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Pre- and Post-Transplant Quantification of Measurable ("Minimal") Residual Disease via Multiparameter Flow Cytometry in Adult Acute Myeloid Leukemia

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Abstract

Measurable ("minimal") residual disease (MRD) before or after hematopoietic cell transplantation (HCT) identifies adults with AML at risk of poor outcomes. Here, we studied whether peri-transplant MRD dynamics can refine risk assessment. We analyzed 279 adults receiving myeloablative allogeneic HCT in first or second remission who survived at least 35 days and underwent 10-color multiparametric flow cytometry (MFC) analyses of marrow aspirates before and 28±7 days after transplantation. MFC-detectable MRD before (n=63) or after (n=16) transplantation identified patients with high relapse risk and poor survival. Forty-nine patients cleared MRD with HCT conditioning, whereas 2 patients developed new evidence of disease. The 214 MRD^{neg}/MRD^{neg} patients had excellent outcomes, whereas both MRD^{neg}/MRD^{pos} patients died within 100 days following transplantation. For patients with pre-HCT MRD, outcomes were

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CONFLICT OF INTEREST

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poor regardless of post-HCT MRD status, although survival beyond 3 years was observed among the 58 patients with decreasing but not the 7 patients with increasing peri-HCT MRD levels. In multivariable models, pre-HCT but not post-HCT MRD was independently associated with OS and RR. These data indicate that MRD^{pos} patients before transplantation have a high relapse risk regardless of whether or not they clear MFC-detectable disease with conditioning and should be considered for pre-emptive therapeutic strategies.

INTRODUCTION

For many adults with acute myeloid leukemia (AML), allogeneic hematopoietic cell transplantation (HCT) is an integral component of curative-intent therapy.[1–4] A large number of prospective studies, primarily using donor vs. no-donor comparisons, indicate that allogeneic HCT leads to better disease control and superior long-term outcomes than alternative treatments for several categories of AML patients transplanted in morphologic complete remission (CR).[3] However, outcomes vary considerably among such patients, with the depth of remission at the time of transplantation being a critical determinant for the risk of post-transplant disease recurrence. Specifically, investigations from others and our institution have demonstrated that the presence of submicroscopic amounts of “minimal” (or, perhaps more appropriately coined, “measurable”[5]) residual disease (MRD) before HCT is strongly and independently associated with increased relapse risk and shorter survival in AML patients undergoing allogeneic HCT in morphologic CR.[6–8]

Several studies have also shown that post-HCT MRD, detected by polymerase chain reaction (PCR), multiparameter flow cytometry (MFC), or (as a surrogate) levels of mixed chimerisms identify patients at high risk of relapse and poor outcome.[6–8] In contrast, very little information is available regarding the prognostic significance of peri-transplant MRD dynamics in these patients. Since bone marrow staging studies with MFC assessment for MRD are routinely obtained not only before but also at approximately day +28 following transplantation at our institution, we had the opportunity to study the relationship between peri-HCT MRD dynamics and post-transplant outcomes in a large patient cohort of consecutive patients who underwent myeloablative allogeneic HCT from a peripheral blood or bone marrow donor between 2006 and 2014. We asked whether persistence or disappearance of MRD might identify cohorts of patients in whom post-transplant therapy was particularly indicated or unnecessary.

PATIENTS AND METHODS

Study cohort

Adults 18 years of age or older with AML were included in this retrospective study if they underwent their first allogeneic HCT after myeloablative conditioning with peripheral blood or bone marrow as a stem cell source while in first or second morphologic CR or CR with incomplete blood count recovery (CRi)[1,9] irrespective of MFC-detectable MRD. We included all such patients if they underwent pre-HCT work up from April 2006 (when a refined ten-color MFC-based MRD detection method was first introduced in the clinical hematopathology service at our institution and utilized routinely in all patients) until

October 2014, an 8.5 year time period over which only minor changes were made to the MFC MRD detection panel. 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 April 24, 2015.

Classification of disease risk and treatment response

We used the 2008 WHO criteria to define AML[10] and the refined United Kingdom Medical Research Council/National Cancer Research Institute (MRC/NCRI) criteria to assign cytogenetic risk.[11] We did not include molecular data to refine disease risk. Data on the mutational status of *NPM1* at initial diagnosis were only available on 103 patients (19 mutated, 84 wild-type), while information on the presence of *FLT3* internal tandem duplication (ITD) at initial diagnosis was only available for 114 patients (41 *FLT3*-ITD, 73 wild-type). The persistence of previously documented mutations in *NPM1* and *FLT3*, as well as other genes, was not part of the routine MRD assessment in our clinical hematopathology laboratory and was only inconsistently determined in bone marrow aspirates obtained before HCT as well as around 28 days after transplantation, which precluded inclusion in our analyses. 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.[12,13] Treatment responses were categorized as proposed by international expert panels.[1,9]

MFC detection of MRD

Ten-color MFC was performed in all patients as a routine clinical test on bone marrow aspirates obtained as baseline assessment before HCT as well as around 28 days after transplantation. As described previously, a panel consisting of three antibody combinations recognizing CD4, CD5, CD7, CD13, CD14, CD15, CD16, CD19, CD33, CD34, CD38, CD45, CD56, CD64, CD71, CD117, CD123, and HLA-DR was used for MRD detection,[12–15] and up to 1 million events per tube were acquired on a custom-built LSRII. Data compensation and analysis was subsequently performed using software developed in our laboratory (WoodList). MRD was identified by visual inspection as a cell population showing deviation (typically seen in more than one antigen) from the normal patterns of antigen expression found on specific cell lineages at specific stages of maturation as compared with either normal or regenerating marrow based on the tested antibody panel.[12–15] This approach was required due to the predominance of referred patients in our study cohort for whom the abnormal immunophenotype was not available or useful. Specifically in this cohort, only 137 of 279 patients (49%) had one or more positive flow cytometric studies in our laboratory prior to the pre-transplant sample evaluation. In these patients, abnormalities observed in the pre- and post-HCT bone marrow specimen were compared those seen in prior studies. This MRD detection approach is estimated to be applicable to roughly 90% of AML patients, as assessed previously by concordance of concurrent FISH and flow cytometric assay results.[16] The sensitivity of the MFC MRD assay varies with the type of phenotypic aberrancy and immunophenotypes of normal cells

in the background populations. Therefore, the assay does not have uniform sensitivity across all cases but is able to detect MRD when present in the large majority of cases down to a level of 0.1% and in progressively smaller subsets of patients as the level of MRD decreases below that level. When identified, the abnormal population was quantified as a percentage of the total $CD45^+$ white cell events. Any measurable level of MRD was considered positive.[12–15] The results from MFC assessments of MRD were available to the transplant teams.

