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A Biologic Definition of Burkitt's Lymphoma from Transcriptional and Genomic Profiling

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ABSTRACT

BACKGROUND

The distinction between Burkitt's lymphoma and diffuse large-B-cell lymphoma is unclear. We used transcriptional and genomic profiling to define Burkitt's lymphoma more precisely and to distinguish subgroups in other types of mature aggressive B-cell lymphomas.

METHODS

We performed gene-expression profiling using Affymetrix U133A GeneChips with RNA from 220 mature aggressive B-cell lymphomas, including a core group of 8 Burkitt's lymphomas that met all World Health Organization (WHO) criteria. A molecular signature for Burkitt's lymphoma was generated, and chromosomal abnormalities were detected with interphase fluorescence in situ hybridization and array-based comparative genomic hybridization.

RESULTS

We used the molecular signature for Burkitt's lymphoma to identify 44 cases: 11 had the morphologic features of diffuse large-B-cell lymphomas, 4 were unclassifiable mature aggressive B-cell lymphomas, and 29 had a classic or atypical Burkitt's morphologic appearance. Also, five did not have a detectable *IG-myc* Burkitt's translocation, whereas the others contained an *IG-myc* fusion, mostly in simple karyotypes. Of the 176 lymphomas without the molecular signature for Burkitt's lymphoma, 155 were diffuse large-B-cell lymphomas. Of these 155 cases, 21 percent had a chromosomal breakpoint at the *myc* locus associated with complex chromosomal changes and an unfavorable clinical course.

CONCLUSIONS

Our molecular definition of Burkitt's lymphoma clarifies and extends the spectrum of the WHO criteria for Burkitt's lymphoma. In mature aggressive B-cell lymphomas without a gene signature for Burkitt's lymphoma, chromosomal breakpoints at the *myc* locus were associated with an adverse clinical outcome.

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Burkitt's lymphoma and diffuse large-B-cell lymphoma are mature aggressive B-cell lymphomas. If left untreated, they follow a rapid clinical course and are fatal within months. Burkitt's lymphoma is a distinct entity that includes endemic and sporadic types and cases associated with immunodeficiency or immunosuppression.[1] With the use of chemotherapy regimens that involve methotrexate and cytarabine, cure rates for sporadic Burkitt's lymphoma approach 90 percent in children and 70 percent in adults.[2] Diffuse large-B-cell lymphoma, by contrast, is biologically and clinically heterogeneous and comprises five morphologic variants and three subtypes.[3] Treatment with a combination of chemotherapy based on cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and the monoclonal antibody rituximab can induce lengthy remissions in many patients.[4] Approximately 30 percent of patients with diffuse large-B-cell lym-

Table 1. Morphologic, Immunohistochemical, Genetic, and Molecular Characteristics of Lymphomas.*

Characteristic	Lymphoma					P Value†
	All	mBL	Non-mBL	Intermediate	Non-mBL plus Intermediate	
	number (percent)					
Total	220	44	128	48	176	
Age at diagnosis						<0.001
<60 Yr	100 (46)	40 (91)	39 (31)	21 (45)	60 (34)	
≥60 Yr	118 (54)	4 (9)	88 (69)	26 (55)	114 (66)	
Sex						0.19
Female	91 (43)	13 (30)	56 (45)	22 (47)	78 (46)	
Male	123 (57)	30 (70)	68 (55)	25 (53)	93 (54)	
Morphologic diagnosis						<0.001
Burkitt's lymphoma (core group)	8 (4)	8 (18)	0	0	0	
Atypical Burkitt's lymphoma‡	28 (13)	21 (48)	3 (2)	4 (8)	7 (4)	
Diffuse large-B-cell lymphoma	165 (75)	11 (25)	115 (90)	39 (81)	154 (88)	
Mature aggressive B-cell non-Hodgkin's lymphoma, unclassifiable	18 (8)	4 (9)	9 (7)	5 (10)	14 (8)	
Burkitt's lymphoma–leukemia	1 (<1)	0	1 (1)	0	1 (1)	
CD10 expression§						<0.001
Absence	115 (55)	0	95 (79)	20 (43)	115 (69)	
Presence	94 (45)	42 (100)	26 (21)	26 (57)	52 (31)	
BCL6 expression§						0.043
Absence	33 (17)	0	26 (23)	7 (15)	33 (21)	
Presence	165 (83)	39 (100)	87 (77)	39 (85)	126 (79)	
BCL2 expression§						<0.001
Absence	61 (29)	33 (79)	20 (16)	8 (17)	28 (16)	
Presence	153 (71)	9 (21)	104 (84)	40 (83)	144 (84)	
Ki-67 score§						<0.001
<95%	162 (76)	15 (34)	107 (88)	40 (85)	147 (87)	
≥95%	51 (24)	29 (66)	15 (12)	7 (15)	22 (13)	
ABC or GCB signature¶						<0.001
ABC	58 (26)	0	50 (39)	8 (17)	58 (33)	
GCB	119 (54)	40 (91)	45 (35)	34 (71)	79 (45)	
Unclassified	43 (20)	4 (9)	33 (26)	6 (13)	39 (22)	

phoma, however, have disease that is resistant to this treatment or relapse soon after receiving it.[2,5]

The distinction between Burkitt's lymphoma and diffuse large-B-cell lymphoma is not reliably reproducible with the use of the current criteria of morphology, immunophenotype, and genetic abnormalities.[6,7] The Burkitt's translocation or its variants, which juxtapose the locus of the *myc* oncogene and one of the three immunoglobulin (*IG*) loci, are present in almost all Burkitt's lymphomas.[8,9] Nevertheless, *myc* translocations are not specific for Burkitt's lymphoma since they also occur in other lymphomas, including diffuse large-B-cell lymphoma. In the latter, chromosomal breakpoints at the *myc* locus are recurrently associated with non-*IG* partner loci and complex chromosomal alterations.[10-17]

The imprecise distinction between Burkitt's lymphoma and diffuse large-B-cell lymphoma on diagnosis may lead to the inadequate treatment of some patients with a mature aggressive B-cell lymphoma. Studies involving gene-expression profiling indicate that diffuse large-B-cell lymphomas comprise two or more main biologic subgroups with different clinical behaviors.[18-21] Until now, however, to our knowledge there has been no signature of gene expression that distinguishes Burkitt's lymphoma from diffuse large-B-cell lymphoma.

