REVIEW ARTICLE

GENOMIC MEDICINE

Molecular Diagnosis of the Hematologic Cancers

Louis M. Staudt, M.D., Ph.D.

THE DIAGNOSIS OF THE HEMATOLOGIC CANCERS PRESENTS A DAUNTING challenge. The many stages of normal hematopoietic differentiation give rise to a number of biologically and clinically distinct cancers. Inherited DNA-sequence variants do not appear to have a prominent causative role; rather, these diverse cancers are typically initiated by acquired alterations to the genome of the cancer cell, such as chromosomal translocations, mutations, and deletions. The diagnosis of the hematologic cancers is commonly based on morphologic evaluation supplemented by analysis of a few molecular markers. However, in some diagnostic categories defined in this fashion, the response of patients to treatment is markedly heterogeneous, arousing the suspicion that there can be several molecularly distinct diseases within the same morphologic category.

Gene-expression profiling is a genomics technique that has proved effective in deciphering this biologic and clinical diversity. The approach relies on the fact that only a fraction of the genes encoded in the genome of each cell are expressed — that is, actively transcribed into messenger RNA (mRNA) (Fig. 1A). The abundance of mRNA for each gene depends on a cell's lineage and stage of differentiation, on the activity of intracellular regulatory pathways, and on the influence of extracellular stimuli. To a large extent, the complement of mRNAs in a cell dictates its complement of proteins, and consequently, gene expression is a major determinant of the biology of normal and malignant cells.

In the process of expression profiling, robotically printed DNA microarrays are used to measure the expression of tens of thousands of genes at a time; this creates a molecular profile of the RNA in a tumor sample[1] (Fig. 1B). A variety of analytic techniques are used to classify cancers on the basis of their gene-expression profiles.[2,3] There are two general approaches. In an unsupervised approach, pattern-recognition algorithms are used to identify subgroups of tumors that have related gene-expression profiles (Fig. 2A). In a supervised approach, statistical methods are used to relate gene-expression data and clinical data (Fig. 2B). These methods have revealed unexpected subgroups within the diagnostic categories of the hematologic cancers that are based on morphology and have demonstrated that the response to therapy is dictated by multiple independent biologic features of a tumor. This is not a comprehensive review of hematologic cancers; rather, it will provide examples of how gene-expression profiling has been used to provide a framework for the molecular diagnosis of these cancers.

From the Metabolism Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Md. Address reprint requests to Dr. Staudt at the Metabolism Branch, NCI, Bldg. 10, Rm. 4N114, NIH, 9000 Rockville Pike, Bethesda, MD 20892, or at lstaudt@mail.nih.gov.

MOLECULAR DIAGNOSIS OF NON-HODGKIN'S LYMPHOMA

DIFFUSE LARGE-B-CELL LYMPHOMA

Some cases of diffuse large-B-cell lymphoma respond well to multiagent chemotherapy,[5] but this lymphoma nonetheless remains a perplexing clinical puzzle, since roughly

60 percent of cases are incurable. This observation raises the possibility that this single diagnostic category may harbor more than one molecular disease.

The gene-expression profiles of lymph-node–biopsy specimens from patients with morphologically identical diffuse large-B-cell lymphoma show pronounced variability, with no common set of genes expressed in all cases.[4,6,7] To make sense of this variability, genes were classified into expression signatures[8] — that is, groups of genes with similar patterns of expression in a set of samples. Some signatures include genes expressed in a particular type of cell or stage of differentiation, whereas other signatures include genes expressed during a particular biologic response, such as cellular proliferation or the activation of a cellular signaling pathway.

One gene-expression signature that varies markedly among diffuse large-B-cell lymphomas is the germinal-center B-cell signature.[4,6] This signature characterizes B cells that are responding to a foreign antigen within the germinal-center microenvironment of secondary lymphoid organs. Among biopsy samples from patients with diffuse large-B-cell lymphoma, three biologically and clinically distinct subgroups have been identified[4,6] (Fig. 3A). The germinal-center B-cell–like subgroup (approximately 50 percent of cases) has high levels of expression of germinal-center B-cell signature genes, whereas the other two subgroups of diffuse large-B-cell lymphoma — termed activated B-cell–like and type 3 — do not. The activated B-cell–like subgroup (approximately 30 percent of cases) instead expresses genes that are induced by mitogenic stimulation of blood B cells. The type 3 subgroup does not express genes characteristic of the other two subgroups and may yet be found to be heterogeneous. These findings suggest that the subgroups of diffuse large-B-cell lymphoma arise from different stages of normal B-cell development.

