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## **Diagnostic and prognostic markers of cutaneous lymphomas**

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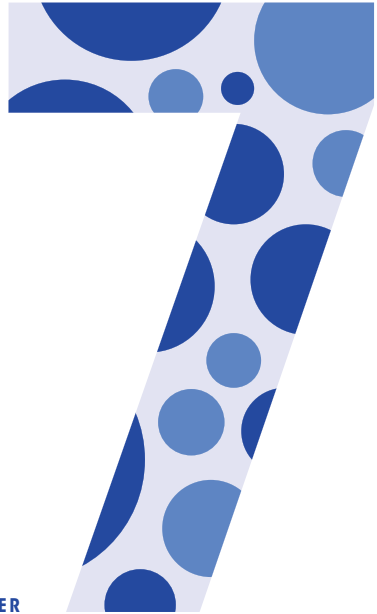


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CHAPTER

## **HIGH PREVALENCE OF *MYD88* AND *CD79B* MUTATIONS IN INTRAVASCULAR LARGE B-CELL LYMPHOMA**

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## HIGH PREVALENCE OF *MYD88* AND *CD79B* MUTATIONS IN INTRAVASCULAR LARGE B-CELL LYMPHOMA

Intravascular large B-cell lymphoma (IVLBCL) is a rare variant of extranodal diffuse large B-cell lymphoma (DLBCL). It is characterized by proliferation of blastic, neoplastic B cells within the lumina of small- or intermediate-sized blood vessels and capillaries.<sup>1</sup> IVLBCL may potentially involve any organ and is often widely disseminated. Two major patterns have been recognized. In Western patients, predominantly the skin and the central nervous system (CNS) are involved, whereas in Asian countries, the disease often involves multiple other organs and is accompanied by hemophagocytosis.<sup>2</sup> Additionally, a cutaneous variant, with skin-limited disease at time of diagnosis, has been described in younger, Western women.<sup>3</sup> Standard treatment of IVLBCL consists of rituximab-containing chemotherapeutic regimens, demonstrating an estimated 3-year overall survival (OS) of 60 to 81%.<sup>4,5</sup>

Previous patient reports and small case series have identified various translocations, sporadically involving an oncogene, such as *BCL2*, *BCL6* or *cyclin D1*, but none occurred in >1 patient.<sup>6-9</sup> In 1 case, RNA expression profiling was similar to the non-germinal center B-cell (non-GCB) subtype of DLBCL.<sup>10</sup> However, the mutational profile of IVLBCL is still unknown. Because of the phenotypic overlap with non-GCB DLBCL and frequent presentation in the skin and CNS, we hypothesized that IVLBCL may harbor NF- $\kappa$ B-activating mutations.<sup>11-13</sup> Therefore, we investigated the prevalence of a subset of DLBCL-associated genetic alterations in patients with IVLBCL and explored their association with OS.

For this retrospective study, we evaluated a unique and clinically well-annotated cohort of 25 patients who were diagnosed with IVLBCL according to the latest World Health Organization criteria in participating university and nonacademic regional hospitals in The Netherlands between 1985 and 2017.<sup>1</sup> Central review was performed in the Leiden University Medical Center by A.M.R.S., P.M.J., and A.H.G.C. The study was performed in accordance with the Dutch Code Proper Secondary Use of Human Tissue and approved by the medical ethics committee

of the Leiden University Medical Center (B16.048). Clinical data are summarized in Table 1. The group consisted of 16 women and 9 men with a median age at diagnosis of 64 (range, 49 to 85) years. The higher percentage of women in our cohort may be explained by the presence of 6 patients with skin-limited disease, which has a known female predominance.<sup>3</sup> B symptoms were recorded in 18 of 22 (82%) patients. In total, 12 patients (48%) presented with skin lesions (Figure 1A-B) of whom 6 showed no signs of other affected organs on routine staging procedures. Other commonly involved organs were the bone marrow (8/24; 33%), followed by the brain and lungs (7/24; 29%). Nine patients had Ann-Arbor stage IE (skin, brain, or kidney) and 15 patients had stage IV disease. Three cases were diagnosed at autopsy and staging was not performed in 1 patient who declined additional diagnostic procedures.