Our aim was to establish a molecular definition of Burkitt's lymphoma and to search for other clinically relevant subgroups of diffuse large-B-cell lymphoma. For this purpose, we performed gene-expression and genomic profiling of 220 mature

Table 1. (Continued.)

Characteristic	Lymphoma					P Value†
	All	mBL	Non-mBL	Intermediate	Non-mBL plus Intermediate	
	number (percent)					
myc partner‖						<0.001
IG-myc	59 (28)	38 (88)	5 (4)	16 (33)	21 (12)	
Non–*IG-myc*	15 (7)	1 (2)	4 (3)	10 (21)	14 (8)	
myc-negative	140 (65)	4 (9)	114 (93)	22 (46)	136 (80)	
***IGH-BCL2* fusion‖**						0.033
Absent	192 (88)	43 (98)	111 (89)	38 (79)	149 (86)	
Present	25 (12)	1 (2)	14 (11)	10 (21)	24 (14)	
***BCL6* breakpoint‖**						<0.001
Absent	177 (83)	43 (100)	94 (76)	40 (85)	134 (79)	
Present	36 (17)	0	29 (24)	7 (15)	36 (21)	
Chromosomal complexity score**						<0.001
Low (<6)	74 (40)	31 (79)	30 (29)	13 (31)	43 (29)	
High (≥6)	111 (60)	8 (21)	74 (71)	29 (69)	103 (71)	
Genetic group						<0.001
myc-simple	35 (17)	29 (76)	0	6 (13)	6 (4)	
myc-complex	33 (16)	5 (13)	9 (7)	19 (40)	28 (16)	
myc-negative	140 (67)	4 (11)	114 (93)	22 (47)	136 (80)	

* Percentages were calculated on the basis of the number of cases that could be evaluated; data were not available for all cases. Percentages may not total 100 because of rounding. mBL denotes molecular Burkitt's lymphoma.
† P values were calculated with the use of Fisher's exact test or the chi-square test and refer to differences between the mBL cases and the non-mBL and intermediate cases.
‡ The diagnosis was based on a Burkitt-like morphology or a deviant immunophenotype.
§ The expression of CD10, BCL6, BCL2, and Ki-67 was determined by immunohistochemical analysis.
¶ The signature of activated B-cell–like (ABC) lymphomas and germinal-center B-cell–like (GCB) lymphomas was assigned according to a modified classifier from Wright et al.[29]
‖ The *myc* and *BCL6* breakpoints and the *IGH-BCL2* and *myc* fusions were analyzed by interphase fluorescence in situ hybridization.
** Chromosomal complexity was determined with array-based comparative genomic hybridization.

aggressive B-cell lymphomas that had been diagnosed by a panel of expert hematopathologists.

METHODS

This study was conducted from July 2003 through November 2005. It was approved by the local ethics commission (Charité University Hospital, Berlin). The data discussed are available from the Gene Expression Omnibus of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/geo/) through GEO accession number GSE4475.

GENE EXPRESSION AND GENETIC ANALYSES

RNA and DNA were extracted from frozen sections (Qiagen). Affymetrix U133A GeneChip hybridization of all 220 specimens was performed in accordance with the manufacturer's recommendations, involving 5 μg of total RNA. We also performed array-based comparative genomic hybridization in 185 cases, applying a BAC/PAC array containing 2799 DNA fragments.[22,23] The number of imbalances per case was determined as an indicator of genetic complexity (explained in detail in the Supplementary Appendix, available with the full text of this article at www.nejm.org).

Interphase fluorescence in situ hybridization was performed on frozen or paraffin-embedded tissues from 217 cases with the use of probes for *IGH*, *IGK*, *IGL*, *myc*, *BCL6* and *BCL2* loci[24-26] (see the Supplementary Appendix). Tumor-biopsy specimens in which *myc* was fused to *IGH*, *IGK*, or *IGL* were referred to as "*IG-myc*"; lymphomas with *myc* breakpoints without fusion of *myc* to an *IG* locus were called "non–*IG-myc*."

ANALYSIS OF MICROARRAY DATA

Probe intensities were normalized according to a variance-stabilization method.[27] Gene-expression levels were estimated by fitting an additive model according to Irizarry et al.[28] Germinal-center and activated B-cell–like lymphomas were diagnosed according to the method of Wright et al.[29] (Further details and algorithms underlying the core-group extension are given in the Supplementary Appendix.) The raw gene-expression data are available at www.ncbi.nlm.nih.gov/geo.

STATISTICAL ANALYSIS

The Mann–Whitney U test, chi-square test, Fisher's exact test, and log-rank test were used to test for differences among groups. Survival was calculated from the day of diagnosis until death or until the end of follow-up. The Cox proportional-hazards model was used to analyze prognostic factors. Since not all variables of the international prognostic index were available for all patients, the age at diagnosis and Ann Arbor stage were used to adjust for known prognostic factors.[30]

RESULTS

PATIENTS AND DIAGNOSES

Biopsy specimens of 220 mature aggressive B-cell lymphomas (i.e., classic Burkitt's lymphomas, atypical Burkitt's lymphomas, and diffuse large-B-cell lymphomas), in which at least 70 percent of all cells were tumor cells, were included in this retrospective study. All specimens were reviewed by a panel of expert hematopathologists using the criteria of the World Health Organization (WHO) (provided in the Supplementary Appendix).