Statistical analysis

Unadjusted probabilities of overall survival (OS) and relapse-free survival (RFS) were estimated using the Kaplan-Meier method, and probabilities of NRM and relapse 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. Cox regression and competing risk regression models were used to assess covariate associations with outcomes. Multivariable models included the following factors: age at the time of HCT, 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 (recovered *vs.* not recovered). Missing cytogenetic risk and karyotype were accounted for as separate categories. Categorical patient characteristics were compared between individual patient groups using Fisher's exact tests, and continuous characteristics were compared with Kruskal-Wallis tests. Statistical analyses were performed using STATA (StataCorp LP, College Station, TX) and R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of study cohort

We identified 311 adults undergoing myeloablative HCT in first or second morphologic remission between May 2006 and October 2012 who had detailed pre-transplant pathological studies available for retrospective analysis. Two patients died within the first 35 days after transplantation. Among the remaining 309 patients, 279 (90.3%) underwent protocol-driven early bone marrow staging studies between post-transplant days +21 and +35. Of these 279 patients, 63 (23%) had MRD by MFC (i.e. were MRD^{pos}) at the pre-transplant baseline disease assessment, whereas 216 (77%) had no flow cytometric evidence of MRD (i.e. were MRD^{neg}) before transplantation. As we have shown in previous studies,[13,15] the outcomes for patients in first and second CR are similar at our institution (Figure 1), and these patients were therefore combined for subsequent analyses. The characteristics of these 279 patients and details of their transplants stratified by pre-HCT MRD status are summarized in Table 1. There were several statistically significant differences between patients who were in MRD^{neg} CR at the time of transplantation and those who were in MRD^{pos} CR. Specifically, MRD^{neg} patients were younger (P=0.031), less likely were male (P=0.031), less likely had adverse-risk cytogenetics rather than intermediate-or favorable-risk cytogenetics (P<0.001), less likely had secondary leukemias (P=0.009), had a longer remission duration before transplantation (P=0.004), and less likely

had persistently abnormal cytogenetic studies in the pre-transplant evaluation compared to MRD^{pos} patients ($P<0.001$).

Relationship between pre- or post-HCT MRD status and outcome

For the first set of analyses, we assessed the relationship between disease status and post-HCT outcome by using either the pre-HCT or the post-HCT MRD status in an isolated fashion, i.e. by not taking peri-HCT MRD dynamics into account. At the early post-HCT disease reassessment between day +21 and day +35, 263 patients were MRD^{neg}. Only 16 patients had MFC-detectable evidence of MRD; of these, 5 patients had morphologic and/or cytogenetic evidence of disease recurrence, whereas in the other 11 patients, patients had MRD by MFC but met morphologic and cytogenetic criteria for remission. By the day of data cut-off, 94 of the 279 patients have relapsed of whom 79 have died. Twenty-nine patients experienced NRM, for a total of 108 deaths. The median follow-up time after HCT in the 171 patients alive at last contact was 36 (range 3–99) months (for MRD^{neg} patients before HCT [n=160]: 36 [3–99] months; for MRD^{pos} patients before HCT [n=11]: 36 [78–97] months). Consistent with our previous analyses, the 63 patients with MRD before HCT had significantly shorter OS and RFS and higher risk of relapse than the 216 MRD^{neg} patients ($P<0.001$, Figure 2A, B and Table 2). Likewise, the 16 patients with MRD at the early post-HCT disease reassessment between day +21 and day +35 had significantly shorter OS and RFS and higher risk of relapse than the 263 MRD^{neg} patients ($P<0.001$, Figure 2C, D and Table 2).

Relationship between peri-HCT MRD dynamics and post-HCT outcome

In a second set of analyses, we then assessed the relationship between peri-HCT disease burden dynamics and post-HCT outcome. In our cohort of 279 patients, 49 patients cleared MRD-level disease between the pre- and post-HCT disease assessment, whereas 2 patients developed new evidence of disease; both of these patients had morphologic evidence of disease recurrence. This resulted in 214 patients (77%) who had no MFC evidence of MRD before and after HCT (i.e. were MRD^{neg}/MRD^{neg}), 2 patients (0.7%) who were MRD^{neg}/MRD^{pos}, 49 patients (18%) who were MRD^{pos}/MRD^{neg}, and 14 patients (5%) who were MRD^{pos}/MRD^{pos}. Of the 65 patients with detectable MRD before and/or after transplantation, 58 had decreasing levels of MRD ("MRD^{decr}") over the peri-HCT period, whereas 7 patients had increasing MRD levels ("MRD^{incr}") around the time of transplantation. As depicted in Figure 3 and Table 2, the 214 MRD^{neg}/MRD^{neg} patients had the best long-term outcomes, with 3-year OS and RFS estimates of 76% (69–82%) and 71% (65–78%), respectively, and a cumulative incidence of relapse of 22% (16–28%) at 3-years. In contrast, both MRD^{neg}/MRD^{pos} patients died within 100 days transplant.

For patients who were MRD^{pos} before transplantation, outcomes were relatively poor regardless of whether or not they had persistent MRD around day +28 after transplantation. Specifically, at 3 years, MRD^{pos}/MRD^{neg} patients had an estimated OS and RFS of 29% (18–45%) and 18% (10–34%), and a relapse risk of 65% (51–79%), whereas these 3-year estimates were 19% (6–60%) for OS, 14% (4–52%) for RFS, and 79% (55–100%) for relapse risk for MRD^{pos}/MRD^{pos} patients, respectively. However, long-term survival was only observed among MRD^{decr} patients (at 3 years: 29% [19–44%] for OS, 19% [10–33%]

for RFS, and 66% [53–79%] for relapse risk), whereas all MRD^{incr} patients died, with a median of 125 (range: 43–836) days following transplant (Figure 4).

Pre-HCT and post-HCT MRD status as independent prognostic factors

Lastly, we fit regression models to assess the effect of pre- and post-HCT MRD status after accounting for the covariates noted in *Patients & Methods*, we developed multivariable models for OS, RFS, relapse, and NRM. These models indicated that independent of these covariates being MRD^{neg} before transplantation was associated with better outcomes (longer OS and PFS as well as lower risk of relapse) than being MRD^{pos} pre-HCT, whereas there were no significant associations between pre-HCT MRD status and NRM (Table 3). On the other hand, there were no statistically significant associations between post-HCT MRD status and OS, RFS, relapse, or NRM.

