Human Cancer Biology

Recurrent Mutations of MYD88 and TBL1XR1 in Primary Central Nervous System Lymphomas

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Abstract

Purpose: Our objective was to identify the genetic changes involved in primary central nervous system lymphoma (PCNSL) oncogenesis and evaluate their clinical relevance.

Experimental Design: We investigated a series of 29 newly diagnosed, HIV-negative, PCNSL patients using high-resolution single-nucleotide polymorphism (SNP) arrays (n = 29) and whole-exome sequencing (n = 4) approaches. Recurrent homozygous deletions and somatic gene mutations found were validated by quantitative real-time PCR and Sanger sequencing, respectively. Molecular results were correlated with prognosis.

Results: All PCNSLs were diffuse large B-cell lymphomas, and the patients received chemotherapy without radiotherapy as initial treatment. The SNP analysis revealed recurrent large and focal chromosome imbalances that target candidate genes in PCNSL oncogenesis. The most frequent genomic abnormalities were (i) 6p21.32 loss (HLA locus), (ii) 6q loss, (iii) CDKN2A homozygous deletions, (iv) 12q12-q22, and (v) chromosome 7q21 and 7q31 gains. Homozygous deletions of PRMD1, TOX, and Dock5 and the amplification of HDAC9 were also detected. Sequencing of matched tumor and blood DNA samples identified novel somatic mutations in MYD88 and TBL1XR1 in 38% and 14% of the cases, respectively. The correlation of genetic abnormalities with clinical outcomes using multivariate analysis showed that 6q22 loss (P = 0.006 and P = 0.01) and CDKN2A homozygous deletion (P = 0.02 and P = 0.01) were significantly associated with shorter progression-free survival and overall survival.

Conclusions: Our study provides new insights into the molecular tumorigenesis of PCNSL and identifies novel genetic alterations in this disease, especially MYD88 and TBL1XR1 mutations activating the NF-kB signaling pathway, which may be promising targets for future therapeutic strategies. Clin Cancer Res; 18(19): 1–9. ©2012 AACR.

Introduction

Primary central nervous system lymphomas (PCNSL) are extranodal non-Hodgkin lymphomas that arise within the brain or the eyes and account for approximately 3% of all primary brain tumors. In immunocompetent patients, the vast majority of PCNSLs (90%) are classified as diffuse large B-cell lymphomas (DLBCL) according to the World Health Organization (WHO) criteria (1). In addition, they are

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-12-0845

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Epstein–Barr virus negative, in contrast with the PCNSLs observed in immunocompromised patients. The standard initial treatment relies on high-dose methotrexate (MTX)–based polychemotherapy with or without whole-brain radiotherapy (2). Little is known about the molecular pathogenesis of PCNSL. This is probably related to the lack of biologic material to study; indeed, most PCNSL samples are obtained from stereotactic biopsies. The PCNSL patients have a median overall survival (OS) ranging from 2 to 5 years, and it is still unclear whether their less favorable outcomes compared with those of patients with systemic DLBCL are linked to the organ-specific microenvironment of the lymphoma or whether they reflect a specific, aggressive, intrinsic biologic behavior. Systemic DLBCLs have been classified into 2 main prognostic subgroups according to their gene expression profile: “germinal center B-cell-like type” (GC) and the “activated B-cell-like type” (ABC; ref. 3). We and others have previously shown that PCNSL can be distinguished from systemic DLBCL in that it expresses both GC-like and ABC-like features, suggesting that it represents an overlapping histogenetic time point of B-cell differentiation that corresponds to an early post-GC stage (4, 5). Several genes and mRNAs have been reported to be highly differentially expressed between PCNSL and systemic DLBCL (6–8). However, it remains unknown whether these expression signatures represent molecular pathways that are specific to PCNSL (9). Studies searching for genomic alterations in PCNSL are scarce. Recently, high-resolution genomic arrays and whole-genome sequencing have been shown to be efficient approaches to achieve comprehensive analyses of chromosome imbalances and gene mutations in solid cancers and hematologic malignancies, including systemic DLBCLs (10–12). These techniques were used to investigate a series of 29 PCNSL samples in this study. Several chromosome regions and genes were identified as being frequently abnormal, suggesting that they may play important roles in PCNSL tumorigenesis. The predictive and prognostic values of these variants were analyzed.

