Clinical Cancer Research

MYD88 L265P Mutations, But No Other Variants, Identify a Subpopulation of DLBCL Patients of Activated B-cell Origin, Extranodal Involvement, and Poor Outcome

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Abstract

Purpose: Mutations in *MYD88* are found in different lymphoproliferative disorders associated with particular biologic characteristics and clinical impact. The aim of this study was to analyze the incidence of *MYD88* mutations and its clinical impact in diffuse large B-cell lymphoma (DLBCL).

Experimental Design: The incidence, clinicobiological features, and outcome of 213 patients (115 M/98 F; median age, 65 years) with DLBCL treated with immunochemotherapy in a single institution according to *MYD88* mutational status as assessed by an allele-specific PCR assay were analyzed. The cell of origin (COO) was determined in 129 cases by gene expression.

Results: *MYD88* mutations were found in 47 cases (22%), including L265P in 39 and S219C and M232F in 4 cases, respectively. Patients with *MYD88* L265P were older, presenting frequent extranodal involvement, and mostly corresponded to activated B-cell like (ABC) subtype, whereas no preference in COO was observed in patients with other *MYD88* mutations. Five-year overall survival (OS) for *MYD88* wild-type, *MYD88* L265P, and other variants was 62%, 52%, and 75%, respectively ($P = 0.05$). International Prognostic Index (IPI) (HR, 2.71; $P < 0.001$) and *MYD88* L265P (HR, 1.786; $P = 0.023$) were independent variables predicting OS in the multivariate analysis. However, *MYD88* L265P lost its independent value when COO was included in the model.

Conclusions: Our findings indicate that *MYD88* L265P mutations, but no other variants, identify a subgroup of DLBCL mainly of ABC origin, with extranodal involvement and poor outcome. *Clin Cancer Res; 22(11); 2755–64. ©2016 AACR.*

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease in terms of morphology, immunophenotyping, gene expression profile (GEP), oncogenic aberrations, clinical presentation, and outcome (1–3). Two major subtypes related to the different cell of origin (COO) of the tumor have been distinguished according to GEP: germinal center B-cell like (GCB) and activated B-cell like (ABC). Each lymphoma subtype bears a phenotypic resemblance to B cells at a particular stage of differentiation (known as COO). The germinal center B cell is the normal counterpart of GCB subtype, whereas ABC subtype resembles postgerminal center plasmablasts (1, 4, 5). In addition, they are associated with distinct genetic lesions and oncogenic pathways in tumor development (2, 6, 7). GEP studies have shown that the unfavorable prognosis of ABC DLBCL subtype most likely relies on the constitutive activation of NF-κB transcription complex-blocking apoptosis (7–11). A variety of signaling pathways are capable of inducing NF-κB transcription complex, including B-cell receptor (BCR), CD40, and Toll-like receptor (TLR; refs. 8–14). Oncogenic *CARD11*, *CD79A/B*, and *TNFAIP3* (also known as *A20)* mutations activate NF-κB by BCR signaling. Inactivation of *TNFAIP3* can activate NF-κB downstream of other pathways besides BCR signaling. Moreover, myeloid differentiation primary response gene 88 (*MYD88)* mutations may activate this pathway through TLR signaling (8–11, 15).

MYD88 is an adaptor protein of the Toll-like and IL1 receptors that participates in the innate immune response and plays a crucial role in the homeostasis of human B cells (16). After TLR's activation, MYD88 phosphorylates and subsequently recruits IL1R-associated kinases (IRAK) and other downstream proteins, such as TRAF6, resulting in NF-κB, JAK kinase/STAT3 activation, and secretion of IL6, IL10, and IFNβ. Ngo and colleagues described that about 39% of ABC DLBCLs had *MYD88* mutations, whereas they were rare in GCB cases (11). *MYD88* has also been recurrently found mutated at different frequencies in several lymphoid neoplasms, including the majority of Waldenström macroglobulinemias (90%), in 69% of primary cutaneous leg-type DLBCL, in 38% to 50% of central nervous system

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doi: 10.1158/1078-0432.CCR-15-1525

Translational Relevance

Diffuse large B-cell lymphoma (DLBCL) includes at least two different subtypes according to cell of origin (COO): germinal center and postgerminal/activated (ABC), with the latter showing poorer outcome probably due to constitutive NF-κB antiapoptotic pathway activation. *MYD88* mutations were first described in 39% of DLBCL, basically restricted to ABC subtype as gain-of-function mutations resulting with increased NF-κB activation. In the present study, we show that *MYD88* L265P mutations are seen mostly, but not exclusively, in ABC subtype, whereas other variants do not present COO preference. In addition, the poor prognosis of *MYD88* mutations is restricted to L265P but not to other mutations that show even a better outcome than wild-type *MYD88*. However, the clinical impact of *MYD88* L265P was not independent from COO. In conclusion, knowledge of *MYD88* mutational status could be useful in the management of DLBCL patients with the future introduction of drugs targeting the TLR/*MYD88* pathway.

lymphomas, in 9% of MALT lymphomas, and in approximately 3% of patients with chronic lymphocytic leukemia (CLL; refs. 11, 17–35). *MYD88* L265P mutation is the most frequent and most oncogenic form in DLBCL, although a gain of function triggering an increased production of chemokines has also been demonstrated for other variants (11, 34, 36).

The clinical impact and prognostic value of *MYD88* mutations are variable among different lymphoproliferative disorders. Most likely, this is due to the fact that the different cellular pathways affected by *MYD88* mutation have different relevance for cell survival according to the stage of B-cell maturation, the genetic/epigenetic mechanisms involved in each type of tumor, and the tumor cell interaction with the microenvironment. Thus, CLL patients with mutated *MYD88* show a favorable prognosis (35). In DLBCL, *MYD88* mutations have been related to specific extranodal sites (particularly the so-called immune-privileged territories) and might be associated with unfavorable outcome (37). However, very few data are available in patients treated in the rituximab era with information on gene expression-COO and the different mutational variants of *MYD88*. The aim of the present study was to assess the *MYD88* mutational status in a series of patients with *de novo* DLBCL homogeneously treated with immunochemotherapy in a single institution in order to determine its relationship with initial clinical and biologic features, including COO, as well as its impact on response to therapy and survival.