Clinical data were available for 146 patients (median year of collection, 1994) who had received a variety of primary treatments. The median follow-up was 60 months (range, 0 to 209). Characteristics of the tumors and patients are given in Tables 1 and 2, respectively, and in Tables S1, S2, and S3 of the Supplementary Appendix.

MOLECULAR SIGNATURE OF BURKITT'S LYMPHOMA

To derive a molecular signature of Burkitt's lymphoma, we devised a computational algorithm called "core-group extension." Given a predefined core group of expression profiles, the algorithm identifies additional cases that have a similar pattern of gene expression and provides a gene-expression signature for the extended group. The method is described in detail in the Supplementary Appendix.

The core group consisted of eight cases that satisfied the WHO criteria for Burkitt's lymphoma (a consensus histologic classification of classic or atypical Burkitt's lymphoma [Fig. 1A], CD20+, BCL6+, CD10+, BCL2–, CD5–, Ki-67 score ≥95 percent, IG-myc+). We applied core-group extension to a training set of 105 lymphomas and identified 58 genes that constituted the molecular Burkitt's lymphoma (mBL) signature (see the Supplementary Appendix). Each case was assigned an mBL-signature index score between 0 and 1, with a higher score reflecting a greater similarity of

Table 2. Clinical Characteristics of the Patients.*

Characteristic	Lymphoma					P Value†
	All	mBL	Non-mBL	Intermediate	Non-mBL plus Intermediate	
	number (percent)					
Total	146	28	87	31	118	
Age at diagnosis						<0.001
<60 Yr	66 (45)	27 (96)	26 (30)	13 (42)	39 (33)	
≥60 Yr	80 (55)	1 (4)	61 (70)	18 (58)	79 (67)	
Ann Arbor stage						0.05
I or II	61 (45)	17 (63)	26 (33)	18 (62)	44 (41)	
III or IV	74 (55)	10 (37)	53 (67)	11 (38)	64 (59)	
Sex						0.02
Female	62 (42)	6 (21)	40 (46)	16 (52)	56 (47)	
Male	84 (58)	22 (79)	47 (54)	15 (48)	62 (53)	
Morphologic appearance						0.004
Burkitt's lymphoma (core group)	5 (3)	5 (18)	0	0	0	
Atypical Burkitt's lymphoma‡	19 (13)	15 (54)	1 (1)	3 (10)	4 (3)	
Diffuse large-B-cell lymphoma	114 (78)	7 (25)	81 (93)	26 (84)	107 (91)	
Mature aggressive B-cell non-Hodgkin's lymphoma, unclassifiable	8 (6)	1 (3)	5 (6)	2 (6)	7 (6)	
Lesions						0.01
Extranodal lesions only	18 (14)	3 (12)	8 (11)	7 (26)	15 (15)	
Nodal lesions only	63 (50)	7 (28)	42 (58)	14 (52)	56 (56)	
Nodal and extranodal lesions	44 (35)	15 (60)	23 (32)	6 (22)	29 (29)	
B symptoms						0.10
Absent	65 (60)	12 (80)	37 (51)	16 (73)	53 (56)	
Present	44 (40)	3 (20)	35 (49)	6 (27)	41 (44)	
Chemotherapy						<0.001
ALL-like§	26 (19)	21 (84)	2 (2)	3 (10)	5 (5)	
CHOP-like	82 (61)	3 (12)	61 (75)	18 (62)	79 (72)	
Other	27 (20)	1 (4)	18 (22)	8 (28)	26 (24)	
Radiotherapy						0.80
No	96 (76)	19 (79)	58 (78)	19 (66)	77 (75)	
Yes	31 (24)	5 (21)	16 (22)	10 (34)	26 (25)	
Rituximab treatment						0.04
No	116 (89)	27 (100)	61 (84)	25 (93)	86 (86)	
Yes	14 (11)	0	12 (16)	2 (7)	14 (14)	
Response to treatment‖						0.12
Complete remission	50 (48)	14 (74)	24 (39)	12 (52)	36 (42)	
Complete remission, unconfirmed	18 (17)	3 (16)	12 (19)	3 (13)	15 (18)	
No change	2 (2)	0	1 (2)	1 (4)	2 (2)	
Partial response	14 (13)	0	11 (18)	3 (13)	14 (16)	
Progress	20 (19)	2 (11)	14 (23)	4 (17)	18 (21)	

* Percentages were calculated on the basis of the number of cases that could be evaluated; data were not available for all cases. Percentages may not total 100 because of rounding. CHOP denotes cyclophosphamide, doxorubicin, vincristine, and prednisone, and mBL molecular Burkitt's lymphoma.

† P values were calculated with the use of Fisher's exact test or the chi-square test and refer to differences between the mBL cases and the non-mBL and intermediate cases.

‡ The diagnosis was based on a Burkitt-like morphology or a deviant immunophenotype.

§ The acute lymphoblastic leukemia (ALL) chemotherapy protocol is also known as the non-Hodgkin's lymphoma Berlin–Frankfurt–Münster (NHL-BFM) protocol.

‖ Response to treatment was classified according to the Cheson criteria.