The notion that the gene-expression subgroups represent pathogenetically distinct types of diffuse large-B-cell lymphoma has been strongly supported by analysis of recurring chromosomal abnormalities in this cancer.[4,10] The t(14;18) translocation involving the *BCL2* gene and the amplification of the *c-rel* gene on chromosome 2p are recurrent oncogenic events in germinal-center B-cell–like diffuse large-B-cell lymphoma, but they never occur in the other subgroups. Activation of the nuclear factor-κB signaling pathway is a feature of the activated B-cell–like subgroup but not the other subgroups, and in-

terference with this pathway selectively kills this type of diffuse large-B-cell lymphoma.[11]

The subgroups defined with the use of gene-expression signatures are clinically distinct as well: patients with the germinal-center B-cell–like form have a higher rate of overall survival five years after chemotherapy than do patients in the other subgroups[4,6] (Fig. 3A). This clinical distinction based on gene-expression profiles was evident even after the patients were classified according to the International Prognostic Index,[4,6] a well-established predictor of outcome in diffuse large-B-cell lymphoma.[12]

PREDICTING THE CLINICAL OUTCOME
The example of diffuse large-B-cell lymphoma demonstrates how an unsupervised analysis of gene-expression data can reveal clinically distinct subgroups of tumors. In the complementary, supervised approach, clinical data are used to identify genes whose patterns of expression are correlated with the length of survival after diagnosis or with the likelihood that therapy will be curative. This approach

Figure 1 (facing page). Differential Expression of Messenger RNA (mRNA) by Different Types of Cells (Panel A) and Gene-Expression Profiling Using DNA Microarrays (Panel B).

In Panel A, different types of cells, exemplified by a myocyte and a lymphocyte, express a distinct set of mRNAs from their genomes. Although the myocyte and lymphocyte possess the same inherited genomic DNA, distinct regulatory networks inside each cell cause different genes to be expressed as mRNA. The genes that encode myosin and immunoglobulin are among the most differentially expressed genes between these two types of cells. The mRNAs for other genes may be present in both types of cells, but at different levels, which may also affect the biology of the cells. Panel B shows the technique of gene-expression profiling, which uses DNA microarrays. First, mRNA is extracted from a cell and copied enzymatically to create a fluorescent complementary DNA (cDNA) probe representing the expressed genes in the cell. This probe is then incubated on the surface of a DNA microarray, which contains spots of DNA derived from thousands of distinct human genes. During the incubation, each cDNA molecule in the probe hybridizes to the microarray spot that represents its respective gene. The extent of hybridization of fluorescent cDNAs to each microarray spot is quantitated with use of a scanning fluorescence microscope. The levels of expression of more than 20,000 genes — in this example, the genes for myosin and immunoglobulin — can be measured in a single DNA-microarray experiment.
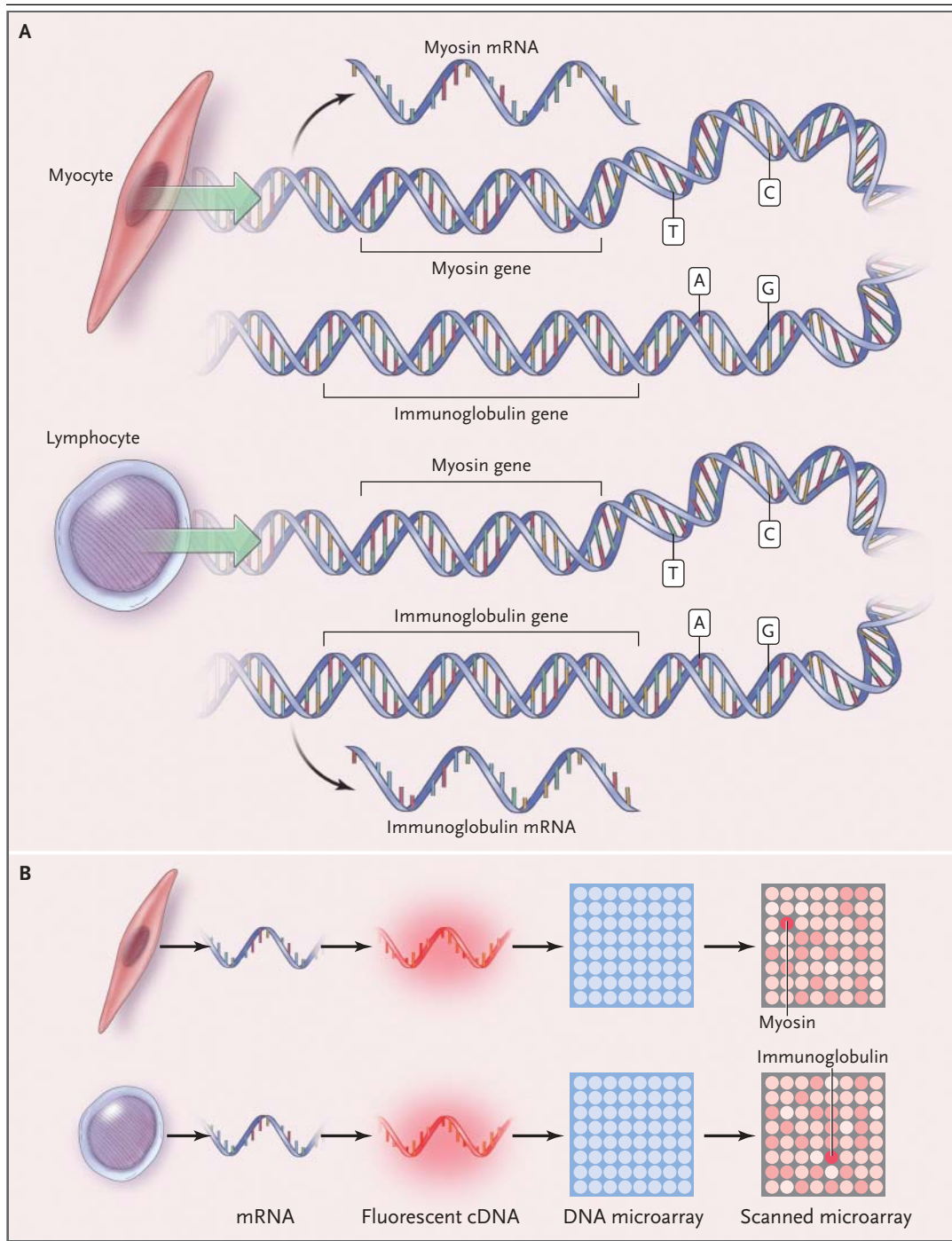
has been used to develop robust predictors of prognosis in mantle-cell lymphoma[13] and diffuse large-B-cell lymphoma.[4,7]

