A Recurrent Immunophenotype at Diagnosis Independently Identifies High Risk Pediatric AML: A Report from the Children's Oncology Group Trial AAML0531

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Background: Risk stratification of pediatric patients with acute myeloid leukemia (AML) combines cytogenetics and molecular markers at diagnosis with detection of measurable residual disease (MRD) by multidimensional flow cytometry (MDF) after initial induction chemotherapy (EOI1). However, using MDF to distinguish between favorable and poor risk groups by diagnostic immunophenotype has not been sufficiently reproducible for clinical implementation.

Objective: We sought to characterize a recurrent, unique diagnostic immunophenotype identified among patients treated on Children's Oncology Group trial AAML0531 and determine the clinical characteristics, frequency, and outcome of patients with this immunophenotype in a large cohort.

Patients and Methods: Of the 1022 newly diagnosed pediatric patients with *de novo* AML who enrolled in protocol AAML0531 (exclusive of patients with promyelocytic leukemia and Down syndrome), 812 who submitted a specimen for MDF at diagnosis and gave consent to MRD testing were eligible for the study.

Results: A recurrent diagnostic phenotype was identified by a combination of high intensity CD56 expression, a lack of HLA-DR, dim to negative CD38 expression and dim to negative CD45 expression (designated as RAM phenotype). At diagnosis 19 patients (2.3%) expressed this phenotype and MRD was subsequently detected by MDF in 16/19 patients at EOI1 at a level of 0.02%-41% (median 0.3). Eleven patients with this phenotype submitted bone marrow specimens for MDF analysis at all 3 additional monitoring time points during therapy and in 8 patients MDF analysis revealed disease persistence throughout treatment. The experimental cohort (RAM cohort, N=19) and 2 control cohorts, one consisting of all other patients with CD56 positive AML (CD56+ control, N=166) and the other of patients with CD56 negative AML (CD56– control, N=627), were analyzed for clinical outcomes.

Patients in the RAM cohort were significantly younger with a median age at diagnosis of 1.26 years [range 0.75-16.89] than those in the CD56+ control (median 7.57 years [range 0.01–29.8], $p=0.002$) and the CD56– control (median 10.64 [range 0.02-23.94], $p<0.001$). All patients in the RAM cohort were classified as standard risk per study protocol compared to 55% ($p<0.001$) for the CD56+ control and 46% ($p<0.001$) for the CD56– control. Patients in the RAM cohort were FLT3-ITD- and wild type for *CEBPA*, *NPM1*, and *WT1*. Of them, 7 had normal cytogenetics, 1 had trisomy 8, and 11 had other cytogenetic abnormalities not associated with prognostic subgroups, including the 11q23/MLL rearrangement. According to morphology, patients in the RAM cohort were more often classified as having megakaryoblastic AML (38%) compared to the CD56+ control (2.0%, $p<0.001$) and the CD56– control (4.0%, $p<0.001$).

MDF analysis detected MRD in 84% of RAM cohort compared to 29% ($p<0.001$) for the CD56+ control and 33% ($p<0.001$) for the CD56– control cohorts. MRD detected at EOI1 was compared to morphologic complete remission (CR). Morphologic CR at EOI1 was achieved in 11 (58%) patients in the RAM cohort (58%) versus 71% of patients in the CD56+ ($p=0.250$) and 74% of patients in the CD56– ($p=0.116$) control cohorts. Patients in the RAM cohort had a 3-year event free survival (EFS) of 16% versus 52% for those in the CD56+ ($p=0.003$) and 51% for those in the CD56– ($p<0.001$) control cohorts. The 3-year overall survival (OS) for patients in the RAM cohort was 26% versus 66% for those in the CD56+ ($p<0.001$) and 70% for those in the CD56– ($p<0.001$) control cohorts. Multivariable Cox regression analysis revealed that the diagnostic RAM immunophenotype was an independent predictor of adverse outcome (hazard ratios [and 95% confidence intervals]: OS= 3.4 (1.84 – 6.18), relapse risk from CR=4.97 (2.67 – 9.24), DFS from CR=4.27 (2.04 – 8.95)).

Conclusions: A definitive diagnostic phenotype is identified that is highly predictive of poor outcome in pediatric AML. The survival of patients with the RAM phenotype is similar to or worse than those with high-risk molecular features. It occurs in 10% of infants (<1 year old) with AML who lack known risk factors, do not respond to standard therapy, and have extremely poor outcomes. This phenotype can be identified rapidly at diagnosis, allowing clinicians to predict failure before initiating therapy, and thereby allowing administration of more effective alternative therapies and minimizing exposure to non-efficacious treatment.