DISCUSSION

Approximately 20–25% of adults with AML who present for allogeneic HCT while in first or second morphologic remission have flow cytometric evidence of MRD at the time of pre-transplant disease staging.[12–15] Consistent with our previous reports[12,14,15] and data from other investigators,[17–21] MRD^{pos} patients in the current study were found to have a substantially increased risk of post-HCT relapse (cumulatively approaching 65–70% after 3 years) and lower survival (3-year estimate of about 25%) compared to patients who underwent transplantation in MRD^{neg} remission (at 3 years: relapse risk of 20–25% and survival of about 75%). Hence, our current results confirm the value of pre-HCT MRD detection as a means of identifying a subset of patients with AML who are at high risk of poor outcome following myeloablative allogeneic HCT.

As a novel finding, the data from this study indicate that myeloablative conditioning is able to suppress the burden of leukemia cells below the current level of MRD detection in the majority of patients who present with MRD^{pos} remission at the time of transplantation. Specifically, in our cohort, 49 out of the 63 patients (78%) who were MRD^{pos} during the pre-HCT disease staging examination had no MFC evidence of MRD 28±7 days after transplantation. Yet, while this conversion from MRD^{pos} to MRD^{neg} may be reassuring to treating physicians and patients alike, our data suggest that such patients nevertheless have poor outcomes. Indeed, the cumulative incidence of relapse of 65% at 3 years in these MRD^{pos}/MRD^{neg} patients appears very similar to the approximately 80% relapse risk in the 14 patients who were MRD^{pos} both before and after transplantation, although the power to detect any statistically significant difference in outcomes between these two groups is very low given the small sample sizes of these patient subsets.

Our data also show that an increase in MRD burden over the peri-transplant period is uncommon in AML patients undergoing myeloablative allogeneic HCT in morphologic remission. In our cohort of 279, we observed increasing MRD levels only in 7 (3%) patients, including 2 patients who converted from MRD^{neg} to MRD^{pos} over the transplant period, limiting our abilities to obtain precise outcome estimates. Nevertheless, these 7 patients had particularly adverse outcomes, with a 100% risk of relapse by 3 years and a 3-year survival of 0% in our cohort.

From a practical perspective, an important question to address is what the relative value is of pre- and post-HCT MRD assessments by flow cytometry? The data from this study suggest that pre-HCT MRD assessments may be the most helpful for risk stratification as they identify a larger proportion of patients at high risk of post-HCT relapse (in our cohort: 63/279 [23%] were MRD^{pos} before transplantation but only 16/279 [6%] on day 28±7 after transplantation). If MRD information were only obtained after transplantation, a large portion of the high-risk patients would be missed. The notion that pre-HCT MRD assessments are more helpful for risk stratification than post-HCT MRD assessments is supported by our multivariable analyses, in which only pre-HCT MRD status but not post-HCT MRD status was independently associated with OS, RFS, and RR if both MRD determinations and several other potential risk factors were included in the models. Nevertheless, our data also suggest that the risk-assessment of AML patients undergoing HCT in morphologic remission can be refined if both pre- and post-HCT MRD measurements are considered, although this refinement will affect only a small number of patients. Specifically, if a patient is MRD^{neg} before transplantation, the likelihood of remaining MRD^{neg} over the transplant period is very high. In our cohort, only 2 out of 216 patients (1%) showed new evidence of leukemia on day 28±7 after transplantation. One might argue that this very low chance of conversion to an MRD^{pos} status may not justify the repetition of MRD testing early after transplantation. Likewise, for patients who are MRD^{pos} before transplantation, peri-HCT MRD dynamics would allow the identification of a relatively small subset of patients (in our cohort: 14/63 [22%]) who remain MRD positive. However, their outcomes overall appear only slightly worse than those for patients who convert to a MRD^{neg} state (thus, post-transplant MRD assessment provides little disease risk refinement) after transplantation except in the small fraction of patients (in our cohort: 5/63 [8%]) who experience an increase of MRD levels over the transplant period and have particularly poor survival expectations.

At our institution, patients with AML in CR are routinely assigned to myeloablative 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. Although perceived as indicator of high relapse risk, the presence of MRD typically plays no major role in the selection of the type of preparative regimen and/or immunosuppression. When residual disease was detected at the submicroscopic or microscopic level, no uniform treatment approach was pursued. Strategies employed included expedited withdrawal of immunosuppressive agents with or without use of donor lymphocyte infusions, treatment with hypomethylating and other molecularly targeted agents, or administration of intensive chemotherapy. The fact that the pre- and post-HCT MRD status of all patients was known to the transplant physicians offers the possibility of bias in our study results if treatment protocols were modified as a result of positive pre- and/or post-HCT MRD measurements. If effective, however, such maneuvers would be expected to decrease (rather than increase) the magnitude of difference in relapse risk and survival between MRD^{pos} and MRD^{neg} patients while potentially increasing the NRM risk for MRD^{pos} patients.

MRD detection in AML is complicated by the genetic and molecular disease heterogeneity, disease evolution over the course of the illness, and the possible emergence of minor

subclones at the time of recurrence.[22,23] So far, no single approach has emerged as clearly superior, and uncertainties persist in terms of optimal targets, assay platform, monitoring scheme, thresholds to declare MRD positivity, and data analysis.[24,25] In the current as well as several previous investigations,[12–15] we an MFC-based approach served to detect and quantify MRD that relies on the identification of a cell population showing a deviation from antigen expression patterns seen on hematopoietic cells in normal or regenerating marrow. As an advantage, this approach does not require the availability of a previously determined leukemia-associated immunophenotype and is applicable to approximately 90% of AML patients. As a potential disadvantage, the assay does not have a uniform sensitivity across all cases, and the sensitivity may not reach that obtained with optimized polymerase-chain reaction (PCR)-based methods. If individual fusion genes or mutations are measured, however, the latter methods may either fail to accurately measure clearance of AML cells or only be useful for defined, small subsets of patients. Clearance of molecular abnormalities in assays that are based on whole-genome or exome sequencing may provide an approach that can measure MRD more universally in AML patients with high sensitivity.[26] It is likely that such assays will soon be tested for the monitoring of MRD of AML patients undergoing allogeneic transplantation, and future studies will need to assess to what degree, if any, they provide clinically more useful information than MFC-based MRD assays. Likewise, flow cytometric methods have been developed that, rather than quantifying bulk cells with abnormal immunophenotype, quantify immature cell populations that are enriched for putative leukemic stem cells (LSCs).[27–31] A recent study has indicated that MFC detection of LSC populations is less frequent than MFC detection of MRD in AML patients undergoing allogeneic HCT but is sometimes identified in patients who could not be monitored by a conventional MFC-based MRD assay.[31] In this study of a relatively small set of adults undergoing either myeloablative or reduced-intensity conditioning, MFC-based LSC detection provided more robust prognostic information than a conventional flow cytometric MRD assay.[31] It is therefore conceivable that LSC detection assays could further refine (or replace) peri-HCT MRD assays to risk stratify AML patients undergoing myeloablative HCT.

