

EDITORIAL



Gene-Expression Profiling in Acute Myeloid Leukemia

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The past three decades have seen considerable advances in our understanding of the underlying mechanisms of acute myeloid leukemia (AML) and dramatic improvements in treatment. At present, decisions about therapy are largely based on prognostic factors identified at the time of diagnosis or shortly thereafter. These features include age, the karyotype of the leukemic clone, the initial leukocyte count, and the response to induction chemotherapy. Karyotypic analysis is particularly important, because it not only provides a key prognostic indicator but also serves to identify biologically distinct subgroups of AML, which in some instances require specific types of treatment.¹ All-*trans*-retinoic acid and arsenic trioxide, for example, have revolutionized the treatment of acute promyelocytic leukemia characterized by t(15;17)(q22;q21).²

Effective risk stratification is essential in patients with newly diagnosed AML so that informed decisions can be made about the need for allogeneic stem-cell transplantation. There is a growing consensus that patients who have AML with prognostically favorable karyotypic abnormalities (i.e., t(15;17), t(8;21), or inv(16), or its variant t(16;16)) do not benefit from the routine use of transplantation during first complete remission, because any therapeutic advantage is offset by the risk of transplantation-related complications and death.³ By contrast, allogeneic stem-cell transplantation is considered the treatment of choice for young adults who have AML with prognostically adverse cytogenetic features (i.e., -5/del(5q), -7, abnormal 3q, t(9;22), or a complex karyotype), because the outcome of conventional chemotherapy is poor in such patients. However, since allogeneic stem-cell transplantation is not feasible or indeed curative in all patients with high-risk AML, there is a critical need for

a greater understanding of the biology of such leukemias.

The most appropriate treatment for standard-risk AML, which accounts for over half the cases of this disease, has not been firmly established. This subgroup is difficult to classify, because it includes cases with various numerical and structural cytogenetic abnormalities that occur infrequently, making it difficult to determine their prognostic significance.¹ Moreover, cytogenetic analysis provides no clues in cases of AML with a normal karyotype, which account for the majority of cases in the standard-risk group.

Molecular events implicated in the pathogenesis of AML include activating mutations in genes encoding tyrosine kinases, such as *fms*-like tyrosine kinase 3 (*FLT3*), *c-KIT*, and *N-RAS*, and mutations in genes encoding transcription factors involved in normal hematopoiesis, such as *AML1*, *GATA1*, and *CCAAT/enhancer binding protein alpha* (*CEBPA*).^{4,5} The observation that chimeric fusion genes generated by chromosomal translocations commonly coexist with a defined spectrum of mutations affecting tyrosine kinases has led to the notion of cooperating genetic lesions, which couple a block in differentiation with enhanced cellular proliferation.⁴ It is clear that molecular screening for mutations in *FLT3* and *CEBPA* can distinguish subgroups of patients with standard-risk AML who have different risks of relapse, but whether any given genetic lesion defines a clinically relevant subgroup of AML is uncertain.

The studies by Bullinger et al.⁶ and Valk et al.⁷ reported in this issue of the *Journal* highlight some of the exciting opportunities that gene-expression profiling promises to provide for advancing our understanding of AML and further refining predic-

tions of the clinical outcome. In the large study by Valk et al., which evaluated 285 unselected patients with AML encompassing a wide spectrum of the heterogeneous cytogenetic and molecular abnormalities of the disease, only 16 distinct gene-expression profiles, or clusters, were revealed by unsupervised analysis. (This term and others are explained in the glossaries that accompany the articles by Valk et al. and Bullinger et al.) Although no such study can possibly include all known molecular lesions, and hence, additional clusters are likely to exist, the work of Valk et al. does raise the interesting possibility that AML may not be as heterogeneous as previously thought. Their results support the notion of the functional redundancy of the molecular lesions in AML, in the sense that different molecular lesions may enhance self-renewal or block differentiation of the leukemic clone (or do both) to the same degree. Such redundancy suggests that there may be relatively few pathways to the development of the leukemic phenotype.⁴

A number of clusters corresponded to well-recognized cytogenetically and molecularly defined entities such as t(15;17), which leads to *PML-RARA*; t(8;21), which leads to *AML1-ETO*; and inv(16), which leads to *CBFB-MYH11* — findings that are in accord with those of Bullinger et al. and other studies^{8,9} and that support the listing of these subgroups as separate entities in the World Health Organization (WHO) classification.¹⁰ However, translocations involving the *MLL* locus at 11q23, which are also distinguished in the WHO classification, were not restricted to a single cluster. Notably, gene-expression profiles of normal CD34+ hematopoietic progenitors clustered with AML cases that had adverse features, raising the interesting possibility that the poor prognosis for patients with such leukemias is indicative of the transformation of primitive progenitors. Overall, the two articles, together with previous studies, clearly demonstrate that gene-expression profiling allows highly accurate identification of genetically defined subtypes of AML and that microarray analyses may one day serve as a diagnostic tool.^{8,9,11}

Of major importance is the potential for gene-expression profiling to provide prognostic information beyond that obtained with current methods. Valk et al. reported significant differences in the outcome among the five most common unsupervised clusters in their series; this finding is not unexpected, given the close correlation of such clusters with

cytogenetically and molecularly defined lesions that are known to have independent prognostic significance.

