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, 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. 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. 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.

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

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
Normal neutrophil kinetics

Marrow
Blood: Circulation and marginal pools
Tissues:

Schematic view of neutrophil kinetics. Neutrophil kinetics divides into 3 phases: marrow, blood, and tissues. In the marrow, developing neutrophils spend about half their time in the mitotic pool as myeloblasts, promyelocytes, myelocytes. The time to transit this pool is the mitotic pool transit time (MPTT). They then enter the postmitotic pool, becoming sequentially metamyelocytes, bands, and then neutrophils. The time for the neutrophil to pass through these stages is referred to as the postmitotic pool transit time (PMPTT). Neutrophils in the circulation are about equally divided between the circulating and marginal pools. Neutrophils leave the circulation and enter the tissues at inflammatory sites or become effete cells and are removed by macrophages in the spleen, marrow, and other tissues.

description of the morphology of stained blood cells and leukocyte changes with infections.3 For the next half century, most of the noteworthy reports in this field were descriptive, for example, conditions associated with neutropenia or neutrophilia, until the advent of radioisotopic studies in the “atomic age” of the 1950s.

As is well known, ionizing radiation and toxic chemicals, for example, external radiation and nitrogen mustard, have major effects on blood cell counts, rapidly causing neutropenia. It was primarily these observations that led researchers to conclude that neutrophils must have a very short life span. These same observations prompted hematologists to begin to study the use of radioactive isotopes, particularly phosphorus 32 (32P), as a tracer to study blood cell formation. In 1954, the Norwegian investigator Ottesen reported that the time required for 32P-labeled neutrophils to transit the postmitotic neutrophil compartment in the marrow and enter the blood was about 5 to 6 days.4 These studies were done by infusion of the isotope to label DNA of dividing cells and then serial separation of blood neutrophils after the infusion. This work also suggested that neutrophils rapidly leave the blood and disappear into the tissues. More sophisticated experiments in dogs and in humans soon followed, confirming these findings.5 Cartwright, Athens, and Wintrobe then led a decade of excellent work using the 32P-labeled disopropyl fluorophosphate (DFP) to study neutrophil kinetics.6 They established values for pool sizes of neutrophils and their precursors from the marrow to the blood and tissues and also the concept and relative proportions of the circulating and marginal blood neutrophil pools. Subsequently, Dancey, Deubelbeiss, Harker, and Finch conducted the most comprehensive and quantitative studies of neutrophil kinetics ever performed in humans using 59Fe, 32P DFP, 3H thymidine and carefully performed bone marrow biopsies.7 This work established norms for neutrophil production and turnover and showed that at least the late phase of neutrophil production is very efficient; that is, cells entering the myelocyte-metamyelocyte stage will mature to become blood neutrophils. The methods established by these investigators were later used to show how myeloid growth factors increase neutrophil production, accelerate the release of neutrophils from the marrow to the blood, and prolonged the blood half-life of these cells.8

Over recent years, increasing concerns have arisen about the use of radioactive tracers in human investigations, particularly the use of even tracer amounts of 32P. Many of the radioisotopes previously used have become unavailable for clinical research. The search for alternatives with less potential hazard continues.99Tc is used in nuclear medicine as a label for neutrophils to find sites of infection, but it is not suitable for research studies of neutrophil kinetics because the necessary separation and washing of the neutrophils is sufficiently damaging to the cells to disturb their natural transit in the blood. By contrast, 32P DFP (used in Craddock et al5 and Cartwright et al) or 3H DFP (used in Price et al) labels the serine proteases of neutrophils selectively, so that the labeling can be done with whole blood, protecting the cells from the damaging effects of washing. Neither of the isotopes is currently available to study neutrophil kinetics.

In 2010, Pillay et al reported studies using an infusion of deuterium labeled “heavy” water to study neutrophil kinetics.9 The methods for the clinical study were similar to those of previous investigators (Ottesen, Craddock, Athens, and Price). It was surprising that this new study reported a far longer blood half-life for neutrophils than any of the previous studies. Li et al raised questions about the assumptions made in analyzing the data and offered an alternative approach to analyzing the data which yielded results similar to those previously published.10 In this issue, Lahoz-Beneytez et al report a new study, using deuterium-labeled glucose and deuterium-labeled water. They measured the neutrophil emergence time and got identical results to those of Ottesen and the other investigators. Analyzing data using a 2-compartment model, they confirmed the old concepts of a large marrow pool of myeloid cells and a much smaller blood pool with rapid turnover.

