.3%

	Nonmyeloablative HCT (n=86)		Myeloablative HCT (n=155)	
	MRD^{neg} (n=65)	**MRD^{pos} (n=21)**	**MRD^{neg} (n=125)**	**MRD^{pos} (n=30)**
HCT Comorbidity Index				
0	6.2%	9.5%	20.0%	20.0%
1–2	23.1%	28.6%	37.6%	30.0%
≥3	69.2%	61.9%	42.4%	46.7%
Missing	1.5%	0%	0%	0%
Median abnormal blasts by MFC (range)	---	0.40% (0.02–4.8%)	---	0.28% (0.007–7.8%)
Unrelated Donor	69.2%	61.9%	57.6%	70.0%
Median Donor Age (range), years	34.3 (18.1–76.6)	42.1 (20.2–70.2)	40.2 (17.6–69.8)	39.6 (19.1–62.4)
Male Donor	46.2%	66.7%	56.8%	66.7%
Conditioning Regimen				
L-TBI ± Flu or Clo	100%	100%	0%	0%
Bu/Cy ± L-TBI	0%	0%	36.0%	46.7%
BU/FLU	0%	0%	22.4%	13.3%
H-TBI/Cy ± Flu	0%	0%	8.0%	10.0%
H-TBI/Tepa/Flu	0%	0%	3.2%	0%
Treo/Flu ± L-TBI	0%	0%	28%	20.0%
Flu/Radiolabeled Ab/L-TBI ± Cy	0%	0%	2.4%	10.0%
Source of Stem Cells				
PBSC	98.5%	95.2%	75.2%	70.0%
BM	1.5%	4.8%	24.8%	30.0%
GVHD Prophylaxis				
Calcineurin Inhibitor + Methotrexate	0%	0%	80.8%	76.7%
Calcineurin Inhibitor + MMF ± Rapa	96.9%	85.7%	2.4%	10.0%
Cy ± Calcineurin Inhibitor	0%	0%	13.6%	13.3%
Other	3.1%	14.3%	4.2%	0%

* ANC ≥1,000/μL and platelets ≥100,000/μL

Abbreviations: Ab, antibody; BM, bone marrow; Bu, busulfan; Clo, clofarabine; Cy, cyclophosphamide; Flu, fludarabine; H-TBI, high-dose total body irradiation; HCT, hematopoietic cell transplantation; L-TBI, low-dose total body irradiation; MFC, multiparameter flow cytometry; MMF, mycophenolate mofetil; PBSC, peripheral blood stem cells; Rapa, rapamycin; Tepa; thiotepa; Treo, treosulfan; WBC, total white blood cell count.

TABLE 2

Outcome Probabilities Stratified by MRD Status and Conditioning Type

	Nonmyeloablative HCT (n=86)		Myeloablative HCT (n=155)	
	MRD^{neg} (n=65)	**MRD^{pos} (n=21)**	**MRD^{neg} (n=125)**	**MRD^{pos} (n=30)**
Overall Survival at 3 Years	48% (35–60%)	41% (20–61%)	76% (67–83%)	25% (11–42%)
Disease-free Survival at 3 Years	42% (30–54%)	33% (15–53%)	71% (62–78%)	13% (4–28%)
Cumulative Incidence of Relapse at 3 Years	28% (18–39%)	57% (34–75%)	22% (15–30%)	63% (44–78%)
Cumulative Incidence of Non-Relapse Mortality at 3 Years	30% (19–41%)	10% (2–26%)	7% (4–13%)	23% (10–39%)
Grade 3/4 Acute GVHD at 100 Days	9% (4–18%)	0%	10% (6–17%)	27% (13–43%)
Chronic GVHD at 18 Months	58% (45–69%)	38% (18–58%)	51% (41–59%)	30% (15–47%)