Mantle-cell lymphoma constitutes approximately 8 percent of cases of non-Hodgkin's lymphomas but a much larger fraction of deaths from lympho-
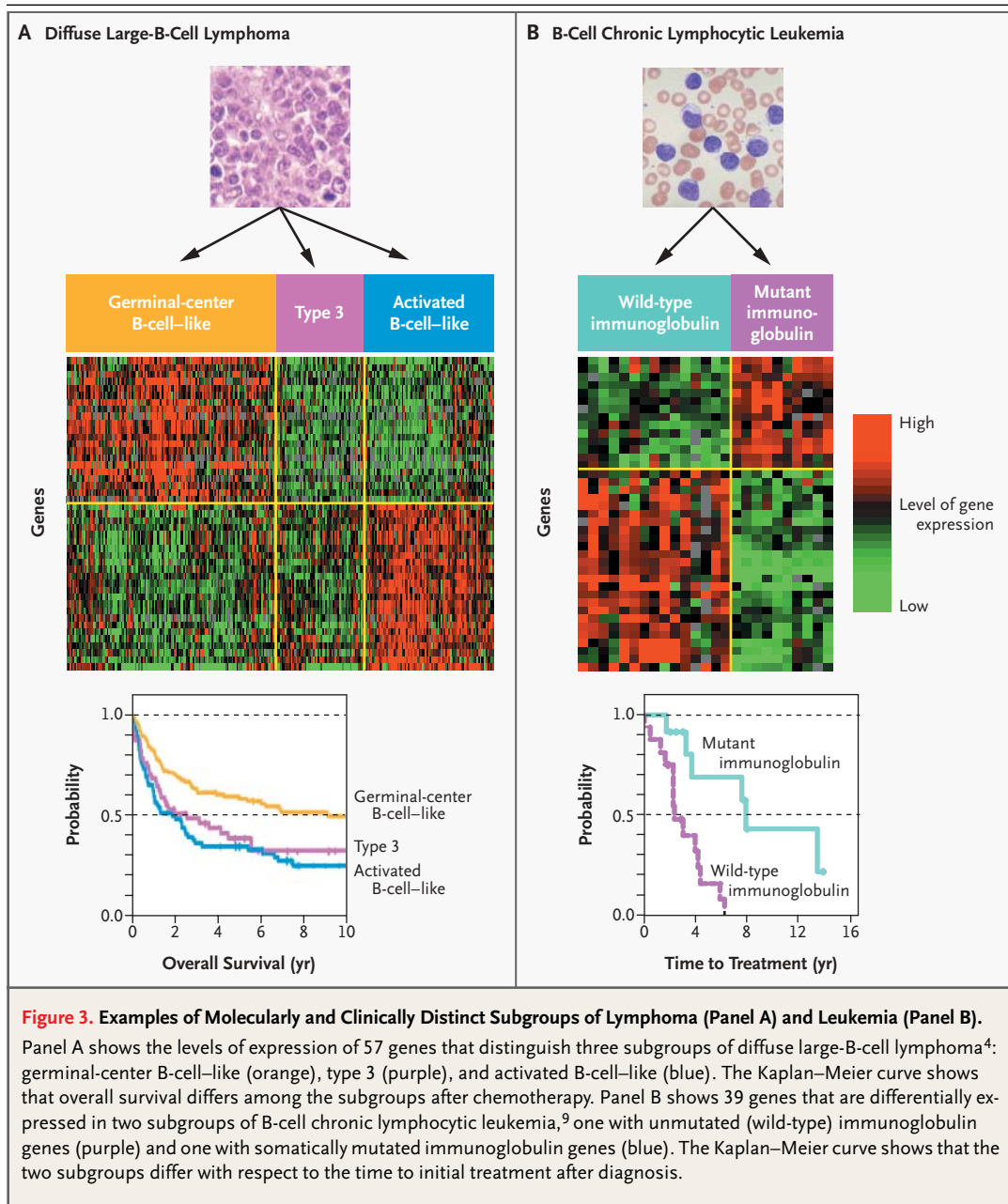
ma, since current therapy is not curative. The length of survival among patients with mantle-cell lymphoma is quite variable, ranging from less than 1 year to more than 10 years.[13] Gene-expression profiling revealed a strong association between the expression of genes in the "proliferation" signature and surviv-

