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Pre-transplant Bone Marrow Monocytic Myeloid-Derived Suppressor Cell Frequency Is Not Associated with Outcome after Allogeneic Hematopoietic Cell Transplantation for Acute Myeloid Leukemia in Remission

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Outcomes in acute myeloid leukemia (AML) vary widely (1). Current prognostic models cannot fully capture this diversity (2, 3), prompting interest in identifying additional factors to refine outcome prediction. Mounting evidence from other cancers suggests myeloid-derived suppressor cells (MDSCs) may be such a factor. Different types of MDSCs, including polymorphonuclear MDSCs (PMN-MDSCs; typically characterized as CD14 negative and CD11b, CD15 or CD66b positive) and monocytic MDSCs (M-MDSCs; typically characterized as CD14 positive and HLA-DR low/negative), have been studied as modulators of the anti-cancer immune response (4). Expansion of MDSCs has been associated with poor prognosis in some solid tumors (5). MDSCs may also play a role in the pathogenesis and/or prognosis of hematologic malignancies. For example, M-MDSCs expansion has been associated with poor outcome in myeloma (6) while MDSC signaling modulation can improve myelodysplastic hematopoiesis *in vitro* (7). Less is known about the role(s) of MDSCs in AML. Not felt to be derived from the malignant clone, MDSCs are

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expanded in AML vs. healthy patients possibly because of leukemia-microenvironment interactions (8). A small study also suggested MDSC frequencies are associated with disease burden after AML chemotherapy (9) but larger studies on the prognostic role of MDSCs in AML are lacking. We hypothesized that in AML patients undergoing hematopoietic cell transplantation (HCT), leukemia burden pre-HCT would be associated with MDSC frequency and that elevated MDSC frequency could portend poor post-HCT outcomes in AML just as it portends poor prognosis in a variety of other malignancies (5–7). Here, we used a large cohort of adults with AML (based on 2016 WHO criteria (10)) undergoing allogeneic HCT in first morphologic complete remission (CR) or CR with incomplete hematologic recovery (CRi) (1) to test this hypothesis and examine the associations between M-MDSC frequency, pre-transplant measurable ('minimal') residual disease (MRD) and post-HCT outcome.

Adults ≥18 years of age were included if they underwent a first allograft after myeloablative or nonmyeloablative conditioning with peripheral blood or bone marrow (BM) as stem cell source from 4/2006 until 10/2014. We used the refined MRC/NCRI criteria to assign cytogenetic risk (11). Secondary leukemia was defined as AML following an antecedent hematologic disorder or prior chemotherapy/radiotherapy treatment (12). All patients were treated on Institutional Review Board (IRB)-approved protocols or standard treatment plans and gave consent in accordance with the Declaration of Helsinki. Follow-up was current as of February 16, 2018.

Ten-color multiparameter flow cytometry (MFC) was performed routinely on BM aspirates with a panel of three antibody combinations as part of the pre-transplant work-up (13, 14). When identified, an abnormal population was quantified as a percentage of the total CD45⁺ cells. Any level of residual disease was considered MRD^{pos} (13). The results from MFC assessment of MRD were available to the transplant teams. In IRB-approved retrospective analyses, pre-transplant MFC data from the BM aspirates were used to quantify M-MDSCs (available antibody combinations did not allow quantification of PMN-MDSCs). M-MDSCs were also quantified in the peripheral blood if specimens were analyzed by MFC. Monocytes were identified by side scatter properties and CD14 positivity, and M-MDSCs quantified as the proportion of the cells with low or negative expression for HLA-DR (15), using mature myeloid cells as an internal negative control for HLA-DR (Supplementary Figure 1).

For statistical analysis, overall survival (OS) and relapse-free survival (RFS) were estimated using the Kaplan-Meier method. Probabilities of non-relapse mortality (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. Associations with OS and RFS were assessed using Cox regression, while proportional subdistribution hazard models that account for competing risks assessed associations with relapse and NRM. Categorical characteristics were compared between individual patient groups using chi-square tests, and continuous characteristics were compared with Kruskal-Wallis 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), R (R Foundation for Statistical Computing, Vienna, Austria) and Prism (GraphPad Software, San Diego, CA).

We identified 349 adults meeting our study eligibility criteria for whom pre-transplant MRD testing was performed and follow-up data were available. Of these, 8 did not have sufficient data for identification of BM M-MDSCs and were excluded from further analysis. Baseline characteristics of the remaining 341 patients are summarized in Supplementary Table 1. When expressed as a proportion of all viable cells in the sample, the BM M-MDSC frequency ranged from <0.001% to 6.53%, with a median of 0.175% (25th percentile: 0.063%; 75th percentile: 0.397%; Figure 1A). Patients with lower (i.e. <median) frequency of BM M-MDSCs were less likely male ($p=0.01$) and more likely to have recovered neutrophil and platelet counts ($p=0.002$) than patients with higher (i.e. median) frequency of BM M-MDSCs. This relationship between M-MDSC frequency and blood count recovery could result either from a suppressive effect of M-MDSCs on marrow hematopoiesis, or stem from the fact that, as a component of the nuclear cells in the bone marrow, M-MDSCs make up a relatively higher percentage of the overall cell number in hypocellular marrows. All other examined characteristics, including age, WBC and cytogenetic risk at diagnosis, type of AML, median CR duration before HCT, donor type, and conditioning intensity were similar between individuals with lower and higher frequency of BM M-MDSCs.