In summary, our data confirm that adult AML patients who are in morphologic remission and have no MFC evidence of MRD at the time of myeloablative allogeneic HCT are at very low risk of early post-HCT emergence of MRD and have an expected cure rate of approximately 75%. In contrast, patients who have MRD before transplantation have significantly worse survival expectations regardless of whether or not they clear MFC-measurable disease within the first 28 days after transplantation, and in addition, long-term survival is only found among those patients with decreasing MRD levels over the peri-transplant period. Together, these data suggest that all patients with pre-HCT MRD should be considered for pre-emptive therapeutic strategies given their high risk of disease recurrence regardless of the early post-HCT MRD information. Such strategies could include the administration of additional pre-transplant chemotherapy, intensified conditioning regimens, reduced use of immunosuppressive medications, and/or administration of post-transplant chemo- or immunotherapeutics, ideally in the setting of controlled clinical trials of sufficient power and adequate follow-up time. One important goal of these investigations should be to establish (or refute) the value of MRD-directed

therapy in AML patients undergoing myeloablative HCT. Efforts to harmonize MRD assays across institutions will hopefully facilitate such investigations and implementation of findings in clinical practice.

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References

1. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010; 115(3):453–474. [PubMed: 19880497]
2. Gupta V, Tallman MS, Weisdorf DJ. Allogeneic hematopoietic cell transplantation for adults with acute myeloid leukemia: myths, controversies, and unknowns. Blood. 2011; 117(8):2307–2318. [PubMed: 21098397]
3. Cornelissen JJ, Gratwohl A, Schlenk RF, Sierra J, Bornhäuser M, Juliusson G, et al. The European LeukemiaNet AML Working Party consensus statement on allogeneic HSCT for patients with AML in remission: an integrated-risk adapted approach. Nat Rev Clin Oncol. 2012; 9(10):579–590. [PubMed: 22949046]
4. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015; 373(12): 1136–1152. [PubMed: 26376137]
5. Goldman JM, Gale RP. What does MRD in leukemia really mean? Leukemia. 2014; 28(5):1131. [PubMed: 24170026]
6. Campana D, Leung W. Clinical significance of minimal residual disease in patients with acute leukaemia undergoing haematopoietic stem cell transplantation. Br J Haematol. 2013; 162(2):147–161. [PubMed: 23654352]
7. Hourigan CS, Karp JE. Minimal residual disease in acute myeloid leukaemia. Nat Rev Clin Oncol. 2013; 10(8):460–471. [PubMed: 23799371]
8. Buckley SA, Appelbaum FR, Walter RB. Prognostic and therapeutic implications of minimal residual disease at the time of transplantation in acute leukemia. Bone Marrow Transplant. 2013; 48(5):630–641. [PubMed: 22825427]
9. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol. 2003; 21(24):4642–4649. [PubMed: 14673054]
10. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009; 114(5):937–951. [PubMed: 19357394]
11. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010; 116(3):354–365. [PubMed: 20385793]
12. Walter RB, Gyurkocza B, Storer BE, Godwin CD, Pagel JM, Buckley SA, et al. Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. Leukemia. 2015; 29(1):137–144. [PubMed: 24888275]

13. Araki D, Wood BL, Othus M, Radich JP, Halpern AB, Zhou Y, et al. Allogeneic hematopoietic cell transplantation for acute myeloid leukemia: is it time to move toward a minimal residual disease-based definition of complete remission. J Clin Oncol. 2016 in press.
14. Walter RB, Gooley TA, Wood BL, Milano F, Fang M, Sorror ML, et al. Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. J Clin Oncol. 2011; 29(9):1190–1197. [PubMed: 21282535]
15. Walter RB, Buckley SA, Pagel JM, Wood BL, Storer BE, Sandmaier BM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. Blood. 2013; 122(10):1813–1821. [PubMed: 23847197]
16. Fang M, Storer B, Wood B, Gyurkocza B, Sandmaier BM, Appelbaum FR. Prognostic impact of discordant results from cytogenetics and flow cytometry in patients with acute myeloid leukemia undergoing hematopoietic cell transplantation. Cancer. 2012; 118(9):2411–2419. [PubMed: 21928360]
17. Maurillo L, Buccisano F, Del Principe MI, Del Poeta G, Spagnoli A, Panetta P, et al. Toward optimization of postremission therapy for residual disease-positive patients with acute myeloid leukemia. J Clin Oncol. 2008; 26(30):4944–4951. [PubMed: 18606980]
18. Grubovikj RM, Alavi A, Koppel A, Territo M, Schiller GJ. Minimal residual disease as a predictive factor for relapse after allogeneic hematopoietic stem cell transplant in adult patients with acute myeloid leukemia in first and second complete remission. Cancers (Basel). 2012; 4(2):601–617. [PubMed: 24213327]
19. Anthias C, Dignan FL, Morilla R, Morilla A, Ethell ME, Potter MN, et al. Pre-transplant MRD predicts outcome following reduced-intensity and myeloablative allogeneic hemopoietic SCT in AML. Bone Marrow Transplant. 2014; 49(5):679–683. [PubMed: 24510069]
20. Bastos-Oreiro M, Perez-Corral A, Martínez-Laperche C, Bento L, Pascual C, Kwon M, et al. Prognostic impact of minimal residual disease analysis by flow cytometry in patients with acute myeloid leukemia before and after allogeneic hemopoietic stem cell transplantation. Eur J Haematol. 2014; 93(3):239–246. [PubMed: 24702162]
21. Rossi G, Carella AM, Minervini MM, di Nardo F, Waure C, Greco MM, et al. Optimal time-points for minimal residual disease monitoring change on the basis of the method used in patients with acute myeloid leukemia who underwent allogeneic stem cell transplantation: a comparison between multiparameter flow cytometry and Wilms' tumor 1 expression. Leuk Res. 2015; 39(2): 138–143. [PubMed: 25498507]
22. Zeijlemaker W, Gratama JW, Schuurhuis GJ. Tumor heterogeneity makes AML a "moving target" for detection of residual disease. Cytometry B Clin Cytom. 2014; 86(1):3–14. [PubMed: 24151248]
23. Graubert TA, Brunner AM, Fathi AT. New molecular abnormalities and clonal architecture in AML: from reciprocal translocations to whole-genome sequencing. Am Soc Clin Oncol Educ Book. 2014:e334–340. [PubMed: 24857122]
24. Grimwade D, Freeman SD. Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"? Blood. 2014; 124(23):3345–3355. [PubMed: 25049280]
25. Hokland P, Ommen HB, Mulé MP, Hourigan CS. Advancing the minimal residual disease concept in acute myeloid leukemia. Semin Hematol. 2015; 52(3):184–192. [PubMed: 26111465]
26. Klco JM, Miller CA, Griffith M, Petti A, Spencer DH, Ketkar-Kulkarni S, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. JAMA. 2015; 314(8):811–822. [PubMed: 26305651]
27. van Rhenen A, Moshaver B, Kelder A, Feller N, Nieuwint AW, Zweegman S, et al. Aberrant marker expression patterns on the CD34+CD38- stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell compartment both at diagnosis and in remission. Leukemia. 2007; 21(8):1700–1707. [PubMed: 17525725]
28. Goardon N, Marchi E, Atzberger A, Quek L, Schuh A, Soneji S, et al. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell. 2011; 19(1):138–152. [PubMed: 21251617]