Figure 2. Molecular Diagnosis of Cancer by Gene-Expression Profiling with the Use of Unsupervised (Panel A) and Supervised (Panel B) Pattern-Recognition Algorithms.

Panel A shows the discovery of cancer subgroups with the use of an unsupervised pattern-recognition algorithm. The expression of genes, as determined by DNA-microarray analysis, is depicted in a tabular format. Each row represents data for a particular human gene, and each column represents the expression of genes in a single biopsy sample (arrows). Highly expressed genes are shown in shades of red, and less highly expressed genes are shown in shades of green, according to the color scale shown. Before the analysis, no pattern is apparent (left-hand panel). A mathematical algorithm, termed "hierarchical clustering,"[2] is applied to the gene-expression data to search for a pattern (right-hand panel). This algorithm first rearranges the genes (in rows) so that genes with related patterns of expression are clustered. The algorithm next rearranges the samples (in columns) so that samples that have related expression of these genes are clustered. In this example, the hierarchical-clustering algorithm identified a clear subgroup of three tumor samples (on the far right-hand side) whose pattern of gene expression is distinct. Panel B shows how a supervised statistical algorithm is used to identify genes with patterns of expression that predict the clinical outcome. For each gene on the microarray, expression data from tumors are correlated with overall survival data from the corresponding patients. The example shows two genes with patterns of expression that are correlated with survival after chemotherapy for diffuse large-B-cell lymphoma.[4] A high level of expression of gene A is associated with extended survival, whereas a high level of expression of gene B is associated with short survival. Neither gene has a pattern of expression that is perfectly correlated with survival, illustrating that the clinical outcome is independently influenced by multiple molecular and clinical variables.[4]

al in mantle-cell lymphoma.[13] The proliferation signature includes genes that are more highly expressed in dividing cells than in quiescent cells (Fig. 4A). The quartile of patients with the highest level of proliferation-signature expression had a median survival of 6.7 years, whereas the quartile with the lowest level of expression had a median survival