Materials and Methods

Patients and tumor samples
A total of 29 paired PCNSL frozen tumor and blood samples were obtained from 10 institutions. All the tumors were obtained in HIV-negative patients at diagnosis and classified as CD20+DLBCLs on the basis of morphology and immunohistochemistry according to the WHO classification (1). Clinical records were reviewed retrospectively; all the patients were newly diagnosed and systemic lymphoma was excluded by extensive work-up including at the minimum a body–computer tomography (CT) scan or a positron emission tomography (PET)–scan and a bone marrow biopsy. All the patients received chemotherapy without whole brain radiotherapy (WBRT) as initial treatment according to Anocel (Association des Neuronoalogues d’Expression Française) protocols. Clinical data and molecular genetic results were analyzed retrospectively. Written consent of patients was obtained for sample collection and genetic analysis in the setting of research work.

DNA isolation and SNP array
Tumor DNA from cryopreserved samples was extracted using the DNA Midi Kit (QIAGEN) according to the manufacturer’s instructions. The DNA was extracted from blood sample by conventional saline method. The DNA was quantified using the NanoVue spectrophotometer and qualified on gel agarosis. Tumor DNA was run upon Infinium Illumina Human 610-Quad SNP array (Illumina). Array processing, using 250 ng of tumor DNA, was outsourced to Integragen. Extracted data using Feature Extraction software were imported and analyzed using Nexus 5.1 (Biodiscovery).

Quantitative real-time PCR
Homozygous deletions were validated using TaqMan Copy Number Assay. Primers and a FAM-labeled minor groove binder TaqMan probe testing CDKN2A (Hs02736179_cn), PRDM1 (Hs02133894_cn), DOCK5 (Hs01042688_cn), TOX (Hs01944817_cn), and primers and VIC-labeled minor groove binder TaqMan probe testing reference gene RNASE P (ref.: 4403326, Applied Biosystems) were used to assess copy number status. All assays were carried in duplicate on LightCycler480 Multiwell Plate 96. Gene copy number status was determined using the method of 2−ΔΔCt.

Targeted-exome sequencing
Genomic DNA was captured using Agilent in-solution enrichment methodology (SureSelect Human All Exon Kits Version 2, Agilent) with their biotinylated oligonucleotides

Translational Relevance

Biology of primary central nervous system lymphoma (PCNSL) is poorly understood because of the rarity of the disease and the scare availability of biopsy-based tumor tissue. Better knowledge of PCNSL genomic landscape would contribute to improve the management of this tumor. To this end, we conducted a comprehensive genome wide DNA profiling on the basis of single-nucleotide polymorphism–based microarray and used for the first time in PCNSL a whole-exome sequencing approach. Recurrent alterations were identified, including MYD88 and TBL1XR1 mutations, which represent novel potential promising targets in PCNSL for future treatments, as they both might activate the NF-κB signaling pathway. Interestingly, other alterations, that is, CDKN2A homozygous deletion and chromosome 6q22 loss, were correlated through multivariate analysis with poor outcome and warrant to be evaluated as prognostic biomarkers in prospective trials. Our study provides new insights into PCNSL pathogenesis and support further investigations with important clinical relevance.
probes library (Human All Exon v2-46 Mb, Agilent), followed by paired-end 75b massively parallel sequencing on Illumina GAIIx (13). Sequence capture, enrichment, and elution were conducted according to manufacturer's instructions and protocols (SureSelect, Agilent). Each eluted-enriched DNA sample was sequenced on an Illumina GAIIx as paired-end 75b reads. Image analysis and base calling was conducted using Illumina Real Time Analysis Pipeline version 1.8 with default parameters. The bioinformatics analysis of sequencing data was on the basis of the Illumina pipeline (CASAVA1.7). CASAVA conducts alignment of a sequencing run to a reference genome (hg19), calls the SNPs on the basis of the allele calls and read depth, and detects variants (SNPs and Indels). The alignment algorithm used is ELANDv2 (conducts multiseed and gapped alignments). Only the positions included in the bait coordinates are conserved. Genetics variation annotation was conducted on the basis of in-house pipeline, consisting on genes annotation (RefSeq), known polymorphisms (dbSNP 131, 1,000 Genome) followed by a mutation characterization. For each position, the exomic frequencies were determined from all the exomes already sequenced at Integragen, and the exome results provided by HapMap. We also realize a coverage/depth statistical analysis for each bait.

