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One giant leap for pediatric AMKL

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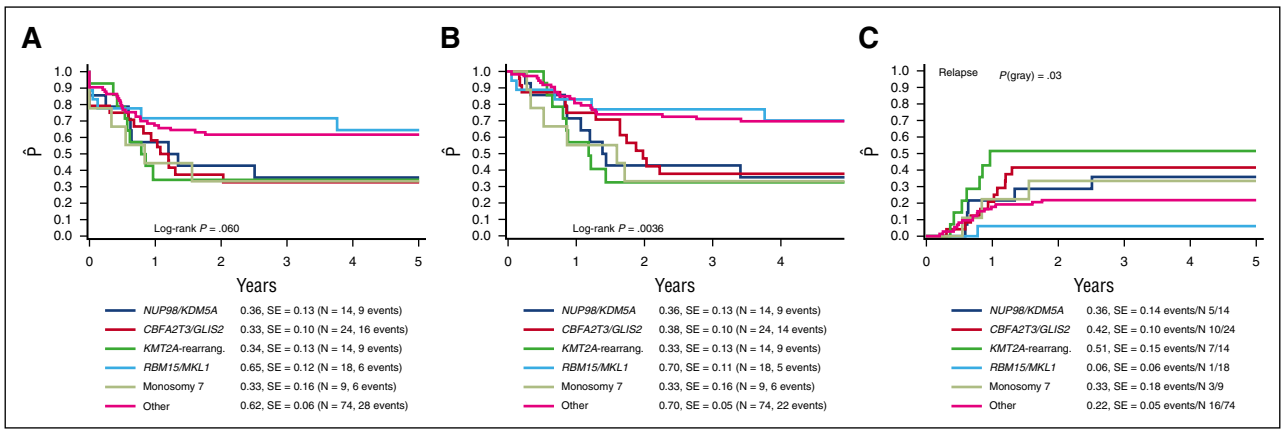
In this issue of *Blood*, de Rooij et al report, through the largest-ever molecular assessment in a multinational cohort, the prognostic significance of several recurrent genetic aberrations in children with acute megakaryoblastic leukemia (AMKL).[1]

Children without trisomy 21/Down syndrome who develop AMKL represent a small subset of pediatric acute myeloid leukemia (AML). To date, the rarity of the disease and limitations of traditional karyotyping to identify potential drivers of megakaryocytic differentiation have precluded a reliable biologic approach to risk stratification. Enter the promise, and potential pitfalls, of genetic sequencing.

Cytogenetic abnormalities resulting from cryptic fusions of NUP98 (a nucleoporin gene located at 11p15) and KMD5A (formerly known as JARID1A; the retinoblastoma-binding protein 2 located at 12p13) have been reported by the authors, and others, in pediatric AMKL.[2] Transcripts from cryptic inversions of chromosome 16 (resulting in the chimeric genes fused with the ETO family repressor CBFA2T3) have also been recently described. These new genetic associations join more familiar players like KMT2A, the lysine-K–specific methyltransferase associated with mixed lineage leukemia translocations, and the t(1;22)-associated RBM15/MKL1 gene, resulting from fusions of the RBM15 RNA recognition motif at 1p13 and the MKL1 nuclear-binding scaffold at 22q13.[3] There remains, however, little consensus as to whether these genetic aberrations can inform prognosis or therapy. In the first study of its size and scope, de Rooij and colleagues help fill this knowledge gap with a comprehensive molecular analysis of 153 pediatric patients from the Associazione Italiana Ematologica Oncologia Pediatrica (Italian Association of Pediatric Hematology and Oncology), the Berlin-Frankfurt-Münster Study Group (BFM-SG), the Children's Oncology Group, the Dutch Childhood Oncology Group, and Hôpital Saint-Louis. Using polymerase chain reaction–specific primers validated by direct sequencing, they identify the frequency of these aberrations and, in a multivariate analysis, determine whether they constitute independent risk factors for event-free (EFS), overall (OS), and relapse-free (RFS) survival (see figure) with a median follow-up of 67 months.

As efforts accelerate to apply up-front, high-throughput genomic screening to pediatric malignancies, the significance of this study highlights the simple and fundamental importance of sample size. The large cohort, relative to the rare incidence of pediatric AMKL, allows the authors to identify, in nearly half of the patients analyzed (46%), mutually exclusive poor-prognosis aberrations in NUP98/KMD5A, KMT2A, and CBFA2T3. They also confirm, using sequencing methods, the poor prognosis imparted by monosomy 7 reported by Inaba and colleagues using traditional karyotyping in their landmark retrospective study of 490 pediatric AMKL patients in the International BFM (I-BFM) Study Group.[4]