Patients and Methods

Patients' selection and characteristics

Two-hundred thirteen patients (115 M/98 F; median age, 65 years), consecutively diagnosed with DLBCL according to the World Health Organization (WHO) classification (3) in a single institution between 2002 and 2012, were selected for the current study. Availability of the histologic material was the only criterion of inclusion. Patients with a recognized disease phase of follicular lymphoma or another type of indolent lymphoma with subsequent transformation into a DLBCL, as well as those with immunodeficiency-associated tumors, posttransplant lymphoproliferative disorders, and with intravascular, primary central nervous system, primary effusion, or primary mediastinum lymphomas were excluded. Staging procedures included patient history and physical examination; performance status according to the Eastern Cooperative Oncology Group (ECOG) scale; presence of B symptoms (fever, night sweats, weight loss) and bulky disease (defined as a tumor diameter >7 cm); blood cell counts; serum biochemistry, including lactate dehydrogenase (LDH) and β2-microglobulin (β2m) levels; chest, abdomen, and pelvis computerized tomography scans; PET/CT and bone marrow biopsy. Posttherapy restaging consisted of a repetition of the abnormal previous tests and/or biopsies. Response was assessed according to conventional criteria (38).

Main clinicobiological variables were recorded and analyzed according to *MYD88* mutational status. In Table 1, the main characteristics of patients are listed. All patients were treated in the rituximab era, although in 16 cases, rituximab was discontinued mostly due to drug intolerance. Rituximab associated

Table 1. Clinicobiological features of 213 patients with diffuse large B-cell lymphoma

	N (%)
Patients	213 (100)
Gender	
Male	115 (54)
Female	98 (46)
Age	
Median (range)	65 (17–91)
≤ 60 years	89 (42)
Gene rearrangements	
BCL2 rearranged	24/129 (19)
BCL6 rearranged	26/120 (22)
MYC rearranged	12/129 (9)
MALT1 rearranged	1/85 (1)
IHC	
BCL2 expression	113/169 (67)
BCL6 expression	132/191 (69)
MUM1 expression	84/175 (48)
MYC expression	45/94 (48)
MYC (+)/BCL2 (+) coexpression	25/71 (35)
Ki67 index ≥80%	100/166 (60)
COO ($N = 129$)	
GCB	61 (47)
ABC	54 (42)
Unclassified	14 (11)
IHC Hans' algorithm ($N = 186$)	
GCB	96 (52)
Non-GCB	90 (48)
B symptoms	88 (43)
ECOG performance status ≥2	72 (35)
Bulky disease (>7 cm)	55 (27)
Primary extranodal	52 (25)
Extranodal involvement	113 (54)
Bone marrow (+)	32 (16)
Ann Arbor stage III–IV	116 (56)
High serum LDH	91 (46)
High serum β2m	103 (57)
International prognostic index	
Low risk	65 (32)
Low intermediate risk	49 (24)
High intermediate risk	47 (23)
High	42 (21)
Response to therapy	
Complete response	151 (72)
Partial response	19 (9)
No response	40 (19)

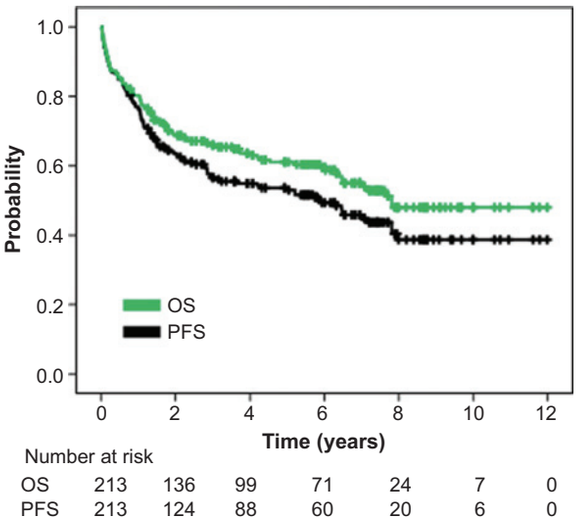

Figure 1.
PFS and OS of 213 patients with DLBCL.

with cyclophosphamide, adriamycin, vincristine, and prednisone (R-CHOP) was administered to most patients ($N = 183$, 87%). Response to treatment was as follows: complete response (CR), 151 cases (72%); partial response (PR), 19 (9%); and stable disease or progression, 40 (19%). The median follow-up for surviving patients was 6.0 years (range, 0.78–12). Five-year progression-free survival (PFS) of the series was 53% [95% confidence interval (CI), 46%–60%]. Ninety-one patients eventually died during the follow-up, with a 5-year overall survival (OS) of 61% (95% CI, 54%–68%). PFS and OS curves are depicted in Fig. 1. The study was performed according to the guidelines of the Ethic committee of Hospital Clínic of Barcelona. Informed consent to use both clinical data and histologic material was obtained in accordance with the Declaration of Helsinki.

Tissue microarray construction, histologic review, immunohistochemistry, and FISH studies

The diagnosis of DLBCL was based on the WHO classification criteria (3). We assessed the proportion of tumor cells in all cases in order to guarantee a minimum of 40% of tumor presence. Tissue microarrays were constructed using a tissue arrayer (MTA I; Beecher Instruments) and included two 1-mm representative cores of each case. Standard methods for tissue fixation (10% buffered formalin) and processing were used. The panel of antibodies included common B- and T-cell markers, as well as CD10 (clone 56C6), MUM1/IRF4 (clone MUM1p), BCL2 (clone 124), and Ki-67 (clone MIB-1) all from Dako, BCL6, kindly provided by Dr. Roncador (Centro Nacional de Investigaciones Oncologicas, Madrid, Spain), and MYC (clone Y69; Epitomics). The conditions for all these antibodies and their evaluation were as previously described and followed the guidelines recommended by the Lunenburg Lymphoma Biomarker Consortium (39, 40). The thresholds herein used for dichotomizing protein expression were based on these references and were as follows: MYC $\geq$40%; BCL2 $\geq$50%; BCL6 $\geq$50%; and Ki67 index $\geq$80%. Algorithm of Hans was used to assess COO by immunohistochemistry (IHC) in 186 cases (41).

Split FISH for *BCL2*, *BCL6*, *MALT1*, and *MYC* was performed using probes and a FISH accessory kit following the manufacturer's recommendations (DAKO). The cut-off values for the interphase FISH analyses were established following Ventura's criteria (42). Tonsil sections were used as controls, and for each sample, 100 to 200 evaluable nuclei with complete FISH signals were scored. For the break-apart probes, the cut-off values were 3% for the detection of the rearrangements and 6% to detect gains. For the dual fusion probe, a cutoff of 11% for both structural and numerical abnormalities was considered (43).

