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Sandeep S. Dave
National Institutes of Health, Bethesda, MD

Kai Fu
University of Nebraska Medical Center, kfu@unmc.edu

George W. Wright
National Institutes of Health, Bethesda, MD

Lloyd T. Lam
National Institutes of Health, Bethesda, MD

Philip Kluin
University of Groningen

ORIGINAL ARTICLE

Molecular Diagnosis of Burkitt's Lymphoma

Sandeep S. Dave, M.D., Kai Fu, M.D., Ph.D., George W. Wright, Ph.D., Lloyd T. Lam, Ph.D., Philip Kluin, M.D., Evert-Jan Boerma, B.S., Timothy C. Greiner, M.D., Dennis D. Weisenburger, M.D., Andreas Rosenwald, M.D., German Ott, M.D., Hans-Konrad Müller-Hermelink, M.D., Randy D. Gascoyne, M.D., Jan Delabie, M.D., Lisa M. Rimsza, M.D., Rita M. Braziel, M.D., Thomas M. Grogan, M.D., Elias Campo, M.D., Elaine S. Jaffe, M.D., Bhavana J. Dave, Ph.D., Warren Sanger, Ph.D., Martin Bast, B.S., Julie M. Vose, M.D., James O. Armitage, M.D., Joseph M. Connors, M.D., Erlend B. Smeland, M.D., Ph.D., Stein Kvaloy, M.D., Ph.D., Harald Holte, M.D., Ph.D., Richard I. Fisher, M.D., Thomas P. Miller, M.D., Emilio Montserrat, M.D., Wyndham H. Wilson, M.D., Ph.D., Manisha Bahl, B.S., Hong Zhao, M.S., Liming Yang, Ph.D., John Powell, M.S., Richard Simon, D.Sc., Wing C. Chan, M.D., and Louis M. Staudt, M.D., Ph.D., for the Lymphoma/Leukemia Molecular Profiling Project

ABSTRACT

BACKGROUND

The distinction between Burkitt's lymphoma and diffuse large-B-cell lymphoma is crucial because these two types of lymphoma require different treatments. We examined whether gene-expression profiling could reliably distinguish Burkitt's lymphoma from diffuse large-B-cell lymphoma.

METHODS

Tumor-biopsy specimens from 303 patients with aggressive lymphomas were profiled for gene expression and were also classified according to morphology, immunohistochemistry, and detection of the t(8;14) c-*myc* translocation.

RESULTS

A classifier based on gene expression correctly identified all 25 pathologically verified cases of classic Burkitt's lymphoma. Burkitt's lymphoma was readily distinguished from diffuse large-B-cell lymphoma by the high level of expression of c-*myc* target genes, the expression of a subgroup of germinal-center B-cell genes, and the low level of expression of major-histocompatibility-complex class I genes and nuclear factor-κB target genes. Eight specimens with a pathological diagnosis of diffuse large-B-cell lymphoma had the typical gene-expression profile of Burkitt's lymphoma, suggesting they represent cases of Burkitt's lymphoma that are difficult to diagnose by current methods. Among 28 of the patients with a molecular diagnosis of Burkitt's lymphoma, the overall survival was superior among those who had received intensive chemotherapy regimens instead of lower-dose regimens.

CONCLUSIONS

Gene-expression profiling is an accurate, quantitative method for distinguishing Burkitt's lymphoma from diffuse large-B-cell lymphoma.

From the National Cancer Institute (S.S.D., G.W.W., L.T.L., E.S.J., W.H.W., M.B., H.Z., R.S., L.M.S.) and the Center for Information Technology (L.Y., J.P.), National Institutes of Health, Bethesda, Md.; University of Nebraska Medical Center, Omaha (K.F., T.C.G., D.D.W., B.J.D., W.S., M.B., J.M.V., J.O.A., W.C.C.); Groningen University Medical Center, University of Groningen, Groningen, the Netherlands (P.K., E.-J.B.); University of Würzburg, Würzburg, Germany (A.R., G.O., H.-K.M.-H.); British Columbia Cancer Agency, Vancouver, B.C., Canada (R.D.G., J.M.C.); Norwegian Radium Hospital, Norway Hospital Clinic, Oslo (J.D., E.B.S., S.K., H.H.); Southwest Oncology Group (L.M.R., R.M.B., T.M.G., R.I.F., T.P.M.); University of Arizona Cancer Center, Tucson (L.M.R., T.M.G., T.P.M.); Oregon Health and Science University, Portland (R.M.B.); University of Barcelona, Barcelona (E.C., E.M.); University of Oslo, Oslo (E.B.S.); and James P. Wilmot Cancer Center, University of Rochester School of Medicine, Rochester, N.Y. (R.I.F.). Address reprint requests to Dr. Staudt at the Metabolism Branch, CCR, NCI, Bldg. 10, Rm. 4N114, NIH, 9000 Rockville Pike, Bethesda, MD 20892, or at lstaudt@mail.nih.gov.

