

Molecular Classification of Acute Myeloid Leukemia: Are We There Yet?

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The treatment of acute myeloid leukemia (AML) has made incremental advances during the last few decades, but the outcomes are still fairly grim. Combination chemotherapy can cure approximately 25% of patients. The strongest prognostic factor remains cytogenetics. Patients with “good-risk” cytogenetics have the relatively favorable outcome of disease-free survival (DFS) of approximately 60%; on the opposite side of the spectrum, patients with “unfavorable” cytogenetics have survival of about 10%.¹ The majority of patients fall into the intermediate-risk group, and most of these patients have no abnormal chromosomes at diagnosis. This group comprises approximately 40% of all adult patients, and their DFS is about 25%.

Once patients experience relapse, the outcome is especially grim. About half of these patients can be reinduced into a second remission, but conventional chemotherapy will cure few, if any, of these patients. Allogeneic transplantation can cure about 25% of patients placed into a second remission, and fewer if they undergo transplantation in continued relapse. In contrast, transplantation can cure 60% of patients who undergo transplantation in first remission, but the issue remains as to how patients should be selected to undergo transplantation early, and who should get continued chemotherapy with the expectation of a reasonable response. Whereas the cytogenetic risk may help guide that decision for those at the extremes (good or bad risk), what does the clinician do with the considerable number of patients with normal cytogenetics?

The promise of using genetics as a deeper approach to understanding the heterogeneity of AML is enormous, but the delivery to the clinic has made slow progress. There are a number of genetic lesions that have been found to be potentially significant in AML. Mutations to the signaling transduction pathway are common in 60% to 70% of AML patients.^{2,3} The first described were point mutations to the guanine triphosphatase protein RAS (principally n-RAS), that are found in 10% to 20% of AML patients.^{4,5} Mutations in the upstream receptor tyrosine kinases can be found in more than 40% of patients, including point mutations in *fms* and *kit*, but predominately in *FLT3*, which increase in frequency from 10% in pediatric patients to 40% in elderly AML patients.

Mutations in the *FLT3* gene have been shown in several studies to be a strong prognostic factor in both pediatric and adult AML.^{6,7} *FLT3* mutations occur in two varieties. The most common is the internal tandem duplication, a head-to-tail duplication of 20 to 300 base pairs in the exons coding for the juxtamembrane region of the molecule. This structural change alters the biology of the *FLT3* receptor from normally signaling through ligand-dependent dimerization to that of

ligand-independent activation. Tyrosine kinase activation of *FLT3* also occurs in 5% to 10% of patients through point mutations in the activating loop of the kinase domain of the molecule.⁸

There is an emerging complexity of the relationship of *FLT3* mutations and prognosis that is an important object lesson. Though in vitro studies suggest that *FLT3-ITD* and *FLT3-ALM* mutations similarly alter signal transduction, *FLT3-ITD* mutations are clearly associated with a poor prognosis, whereas this association is less clear in patients with *FLT3* point mutations.^{7,8} Moreover, not all *FLT3-ITD* mutations are created equal. Some patients with *FLT3-ITD* are in a heterozygous state—that is, one mutated, and one wild-type allele. Other patients have clones that are homozygous for the mutated allele. Several studies have demonstrated that the higher the allelic ratio (meaning more mutated *v* wild-type allele), the worse the prognosis.^{7,9-11} The prognostic power of *FLT3* is increased by combining it with the recently described mutation status of the nucleophosmin (*NPM1*) gene. Mutations in *NPM1* are the most common mutations detected in AML, occurring in 40% of patients.¹²⁻¹⁴ The combination of a wild-type *FLT3* with a mutated *NPM* provides the best prognosis, whereas the worst prognosis is in patients with the *FLT3* mutation and wild-type *NPM*.^{14,15}

These associations, along with the emerging field of classification using gene expression, where outcome is associated with the expression of tens to thousands of genes,^{16,17} offer a destination on the long road trip from bench to bedside. New technology and more gene discoveries yield more and more potential candidates to be tested. Predictably, clinicians and scientists are left to say, “Are we there yet?” Two articles in this issue of *Journal of Clinical Oncology* merge onto this road, further refining the genetic risk of AML based on gene mutations.

