

ORIGINAL ARTICLE

Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma

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ABSTRACT

BACKGROUND

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Diffuse large B-cell lymphomas (DLBCLs) are phenotypically and genetically heterogeneous. Gene-expression profiling has identified subgroups of DLBCL (activated B-cell–like [ABC], germinal-center B-cell–like [GCB], and unclassified) according to cell of origin that are associated with a differential response to chemotherapy and targeted agents. We sought to extend these findings by identifying genetic subtypes of DLBCL based on shared genomic abnormalities and to uncover therapeutic vulnerabilities based on tumor genetics.

METHODS

Drs. Schmitz, Wright, Huang, and Johnson contributed equally to this article.

We studied 574 DLBCL biopsy samples using exome and transcriptome sequencing, array-based DNA copy-number analysis, and targeted amplicon resequencing of 372 genes to identify genes with recurrent aberrations. We developed and implemented an algorithm to discover genetic subtypes based on the co-occurrence of genetic alterations.

RESULTS

We identified four prominent genetic subtypes in DLBCL, termed MCD (based on the co-occurrence of *MYD88*^{L265P} and *CD79B* mutations), BN2 (based on *BCL6* fusions and *NOTCH2* mutations), N1 (based on *NOTCH1* mutations), and EZB (based on *EZH2* mutations and *BCL2* translocations). Genetic aberrations in multiple genes distinguished each genetic subtype from other DLBCLs. These subtypes differed phenotypically, as judged by differences in gene-expression signatures and responses to immunochemotherapy, with favorable survival in the BN2 and EZB subtypes and inferior outcomes in the MCD and N1 subtypes. Analysis of genetic pathways suggested that MCD and BN2 DLBCLs rely on “chronic active” B-cell receptor signaling that is amenable to therapeutic inhibition.

CONCLUSIONS

We uncovered genetic subtypes of DLBCL with distinct genotypic, epigenetic, and clinical characteristics, providing a potential nosology for precision-medicine strategies in DLBCL. (Funded by the Intramural Research Program of the National Institutes of Health and others.)

N Engl J Med 2018;378:1396-407.

DOI: 10.1056/NEJMoa1801445

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GENE-EXPRESSION PROFILING DEFINED the activated B-cell–like (ABC) and germinal-center B-cell–like (GCB) subgroups of diffuse large B-cell lymphoma (DLBCL), leaving approximately 10 to 20% of cases “unclassified.”^{1,2} This phenotypic distinction is associated with overall survival after standard therapy with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapy³ and predicts response of relapsed or refractory DLBCL to ibrutinib, an inhibitor of B-cell receptor–dependent nuclear factor κ B (NF- κ B) activation.⁴ Responses to ibrutinib were especially frequent in ABC tumors that have both a mutation in *CD79B*, encoding a B-cell receptor subunit, and the *MYD88*^{L265P} mutation — a finding that suggests that tumor genotype could influence response.

Genomic sequencing studies in DLBCL have focused on recurrent mutations in individual genes, revealing oncogenic mechanisms (reviewed by Shaffer et al.⁵). Functional genomic screening has provided a complementary view of regulatory pathways that are essential for the proliferation and survival of DLBCL cells.^{6–8} Here, we undertook a multiplatform analysis of structural genomic abnormalities and gene expression in DLBCL biopsy samples. We hypothesized that this hybrid approach might yield a nosology of DLBCL based on shared genetic pathogenesis that could aid in our understanding of treatment response and identify therapeutic vulnerabilities.

METHODS

PROCEDURES

Fresh-frozen DLBCL biopsy samples were analyzed by means of exome and transcriptome sequencing, deep amplicon resequencing of 372 genes, and DNA copy-number analysis (Fig. S1A in Supplementary Appendix 1, available with the full text of this article at NEJM.org). Most biopsies were performed before treatment (96.5%), and the biopsy samples included ABC cases (51.4%), GCB cases (28.6%), and unclassified cases (20.0%). Because most biopsy samples lacked matched normal DNA, we developed a tumor-only mutation-calling pipeline. Using sequencing data from 48 cases with matched normal DNA, we created and validated a random forest-based model of somatic mutations, which

predicted that 94% of the mutations called by our tumor-only pipeline are somatic (see the Methods section in Supplementary Appendix 1).

STATISTICAL ANALYSIS

P values relating discrete variables to each other were calculated with the use of a Fisher’s exact test. P values for survival were calculated from a Cox proportional-hazards score test. The score on the International Prognostic Index (IPI; range, 0 to 5, with higher scores indicating greater risk) was treated as a categorical variable: low (score of 0 or 1), intermediate (score of 2 or 3), or high (score of 4 or 5). P values for the differences in signature averages between DLBCL genetic subtypes were derived from Student t-tests. All P values reported are two-sided.

RESULTS

GENETIC DISTINCTIONS AMONG GENE-EXPRESSION SUBGROUPS

Multiplatform genomic analysis of 574 DLBCL biopsy samples identified genes that were altered at significantly different frequencies ($P < 0.01$) in ABC and GCB cases (Fig. 1A; and Fig. S1B in Supplementary Appendix 1 and Tables S1 through S8 in Supplementary Appendix 2, available at NEJM.org). Because the genetic composition of unclassified DLBCL is unknown, we enriched for these cases (20.0% in our cohort, as compared with 11.3% in a population-based cohort⁹). Among common genetic features in unclassified DLBCL, *NOTCH2* mutations and *BCL6* fusions significantly co-occurred ($P = 2.78 \times 10^{-12}$) and distinguished unclassified from other DLBCLs (Fig. 1B, and Fig. S1C and S1D in Supplementary Appendix 1). Unclassified cases were enriched for mutations targeting *SPEN*, an inhibitor of NOTCH-dependent gene expression, and 30.4% of cases had *NOTCH2* or *SPEN* mutations (Fig. 1B, and Fig. S1C in Supplementary Appendix 1). We additionally identified gain-of-function *NOTCH1* mutations in 19 cases (Fig. S1C in Supplementary Appendix 1). However, these were primarily in ABC cases (95%) and never co-occurred with *NOTCH2* or *SPEN* mutations, which suggests that *NOTCH1* and *NOTCH2* contribute to distinct pathogenetic pathways.