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Burkitt's lymphoma is an aggressive B-cell lymphoma characterized by a high degree of proliferation of the malignant cells and deregulation of the c-*myc* gene.[1] Distinguishing between Burkitt's lymphoma and diffuse large-B-cell lymphoma is critical because the management of these two diseases differs. Whereas relatively low-dose chemotherapy regimens such as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) are typically used to treat diffuse large-B-cell lymphoma, they are inadequate for Burkitt's lymphoma,[2,3] for which intensive chemotherapy regimens are required.[4-8] Furthermore, prophylactic intrathecal chemotherapy or systemic chemotherapy that crosses the blood–brain barrier is unnecessary in most cases of diffuse large-B-cell lymphoma; such chemotherapy is essential for treating Burkitt's lymphoma, however, because of the high risk of involvement of the central nervous system.[2,9]

The diagnosis of Burkitt's lymphoma relies on morphologic findings, immunophenotyping results, and cytogenetic features.[1] However, diffuse large-B-cell lymphoma and Burkitt's lymphoma can have overlapping morphologic and immunophenotypic features, and the characteristic t(8;14) translocation of Burkitt's lymphoma[10-12] also occurs in 5 to 10 percent of cases of diffuse large-B-cell lymphoma.[13] Because diffuse large-B-cell lymphoma is more than 20 times as common as Burkitt's lymphoma,[14] a lymphoma with a t(8;14) translocation can present a diagnostic problem.

The term "Burkitt-like lymphoma" has been used for cases that have some features in common with Burkitt's lymphoma. However, the most recent guidelines of the World Health Organization (WHO)[1] eliminate Burkitt-like lymphoma as a separate diagnostic category. Burkitt-like lymphoma is now considered synonymous with the term "atypical Burkitt's lymphoma," which is reserved for cases that have the genetic abnormality and immunophenotype of Burkitt's lymphoma but have atypical morphologic features. It is not clear whether atypical Burkitt's lymphoma is a biologically distinct entity or a morphologic variant of Burkitt's lymphoma.

In the present study, we investigated whether gene-expression profiling could reliably distinguish Burkitt's lymphoma from diffuse large-B-cell lymphoma. We hypothesized that analysis of the molecular features of Burkitt's lymphoma would permit a more accurate and reproducible diagnosis than would the use of standard pathological methods.

METHODS

STUDY POPULATION

The patients were studied according to a protocol approved by the institutional review board of the National Cancer Institute. Tumor-biopsy specimens were obtained from 71 patients who had not previously received treatment for lymphoma, who were negative for the human immunodeficiency virus, and who had received the diagnosis of sporadic Burkitt's lymphoma (54 patients) or Burkitt-like lymphoma (17 patients) between 1986 and 2004 at seven institutions in Europe and North America. The institutions are members of an international consortium, the Lymphoma/Leukemia Molecular Profiling Project.

We also studied 232 tumor-biopsy specimens from patients with the diagnosis of diffuse large-B-cell lymphoma, 223 of which have been used in previous investigations.[15,16] Nine cases of diffuse large-B-cell lymphoma were high-grade and had a Ki-67 score (a measure of lymphoma-cell proliferation) of nearly 100 percent. The cases of diffuse large-B-cell lymphoma were further subdivided on the basis of gene expression into one of the three main subgroups — activated B-cell–like, germinal-center B-cell–like, and primary mediastinal — or were declared to be unclassified, as previously described.[15-17]

All cases were reviewed anew by an expert panel of eight hematopathologists according to the current criteria of the WHO[1] for morphologic features, immunophenotype, and cytogenetic findings (including the presence or absence of a c-*myc* translocation). Specifically, tumor-biopsy specimens classified as Burkitt's lymphoma had a c-*myc* translocation, a morphologic profile consistent with Burkitt's lymphoma, a Ki-67 score of more than 90 percent, and immunohistochemical evidence of CD10 or BCL6, or both, in the tumor cells. Cases of diffuse large-B-cell lymphoma were classified on the basis of morphologic criteria and a B-cell immunophenotype. A detailed description of the pathology review is provided in the Supplementary Appendix, available with the full text of this article at www.nejm.org.

The regimens used to treat Burkitt's lymphoma were classified as either CHOP-like regimens (CHOP[18] or cyclophosphamide, mitoxantrone, vin-

cristine and prednisone [CNOP][19]) or intensive regimens (Berlin–Frankfurt–Münster[6]; cyclophosphamide, doxorubicin, high-dose methotrexate or ifosfamide, etoposide, and high-dose cytarabine[4]; or intensive chemotherapy regimens combined with autologous stem-cell transplantation). Fluorescence in situ hybridization (Vysis) to detect c-*myc* or *BCL2* translocation was performed on some specimens.

GENE-EXPRESSION PROFILING

We performed gene-expression profiling of all biopsy specimens using a custom oligonucleotide microarray with 2524 unique genes that are expressed differentially among the various forms of non-Hodgkin's lymphoma; a subgroup of specimens was also profiled on Affymetrix U133 Plus 2.0 arrays. The primary gene-expression profiling data are available from the Gene Expression Omnibus of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/geo) through GEO accession number GSE4732 or at http://llmpp.nih.gov/BL.

IDENTIFICATION OF c-*myc* TARGET GENES BY RNA INTERFERENCE

The OCI-Ly10 diffuse large-B-cell lymphoma cell line was transfected with small interfering RNA targeting the c-*myc* gene (Smart Pool, Dharmacon). Gene expression in transfected cells was compared with that in control sham-transfected cells with the use of Lymphochip DNA microarrays.[20] Genes were defined as c-*myc* target genes if they were down-regulated at least 40 percent at two or more times after transfection with small interfering RNA and if the expression levels of messenger RNA (mRNA) were correlated ($r>0.4$ across all lymphoma biopsy specimens) with those of c-*myc* mRNA.