of 0.8 year (Fig. 4A). The variable survival of patients with mantle-cell lymphoma is therefore largely dictated by a single aspect of tumor biology, the rate of cell division, which can be quantitated by gene-expression profiling.
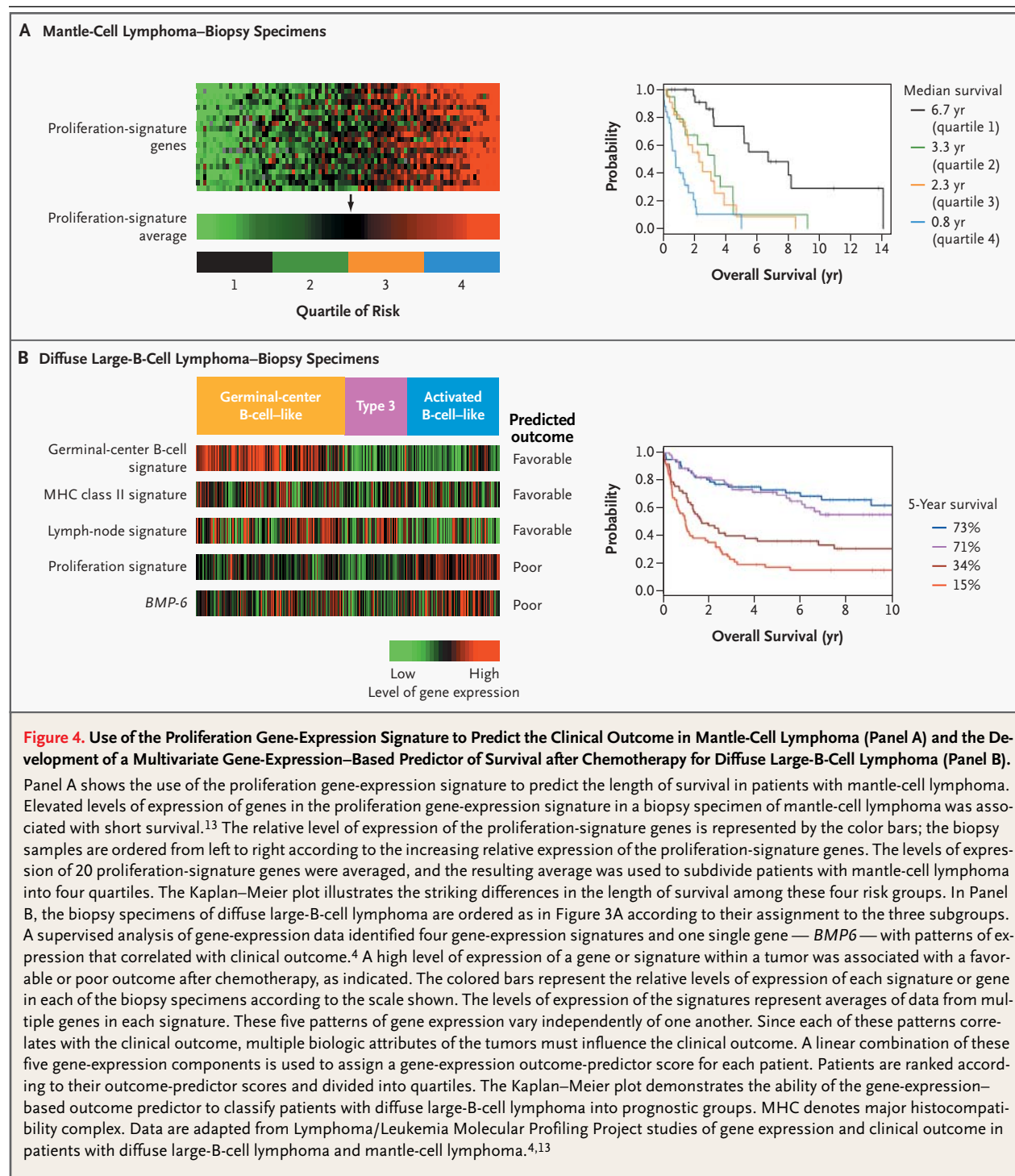
Although the subgroups of diffuse large-B-cell lymphoma have distinct survival rates, the statistical

Figure 3. Examples of Molecularly and Clinically Distinct Subgroups of Lymphoma (Panel A) and Leukemia (Panel B).
Panel A shows the levels of expression of 57 genes that distinguish three subgroups of diffuse large-B-cell lymphoma[4]: germinal-center B-cell–like (orange), type 3 (purple), and activated B-cell–like (blue). The Kaplan–Meier curve shows that overall survival differs among the subgroups after chemotherapy. Panel B shows 39 genes that are differentially expressed in two subgroups of B-cell chronic lymphocytic leukemia,[9] one with unmutated (wild-type) immunoglobulin genes (purple) and one with somatically mutated immunoglobulin genes (blue). The Kaplan–Meier curve shows that the two subgroups differ with respect to the time to initial treatment after diagnosis.

approach of supervised analysis identified additional molecular differences among the tumors that can account for much of the remaining heterogeneity in survival[4,7] (Fig. 4B). This approach demonstrated that at least five distinct features of diffuse large-B-cell lymphomas influence the response to chemotherapy.[4] Specifically, the levels of expression of the germinal-center B-cell signature, the proliferation signature, the major-histocompatibility-complex (MHC) class II signature, and the lymph-node signature were predictive of the clinical outcome, as was the level of expression of *BMP6*, a gene that does not belong to a defined expression signature. As in mantle-cell lymphoma, expression of the proliferation signature predicted a poor outcome. Predictive genes in two other signatures suggest that the host immune response has an important role in curative responses to chemotherapy. Expression of the lymph-node–signature genes reflects the nontumor cells in the diffuse large-B-cell lymphoma–biopsy