We next investigated whether genetic aberrations were correlated with the ABC–GCB predic-

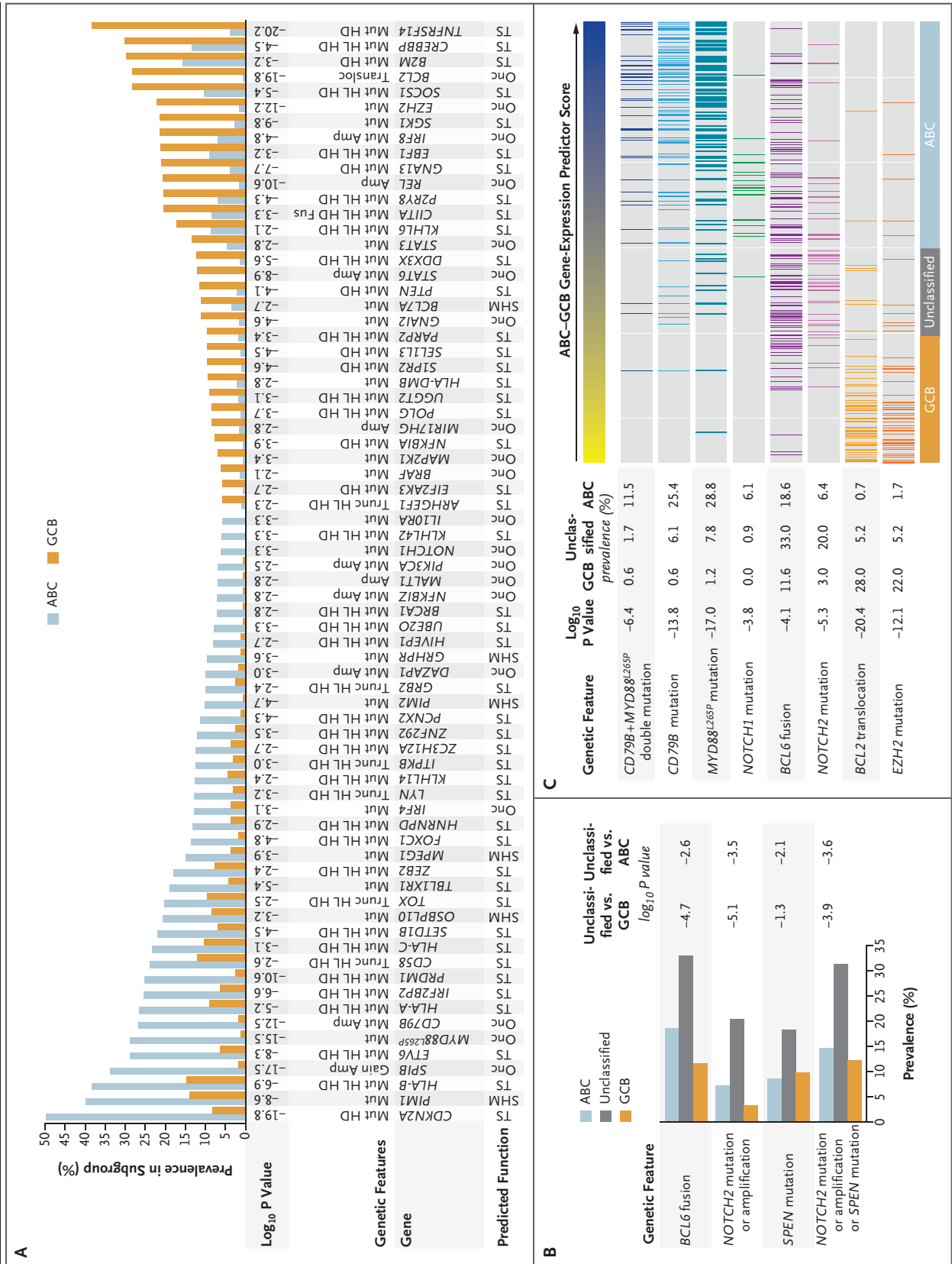


Figure 1 (facing page). Relationship between Gene-Expression Subgroups and Genetic Alterations.

Panel A shows genetic aberrations that distinguish the activated B-cell–like (ABC) and germinal-center B-cell–like (GCB) subgroups of diffuse large B-cell lymphoma (DLBCL). Shown is the prevalence of the indicated genetic abnormalities in 72 genes in ABC and GCB, along with the \log_{10} P value for the difference in prevalence between the two subgroups. Putative assignment as an oncogene (Onc), tumor suppressor (TS), or target of aberrant somatic hypermutation (SHM) is shown. Amp denotes amplification, Fus gene fusion, Gain single-copy gain, HD homozygous deletion, HL heterozygous loss, Mut mutation, Transloc translocation, and Trunc protein-truncating mutation. Panel B shows genetic lesions that are associated with unclassified DLBCL. Shown is the prevalence of the indicated genetic aberrations in the gene-expression subgroups, along with the \log_{10} P values for the differences between unclassified and either ABC or GCB. Panel C shows the correlation between genetic abnormalities and the ABC–GCB gene-expression predictor score. The ABC–GCB predictor score is a quantitative metric used to assign DLBCL cases to the indicated gene-expression subgroups. High values (blue) are on the ABC end of the spectrum, and low values (yellow) are on the GCB end of the spectrum. The \log_{10} P value of the correlation between the predictor score and the presence of the indicated aberrations is shown.

tor score, a quantitative gene-expression metric with low values for the most GCB-like cases and high values for the most ABC-like (Fig. 1C). *NOTCH2* mutations and *BCL6* fusions were concentrated in the center of this distribution, a finding consistent with their enrichment in unclassified DLBCL. In contrast, cases with *CD79B* and *MYD88^{L265P}* mutations were enriched at the far ABC end of the spectrum, with significant co-occurrence of these abnormalities ($P=2.81\times 10^{-11}$) (Fig. 1C). Although most *NOTCH1* mutant cases were ABC DLBCL, none had *CD79B* or *MYD88^{L265P}* mutations and their predictor scores were significantly lower than those of the *CD79B*–*MYD88^{L265P}* double-mutant cases ($P=0.006$) (Fig. 1C). On the GCB end of the spectrum, *EZH2* mutations and *BCL2* translocations significantly co-occurred ($P=6.39\times 10^{-14}$) and were present in cases with the lowest predictor scores (Fig. 1C). Together, these analyses suggest that the gene-expression subgroups may have distinct genetic subtypes.