STATISTICAL ANALYSIS

Three pairwise Bayesian compound covariate classifiers were constructed — one between Burkitt's lymphoma and each of the three diffuse large-B-cell lymphoma subgroups: activated B-cell–like, germinal-center B-cell–like, and primary mediastinal — as previously described.[16,17,21] Each

Table 1. Classification of Cases.*

Original Diagnosis	Total No. of Cases	Pathological Diagnosis†	Total No. of Cases	Molecular Diagnosis	Total No. of Cases
Burkitt's lymphoma or Burkitt-like lymphoma	71	Classic Burkitt's lymphoma	25	Burkitt's lymphoma	25
		Atypical Burkitt's lymphoma	20	Burkitt's lymphoma	19
				DLBCL	1
		DLBCL	20	Burkitt's lymphoma	7
				DLBCL	13
		High-grade lymphoma, NOS	6	DLBCL	5
				Burkitt's lymphoma	1
DLBCL	223	DLBCL	223	Activated B-cell–like DLBCL	78
				Germinal-center B-cell–like DLBCL	82
				Primary mediastinal DLBCL	33
				Unclassified DLBCL	30
High-grade DLBCL	9	DLBCL	9	Activated B-cell–like DLBCL	6
				Germinal-center B-cell–like DLBCL	2
				Burkitt's lymphoma	1

* DLBCL denotes diffuse large-B-cell lymphoma, and NOS not otherwise specified.
† The pathological diagnosis is according to the current criteria of the World Health Organization[1] for morphologic, immunophenotype, and cytogenetic findings.

pairwise comparison was carried out in two stages, with different sets of genes for each stage, to create the compound covariate classifier. In the first stage, c-*myc* and c-*myc* target genes were used; in the second stage, the 100 genes with the most significant t-statistic differentiating Burkitt's lymphoma from each subgroup of diffuse large-B-cell lymphoma were used, excluding the genes used in the first stage. For a tumor-biopsy specimen to be classified as Burkitt's lymphoma, it had to be classified as Burkitt's lymphoma in both stages in each of the three pairwise comparisons. Statistical procedures are described in detail in the Supplementary Appendix.

RESULTS

STUDY POPULATION

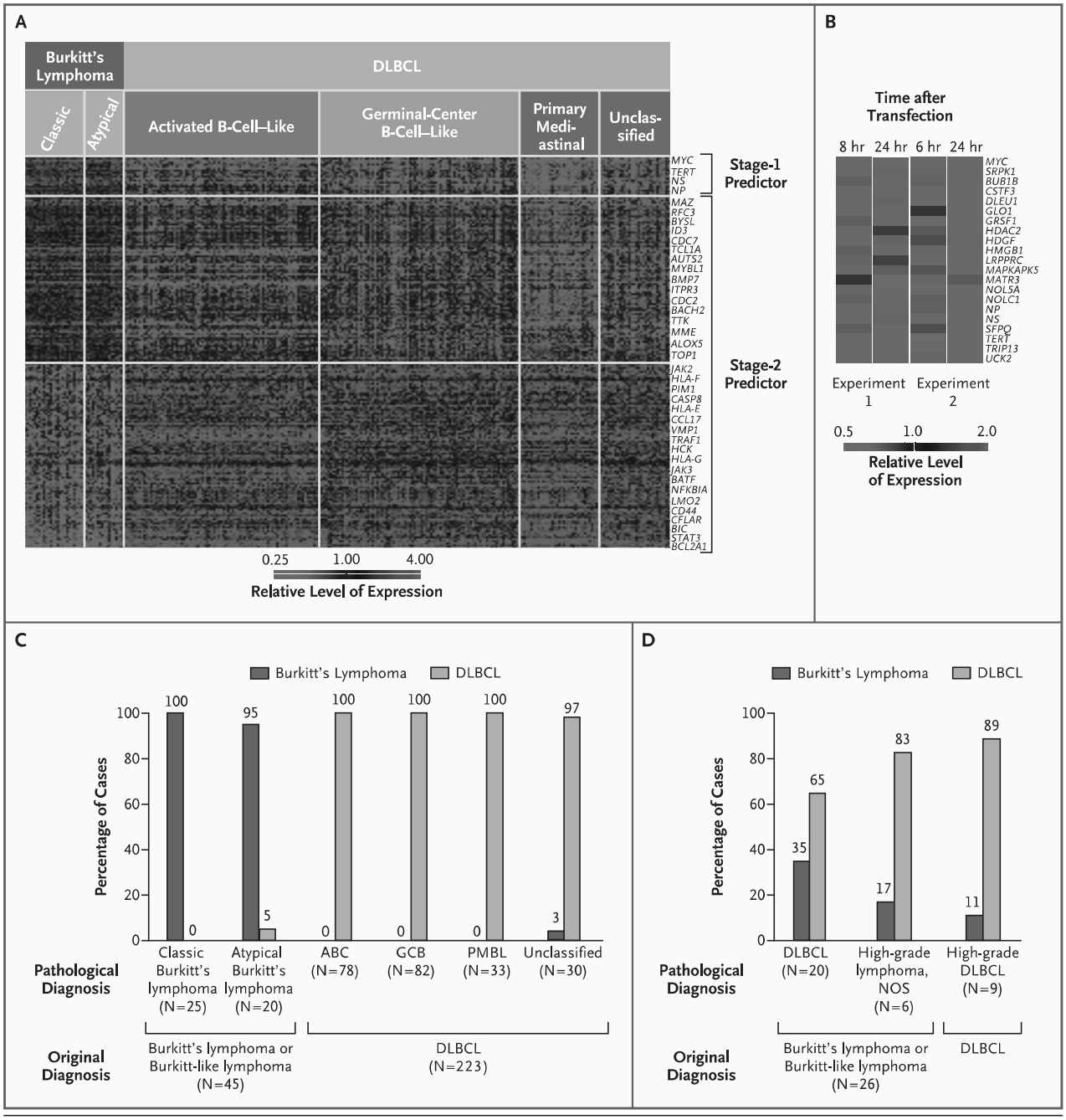
Of the 45 tumor-biopsy specimens verified to be classic or atypical Burkitt's lymphoma by the pathology review, 48 percent were from children (age range, 2.9 to 18 years) and 52 percent were from adults (age range, 18 to 73 years). The median follow-up was 1.6 years for all patients and 4.9 years for patients who were still alive at the end of the study. Fluorescence in situ hybridization for c-*myc* translocation was successfully performed in 67 of the 71 specimens originally diagnosed as Burkitt's lymphoma or Burkitt-like lymphoma, including all specimens in which Burkitt's lymphoma was not ruled out by immunohistochemical or morphologic findings. Of these 71 specimens, 52 were found to be positive for the translocation. *BCL2* translocations were found in 7 of the 44 specimens of Burkitt's and Burkitt-like lymphoma that were tested for them. Among the 232 patients with diffuse large-B-cell lymphoma, the median age at diagnosis was 61.5 years (range, 9 to 92). The median follow-up was 2.5 years (6.8 years for survivors). We successfully performed fluorescence in situ hybridization for the c-*myc* translocation in 87 specimens of diffuse large-B-cell lymphoma; 6 were positive for the translocation.

