and show that zebrafish CXCR4 localizes to the cell surface and internalizes in response to human SDF-1, whereas CXCR4\(^{WHIM}\) fails to internalize when exposed to SDF-1.

Walters et al subsequently generate transgenic zebrafish using the myeloperoxidase promoter driving neutrophil-specific expression of the GFP-CXCR4\(^{WHIM}\) allele to show aggregation of neutrophils at the sites of hematopoiesis and other sites of high SDF-1 expression in zebrafish embryos. Knocking down the expression of SDF-1 using morpholino antisense oligonucleotides causes the dispersal of CXCR4\(^{WHIM}\) neutrophils. In contrast, ectopic expression of SDF-1 leads to neutrophil aggregation at these sites. Together, these results show that CXCR4\(^{WHIM}\) neutrophils accumulate and remain at sites of high SDF-1 expression in vivo.

The transparency of zebrafish embryos allows for the direct visualization of the vasculature in live animals without perturbation. When neutrophil motility is assayed in transgenic embryos expressing GFP-CXCR4\(^{WHIM}\), the GFP-positive cells display greatly reduced 3-dimensional motility compared with GFP-CXCR4 controls. The GFP-CXCR4\(^{WHIM}\) animals are also severely neutropenic, like human WHIM patients, with virtually no neutrophils in circulation.

The aggregation of zebrafish CXCR4\(^{WHIM}\) neutrophils and their failure to circulate in an SDF-1-dependent manner make it a useful model for WHIM syndrome. The question of clinical importance is how the CXCR4\(^{WHIM}\) neutrophils respond to inflammation. By clipping the tailfin, which stimulates neutrophil migration to the wound site, the authors show that the CXCR4\(^{WHIM}\) neutrophils fail to migrate to the wound. When SDF-1 expression is inhibited, migration occurs. This is not, however, a trivial result because Walters et al observe that the CXCR4\(^{WHIM}\) neutrophils respond to the tailfin clip by increasing cellular protrusion and localized motility. This implies that the neutrophils are not deaf to inflammatory signals, but rather that their motility is in some way restricted by the CXCR4\(^{WHIM}/SDF-1\) interaction. This has not been previously demonstrated in vivo, and it may be an important factor for understanding the excessive neutrophil proliferation and apoptosis in the marrow of WHIM patients.

Walters et al have also begun to dissect the pathway regulating neutrophil motility using a photo-activated Rac GTPase, a downstream component of the SDF-1 signaling pathway. Earlier work suggested WHIM neutrophils are defective in actin polarization. Walters and colleagues used this Rac allele to drive actin polymerization, which in turn allowed neutrophil migration to wound sites. These experiments demonstrate a more direct cause-and-effect link between CXCR4 function and the machinery for cellular motility. It is nicely consistent with the observation that CXCR4\(^{WHIM}\) neutrophils do, in fact, respond to inflammatory signals, but they cannot mobilize.

The zebrafish WHIM syndrome model presented by Walters et al demonstrates in vivo that truncated alleles of CXCR4, together with its ligand SDF-1, are essential drivers of neutrophil retention in WHIM syndrome. They demonstrate in vivo that the CXCR4 alleles found in WHIM syndrome are a dominant "on" receptor that still requires its ligand, SDF-1, for activation. This "activation" is a STOP signal preventing actin polymerization, and hence retards neutrophil motility out of the marrow into the periphery.

Walters and colleagues are now positioned to use their zebrafish system to investigate how CXCR4\(^{WHIM}/SDF-1\) interactions affect neutrophil homeostasis. The signals driving myelokathexis in WHIM patients remains an important unsolved mystery. This zebrafish WHIM model now provides a tool to screen for agents that either enhance or inhibit neutrophil migration from the bone marrow. The recent identification of zebrafish G-CSF will further enhance the value of this system.

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Comment on Bachas et al, page 2752

Personalized medicine for AML?

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Almost a decade ago, Gilliland proposed a model of leukemogenesis for acute myeloid leukemia (AML).1 In this model, AML results from activating mutations in genes that confer increased proliferation and survival capabilities (class I mutations) acting in concert with chromosomal abnormalities or gene mutations that block differentiation and subsequent apoptosis (class II mutations). In this issue of Blood, Bachas and colleagues present data that clearly demonstrate mutational shifts in class I/II mutations that occur between diagnosis and relapse in children with AML.2 The authors suggest that these findings may allow for personalized treatment.

As a result of highly collaborative and continuous clinical research involving children with AML, significant advances in disease-free survival have been achieved over the past several decades.3 Modern AML therapy is based upon the principles of combination of multiple effective agents, dose intensity, risk-adapted use of allogeneic hematopoietic stem cell transplantation, and improved supportive care. However, despite extensive efforts to develop new agents for the treatment of AML, current therapy is still based on the use of...
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.2 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,3 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.

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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.
Personalized medicine for AML?

Franklin O. Smith