A GENETIC CLASSIFIER FOR DLBCL

To identify genetic subtypes in DLBCL, we created an automated method that starts with a set of seed classes and iteratively moves cases into and

out of the classes to optimize a genetic distinctiveness metric (see the Methods section and Fig. S2 in Supplementary Appendix 1). We chose four seeds as follows: *CD79B*–*MYD88^{L265P}* double mutation, *NOTCH2* mutation or *BCL6* fusion in ABC or unclassified DLBCL, *NOTCH1* mutation, and *EZH2* mutation or *BCL2* translocation. The algorithm converged on genetic subtypes that we term MCD (71 cases, from the *MYD88^{L265P}*–*CD79B* seed), BN2 (98 cases, from the *BCL6*–*NOTCH2* seed), N1 (19 cases, from the *NOTCH1* seed), and EZB (69 cases, from the *EZH2*–*BCL2* seed). A separate algorithm that used random forest methods produced significantly overlapping genetic subtypes ($P<1\times 10^{-105}$) (see the Methods section in Supplementary Appendix 1). Among non-subtyped “other” cases, the only gene that was mutated in more than 10% of cases and was significantly enriched in this subset was *TET2* (10.5% prevalence, $P=0.03$). Thus, additional genetic subtypes were not apparent in our data set but may emerge from the study of larger DLBCL cohorts.

The MCD and N1 subtypes were dominated by ABC cases, EZB included mostly GCB cases, and BN2 had contributions from all three gene-expression subgroups (Fig. 2A). Overall, we classified 44.8% of our samples into these genetically “pure” subtypes of DLBCL and recognize that non-subtyped cases may share genetic features as well as etiologic factors with the genetic subtypes (Fig. 2B). Because we deliberately skewed our sample cohort toward ABC and unclassified cases, we modeled the expected prevalence of the genetic subtypes using a recent population-based analysis of the prevalence of ABC, GCB, and unclassified DLBCL.⁹ On the basis of the gene-expression predictor classifications of MCD, BN2, N1, and EZB cases, we estimate that these genetic subtypes would comprise 46.6% of cases (Fig. 2C).

To explore how this subtype classification might be implemented clinically, we created a subtype predictor using mutations in 50 genes and translocations of *BCL2* and *BCL6* (see the Methods section in Supplementary Appendix 1). In 10-fold cross-validation testing, the predictor was 94.8% accurate. A related predictor that included amplifications and homozygous deletions achieved 97.5% accuracy. Thus, next-generation sequencing tests for this subtype distinction would be feasible.

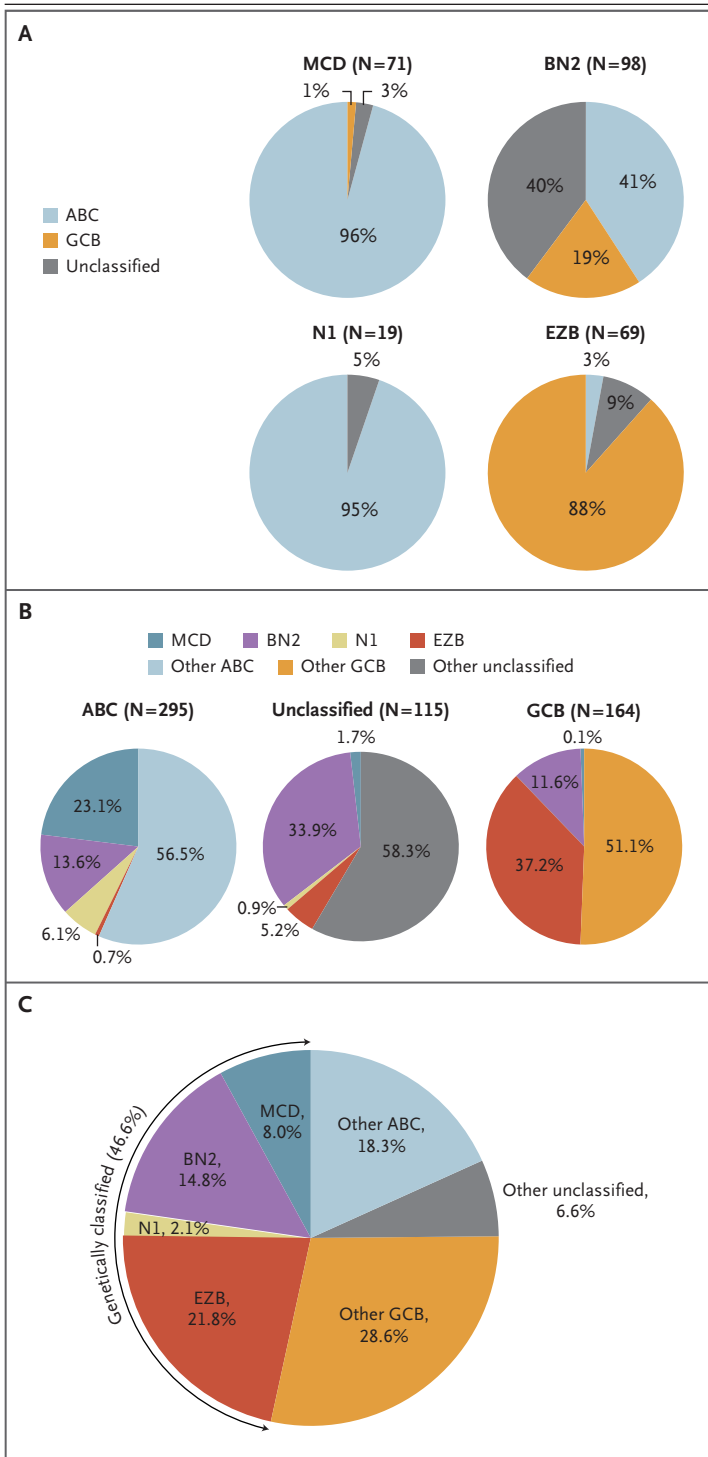


Figure 2. Genetic Aberrations That Distinguish Genetic Subtypes of DLBCL.