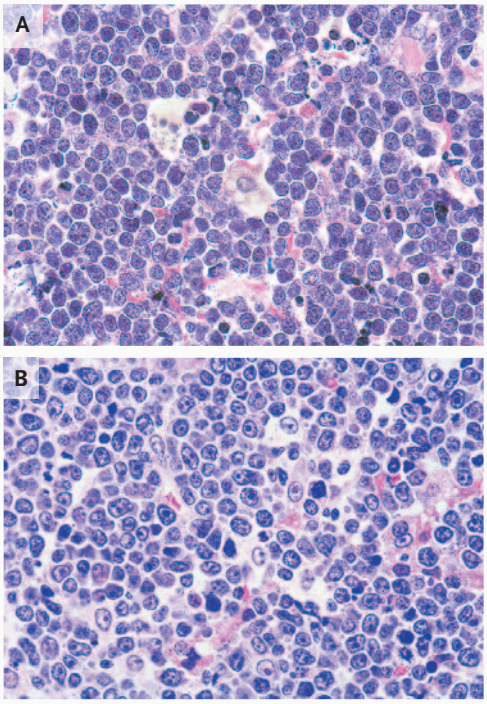


Figure 1. Histomorphologic Appearance of Cases with an mBL Gene Signature (Hematoxylin and Eosin).

Panel A shows the classic morphologic appearance of Burkitt's lymphoma; cytogenetically, this case was classified as a lymphoma with an *IG-myc* fusion and a low chromosomal complexity score (<6) that does not have an *IGH-BCL2* fusion or a *BCL6* breakpoint (called "myc-simple"). Panel B shows the morphologic appearance of diffuse large-B-cell lymphoma; cytogenetically, this case was classified as myc-simple.

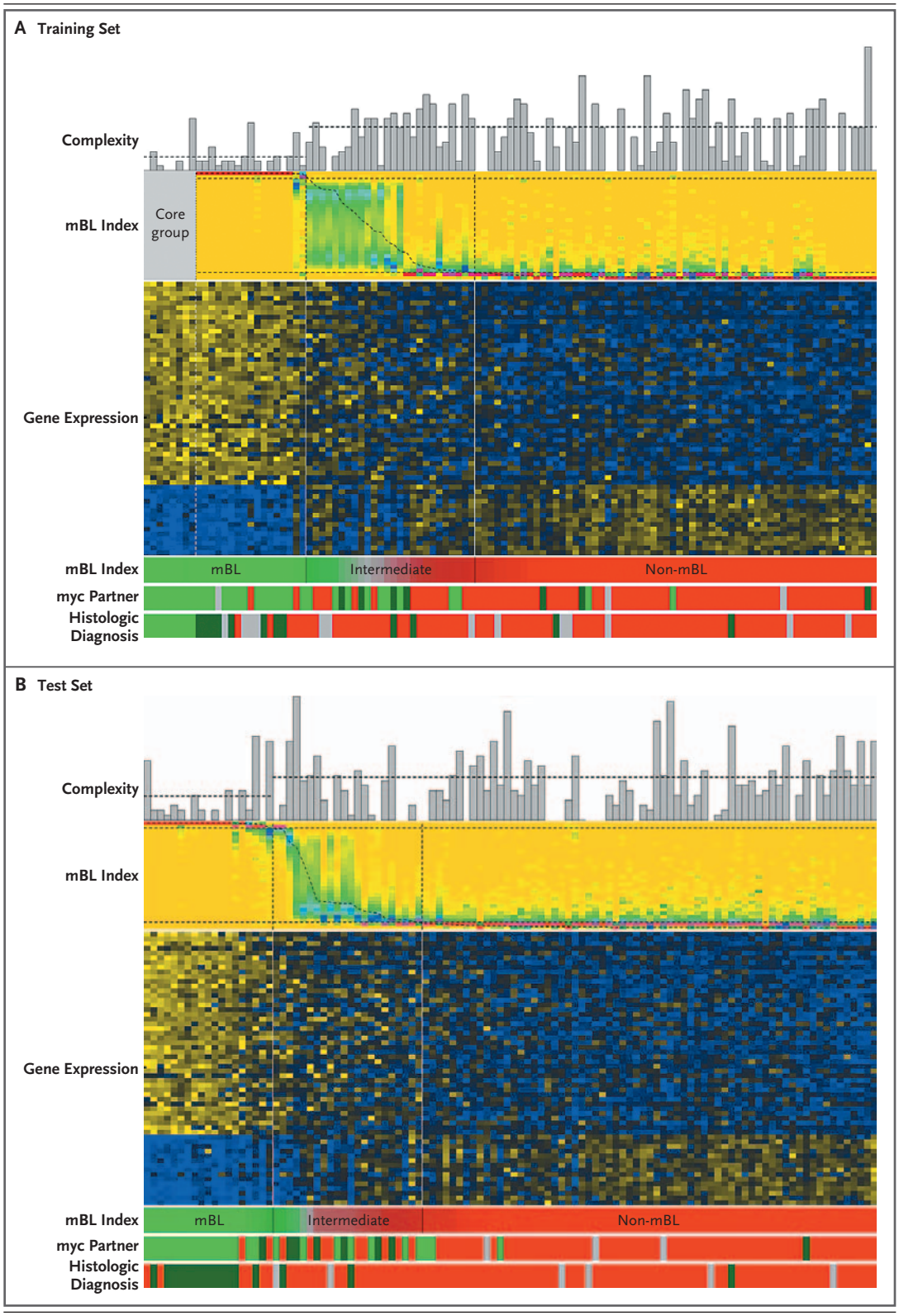
Figure 2 (facing page). Identification by Core-Group Extension of Cases with an mBL Signature.