Histologic examination of the diagnostic formalin-fixed, paraffin-embedded biopsies showed the presence of large blastic cells within the lumina of blood vessels. For 1 patient with skin-limited disease, only a skin biopsy of relapsed IVLBCL 6 years after diagnosis was available. Immunohistochemistry with antibodies against CD20, CD10, BCL6, MUM1, BCL2, IgM, MYC, CD5, cyclin D1, and CD30 was performed according to routine procedures (Table 1). In all cases, the tumor cells expressed CD20. Most cases were positive for BCL2 (23/24; 96%), IgM (21/23; 91%), and MUM1 (18/24; 75%), while expression of CD5 (13/25; 52%), MYC (15/22; 68%), and BCL6 (14/24; 58%) was variable. CD10 was weakly positive in 2 (8%) cases and all cases were negative for CD30 and cyclin D1. Expression of BCL2 and MUM1 in the vast majority of cases corresponded with that in previous studies<sup>14</sup> and, together with expression of IgM, strongly suggest an activated B-cell phenotype. Also, aberrant co-expression of CD5 in a subset of patients with IVLBCL has been reported, without known clinical significance.<sup>14</sup> Fluorescence *in situ* hybridization for *MYC* using Vysis Dual Color Break Apart Probes (Abbott) showed no rearrangement of the *MYC* gene in 15 evaluated patients, and *in situ* hybridization for Epstein-Barr virus early RNA was negative in 24 patients.

**Table 1.** Overview of clinical characteristics, treatment, follow-up, and results of 25 patients with intravascular large B-cell lymphoma

<b>Sex</b>	
Men	9/25 (36%)
Women	16/25 (64%)
<b>Age at diagnosis, years</b>	
Median	64
Range	49-85
<b>Presence of B symptoms</b>	18/22 (82%)
<b>Disease localization</b>	
Skin	12/25 (48%)
Bone marrow	8/24 (33%)
Brain	7/24 (29%)
Lung	7/24 (29%)
Spleen	5/24 (21%)
Liver	4/24 (17%)
Lymph nodes	4/24 (17%)
Kidney	3/24 (13%)
Other (thyroid, testis, [cardiac] muscle, soft tissue)	5/24 (21%)
<b>Skin-limited disease</b>	6/23 (26%)
<b>Ann Arbor stage<sup>^</sup></b>	
IE (skin, brain, kidney)	9/24 (38%)
IV	15/24 (63%)
<b>First-line therapy<sup>*</sup></b>	
Immunochemotherapy <sup>#</sup>	6/22 (27%)
Chemotherapy <sup>§</sup>	6/22 (27%)
Immunotherapy (rituximab only)	1/22 (5%)
Radiotherapy	3/22 (14%)
Supportive care with/without steroids	5/22 (23%)
Surgery + watchful waiting (subcutaneous lipoma)	1/22 (5%)
<b>Second-line therapy</b>	
Immunochemotherapy <sup>#</sup>	2/22 (9%)
Chemotherapy <sup>§</sup>	1/22 (5%)
Radiotherapy	2/22 (9%)
<b>Survival status</b>	
Died, disease related	14/25 (56%)
Died, disease unrelated	5/25 (20%)
Alive	6/25 (24%)

**Table 1.** Overview of clinical characteristics, treatment, follow-up, and results of 25 patients with intravascular large B-cell lymphoma (continued)

<b>Median overall survival time, years<sup>§</sup></b>	1.68
<b>Immunohistochemistry</b>	
CD20	25/25 (100%)
CD10	2/25 (8%)
BCL6	14/24 (58%)
MUM1	18/24 (75%)
BCL2	23/24 (96%)
IgM	21/23 (91%)
MYC	15/22 (68%)
CD5	13/25 (52%)
Cyclin D1	0/25 (0%)
CD30	0/24 (0%)
<b>EBV infection (EBER ISH)</b>	0/24 (0%)
<b>MYC rearrangement (FISH)</b>	0/15 (0%)
<b>Mutations</b>	
MYD88 (exon 3/exon 5)	11/25 (44%)
CD79B (exon 5/exon 6)	6/23 (26%)
CD79A (exon 5)	0/24 (0%)
CARD11 (exon 6)	0/24 (0%)
EZH2 (exon 16)	1/25 (4%)

Abbreviations: EBER ISH, Epstein-Barr virus early RNA in situ hybridization; FISH, fluorescence in situ hybridization with break apart probes for MYC.

