inside blood

the mutant genes present at diagnosis and causally implicated in disease pathogenesis will be harbingers of molecular relapse. This principle has been thoroughly validated, for example, in the context of *BCR/ABL* in CML. But 2 articles in this issue indicate that *FLT3*/ITD may not be a suitable marker for MRD in a subset of AML patients. Shih et al (page 2387) and Kottaridis et al (page 2393) report the fascinating observation that some *FLT3*/ITD-positive patients at diagnosis do not have detectable *FLT3*/ITDs at relapse, and the converse may also be true. Furthermore, a cohort of patients had several *FLT3*/ITD variants detectable at diagnosis, with selection for one of the variants at time of relapse.

These observations have several important implications. First, use of *FLT3*/ITD for MRD detection must be employed with caution and will not be of value in detecting molecular relapse in all AML patients that are *FLT3*/ITD positive at diagnosis. Second, the data indicate that *FLT3*/ITD is probably a secondary event in clonal evolution of at least some AML. Viewed from another perspective, these data provide further support for 2-hit pathogenesis of AML in which activating mutations in *FLT3* confer a proliferative and/or survival benefit to leukemia clones. Third, the data raise the question of whether *FLT3*/ITD inhibition using small molecule inhibitors, analogous to imatinib, will be an effective therapy for AML or will simply select for a parent clone with an alternative second mutation that confers a proliferative signal. Indeed, one patient with *FLT3*/ITD at diagnosis had "substituted" a clone with an activating mutation in *RAS* for the *FLT3*/ITD. It seems likely (and comes as no surprise given the more modest response rates to imatinib for CML blast crisis) that *FLT3*/ITD inhibitors will need to be combined with other agents for effective therapy of AML. Finally, *FLT3*, *RAS*, and *KIT* may each be activated by mutation in AML and confer proliferative and survival signals, but collectively account for perhaps one-half of all AML cases. In those cases in which *FLT3*, *RAS*, or *KIT* are not mutant, it seems likely that other mutations must confer a proliferative signal to cells. Identification of these genes will be important in the future, as they may also be targets for small molecule inhibition.

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Epo on demand

The development of safe and effective gene therapy poses many formidable challenges. Some biomedical applications such as the hemoglobinopathies require that the gene product be expressed in a specific cell type, for example, erythroid progenitors. No such constraint pertains when the gene product circulates in the plasma. But in many cases, the level of the therapeutic protein needs to be tightly regulated. Perhaps the most compelling example is the hotly pursued goal of treating diabetes by a vector in which insulin gene expression responds appropriately to changes in blood glucose. The closest hematologic counterpart is the development of a vector in which erythropoietin (Epo) production is induced by subtle physiologic decreases in intracellular oxygen tension. Unlike the insulin gene, wherein the transcriptional response to glucose is complex and not well understood, the hypoxic induction by Epo depends upon a single crucial hypoxia inducible (transcription) factor (HIF) that is regulated by an elegant system of oxygen sensing and signal transduction, shared by all cells.

Binley and colleagues (page 2406) exploit this transcriptional servomechanism. They show quite convincingly that intramuscular injection of an adeno-associated virus harboring an *EPO* gene driven by a promoter containing HIF-response elements can cure severe anemia in mice in which endogenous Epo production has been nearly eliminated. Normal hematocrit levels are maintained for 7 months or more after initiation of treatment. The remarkably tight oxygen-dependent regulation of the transduced *EPO* gene is borne out by the total lack of change in hematocrit when this vector is injected into healthy animals.

This strategy could lead to a much-improved way of administering recombinant human Epo (rhEpo) to patients. Long-term treatment with thrice-weekly rhEpo is very costly, particularly in patients who require relatively high doses. A key benefit of a physiologically regulated therapeutic gene is that enhanced production of Epo will continue until the anemia is corrected and the tissue depot in which the vector has been injected no longer senses hypoxia. But one note of caution seems warranted. Mother Nature placed the major site of Epo production in the subcortex of the kidney for a very good reason. At this site, wide fluctuations in oxygen tension are dampened, and therefore Epo production properly responds to total body hypoxia rather than to local vicissitudes. Intramuscular injection of a regulatable *EPO* gene could result in untoward increases in Epo expression from local hypoxia induced by exercise. Further studies in larger animal models are needed to investigate this potential problem and circumvent it.

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