MOLECULAR DIAGNOSIS OF BURKITT'S LYMPHOMA

We examined the patterns of gene expression in the biopsy specimens from patients who had received a diagnosis of Burkitt's lymphoma (54 patients), Burkitt-like lymphoma (17 patients), or high-grade diffuse large-B-cell lymphoma (9 patients). These cases were reviewed anew by our panel of expert hematopathologists according to current criteria of the WHO,[1] which include morphologic, immunophenotype, and cytogenetic findings (the presence or absence of a c-*myc* translocation). During this process, the 71 cases of Burkitt's or Burkitt-like lymphomas were reclassified as classic Burkitt's lymphoma (25 cases), atypical Burkitt's lymphoma (20 cases), diffuse large-B-cell lymphoma (20 cases), or other high-grade lymphomas that could not be classified according to the criteria (called "not otherwise specified"; 6 cases) (Table 1). Hereafter, the pathological diagnosis was considered the standard against which the performance of the molecular diagnosis based on the pattern of gene expression was compared. In addition, we studied 223 previously characterized cases of diffuse large-B-cell lymphoma. The non–high-grade cases were sub-

Figure 1 (facing page). A Molecular Classifier of Burkitt's Lymphoma.

Panel A shows the difference in gene expression between Burkitt's lymphoma and diffuse large-B-cell lymphoma (DLBCL) derived from DNA-microarray analysis. The relative levels of gene expression are depicted according to the color scale shown. The genes analyzed in stage 1 of constructing the classifier include c-*myc* and its target genes. The 196 genes analyzed in stage 2 of constructing the classifier include additional genes that distinguish Burkitt's lymphoma from the three subgroups of DLBCL. Only specimens for which the diagnoses based on the pathology review and molecular analysis of gene expression agreed are shown. Panel B shows the list of c-*myc* target genes identified with the use of RNA interference. The OCI-Ly10 DLBCL cell line was transfected with small interfering RNA targeting the c-*myc* gene. We compared the gene expression of the transfected cells with that of control cells by DNA-microarray analysis at various hours after transfection in two separate experiments. The levels of gene expression relative to that of control cells are depicted according to the color scale shown; down-regulation is depicted in shades of green; up-regulation is shown in shades of red (see the Methods section). Panel C depicts the diagnostic performance of the molecular classifier based on gene expression — as compared with the original diagnosis and the pathological diagnosis — according to leave-one-out cross-validation analysis. Panel D depicts the molecular classification of the 26 specimens originally diagnosed as Burkitt's lymphoma or Burkitt-like lymphoma that were diagnosed on pathology review as either DLBCL or high-grade lymphoma not otherwise specified (NOS) and the nine specimens that were originally diagnosed as high-grade DLBCL and were verified as such on pathology review. The molecular diagnosis sometimes disagreed with the pathological diagnosis (red bars in Panel D).



classified according to the pattern of gene expression into three subgroups — activated B-cell–like, germinal-center B-cell–like, and primary mediastinal — or were declared "unclassified."[15-17]

To develop a diagnostic test based on the gene-expression profile of Burkitt's lymphoma, we initially focused only on the 45 cases that were originally diagnosed as Burkitt's lymphoma and confirmed as such by the pathological review. By selecting genes that were differentially expressed in these 45 cases and among the three subgroups of diffuse large-B-cell lymphoma (Fig. 1A), we created a classifier, based on gene expression, that distinguished Burkitt's lymphoma from diffuse large-B-cell lymphoma. Given the central role of c-*myc* deregulation in Burkitt's lymphoma, we identified a set of c-*myc* target genes by using RNA interference (Fig. 1B) and treated this set sepa-

rately in our classification algorithm. The classifier also included many other genes that reflected biologic differences between Burkitt's lymphoma and diffuse large-B-cell lymphoma.

Leave-one-out cross-validation was used to estimate the performance of the classifier.[22-24] All 25 cases identified on pathology review as classic Burkitt's lymphoma were classified correctly on the basis of gene expression (Fig. 1C). The cases of atypical Burkitt's lymphoma and classic Burkitt's lymphoma identified on pathology review could not be distinguished on the basis of gene expression (Fig. 1A); the algorithm also classified 19 of the 20 cases of atypical Burkitt's lymphoma as Burkitt's lymphoma. The cases for which the molecular and pathological diagnoses were in agreement are referred to hereafter as "Burkitt's lymphoma–concordant cases." The diagnoses based on the classifier were in perfect agreement with the pathological diagnoses of diffuse large-B-cell lymphoma, irrespective of their subclassifications of activated B-cell–like, germinal-center B-cell–like, and primary mediastinal diffuse large-B-cell lymphoma. All but 1 of the 30 unclassified diffuse large-B-cell lymphomas were molecularly classified as diffuse large-B-cell lymphoma (Fig. 1C).