<sup>^</sup>Staging procedures were not performed in 1 patient with skin involvement.

<sup>\*</sup>Three patients were diagnosed at autopsy.

<sup>#</sup>Immunochemotherapy consisted in first-line of R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; n=4), R-CHOP with methotrexate (n=1), and R-CVP (rituximab plus cyclophosphamide, vincristine, and prednisone; n=1), and in second-line of R-CHOP (n=1) and R-DHAP (rituximab plus dexamethasone, cytarabine, and cisplatin; n=1).

<sup>§</sup>Chemotherapy consisted in first-line of CHOP or CHOP-like regimens (n=4), MBVP (methotrexate, camustine, teniposide, and prednisone; n=1), and vincristine with steroids (n=1), and in second-line of IMVP (ifosfamide, methotrexate, etoposide, and prednisone) and DHAP followed by autologous stem-cell transplantation (n=1).

<sup>§</sup>Patients diagnosed at autopsy were excluded.

Additionally, we performed targeted next-generation sequencing covering hotspot regions of the genes *MYD88* (exon 3/exon 5), *CD79B* (exon 5/exon 6), *CD79A* (exon 5), *CARD11* (exon 6), and *EZH2* (exon 16). As previously described, DNA of microdissected tumor cells was fully automatically isolated<sup>15</sup> and sequenced<sup>16</sup> with a single primer pool with Ampliseq amplicons covering hotspot regions of the selected genes. Pathogenic variants (class 5) and possibly pathogenic variants (class 4) with a variant allele frequency of  $\geq 5\%$  were reported. In 11 of 25 patients (44%), *MYD88* L265P mutations (NM\_001172567) were detected. In 6 of 23 (26%) patients, mutations in *CD79B* Y196 (NM\_001039933) were present, with co-occurrence of mutated *MYD88* in 5 of these patients (Figure 1C). One patient had an *EZH2* Y646F mutation (NM\_004456), and no mutations were identified in the target regions of *CD79A* and *CARD11*. In 1 patient with a relapse after R-CHOP, the *MYD88* L265P mutation was also identified in circulating free tumor DNA in the peripheral blood using digital droplet polymerase chain reaction (Bio-Rad). An overview of the results is presented in Table 1. Associated clinical and molecular features for each patient are listed in Supplemental Table 1.

To the best of our knowledge, this is the first study to report a mutational analysis of IVLBCL, revealing a high prevalence of *MYD88* L265P mutations (44%) and *CD79B* Y196 mutations (26%). In DLBCL, activating mutations in *MYD88* and/or *CD79B* have been identified as important molecular drivers of the Toll-like receptor and B-cell receptor pathways, respectively, resulting in constitutive activation of NF- $\kappa$ B signaling.<sup>17,18</sup> *MYD88* mutations in particular are a hallmark of DLBCL presenting at immune-privileged sites, such as the testes and CNS, and primary cutaneous DLBCL leg type, with percentages up to 80%, in contrast to DLBCL in other sites (17%).<sup>12,13,19,20</sup> Also, co-occurrence of *MYD88* and *CD79B* mutations is a common phenomenon.<sup>11,13,17</sup> Our results suggest that *MYD88* and/or *CD79B* mutations are important molecular oncogenic drivers of IVLBCL.