Panel A shows the results for the training set of 105 cases, and Panel B the results for the test set of 107 cases. Genomic complexity is described in the bar plots at the top of the panels, with complexity increasing with height; the dotted horizontal line represents the mean complexity of each group. The second plot shows the stability of the core-group extension with respect to random perturbations of the core-group data (bootstrap analysis). The frequency of the perturbed mBL-signature index scores (from 0 to 1, bottom of plot to top) obtained from 1000 runs of the algorithm is indicated by color (very low frequency, orange; low, yellow; medium, green; high, blue; very high, red). The vertical lines delineate the three groups of lymphomas (mBL, intermediate, and non-mBL) — as well as the core group of cases (Panel A) — and the dashed horizontal lines indicate the index-score cutoffs defining the mBL group (0.95) and the non-mBL group (0.05). Among the mBL cases, the index score is close to 1 for all bootstrap perturbations, whereas in the non-mBL group it is near 0, demonstrating the stability of the signature. The mBL-signature index scores resulting from the nonbootstrapped signatures are represented as a dashed curve. Below, the heat map shows the gene-expression levels of the 58 mBL-signature genes, with 1 gene shown per row. Bright blue indicates a low level of expression (3 SD below the average of all cases), bright yellow indicates a high level of expression (3 SD above the average), and black the average level of expression across all samples. The cases are ordered from left to right on the basis of decreasing mBL-signature index score, given below the heat map. Green represents a high index score (mBL), and red a low index score (non-mBL). The color gradient in the intermediate group highlights the continuous transition of the index score between the mBL and non-mBL cases. The *myc* translocation partners are shown according to type: *IG-myc* fusion (bright green), non–*IG-myc* fusion (dark green), *myc*-breakpoint absent (red), and no data available (gray). Finally, the histologic diagnosis is shown at the bottom. Bright green indicates Burkitt's lymphoma in the core group; dark green, atypical Burkitt's lymphoma; red, diffuse large-B-cell lymphoma; and gray, unclassifiable mature aggressive B-cell lymphoma.

gene expression in the sample to that in the core group. Cases with an index score greater than 0.95 were classified as mBL, and those with an index score of less than 0.05 were designated non–molecular Burkitt's lymphoma (non-mBL). The remaining cases were considered intermediate (Fig. 2). By rerunning the core-group extension algorithm 1000 times with random perturbations of the core-group data (bootstrapping), we evaluated the stability of the mBL signature (Fig. 2). Although the mBL signature was stable at the extremes, the index scores between mBL cases and non-mBL cases were continuous (Fig. 2). Results obtained in an independent test set of 107 cases were consistent with the training set with regard to the histologic and genetic characteristics of the mBL and non-mBL cases and the size and stability during bootstrap validation (Fig. 2).

FEATURES OF MATURE AGGRESSIVE B-CELL LYMPHOMAS WITH THE mBL SIGNATURE

We identified 36 lymphomas with an mBL-signature index score of greater than 0.95 in addition to the 8 core Burkitt's lymphomas, for a total of 44 mBL cases. The 8 core cases were similar to



the additional 36 cases with regard to age distribution and genetic features (Table 1, and Table S1 in the Supplementary Appendix), as well as clinical course. Of the additional 36 mBL cases, 21 were categorized as atypical Burkitt's lymphomas because of their Burkitt-like morphology or their deviant immunophenotype. It is important to note that 11 of these 36 cases had the distinctive morphologic appearance of diffuse large-B-cell lymphoma (Fig. 1B). The remaining four mBL cases had the morphologic appearance of mature aggressive B-cell lymphoma but could not be further classified histologically. With regard to immunophenotype, CD10 and BCL6 were consistently expressed in the 42 and 39 mBL cases, respectively, that could be evaluated. BCL2 was detected at a low level in seven of these mBL cases and at a high level in two.

FEATURES OF MATURE AGGRESSIVE B-CELL LYMPHOMAS WITHOUT THE mBL SIGNATURE

Of all 220 lymphomas, 176 had an mBL-signature index score of less than 0.95. Of these 176 cases, 128 had an mBL-signature index score of less than 0.05 and were thus assigned to the non-mBL group. The remaining 48 cases had an mBL-signature index score between 0.05 and 0.95 and thus could not be assigned unambiguously to the mBL or non-mBL group. These cases were assigned to the intermediate group, representing the transition zone between the mBL and non-mBL groups.

With few exceptions (12 cases), the histologic diagnosis in the non-mBL cases was diffuse large-B-cell lymphoma (Table 1). The histologic diagnosis of 39 (81 percent) of the intermediate cases was also diffuse large-B-cell lymphoma. Non-mBL and intermediate cases showed strong concordance regarding age distribution, immunophenotype, growth fraction (Ki-67 score), and chromosomal complexity (Table 1).

GENETIC ABERRATIONS AND GENE EXPRESSION

We were able to evaluate 43 mBL cases for the presence of *myc* translocations by using fluorescence in situ hybridization. All but five cases (88 percent) carried an *IG-myc* fusion and one of these five had both non–*IG-myc* and *IGH-BCL2* fusions. In the 38 mBL cases with *IG-myc* fusion, *IGH-BCL2* fusion and *BCL6* breakpoints were absent. The average chromosomal complexity score was low in the 38 mBL cases with *IG-myc* fusion but was high in the 5 mBL cases without *IG-myc* fusion (median complexity score, 2 vs. 9; P<0.001).

The frequency of *myc* breakpoints (regardless of translocation partner) was significantly lower in the intermediate and non-mBL groups (present in 35 of the 171 cases that could be evaluated [20 percent]) than in the mBL group (present in 39 of 43 cases [91 percent], P<0.001) (Table 1). It is remarkable that *myc* breakpoints were common in the intermediate group (26 of 48 cases [54 percent]), whereas they were uncommon in the non-mBL group (9 of 123 cases [7 percent]). Non-*IG* partners were frequently involved in *myc* translocation in both the intermediate group (10 of 26 cases [38 percent]) and the non-mBL group (4 of 9 cases [44 percent]). Among the 35 *myc*-positive intermediate and non-mBL cases, 16 (46 percent) had a concurrent *IGH-BCL2* fusion, *BCL6* breakpoint, or both. The chromosomal complexity score was significantly higher in the intermediate and non-mBL groups than in the mBL group (median complexity score, 8.5 vs. 2; P<0.001) regardless of the presence of *myc* breakpoints (median complexity score, 7.5) or absence of *myc* breakpoints (median complexity score, 9).