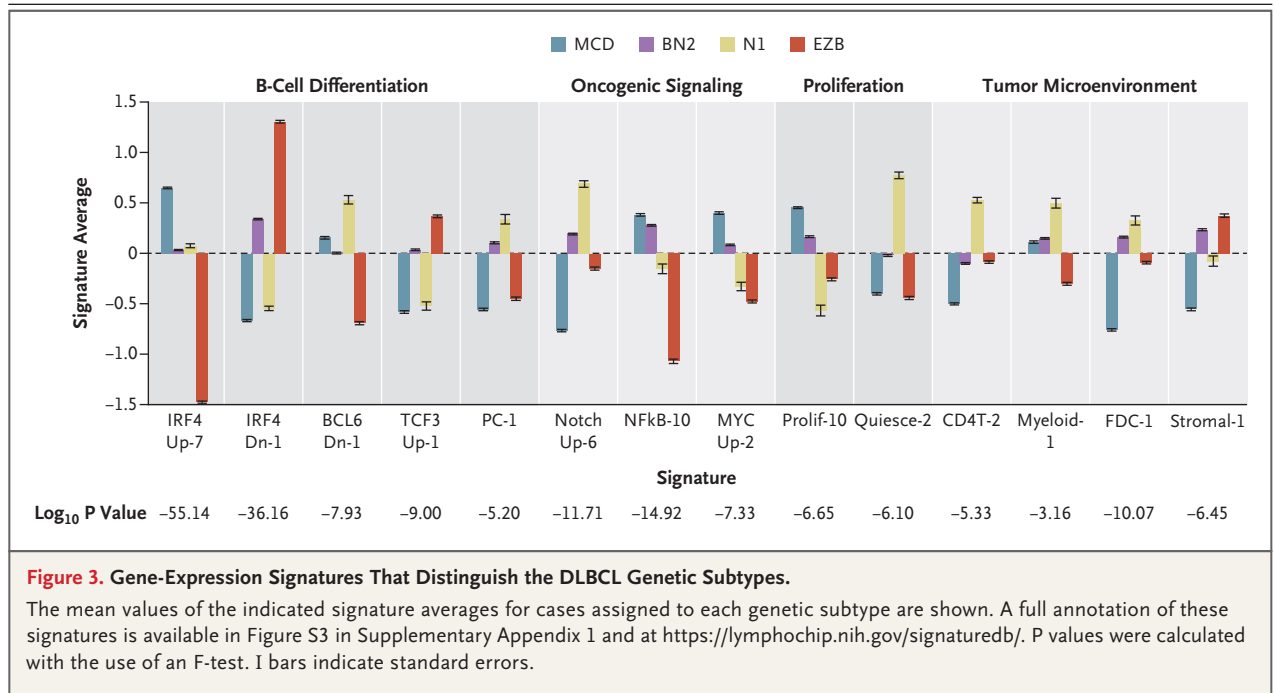
Panel A shows the distribution of gene-expression subgroups within genetic subtypes, termed MCD (based on the co-occurrence of *MYD88*^{L265P} and *CD79B* mutations), BN2 (based on *BCL6* fusions and *NOTCH2* mutations), N1 (based on *NOTCH1* mutations), and EZB (based on *EZH2* mutations and *BCL2* translocations). Panel B shows the distribution of genetic subtypes within gene-expression subgroups. In Panels A and B, the number of cases of DLBCL is shown in parentheses. Panel C shows the predicted prevalence of the indicated DLBCL subsets in a population-based cohort.⁹

with 42% bearing both abnormalities. MCD had frequent gain or amplification of *SP1B*, encoding a transcription factor that, with *IRF4*, defines the ABC phenotype and promotes plasmacytic differentiation.¹⁰ Full plasmacytic differentiation is blocked in MCD by mutations that inactivate *BLIMP1* (*PRDM1*).^{11,12} Known tumor suppressors in MCD include *CDKN2A*, *ETV6*, *BTG1*, and *BTG2*, and putative tumor suppressors include *TOX*, *SETD1B*, *FOXO1*, *TBL1XR1*, and *KLHL14*. The tumor suppressor *TP53* was mutated significantly less often in MCD than in other subtypes. Immune editing appears prominent in MCD genomes, with 76% acquiring a mutation or deletion of *HLA-A*, *HLA-B*, or *HLA-C* and 30% acquiring truncating mutations targeting *CD58*, an activator of natural killer cells.¹³

BN2 was dominated by NOTCH pathway aberrations, with 73% acquiring a *NOTCH2* mutation or amplification, *SPEN* mutation, or mutation in *DTX1*, a NOTCH target gene. Many *SPEN* mutant BN2 cases (50%) lacked *NOTCH2* aberrations, which suggests that NOTCH2 ligand-induced signaling may play a role in BN2. *BCL6* fusion, the other BN2 hallmark, occurred in 73% of cases. *BCL6* fusions were enriched in cases with *NOTCH2*, *SPEN*, or *DTX1* lesions to a significantly greater extent in BN2 than in non-BN2 cases (72% vs. 15%, $P=2.31 \times 10^{-10}$), a finding that suggests oncogenic cooperation between these pathways in BN2.

Genetic aberrations targeting regulators of the NF- κ B pathway were a prominent feature of BN2. Lesions targeting the NF- κ B negative regulator A20 (*TNFAIP3*) or its partner TNIP1 were common (55%). Two components of the B-cell receptor-dependent NF- κ B pathway, protein kinase C beta (*PRKCB*) and *BCL10*, were altered by muta-

A total of 79 genes with aberrations that characterized each genetic subtype are shown in Figure S2B in Supplementary Appendix 1. *MYD88*^{L265P} or a *CD79B* aberration (mutation or amplification) was present in 82% of MCD cases,



tions or amplifications in 47% of BN2 cases. Other likely gain-of-function events included mutations targeting cyclin D3 and CXCR5, whereas inactivating lesions targeting the immune regulator CD70 suggested immune escape.

N1 was characterized by *NOTCH1* mutations and aberrations targeting transcriptional regulators of B-cell differentiation (IRF4, ID3, and BCOR), which may contribute to its plasmacytic phenotype (see below). *TNFAIP3* mutations in N1 could reinforce this phenotype by fostering NF-κB-induced IRF4 expression.

EZB was enriched for most genetic events previously ascribed to GCB DLBCL, including *BCL2* translocation, *EZH2* mutation, and *REL* amplification, as well as inactivation of the tumor suppressors *TNFRSF14*, *CREBBP*, *EP300*, and *KMT2D*. The germinal-center homing pathway involving *S1PR2* and *GNA13*¹⁴ was disrupted in 38% of EZB cases. Janus-associated kinase–signal transducers and activators of transcription (JAK-STAT) signaling may have been promoted in 49% of cases by a *STAT6* mutation or amplification or by a mutation or deletion targeting *SOCS1*, a JAK-STAT negative regulator. Phosphatidylinositol 3 (PI3) kinase–mammalian target of rapamycin signaling may have been activated in 23% of cases by *MTOR* mutations or amplification of *MIR17HG*, encoding a microRNA targeting *PTEN*. Immune editing

may also sculpt EZB genomes since 39% acquired lesions in the major histocompatibility complex class II pathway genes *CIITA* and *HLA-DMA*.