Figure 4. **Use of the Proliferation Gene-Expression Signature to Predict the Clinical Outcome in Mantle-Cell Lymphoma (Panel A) and the Development of a Multivariate Gene-Expression–Based Predictor of Survival after Chemotherapy for Diffuse Large-B-Cell Lymphoma (Panel B).**

Panel A shows the use of the proliferation gene-expression signature to predict the length of survival in patients with mantle-cell lymphoma. Elevated levels of expression of genes in the proliferation gene-expression signature in a biopsy specimen of mantle-cell lymphoma was associated with short survival.[13] The relative level of expression of the proliferation-signature genes is represented by the color bars; the biopsy samples are ordered from left to right according to the increasing relative expression of the proliferation-signature genes. The levels of expression of 20 proliferation-signature genes were averaged, and the resulting average was used to subdivide patients with mantle-cell lymphoma into four quartiles. The Kaplan–Meier plot illustrates the striking differences in the length of survival among these four risk groups. In Panel B, the biopsy specimens of diffuse large-B-cell lymphoma are ordered as in Figure 3A according to their assignment to the three subgroups. A supervised analysis of gene-expression data identified four gene-expression signatures and one single gene — *BMP6* — with patterns of expression that correlated with clinical outcome.[4] A high level of expression of a gene or signature within a tumor was associated with a favorable or poor outcome after chemotherapy, as indicated. The colored bars represent the relative levels of expression of each signature or gene in each of the biopsy specimens according to the scale shown. The levels of expression of the signatures represent averages of data from multiple genes in each signature. These five patterns of gene expression vary independently of one another. Since each of these patterns correlates with the clinical outcome, multiple biologic attributes of the tumors must influence the clinical outcome. A linear combination of these five gene-expression components is used to assign a gene-expression outcome-predictor score for each patient. Patients are ranked according to their outcome-predictor scores and divided into quartiles. The Kaplan–Meier plot demonstrates the ability of the gene-expression–based outcome predictor to classify patients with diffuse large-B-cell lymphoma into prognostic groups. MHC denotes major histocompatibility complex. Data are adapted from Lymphoma/Leukemia Molecular Profiling Project studies of gene expression and clinical outcome in patients with diffuse large-B-cell lymphoma and mantle-cell lymphoma.[4,13]

specimen, including activated macrophages, natural killer cells, and stromal cells. A high level of expression of these genes predicts a favorable clinical outcome, suggesting that this reactive immune response is beneficial. The MHC class II signature includes genes encoding components of this critical antigen-presentation–protein complex, and decreased expression of these genes predicts a poor outcome. These findings suggest that some tumors may evade the immune response by down-regulating their antigen-presentation capacity.

These expression signatures can be combined to form a multivariate predictor of survival after chemotherapy for diffuse large-B-cell lymphoma.[4] With the use of this approach, half the patients can be placed into a favorable-risk group, with a five-year survival rate of more than 70 percent; one quarter can be assigned to a poor-risk group, with a five-year survival rate of 15 percent; and the remaining patients are in an intermediate-risk group, with a five-year survival rate of 34 percent (Fig. 4B).

MOLECULAR DIAGNOSIS OF LEUKEMIAS

ACUTE LEUKEMIAS

The molecular diagnosis of leukemias began with the recognition and analysis of recurrent chromosomal translocations.[14,15] The genes discovered at the translocation break points have drawn attention to critical regulatory pathways in hematopoietic cells that can cause cancer when they are dysregulated. In many acute leukemias, translocations fuse genes that reside on the two partner chromosomes, creating a chimeric gene with novel oncogenic properties.

Chromosomal translocations have been used to identify patients with acute leukemia with distinct clinical outcomes.[16,17] In acute myeloid leukemia (AML), for instance, the presence of a t(8;21) translocation or a chromosome 16 inversion identifies patients with a comparatively good prognosis, whereas the t(9;22) translocation is associated with a poor outcome.[17] It is important to note that chromosomal translocations have been used to identify patients who will benefit from intensifying the dose of chemotherapy.[18-20]

Despite these prognostic and therapeutic successes, chromosomal translocations account for only part of the varied clinical behavior of acute leukemia, for several reasons. First, other genetic aberrations can be functionally equivalent to a transloca-

tion,[21,22] thus diminishing the prognostic power of a translocation as a single variable. Second, additional oncogenic abnormalities may accumulate in a leukemia that alter its responsiveness to therapy. For example, mutations in the gene encoding the flt3 receptor tyrosine kinase have been associated with response to treatment in patients with AML.[23-26] Furthermore, flt3 mutations that activate the kinase are present in some cases of acute lymphoblastic leukemia (ALL) with a t(4;14) translocation, rendering them susceptible to killing by flt3 inhibitors.[27] Finally, a sizable fraction of the acute leukemias have none of the defined recurrent translocations.[16,17]