We further tested the Burkitt's lymphoma classifier by dividing the cases depicted in Figure 1C into equally sized training and test sets. The algorithm was generated with the use of data from the training set and was applied to the test set. The results of this analysis agreed with those of the leave-one-out cross-validation analysis in 99 percent of the cases in the test set, confirming that the algorithm effectively distinguishes Burkitt's lymphoma from diffuse large-B-cell lymphoma.

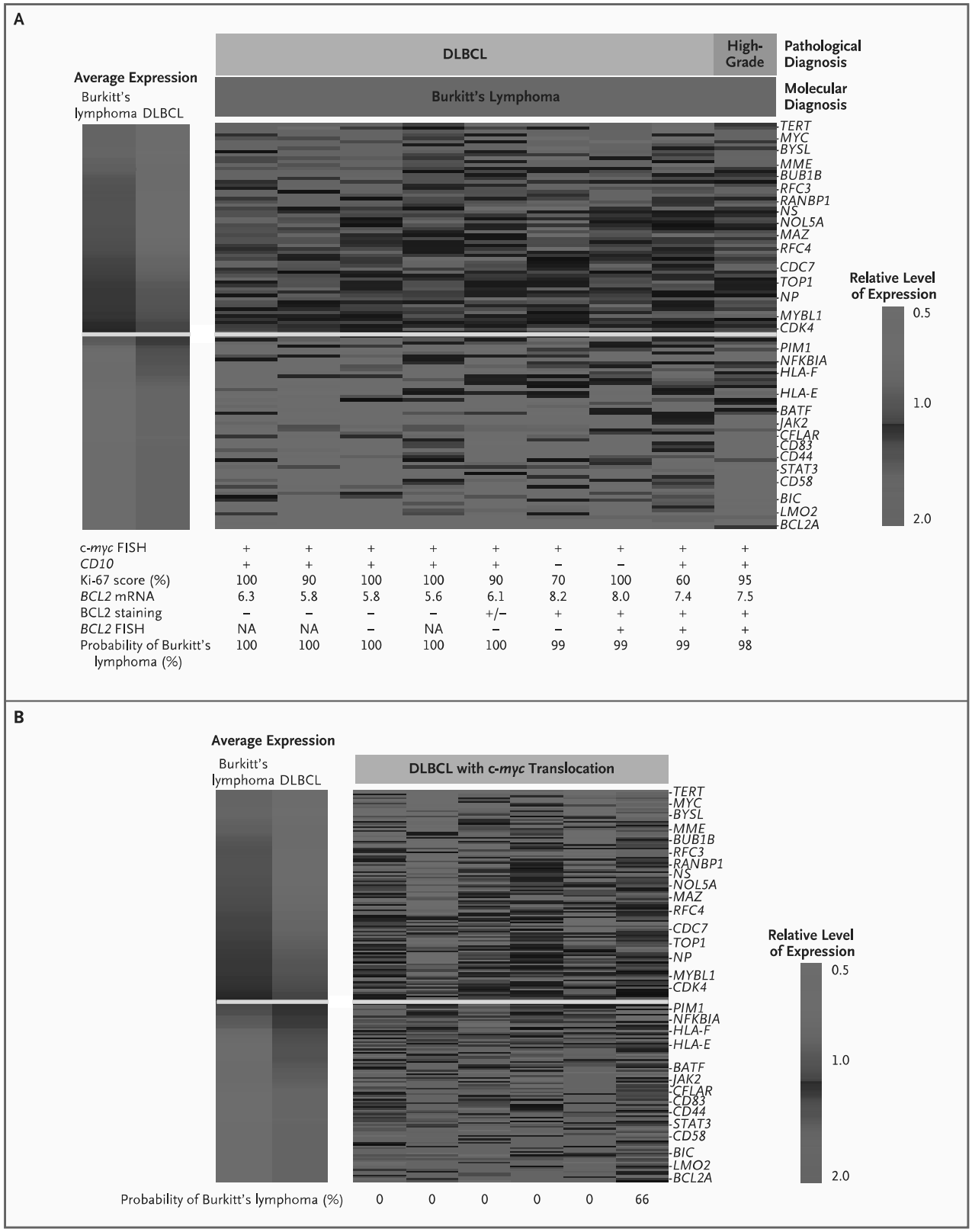
We next used the DNA microarrays to classify the cases that were originally diagnosed as Burkitt's lymphoma or Burkitt-like lymphoma but were reclassified during pathology review as either diffuse large-B-cell lymphoma (20 cases) or high-grade lymphoma, not otherwise specified (6 cases) (Fig. 1D). The gene-expression profile was not in accord with the pathological diagnosis in 8 of these 26 cases (31 percent). We also analyzed the nine cases that had originally been diagnosed as high-grade diffuse large-B-cell lymphoma and were verified on pathology review as such; one of these was molecularly classified as Burkitt's lymphoma.

Figure 2 (facing page). Performance of a Molecular Classifier of Burkitt's Lymphoma for Cases with Conflicting Diagnoses.