DNA extraction and assessment of *MYD88* mutations

DNA was isolated from frozen and paraffin tissue sections with the QIAamp DNA mini Kit (Qiagen) according to the manufacturer's instructions. Screening for the most frequent *MYD88* mutations (L265P, M232T, S219C, V217F, and S222R) was performed using an allele-specific PCR assay achieved by Amplification Refractory Mutation System (ARMS) technology (qBiomarker Somatic PCR Assay; Qiagen). This technique is based on the discrimination by *Taq*polymerase between a match and a mismatch at the 3' end of a PCR primer. The method allows reproducible detection of *MYD88* mutations in up to 1% of tumor DNA diluted in wild-type DNA. Cutoff for considering a case as mutated was Ct> 37. In *MYD88* L265P cases, a relative quantitation analysis was done using the DLBCL ABC OCI Ly3 cell line as a calibrator.

COO assessment by gene expression–based assay

COO by gene expression (GE-COO) could be determined in 129 samples. In 60 cases, gene expression was assessed by using Affymetrix HG U133 plus 2.0 gene expression arrays as previously described (5, 10, 44). All samples predicted as ABC DLBCL at higher than 90% were called ABC DLBCL. The samples that showed less than 10% of probability of being called ABC DLBCL were classified as GCB DLBCL. All the other cases were considered unclassified DLBCL. In addition, other 69 tumors were classified by a NanoString-based technology (45).

Statistical methods

Differences in the clinicobiological features between patients with mutated or unmutated *MYD88* were analyzed by using χ^2 test, Student *t* test, or nonparametric tests when necessary. The actuarial survival analysis was performed by the Kaplan and Meier method and differences assessed by the log-rank test. To evaluate the prognostic impact of different variables, including *MYD88* mutational status in PFS and OS, a multivariate analysis was performed with the stepwise proportional hazards model (Cox model; ref. 46).The replicability of results of the Cox analyses was assessed by bootstrap resampling. One thousand samples were built through random extractions with reposition, so that in each sample, a given patient may either not be represented at all or represented once, twice, or more times. The parameters assessed by resampling were the *P* value of HR in the Cox regression. Bootstrap resampling allows verifying that the predictive value of *MYD88* was not critically dependent on the particular composition of the present series. All tests were two-tailed, and *P* values <0.05 were considered statistically significant.

Results

MYD88 mutations

MYD88 mutations were found in 47 (22%) cases. The most frequent mutation was L265P detected in 39 cases (83% of mutated

Table 2. COO and other biologic features of 213 patients with DLBCL according to *MYD88* mutational status

	MYD88 **wild-type; *N* (%)**	***MYD88*** **L265P; *N* (%)**	***MYD88*** **other mutations, *N* (%)**
Patients	166	39	8
COO ($N = 129$)			
GCB	53 (55)	4 (17)	4 (50)
ABC	32 (33)	18 (75)[a]	4 (50)
Unclassified	12 (12)	2 (8)	0
IHC Hans' algorithm ($N = 186$)			
GCB	80 (55)	10 (30)	6 (75)
Non-GCB	65 (45)	23(70)[a]	2 (25)
MYC rearrangement ($N = 129$)	11 (6.6)	1 (2.6)	0
BCL2 rearrangement ($N = 129$)	21 (21)	2 (8)[b]	1 (17)
BCL6 rearrangement ($N = 120$)	20 (21)	4 (18)	2 (40)
BCL2 expression ($N = 169$)	84 (66)	26 (74)	3 (43)
BCL6 expression ($N = 191$)	98 (68)	27 (71)	7 (87.5)
MUM1 expression ($N = 175$)	62 (47)	20 (57)	2 (29)
MYC expression ($N = 94$)	21 (29)	4 (22)	0
MYC/BCL2 coexpression ($N = 71$)			
MYC (−)/BCL2 (−)	19 (35)	3 (19)	0
MYC (+)/BCL2 (−) or MYC (−)/BCL2 (+)	13 (24)	10 (62)	1 (100)
MYC (+)/BCL2 (+)	22 (41)	3 (19)[a]	0
Ki67 index ≥80% ($N = 166$)	77 (60)	22 (73)[a]	1 (12.5)

[a]$P < 0.05$.
[b]$P = 0.053$.

cases; 18.3% of the whole series; Table 2). The other eight mutated cases corresponded to S219C and M232F mutations (four cases each). Two tumors showed a double mutation status: L265P/S219C and L265P/M232F and were considered part of the L265P group. No V217F or S222R mutations were observed in the present series.

MYD88 mutations in DLBCL subtypes

The clinical and biologic characteristics of the DLBCL cases according to the mutational status of *MYD88* are shown in Table 2. The distribution according to GE-COO was as follows: 61 cases (47%) were assigned to GCB type and 54 (42%) to ABC type, whereas 14 (11%) remained unclassified. Among these 129 cases with available information on GE-COO, 32 tumors (25%) carried *MYD88* mutations. As expected, *MYD88* mutations were more frequently found in ABC DLBCL subtype ($N = 22$, 69%) but were also detected in eight GCB subtype (25%) and two (6%) unclassified. Interestingly, L265P mutation was significantly more frequent in ABC than in GCB-DLBCL (82% vs. 18%, respectively; $P = 0.0003$). No significant relationship between the other mutations and GCB or ABC DLBCL subtypes was observed. S219C/M232F mutations ($N = 8$) were detected in 4 ABC DLBCL and 4 GCB DLBC patients with no differences in the type of mutation (two S219C and two M232F cases in both subtypes).

We analyzed the allelic burden according to the COO only in the *MYD88* L265P cases due to the lack of calibrator in the other mutations. The frequency for the mutant allele burden varied between 0.05% and 100% (median, 21%). The distribution by quartiles was as follows: Q1: 0.05–0.28; Q2: 0.29–20.9; Q3: 21.0–54.4; Q4: 54.5–100. The proportion of *MYD88*-mutated samples with allelic burden higher than the median (21%) was 89% (16/18 cases) for ABC subtype and 0% (0/4 cases) for GCB DLBCL ($P = 0.002$). In addition, allelic burden <1% was found in three of four GCB tumors showing *MYD88* L265P mutation.

By using Hans IHC algorithm, 96 tumors were GCB (52%) and 90 non-GCB (48%). The relationship between *MYD88* mutations and the DLBCL subtypes identified by Hans IHC algorithm was also analyzed (Table 2). As expected, there were discrepancies in COO between GEP and IHC, 29% and 23% for GCB and ABC, respectively (Supplementary Table S1). L265P-mutated cases ($N = 33$) were distributed as follows: 10 GCB DLBCL (30%) and 23 non–GCB DLBCL (70%).