Gene-expression profiling has been used as an alternative approach to mapping chromosomal translocations. In pediatric B-cell ALL, gene-expression signatures have been identified that correlate with six different chromosomal abnormalities.[28,29] These gene-expression signatures can be combined with the use of statistical algorithms to predict chromosomal abnormalities with 96 to 100 percent accuracy.[29] Likewise, in adult AML, a gene-expression–based predictor has been created that can identify three different chromosomal translocations with a high rate of accuracy.[30] Gene-expression predictors can also identify patients with AML who have isolated trisomy 8.[31] These encouraging results demonstrate that DNA microarrays can be used to diagnose most chromosomal abnormalities in acute leukemias and could potentially substitute for the multiple diagnostic tests for these abnormalities that are currently required.

An oncogene likely to be causally related to T-cell ALL can be dysregulated by chromosomal translocations in some cases but by alternative mechanisms in others.[22] For example, the HOX11 oncogene is involved in recurrent but infrequent translocations in T-cell ALL, but gene-expression profiling revealed that some cases of T-cell ALL overexpress HOX11 without any detectable chromosomal abnormalities in this gene. All leukemias that overexpress HOX11 have a common gene-expression signature, suggesting that they are biologically similar. Most important, patients with leukemias that overexpress HOX11 have a favorable outcome, as compared with patients with other types of T-cell ALL, whether or not the overexpression is due to translocation, indicating the clinical superiority of expression profiling[22] over identification of the translocation.

Two adverse events after the treatment of acute leukemias are relapse and the development of sec-

ondary leukemias. In B-cell ALL, gene-expression profiling at the time of diagnosis provided information that could predict which patients would relapse and which would remain in continuous complete remission.[29] Interestingly, no patterns of gene expression have been found to predict relapse in all subtypes of ALL. Rather, relapse was predicted by the expression of different genes in each leukemic subtype, emphasizing once again their divergent biologic characteristics. Secondary AML arises as a consequence of treatment in some patients with ALL, and this complication could also be predicted on the basis of gene-expression profiling in the subgroup of B-cell ALL with the t(12;21) translocation.[29] Although these predictors of clinical outcome will need to be validated in independent data sets, these findings suggest that treatment stratification based on gene-expression profiling can be initiated at the time of the initial diagnosis of ALL.

CHRONIC LYMPHOCYTIC LEUKEMIA

The most common leukemia in humans — chronic lymphocytic leukemia (CLL) — is an indolent but inexorable disease with no cure. Studies of immunoglobulin gene mutations in CLL cells raised the intriguing hypothesis that CLL might be two distinct diseases.[32,33] The presence of somatic mutations in the immunoglobulin genes of CLL cells defined a group of patients who had stable or slowly progressing disease requiring late or no treatment. By contrast, the absence of immunoglobulin gene mutations in CLL cells defined a group of patients who had a progressive clinical course requiring early treatment. These two subtypes of CLL may also differ with respect to oncogenic mechanisms, since deletion of the ATM locus on chromosome 11q is associated with the absence of immunoglobulin gene mutations in CLL[34-36] and with shortened survival in some patients.[37]

Despite these clinical and molecular differences between the subtypes of CLL, gene-expression profiling revealed that CLL cells express a common gene-expression signature that differentiates this form of leukemia from other lymphoid cancers and from normal lymphoid subpopulations.[9,38] This signature is shared by all cases of CLL, irrespective of the immunoglobulin gene mutation status, sug-

gesting that CLL should be considered a single disease entity.

Nonetheless, given the clear clinical differences between the two subtypes of CLL, a hunt was made for genes that correlated with this distinction.[9,38] Roughly 160 genes were found whose levels of expression differed significantly between the two subtypes[9] (Fig. 3B). Expression of the single most discriminating gene, ZAP-70, distinguished these two subtypes with 93 percent accuracy.[9,39] Whereas analysis of the immunoglobulin gene sequence would be a challenging and expensive test to introduce into routine clinical practice, a quantitative reverse-transcriptase–polymerase-chain-reaction assay or protein-based assay for the expression of ZAP-70 is feasible.[39,40]

TRANSLATING MOLECULAR DIAGNOSIS INTO A CLINICAL REALITY

What form of technology will be used for the molecular diagnosis of cancer in the future? Our experience with gene-expression profiling has taught us two clear lessons: multiple genes need to be studied to distinguish most types of cancer, and quantitative measurement of molecular differences among tumors results in clinically important diagnostic and prognostic distinctions. An important goal will therefore be to develop a platform for routine clinical diagnosis that can quantitatively measure the expression of a few hundred genes. Such a diagnostic platform would allow us quickly to translate what we have learned about important molecular subgroups within each hematologic cancer. As we design new clinical trials, however, we must include genomic-scale gene-expression profiling in order to identify the genes that influence the response to the agents under investigation. In this fashion, we can iteratively refine the molecular diagnosis of the hematologic cancers on the basis of new advances in treatment and thus eventually reach the goal of tailored therapies for molecularly defined diseases.

