

with NPM-ALK, and this phosphorylation correlated with the increased stability of a number of mRNAs encoding critical growth-promoting proteins such as c-MYC and cyclins A2, B1, D1, and D3. Biologically, this posttranscriptional enhancement of mRNA stability was associated with an increased survival of cells in which transcription had been experimentally arrested by actinomycin D.

These data are consistent with recent results from a number of investigators indicating that the mRNA-binding properties of AU-BPs including AUF1/hnRNP D can be modified by various posttranslational modifications such as ubiquitinylation, methylation, and phosphorylation. The observations of Fawal et al show that NPM-ALK increases the stability of otherwise short-lived mRNAs, thus contributing to enhanced proliferation, survival, and oncogenesis. Now established for NPM-ALK, it will be important to examine other oncogenic kinases and AU-BPs for a similar functional interplay; if identified, such a mechanistic relationship could

represent an important, heretofore unrecognized, paradigm underlying oncogenic transformation in not only hematopoietic but perhaps other malignancies as well. ■

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## NEOPLASIA

Comment on Pollard et al, page 2764

# FLT3: the root of the problem

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Most AML patients with FLT3/ITD mutations have poor outcomes. This article by Pollard and colleagues suggests that the explanation, not surprisingly, may be found at the root of the problem, the leukemia stem cell.

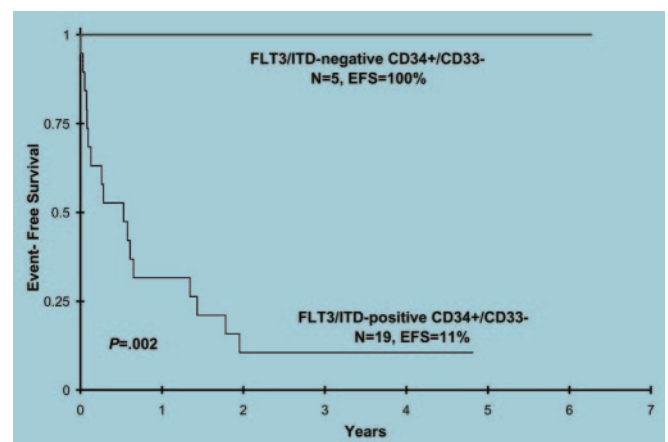
Acute myeloid leukemia (AML) is organized into a hierarchy that in some respects parallels normal hematopoiesis.<sup>1</sup> Primitive hematopoietic precursor cells acquire mutations that eventually result in transformation to leukemia. The exact stem or progenitor cell that eventually becomes the leukemia stem cell probably varies from case to case, but the more primitive it is, the less curable the leukemia is likely to be.

FLT3/internal tandem duplication (ITD) mutations have emerged as the most common molecular abnormality in AML, and only a minority of patients harboring these mutations can be cured.<sup>2</sup> It has been postulated that FLT3 mutations are relatively late hits in leukemogenesis because they are not by themselves capable of induc-

ing transformation and are occasionally lost (or acquired) at relapse.<sup>3,4</sup> Nonetheless, previous work using nonobese diabetic/severe combined immunodeficient (NOD/SCID) engraftment and polymerase chain reaction (PCR) assays of CD34<sup>+</sup>/CD38<sup>-</sup> fractions of AML samples suggests that FLT3 mutations are present in leukemia stem cells.<sup>5</sup> The findings of Pollard and col-

leagues, in this issue of *Blood*, offer another opportunity to understand how FLT3 mutations fit into the stem cell paradigm of leukemia.

These authors examined the FLT3 mutation status of 24 diagnostic AML samples from pediatric patients harboring FLT3/ITD mutations. Their purpose was to determine if the FLT3 mutations were present in mature versus immature myeloid cell populations and whether this affected the clinical outcome. They sorted the samples into CD34<sup>+</sup>/CD33<sup>-</sup> (more primitive) or CD34<sup>+</sup>/CD33<sup>+</sup> (less primitive) progenitor cell fractions and then used those cells to generate granulocyte-macrophage colony-forming units (CFU-GMs) and erythroid burst-forming units (BFU-Es) in colony assays. Using patient-specific FLT3 primers, PCR was used to determine mutation status of the sorted cells and of CFU-GMs and BFU-Es plucked from the colony plates. They were able to detect the FLT3 mutations in the more differentiated CD34<sup>+</sup>/CD33<sup>+</sup> cells from all 24 of the samples. However, 5 of the 24 samples had no detectable mutation in the less mature CD34<sup>+</sup>/CD33<sup>-</sup> cells or in any of the colonies. In other words, the early progenitor cells of these 5 patients lacked the FLT3 mutations. The other 19 patients did harbor the mutations within the CD34<sup>+</sup>/CD33<sup>-</sup> fraction, and in some cases the mutations were present in both CFU-GM and BFU-E colonies, implying derivation from a precursor with bilineage potential. Strikingly, the 5 patients in whom the mutation was present in only the more differentiated cell fraction had



