cytarabine and anthracyclines. Although beneficial for many children, these old regimens are relatively nonselective and are associated with significant treatment-related toxicity.

The past decade has seen extraordinary advances in our understanding of the molecular events leading to AML. Continuously improving molecular tools and technologies now make it possible to characterize specific genetic alterations in AML blasts. This molecular information allows for improved classification of AML, a better understanding of the molecular mechanisms that underlie the development of leukemia, an expanded ability to monitor for minimal residual disease, and also presents an exciting opportunity for the development of novel agents directed toward aberrant molecular targets.4 Thus, the improved ability to assess risk of relapse based upon relevant molecular features of AML blasts and risk factors inherent to the child’s ability to tolerate therapy (eg, host gene polymorphisms), and the potential for the addition of targeted agents to conventional chemotherapy, now present us with the possibility of personalized therapy for AML.

Bachas and colleagues assessed the mutational status of FLT3, N-RAS/K-RAS, KIT, WT1, CEBPa, PTPN11, and NPM1 in children with AML who had paired diagnostic and relapse leukemia samples.5 Overall, the mutational status changed in 38% of children between diagnosis and relapse, with 61% of patients having a mutational shift if a mutation was present at either diagnosis or relapse. Interestingly, the presence or gain of a class I/II mutation was associated with a shorter time to relapse in these children, while a longer time to relapse was seen in children who had an absence or loss of a class I/II mutation.

The consequences of this study are 3-fold. First, these results underscore the complexity and heterogeneity of AML at both diagnosis and relapse. Second, because molecular abnormalities are increasingly used to monitor patients for the presence of minimal residual disease, such clear demonstration of these mutational shifts serves as a cautionary note to clinicians. Finally, these molecular abnormalities may serve as targets for molecularly targeted therapy, allowing treatment to be personalized based upon mutations that are defined to be present at diagnosis and/or relapse. Most of the world’s cooperative cancer groups have ongoing efforts to test novel agents directed toward defined molecular targets. For example, based upon considerable data regarding the significant prognostic value of FLT3 internal tandem duplications with a high allelic ratio in children and adults,6 there continues to be great interest and enthusiasm for the development of FLT3 inhibitors, despite some recent disappointing results.

However, our optimism, excitement and enthusiasm for molecularly targeted and individualized therapies must be tempered by a healthy respect for the significant challenges inherent with this strategy. Among the most difficult of these challenges is an increasing awareness that many molecular targets may not, in the end, be clinically relevant. And for targets that are truly critical to the survival of the leukemia stem cell, inhibition of multiple molecular targets may be required for an impact on clinical outcome because of the ability of the cancer cell to escape 1 pathway with an alternative. The testing of multiple new agents on the backbones of conventional therapies will present numerous serious challenges in the design of clinical trials, in addition to the challenges inherent in working with more than a single drug company within the same study.

Furthermore, clinical trialists and biostatisticians must face a rapidly expanding number of groups of molecularly defined patients with increasingly small numbers of patients in each group. AML is defined by its molecular heterogeneity. Therefore, taken to its extreme, personalized therapy will ultimately result in sample sizes of 1, with each patient having a unique combination of molecular features characterizing his or her leukemia. Presently, it is not clear how the safety and efficacy of such highly personalized therapies can be appropriately assessed, especially for rare and highly heterogeneous diseases like pediatric AML.

Harold Varmus, director of the National Cancer Institute, was recently quoted as saying: “Genomics is a way to do science, not medicine.” This provocative statement reminds us of the extraordinary power of genomics to identify the profound complexity of most human diseases. It also serves as a reality test for how difficult it is to translate highly complex genetic information, such as the mutations defined in the study by Bachas et al, into effective treatments for diseases like AML. Most importantly, however, Varmus’ statement should be viewed as a challenge. If his conjecture is true, why must it remain so? We must, as a clinical and scientific community, help to effect significant transformational changes in every aspect of the drug development process to capitalize on the extraordinary molecular knowledge now available to us in a way that positively impacts the outcome of patients.

Conflict-of-interest disclosure: F.O.S. serves on data and safety monitoring committees for Seattle Genetics and is a member of scientific advisory committees for Pfizer.

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Comment on Devlin et al, page 2826

Finding a diamond in the (mouse is) rough

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In this issue of Blood, Devlin and colleagues use a new strategy to create a mouse model for the inherited bone marrow failure syndrome, DBA. The result, while recapitulating certain aspects of the disease and representing a positive step forward, also demonstrates that significant hurdles remain in faithfully creating a mammalian model for DBA.
Data from Devlin et al. that show both similarities and differences between hematologic parameters observed for RPS19R62W transgenic mice and DBA patients. Left panel: Similarities include granulocyte/macrophage precursors less affected than erythrocyte lineages. Right panel: Differences include binucleate erythroblasts not commonly found in circulation of DBA patients.

Diamond Blackfan anemia (DBA) is a rare hypoplastic anemia that typically presents within the first year of life. In addition to the bone marrow failure, DBA patients also manifest with a heterogeneous constellation of congenital anomalies. Nine affected genes have been identified in approximately 50% of DBA patients, all of which encode ribosomal proteins. Thus, DBA is the first human disease clearly established as a ribosomopathy. Although substantial progress has been made in the past few years demonstrating that haploinsufficiency for ribosomal proteins and subsequent adverse affects on ribosome assembly contribute to the underlying molecular basis of DBA, many questions remain regarding disease pathophysiology. Of particular note, the exquisite sensitivity of the developing erythron to suboptimal levels of ribosomal proteins and the timing of disease presentation in the first year of life remain unexplained.

