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Science 286, 531 (1999);
DOI: 10.1126/science.286.5439.531

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Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring


Although cancer classification has improved over the past 30 years, there has been no general approach for identifying new cancer classes (class discovery) or for assigning tumors to known classes (class prediction). Here, a generic approach to cancer classification based on gene expression monitoring by DNA microarrays is described and applied to human acute leukemias as a test case. A class discovery procedure automatically discovered the distinction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) without previous knowledge of these classes. An automatically derived class predictor was able to define the class of new leukemia cases. The results demonstrate the feasibility of cancer classification based solely on gene expression monitoring and suggest a general strategy for discovering and predicting cancer classes for other types of cancer, independent of previous biological knowledge.

The challenge of cancer treatment has been to target specific therapies to pathogenetically distinct tumor types, to maximize efficacy and minimize toxicity. Improvements in cancer classification have thus been central to advances in cancer treatment. Cancer classification has been based primarily on morphological appearance of the tumor, but this has serious limitations. Tumors with similar histopathological appearance can follow significantly different clinical courses and show different responses to therapy. In a few cases, such clinical heterogeneity has been explained by dividing morphologically similar tumors into subtypes based on distinct pathogeneses. Key examples include the subdivision of acute leukemias, non-Hodgkin’s lymphomas, and childhood “small round blue cell tumors” (tumors with variable response to therapy (1) that are now molecularly subclassified into neuroblastomas, rhabdomyosarcoma, Ewing’s sarcoma, and other types (2)). For many more tumors, important subtypes are likely to exist but have yet to be defined by molecular markers. For example, prostate cancers of identical grade can have widely variable clinical courses, from indolence over decades to explosive growth causing rapid patient death. Cancer classification has been difficult in part because it has historically relied on specific biological insights, rather than systematic and unbiased approaches for recognizing tumor subtypes.

Here we describe such an approach based on global gene expression analysis.

We divided cancer classification into two challenges: class discovery and class prediction. Class discovery refers to defining previously unrecognized tumor subtypes. Class prediction refers to the assignment of particular tumor samples to already-defined classes, which could reflect current states or future outcomes.

We chose acute leukemias as a test case. Classification of acute leukemias began with the observation of variability in clinical outcome (3) and subtle differences in nuclear morphology (4). Enzyme-based histochemical analyses were introduced in the 1960s to demonstrate that some leukemias were periodic acid-Schiff positive, whereas others were myeloperoxidase positive (5). This provided the first basis for classification of acute leukemias into those arising from lymphoid precursors (acute lymphoblastic leukemia, ALL) or from myeloid precursors (acute myeloid leukemia, AML). This classification was further solidified by the development in the 1970s of antibodies recognizing either lymphoid or myeloid cell surface molecules (6). Most recently, particular subtypes of acute leukemia have been found to be associated with specific chromosomal translocations—for example, the t(12;21)(p13;q22) translocation occurs in 25% of patients with ALL, whereas the t(8;21)(q22;q22) occurs in 15% of patients with AML (7).

Although the distinction between AML and ALL has been well established, no single test is currently sufficient to establish the diagnosis. Rather, current clinical practice involves an experienced hematopathologist’s interpretation of the tumor’s morphology, histochemistry, immunophenotyping, and cytogenetic analysis, each performed in a separate, highly specialized laboratory. Although usually accurate, leukemia classification remains imperfect and errors do occur.

Distinguishing ALL from AML is critical for successful treatment; chemotherapy regimens for ALL generally contain corticosteroids, vincristine, methotrexate, and L-asparaginase, whereas most AML regimens rely on a backbone of daunorubicin and cytarabine (8). Although remissions can be achieved using ALL therapy for AML (and vice versa), cure rates are markedly diminished, and unwarranted toxicities are encountered.

We set out to develop a more systematic approach to cancer classification based on the simultaneous expression monitoring of thousands of genes using DNA microarrays (9). It has been suggested (10) that such microarrays could provide a tool for cancer classification. Microarray studies to date (11), however, have primarily been descriptive rather than analytical and have focused on cell culture rather than primary patient material, in which genetic noise might obscure an underlying reproducible expression pattern.