**Sanger sequencing**

Primers and conditions for PCR used for MYD88, PIM1, and TBL1XR1 sequencing are available upon request. The PCR products were purified using NucleoFast PCR (Macherey Nagel). The Big-Dye Terminator Cycle sequencing Ready Reaction (PerkinElmer) was used for the sequencing reactions. Both forward and reverse chains were analyzed on ABI Prism 3730 Genetic Analyzer (Perkin Elmer).

**Statistical analysis**

Progression-free survival (PFS) and OS were calculated from the first day of treatment. The survival distributions were estimated by the method of Kaplan–Meier. The Log-Rank test was used in univariate analysis to compare PFS and OS across groups. The Cox proportional hazards model was used to estimate univariate HRs and 95% CIs presented in Table 4. Variables that were found to be significant in univariate analysis with the Log-Rank test were included in a multivariate stepwise Cox regression model. Because age and Karnofsky performance status are known to be important prognostic factors, they were also introduced in the model, although they were not significant in univariate analysis. All tests were 2-sided. P values less than 0.05 were considered statistically significant. All analyses were conducted with the SAS software version 9.2 (SAS Institute).

**Results**

**Chromosome imbalances**

Nine chromosome regions were recurrently imbalanced in at least 20% of the cases (Fig. 1 and Table 1). The most frequent deletion was 6p21.32 (79%) corresponding to the HLA locus. Three minimal common regions of deletion were identified on chromosome arm 6q: (i) 6q14.1-q16.3 (27%), (ii) 6q16.3 (37%), and (iii) 6q21.1-q25 (34%). Chromosome 12q12-q22 (27%), 7q21.11-q21.12 (20%), and 7q31.1-q31.2 (20%) were the most frequent material gained.

**Homozygous gene deletion and gene amplification**

CDKN2A was by far the most frequently homozygously deleted gene (13 cases, 45%). Other genes were homozygously deleted at a lower rate (Table 2): DOCK5 (3 cases), PRDM1 (2 cases), TOX (1 case). High-level amplifications involved the 7p21.1 region targeting HDAC9 (1 case). CDKN2A, TOX, PRM1, and DOCK5 homozygous deletion were validated using qPCR.

**Somatic gene mutations**

High quality and large amount (3 μg) of DNA were requested for the whole-exome sequencing experiments. From the cohort of 29 samples, 4 tumor DNA and 4 matched blood DNA fulfilling these criteria were selected for targeted-exome capture. Whole-exome sequencing