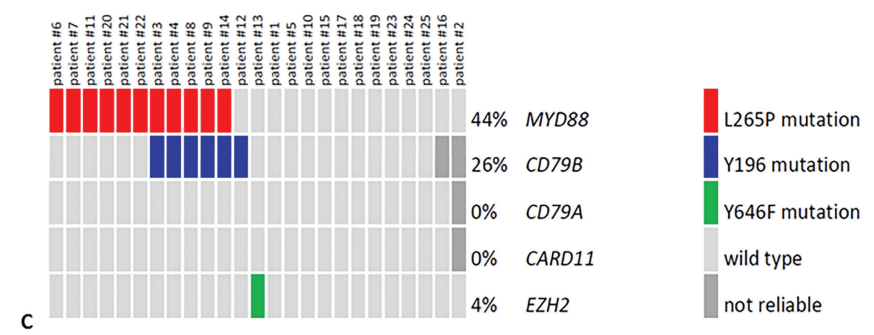
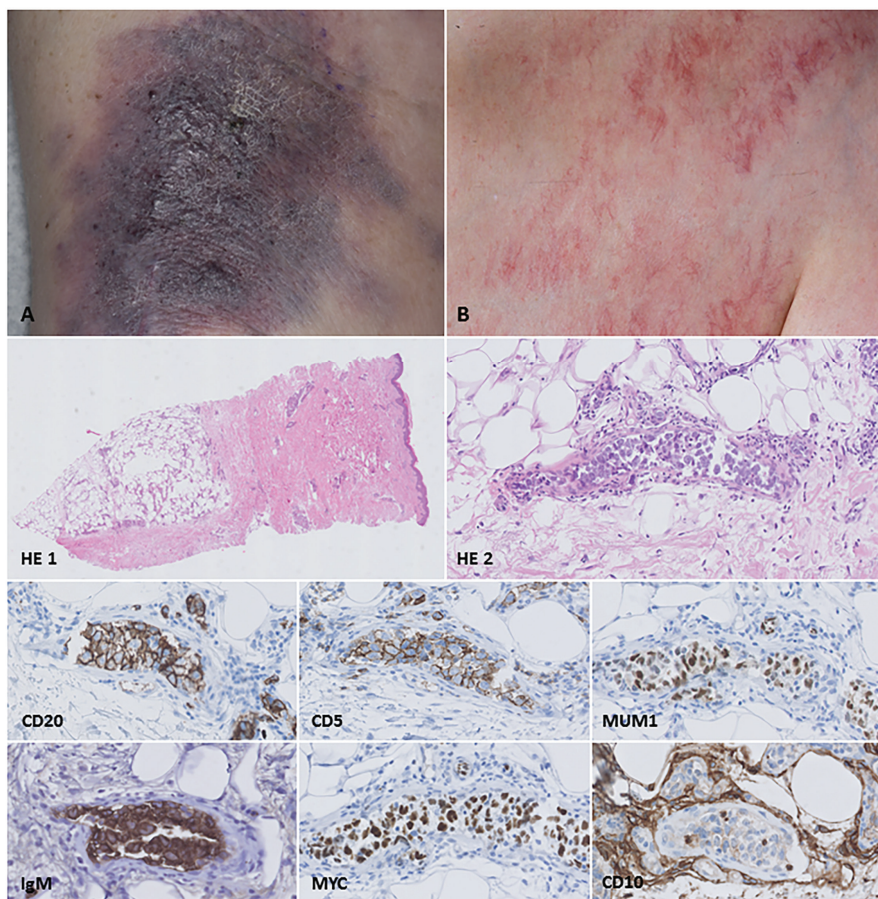
As for the exploratory analysis of survival, Kaplan-Meier curves are shown in Supplemental Figure 1. Because a significant number of patients received only supportive care or were treated in the prerituximab era, the 3-year OS in our cohort (43%) was lower than previously reported (60 to 81%).<sup>4,5</sup> In line with



literature, patients with skin-limited disease showed a trend in superior 5-year disease-specific survival compared with patients with systemic disease (Log-rank,  $p=0.06$ ).<sup>3</sup> Mutational status of *MYD88* and/or *CD79B* did not seem to influence disease-specific survival (Log-rank,  $p=0.64$ ), as was previously shown for other extranodal DLBCL.<sup>12,21,22</sup> Additionally, *MYD88/CD79B* mutations were detected in patients with skin-limited disease as well as those with systemic disease. Given our small patient number and heterogeneity of clinical characteristics and treatment regimens, our results indisputably need validation in larger, homogeneous (i.e. R-CHOP-treated) IVLBCL cohorts, together with a broader evaluation of genetic aberrations, including other NF- $\kappa$ B-activating mutations, such as *TNFAIP3*.