MYD88 mutational status and other oncogenic alterations in DLBCL

The relationship between *MYD88* mutations and rearrangements of *MYC*, *BCL2*, and *BCL6* is detailed in Table 2. *BCL2* rearrangements were less frequently found in *MYD88* mutated than unmutated DLBCL (8% vs. 21%, respectively; $P = 0.05$). We observed seven cases with double hit and one triple hit, being all of them *MYD88* wild-type. No other statistically significant correlation was observed.

The association between *MYD88* mutations and the protein expression of MYC, BCL2, BCL6, and MUM1 is listed in Table 2. Moreover, we analyzed the coexpression of MYC and BCL2 according to *MYD88* mutational status. This particular pattern was observed in 41% of *MYD88* wild-type versus 18% of *MYD88*-mutated cases ($P = 0.046$). Sixty percent of cases had high Ki67 proliferative index (≥80%). Among *MYD88* wild-type cases, high Ki67 was observed in 61%, whereas in *MYD88* L265P and other *MYD88* mutations, such proportion was 73% and 12%, respectively ($P = 0.008$).

MYD88 mutational status, cytokine expression, and serum cytokine levels

NF-κB signaling activation due to *MYD88* mutation ends up with IL6, IL10, and IFNβ secretion. Thus, *MYD88* mutational status was correlated with tumor expression of IL6, IL10, TNFα, and IL1β with no significant associations (Supplementary Table S2). There were no differences according to *MYD88* in IL6, IL2-R, and TNF serum levels (Supplementary Table S3).

Clinical impact of *MYD88* mutational status

Main clinical features of all DLBCL patients according to the *MYD88* mutational status are listed in Table 3. Patients with

Table 3. Clinical features of 213 patients with DLBCL according to *MYD88* mutational status

	MYD88 **wild-type; *N* (%)**	***MYD88*** **L265P; *N* (%)**	***MYD88*** **other mutations; *N* (%)**
Patients	166 (78)	39 (18)	8 (4)
Gender			
Male	89 (77)	22 (19)	4 (4)
Female	77 (79)	17 (17)	4 (4)
Age			
Median (range)	63 (17–91)	69 (18–91)	69 (34–82)
> 60 years	88 (53)	31 (79)[a]	5 (62)[b]
B symptoms	70 (42)	12 (32)	6 (75)[c]
ECOG performance status ≥ 2	56 (35)	11 (29)	5 (63)
Bulky disease (>7 cm)	42 (27)	11 (29)	2 (29)
Primary extranodal	39 (24)	11 (29)	2 (25)
Extranodal involvement	83 (51)	27 (69)[a]	3 (37)[c]
BM (+)	22 (14)	9 (23)	1 (12)
CNS (+)	2 (1.2)	0	0
Testis (+)	1 (0.6)	4 (10)	0[b]
Breast (+)	1 (0.6)	2 (5)	0[d]
Ann Arbor stage			
I–II	73 (45)	15 (38)	4 (50)
III–IV	88 (55)	24 (62)	4 (50)
High serum LDH	65 (42)	24 (61)[a]	2 (25)[b]
High serum β2m	75 (54)	23 (64)	5 (71)
International prognostic index			
Low risk	52 (33)	11 (29)	2 (25)
Low intermediate risk	40 (25)	6 (16)	3 (37.5)
High intermediate risk	36 (23)	10 (26)	1 (12.5)
High risk	29 (19)	11 (29)	2 (25)
Response to therapy			
Complete response	118 (72)	26 (67)	7 (87)
Partial response	17 (10)	2 (5)	0
No response	28 (18)	11 (28)	1 (13)
Relapse/progression	85 (51)	23 (59)	2 (25)
5-year PFS (%)	54	44	75[d]
5-year OS (%)	62	52	75[b]

[a]$P < 0.05$ considering *MYD88* L265P vs. remainders. All the comparisons are among the three groups except specifically indicated.
[b]$P < 0.05$.
[c]$P < 0.08$.
[d]$P = 0.09$.

MYD88 L265P were older, had higher serum LDH levels, and had more frequently extranodal involvement, particularly testis and breast. *MYD88* mutation was observed in only 1 of 23 patients with gastrointestinal involvement. No significant differences were observed in terms of specific location of disease or survival when only non-MYC/BCL2 coexpression cases were considered. On the contrary, patients with *MYD88* mutations other than L265P showed a trend for exclusive nodal disease (exclusive nodal involvement: 31%, 49%, and 63% for *MYD88* L265P, *MYD88* wild-type, and *MDY88* other than L265P, respectively; $P = 0.06$).

Treatment administration did not differ according to *MYD88* status. CR rates are listed in Table 3 and were not significantly different based on *MYD88*. One hundred and ten patients eventually experienced failure, including 85 of 166 cases *MYD88* wild type (51%), 23 of 39 *MYD88* L265P (59%), and 2 of 8 *MYD88* other (25%). Five-year PFS was 54% (95% CI, 46%–62%), 44% (95% CI, 28%–60%), and 75% (95% CI, 45%–100%), respectively ($P = 0.09$; Fig. 2A). Variables predicting poor PFS were older age, presence of B symptoms, poor performance status, bulky disease, extranodal involvement >1 site, advanced Ann Arbor stage, high serum LDH, high serum β2m, high-risk IPI, high tumor BCL2 expression, and ABC subtype ($P < 0.04$ in all cases). PFS according to *MYD88* mutational status for GCB and ABC subtypes in 115 patients with information available is shown in Fig. 2B and C. The most important variables predicting PFS in the Cox multivariate analysis model with 110 patients were IPI (low vs. intermediate vs. high-risk; HR, 2.915; *P*, 0.002) and COO (GCB vs. ABC; HR, 2.481; *P*, 0.002).