Panel A shows the gene expression of the nine Burkitt's lymphoma–discrepant cases (for which the pathological diagnosis and the molecular diagnosis did not agree). The expression of classifier genes for Burkitt's lymphoma in these specimens is compared with the average expression of these genes in Burkitt's lymphoma and diffuse large-B-cell lymphoma (DLBCL). The relative gene expression is depicted according to the color scale shown. For each specimen, the immunophenotype, the Ki-67 score, expression of *BCL2* mRNA (values are shown on a base-2 log scale), and fluorescence in situ hybridization (FISH) for translocation in c-*myc* or *BCL2* are given at the bottom; a plus sign denotes presence, a minus sign absence, and NA not available. The BCL2 staining data are the result of immunohistochemical assays for BCL2 protein. One case had equivocal BCL2 protein staining, denoted by the plus–minus sign; this case was considered to be BCL2-negative in the analysis. Also shown is the probability that each specimen is Burkitt's lymphoma, on the basis of gene expression. Panel B illustrates the expression of the classifier genes for Burkitt's lymphoma in the six specimens of diffuse large-B-cell lymphoma known to have a translocation involving the c-*myc* gene, as compared with the average level of expression in Burkitt's lymphoma and diffuse large-B-cell lymphoma. Also shown is the probability that each specimen is Burkitt's lymphoma, on the basis of gene expression.

Thus, nine cases with a pathological diagnosis of either diffuse large-B-cell lymphoma or high-grade lymphoma not otherwise specified had a gene-expression profile consistent with Burkitt's lymphoma (Fig. 1D); these cases are referred to hereafter as "Burkitt's lymphoma–discrepant cases."

The Burkitt's lymphoma–discrepant cases could be readily distinguished from all subgroups of diffuse large-B-cell lymphoma on the basis of gene expression. The probability that these cases were Burkitt's lymphoma according to gene-expression profiles was 98 to 100 percent (Fig. 2A). The validity of the molecular diagnosis of Burkitt's lymphoma in these nine cases was supported by the presence of a t(8;14) c-*myc* translocation in all of them. Four of these cases expressed relatively high levels of *BCL2* mRNA and protein, and three had a t(14;18) translocation in addition to the t(8;14). The remaining five Burkitt's lymphoma–discrepant cases were BCL2-negative and were indistinguishable from Burkitt's lymphoma on the basis of gene expression.



We next examined whether the molecular classifier could be used to distinguish Burkitt's lymphoma from diffuse large-B-cell lymphoma bearing a c-*myc* translocation. It was consistent with previous reports that 7 percent of the cases originally diagnosed as diffuse large-B-cell lymphoma (6 of the 87 cases tested) had a c-*myc* translocation. The gene-expression profiles of these cases were distinct from those of Burkitt's lymphoma (Fig. 2B); all had profiles of diffuse large-B-cell lymphoma (four germinal-center B-cell–like and two activated B-cell–like). Five of these six cases had a gene-expression profile that resulted in a probability of 0 percent for a diagnosis of Burkitt's lymphoma; one had a probability of 66 percent, which may represent a rare genetic overlap between Burkitt's lymphoma and diffuse large-B-cell lymphoma.

BIOLOGIC DIFFERENCES BETWEEN BURKITT'S LYMPHOMA AND DIFFUSE LARGE-B-CELL LYMPHOMA

To elucidate the biologic mechanisms that distinguish Burkitt's lymphoma from diffuse large-B-cell lymphoma, we used hierarchical clustering[25] to organize the Burkitt's lymphoma classifier genes (see Fig. 1 in the Supplementary Appendix). This method revealed four prominent clusters of coordinately expressed genes, which we term gene-expression "signatures," because they reflect specific biologic processes.[26]

The c-myc protein and its target genes constituted one signature, which was more highly expressed in Burkitt's lymphoma than in diffuse large-B-cell lymphoma (Fig. 3A). The second signature included genes that were expressed in normal germinal-center B cells. The subgroup of these genes that was expressed more highly in Burkitt's lymphoma than in germinal-center B-cell–like diffuse large-B-cell lymphoma is termed the "BL-high" signature (Fig. 3B). The third signature included major-histocompatibility-complex (MHC) class I genes, and the fourth included nuclear factor-κB (NF-κB) target genes.[27] The third and fourth signatures were expressed at lower levels in Burkitt's lymphoma than in diffuse large-B-cell lymphoma (Fig. 3C and 3D).