29. Gerber JM, Smith BD, Ngwang B, Zhang H, Vala MS, Morsberger L, et al. A clinically relevant population of leukemic CD34(+)CD38(−) cells in acute myeloid leukemia. Blood. 2012; 119(15): 3571–3577. [PubMed: 22262762]

30. Terwijn M, Zeijlemaker W, Kelder A, Rutten AP, Snel AN, Scholten WJ, et al. Leukemic stem cell frequency: a strong biomarker for clinical outcome in acute myeloid leukemia. PLoS One. 2014; 9(9):e107587. [PubMed: 25244440]

31. Bradbury C, Houlton AE, Akiki S, Gregg R, Rindl M, Khan J, et al. Prognostic value of monitoring a candidate immunophenotypic leukaemic stem/progenitor cell population in patients allografted for acute myeloid leukaemia. Leukemia. 2015; 29(4):988–991. [PubMed: 25425198]

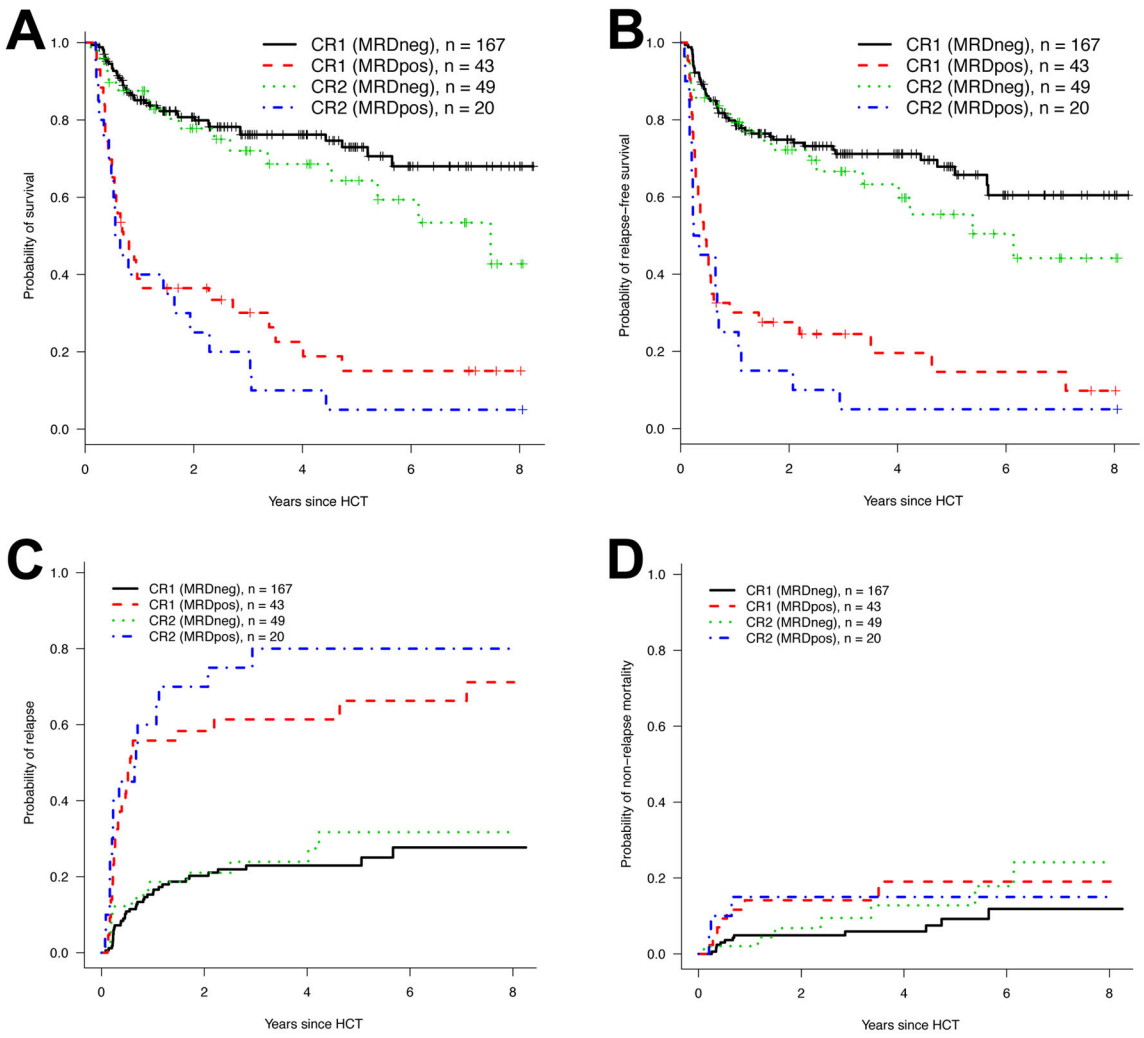

Figure 1. Association between pre-transplant disease status and outcome for 279 AML patients undergoing myeloablative HCT while in morphologic remission

Estimates of **(A)** overall survival, **(B)** relapse-free survival, **(C)** cumulative risk of relapse, and **(D)** cumulative risk of non-relapse mortality following myeloablative allogeneic HCT for adults with AML who underwent bone marrow assessments with MFC before as well as on day +28 (± 7 days) after transplantation. Outcome estimates are shown individually for patients in MRD^{neg} remission 1 (n=167), MRD^{neg} remission 2 (n=49), MRD^{pos} remission 1 (n=43), and MRD^{pos} remission 2 (n=20) at the time of transplantation, respectively.