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**Figure 1.** Overview of the genomic aberrations found in 29 PCNSL using SNP. Bars at the right of the chromosomes, gains and bars at the left, losses.
revealed 33,628 base substitutions involving 8,991 genes and 3,467 insertions and deletions involving 2,683 genes in tumor DNA. SNP reported in the Hapmap, 1,000 genomes and ncbi refseq databases were removed. Comparison of tumor and matched normal lymphocyte genome showed 1,678 somatic mutations involving 1,424 genes and 71 small insertions and deletions involving 65 genes. We focused on somatic mutations changing protein-coding sequences and found in at least 2 of 4 tumors investigated. We identified 12 recurrent nonsynonymous mutations and 2 insertions involving 7 and 2 genes, respectively. Finally after Sanger sequencing, 5 somatic mutations in 3 genes (MYD88, TBL1XR1, and PIM1) were validated. To specify the incidence of these gene mutations, we screened the 29 frozen sample DNAs of the series by standard Sanger sequencing. Two additional mutations in TBL1XR1 were identified in 2 cases. Altogether, MYD88, TBL1XR1, and PIM1 showed nonsynonymous mutations in 38%, 14%, and 3% of cases, respectively (Table 3).

**Correlation between molecular genetic alterations and patient outcome**

The cohort comprised 15 men and 14 women, median age was 65 years (range: 23 to 81) and the median Karnofsky performance status (KPS) was 70 (range: 40 to 100). Seventeen patients had multiple enhancing lesions on MRI. All but 1 received high dose MTX-based polychemotherapy without WBRT as initial treatment: MTX 3 g/m²–lomustine–procarbazine/C6 prophylactic intrathecal chemotherapy (n = 11); MTX 3.5 g/m²–procarbazine–vincristine–cytarabine (n = 8); MTX 3.5 g/m²–temozolomide (n = 9), temozolomide (n = 1). At the time of analysis, the median follow-up was 79 months. The 3-year PFS and OS were 40% (95% CI, 22 to 58) and 61% (95% CI, 41 to 76), respectively. Salvage treatments in the 22 relapsing or refractory patients were: chemotherapy alone (n = 12), WBRT with or without chemotherapy (n = 5), palliative care (n = 5). Age (<60 vs. ≥60 years), KPS (<70 vs. ≥70), number of enhancing lesions on MRI (single vs. multiple), initial chemotherapy regimen (with or without cytarabine), and the most frequent or relevant genomic alterations were investigated for prognostic significance: 6p21.32 loss (HLA-DRB5 locus), 6q21.3 gain, CDKN2A homozygous deletions, MYD88 and TBL1XR1 mutations. Chromosome 6q loss is complex in our series with 3 minimal common regions of deletions. To assess its previously reported adverse prognostic significance on survival (14), we have tested the chr6:128,591,541-128,845,913 region [including

<table>
<thead>
<tr>
<th>Chromosome region</th>
<th>Cytoband</th>
<th>Size (Mb)</th>
<th>Event</th>
<th>Cases, n (%)</th>
<th>Genes, n</th>
<th>Gene symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr6:32,561,968-32,616,499</td>
<td>p21.32</td>
<td>0.1</td>
<td>Loss</td>
<td>23 (79)</td>
<td>1</td>
<td>HLA-DRB5</td>
</tr>
<tr>
<td>chr6:32,611,800-33,081,054</td>
<td>p21.32</td>
<td>0.5</td>
<td>Loss</td>
<td>10 (34)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>chr6:102,757,131-104,010,647</td>
<td>q16.3</td>
<td>1.3</td>
<td>Loss</td>
<td>11 (37)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>chr6:77,085,561-102,662,047</td>
<td>q14.1-q16.3</td>
<td>25.6</td>
<td>Loss</td>
<td>8 (27)</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>chr6:104,992,890-151,197,385</td>
<td>q21-q25.1</td>
<td>46.2</td>
<td>Loss</td>
<td>10 (34)</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>chr7:83,113,802-86,268,286</td>
<td>q21.11-q21.12</td>
<td>3.2</td>
<td>Gain</td>
<td>6 (20)</td>
<td>4</td>
<td>SEMA3E; SEMA3A, SEMA3D, GRM3</td>
</tr>
<tr>
<td>chr7:113,788,480-115,513,297</td>
<td>q31.1-q31.2</td>
<td>1.7</td>
<td>Gain</td>
<td>6 (20)</td>
<td>3</td>
<td>FOXP2, MDFIC, TFEC</td>
</tr>
<tr>
<td>chr12:37,332,160-91,334,471</td>
<td>q12-q22</td>
<td>54.0</td>
<td>Gain</td>
<td>8 (27)</td>
<td>452</td>
<td></td>
</tr>
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</table>