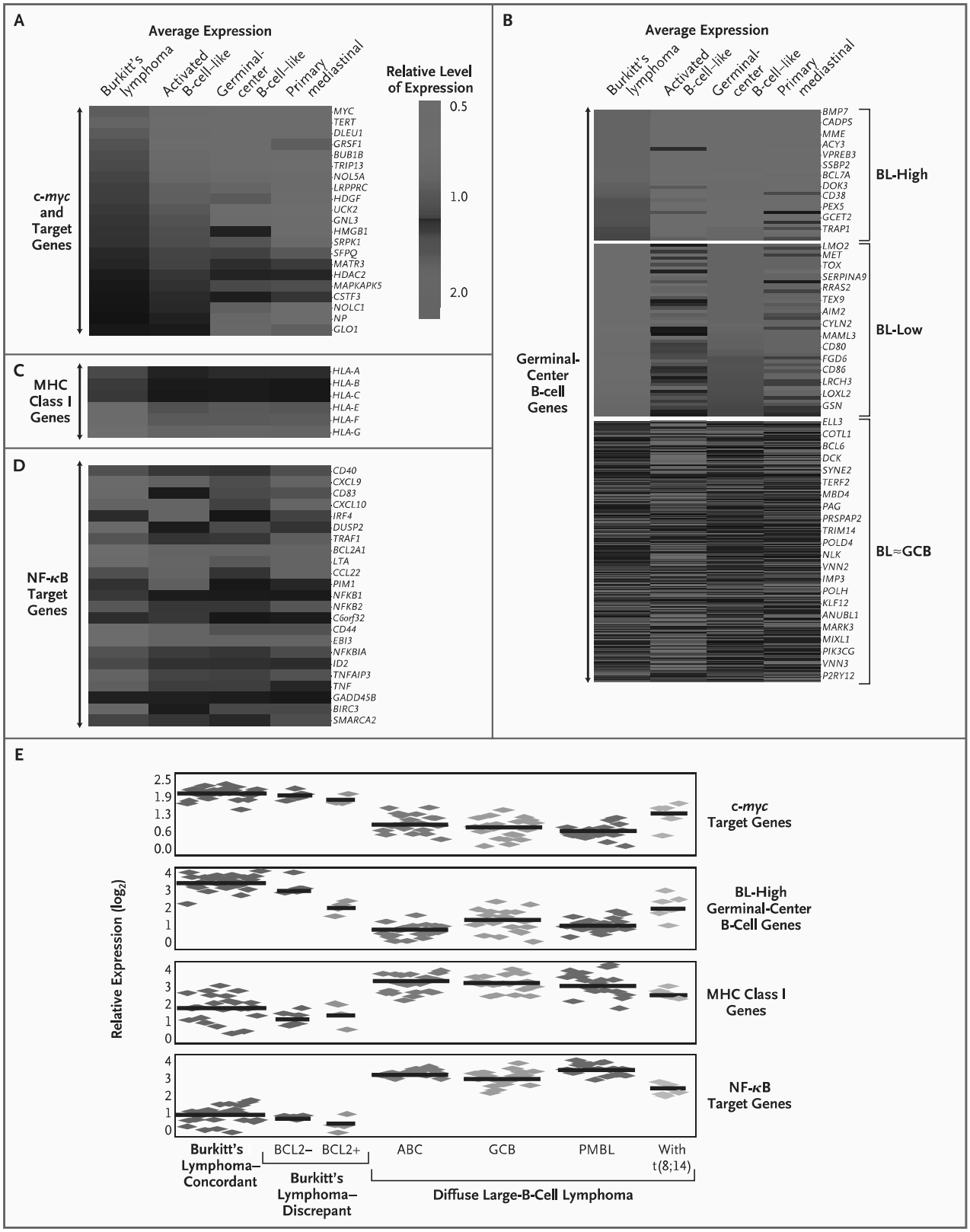
We averaged the expression levels of the genes in each signature and plotted these signature averages according to the molecular diagnosis of the cases (Fig. 3E). The Burkitt's lymphoma cases, including Burkitt's lymphoma–discrepant cases, were readily distinguished from diffuse large-B-cell lymphoma specimens ($P<0.001$). The BCL2-positive Burkitt's lymphoma–discrepant cases had a lower level of expression of the BL-high germinal-center B-cell signature than did the Burkitt's lymphoma–concordant cases (Fig. 3E); the same was true of two Burkitt's lymphoma–concordant cases with a t(14;18) translocation (data not shown). Diffuse large-B-cell lymphomas with a c-*myc* t(8;14) translocation were clearly distinguishable from Burkitt's lymphoma with respect to the expression of each signature.

Figure 3 (facing page). Relative Expression of Gene-Expression Signatures.

The average relative expression of genes that distinguish Burkitt's lymphoma from each subgroup of diffuse large-B-cell lymphoma (activated B-cell–like, germinal-center B-cell–like, and primary mediastinal) are categorized into gene-expression signatures: c-*myc* and its target genes (Panel A); genes that are expressed in normal germinal-center B cells (Panel B) and are expressed more highly (BL-high), less highly (BL-low), or equivalently (BL-GCB) in Burkitt's lymphoma than in germinal-center B-cell–like diffuse large-B-cell lymphoma; MHC class I genes (Panel C); and genes targeted by the NF-κB signaling pathway[27] (Panel D). Relative gene expression is depicted according to the color scale shown. We defined germinal-center B-cell signature genes as those that were overexpressed in normal germinal-center B cells, as compared with blood B cells, but that were not merely associated with cellular proliferation (see the Supplementary Appendix for details). The "BL-high" genes were expressed at levels twice as high in Burkitt's lymphoma as in germinal-center B-cell–like diffuse large-B-cell lymphoma ($P<0.001$). The "BL-low" genes were expressed at levels twice as high in germinal-center B-cell–like diffuse large-B-cell lymphoma as in Burkitt's lymphoma ($P<0.001$). The expression levels of the "BL-GCB" genes did not differ significantly between the two lymphomas. In Panel E, each diamond represents the average expression of one of the four gene-expression signatures for one biopsy specimen, shown according to the molecular diagnosis. Each bar represents the average for the diagnosis, as $\log_2$ values over the indicated range. Burkitt's lymphoma–discrepant specimens had signature averages that were readily distinguished from those of specimens belonging to the three diffuse large-B-cell lymphoma subgroups ($P<0.001$).

SURVIVAL

We analyzed data from the 28 children and adults with a molecular diagnosis of Burkitt's lymphoma for whom complete clinical information was available. Overall survival was markedly longer



among those who received intensive chemotherapy regimens than among those who received CHOP-like regimens (P=0.005) (Fig. 4). Of seven patients with discrepant Burkitt's lymphoma who could be evaluated, five had received CHOP-like regimens and none survived beyond two years. Both of the remaining two patients had received intensive regimens, and one lived more than five years after diagnosis; the other died nine months after diagnosis.