TABLE 3

Cox Regression Models for Relationship between MRD Status and HCT Type

	All (n=241)	Nonmyeloablative HCT (n=86)	Myeloablative HCT (n=155)
Overall Survival			
MRD Status			
MRD^{neg}	1 (Reference)	1 (Reference)	1 (Reference)
MRD^{pos}	2.69 (1.78–4.07), *P*<0.001	1.27 (0.67–2.41), *P*=0.457	5.11 (2.94–8.88), *P*<0.001
HCT Type			
Myeloablative	1 (Reference)	N/A	N/A
Nonmyeloablative	1.63 (1.10–2.42), *P*=0.015		
***P*-value for interaction**	0.001	N/A	N/A
Disease-Free Survival			
MRD Status			
MRD^{neg}	1 (Reference)	1 (Reference)	1 (Reference)
MRD^{pos}	3.41 (2.29–5.06), *P*<0.001	1.64 (0.90–3.00), *P*=0.106	6.62 (3.89–11.26), *P*<0.001
HCT Type			
Myeloablative	1 (Reference)	N/A	N/A
Nonmyeloablative	1.47 (1.02–2.13), *P*=0.040		
***P*-value for interaction**	0.001	N/A	N/A
Relapse			
MRD Status			
MRD^{neg}	1 (Reference)	1 (Reference)	1 (Reference)
MRD^{pos}	4.56 (2.84–7.34), *P*<0.001	2.83 (1.38–5.81), *P*=0.005	7.22 (3.85–13.54), *P*<0.001
HCT Type			
Myeloablative	1 (Reference)	N/A	N/A
Nonmyeloablative	1.11 (0.70–1.77), *P*=0.645		
***P*-value for interaction**	0.169	N/A	N/A
Non-Relapse Mortality			
MRD Status			

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	All (n=241)	Nonmyeloablative HCT (n=86)	Myeloablative HCT (n=155)
MRD^{neg}	1 (Reference)	1 (Reference)	1 (Reference)
MRD^{pos}	1.77 (0.83–3.80), *P*=0.140	0.46 (0.11–1.95), *P*=0.289	5.36 (1.99–14.44), *P*<0.001
HCT Type			
Myeloablative	1 (Reference)	N/A	N/A
Nonmyeloablative	2.56 (1.35–4.85), *P*=0.004		
***P*-value for interaction**	0.002	N/A	N/A

TABLE 4

Multivariate Cox Regression Models, Entire Study Cohort (n=241)

	Overall Mortality	Failure for DFS	Relapse	NRM
MRD Status				
Negative (n=190)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Positive (n=51)	2.16 (1.34–3.49), *P*=0.002	3.24 (2.03–5.17), *P*<0.001	4.51 (2.57–7.90), *P*<0.001	1.46 (0.59–3.60), *P*=0.408
HCT Type				
Myeloablative (n=155)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Nonmyeloablative (n=86)	1.58 (0.95–2.62), *P*=0.079	1.48 (0.92–2.38), *P*=0.109	1.22 (0.68–2.19), *P*=0.495	2.06 (0.87–4.87), *P*=0.099
Age (per 10 years)	0.95 (0.79–1.15), *P*=0.615	0.90 (0.76–1.06), *P*=0.192	0.84 (0.69–1.01), *P*=0.068	1.07 (0.77–1.49), *P*=0.705
HCT Comorbidity Index				
0 (n=37)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
1–2 (n=78)	0.79 (0.42–1.46), *P*=0.449	0.74 (0.41–1.34), *P*=0.324	1.03 (0.48–2.20), *P*=0.942	0.42 (0.16–1.14), *P*=0.087
≥3 (n=125)	0.81 (0.45–1.45), *P*=0.478	0.87 (0.50–1.52), *P*=0.633	1.13 (0.55–2.34), *P*=0.743	0.57 (0.24–1.35), *P*=0.198
Cytogenetic Risk Group				
Intermediate/favorable (n=170)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Adverse (n=64)	1.98 (1.15–3.41), *P*=0.014	1.66 (1.00–2.73), *P*=0.049	1.49 (0.82–2.71, *P*=0.192	2.05 (0.80–5.20), *P*=0.133
Type of AML				
De novo (n=144)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Secondary (n=97)	1.34 (0.87–2.07), *P*=0.185	1.05 (0.69–1.59), *P*=0.816	0.95 (0.56–1.60), *P*=0.841	1.28 (0.64–2.57), *P*=0.490
Number of Chemotherapy Courses before HCT	1.11 (0.95–1.30), *P*=0.181	1.16 (1.01–1.34), *P*=0.042	1.20 (1.00–1.44), *P*=0.049	1.07 (0.84–1.37), *P*=0.579
Pre-HCT Karyotype				
Normalized (n=112)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Not normalized (n=31)	1.34 (0.71–2.54), *P*=0.361	1.08 (0.59–1.96), *P*=0.802	1.08 (0.54–2.18), *P*=0.828	1.19 (0.38–3.71), *P*=0.764
Pre-HCT Blood Counts*				
Recovered (n=198)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Not recovered (n=43)	1.10 (0.65–1.88), *P*=0.713	1.30 (0.79–2.12), *P*=0.301	1.18 (0.62–2.24), *P*=0.611	1.65 (0.76–3.61), *P*=0.207

* Recovered: ANC ≥1,000/μL and platelets ≥100,000/μL; not recovered: ANC <1,000/μL and/or platelets <100,000/μL

Number of events: deaths=101; relapses=78; deaths without prior relapse=39