On the basis of these data, we distinguished three main cytogenetic groups within the mature aggressive B-cell lymphomas. The first is called "myc-simple": lymphomas with *IG-myc* fusions and a low chromosomal complexity score (<6) that do not have *IGH-BCL2* fusions and *BCL6* breakpoints. The second is called "myc-complex": all lymphomas with non–*IG-myc* fusions or all lymphomas with *IG-myc* fusions that have a high chromosomal complexity score (≥6), an *IGH-BCL2* fusion, or *BCL6* breakpoint, or any combination of these. The third is called "myc-negative," comprising *myc*-negative lymphomas. The mBL group predominantly consisted of myc-simple lymphomas; the non-mBL group predominantly consisted of myc-negative lymphomas. In contrast, the intermediate group contained most of the myc-complex cases but also occasional myc-simple and several myc-negative cases (Table 1).

CORRELATION OF MOLECULAR AND CLINICAL FEATURES

No significant differences were observed regarding the morphologic characteristics, immunophenotype, or gene-expression pattern between the 146 patients with survival data and the 74 patients

without or with incomplete clinical information (Table S2 and Fig. S1 in the Supplementary Appendix). The clinical data available for the 146 patients (Fig. S1 in the Supplementary Appendix) suggest that the tissue specimens were obtained during a period of relapse in less than 10 percent; this percentage was assumed to be representative of those without clinical information.

Patients with lymphomas classified as mBL or myc-simple had a significantly better five-year survival rate than patients with non-mBL or intermediate lymphomas (75 percent vs. 39 percent, P=0.003 [Fig. 3]) or with myc-complex or myc-negative lymphomas (70 percent vs. 41 percent, P=0.005 [Fig. S3 in the Supplementary Appendix]). However, the results of multivariate regression analysis showed that even if we ignored the different treatments received by these patients, the favorable outcome among patients with mBL or myc-simple lymphomas could largely be explained by the onset of the disease at a young age and the limited stage of the disease (Tables 2 and 3).

Among the 82 patients with non-mBL or intermediate lymphomas for whom clinical information was available, the presence of a *myc* breakpoint, as compared with its absence, was associated with a poor five-year survival rate (15 percent vs. 44 percent) (Fig. S2 in the Supplementary Appendix). In the non-mBL and intermediate groups, the presence of a *myc* breakpoint — which occurred mainly in myc-complex lymphomas (82 percent) — was associated with a significantly worse survival rate, independently of Ann Arbor stage and age (hazard ratio for death, 2.85; 95 percent confidence interval, 1.43 to 5.68; P=0.003) (Table 3 and Fig. S3 in the Supplementary Appendix).

When we applied the signature for activated B-cell–like or germinal-center B-cell–like large-B-cell lymphomas described by Rosenwald et al.[19] to our non-mBL and intermediate cases, we found that lymphomas with a germinal-center B-cell–like signature were associated with a significantly better five-year survival rate than activated B-cell–like lymphomas (51 percent vs. 12 percent, P=0.003) (Fig. S4 in the Supplementary Appendix). The hazard ratio for death for lymphomas carrying a germinal-center B-cell–like signature as compared with activated B-cell–like signature (1.79; 95 percent confidence interval, 0.94 to 3.42; P=0.08) was in the same range as that reported by Rosenwald et al.[19] (Table 3).

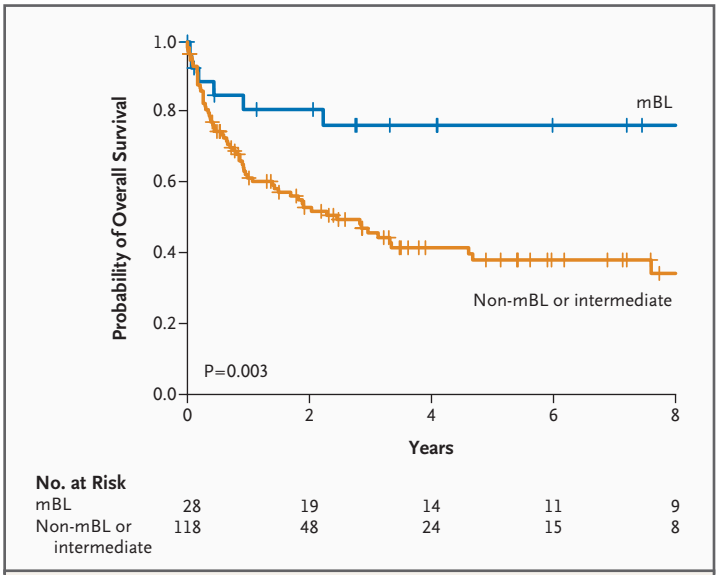


Figure 3. Kaplan–Meier Estimates of Survival According to the mBL Signature.
Overall survival among patients with an mBL-signature index score greater than 0.95 was significantly greater than that among the patients with non-mBL or intermediate lymphoma (P=0.003 by the log-rank test). Tick marks denote patients alive at the time of last follow-up.

DISCUSSION

The distinction between Burkitt's lymphoma and diffuse large-B-cell lymphoma is clinically important, because these lymphomas are treated with different chemotherapeutic protocols and differ in their outcome.[31] Even with the use of current diagnostic criteria, the distinction is not precise; agreement among expert hematopathologists on the pathological diagnosis of classic Burkitt's lymphoma, atypical Burkitt's lymphoma, and diffuse large-B-cell lymphomas is only 53 percent.[6,7] Our results provide a molecular definition of Burkitt's lymphoma that reliably and reproducibly distinguishes it from other mature aggressive B-cell lymphomas.