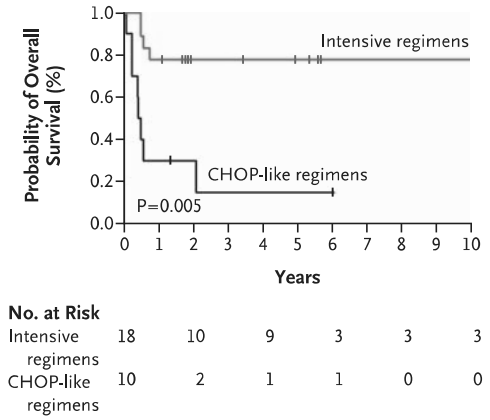


Figure 4. Kaplan–Meier Survival Estimates among Patients with Burkitt's Lymphoma and Diffuse Large-B-Cell Lymphoma.

The analysis includes the 28 children and adults with a molecular diagnosis of Burkitt's lymphoma for whom complete clinical information was available, according to the treatment received. Tick marks denote patients who were alive at the time of last follow-up.

DISCUSSION

A diagnostic test based on gene-expression profiling identified all 25 cases of classic Burkitt's lymphoma that had been verified by an expert panel of hematopathologists. Our study revealed substantial difficulty in rendering a reproducible diagnosis of Burkitt's lymphoma with the use of current pathological methods. Among the cases that were submitted for our analysis as either Burkitt's lymphoma or Burkitt-like lymphoma, more than one third were assigned a different diagnosis by the expert panel. Moreover, nine aggressive lymphomas that were diagnosed as diffuse large-B-cell lymphoma or high-grade lymphoma by the panel were classified as Burkitt's lymphoma on the basis of gene-expression profiles. These cases had all the gene-expression features of Burkitt's lymphoma, suggesting that they are actually cases of Burkitt's lymphoma that cannot be reliably diagnosed by current methods. These cases constituted 17 percent of the 53 specimens that had a molecular profile of Burkitt's lymphoma.

In line with previous studies,[3,4,8,28] we found that patients with a molecular diagnosis of Burkitt's lymphoma had a poor outcome with standard chemotherapy regimens yet had a good response to intensive regimens. Intensive regimens are more frequently associated with treatment-related complications than standard regimens and are therefore not appropriate as initial therapy for diffuse large-B-cell lymphoma. Therefore, the ability of the classifier to distinguish Burkitt's lymphoma from diffuse large-B-cell lymphoma could improve clinical decision making.

The translocation of the c-*myc* gene and its consequent deregulation is a key oncogenic event in the development of Burkitt's lymphoma. Accordingly, the signature of the c-*myc* target genes distinguished Burkitt's lymphoma from diffuse large-B-cell lymphoma. However, c-*myc* translocations also occur in 5 to 10 percent of diffuse large-B-cell lymphomas. Given the much higher incidence of diffuse large-B-cell lymphoma than Burkitt's lymphoma, most aggressive lymphomas with a c-*myc* translocation are clearly diffuse large-B-cell lymphoma. It is therefore notable that our classifier based on gene expression did not diagnose any of the six cases of diffuse large-B-cell lymphoma bearing a c-*myc* translocation as Burkitt's lymphoma.

Burkitt's lymphoma and diffuse large-B-cell lymphoma were found to differ with respect to the signature of the c-*myc* target genes as well as the other three gene-expression signatures. Though Burkitt's lymphoma and germinal-center B-cell–like diffuse large-B-cell lymphoma both originate from germinal-center B cells,[29,30] the expression of a subgroup of germinal-center B-cell genes distinguished Burkitt's from diffuse large-B-cell lymphomas. NF-κB target genes were expressed at lower levels in Burkitt's lymphoma than in any of the diffuse large-B-cell lymphoma subgroups; it is unclear whether this is due to differences in the malignant cells or in the tumor-infiltrating immune cells. Burkitt's-lymphoma tumors expressed MHC class I genes at very low levels as compared with tumors of diffuse large-B-cell lym-

phoma. Previous studies have documented the loss of MHC class I molecules in some cell lines derived from Burkitt's lymphoma,[31] but the mechanism underlying this down-modulation is unclear.

The gene-expression signatures that distinguish Burkitt's lymphoma from diffuse large-B-cell lymphoma provide insight into the nine Burkitt's lymphoma–discrepant cases. The five Burkitt's lymphoma–discrepant cases that were BCL2-negative were indistinguishable from the Burkitt's lymphoma–concordant cases in the expression of all four signatures. Therefore, these cases bear all the molecular hallmarks of Burkitt's lymphoma but cannot be diagnosed with the use of current methods. Interestingly, Burkitt's lymphoma–discrepant cases that were BCL2-positive resembled Burkitt's lymphoma–concordant cases with respect to three signatures but had a lower level of expression of the BL-high germinal-center B-cell signature, a phenotype that was also observed in the two Burkitt's lymphoma–concordant cases that were BCL2-positive. Cases with both the t(8;14) and t(14;18) translocations have been described previously as being very aggressive and associated with a poor prognosis.[32] Our data confirm that CHOP-like regimens are not adequate to treat such cases. A thorough characterization of more such cases will be needed in order to ascertain whether they represent a variant of Burkitt's lymphoma or have a separate pathogenesis.

In summary, the molecular classifier of Burkitt's lymphoma based on gene expression provides a quantitative and reproducible diagnosis of Burkitt's lymphoma that is superior to the best current diagnostic methods. It could be used to enhance diagnostic accuracy for this curable lymphoma.

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