We began with class prediction: How could one use an initial collection of samples belonging to known classes (such as AML and ALL) to create a “class predictor” to classify new, unknown samples? We developed an analytical method and first tested it on distinctions that are easily made at the morphological level, such as distinguishing normal kidney from renal cell carcinoma (12). We then turned to the more challenging problem of distinguishing acute leukemias, whose appearance is highly similar.

Our initial leukemia data set consisted of 38 bone marrow samples (27 ALL, 11 AML) obtained from acute leukemia patients at the time of diagnosis (13). RNA prepared from bone marrow mononuclear cells was hybridized to high-density oligonucleotide microarrays, produced by Affymetrix and containing 6081 human genes (14). For each gene, we obtained a quantitative expression level. Samples were subjected to a priori quality control standards regarding the amount of labeled RNA and the quality of the scanned microarray image (15).

The first issue was to explore whether
there were genes whose expression pattern was strongly correlated with the class distinction to be predicted. The 6817 genes were sorted by their degree of correlation (16). To establish whether the observed correlations were stronger than would be expected by chance, we developed a method called “neighborhood analysis” (Fig. 1A). Briefly, one defines an “idealized expression pattern” corresponding to a gene that is uniformly high in one class and uniformly low in the other. One tests whether there is an unusually high density of genes “nearby” (that is, similar to) this idealized pattern, as compared to equivalent random patterns.

For the 38 acute leukemia samples, neighborhood analysis showed that roughly 1100 genes were more highly correlated with the AML-ALL class distinction than would be expected by chance (Fig. 2) (17). This suggested that classification could indeed be based on expression data.

The second issue was how to use a collection of known samples to create a “class predictor” capable of assigning a new sample to one of two classes. We developed a procedure that uses a fixed subset of “informative genes” (chosen based on their correlation with the class distinction) and makes a prediction on the basis of the expression level of these genes in a new sample. Each informative gene casts a “weighted vote” for one of the classes, with the magnitude of each vote dependent on the expression level in the new sample and the degree of that gene’s correlation with the class distinction (Fig. 1B) (18, 19). The votes were summed to determine the winning class, as well as a “prediction strength” (PS), which is a measure of the margin of victory that ranges from 0 to 1 (20). The sample was assigned to the winning class if PS exceeded a predetermined threshold, and was otherwise considered uncertain. On the basis of previous analysis, we used a threshold of 0.3 (21).

The third issue was how to test the validity of class predictors. We used a two-step procedure. The accuracy of the predictors was first tested by cross-validation on the initial data set. (Briefly, one withholds a sample, builds a predictor based only on the remaining samples, and predicts the class of the withheld sample. The process is repeated for each sample, and the cumulative error rate is calculated.) One then builds a final predictor based on the initial data set and assesses its accuracy on an independent set of samples.

We applied this approach to the 38 acute leukemia samples. The set of informative genes to be used in the predictor was chosen to be the 50 genes most closely correlated with AML-ALL distinction in the known samples. The parameters of the predictor were determined by the expression levels of these 50 genes in the known samples. The predictor was then used to classify new samples, by applying it to the expression levels of these genes in the sample.

The 50-gene predictors derived in cross-validation tests assigned 36 of the 38 samples as either AML or ALL and the remaining two as uncertain (PS < 0.3) (22). All 36 predictions agreed with the patients’ clinical diagnosis. We then created a 50-gene predictor on the basis of all 38 samples and applied it to an independent collection of 34 leukemia samples. The specimens consisted of 24 bone marrow and 10 peripheral blood samples (23). In total, the predictor made strong predictions for 29 of the 34 samples, and the accuracy was 100%. The success was notable because the collection included a much broader range of samples, including samples from peripheral blood rather than bone marrow, from childhood AML patients, and from different reference laboratories that used different sample preparation protocols. Overall, the prediction strengths were quite high (median PS = 0.77 in cross-validation and 0.73:

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**Fig. 1.** Schematic illustration of methodology. (A) Neighborhood analysis. The class distinction is represented by an “idealized expression pattern” c, in which the expression level is uniformly high in class 1 and uniformly low in class 2. Each gene is represented by an expression vector, consisting of its expression level in each of the tumor samples. In the figure, the data set is composed of six AMLs and six ALLs. Gene $g_1$ is well correlated with the class distinction, whereas $g_2$ is poorly correlated. Neighborhood analysis involves counting the number of genes having various levels of correlation with c. The results are compared to the corresponding distribution obtained for random idealized expression patterns $c^\prime$, obtained by randomly permuting the coordinates of c. An unusually high density of genes indicates that there are many more genes correlated with the pattern than expected by chance. The precise measure of distance and other methodological details are described in (16, 17) and on our Web site (www.genome.wi.mit.edu/MPR). (B) Class predictor. The prediction of a new sample is based on “weighted votes” of a set of informative genes. Each such gene $g_i$ votes for either AML or ALL, depending on whether its expression level $x_i$ in the sample is closer to $\mu_{AML}$ or $\mu_{ALL}$ (which denote, respectively, the mean expression levels of AML and ALL in a set of reference samples). The magnitude of the vote is $w_i x_i$, where $w_i$ is a weighting factor that reflects how well the gene is correlated with the class distinction and $v_i = |x_i - (\mu_{AML} + \mu_{ALL})/2|$ reflects the deviation of the expression level in the sample from the average of $\mu_{AML}$ and $\mu_{ALL}$. The votes for each class are summed to obtain total votes $V_{AML}$ and $V_{ALL}$. The sample is assigned to the class with the higher vote total, provided that the prediction strength exceeds a predetermined threshold. The prediction strength reflects the margin of victory and is defined as $(V_{win} - V_{lose})/(V_{win} + V_{lose})$, where $V_{win}$ and $V_{lose}$ are the respective vote totals for the winning and losing classes. Methodological details are described in (19, 20) and on the Web site.
in independent test) (Fig. 3A). The average prediction strength was lower for samples from one laboratory that used a very different protocol for sample preparation. This suggests that clinical implementation of such an approach should include standardization of sample preparation.

The choice to use 50 informative genes in the predictor was somewhat arbitrary. The number was well within the total number of genes strongly correlated with the class distinction (Fig. 2), seemed likely to be large enough to be robust against noise, and was small enough to be readily applied in a clinical setting. In fact, the results were insensitive to the particular choice: Predictors based on between 10 and 200 genes were all found to be 100% accurate, reflecting the strong correlation of genes with the AML-ALL distinction (24).

The list of informative genes used in the AML versus ALL predictor was highly instructive (Fig. 3B). Some, including CD11c, CD33, and MB-1, encode cell surface proteins for which monoclonal antibodies have been demonstrated to be useful in distinguishing lymphoid from myeloid lineage cells (25). Others provide new markers of acute leukemia subtype. For example, the leptin receptor, originally identified through its role in weight regulation, showed high relative expression in AML. The leptin receptor was recently demonstrated to have antiapoptotic function in hematopoietic cells (26). Similarly, the zyxin gene has been shown to encode a LIM domain protein important in cell adhesion in fibroblasts, but a role in hematopoesis has not been reported (27).

We had expected that the genes most useful in AML-ALL class prediction would simply be markers of hematopoietic lineage, and would not necessarily be related to cancer pathogenesis. However, many of the genes encode proteins critical for S-phase cell cycle progression (Cyclin D3, Opf18, and MCM3), chromatin remodeling (RbAp48 and SNF2), transcription (TFIIIEβ), and cell adhesion (zyxin and CD11c) or are known oncogenes (c-MYB, E2A and HOXA9). In addition, one of the informative genes encodes topoisomerase II, which is the principal target of the antileukemic drug etoposide (28). Together, these data suggest that genes useful for cancer class prediction may also provide insight into cancer pathogenesis and pharmacology.