REFERENCES

1. Staudt LM, Brown PO. Genomic views of the immune system. Annu Rev Immunol 2000;18:829-59.

2. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998;95:14863-8.

3. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531-7.

4. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002; 346:1937-47.

5. DeVita VT Jr, Canellos GP, Chabner B, Schein P, Hubbard SP, Young RC. Advanced diffuse histiocytic lymphoma, a potentially curable disease. Lancet 1975;1:248-50.

6. 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.

7. Shipp MA, Ross KN, Tamayo P, et al. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nat Med 2002; 8:68-74.

8. Shaffer AL, Rosenwald A, Hurt EM, et al. Signatures of the immune response. Immunity 2001;15:375-85.

9. Rosenwald A, Alizadeh AA, Widhopf G, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001;194:1639-47.

10. Huang JZ, Sanger WG, Greiner TC, et al. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. Blood 2002;99:2285-90.

11. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med 2001;194:1861-74.

12. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 1993;329:987-94.

13. Rosenwald A, Wright G, Wiestner A, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell 2003;3:185-97.

14. Rowley JD. The critical role of chromosome translocations in human leukemias. Annu Rev Genet 1998;32:495-519.

15. Nowell PC. Progress with chronic myelogenous leukemia: a personal perspective over four decades. Annu Rev Med 2002;53: 1-13.

16. Ferrando AA, Look AT. Clinical implications of recurring chromosomal and associated molecular abnormalities in acute lymphoblastic leukemia. Semin Hematol 2000; 37:381-95.

17. Mrozek K, Heinonen K, Bloomfield CD. Clinical importance of cytogenetics in acute myeloid leukaemia. Best Pract Res Clin Haematol 2001;14:19-47.

18. Bloomfield CD, Lawrence D, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res 1998;58:4173-9.

19. Ayigad S, Kuperstein G, Zilberstein J, et al. TEL-AML1 fusion transcript designates a favorable outcome with an intensified protocol in childhood acute lymphoblastic leukemia. Leukemia 1999;13:481-3.

20. Maloney K, McGavran L, Murphy J, et al. TEL-AML1 fusion identifies a subset of children with standard risk acute lymphoblastic leukemia who have an excellent prognosis when treated with therapy that includes a single delayed intensification. Leukemia 1999; 13:1708-12.

21. Pabst T, Mueller BU, Zhang P, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet 2001;27:263-70.

22. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 2002;1: 75-87.

23. Nakao M, Yokota S, Iwai T, et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 1996;10: 1911-8.

24. Hayakawa F, Towatari M, Kiyoi H, et al. Tandem-duplicated Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. Oncogene 2000;19:624-31.

25. Yamamoto Y, Kiyoi H, Nakano Y, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood 2001;97:2434-9.

26. Gilliland DG, Griffin JD. Role of FLT3 in leukemia. Curr Opin Hematol 2002;9: 274-81.

27. Armstrong SA, Kung AL, Mabon ME, et al. Inhibition of FLT3 in MLL: validation of a therapeutic target identified by gene expression based classification. Cancer Cell 2003; 3:173-83.

28. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2001;30:41-7.

29. Yeoh E-J, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002;1:133-43.

30. 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.

31. Virtaneva K, Wright FA, Tanner SM, et al. Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. Proc Natl Acad Sci U S A 2001; 98:1124-9.

32. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840-7.

33. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94:1848-54.

34. Stankovic T, Stewart GS, Fegan C, et al. Ataxia telangiectasia mutated-deficient B-cell chronic lymphocytic leukemia occurs in pregerminal center cells and results in defective damage response and unrepaired chromosome damage. Blood 2002;99:300-9.

35. Krober A, Seiler T, Benner A, et al. V(H) mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. Blood 2002;100: 1410-6.

36. Oscier DG, Gardiner AC, Mould SJ, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood 2002;100:1177-84.

37. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910-6.

38. Klein U, Tu Y, Stolovitzky GA, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 2001;194:1625-38.

39. Wiestner A, Rosenwald A, Barry TS, et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood (in press).

40. Crespo M, Bosch F, Villamor N, et al. ZAP-70 as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med 2003;348:1764-75.

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