**Table 2. Homozygous deletions (HD) and high copy gains (HCG) found in 29 PCNSLs**

<table>
<thead>
<tr>
<th>Cytoband</th>
<th>Genes (n)</th>
<th>Candidate gene</th>
<th>Gene product</th>
<th>Biologic function</th>
<th>Event</th>
<th>Cases (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6q21</td>
<td>1</td>
<td>PRDM1</td>
<td>PR domain zinc finger protein 1 Transcriptional repressor</td>
<td>B-cell differentiation into plasma cell</td>
<td>HD</td>
<td>2</td>
</tr>
<tr>
<td>8p21.2</td>
<td>1</td>
<td>DOCK5</td>
<td>Dedicator of cytokinesis 5</td>
<td>Guanine nucleotide exchange factor for GTPases</td>
<td>HD</td>
<td>3</td>
</tr>
<tr>
<td>8q12.1</td>
<td>1</td>
<td>TOX</td>
<td>Thymocyte selection-associated high mobility group box 1</td>
<td>B-cell differentiation</td>
<td>HD</td>
<td>1</td>
</tr>
<tr>
<td>9p21</td>
<td>1</td>
<td>CDKN2A</td>
<td>Cyclin-dependent kinase inhibitor 2A</td>
<td>Cell-cycle G1, control</td>
<td>HD</td>
<td>13</td>
</tr>
<tr>
<td>7p21.1</td>
<td>1</td>
<td>HDAC9</td>
<td>Histone deacetylase 9</td>
<td>Chromatin condensation</td>
<td>HCG</td>
<td>1</td>
</tr>
</tbody>
</table>
the RP11-151E20 and CTD-2378A7 BACs (14)]. In univariate analysis, only 6q22 loss ($P = 0.006$; $P = 0.006$) and CDKN2A homozygous deletion ($P = 0.01$; $P = 0.004$) were associated with significantly shorter PFS and OS. In multivariate analysis, age > 60 years ($P = 0.009$ and $P = 0.03$), 6q22 loss ($P = 0.006$ and $P = 0.01$), and CDKN2A deletion ($P = 0.02$ and $P = 0.01$) were significantly associated with poor PFS and OS, respectively (Table 4 and Fig. 2A–D).

**Discussion**

To our knowledge, the present study is the most comprehensive analysis of genetic alterations in PCNSL to date, using a whole-exome sequencing approach and SNP. We reported novel recurrent somatic gene mutations of MYD88 and TBL1XR1 in PCNSL. We identified focal genetic abnormalities with new putative candidate genes in PCNSL oncogenesis. We also confirmed and extended reports from previous studies that chromosome 6p21.32 deletion, chromosome 6q loss, and CDKN2A homozygous deletion are frequent genetic changes in PCNSL. Finally, the correlation of genetic profiles with clinical outcomes through multivariate analysis showed that 6q22 and CDKN2A deletions were associated with a significant unfavorable impact on prognostic both in term of PFS and OS.

Whole-genome sequencing strategies have successfully identified critical genes in the pathogenesis of several