These complex aspects of disease pathophysiology cannot be adequately assessed in cell culture models of DBA, leaving the field crying out for mammalian models. The mouse models created to date, however, have significant limitations. The majority of mutations identified in ribosomal protein genes responsible for DBA are loss-of-function mutations including complete gene deletions. Thus, the most straightforward mouse model for DBA would involve a gene knockout, a strategy initially used for the RPS19 gene by Matsson et al. Unfortunately, the hematologic profile of heterozygous mice showed no abnormalities, whereas the homozygous knockout displayed early embryonic lethality. More recently, mutations in mouse RPS19 and a second ribosomal protein gene RPS20 were identified in a forward genetic screen for dark-skinned mutants. In contrast to the previous attempt at a mouse model of DBA, mice harboring the dark-skinned mutations in RPS19 exhibited a mild anemia. A limited characterization of the hematologic properties of these mice revealed a relatively normal bone marrow morphology with some evidence of increased apoptosis in lin<sup>−</sup>e<sup>−</sup> cKit<sup>−</sup> progenitors. Further work is necessary in the dark-skinned mouse model to determine the extent to which the underpinnings of the mild anemia observed reflect the situation in DBA patients.

Intriguingly, another mouse model that captures both some of the hematologic characteristics of DBA as well as some of its congenital anomalies lacks the FLVCR gene encoding a heme transporter present in erythroid progenitors and other cell types. Mutations in FLVCR have not, however, been found in DBA patients, suggesting that expression of genes like FLVCR could play a role in disease pathophysiology downstream of pathogenic mutations in ribosomal protein genes.

In the new mouse model of DBA presented in this issue of Blood, Devlin et al express an RPS19 allele harboring a missense mutation (RPS19R62W) found in 4 DBA pedigrees. This mutant form of RPS19 induces hematologic phenotypes in mice with 2 normal copies of RPS19, indicating that it behaves in a dominant-negative fashion. Initial attempts to express the RPS19R62W transgene in mice resulted in early embryonic lethality. To circumvent early embryonic lethality, the authors devised a strategy to delay expression of the transgene to later stages of embryogenesis. Many of these mice die of severe anemia in utero or shortly after weaning. Survivors show a mild anemia with evidence of extramedullary compensation. Other hematopoietic lineages appear normal. Analysis of erythroid development in these mice shows a significant reduction in BFU-E and CFU-E and a 10-fold decrease in circulating reticulocytes. There was no effect on CFU-GM. Thus, these new transgenic mice recapitulate a number of hematologic parameters observed in DBA patients (see left panel of figure). And yet, there are still significant differences. Similar to other mouse models, the anemia observed in the RPS19R62W mice is mild relative to that observed in humans. The differences in anemia severity may be linked to compensating mechanisms in mice. Another distinct feature of the current mouse model is effects later in erythroid development resulting in the accumulation of binucleate erythroblasts and other evidence of improper erythroid maturation that is not observed in the DBA patient population (see right panel of figure). The binucleate cells may be a consequence of the RPS19R62W transgene inducing an arrest at the G2/M phase of the cell cycle as opposed to the G1/S arrest observed in patient cells. The basis for this difference is unknown, but it is worthwhile pointing out that the block in pre-rRNA processing observed in cells from the RPS19R62W transgenic mice is not the same as that observed in cells from patients with mutations in RPS19.

In summary, the article by Devlin and colleagues represents a significant step toward the goal of faithfully producing a mouse model of Diamond Blackfan anemia. Moreover, the present work indicates how challenging the road has been in creating a mammalian model to study complex developmental aspects of DBA pathophysiology.

Conflict-of-interest disclosure: The authors declare no competing financial interests.
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Comment on Ni et al, page e66
Shear stress: devil’s in the details

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In this issue of Blood, Ni et al use an in vivo mouse model of disturbed flow that results in accelerated atherosclerosis to identify novel mechanosensitive genes.1 Despite the recent emphasis on in vitro mechanosensitive genes identified in Ni et al’s mouse model have been previously reported using in vitro HUVEC shear stress experiments. Furthermore, several genes identified in the current study had not been identified as flow-responsive in any previous publication. It is not unusual to find discrepancies in the microarray results of flow experiments from different laboratories; after all, different in vitro systems and waveforms are often used, cells are exposed to different culture conditions and derived from different species or from different vascular beds. A fascinating cause of endothelial functional heterogeneity is the anatomical origin of the cells (vascular bed, vessel type and size).7 There is also evidence that prolonged culture of EC can result in gene-specific loss of response to flow. Should these variables result in only a 50% identity of the genes shown to be flow-regulated? How do we determine experimental artifact from true positive/negative changes? Even the extracellular matrix can affect response to flow. The extracellular matrix below EC is normally composed primarily of collagen IV and laminin, but fibronectin is deposited in proatherogenic parts of vessels and could potentiate atherosclerosis.8 This is particularly noteworthy as fibronectin-coated dishes are commonly used in in vitro shear stress experiments.

Ni and colleagues also compared their results to previous in vivo efforts in the porcine model where gene expression in linear and curved segments of the pig aorta were compared. Surprisingly, of the 42 genes identified to be mechanosensitive in this study, only 2 (KLF4 and eNOS) were identified in the porcine results is likely to be the differential

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