Why is this study important? Understanding normal hematopoietic cell production is a foundation for understanding blood diseases (see figure). Proper diagnosis and treatment of neutropenia and neutrophilia rest on a physiological understanding of the normal process and what can go wrong with it. This new report by Lahoz-Beneytez et al “clears the air” from a previous report with good data but a flawed analysis. From another perspective, there is a common tendency to cite the latest report on a research topic, rather than an older study. As a “seasoned” investigator, it is satisfying to see that in this
The power of cord blood cells

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In this issue of Blood, Michel et al1 showed in a well-designed randomized controlled study that single unrelated cord blood transplantation (UCBT), with adequate cell dose, remains the standard of care. The single-unit UCBT arm had good survival (~70%), low transplantation-related mortality (TRM: 5-6%), and a lower rate of extensive chronic graft-versus-host disease (GVHD) compared with the double-unit transplantation arm. This study provides important information on optimal cord blood donor selection. In addition, this study, as well as data from other recent reports, shows that immune reconstitution after UCBT is excellent (provided no antithymocyte globulin [ATG] is given). Furthermore, by refining and individualizing the conditioning regimens used for UCBT, the survival chances may improve further.

Historically, the limited number of hematopoietic cells in a single cord blood unit was believed to result in delayed hematopoietic recovery and higher mortality in larger recipients. It was hypothesized that the greater numbers of hematopoietic cells in 2 units of cord blood would improve outcomes. Some registry and single center data suggested that relapse rate was lower after double-unit transplantation compared with single-unit transplantation, resulting in inferior disease-free survival after single-unit transplantation.2 Obviously, randomized controlled trials, as in this article, are of great interest and importance to either prove or reject this hypothesis.

The primary end point in this study was transplantation failure, which was hypothesized to be higher in the single-unit arm compared with the double-unit arm (40% vs 20%, respectively). The study, however, failed to prove this; survival was similar (~70%) in both arms. Although the acute GVHD rates were similar between the 2 arms, extensive chronic GVHD was more frequent in the double-unit arm (P = .02). These results confirm a recent similar report by Wagner et al,3 in which it was hypothesized to be higher in the single-unit arm compared with the double-unit arm (40% vs 20%, respectively). The study, however, failed to prove this; survival was similar (~70%) in both arms. Although the acute GVHD rates were similar between the 2 arms, extensive chronic GVHD was more frequent in the double-unit arm (P = .02). These results confirm a recent similar report by Wagner et al;4 double-unit UCBT fails to provide benefit above adequately dosed single-unit transplants, and toxicity was higher after double-unit UCBT: higher acute GVHD2-4 and extensive chronic GVHD.

Michel et al suggest that double-unit UCBT after fludarabine + cyclophosphamide + total body irradiation (without ATG) may result in a lower relapse probability (P = .05). This, however, needs further analyses, as the greater HLA mismatch in the double-unit arm may be an alternative explanation for this as well. Others, including the recent report by Wagner et al, have reported on the potential beneficial effect of greater HLA mismatch on relapse.3,4 In Wagner et al, there was even a survival advantage for patients receiving a 4/6-matched cord blood unit compared with patients receiving a 5-6/6 matched unit (P = .03). This is an intriguing feature specifically associated with cord blood cells, as multiple mismatches using T-repleted unrelated volunteer donor cells would be associated with very poor survival chances. Future studies should focus on identifying the optimal mismatch mediating the strongest antileukemic activity. This may result in better disease control; eg, recently high predicted indirectly recognizable HLA epitopes in class I was found to promote antileukemia responses after UCBT.5 That cord blood T cells mediate enhanced antitumor effects, compared with adult peripheral blood T cells, was also recently shown in a xenograft Epstein-Barr virus lymphoma model.6 For the optimal effect, prompt immune reconstitution is essential. The use of ATG in the conditioning is considered to be the major limitation for prompt immune reconstitution after UCBT. Although ATG is given prior to UCBT, it will give exposure after UCBT, as the half-life of ATG is long (days to weeks).

Recent studies of ATG have shown that ATG and cord blood T cells mediate enhanced antitumor effects, compared with adult peripheral blood T cells, was also recently shown in a xenograft Epstein-Barr virus lymphoma model.6 For the optimal effect, prompt immune reconstitution is essential. The use of ATG in the conditioning is considered to be the major limitation for prompt immune reconstitution after UCBT. Although ATG is given prior to UCBT, it will give exposure after UCBT, as the half-life of ATG is long (days to weeks).

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Tracers for tracing neutrophils

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