The methodology of class prediction can be applied to any measurable distinction among tumors. Importantly, such distinctions could concern a future clinical outcome—such as whether a prostate cancer turns out to be indolent or a breast cancer responds to a given chemotherapy. We explored the ability to predict response to chemotherapy among the 15 adult AML patients who had been treated with an anthracycline-cytarabine regimen and for whom long-term clinical follow-up was available (29). Eight patients failed to achieve remission after induction chemotherapy, while the remaining seven remained in remission for 46 to 84 months. Neighborhood analysis found no striking excess of genes correlated with response to chemotherapy, in contrast to the situation for the AML-ALL distinction, and class predictors that used 10 to 50 genes were not highly accurate in cross-validation. We thus found no evidence of a strong multigene expression signature correlated with clinical outcome, although this could reflect the relatively small sample size. Nonetheless, we examined the most highly correlated genes for potential biological significance. The single most highly correlated gene out of the 6817 genes was the homeobox gene HOXA9, which was overexpressed in patients with treatment failure. Notably, HOXA9 is rearranged by a t(7; 11)(p15;p15) chromosomal translocation in a rare subset of AML patients, who tend to have poor outcomes (30). Furthermore, HOXA9 overexpression has been shown to transform myeloid cells in vitro and to cause leukemia in animal models (31). A general role for HOXA9 expression in predicting AML outcome has not been previously suggested. Larger studies will be needed to test this hypothesis.

We next turned to the question of class discovery. The initial identification of cancer classes has been slow, typically evolving through years of hypothesis-driven research. We explored whether cancer classes could be discovered automatically. For example, if the AML-ALL distinction were not already known, could it have been discovered simply on the basis of gene expression?

Class discovery entails two issues: (i) developing algorithms to cluster tumors by gene expression and (ii) determining whether putative classes produced by such clustering algorithms are meaningful—that is, whether they reflect true structure in the data rather than simply random aggregation.

To cluster tumors, we used a technique called self-organizing maps (SOMs), which is particularly well suited to the task of identifying a small number of prominent classes in a data set (32). In this approach, the user specifies the number of clusters to be identified. The SOM finds an optimal set of “centroids” around which the data points appear to aggregate. It then partitions the data set, with each centroid defining a cluster consisting of the data points nearest to it.

We applied a two-cluster SOM to automatically group the 38 initial leukemia samples into two classes on the basis of the expression pattern of all 6817 genes (33). We first evaluated the clusters by comparing them to the known AML-ALL classes (Fig. 4A). The SOM paralleled the known classes closely: Class A1 contained mostly AML (24 of 25 samples) and class A2 contained mostly AML (10 of 13 samples). The SOM was thus quite effective, albeit not perfect, at automatically discovering the two types of leukemia.

We then considered how one could evaluate such putative clusters if the “right” answer were not already known. We reasoned...
that class discovery could be tested by class prediction: If putative classes reflect true structure, then a class predictor based on these classes should perform well.

To test this hypothesis, we evaluated the clusters A1 and A2. We constructed predictors to assign new samples as “type A1” or “type A2.” Predictors that used a wide range of different numbers of informative genes performed well in cross-validation. For example, a 20-gene predictor gave 34 accurate predictions with high prediction strength, one error, and three uncertain (34). The one “error” was the assignment of the sole AML sample in class A1 to class A2, and two of the three uncertain were ALL samples in class A2. The cross-validation thus not only showed high accuracy, but actually refined the SOM-defined classes: With one exception, the subset of samples accurately classified in cross-validation were those perfectly subdivided by the SOM into ALL and AML classes. The results suggest an iterative procedure for refining clusters, in which an SOM is used to initially cluster the data, a predictor is constructed, and samples not correctly predicted in cross-validation are removed. The edited data set could then be used to generate an improved predictor to be tested on an independent data set (35).

We then tested the class predictor of the A1-A2 distinction on the independent data set. In the general case of class discovery, predictors for novel classes cannot be assessed for “accuracy” on new samples, because the “right” way to classify the independent samples is not known. Instead, however, one can assess whether the new samples are assigned a high prediction strength. High prediction strengths indicate that the structure seen in the initial data set is also seen in the independent data set: The prediction strengths, in fact, were quite high: The median PS was 0.61, and 74% of samples were above threshold (Fig. 4B). To assess these results, we performed the same analyses with random clusters. Such clusters consistently yielded predictors with poor accuracy in cross-validation and low prediction strength on the independent data set (Fig. 4B). On the basis of such analysis (36), the A1-A2 distinction can be seen to be meaningful, rather than simply a statistical artifact of the initial data set. The results thus show that the AML-ALL distinction could have been automatically discovered and confirmed without previous biological knowledge.