Ninety-one patients died after a median follow-up of 6.0 years, including 67 of 166 (40%) with *MYD88* wild-type, 22 of 39 (56%) *MYD88* L265P (56%), and 2 of 8 (25%) *MYD88* other than L265P. Five-year OS was 62% (95% CI, 55%–70%), 52% (95% CI, 36%–68%), and 75% (95% CI, 45%–100%), respectively ($P = 0.05$; Fig. 3A). In the 39 cases carrying the L265P mutation, the presence of mutation at low tumor burden maintained its unfavorable prognostic significance. Other variables associated with poor OS were older age, presence of B symptoms, poor performance status, bulky disease, extranodal involvement >1 site, advanced Ann Arbor stage, high serum LDH, high serum β2m, high-risk IPI, high tumor BCL2 expression, and ABC subtype ($P < 0.03$ in all cases). OS according to *MYD88* mutational status for GCB and ABC subtypes, as assessed by gene expression, is depicted in Fig. 3B and C, respectively. A multivariate analysis was performed including IPI (low vs. intermediate vs. high-risk), *MYD88* L265P (unmutated vs. mutated), and *MYD88* other than L265P (unmutated vs. mutated). In the final model with 203 patients, IPI (HR, 2.71; $P < 0.001$) and *MYD88* L265P (HR, 1.786; $P = 0.023$) were independent variables predicting OS. At the resampling test of replicability for validating the results, *MYD88* L265P was selected as an independent predictor of OS in 79% of the

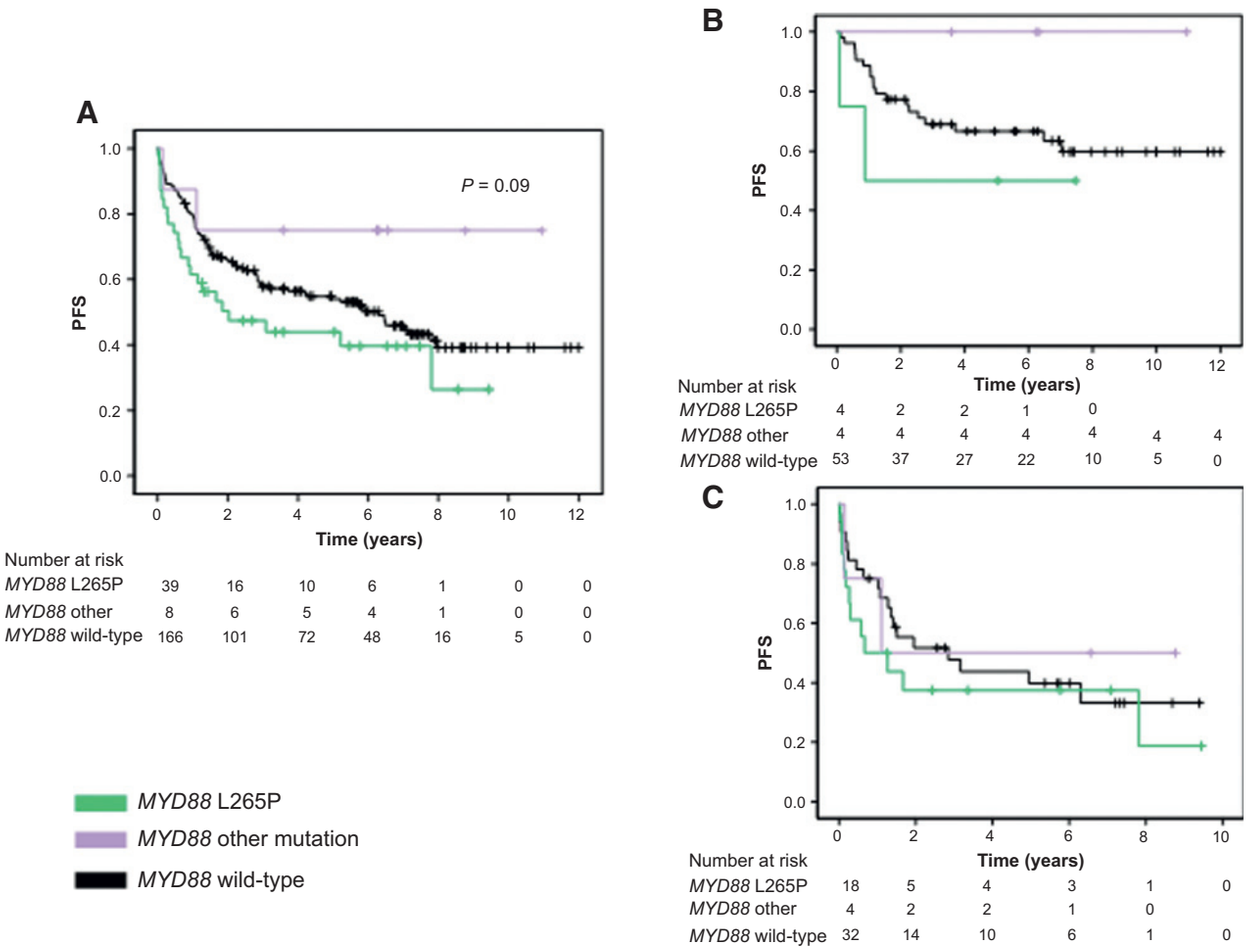

Figure 2.
PFS according to *MYD88* mutational status (A) of the whole series ($N = 213$), (B) in patients with germinal center B-cell DLBCL ($N = 61$), and (C) in patients with activated B-cell DLBCL ($N = 54$).

1,000 bootstrap samples, whereas IPI was selected in 95%. The same analysis was performed including COO (GCB vs. ABC). In the model with 110 patients, only IPI (HR, 3.401; *P*, 0.001) and COO (HR, 2.841; *P*, 0.002) maintained the prognostic value for OS.

Discussion

DLBCL is a clinical and biologically heterogeneous disease. Although many patients are currently cured with immunochemotherapy, a considerably proportion is still refractory to treatment or eventually relapse. Nowadays, to elucidate the biologic background of patients with unfavorable outcome is a key objective. Gene expression methods can stratify DLBCL according to the COO into GCB or ABC subtypes. In addition, next-generation sequencing of whole-exome sequences has provided the genetic landscape of this lymphoma. In the ABC subtype, the major signaling alterations are related to the constitutive activation of the NF-κB pathway through chronic stimulation of the BCR, CD40, and TLR pathways. However, the GCB subtype is definitely less dependent upon the regulation of any particular pathway. *MYD88* mutation that promotes both NF-κB and JAK/STAT signaling conferring a selective advantage in cell survival (11) is the most frequent genetic alteration found in ABC DLBCLs and is associated with a poor outcome (27,37). The identification and characterization of the molecular basis of the dismal prognosis of patients with *MYD88*-mutant DLBCL could provide the rationale for new treatment targets in the involved pathways. Thus, *MYD88* could emerge as a key biomarker in ABC DLBCL (23, 25, 47–49).

We observed *MYD88* mutations in 22% of the whole series, being L265P the most frequent one (39 of 47 cases, 83%). These figures are similar to previously reported studies ranging from 14% to 39% (11, 23, 50), but higher than others (around 10%; refs. 24, 25, 27, 37), which may be due to the technique used. Next-generation sequencing techniques at low coverage are probably less sensitive than allele-specific PCR. Whether or not the presence of mutations at lower burden has prognostic impact is of high clinical interest. It is of note that in the present series, the clinical impact of *MYD88* mutation was maintained even at low allelic burden, whereas with ARMS technology, we can detect less than 1% of tumor cells.