From a therapeutic perspective, our results suggest that a substantial number of IVLBCL patients may be amenable to treatments targeting NF- $\kappa$ B signaling. Recent studies have shown that patients with DLBCL with *MYD88* and/or *CD79B* mutations are more sensitive to treatment with ibrutinib, a Bruton tyrosine kinase inhibitor that blocks the NF- $\kappa$ B pathway.<sup>23-25</sup> Additional studies are required to assess whether patients with IVLBCL may also benefit from these therapeutic approaches.

In conclusion, this is the first study to identify a high prevalence of *MYD88* L265P and/or *CD79B* Y196 mutations as potential oncogenic drivers in patients with IVLBCL. This highlights the importance of investigating the mutational status of *MYD88* and *CD79B* in larger, homogeneous cohorts and exploring the efficacy of molecularly targeted agents for these patients.



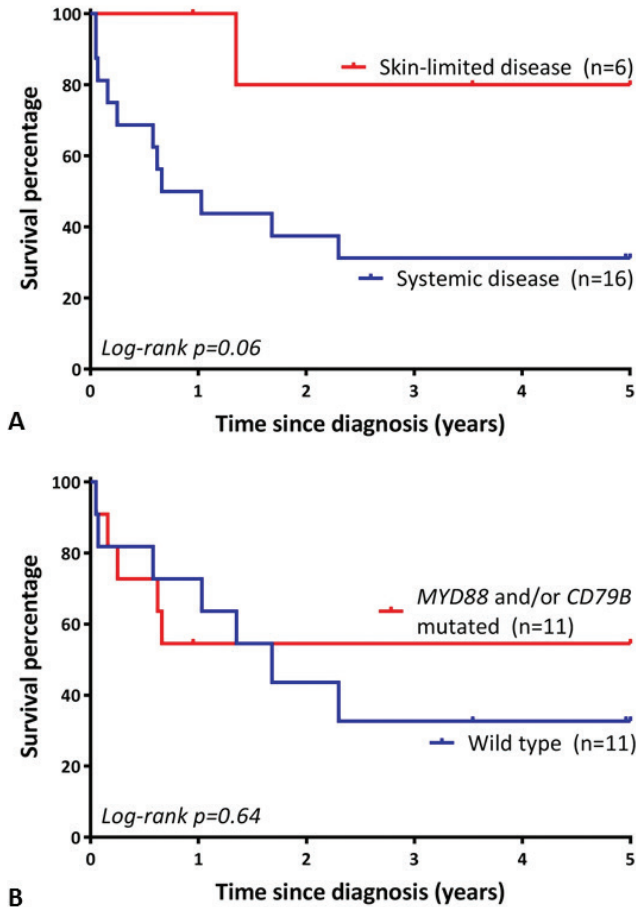
**Figure 1.** Intravascular large B-cell lymphoma: skin lesions, histology, mutational status, and survival analysis. Skin lesions may present as bluish indurated plaques (A) or generalized telangiectasias (B). Histology of a representative skin biopsy shows a dermal blood vessel with intraluminal clustering of large, blastic cells (HE 1; HE 2), positive for CD20 (clone L26 from Dako, diluted 1:800), CD5 (clone 4C7 from Dako, diluted 1:400), MUM1 (clone MUM1p from Dako, diluted 1:100), IgM (polyclonal, from Dako, diluted 1:500), and MYC (clone Y69 from ABCAM, diluted 1:100), and negative for CD10 (clone 56C6 from Dako, diluted 1:20). Original magnification: x50 for HE 1 and x400 for HE 2, CD20, CD5, MUM1, IgM, MYC, and CD10. (C) OncoPrinter plot of the targeted next-generation sequencing data shows an *MYD88* L265P mutation (NM\_001172567) in 44%, a *CD79B* Y196 (NM\_001039933) in 26%, and an *EZH2* Y646F (NM\_004456) in 4% of the patients. Patient numbers correspond to those in the Supplemental Table 1. In patients 16 and 2, the sequencing data of *CD79B* resp. *CD79A/B* and *CARD11* was not reliable because of low read count (<100 reads).