To examine the relationship between BM M-MDSC frequency and circulating numbers of M-MDSC, we took advantage of data from 39 patients (24 MRD^{neg}, 15 MRD^{pos}) who underwent same-day MFC analysis of paired peripheral blood (PB) and BM specimens. Linear regression demonstrated a statistically significant but weak correlation between PB and BM M-MDSC frequency (Figure 1B, $p<0.0001$, $R^2=0.67$). There was no statistically significant linear relationship between absolute PB M-MDSC number (obtained by multiplying PB M-MDSC frequency with PB white blood cell count) and BM M-MDSC frequency ($p=0.08$, $R^2=0.08$). Thus, the frequency or absolute number of PB M-MDSCs cannot be used as an accurate surrogate for the frequency of BM M-MDSCs.

Defining M-MDSCs as a population characterized by positivity for CD33 and CD11b and low positivity or negativity for HLA-DR, Sun and colleagues reported an association between MDSC frequency and MRD after AML therapy (9). We examined the relationship between MRD status and M-MDSC frequency in our cohort in two ways. First, we examined the frequency of MRD^{pos} disease in patients stratified by M-MDSC frequency and found that the proportion of MRD^{pos} patients was similar among those with lower vs. higher BM M-MDSC frequency (19% vs. 25%, $p=0.25$). Second, we examined whether M-MDSC frequency varied in patients stratified by MRD status. While MRD^{neg} patients ($n=267$) differed statistically significantly from MRD^{pos} patients ($n=74$) with regard to several characteristics such as higher likelihood of favorable disease risk and lower likelihood of secondary AML, there was only a statistically non-significant trend toward lower BM M-MDSC frequency in MRD^{neg} patients (0.16% [<0.001 –5.61%] vs. 0.22% [<0.001 –6.53%], $p=0.09$; Figure 1C and Supplemental Table 2).

Finally, we examined the association between BM M-MDSC frequency and post-HCT outcome. There were 166 deaths, 121 relapses, and 63 NRM events contributing to the

probability estimates for OS, RFS, relapse, and NRM. The median follow-up time after HCT among all survivors was 5.8 years (range 2.0–11.4). The time was slightly longer for survivors with lower compared to those with higher BM M-MDSC frequency (6.1 [2.6–11.4] vs. 5.1 [2.0–11.3] years; $p=0.0076$). Stratified by the median BM M-MDSC frequency, the 3-year estimates of OS were similar for patients with lower and higher pre-HCT M-MDSCs (58.2% [51.3–66.1%] vs. 57.6% [50.6–65.6%]); Figure 1D), as were 3-year estimates for RFS (53.5% [46.5–61.6%] vs. 48.3% [41.4–56.5%]); Figure 1E), 3-year estimates of the cumulative incidence of relapse (31.8% [24.7–38.9%] vs. 34.6% [27.5–41.7%]); Figure 1F), and 3-year estimates of the cumulative incidence of NRM (14.7% [9.4–20.0%] vs. 17.0% [11.3–22.7%]); Figure 1G). We note that as a sensitivity analysis, we also evaluated associations with BM M-MDSC as quantitative covariate, and also using 25th and 75th quantiles to define high versus low M-MDSC frequency; all analyses were consistent with the median analyses presented above. To further assess a possible relationship between BM M-MDSC frequency and transplant outcome, we undertook additional analyses, limiting our dataset to 1) all patients who underwent myeloablative conditioning, and 2) all patients who underwent myeloablative conditioning AND were MRD^{neg} in the pre-HCT work-up. As summarized in Supplementary Table 3, there were no statistically significant differences in outcomes between patients with lower or higher pre-HCT BM M-MDSC frequencies in either one of these 2 subset analyses.

In summary, we report the largest study to date examining the frequency of BM M-MDSC in AML, and the first to our knowledge that examines the prognostic impact of BM M-MDSCs in AML patients undergoing HCT. While we found no evidence for a statistically significant relationship between BM M-MDSC frequency and outcome following HCT, future prospective studies with dedicated flow cytometric detection of both M- and PMN-MDSC may be helpful to further characterize the potential prognostic and predictive importance of MDSCs in AML patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Distribution of M-MDSCs frequency, and association with MRD and clinical outcome among patients undergoing HCT for AML in first CR.

(A) Bone marrow (BM) M-MDSC frequency was measured as a proportion of total viable cells and ranked from low to high among 341 patients. (B) Comparison of peripheral blood (PB) and BM M-MDSC frequencies in 39 patients with availability of same-day MFC analysis of matched PB and BM samples. Linear regression is shown as dashed line ($R^2 = 0.67$) (C) BM M-MDSC frequency was stratified by MRD^{neg} (N=267) or MRD^{pos} (N=74) status. Median and interquartile range are plotted. (D-G) Kaplan-Meier estimates of (D) overall survival, (E) relapse-free survival, (F) cumulative incidence of relapse and (G) non-relapse mortality, stratified by median BM M-MDSC frequency.