While in most of the previous reports, the different *MYD88* mutations are considered as a whole to assess their clinical impact, we herein have shown that the type of mutation could be relevant.

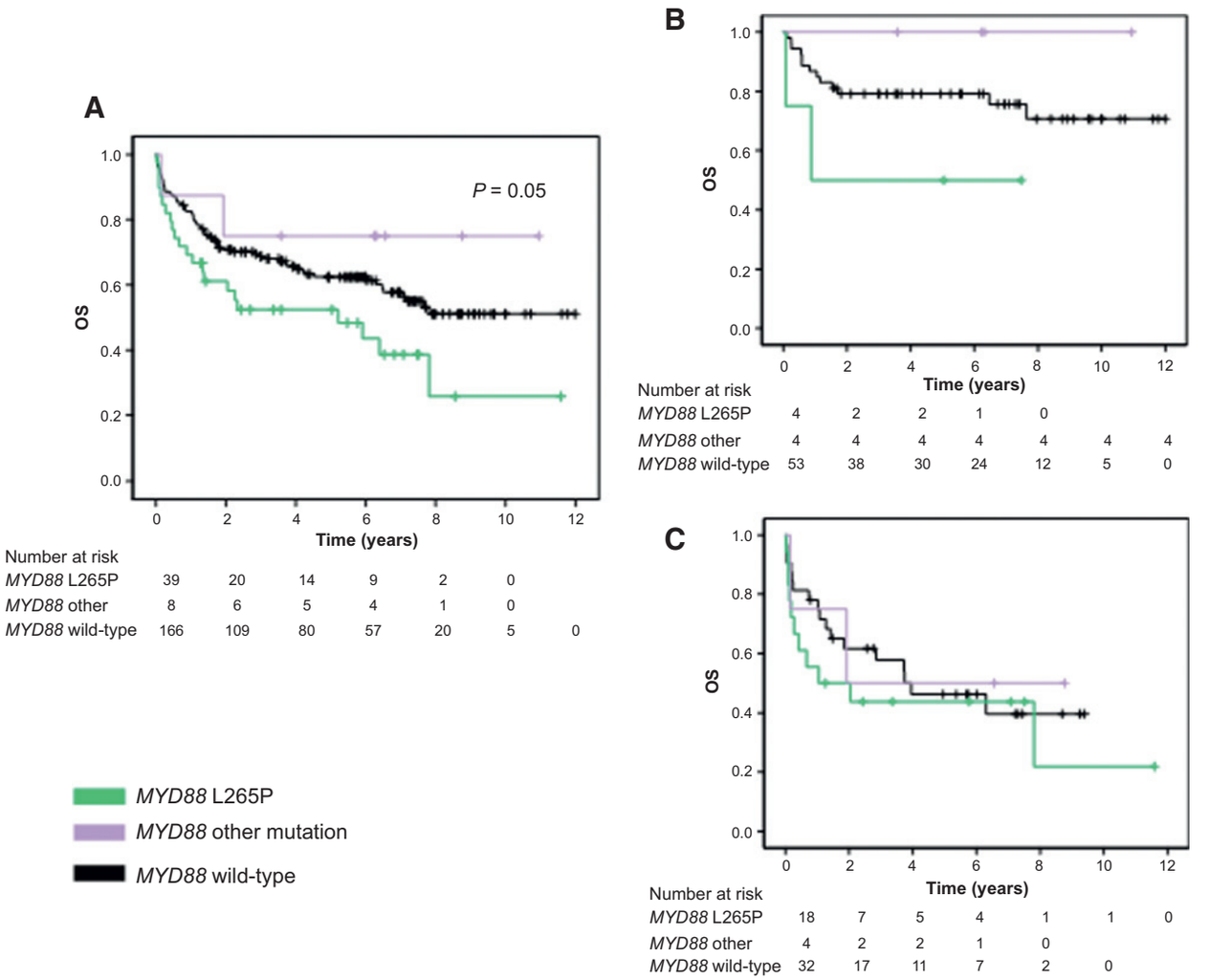

Figure 3.
OS according to MYD88 mutational status (A) of the whole series ($N = 213$), (B) in patients with germinal center B-cell DLBCL ($N = 61$), and (C) in patients with activated B-cell DLBCL ($N = 54$).

As previously described, patients with a *MYD88* L265P mutation are older, mostly of ABC COO, have extranodal involvement, high serum LDH and high Ki67 index, and a poorer OS (Table 3 and Fig. 3; refs. 27, 37, 50). On the other hand, DLBCLs with *MYD88* mutations other than L265P have no COO preference, are typically nodal, and the OS is even better than that of *MYD88* wild type. However, this information is based on a small number of patients and therefore should be confirmed in further studies. Even though the favorable prognosis of *MYD88* mutations different from L265P could be hampered by the low number of patients, our data clearly show that such patients had a considerable better outcome than patients with *MYD88* L265P. Moreover, some biologic data can support this different behavior: in a GCB DLBCL cell line with little endogenous NF-κB, Ngo and colleagues demonstrated that wild-type *MYD88* only modestly activated NF-κB, whereas L265P had the strongest NF-κB activation. Other isoforms, including S219C, M232T, V217F, and S222R, showed much less capacity to activate NF-κB than L265P (11). This circumstance could explain the favorable evolution of some *MYD88*-mutated GCB-DLBCL cases. Patients with *MYD88* L265P showed poor PFS and OS. This may be due to other related variables such as older age or ABC subtype rather than to the mutation itself. Similarly to previous data, in our series, *MYD88* L265P and IPI were independent variables to predict OS (37). However, when including GE-COO in the model, *MYD88* L265P lost its prognostic significance at the multivariate level most likely because the vast majority of *MYD88*-mutated cases were of ABC origin. Interestingly, on the other hand, the coexpression of MYC and BCL2, typically observed in ABC DLBCLs and considered one of the factors of the adverse outcome in this group (51,52), was less frequently seen in our patients with *MYD88* L265P than in wild-type ones (18% vs. 41%, respectively). Moreover, patients with *MYD88* mutation were significantly older than those without the mutation. This is consistent with the higher rate of these mutations in ABC DLBCL and the high frequency of this subtype in older patients (53). As described in other series, patients with *MYD88* L265P frequently had extranodal involvement, including bone marrow, testes, and breast.

Primary central nervous system was not evaluable in the present series because those patients were excluded from the study. It is of note that we did not find especial predilection for gastrointestinal system as previously suggested by others (37, 49, 50).