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## SUPPLEMENTAL DATA



**Supplemental Figure 1.** Survival curves using the Kaplan-Meier method demonstrate a trend in superior 5-year overall survival for patients with skin-limited disease at diagnosis (Log-rank,  $p=0.06$ ; A), and no difference in 5-year overall survival for the mutational status of *MYD88* and/or *CD79B* (Log-rank,  $p=0.64$ ; B). For survival analysis, patients diagnosed at autopsy ( $n=3$ ) were excluded and patients with a follow-up of less than 5 years without an event ( $n=3$ ) were censored. The patient without staging procedures was classified as systemic disease due to severe B symptoms and accompanying poor performance status at diagnosis, and the 2 patients with unknown *CD79B* status were classified according to their *MYD88* status. IBM SPSS Statistics 23 was used for statistical analysis.



**Supplemental Table 1.** Associated clinical, immunohistochemical, and molecular features for each patient

Patient	Gender	Age at diagnosis (y)	Ann-Arbor stage	Involved sites	B-symptoms	Status, follow-up duration (y)	First-line therapy (response); site of relapse → second-line therapy (response)
1	F	78	IE	skin	+	Do, 3.54	RT (PR); skin relapse → RT (PD)
2	F	56	IE	skin	-	D+, 1.35	CHOP (CR); skin/lung relapse → IVMP/DHAP/aSCT (PD)
3	F	63	IE	skin	-	Do, 15.82	CHOP (CR); skin relapse → no treatment (SR)
4	M	61	IE	skin (subcutaneous lipoma)	-	A, 0.95	surgery + watchful waiting (CR)
5	F	66	IE	skin	+	A, 1.35	R-CHOP (CR)
6	F	51	IE	skin	-	A, 29.37	RT (CR); skin relapse → RT (CR)
7	F	85	-	skin (no staging performed)	+	D+, 0.16	supportive care w/ steroids
8	F	63	IV	skin, LN	+	D+, 0.62	R-CHOP (PD); R-DHAP (PD)
9	M	80	IV	skin, muscle, BM	+	Do, 6.17	R-CVP (CR)
10	F	56	IV	skin, lung, brain (choroid)	+	A, 4.96	R-CHOP/MTX (CR)
11	M	61	IV	skin, lung, LN	+	D+, 0.66	CHVMP/BV (PD)
12	F	69	IV	skin, muscle, testis, LN	+	D+, 0.05	supportive care w/o steroids
13	M	57	IV	brain, soft tissue	+	D+, 2.30	supportive care w/o steroids
14	F	73	IV	brain, lung, spleen, liver, kidney, thyroid, myocardium, BM	+	D+, autopsy	-
15	M	73	IV	brain, BM	+	D+, 1.68	R-CHOP (CR); brain relapse → dexamethasone (PD)
16	F	70	IE	brain	+	D+, 0.58	RT cerebrum + dexamethasone
17	F	49	IV	brain, lung, BM	U	A, 8.71	MBVP (PR); R-CHOP (CR)
18	F	53	IV	brain, lung, spleen, liver, kidney	+	D+, 1.03	supportive care w/o steroids
19	F	64	IE	brain	-	D+, autopsy	-
20	M	52	IV	lung, BM	+	A, 10.72	R-CHOP (CR)
21	M	70	IV	lung, BM	U	Do, 10.18	CHOP (CR)
22	M	73	IV	spleen, LN, BM	+	D+, 0.25	rituximab (PD)
23	F	59	IV	spleen, liver	U	D+, 0.07	vincristin + supportive care w/steroids
24	M	74	IV	spleen, liver, BM	+	D+, 0.05	supportive care w/ steroids
25	F	79	IE	kidney	+	Do, autopsy	-

*Abbreviations: F, female; M, male; LN, lymph nodes; BM, bone marrow; U, unknown; Do, died disease unrelated; D+, died disease related; A, alive; RT, radiotherapy; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; IVMP, ifosfamide, mercapto-ethane sulphonate, etoposide, and methotrexate; DHAP, dexamethasone, cytarabine, and cisplatin; aSCT, autologous stem cell transplantation; R-CHOP, CHOP with rituximab; R-DHAP, DHAP with rituximab; R-CVP, rituximab, cyclophosphamide, vincristine, and prednisone; MTX, methotrexate; CHVMP/BV, cyclophosphamide, doxorubicin, teniposide, and prednisone with bleomycin and vincristin; MBVP, methotrexate, teniposide, carmustine, and methylprednisolone; PR, partial remission; PD, progressive disease; CR, complete remission; SR, spontaneous remission; W, weak;*



with an intravascular large B-cell lymphoma.