**Table 3. Genes recurrently mutated in PCNSL**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene product</th>
<th>Function/pathway</th>
<th>Wt codon</th>
<th>Mutant codon</th>
<th>Amino acid change</th>
<th>Mutation type</th>
<th>Exome capture (n = 4)</th>
<th>Validation exome (n = 4)</th>
<th>Whole population (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYD88</td>
<td>Signaling adaptor protein NF-κB and JAK/STAT3 activation</td>
<td>CTG</td>
<td>CCG</td>
<td>L265P</td>
<td>Nonsynonymous</td>
<td>3</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>PIM1</td>
<td>Proviral integration site for MuLVa Ser/Thr kinase</td>
<td>CTC</td>
<td>TTC</td>
<td>L177F</td>
<td>Nonsynonymous</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TBL1XR1</td>
<td>Transducin β-like 1 X-linked receptor 1 Transcriptional regulation of NF-κB and Wnt</td>
<td>GAT</td>
<td>CAT</td>
<td>D421H</td>
<td>Nonsynonymous</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCC</td>
<td>TTC</td>
<td>S419F</td>
<td>Nonsynonymous</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCC</td>
<td>ACC</td>
<td>S419T</td>
<td>Nonsynonymous</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TTT</td>
<td>TCT</td>
<td>F541S</td>
<td>Nonsynonymous</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GTG</td>
<td>GTT</td>
<td>V445V</td>
<td>Synonymous</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Molony murine leukemia virus.*
which is a transcriptional regulator that interacts with TBL1XR1, encodes transducin-
nonymous somatic mutations in found recurrent, potentially functionally relevant, nonsy-
tom 3) signaling and interferon-
k receptor (TIR) domain and would induce a gain of function
mamia (15, 16). The L265P mutation resides in the Toll/IL-1
hematologic malignancies, such as mucosa-associated lym-
tions have also been observed at lower rates in other
sively in the “activated B-cell” type (ABC).
reported the same L265P mutation in
same mutation, L265P. Recently, Ngo and colleagues (16)
encodes IL1 and IL18 receptors. Notably, all PCNSLs harbored the
way after stimulation of the Toll-like receptor and the
a signaling adaptor protein that activates the NF-
ed gene in PCNSL that is currently known.
However, this approach has yet not been used to screen
cancers, including hematologic malignancies (12, 15).
We detected MYD88 mutations in 38% of PCNSL
samples, and MYD88 represents the most frequently mutat-
ed gene in PCNSL that is currently known. MYD88 encodes a
signaling adapter protein that activates the NF-xB pathway
after stimulation of the Toll-like receptor and the
IL1 and IL18 receptors. Notably, all PCNSLs harbored the
same mutation, L265P. Recently, Ngo and colleagues (16)
reported the same L265P mutation in MYD88 as a hot spot
in 29% of systemic DLBCLs, although it occurred exclu-
sively in the “activated B-cell” type (ABC). MYD88 mutations
have also been observed at lower rates in other
hematologic malignancies, such as mucosa-associated lymphoid tissue lymphomas and chronic lymphocytic leukemia (15, 16). The L265P mutation resides in the Toll/IL-1 receptor (TIR) domain and would induce a gain of function by increasing NF-xB activity and enhancing JAK-STAT3 (Janus kinase-signal transducer and activator of transcription 3) signaling and interferon-β production. We also found recurrent, potentially functionally relevant, nonsyn-
onymous somatic mutations in TBL1XR1 in 14% of cases. TBL1XR1 encodes transducin-β-like 1 X-linked receptor 1, which is a transcriptional regulator that interacts with nuclear hormone receptor corepressors (17) and may play a regulatory role in the NF-xB pathway and Wnt-
mediated transcription. Braggio and colleagues (18) recently
described a focal deletion on the 3q26.32 region in 3 of 7
PCNSL samples investigated. Interestingly, this region
encompasses the TBL1XR1 locus. However, to our knowl-
edge, no sequence mutations in this gene have yet to be
described in PCNSL or in systemic DLBCLs (12). Moreover,
1 tumor of our series displayed biallelic inactivation of
TBL1XR1 with a somatic mutation of 1 allele and a deletion of
the other allele suggesting that TBL1XR1 may be inacti-
vated as a tumor suppressor gene. Recurrent deletions and
mutations of TBL1XR1 have also been reported in acute
lymphoblastic leukemias (19, 20). However, the precise
mechanisms by which TBL1XR1 mutations contribute to
tumorigenesis remain unclear. Indeed, TBL1XR1 amplifica-
tion has been shown in breast cancer, and its inactivation
reduced tumor cell invasion in in vitro and in vivo knock-
down experiments in malignant mammary cell lines (21).
These observations might suggest contradictory tumor-spe-
cific roles for TBL1XR1 mutations in oncogenesis. PIM-1, which is located on 6p21.2, encodes a serine/threonine
kinase that may contribute to tumor progression in
many cancers by promoting early transformation, cell