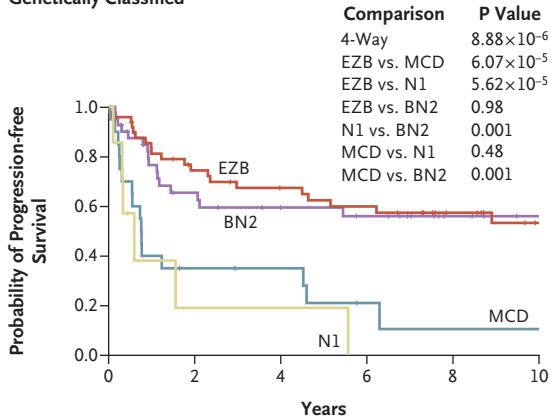
EPIGENETIC ATTRIBUTES OF THE DLBCL GENETIC SUBTYPES

We used RNA-sequencing data to explore phenotypic differences among the DLBCL genetic subtypes, using gene-expression signatures of B-cell differentiation, oncogenic signaling, and the tumor microenvironment¹⁵ (Fig. 3). MCD expressed genes that are transactivated by IRF4, a master regulator of the ABC phenotype.¹⁰ In EZB, a signature of BCL6-repressed genes was low and a signature of TCF3-activated genes was high, implying a germinal-center origin. N1 expressed a plasma-cell signature highly.

Among oncogenic signatures, NOTCH signatures were highest in BN2 and N1. Signatures of B-cell receptor–dependent NF-κB activation were highest in MCD and BN2. Genes induced by MYC were highly expressed in MCD and BN2, as were signatures of proliferation. Conversely, a signature of quiescent cells was high in N1.

With respect to the tumor microenvironment, signatures of T cells, myeloid cells, and follicular dendritic cells were highest in N1. BN2 and EZB cases expressed the Stromal-1 signature highly, which reflects a fibrotic microenviron-

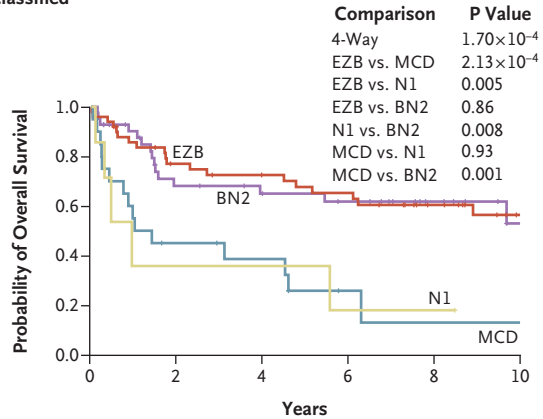
A Progression-free Survival among Patients Whose Tumors Were Genetically Classified



No. at Risk

	0	2	4	6	8	10
MCD	20	6	5	2	1	1
BN2	41	22	17	15	8	5
N1	7	1	1	0	0	0
EZB	49	32	27	24	17	11

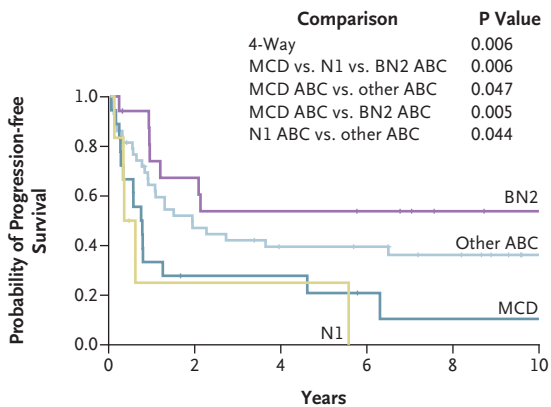
B Overall Survival among Patients Whose Tumors Were Genetically Classified



No. at Risk

	0	2	4	6	8	10
MCD	20	8	6	2	1	1
BN2	42	24	20	18	11	5
N1	7	2	2	1	1	0
EZB	50	34	30	27	19	11

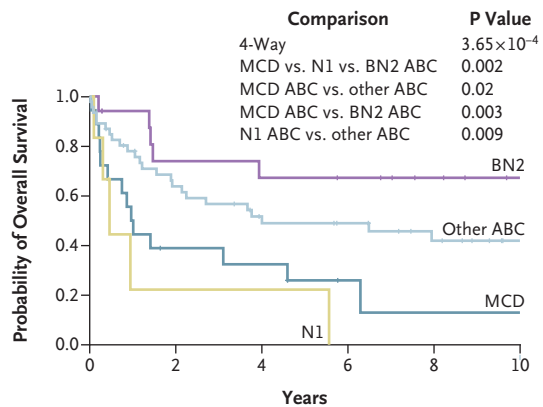
C Progression-free Survival among Patients with ABC Tumors



No. at Risk

	0	2	4	6	8	10
MCD	18	4	4	2	1	1
BN2	17	10	8	7	4	3
N1	6	1	1	0	0	0
Other ABC	43	19	14	13	10	4

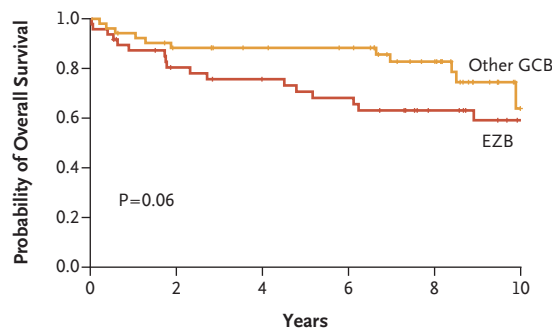
D Overall Survival among Patients with ABC Tumors



No. at Risk

	0	2	4	6	8	10
MCD	18	6	5	2	1	1
BN2	17	11	10	9	6	3
N1	6	1	1	0	0	0
Other ABC	46	27	19	16	11	5

E Overall Survival among Patients with GCB Tumors



No. at Risk

	0	2	4	6	8	10
EZB	47	33	29	26	18	11
Other GCB	52	43	39	37	26	6

Figure 4 (facing page). Relationship between DLBCL Genetic Subtypes and Survival after R-CHOP Chemotherapy.