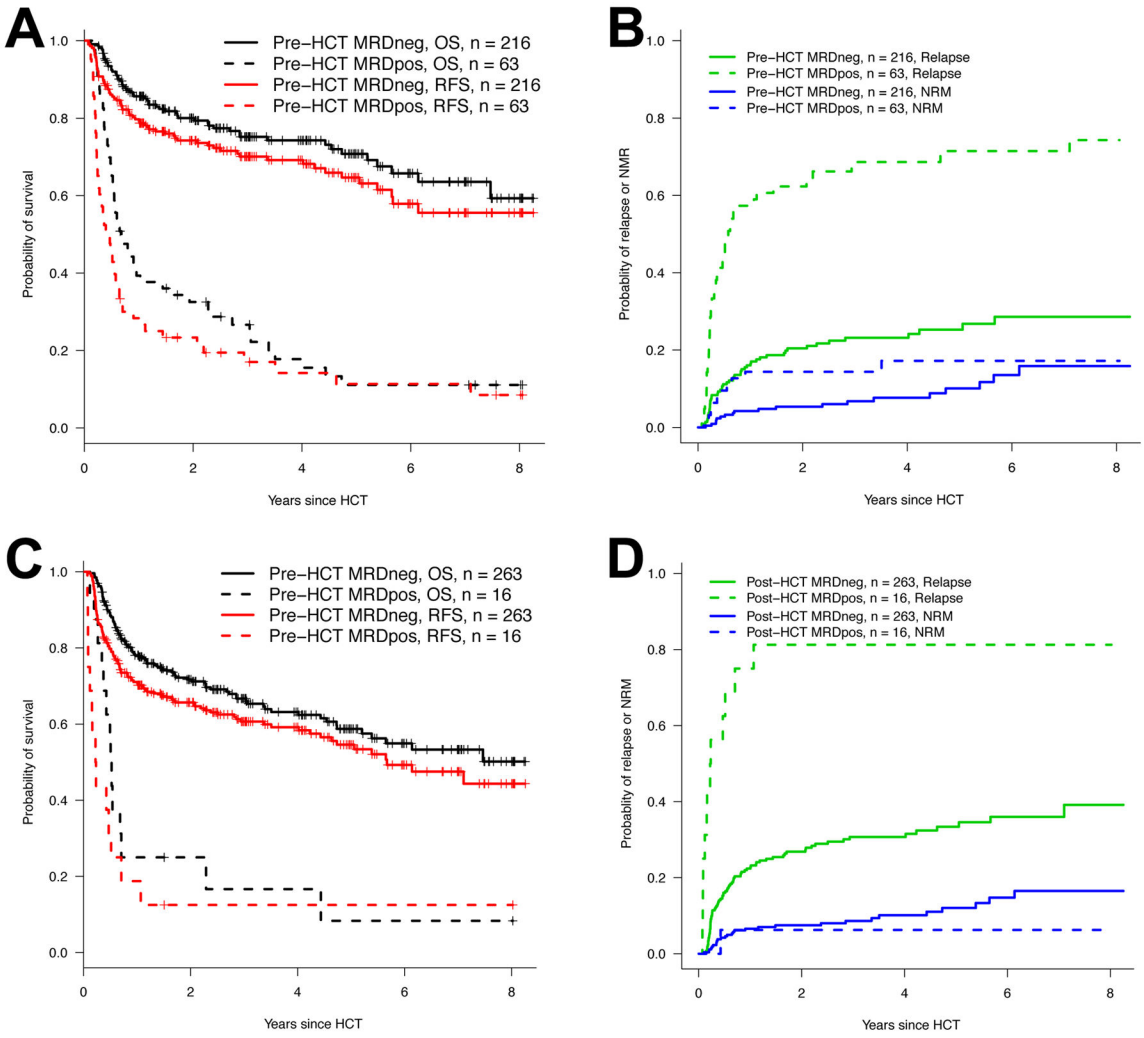

Figure 2. Association between pre-HCT or post-HCT MRD status and outcome following myeloablative HCT

Kaplan-Meier estimates of **(A)** overall survival (OS) and relapse-free survival (RFS) as well as **(B)** cumulative incidences of relapse and non-relapse mortality (NRM), shown individually for patients with (n=63) or without (n=216) MFC-evidence of MRD in the pre-HCT bone marrow examination. Kaplan-Meier estimates of **(C)** OS and RFS as well as **(D)** cumulative incidences of relapse and NRM, shown individually for patients with (n=16) or without (n=263) MFC-evidence of MRD on day +28 (± 7 days) after transplantation.