Table 4. Univariate and multivariate analysis for PFS and OS

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P value (Log-Rank test)</td>
</tr>
<tr>
<td>PFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agea</td>
<td>2.25 (0.88–5.75)</td>
<td>0.08</td>
</tr>
<tr>
<td>KPSb</td>
<td>1.22 (0.48–3.08)</td>
<td>0.67</td>
</tr>
<tr>
<td>Chemotherapyc</td>
<td>1.07 (0.39–2.98)</td>
<td>0.89</td>
</tr>
<tr>
<td>6q22 loss</td>
<td>3.42 (1.36–8.62)</td>
<td>0.006</td>
</tr>
<tr>
<td>6p21.32 loss</td>
<td>2.1 (0.79–5.42)</td>
<td>0.13</td>
</tr>
<tr>
<td>12q12-22 gain</td>
<td>1.01 (0.39–2.63)</td>
<td>0.98</td>
</tr>
<tr>
<td>CDKN2A hd</td>
<td>2.99 (1.24–7.20)</td>
<td>0.01</td>
</tr>
<tr>
<td>MYD88 mut</td>
<td>1.44 (0.61–3.40)</td>
<td>0.41</td>
</tr>
<tr>
<td>TBL1XR1 mut</td>
<td>2.55 (0.82–7.90)</td>
<td>0.09</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agea</td>
<td>2.33 (0.77–7.01)</td>
<td>0.13</td>
</tr>
<tr>
<td>KPSb</td>
<td>1.15 (0.39–3.38)</td>
<td>0.8</td>
</tr>
<tr>
<td>Chemotherapyc</td>
<td>1.15 (0.32–4.713)</td>
<td>0.83</td>
</tr>
<tr>
<td>6q22 loss</td>
<td>3.78 (1.36–10.53)</td>
<td>0.006</td>
</tr>
<tr>
<td>6p21.32 loss</td>
<td>1.43 (0.40–5.26)</td>
<td>0.58</td>
</tr>
<tr>
<td>12q12-22 gain</td>
<td>1.48 (0.50–4.35)</td>
<td>0.47</td>
</tr>
<tr>
<td>CDKN2A hd</td>
<td>4.37 (1.47–12.95)</td>
<td>0.004</td>
</tr>
<tr>
<td>MYD88 mut</td>
<td>1.59 (0.56–4.43)</td>
<td>0.38</td>
</tr>
<tr>
<td>TBL1XR1 mut</td>
<td>2.60 (0.68–9.90)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Variables included in multivariate analysis were: age, KPS, 6q22 loss, and CDKN2A hd.
Mut, mutation; Hd, homozygous deletion; n.s., nonsignificant.

aAge (<60 vs. > or = 60 years).
bKPS (<70 vs. > or = 70 years).
cChemotherapy (with vs. without high intravenous dose cytarabine).
proliferation and potentially angiogenesis (22). PIM1 is a
target proto-oncogene that is reported in DLBCLs with an
aberrant somatic hypermutation and has been proposed as
an important component of the transformation process
(12). A previous study reported mutations in PIM1 in 5% of
ten cases of PCNSL (23). In contrast, we found somatic
mutations in only 2% of cases in our series. Our identifi-
cation of recurrent somatic mutations in MYD88 and
TBL1XR1 have extended the list of genes, such as PRDM1
(24) and CARD11 (25), that when mutated contribute to the
constitutive activation of NF-kB, emphasizing the
importance of this key signaling pathway in PCNSL. Hence,
patients with PCNSL may be likely to benefit from treat-
ments that inhibit the MYD88 signaling pathway, especially
those with tumors bearing the L265P mutation.