The molecular confirmation of poor-prognosis lesions reported in this study will undoubtedly generate additional hypotheses about the role of associated fusions in the unique pathogenesis of myelomegakaryocytic differentiation and fuel the search for druggable targets. For example, several groups, including the authors of this study, have already reported a role for NUP98 fusion oncoproteins in dysregulated HOX gene expression as a proleukemogenic event in AML.[5] Fusion transcripts resulting from recurrent CBFA2T3/GLIS2 abnormalities have been associated with oncogenic Hedgehog and Ras-GTPase activity.[6] Attempts to thwart DNA break repair with poly-ADP ribose polymerase (PARP) inhibitors in KMT2A fusions have revealed that downstream inhibition of HOX genes sensitizes these otherwise resistant cells to PARP killing.[7] The connection de Rooij and colleagues make



Survival curves comparing 4-year (A) EFS, (B) OS, and (C) RFS among pediatric AMKL patients with validated molecular aberrations in NUP98/KDMSA (blue), CBFA2T3/GLIS2 (red), rearrangements of KMT2 (light green), monosomy 7 (lime green), RBM15/MKL1 (light blue), and other (pink), identified as independent prognostic risk factors. See Figure 2D-F in the article by de Rooij et al that begins on page 3424.

between these aberrations and poor prognosis ensures that molecular mechanistic observations made in preclinical models of myeloid neoplasia may be pursued with confidence, on a larger clinical scale, in pediatric AMKL.

Fortunately for children with AMKL, the molecular prognostic news is not all bad. The authors address historically heterogeneous interpretations of inferior EFS in patients with RBM15/MKL1 abnormalities detected by traditional karyotype and conclude, based on the low frequency of early death, that improved supportive care and overall superior outcomes compared with poor-prognosis lesions place these patients at intermediate risk. Based on similar frequencies of hyperdiploidy among traditional cytogenetic categories, the authors conclude that hyperdiploidy is not an independent risk factor. These conclusions highlight the ability of a large-scale molecular study to refine and redefine our conclusions about not only screening and prognostic significance but also disease biology.

As with any study involving retrospective analysis of cryopreserved primary tumors, the amount and quality of starting material can vary. The authors address an important limitation of their study: that sufficient DNA material was inadequate to analyze GATA1 mutation frequency, the major molecular driver of AMKL in Down syndrome,[8] in those patients who did not express the other aberrations studied. One could easily envision that genomic screening and validation for GATA1 mutations in patients in this subset could be accomplished in future studies, and lead to other hypothesis-driven work about differences in GATA1-driven malignant hematopoiesis in patients with and without Down syndrome.

The authors also acknowledge a frequency of missing karyotype data similar to other pediatric AMKL studies. A case of the missing karyotypes appears enriched in this population, possibly related to an increased incidence of myelofibrosis that affects the quantity and quality of bone marrow specimen procurement. The results of this, and many other studies using next-generation sequencing, urge us to consider whether the benefit of precision sequencing that can detect cryptic aberrations outweighs the economy and convenience of traditional karyotyping, and the challenge of "big data" in rare diseases like pediatric AMKL.

Although we may use the results of de Rooij and colleagues to build tomorrow's toolbox of genomic screening, biologic correlative, and targeted therapy studies for pediatric AMKL, we must also ask how they will guide today's treatment conundrums. Here, too, the study provides an answer for future debate. Hematopoietic stem cell transplantation (HSCT) for pediatric AMKL in first complete remission (CR1) remains controversial, particularly in the absence of other poor-prognosis factors such as persistent residual disease or poor-prognosis molecular lesions.[9] Although no statistically significant benefit of HSCT on RFS was demonstrated in this study, future prospective studies may reveal smaller subsets of AMKL patients, with the poor-prognosis genetic lesions they describe, who benefit from HSCT in CR1.

To conclude, in this first intergroup study applying genetic analysis to a large cohort of pediatric AMKL patients, de Rooij and colleagues have taken a giant molecular leap toward precision risk stratification and therapy. Future studies may interface such sequencing data with existing mapping platforms to model the transcriptome of non–Down syndrome AMKL.[10] New mechanistic studies on the contribution of poor-prognosis NUP98/KDM5A, CBFA2T3, and KMT2A aberrations will likely reveal their impact on the biology of minimal residual disease, particularly in the areas of therapeutic resistance and dysregulated hematopoiesis.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Comment on Lahoz-Beneytez et al, page 3431

Tracers for tracing neutrophils

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In this issue of *Blood*, Lahoz-Beneytez et al use deuterium as a nonradioactive tracer to study neutrophil kinetics; their analysis shows that neutrophils originate from a large marrow progenitor pool and have a rapid blood turnover.[1]

For more than 2 centuries, hematologists have tried to understand the dynamics of neutrophil production and the transit of these cells from the marrow through the blood to tissues. Progress in this field occurred only very gradually. In the 1840s, Addison described the formation of pus by white blood cells and Jones linked this phenomenon to leukocyte margination at a site of inflammation.[2] Subsequently, Ehrlich provided the classic