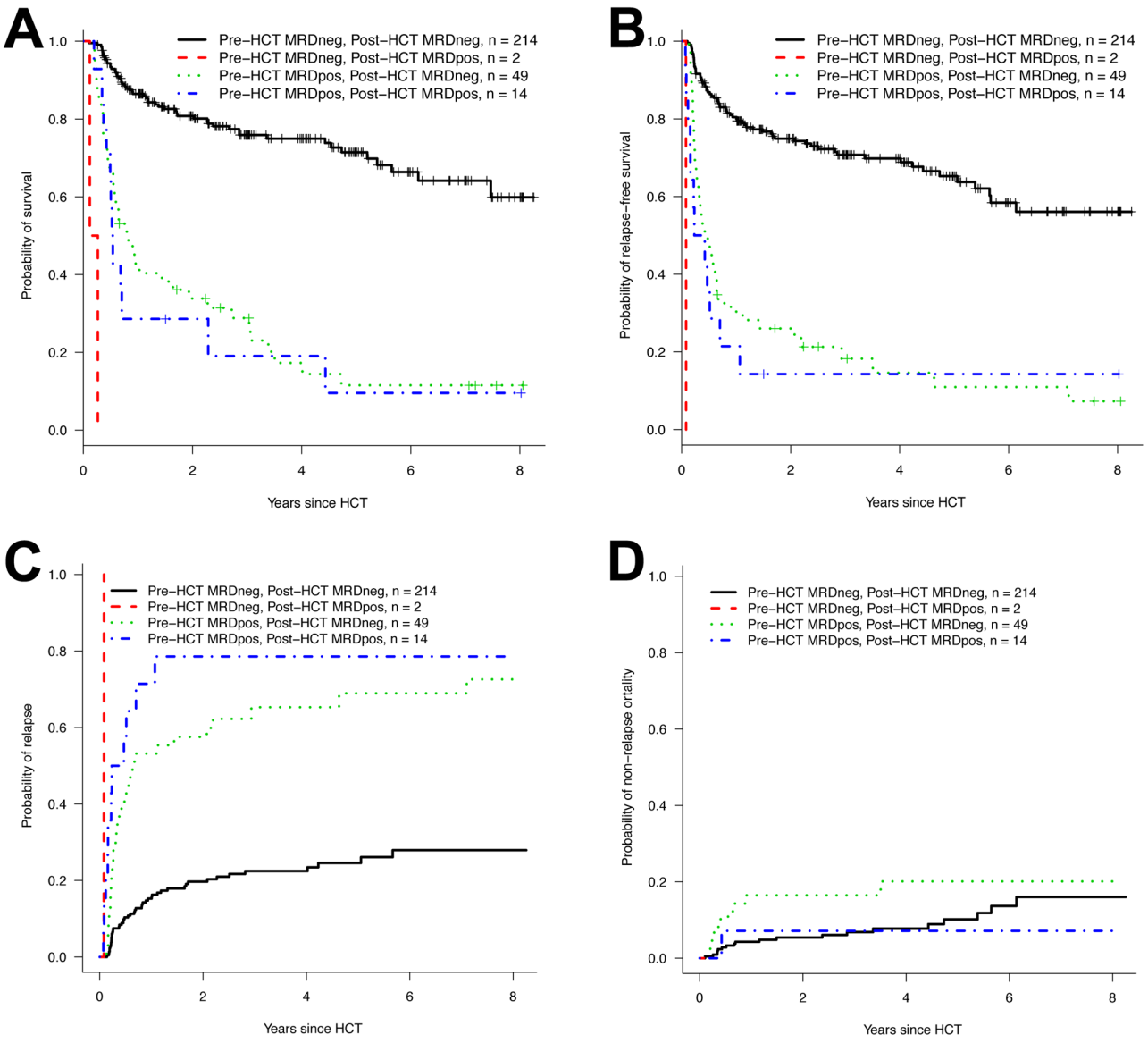

Figure 3. Association between peri-HCT MRD dynamics and outcome for AML patients following myeloablative HCT, stratified by positive/negative MRD status

Kaplan-Meier estimates of **(A)** overall survival and **(B)** relapse-free survival as well as cumulative incidences of **(C)** relapse, and **(D)** non-relapse mortality following myeloablative allogeneic HCT for adults with AML, shown individually for patients with MRD^{neg}/MRD^{neg} (n=214), MRD^{neg}/MRD^{pos} (n=2), MRD^{pos}/MRD^{neg} (n=49) and MRD^{pos}/MRD^{pos} (n=14) disease status in the pre/post-HCT bone marrow assessment.

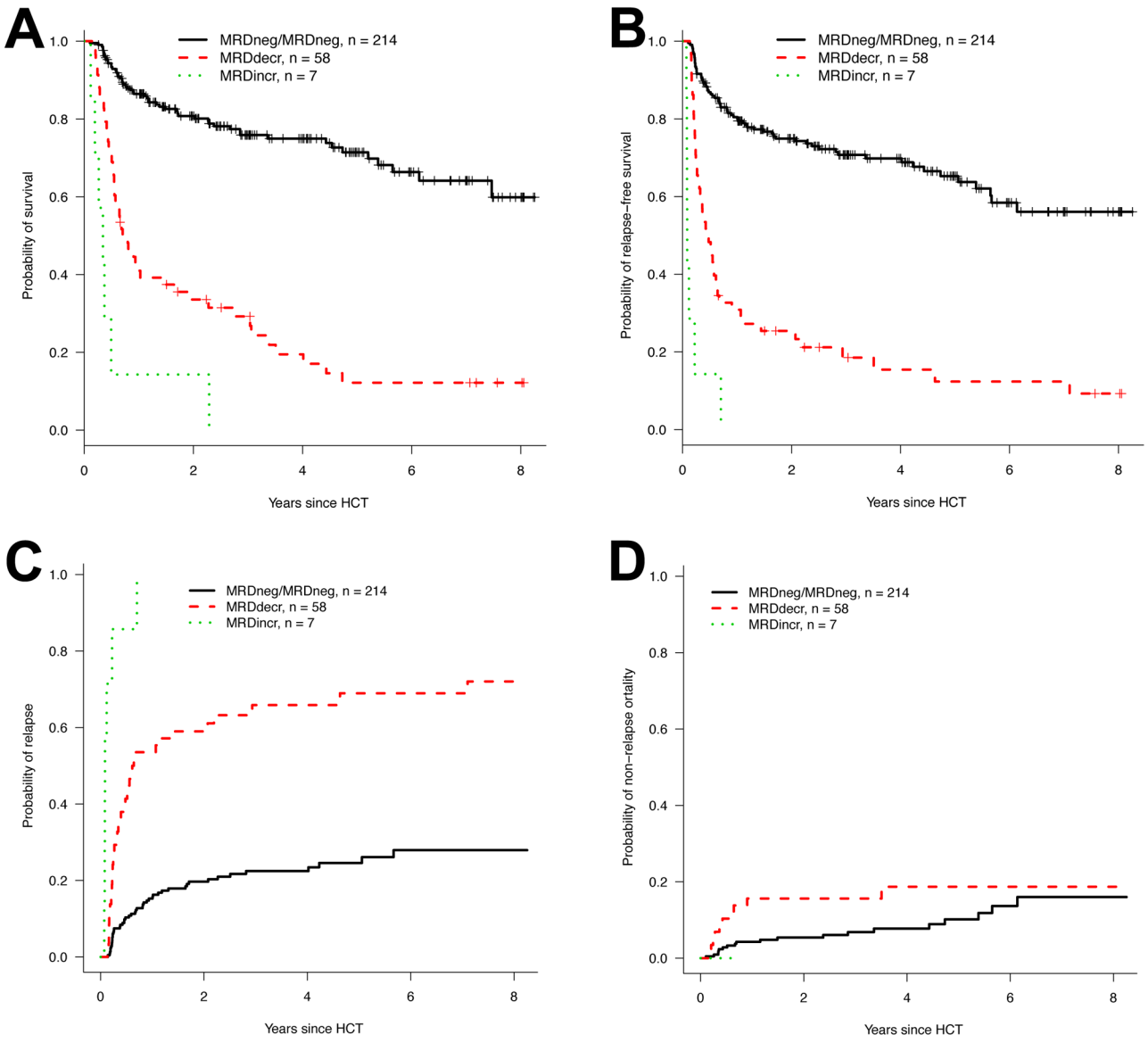

Figure 4. Association between peri-HCT MRD dynamics and outcome for AML patients following myeloablative HCT, stratified by increasing/decreasing MRD levels

Kaplan-Meier estimates of **(A)** overall survival and **(B)** relapse-free survival as well as cumulative incidences of **(C)** relapse, and **(D)** non-relapse mortality following myeloablative allogeneic HCT for adults with AML, shown individually for patients with MRD^{neg}/MRD^{neg} (n=214), MRD^{decr} (n=58), and MRD^{incr} (n=7) disease status in the pre/post-HCT bone marrow assessment.

Leukemia. Author manuscript; available in PMC 2016 August 29.