Of the 220 mature aggressive B-cell lymphomas studied, we identified 44 with a consistent pattern of gene expression that was characteristic of mBL. The distinctive mBL signature consisted of 58 genes, including several target genes of the nuclear factor-κB pathway (i.e., *BCL2A1*, *FLIP*, *CD44*, *NFKBIA*, *BCL3*, and *STAT3*) that are known to distinguish activated B-cell–like or germinal-center B-cell–like lymphomas.[19,29] It is notable that these genes were expressed at lower

Table 3. Results of Multivariate Survival Analyses.

Factor	Hazard Ratio for Death (95% CI)*	P Value
Patients with primary lymphoma and survival data (N=135)†		
Age at diagnosis		<0.001
<60 Yr	1.00	
≥60 Yr	3.00 (1.63–5.53)	
Ann Arbor stage		0.003
I or II	1.00	
III or IV	2.24 (1.31–3.81)	
Molecular diagnosis		0.43
mBL	1.00	
Non-mBL and intermediate	1.47 (0.57–3.81)	
Patients with non-mBL or intermediate lymphoma and survival data (N=82)‡		
Age at diagnosis		0.006
<60 Yr	1.00	
≥60 Yr	2.77 (1.34–5.73)	
Ann Arbor stage		0.02
I or II	1.00	
III or IV	2.16 (1.13–4.14)	
Cell of origin		0.08
Germinal-center B-cell–like	1.00	
Activated B-cell–like	1.79 (0.94–3.42)	
myc Breakpoint		0.003
Absent	1.00	
Present	2.85 (1.43–5.68)	
Patients with non-mBL or intermediate lymphoma and survival data (N=78)§		
Age at diagnosis		0.01
<60 Yr	1.00	
≥60 Yr	2.66 (1.25–5.64)	
Ann Arbor stage		0.09
I or II	1.00	
III or IV	1.79 (0.91–3.54)	
Cell of origin		0.06
Germinal-center B-cell	1.00	
Activated B-cell	1.87 (0.97–3.62)	
Genetic group		0.002
myc-negative	1.00	
myc-complex	3.23 (1.51–6.89)	

* CI denotes confidence interval.
† Eleven patients with primary lymphoma were excluded from the analysis.
‡ Seven patients whose lymphoma could not be classified in either the activated B-cell–like or the germinal-center B-cell–like lymphoma group were excluded from the analysis.
§ Four patients with myc-simple cases were excluded from the analysis.

levels in mBL cases than in cases of germinal-center B-cell–like diffuse large-B-cell lymphoma.

The mBL signature extends the WHO definition of Burkitt's lymphoma[1] to cases with the morphologic characteristics of diffuse large-B-cell lymphoma and expression of BCL2. Yet not all cases with morphologic or immunophenotypical features of Burkitt's lymphoma were classified as mBL. Parallel analyses of genetic features — by means of interphase fluorescence in situ hybridization and array-based comparative genomic hybridization — strongly support the biologic relevance of the mBL signature. Of the 38 mBL cases for which we had genetic data, 29 (76 percent) were classified as myc-simple, irrespective of their morphologic appearance. However, in four mBL cases, no *IG-myc* fusion was detectable, and one case was found to have a non–*IG-myc* translocation.

Clinically, all patients with mBL had a favorable prognosis (five-year survival rate, 75 percent). There was no significant difference in survival between patients whose tumors had a morphologic appearance of classic or atypical Burkitt's lymphoma and those with a morphologic appearance of diffuse large-B-cell lymphoma, irrespective of the presence or absence of a *myc* breakpoint (data not shown). Our results suggest that a molecular diagnosis of Burkitt's lymphoma yields a more precise definition of this entity than do current diagnostic criteria.

Of the 220 mature aggressive B-cell lymphomas, 176 had an mBL-signature index below 0.95 and thus were not classified as mBL. Most of these 176 cases had the morphologic appearance of diffuse large-B-cell lymphoma. In line with a previous report,[19] there was a difference in five-year survival between cases with a germinal-center B-cell–like gene signature and an activated B-cell–like gene signature (51 percent vs. 12 percent). More striking, the presence of breakpoints at the *myc* locus was strongly associated with an unfavorable five-year survival rate, as compared with the absence of such breakpoints (15 percent vs. 44 percent). This association was independent of stage, age, and whether the tumor was classified as activated B-cell–like or germinal-center B-cell–like. In addition, 46 percent of cases with *IG-myc* and non–*IG-myc* breakpoints had concurrent *BCL2* and *BCL6* translocations, suggesting that the *myc* breakpoint arose during clonal evolution and conferred clinical aggressiveness.[32]

In the 208 cases that could be evaluated genetically, the pattern of chromosomal aberrations allowed us to define three cytogenetic groups. The myc-simple group comprises lymphomas with an *IG-myc* fusion and a low number of chromosomal imbalances (complexity score, less than 6). This group included cases in which the Burkitt's translocation is most likely to constitute the primary oncogenic event. The myc-simple cases largely overlapped with the mBL cases and were associated with a favorable clinical outcome. In contrast, myc-complex status was associated with a poor outcome, independently of age and clinical stage (five-year survival rate, 21 percent). The myc-complex cases were common in the intermediate group. We speculate that in these intermediate cases, a primary genetic aberration induced a gene-expression profile that was subsequently shifted toward the mBL profile by a *myc* translocation.

In summary, we have devised a molecular definition of Burkitt's lymphoma by global gene-expression and genomic profiling. Mature aggressive B-cell lymphomas with both the mBL signature and the cytogenetic myc-simple status have a favorable outcome and can be regarded as biologic Burkitt's lymphoma. Mature aggressive B-cell lymphomas with *myc* breakpoints but without an mBL signature are clearly distinct from biologic Burkitt's lymphoma and are associated with a poor clinical outcome. The few cases that met only one of the two criteria for biologic Burkitt's lymphoma require further investigation. The distinction between biologic Burkitt's lymphoma and other mature aggressive B-cell lymphomas with *myc* breakpoints or without them is a prerequisite for planning clinical trials of the treatment of mature aggressive B-cell lymphomas. Such trials should clarify whether Burkitt's lymphomas and aggressive B-cell lymphomas (with *myc* breakpoints or without them) should be treated differently.