CD20	CD10	BCL6	MUM1	BCL2	IgM	MYC	CD5	Cyclin D1	CD30	EBER-ISH	MYC-FISH	MYD88 (exon 3/ exon 5)	CD79B (exon 5/ exon 6)	CD79A (exon 5)	CARD11 (exon 6)	EZH2 (exon 16)	
+	-	-	+	+	(W)	+	+	+	-	-	-	WT	WT	WT	WT	WT	
+	-	-	+	+	(W)	+	+	+	-	-	-	NR	WT	NR	NR	NR	WT
+	-	-	+	+	+	+	+	(P)	-	-	-	NR	L265P	Y196C	WT	WT	WT
+	-	+	+	+	+	-	+	-	-	-	-	L265P	Y196F	WT	WT	WT	
+	+	(W)	+	+	+	+	+	+	-	-	-	WT	WT	WT	WT	WT	
+	-	-	-	-	+	-	-	-	-	-	-	NR	L265P	WT	WT	WT	WT
+	-	+	+	+	(W)	+	+	-	-	-	-	L265P	WT	WT	WT	WT	
+	-	-	-	+	+	-	+	-	-	-	-	L265P	Y196F	WT	WT	WT	
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+	-	+	+	+	+	+	-	-	-	-	-	WT	WT	WT	WT	WT	
+	-	+	+	+	+	+	+	-	-	-	-	L265P	WT	WT	WT	WT	
+	-	+	-	+	+	+	-	-	-	-	-	NR	WT	Y196C	WT	WT	WT
+	-	+	-	+	-	-	-	-	-	-	-	WT	WT	WT	WT	Y646F	
+	+	(W)	-	+	+	+	ND	-	-	ND	-	L265P	Y196S	WT	WT	WT	
+	-	ND	ND	ND	ND	ND	+	-	-	ND	ND	WT	WT	WT	WT	WT	
+	-	+	+	+	+	+	-	-	-	-	ND	WT	NR	WT	WT	WT	
+	-	+	+	+	+	+	+	-	-	-	-	WT	WT	WT	WT	WT	
+	-	+	+	+	+	+	-	-	-	-	-	WT	WT	WT	WT	WT	
+	-	-	-	+	+	-	+	(P)	-	-	-	ND	WT	WT	WT	WT	WT
+	-	+	+	+	+	+	-	-	-	-	-	L265P	WT	WT	WT	WT	WT
+	-	-	+	+	+	+	+	-	-	-	-	NR	L265P	WT	WT	WT	WT
+	-	-	+	+	-	ND	-	-	-	-	-	L265P	WT	WT	WT	WT	WT
+	-	+	+	+	ND	-	+	-	-	-	-	ND	WT	WT	WT	WT	WT
+	-	+	+	+	+	+	+	-	-	-	-	ND	WT	WT	WT	WT	WT
+	-	-	-	+	+	-	-	-	-	-	-	WT	WT	WT	WT	WT	WT

*P*, partial; *ND*, not done; *NR*, not reliable; *WT*, wild type; *EBER-ISH*, Epstein-barr virus early RNA in situ hybridization; *MYC-FISH*, fluorescence in situ hybridization with break-apart probes for *MYC*. Immunohistochemistry was performed with antibodies against CD20 (clone L26 from Dako, diluted 1:800), CD10 (clone 56C6 from Dako, diluted 1:20), BCL6 (clone PG-B6p from Invitrogen, diluted 1:100), MUM1 (clone MUM1p from Dako, diluted 1:100), BCL2 (clone 124 Dako, diluted 1:80), MYC (clone Y69 from ABCAM, diluted 1:100), CD5 (clone 4C7 from Dako, diluted 1:400), Cyclin D1 (clone EP12 from Dako, diluted 1:40), IgM (polyclonal, from Dako, diluted 1:500), and CD30 (clone BerH2 from Dako, diluted 1:100).