Panels A and B show Kaplan–Meier models of progression-free survival and overall survival, respectively, according to DLBCL genetic subtype. Panels C and D show Kaplan–Meier models of progression-free survival and overall survival, respectively, among patients with ABC DLBCL according to genetic subtype and including patients with non-subtyped ABC cases as “other ABC.” Panel E shows a Kaplan–Meier model of overall survival among patients with GCB DLBCL cases belonging to the EZB subtype or not (“other GCB”). R-CHOP denotes rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

ment that is associated with favorable survival in DLBCL after immunochemotherapy.³

CLINICAL ATTRIBUTES OF THE DLBCL GENETIC SUBTYPES

For the survival analysis, we selected all untreated patients with outcome data who received immunochemotherapy (R-CHOP or CHOP-like chemotherapy; 240 patients), including 119 patients whose tumors were classified into one of the genetic subtypes. Our genetic algorithm, which did not use clinical information, was locked down before the analysis of clinical data, allowing us to analyze the relationship between genetic subtypes and survival in this entire cohort. The four subtypes differed significantly in progression-free survival ($P=8.88\times 10^{-6}$) and overall survival ($P=1.70\times 10^{-4}$), with the BN2 and EZB subtypes having much more favorable outcomes than the MCD and N1 subtypes (Fig. 4A and 4B). The predicted 5-year overall survival rates for the MCD, N1, BN2, and EZB subtypes were 26%, 36%, 65%, and 68%, respectively.

Within ABC DLBCL, the MCD, N1, and BN2 genetic subtypes had distinct progression-free survival ($P=0.006$) and overall survival ($P=0.002$) (Fig. 4C and 4D). Patients with MCD had significantly inferior survival as compared with those with BN2, and patients with either MCD or N1 had significantly inferior survival as compared with patients with ABC tumors that were not genetically classified (Fig. 4C and 4D). Within GCB DLBCL, there was a trend toward inferior overall survival among patients with EZB as compared with patients with other GCB tumors ($P=0.06$) (Fig. 4E). The distinction regarding the gene-expression profiling subgroup and the dis-

inction regarding the genetic subtype contributed independently to survival in a multivariate analysis: gene-expression profiling added significantly to a genetic-subtype model ($P=7.91\times 10^{-7}$), and genetic subtype added significantly to a gene-expression profiling model ($P=4.16\times 10^{-4}$).

The IPI score did not vary significantly among the genetic subtypes (Tables S9 and S10 in Supplementary Appendix 2). However, with respect to individual IPI components, patients with EZB tumors had significantly better performance status (greater prevalence of an Eastern Cooperative Oncology Group performance status >1 ; $P=0.001$). A trend toward increased extranodal involvement (>1 site) was a feature of MCD (28% prevalence, as compared with 16% in others; $P=0.051$) and potentially N1 (38%; $P=0.06$). The degree of extranodal involvement in MCD is of interest, given the frequent *CD79B* and *MYD88*^{L265P} mutations in these tumors, which are cardinal features of extranodal lymphomas such as primary central nervous system lymphoma.¹⁶ Moreover, several other genes that were characteristically mutated in MCD tumors are recurrently mutated in primary central nervous system lymphoma (Fig. S4 in Supplementary Appendix 1).

The IPI score was associated with progression-free survival ($P=1.51\times 10^{-6}$) and overall survival ($P=6.05\times 10^{-5}$), as expected. The genetic subtype distinction added significantly to the IPI model of progression-free survival ($P=5.60\times 10^{-4}$) and overall survival ($P=0.001$).

ONCOGENIC PATHWAYS

To gain insight into potential therapeutic strategies for the genetic subtypes of DLBCL, we considered groups of genetic aberrations that target oncogenic signaling pathways (Fig. 5). First, we considered the regulation of proximal B-cell receptor signaling, both positively by mutation or amplification of *CD79B* and *CD79A* (“CD79A/B”) and negatively by known inhibitors of B-cell receptor signaling in normal B cells, including *LAPTM5*,¹⁷ *LYN*,¹⁸ *PTPN6*,¹⁸ *GRB2*,¹⁹ *PRKCD*,²⁰ *DGKZ*,²¹ *SLA*,²² and *MAP4K1*²³ (Fig. 5, and Fig. S5A in Supplementary Appendix 1). Potential loss-of-function lesions targeting negative regulators of B-cell receptor signaling (“BCRnegreg”) were present in 38.5% of cases. They were more prevalent in CD79A/B cases with *MYD88*^{L265P} (56%) than in cases lacking these aberrations (36.4%) ($P=0.02$). This is notable given evidence