We then sought to extend the class discovery by searching for finer subclasses of the leukemias. We used a SOM to divide the samples into four clusters (denoted B1 to B4). We subsequently obtained immunophenotype data on the samples and found that the four classes largely corresponded to AML, T-lineage ALL, B-lineage ALL, and B-lineage ALL, respectively (Fig. 4C). The four-cluster SOM thus divided the samples along

**Fig. 3.** (A) Prediction strengths. The scatter-plots show the prediction strengths (PSs) for the samples in cross-validation (left) and on the independent sample (right). Median PS is denoted by a horizontal line. Predictions with PS < 0.3 are considered as uncertain. (B) Genes distinguishing ALL from AML. The 50 genes most highly correlated with the ALL-AML class distinction are shown. Each row corresponds to a gene, with the columns corresponding to expression levels in different samples. Expression levels for each gene are normalized across the samples so that the mean is 0 and the SD is 1. Expression levels greater than the mean are shaded in red, and those below the mean are shaded in blue. The scale indicates SDs above or below the mean. The top panel shows genes highly expressed in ALL, the bottom panel shows genes more highly expressed in AML. Although these genes as a group appear correlated with class, no single gene is uniformly expressed across the class, illustrating the value of a multigene prediction method. For a complete list of gene names, accession numbers, and raw expression values, see www.genome.wi.mit.edu/MPR.
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We again evaluated these classes by constructing class predictors (37). The four classes could be distinguished from one another, with the exception of B3 versus B4 (Fig 4D). The prediction tests thus confirmed the distinction PS = 0.20 and 0.34, respectively), and about half of the samples fall below the threshold for prediction (PS = 0.3). A total of 100 such random predictors were examined, to calculate the distribution of median PS scores to evaluate the statistical significance of the predictor for A1-A2 (36). (C) Schematic representation of the four-cluster SOM. AML samples are shown as black circles, T-lineage ALL as open squares, and B-lineage ALL as gray squares. T- and B-lineages were differentiated on the basis of cell-surface immunophenotyping. Class B1 is exclusively AML, class B2 contains all eight T-ALLs, and classes B3 and B4 contain the majority of B-ALL samples. (D) Prediction strength (PS) distributions for pair-wise comparison among classes. Cross-validation prediction studies show that the four classes could be distinguished with high prediction scores, with the exception of classes B3 and B4. These two classes could not be easily distinguished from one another, consistent with their both containing primarily B-ALL samples, and suggesting that B3 and B4 might best be merged into a single class.


We compared six normal human kidney biopsies and six kidney tumor biopsies (lymphomas, RCCs) using the methods described for the leukemias. Neighborhood analysis showed a high density of genes correlated with the class distinction during the construction using 50 genes, and the predictions proved to be 100% accurate in cross-validation. The informative genes more highly expressed in normal kidney as compared to various RCCs.[12] We investigated interleukin-1, an inflammatory cytokine responsible for the febrile response experienced by patients with RCC, and CCND1, a D-type cyclin amplified in some cases of RCC.

The initial 38 samples were all derived from bone marrow aspirates performed at the time of diagnosis, before chemotherapy. After informed consent was obtained, mononuclear cells were collected by Ficoll-Hygral (suppl. 1), 38 (1999).