Paschka et al¹⁸ studied the impact of *WT1* mutations in adult AML with normal cytogenetics. *WT1* was originally described as a tumor suppressor associated with Wilms’ tumor, but has been shown to be a transcription factor with cell-context-dependent oncogenic properties.¹⁹ *WT1* is overexpressed in more than 70% of AML patients, and in some acute lymphoblastic leukemia and myeloid blast crisis chronic myelogenous leukemia.^{20,21} Indeed, some investigators have advocated the use of *WT1* expression as a marker of prognosis and residual disease in AML.²² Mutations in *WT1* exon 7 and 9 have been previously reported.^{21,23} In the Paschka et al article, the investigators studied 196 patients, analyzing not only for *WT1* mutations, but also *FLT3*, *NPM*, and *CEBPA* mutations, as well as *ERG* expression, all of which have been

associated with prognosis in AML.²⁴⁻²⁶ *WT1* mutations were found in 11% of patients, and although patients with mutations had similar rates of complete remission compared with patients without *WT1* mutations, the relapse and progression-free survival was remarkably different. Patients with *WT1* mutations had a 3-year DFS of only 13%, whereas patients without *WT1* mutations had a DFS of 50%. This impact of *WT1* was preserved when adjusted for the mutation status of the other potential prognostic factors noted above. The clinical implication is that patients with *WT1* mutations would benefit from aggressive consolidation, for example, allogeneic or autologous transplantation.

The article by Neubauer et al²⁷ studies the prognostic significance of *RAS* mutations in adult AML, investigating the possibility that *RAS* mutations have a differential effect based on cytarabine dose, as is seen with the t(8;21) and inv(16) translocations.²⁸ Patients (N = 185) enrolled onto a study that randomly assigned patients to low-, standard-, or high-dose cytarabine consolidation were retrospectively tested for *RAS* mutations (though it is not clear whether these are n-, k-, and h-*RAS* mutations or a subset of these three); 18% of patients were found to have *RAS* mutations at diagnosis. Overall there was no effect of *RAS* mutations on outcome. However, in the subset of those receiving high-dose cytarabine, patients with *RAS* mutations had fewer relapses compared with patients with wild-type *RAS*. Thus, the cumulative incidence of relapse was 45% for patients with *RAS* mutations, compared with 68% in wild-type *RAS* patients. Moreover, among *RAS* mutants, the relapse rate was far lower in patients receiving high-dose compared with low-dose cytarabine. The clinical implication is that patients with *RAS* mutations will benefit from consolidation with high-dose cytarabine.

Both articles have some limitations, but nothing that undermines the main message of the studies. In the article by Paschka et al, one wonders how *WT1* mutations correlated with *WT1* expression, and how using this and the *FLT3* allelic ratio would have changed the multivariate results. In the article by Neubauer et al, would mutated *RAS* still have significance when correcting for cytogenetic status, or mutations in other key genes? And in both articles, what was the percentage of blasts in the diagnostic samples that harbored *WT1* and *RAS* mutations? The percentage of mutations of *RAS* and *FLT3*, for example, are quite variable at presentation, and one wonders if the association of mutation and outcome is different in patients in whom the mutation is dominant, as opposed to a minority of the leukemia cells.

It is clear that the mutation detection should be a part of most, if not all, clinical trials, as should soon be a part of routine clinical practice. The road ahead to genetic stratification still has its obstacles (and, if one considers the current grant funding situation, there is little money to pay the tolls). First, only a few specialized laboratories have invested in testing the breadth of relevant mutations. Second, it is clear that the relevance of mutations must be taken in the context of the type of mutation (eg, *FLT3-ITD* v *ALM*), other mutations (eg, *FLT3-ITD* and *NPM1*), and the particular therapy. This is problematic for clinical investigators, though it ironically adds job security to those interested in the intersection of genetics and clinical medicine. Nonetheless, for those heckling in the back, wondering when on earth we'll get there, patience, please: I think I see a sign up ahead.

AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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