TABLE 1

Demographic and clinical characteristics of study cohort, stratified by pre-HCT disease status

	MRDneg (n=216)	MRDpos (n=63)	All patients (n=279)	*P*-value
Median age at HCT (range), years	48 (19–71)	51 (18–72)	49 (18–72)	0.031
Male gender	110 (51%)	42 (67%)	152 (54%)	0.031
Median WBC at diagnosis (range), x10^3/μL	10 (0.3–297)	3 (0.3–250)	8.0 (0.3–297)	0.040
Cytogenetic risk, n (%)				<0.001
Favorable	21 (10%)	0 (0%)	21 (8%)	
Intermediate	154 (73%)	37 (61%)	191 (70%)	
Adverse	37 (17%)	24 (39%)	61 (22%)	
Missing	4	2	6	
Secondary AML	49 (23%)	25 (40%)	74 (27%)	0.009
CR status, n (%)				0.18
CR1	167 (77%)	43 (68%)	210 (75%)	
CR2	49 (23%)	20 (32%)	69 (25%)	
Median CR duration before HCT (range), days	104 (7–465)	71 (16–485)	100 (7–485)	0.004
Recovered peripheral blood counts before HCT *	176 (81%)	48 (76%)	224 (80%)	0.37
Routine karyotyping before HCT, n (%)				<0.001
Normalized karyotype	98 (45%)	22 (35%)	120 (43%)	
Abnormal karyotype	20 (9%)	19 (30%)	39 (14%)	
Missing/non-informative data	98 (45%)	22 (35%)	120 (43%)	
Median abnormal blasts by MFC (range)	0 (0–41%)	0.90 (0.007–19.4%)	0 (0–19.4%)	<0.001
Unrelated donor	127 (59%)	38 (60%)	165 (59%)	0.88
Conditioning regimen				0.013
BU/CY ± L-TBI	70 (32%)	22 (35%)	92 (33%)	
BU/FLU, BU/VP16, or BU/CLO	58 (27%)	11 (17%)	69 (25%)	
H-TBI ± CY or FLU	22 (10%)	7 (11%)	29 (10%)	
H-TBI/Tepa/FLU	10 (5%)	1 (2%)	11 (4%)	
Treo/FLU ± L-TBI	46 (21%)	10 (16%)	56 (20%)	

	MRDneg (n=216)	MRDpos (n=63)	All patients (n=279)	*P*-value
FLU/Radiolabeled Ab/L-TBI ± CY	10 (5%)	12 (19%)	22 (8%)	
Stem cell source				0.48
PBSC	174 (81%)	48 (76%)	222 (80%)	
BM	42 (19%)	15 (24%)	57 (20%)	
GVHD prophylaxis				0.018
Calcineurin Inhibitor + Methotrexate	167 (77%)	42 (67%)	209 (75%)	
Calcineurin Inhibitor + MMF	10 (5%)	10 (16%)	20 (7%)	
CY ± Calcineurin Inhibitor ± MMF	29 (13%)	10 (16%)	39 (14%)	
Other	10 (5%)	1 (2%)	11 (4%)	
Post-HCT disease status (at day 28±7 days), n (%)				
MRD^{neg}	214 (99%)	49 (78%)	263 (94%)	
MRD^{pos}	2 (1%)	14 (22%)	16 (6%)	

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

Abbreviations: Ab, antibody; BM, bone marrow; BU, busulfan; CLO, clofarabine; CR1, first CR; CR2, second CR; 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; Tepa; thiotepa; Treo, treosulfan; WBC, total white blood cell count.

TABLE 2

Outcome probabilities stratified by pre- or peri-HCT MRD status

	OS at 3 years	RFS at 3 years	CI of relapse at 3 years	CI of NRM at 3 years
Pre-HCT MRD information only				
Pre-HCT MRD^{neg} (n=216)	75% (69–81%)	70% (64–77%)	23% (17–29%)	7% (4–10%)
Pre-HCT MRD^{pos} (n=63)	27% (17–41%)	17% (10–30%)	69% (56–81%)	14% (5–22%)
Post-HCT MRD information only				
Post-HCT MRD^{neg} (n=263)	67% (61–73%)	61% (55–67%)	31% (25–37%)	9% (6–12%)
Post-HCT MRD^{pos} (n=16)	17% (5–54%)	13% (3–46%)	81% (60–100%)	6% (0–18%)
Pre- and post-HCT MRD information				
MRD^{neg}/MRD^{neg} (n=214)	76% (69–82%)	71% (65–78%)	22% (16–28%)	7% (4–10%)
MRD^{neg}/MRD^{pos} (n=2)	0%	0%	100%	0%
MRD^{pos}/MRD^{neg} (n=49)	29% (18–45%)	18% (10–34%)	65% (51–79%)	16% (5–27%)
MRD^{pos}/MRD^{pos} (n=14)	19% (6–60%)	14% (4–52%)	79% (55–100%)	7% (0–21%)
MRD^{decr} (n=58)	29% (19–44%)	19% (10–33%)	66% (53–79%)	16% (7–25%)
MRD^{incr} (n=7)	0%	0%	100%	0%

TABLE 3

Multivariable regression models for pre-HCT and post-HCT disease status

	Overall mortality	Failure for RFS	Relapse	NRM
Pre-HCT Disease Status				
MRD^{neg} status (n=216)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
MRD^{pos} status (n=63)	4.66 (2.95–7.36), *P*<0.001	4.18 (2.73–6.39), *P*<0.001	3.66 (2.19–6.13), *P*<0.001	2.28 (1.00–5.21), *P*=0.050
Post-HCT Disease Status				
MRD^{neg} status (n=263)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
MRD^{pos} status (n=16)	1.45 (0.72–2.91), *P*=0.296	1.45 (0.72–2.94), *P*=0.300	1.74 (0.73–4.16), *P*=0.210	0.43 (0.04–4.05), *P*=0.460
Age	1.00 (0.98–1.02), *P*=0.875	1.00 (0.98–1.01), *P*=0.605	0.99 (0.97–1.00), *P*=0.120	1.03 (0.99–1.07), *P*=0.120
Cytogenetic Risk Group				
Adverse (n=61)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Intermediate/favorable (n=212)	1.15 (0.67–1.97), *P*=0.617	0.93 (0.57–1.53), *P*=0.774	0.76 (0.44–1.32), *P*=0.330	2.27 (0.74–7.00), *P*=0.150
Type of AML				
De novo (n=74)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Secondary (n=205)	0.98 (0.62–1.55), *P*=0.937	1.15 (0.76–1.75), *P*=0.506	1.11 (0.70–1.78), *P*=0.650	1.11 (0.43–2.88), *P*=0.830
Pre-HCT Karyotype				
Not normalized (n=39)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Normalized (n=120)	0.64 (0.36–1.14), *P*=0.130	0.63 (0.36–1.10), *P*=0.102	0.66 (0.36–1.20), *P*=0.170	0.95 (0.28–3.26), *P*=0.930
Pre-HCT Blood Counts *				
Not recovered (n=55)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Recovered (n=224)	1.17 (0.71–1.93), *P*=0.546	1.00 (0.63–1.58), *P*=0.995	0.75 (0.43–1.30), *P*=0.300	1.43 (0.48–4.29), *P*=0.520

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