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Measurement of minimal residual disease before and after myeloablative hematopoietic cell transplantation for acute leukemia

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Multiparameter flow cytometry (MFC) can identify leukemia-associated immunophenotypes in more than 90% of cases of acute leukemia with detection limits of 10^{-3}–10^{-4}. In order to better understand the potential utility of MFC to measure minimal residual disease (MRD) in the setting of myeloablative hematopoietic cell transplantation (HCT), we studied cohorts of patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) in complete remission (CR) both pre- and post-HCT. Among 253 patients with AML, the 3-year estimates of overall survival were 73% (CR1) and 73% (CR2) for those who were MRD^{neg} and 32% (CR1) and 44% (CR2) for those who were MRD^{pos}, with relapse rates being more than doubled in those who were MRD^{pos} pre-HCT (21% vs 58% for CR1 patients and 19% vs 68% for CR2 patients). The presence of MRD anytime during the first 100 days post-HCT predicted a 6-fold higher risk of subsequent relapse. In 157 patients with ALL, the 3-year overall survivals were 68% for the MRD^{neg} cohort vs 40% for those who were MRD^{pos} pre-HCT, with

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CR, complete remission; CR1, first complete remission; CR2, second complete remission; HCT, hematopoietic cell transplantation; FISH, fluorescence in situ hybridization; GVHD, graft vs host disease; MFC, multiparameter flow cytometry; MRD, minimal residual disease; OS, overall survival.

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probabilities of relapse of 16% in those who were MRD^{neg} vs 33% in the MRD^{pos} group. As in AML, the presence of MRD in the post-transplant setting indicated that the risk of subsequent relapse was high, but not inevitable.

Introduction

Following allogeneic hematopoietic cell transplantation (HCT) using a myeloablative preparative regimen for treatment of acute leukemia in remission, approximately 30% of patients will relapse. Multiparameter flow cytometry (MFC) measurement of minimal residual disease (MRD) in acute leukemia is based on the detection of leukemia-associated immunophenotypes that distinguish malignant from normal cells. With the use of $\geq$7 color flow cytometry, leukemia-associated immunophenotypes can be identified in >90% of cases with detection limits that exceed 10^{-3}–10^{-4}. The broad applicability and high sensitivity of MFC measurement of MRD allowed us to address the question of whether the detection of MRD prior to, and following, allogeneic HCT for acute leukemia predicts subsequent outcome.

Acute myeloid leukemia

Pre-transplant MRD

In an effort to understand the impact of the presence of MRD in patients with acute myeloid leukemia in morphologic complete remission undergoing allogeneic HCT following a myeloablative conditioning regimen, we studied 253 consecutive patients treated at the Fred Hutchinson Cancer Research Center (FHCRC), including 183 transplanted in first complete remission (CR) and 70 transplanted in second CR [1,2]. All had bone marrow examinations performed within 28 days of starting their preparative regimen. The marrows were studied for presence of MRD using ten-color MFC with a panel consisting of three tubes as follows: (1) HLA-DR-Pacific Blue (PB), CD15-FITC, CD33-Phycoerythrin (PE), CD19-PE-Texas Red (PE-TR), CD117-PE-Cy5, CD13-PE-Cy7, CD38-Alexa 594 (A594), CD34-allophycocyanin (APC), CD71-APC-A700 and CD45-APC-H7; (2) HLA-DR-PB, CR64-FITC, CD123-PE, CD4-PE-TR, CD14-PE-Cy5.5, CD13-PE-Cy7, CD38-A594, D34-APC, CD16-APC-A700 and CD45-APC-H7; and (3) CD56-Alexa 488, CD7-PE, CD5-Cy5, CD33-PE-Cy7, CD38-A594, CD34-APC, and CD45-APC-H7. Up to 1 million events per tube were acquired on a custom built LSRII and data compensation and analysis performed using software developed in the laboratory of Brent Wood at our institution.

MRD was detected in 54 of the 253 patients (21.3%). Patients who were MRD^{pos} more likely had AML with unfavorable cytogenetics, more likely had secondary AML, and more likely had incomplete blood count recovery compared to MRD^{neg} patients. Among those patients transplanted in CR1, the 3-year overall survival (OS) was 73% (64%–79%) for MRD^{neg} patients and 32% (17%–48%) for those who were MRD^{pos}. Among those transplanted in CR2, the 3-year OS for MRD^{neg} and MRD^{pos} patients was 73% (57%–83%) and 44% (21%–65%) (Fig. 1A). The 3-year estimates for relapse for CR1 patients were 21% (14%–28%) and 59% (41%–72%) for MRD^{neg} and MRD^{pos} patients, and for those transplanted in CR2 the respective percents were 19% (9%–31%) and 68% (41%–85%) (Fig. 1B). Multivariate models were fit for OS, disease-free survival, relapse and non-relapse mortality using MRD status (MRD^{pos} vs MRD^{neg}), age at HCT, CR status (CR1 vs CR2), cytogenetic risk grouping, type of AML (primary vs secondary), number of induction/consolidation chemotherapy cycles before HCT, CR duration before HCT, pre-transplant peripheral count recovery (CR vs CRi), and conditioning regimen (with vs without total body irradiation) as co-variates. After adjustment for these factors, the hazard ratios of MRD^{pos} vs MRD^{neg} were 2.61 for overall mortality, 3.74 for failure of DFS, 4.90 for relapse, and 1.88 for non-relapse mortality. These results thus demonstrate that presence of MRD pre-transplant as measured by MFC is a powerful independent predictor of outcome of allogeneic myeloablative HCT in the treatment of AML.