Event-free survival for patients with and without FLT3/ITD detection in CD34<sup>+</sup>/CD33<sup>-</sup> progenitor cells. See the complete figure in the article beginning on page 2764.

dramatically better clinical outcomes than the other 19 patients (see figure).

Despite these fascinating findings, the exact role of FLT3 mutations in leukemogenesis and how they affect prognosis are still not clear. In the group of 5 patients with the good outcome as described in this paper, did the FLT3 mutations arise as part of the transformation process in a more committed progenitor cell (much like 15;17 translocations are thought to occur in acute promyelocytic leukemia), resulting in a more curable disease? Or were they late hits in the progenitor cells of an already established leukemia? Just how deep into the roots of the hematopoietic system do these FLT3 mutations go? ■

## ● ● ● PHAGOCYTES

Comment on Pestonjamas et al, page 2814

# Neutrophil chemotaxis: a tail of 2 GTPases

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A study by Pestonjamas and colleagues has identified Rac1, a Rho family GTPase, as an important link between the leading edge of migrating neutrophils and their uropod “tail,” via activation of RhoA and myosin.

Polarization of neutrophils in response to chemoattractants results from a complex series of “directional sensing” events leading to remodeling of the actin and myosin cytoskeleton, which are crucial for directed movement of neutrophils into sites of infection or inflammation.<sup>1,2</sup> The coordinated and spatially distinct activation of different members of the Rho family of Ras GTPases is critical for this response. At the front of the cell, an actin-rich lamellipodium forms, which requires Rac, Cdc42, and phosphoinositides. At the rear of the cell, the RhoA GTPase regulates myosin-based contraction via Rho kinase and myosin light chain kinase in a structure known as the uropod, and the periodic detachment and retraction of uropod are essential for cell motility. A proposed model for the spatial distribution of these signals into “frontness” and “backness” programs, organized by Rac and Rho, respectively, has been proposed by Bourne and colleagues (reviewed in Fenteany and Glogauer,<sup>2</sup> Keymeulen et al,<sup>3</sup> and Wong et al<sup>4</sup>). In this model, mutual negative feedback

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trophils lacking Rac1 form multiple unstable lamellipodia and develop an elongated morphology due to a uropod retraction defect.<sup>1,2</sup> In the current study, the introduction of dominant-negative Rac or dominant-negative Rho into human neutrophils produced a phenotype similar to Rac1-deficient mouse neutrophils. Additional studies showed that Rac, particularly Rac1, activity was coupled to Rho activation, which in turn was critical for myosin II contractility, tail retraction, and chemotaxis. Although genetic deletion studies in the murine system allow a relatively clean assessment of the relative functions of these 2 Rac isoforms, whether Rac1 plays a distinct role in regulating RhoA and uropod formation in human neutrophils remains unresolved. The amounts of Rac1 and Rac2 are similar in mouse neutrophils, whereas only 5% to 10% of the total Rac in human neutrophils is Rac1, with Rac2 accounting for the remainder. In addition, dominant-negative forms of Rac1 or Rac2 act by binding stably to Rac guanine nucleotide exchange factors to block Rac activation and lack selectivity for a particular Rac isoform.

The Cdc42 GTPase may also provide positive regulatory signals for Rho activation,<sup>3</sup> and F-actin formation in the frontness response may help to limit the distribution of activated Rho.<sup>4</sup> The organization of the lipid membrane itself also contributes to RhoA activation.<sup>5</sup> A future challenge is to understand how these multiple underlying molecular events are linked to mediate chemoattractant-induced neutrophil polarization and movement. ■

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