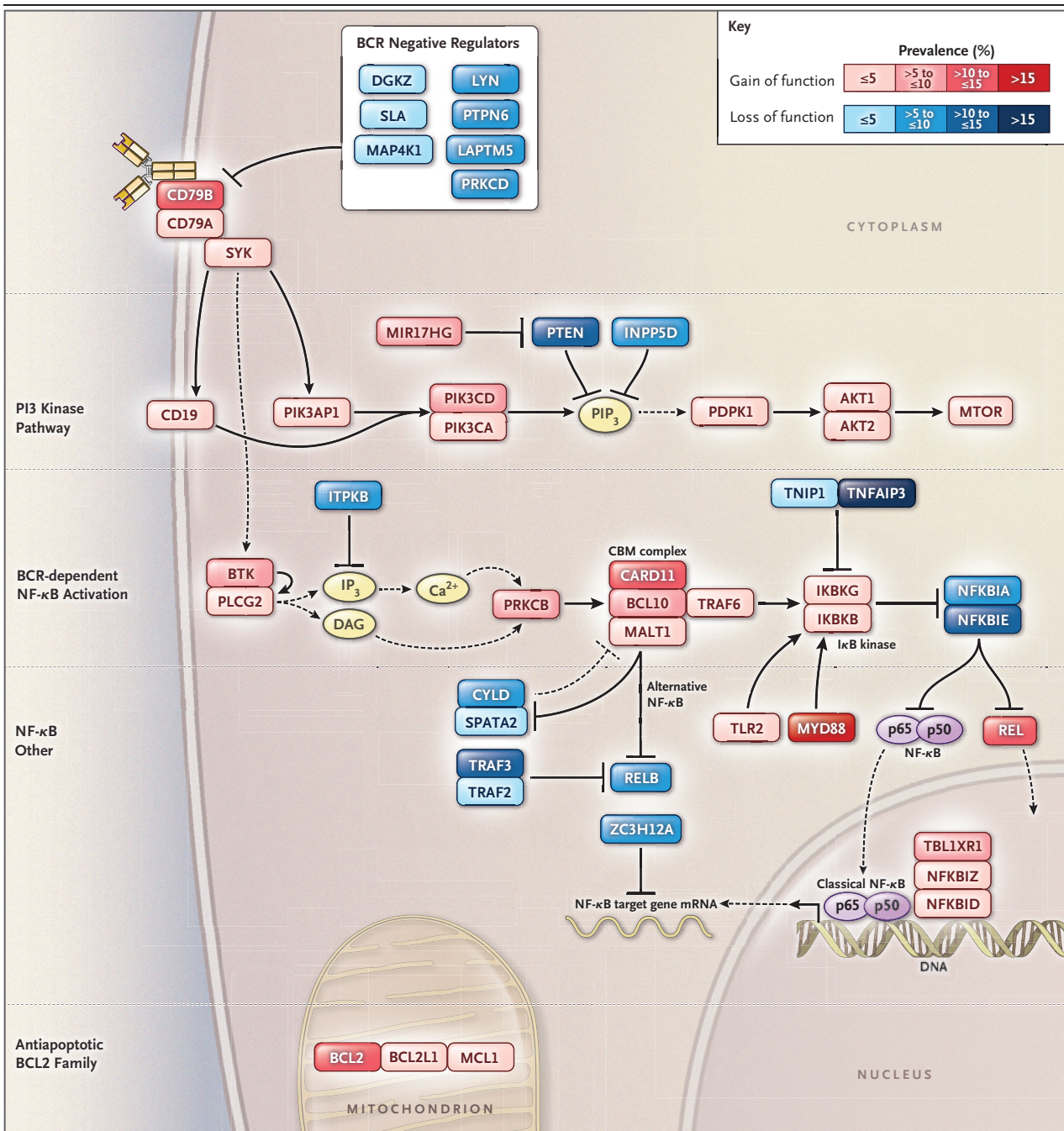


Figure 5. Genetic Aberrations Targeting Oncogenic Signaling Pathways in DLBCL.

Shown is the prevalence of putative gain-of-function or loss-of-function genetic aberrations targeting genes in each indicated oncogenic signaling category. The prevalence of genetic aberrations is indicated by the color scale shown. Genetic aberrations included for each gene are indicated in Figure S5B in Supplementary Appendix 1. BCR denotes B-cell receptor, CBM complex CARD11–BCL10–MALT1 signaling adaptor complex, mRNA messenger RNA, and NF-κB nuclear factor κB.

that aggressive lymphomas with both MYD88^{L265P} and CD79B mutations frequently have a response to ibrutinib and presumably have “chronic active” B-cell receptor signaling.^{4,16,24}

Signaling enzymes and adaptors that promote B-cell receptor–dependent NF-κB activation (“BCR–NF-κB”) were genetically aberrant in 44.9% of cases (Fig. 5, and Fig. S5A and S5B in Supple-

mentary Appendix 1). This B-cell receptor signaling cascade activates I κ B kinase (IKK), which was altered in 8.5% of cases. IKK is negatively regulated by A20 and its partner TNIP1, which were targeted by mutations and deletions in 25.8% of cases. A variety of additional genetic events in 66.2% of cases affected other NF- κ B regulators, such as TLR2 and regnase-1 (*ZC3H12A*), which negatively regulates the stability of NF- κ B-dependent messenger RNA (mRNA).²⁵ The PI3 kinase pathway, which can indirectly activate NF- κ B,²⁶ was genetically altered in 34.3% of cases.

Among the genetic subtypes, BCRnegreg aberrations were most frequent in MCD and least frequent in EZB, but these aberrations occurred in all genetic subtypes, suggesting that constitutive B-cell receptor signaling is a pervasive aspect of DLBCL pathogenesis (Fig. S5B in Supplementary Appendix 1). In addition to BCRnegreg alterations, MCD frequently acquired CD79A/B aberrations and thus was significantly enriched for lesions targeting B-cell receptor proximal signaling (in 75% of cases; $P=3.72\times 10^{-6}$). BN2 was notably enriched for BCR-NF- κ B aberrations (in 66% of cases; $P=3.81\times 10^{-6}$) and IKK regulator aberrations (in 56% of cases; $P=8.34\times 10^{-11}$). These findings support the hypothesis that B-cell receptor-dependent NF- κ B activation is a recurrent feature of MCD and BN2 tumors.

In addition to NF- κ B, survival of DLBCL cells is promoted by antiapoptotic BCL2 family members (*BCL2*, *BCL-X_L* [*BCL2L1*], and *MCL1*), which were targeted by genomic amplification or translocation in 17.4% of cases (Fig. 5, and Fig. S5A and S5B in Supplementary Appendix 1). As expected, *BCL2* mRNA levels were significantly higher in EZB tumors with *BCL2* translocations than in other EZB tumors ($P=0.004$) (Fig. S5C in Supplementary Appendix 1). MCD tumors also had high *BCL2* mRNA expression as compared with other cases ($P=0.009$) (Fig. S5C in Supplementary Appendix 1), a finding that potentially explains why MCD was significantly lacking in amplifications and translocations of antiapoptotic BCL2 family members ($P=1.60\times 10^{-4}$) (Fig. S5A in Supplementary Appendix 1).

DISCUSSION

The genetic framework for DLBCL that we present here provides a new and evolving understanding of the pathogenesis of DLBCL and the molecular attributes that may influence therapeutic

response. Unlike previous genetic investigations that catalogued individual genetic aberrations, our study identified genetic subtypes of DLBCL that differ from one another by many recurrent genetic aberrations. There are two overarching implications of these findings. First, it seems likely that these genetic subtypes have distinct evolutionary histories driven by the stage of B-cell differentiation from which they arise or by their initiating genetic events (or both), which may influence the subsequent acquisition of cooperating genetic lesions. Second, the genetic subtypes had distinct outcomes after immunotherapy and could affect the selection of targeted therapies owing to their distinct oncogenic abnormalities.

The MCD genetic subtype was enriched for many of the genetic changes that have been ascribed previously to the ABC subgroup of DLBCL. MCD included cases with both *MYD88*^{L265P} and *CD79B* mutations, a genotype that has been associated with a response to ibrutinib in relapsed or refractory ABC DLBCL⁴ and is common in primary central nervous system lymphoma, which has an ABC phenotype and which often has a response to ibrutinib.^{16,24} Moreover, MCD tumors had extensive extranodal involvement and acquired mutations in genes that are recurrently mutated in primary extranodal lymphomas. Together, these observations suggest that the pathogenesis of nodal MCD DLBCL is related to that of primary extranodal lymphomas.