Different gene alterations in DLBCL, including *MYC*, *BCL2*, and *BCL6*, were analyzed. The only significant correlation was the expected lower frequency of *BCL2* rearrangement in *MYD88* L265P–mutated tumors. Only one of 39 *MYD88* L265P cases showed *MYC* rearrangement, being in line with a recent publication showing that only one of 37 double-hit DLBCL cases had *MYD88* mutation (49).

Despite notable improvements in survival after the introduction of an anti-CD20 monoclonal antibody (rituximab) to frontline therapy, a proportion of patients still remains refractory or eventually relapses. New treatments targeting specific tumor alterations are currently under development. NF-κB activation and resistance to chemotherapy are well-known molecular pathways that could be overcome by these novel therapies; thus, *MYD88* pathway could represent a key hub to block the viability of tumor cells. Several studies have shown that bortezomib, a proteasome inhibitor, can act synergistically with R-CHOP inhibiting NF-κB and improve the outcome of ABC DLBCL patients (54–56). DLBCLs carrying *MYD88* mutations could be particularly sensitive to those drugs inhibiting either NF-κB or JAK/STAT signaling pathways (57, 58). There are several drugs currently being studied that could target different steps of the pathway, including BTK and PI3K inhibitors. Wilson and colleagues have recently showed 5% of responses to ibrutinib in GCB DLBCL versus 37% in ABC DLBCL due to chronic active BCR signaling in the latter. The authors have hypothesized that ABC DLBCL could arise by two distinct pathogenetic routes, one BCR dependent and other independent. Interestingly, only BCR-dependent cases responded to ibrutinib particularly when *MYD88* L265P mutation was present (59). In this setting, *MYD88* mutational status along with other mutations may be good biomarkers of response to the targeted therapies in the near future.

In summary, *MYD88* mutational status analysis by PCR could help to recognize subgroups of patients with particular features and outcome. The current data highly suggest that *MYD88* L265P mutation highlights a subpopulation of older patients with an ABC origin, specific extranodal involvement, and unfavorable prognosis. On the contrary, *MYD88* mutations other than L265P, also present in the elderly population, may have a favorable outcome. Due to the low incidence of these mutations, current data should be validated in further studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Conception and design: J. Rovira, A. López-Guillermo

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Rovira, K. Karube, A. Enjuanes, L. Colomo, A. Martínez, E. Campo, A. López-Guillermo

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Other (review clinical data and approve the final version of the manuscript): J. Delgado

Acknowledgments

This work was partially developed at the Centro Esther Koplowitz (CEK), Barcelona, Spain. The authors thank the HCB-IDIBAPS Biobank-Tumor Bank and Hematopathology Collection for sample procurement and Laura Jiménez for her technical assistance.

Grant Support

This work was funded in part by PI12/01536 (to A. López-Guillermo), RD12/0036/0023(to A. López-Guillermo), RD12/0036/0004 (to D. Colomer), RD12/0036/0036 (to E. Campo), SAF12-38432 (to E. Campo), AGAUR 2013-SGR-0795 (to E. Campo), AGAUR 2014SGR967 (to D. Colomer), and PIE13/00033 (to E. Campo and A. López-Guillermo) from Instituto de Salud Carlos III, Spanish Ministry of Health. E. Campo is an Academia Researcher of the "Institució Catalana de Recerca I Estudis Avançats" (ICREA) of the Generalitat de Catalunya. J. Rovira was supported by an "Emili Letang" grant (Hospital Clínic).

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Received July 12, 2015; revised November 10, 2015; accepted January 5, 2016; published OnlineFirst January 20, 2016.

References

1. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503–11.
2. Staudt LM, Dave S. The biology of human lymphoid malignancies revealed by gene expression profiling. Adv Immunol 2005;87:163–208.
3. Steven H., Swerdlow EC, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, et al. WHO Classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer (IARC); 2008.
4. Shaffer AL3rd, Young RM, Staudt LM. Pathogenesis of human B cell lymphomas. Annu Rev Immunol 2012;30:565–610.
5. 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.
6. Refaeli Y, Young RM, Turner BC, Duda J, Field KA, Bishop JM. The B cell antigen receptor and overexpression of MYC can cooperate in the genesis of B cell lymphomas. PLoS Biol 2008;6:e152.
7. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. The J Exp Med 2001;194:1861–74.
8. Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, Shen Q, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature 2009;459:717–21.
9. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 2010;463:88–92.
10. Lenz G, Davis RE, Ngo VN, Lam L, George TC, Wright GW, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science 2008;319:1676–9.
11. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, et al. Oncogenically active MYD88 mutations in human lymphoma. Nature 2011;470:115–9.
12. Jeelall YS, Horikawa K. Oncogenic MYD88 mutation drives Toll pathway to lymphoma. Immunol Cell Biol 2011;89:659–60.