The study by Bullinger et al. takes the prognostic potential of gene-expression profiling a step farther. Using sophisticated and novel biostatistical methods, they found that a gene-expression profile based on 133 genes predicted the clinical outcome across cytogenetic risk groups. It will be interesting to determine whether such expression profiles point to pathways that are critical for a response to chemotherapy and whether they are related to the type of hematopoietic progenitor that undergoes leukemic transformation or the mechanisms by which this might occur. It is intriguing that the signatures reported by Bullinger et al. overlap only in part with the predictor genes identified by Yagi et al. in childhood AML.¹² Such studies suggest that small, custom-made arrays or quantitative reverse-transcriptase–polymerase-chain-reaction assays could rapidly provide information needed to tailor therapy for individual patients with AML.

Although Valk et al. and Bullinger et al. used completely different gene-expression array platforms, there is a heartening concordance between their findings and those of previous studies, which suggests that these novel techniques are robust. The consistent finding that the level of expression of some genes is altered as a direct result of chromosomal translocations (e.g., *ETO* in t(8;21) and *MYH11* in inv(16)) raises the possibility that additional targets of chromosomal translocations will be identified through microarray analysis. Indeed, the combined use of gene-expression and DNA-microarray profiling could identify consistent regions of chromosomal loss or gain involved in the primary or cooperating molecular lesions of AML.

Gene-expression profiling has already yielded considerable insights into other hematologic cancers, such as diffuse large-B-cell lymphoma,¹³ and solid tumors, such as breast carcinoma.¹⁴ The studies of AML by Valk et al. and Bullinger et al. set the scene for investigations that will use this technique to answer fundamental questions concerning the biology of AML and its response to therapy. We anticipate further insights in cases of AML with no known molecular markers. Moreover, these types of studies will inform us about associations between genetic lesions that predict a poor outcome and mechanisms of drug resistance. Although the results of gene-expression profiling may predict the

outcome of various cancers, clinically significant increases in overall cure rates are unlikely to be achieved through improvements in risk stratification alone. For this reason, a more ambitious aim of gene-expression profiling should be to characterize the hierarchy of leukemic stem cells and determine the differences between leukemic stem cells and their normal counterparts. This undertaking is fundamental to the development of treatments that can eliminate the neoplastic clone at its source, rather than target the nonclonogenic progeny that constitute the bulk of the tumor burden.

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1. Grimwade D. The clinical significance of cytogenetic abnormalities in acute myeloid leukaemia. *Best Pract Res Clin Haematol* 2001;14:497-529.
2. Mistry AR, Pedersen EW, Solomon E, Grimwade D. The molecular pathogenesis of acute promyelocytic leukaemia: implications for the clinical management of the disease. *Blood Rev* 2003;17:71-97.
3. Burnett AK. Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation? An adult treater's view. *Br J Haematol* 2002;118:357-64.
4. Kelly LM, Gilliland DG. Genetics of myeloid leukemias. *Annu Rev Genomics Hum Genet* 2002;3:179-98.
5. Tenen DG. Disruption of differentiation in human cancer: AML shows the way. *Nat Rev Cancer* 2003;3:89-101.
6. Bullinger L, Döhner K, Bair E, et al. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. *N Engl J Med* 2004;350:1605-16.
7. Valk PJM, Verhaak RGW, Beijten MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med* 2004;350:1617-28.
8. Schoch C, Kohlmann A, Schnittger S, et al. Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. *Proc Natl Acad Sci U S A* 2002;99:10008-13.
9. Debernardi S, Lillington DM, Chaplin T, et al. Genome-wide analysis of acute myeloid leukemia with normal karyotype reveals a unique pattern of homeobox gene expression distinct from those with translocation-mediated fusion events. *Genes Chromosomes Cancer* 2003;37:149-58.
10. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Vol. 3 of World Health Organization classification of tumours. Lyons, France: IARC Press, 2001.
11. Kohlmann A, Schoch C, Schnittger S, et al. Pediatric acute lymphoblastic leukemia (ALL) gene expression signatures classify an independent cohort of adult ALL patients. *Leukemia* 2004;18:63-71.
12. Yagi T, Morimoto A, Eguchi M, et al. Identification of a gene expression signature associated with pediatric AML prognosis. *Blood* 2003;102:1849-56.
13. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403:503-11.
14. van de Vijver MJ, He YD, van 't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002;347:1999-2009.

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