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APPENDIX

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REFERENCES

1. Diebold J, Jaffe ES, Raphael M, Warnke RA. Burkitt lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Vol. 3 of World Health Organization classification of tumours. Lyon, France: IARC Press, 2001:181-4.
2. Divine M, Casassus P, Koscielny S, et al. Burkitt lymphoma in adults: a prospective study of 72 patients treated with an adapted pediatric LMB protocol. Ann Oncol 2005; 16:1928-35.
3. Gatter KC, Warnke RA. Diffuse large B-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Vol. 3 of World Health Organization classification of tumours. Lyon, France: IARC Press, 2001:171-4.
4. Coiffier B. State-of-the-art therapeutics: diffuse large B-cell lymphoma. J Clin Oncol 2005;23:6387-93.
5. Pfreundschuh M, Truemper L, Gill D,

et al. First analysis of the completed Mabthera International (Mint) trial in young patients with low-risk diffuse large B-cell lymphoma (DLBCL): addition of rituximab to a CHOP-like regimen significantly improves outcome of all patients with the identification of a very favorable subgroup with IPI=O and no bulky disease. In: Abstracts of the 46th Annual Meeting of the American Society of Hematology, New Orleans, December 4–7, 2004:157. abstract.
6. Magrath I, Jaffe ES, Bhatia K. Burkitt's lymphoma. In: Knowles DM, ed. Neoplastic hematopathology. Philadelphia: Lippincott Williams & Wilkins, 2001:953-86.
7. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma: the Non-Hodgkin's Lymphoma Classification Project. Blood 1997;89:3909-18.
8. Hecht JL, Aster JC. Molecular biology of Burkitt's lymphoma. J Clin Oncol 2000; 18:3707-21.
9. Boxer LM, Dang CV. Translocations involving c-myc and c-myc function. Oncogene 2001;20:5595-610.
10. Au WY, Horsman DE, Gascoyne RD, Viswanatha DS, Klasa RJ, Connors JM. The spectrum of lymphoma with 8q24 aberrations: a clinical, pathological and cytogenetic study of 87 consecutive cases. Leuk Lymphoma 2004;45:519-28.
11. Knezevich S, Ludkovski O, Salski C, et al. Concurrent translocation of BCL2 and MYC with a single immunoglobulin locus in high-grade B-cell lymphomas. Leukemia 2005;19:659-63.
12. Kramer MH, Hermans J, Wijburg E, et al. Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood 1998;92:3152-62.
13. Au WY, Horsman DE, Viswanatha DS, Connors JM, Klasa RJ, Gascoyne RD. 8q24 Translocations in blastic transformation of mantle cell lymphoma. Haematologica 2000;85:1225-7.
14. Dunphy CH, van Deventer HW, Carder KJ, Rao KW, Dent GA. Mature B-cell acute lymphoblastic leukemia with associated translocations (14;18)(q32;q21) and (8;9)(q24;p13): a Burkitt variant? Arch Pathol Lab Med 2003;127:610-3.
15. Levine EG, Arthur DC, Machnicki J, et al. Four new recurring translocations in non-Hodgkin lymphoma. Blood 1989;74: 1796-800.
16. Gauwerky CE, Huebner K, Isobe M, Nowell PC, Croce CM. Activation of MYC in a masked t(8;17) translocation results in an aggressive B-cell leukemia. Proc Natl Acad Sci U S A 1989;86:8867-71.
17. Nacheva E, Dyer MJ, Fischer P, et al. C-MYC translocations in de novo B-cell lineage acute leukemias with t(14;18) (cell lines Karpas 231 and 353). Blood 1993;82: 231-40.
18. 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.
19. 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.
20. Monti S, Savage KJ, Kutok JL, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. Blood 2005;105:1851-61.
21. Bea S, Zettl A, Wright G, et al. Diffuse large B-cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene-expression-based survival prediction. Blood 2005;106: 3183-90.
22. Schwaenen C, Nessling M, Wessendorf S, et al. Automated array-based genomic profiling in chronic lymphocytic leukemia: development of a clinical tool and discovery of recurrent genomic alterations. Proc Natl Acad Sci U S A 2004;101:1039-44.
23. Fiegler H, Carr P, Douglas EJ, et al. DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. Genes Chromosomes Cancer 2003;36:361-74.
24. Martin-Subero JI, Gesk S, Harder L, Grote W, Siebert R. Interphase cytogenetics of hematological neoplasms under the perspective of the novel WHO classification. Anticancer Res 2003;23:1139-48.
25. Ventura RA, Martin-Subero JI, McParland M, Gesk S, Mason DY, Siebert R. FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. J Mol Diagn 2006;8:141-51.
26. Martin-Subero JI, Harder L, Gesk S, et al. Interphase FISH assays for the detection of translocations with breakpoints in immunoglobulin light chain loci. Int J Cancer 2002;98:470-4.
27. Huber W, von Heydebreck A, Sultmann H, Poustka A, Vingron M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. Bioinformatics 2002;18: Suppl 1:S96-S104.
28. Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4:249-64.
29. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci U S A 2003; 100:9991-6.
30. Shipp MA. Prognostic factors in aggressive non-Hodgkin's lymphoma: who has "high-risk" disease? Blood 1994;83: 1165-73.
31. Pfreundschuh M, Trumper L, Kloess M, et al. Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. Blood 2004;104: 634-41.
32. MacPherson N, Lesack D, Klasa R, et al. Small noncleaved, non-Burkitt's (Burkitt-like) lymphoma: cytogenetics predict outcome and reflect clinical presentation. J Clin Oncol 1999;17:1558-67.