Each gene is represented by an expression vector $\mathbf{v}(g) = (e_1, e_2, \ldots, e_r)$, where $e_i$ denotes the expression level of gene $g$ in $i$th sample in the initial set of samples. $\mathbf{v}(g)$ is normalized by an idealized expression pattern $c = (c_1, c_2, \ldots, c_r)$, where $c_i = 1$ or 0 according to whether the $i$th sample belongs to class 1 or class 2. One can measure “correlation” between a gene and a class distinction in a variety of ways. One can use the Pearson correlation coefficient or the Euclidean distance. We used a measure of correlation, $P(g,c)$, that emphasizes the “signal-to-noise” ratio in using the gene as a predictor. Let $\{u_i(g,r)\}$ and $\{u_i(g,r)\}$ denote the means and SDs of the expression levels of gene $g$ for the samples in class 1 and class 2, respectively. Let $P(g,c) = (u(g,r) - \mu(r)) (\sigma(r) + \sigma(r)),\quad \text{which reflects the difference between the classes relative to the SD within the classes.}$

Large values of $P(g,c)$ indicate a strong correlation between the gene expression and the class distinction, while the sign of $P(g,c)$ being positive or negative corresponds to $g$ being more highly expressed in one class or the other.

Unlike a standard Pearson correlation coefficient, $P(g,c)$ is not confined to the range $[-1, 1]$. Neighborhood $V(g,c)$ and $\bar{P}(g,c)$ of radius $r$ around class 1 and class 2 were defined to be the sets of genes such that $P(g,c) = r$ and $P(g,c) = -r$, respectively. An unusually large number of genes within the neighborhoods indicates that many genes have expression patterns closely correlated with the class predictor.

A permutation test was used to calculate whether the density of genes in a neighborhood was statistically significantly higher than what was expected. We compared the number of genes in the neighborhood to the number of genes in similar neighborhoods around idealized expression patterns corresponding to random class distributions. The percentage of genes that were visible defects in the array (such as scratches). A mathematical procedure used in this paper is available from the Web site for quantitative criteria, or if there were problems with the array. A previously described, with minor modifications [P. Tamayo et al., *Proc. Natl. Acad. Sci. U.S.A.* 96, 2907 (1999); L. Wodicka, H. Dong, M. Mittmann, M. H. Do, D. J. Lockhart, *Nature Biotechnol.* 15, 1359 (1997)]. A complete description of the biochemical and mathematical procedures used in this paper is available through our Web site at www.genome.wi.mit.edu/MHR.

Samples were excluded if they yielded less than 15 µg of biotinylated RNA, if the hybridization was weak (see our Web site for quantitative criteria), or if there were visible defects in the array (such as scratches). A total of 80 leukemia samples were analyzed during the course of the experiments reported here. Of these, eight were excluded on the basis of these a priori quality control criteria. Eight were visible defects in the array (such as scratches). A mathematical procedure used in this paper is available from the Web site for quantitative criteria, or if there were problems with the array. A previously described, with minor modifications [P. Tamayo et al., *Proc. Natl. Acad. Sci. U.S.A.* 96, 2907 (1999); L. Wodicka, H. Dong, M. Mittmann, M. H. Do, D. J. Lockhart, *Nature Biotechnol.* 15, 1359 (1997)]. A complete description of the biochemical and mathematical procedures used in this paper is available through our Web site at www.genome.wi.mit.edu/MHR.

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Sequencing Complex Polysaccharides

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Although rapid sequencing of polynucleotides and polypeptides has become commonplace, it has not been possible to rapidly sequence femto- to picomole amounts of tissue-derived complex polysaccharides. Heparin-like glycosaminoglycans (HLGAGs) were readily sequenced by a combination of matrix-assisted laser desorption ionization mass spectrometry and a notation system for representation of polysaccharide sequences. This will enable identification of sequences that are critical to HLGAG biological activities in anticoagulation, cell growth, and differentiation.

The chemical heterogeneity of polysaccharides, their structural complexity, and the lack of effective tools and methods have seriously limited the development of sequencing approaches that is rapid and practical, like that used for polynucleotides and polypeptides. This limitation is especially relevant in the study of glycosaminoglycan (GAG) complex polysaccharides, which are present at the cell surface and in the extracellular matrix (1, 2). Heparin or heparan sulfate–like glycosaminoglycans (HLGAGs), a subset of GAGs, are currently used clinically as anticoagulants, and this function of HLGAGs has been assigned to a specific pentasaccharide sequence that is responsible for binding to antithrombin III (3). Recent progress in developmental biology, genetics, and other fields has resulted in understanding of structural-function relationships for HLGAGs' modulation of biological activity, in only a few cases is there any structural information regarding sequences (4). Therefore, accelerating our understanding of structural-function relationships for HLGAGs requires the development of rapid yet thorough sequencing methodologies.