13. Lin SC, Lo YC, Wu H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. Nature 2010;465:885–90.
14. Muzio M, Bertilaccio MT, Simonetti G, Frenquelli M, Caligaris-Cappio F. The role of toll-like receptors in chronic B-cell malignancies. Leuk Lymphoma 2009;50:1573–80.
15. Kato M, Sanada M, Kato I, Sato Y, Takita J, Takeuchi K, et al. Frequent inactivation of A20 in B-cell lymphomas. Nature 2009;459:712–6.
16. Rawlings DJ, Schwartz MA, Jackson SW, Meyer-Bahlburg A. Integration of B cell responses through Toll-like receptors and antigen receptors. Nat Rev Immunol 2012;12:282–94.
17. Landgren O, Tageja N. MYD88 and beyond: novel opportunities for diagnosis, prognosis and treatment in Waldenstrom's macroglobulinemia. Leukemia 2014;28:1799–803.
18. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med 2012;367:826–33.
19. Gachard N, Parrens M, Soubeyran I, Petit B, Marfak A, Rizzo D, et al. IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenstrom macroglobulinemia/lymphoplasmacytic lymphomas. Leukemia 2013;27:183–9.
20. Jimenez C, Sebastian E, Chillon MC, Giraldo P, Mariano Hernandez J, Escalante F, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenstrom's macroglobulinemia. Leukemia 2013;27:1722–8.
21. Varettoni M, Zibellini S, Arcaini L, Boveri E, Rattotti S, Pascutto C, et al. MYD88 (L265P) mutation is an independent risk factor for progression in patients with IgM monoclonal gammopathy of undetermined significance. Blood 2013;122:2284–5.
22. Varettoni M, Arcaini L, Zibellini S, Boveri E, Rattotti S, Riboni R, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. Blood 2013;121:2522–8.
23. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet 2011;43:830–7.
24. Lohr JG, Stojanov P, Lawrence MS, Auclair D, Chapuy B, Sougnez C, et al. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. Proc Natl Acad Sci U S A 2012;109:3879–84.
25. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature 2011;476:298–303.
26. Wang CZ, Lin J, Qian J, Shao R, Xue D, Qian W, et al. Development of high-resolution melting analysis for the detection of the MYD88 L265P mutation. Clin Biochem 2013;46:385–7.
27. Choi JW, Kim Y, Lee JH, Kim YS. MYD88 expression and L265P mutation in diffuse large B-cell lymphoma. Hum Pathol 2013;44:1375–81.
28. Pham-Ledard A, Cappellen D, Martinez F, Vergier B, Beylot-Barry M, Merlio JP. MYD88 somatic mutation is a genetic feature of primary cutaneous diffuse large B-cell lymphoma, leg type. J Invest Dermatol 2012;132:2118–20.
29. Gonzalez-Aguilar A, Idbaih A, Boisselier B, Habbita N, Rossetto M, Laurenge A, et al. Recurrent mutations of MYD88 and TBL1XR1 in primary central nervous system lymphomas. Clin Cancer Res 2012;18:5203–11.
30. Montesinos-Rongen M, Godlewska E, Brunn A, Wiestler OD, Siebert R, Deckert M. Activating L265P mutations of the MYD88 gene are common in primary central nervous system lymphoma. Acta Neuropathol 2011;122:791–2.
31. Liu F, Karube K, Kato H, Arita K, Yoshida N, Yamamoto K, et al. Mutation analysis of NF-kappaB signal pathway-related genes in ocular MALT lymphoma. Int J Clin Exp Pathol 2012;5:436–41.
32. Troen G, Warsame A, Delabie J. CD79B and MYD88 mutations in splenic marginal zone lymphoma. ISRN Oncol 2013;2013:252318.
33. Yan Q, Huang Y, Watkins AJ, Kocialkowski S, Zeng N, Hamoudi RA, et al. BCR and TLR signaling pathways are recurrently targeted by genetic changes in splenic marginal zone lymphomas. Haematologica 2012;97:595–8.
34. Puente XS, Pinyol M, Quesada V, Conde L, Ordonez GR, Villamor N, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 2011;475:101–5.
35. Martinez-Trillos A, Pinyol M, Navarro A, Aymerich M, Jares P, Juan M, et al. Mutations in TLR/MYD88 pathway identify a subset of young chronic lymphocytic leukemia patients with favorable outcome. Blood 2014;123:3790–6.
36. Bea S, Valdes-Mas R, Navarro A, Salaverria I, Martin-Garcia D, Jares P, et al. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. Proc Natl Acad Sci U S A 2013;110:18250–5.
37. Fernandez-Rodriguez C, Bellosillo B, Garcia-Garcia M, Sanchez-Gonzalez B, Gimeno E, Vela MC, et al. MYD88 (L265P) mutation is an independent prognostic factor for outcome in patients with diffuse large B-cell lymphoma. Leukemia 2014;28:2104–6.
38. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, et al. Revised response criteria for malignant lymphoma. J Clin Oncol 2007;25:579–86.
39. Colomo L, Lopez-Guillermo A, Perales M, Rives S, Martinez A, Bosch F, et al. Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood 2003;101:78–84.
40. de Jong D, Rosenwald A, Chhanabhai M, Gaulard P, Klapper W, Lee A, et al. Immunohistochemical prognostic markers in diffuse large B-cell lymphoma: validation of tissue microarray as a prerequisite for broad clinical applications–a study from the Lunenburg Lymphoma Biomarker Consortium. J Clin Oncol 2007;25:805–12.
41. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275–82.
42. Ventura RA, Martin-Subero JI, Jones M, McParland J, Gesk S, Mason DY, et al. FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. J Mol Diagn 2006;8:141–51.
43. Valera A, Balague O, Colomo L, Martinez A, Delabie J, Taddesse-Heath L, et al. IG/MYC rearrangements are the main cytogenetic alteration in plasmablastic lymphomas. Am J Surg Pathol 2010;34:1686–94.
44. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med 2008;359:2313–23.
45. Scott DW, Wright GW, Williams PM, Lih CJ, Walsh W, Jaffe ES, et al. Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. Blood 2014;123:1214–7.
46. DR C. Regression models and life-tables. J R Stat Soc Series B (Methodol) 1972;34:187–220.
47. Bohers E, Mareschal S, Bouzelfen A, Marchand V, Ruminy P, Maingonnat C, et al. Targetable activating mutations are very frequent in GCB and ABC diffuse large B-cell lymphoma. Genes Chromosomes Cancer 2014;53:144–53.
48. Jardin F. Next generation sequencing and the management of diffuse large B-cell lymphoma: from whole exome analysis to targeted therapy. Discov Med 2014;18:51–65.
49. Gebauer N, Bernard V, Thorns C, Feller AC, Merz H, Bohers E, et al. Oncogenic MYD88 mutations are rare events in double-hit B-cell lymphomas. Acta Haematol 2014;133:113–5.
50. Kraan W, Horlings HM, van Keimpema M, Schilder-Tol EJ, Oud ME, Scheepstra C, et al. High prevalence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. Blood Cancer J 2013;3:e139.
51. Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30:3452–9.
52. Green TM, Young KH, Visco C, Xu-Monette ZY, Orazi A, Go RS, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30:3460–7.
53. Mareschal S, Lanic H, Ruminy P, Bastard C, Tilly H, Jardin F. The proportion of activated B-cell like subtype among de novo diffuse large B-cell lymphoma increases with age. Haematologica 2011;96:1888–90.
54. Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, Grant N, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood 2009;113:6069–76.

55. Ribrag V, Gisselbrecht C, Haioun C, Salles G, Golfier JB, Ertault M, et al. Efficacy and toxicity of 2 schedules of frontline rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone plus bortezomib in patients with B-cell lymphoma: a randomized phase 2 trial from the French Adult Lymphoma Study Group (GELA). Cancer 2009;115: 4540–6.
56. Ruan J, Martin P, Furman RR, Lee SM, Cheung K, Vose JM, et al. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. J Clin Oncol 2011;29: 690–7.
57. Lam LT, Wright G, Davis RE, Lenz G, Farinha P, Dang L, et al. Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-{kappa}B pathways in subtypes of diffuse large B-cell lymphoma. Blood 2008;111:3701–13.
58. Lim KH, Yang Y, Staudt LM. Pathogenetic importance and therapeutic implications of NF-kappaB in lymphoid malignancies. Immunol Rev 2012;246:359–78.
59. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med 2015;21:922–6.