The BN2 subtype sheds light on unclassified DLBCL, a previously obscure gene-expression subgroup, and is predicted to rely on B-cell receptor-dependent NF- κ B activation and to be responsive to antagonists of B-cell receptor signaling such as the Bruton's tyrosine kinase (BTK) inhibitors. *NOTCH2* mutations link BN2 to marginal-zone lymphoma, which is also responsive to ibrutinib.²⁷⁻²⁹ *BCL6* fusions, the second defining feature of BN2, are common in transformed marginal-zone lymphoma,³⁰ raising the possibility that BN2 arises from an occult marginal-zone lymphoma. N1 differs from BN2 genetically, phenotypically, and clinically, despite functional similarities between *NOTCH1* and *NOTCH2*, suggesting a distinct pathogenesis. Finally, most of the genetic lesions previously associated with GCB DLBCL were concentrated in the EZB subtype, reflecting a shared genetic pathogenesis and distinguishing them from other GCB tumors.

In our cohort, these genetic subtypes differed in their outcomes after immunochemotherapy, which were favorable in BN2 and EZB and poorer in MCD and N1. Moreover, within ABC DLBCL, heterogeneity in clinical outcome can be traced, in part, to genetic heterogeneity, with inferior survival in the MCD and N1 subtypes and favorable survival in the BN2 subtype. Hence, our findings suggest that clinical trials involving patients with ABC DLBCL who received R-CHOP-like chemotherapy should be interpreted in the light of these genetic distinctions. Our survival analysis tested a single hypothesis on the basis of a locked-down genetic-subtype algorithm that did not use the clinical data. Nonetheless, evaluation of the relationship between these genetic subtypes and treatment response in additional cohorts will be important to confirm and extend these findings.

The results of our studies suggest that, in clinical trials, targeted agents in DLBCL could be evaluated in the context of particular genetic subtypes or genetic aberrations that affect the targeted pathway. For example, drugs that target B-cell receptor–dependent NF- κ B activation (e.g., inhibitors of BTK and protein kinase C beta) could be investigated in BN2 and MCD, given their enrichment for genetic aberrations that should activate or augment this signaling. Clinical trials evaluating inhibitors of B-cell receptor proximal signaling (e.g., spleen tyrosine kinase [SYK] inhibitors) or the downstream PI3 kinase pathway could investigate whether response is correlated with lesions that alter negative regulators of B-cell receptor signaling or the B-cell receptor subunits CD79A and CD79B. Differential BCL2 expression could be considered in the

assessment of response to BCL2 inhibitors. Finally, immune-checkpoint inhibitors could be studied in the N1 subtype, given its prominent T-cell gene-expression signature and poor response to R-CHOP.

In summary, our multiplatform genomic analysis builds on the gene-expression classification of DLBCL by the addition of a genetic nosology that may inform DLBCL pathogenesis. Our analysis uncovered an interrelationship between this genetic nosology and oncogenic signaling pathways, suggesting testable therapeutic interventions. From the perspective of precision medicine, selecting treatment for DLBCL on the basis of individual genetic alterations is not optimal since it is likely that constellations of genetic aberrations influence therapeutic response. The genetic subtypes that we define may provide a conceptual edifice on which to develop precision therapies for these aggressive cancers.

Supported by the Intramural Research Program of the National Institutes of Health (NIH), the Center for Cancer Research of the National Cancer Institute (NCI), and an NCI Strategic Partnering to Evaluate Cancer Signatures grant (5U01CA157581-05). Dr. Schmitz was supported by the Dr. Mildred Scheel Stiftung für Krebsforschung (Deutsche Krebshilfe). Dr. Kasbekar was supported by the NIH Oxford–Cambridge Scholars Program and the Washington University in St. Louis Medical Scientist Training Program. Dr. Hodson was a Kay Kendall Leukaemia Fund intermediate research fellow.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Kathleen Calzone, Ina Felau, Bill Wysocki, Michael Fitzsimons, and Wendy Wu for help with Database of Genotypes and Phenotypes and NCI Genomic Data Commons submission; Arati Raziuddin, Xiaolin Wu, and Nina Bubunenka (Frederick National Laboratory for Cancer Research, Leidos Biomedical Research) for help with exome library preparation and NanoString analysis; Norman Gerry (Advanced BioMedical Laboratories) for processing data from the Affymetrix Genome-Wide Human SNP Array 6.0 platform; Michele Ceribelli and Hee Min Yoo for helpful discussions; and Arlene Dyer for administrative assistance.

APPENDIX

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From the Lymphoid Malignancies Branch (R.S., D.W.H., J.D.P., J.Q.W., S.R., M.K., R.M.Y., A.L.S., D.J.H., W. Xiao, X.Y., Y.Y., H.Z., W. Xu, W.H.W., L.M.S.), the Biometric Research Program, Division of Cancer Diagnosis and Treatment (G.W.W.), and the Laboratory of Pathology, Center for Cancer Research (E.S.J., S.P.), National Cancer Institute, and the Office of Intramural Research, Center for Information Technology (C.A.J., X.L., B.Z., W.D.), National Institutes of Health, Bethesda, and the Cancer Research Technology Program, Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Frederick (B.T., J.S., Y.Z., D.R.S.) — all in Maryland; the Department of Pathology, City of Hope National Medical Center, Duarte, CA (W.C.C.); the BC Cancer Agency, Vancouver, BC (R.D.G., J.M.C.), and the University Health Network, Laboratory Medicine Program, Toronto General Hospital and University of Toronto, Toronto (J.D.) — both in Canada; the Hospital Clinic of Barcelona, University of Barcelona, Institute for Biomedical Research

August Pi I Sunyer, Barcelona (E.C., A.L.-G.); the Institute of Pathology, University of Würzburg, and Comprehensive Cancer Center Mainfranken, Würzburg (A.R.), and the Department of Clinical Pathology, Robert-Bosch-Krankenhaus, and Dr. Margarete Fischer-Bosch Institute for Clinical Pharmacology, Stuttgart (G.O.) — all in Germany; the Department of Laboratory Medicine and Pathology, Mayo Clinic, Scottsdale, AZ (L.M.R.); the National Cancer Centre of Singapore, Singapore (K.T.K.W.); Memorial Sloan Kettering Cancer Center (A.D.Z.) and Weill Cornell Medicine (J.P.L.) — both in New York; the Department of Medicine, Washington University School of Medicine, St. Louis (N.L.B.); and the Alliance for Clinical Trials in Oncology, Chicago (A.D.Z., J.P.L., N.L.B.).

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