There are many issues that have limited the development of sequencing techniques for HLGAGs. HLGAGs are chemically complex and heterogeneous, because the HLGAG chain can vary in terms of the number of disaccharide repeat units and possesses, within the disaccharide repeat unit, four potential sites for chemical modification. The basic disaccharide repeat unit of HLGAG is a uronic acid [α-L-iduronic acid (I) or β-D-glucuronic acid (G)] linked 1,4 to α-D-hexosamine (H) (Fig. 1A). Together, the four different modifications (2^4 = 16) for an I or G uronic acid isomer containing disaccharide give rise to 16 × 2 = 32 different plausible disaccharide units for HLGAGs. In contrast, four bases make up DNA, and 20 amino acids make up proteins. With these 32 building blocks, an octasaccharide could have over a million possible sequences, thereby making HLGAGs not only the most acidic but also the most information-dense biopolymers found in nature. There are no methods available to amplify or produce HLGAGs in large amounts, unlike the techniques that are available for DNA or proteins.

To handle the enormous information den-

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29. Treatment failure was defined as failure to achieve a complete remission after a standard induction regimen including 3 days of anthracycline and 7 days of cytarabine. Treatment successes were defined as patients in continuous complete remission for a minimum of 3 years. FAB subclass M3 patients were excluded but samples were otherwise not selected with regard to FAB criteria.
33. The SOM was constructed using our GENECLUSTER software (32), with a variation filter excluding genes with less than fivefold variation across the collection of samples.
34. For testing putative clusters derived from the SOM or chosen at random, we constructed class predictors with various number of genes (ranging from 10 to 100) and selected the one with the highest cross-validation accuracy rate (in this case, 20 genes).
35. A related approach would be to represent each cluster only as the subset of points lying near the centroid of the cluster.
36. Various statistical methods can be used to compare the predictors derived from the SOM-derived clusters with predictors derived from random classes. We compared the median prediction strength. Specifically, 100 predictors corresponding to random classes of comparable size were constructed, and the median PS for each predictor was determined. The performance for the actual predictor was then compared to the distribution of these 100 median PSs, to obtain empirical significance levels. The observed median PS in the initial data set was 0.86, which exceeded the median PS for all 100 random predictors; the empirical significance level was thus <1%. The observed median PS for the independent data set was 0.61, which exceed the median PS for all but 4 of the 100 random permutations; the empirical significance level was thus 4%.
37. Various approaches can be used to test classes C1, C2, . . . , Cn arising from a multine SOM. One can construct predictors to distinguish each pair of classes (Ci versus Cj) or to distinguish each class for the complement of the class (Cn versus Cj). Here we used the pair-wise approach [Ci versus Cj]. For cross-validation, one can restrict attention to samples known to lie in the union of Ci and Cj. For an independent data set, one must examine all samples (because it is unknown which samples lie in the union of Ci and Cj). It may be possible to improve the statistical power of this test by using techniques for multiclass prediction.
38. Thirty-three ALL samples were tested by cross-validation using a 50-gene predictor. Thirty-two of 33 samples were correctly assigned as T-ALL or B-ALL; the remaining sample received a PS < 0.3, and no prediction was therefore made. Details are provided on our Web site.
41. We are grateful to S. Sallan, J. Ritz, K. Loughlin, S. Shurtleff, P. Kourlas, F. Smith, the Cancer and Leukemia Group B, and Children’s Cancer Group for providing valuable patient samples. We thank R. Klausner, D. G. Gilliland, D. Nathan, G. Daley, J. Staunton, M. Angelo, A. Leblanc, P. Lee, Z. Kiliński, G. Acton, and members of the Lander and Golub laboratories for helpful discussions. This work was supported in part by the Leukemia Society of America (T.R.G); the National Institutes of Health and the Leukemia Clinical Research Foundation (C.D.B.); and Affymax, Millennium Pharmaceuticals, and Bristol-Myers Squibb (E.S.L.).