High-resolution genomic arrays (aCGH and SNP) are
powerful tools for the comprehensive analysis of chromo-
some imbalances. To date, only 3 genomic array studies of
PCNSL have been published and were limited to 7, 12, and
19 cases (refs. 18, 26, 27; Supplementary Table S1). Our
study confirmed the main imbalanced region previously
reported and identified novel altered region and candidate
targeted genes. The most frequent changes were losses at
6p21.3 and 6q and gains at 12q12.22. The recurrent loss of
chromosome 6p21.32 involving the HLA locus was
observed in 79% of cases. This feature seems specific to
DLBCLs of immune-privileged sites, including PCNSL and
testicular DLBCLs, and might represent a mechanism of
immune escape from CD8+ and CD4+ cytotoxic T cells via
the downregulation of HLA class II expression (28, 29).
However, few studies have addressed its clinical signifi-
cance. In systemic DLBCLs, 6p21.32 deletion has been
reported in chemotherapy-resistant tumors (30); on the
other hand, the loss of MHC class II gene and protein expression
have been correlated with poor prognosis (31). In this
study, we found no significant correlation between the loss
of 6p21.32 (HLA locus) and outcome. Another recurrent
aberration in PCNSL was the loss of chromosome 6q (27% to
37%), which seems to occur at a higher rate than in systemic
DLBCLs, where a lower frequency has been reported (32, 33).
We found 3 distinct minimal common deletion regions (MCRs),
6q14.1-16.3, 6q16.3, and 6q21-25.1. Several MCRs have been reported in previous studies
(18, 34, 35), suggesting a highly complex rearrangement of
this chromosome, which likely involves more than one
targeted gene. Altogether, up to 4 nonoverlapping MCRs
have been reported to date. Several candidate genes, such as
PRDM1 and PTPRK, have been identified in this region.
PRDM1, located on 6q21, a positive regulatory
domain I protein with a Zinc Finger domain (BLIMP1
protein), which acts as a tumor suppressor (36). PRDM1
inactivation would contribute to lymphomagenesis by
blocking B-cell differentiation into antibody-secreting plas-
ma cells. PRDM1 mutations have been reported in 24% of
systemic DLBCLs (37, 38) and 10% of PCNSLs (4 PRDM1
mutations out of a total of 40 PCNSL samples screened in 2
studies; refs. 24, 26). We observed recurrent homozygous
deletions of PRDM1, which have not been reported else-
where to date, in 2 cases. PTPRK, located on 6q22-23 within
a MCR refined by LOH mapping, encodes a tyrosine phos-
phatase protein that is involved in the regulation of cell
contact and adhesion (39). A loss of PTPRK protein expres-
sion was observed in most of the PCNSLs tested. Further
studies to identify gene mutations and/or rearrangements
are needed to ascertain the involvement of PTPRK in
PCNSL tumorigenesis. Interestingly, the loss of the PTPRK
locus on 6q22 has been associated with a poor outcome
in PCNSL patients in 2 previous studies. However, patient
clinical characteristics were not available (39) or treat-
ments not detailed (14, 39). In the present study, we
observed a significantly shorter PFS and OS in PCNSL
patients with 6q22 loss in multivariate analysis. The most
frequent chromosome gains in our series were 12q12-22,
7q21.11-21.12, and 7q31.1-31.2. The minimal regions of interest on both 12q and 7q should be refined further;
they overlap with loci previously reported to be gained in
PCNSL and encompass many potential candidate genes
(26, 29, 34).

Genomic array techniques detecting also small genomic
alterations, such as gene amplifications and homozygous
deletions, may identify target genes more accurately. In
accordance with previous studies, CDKN2A was the
most frequently deleted gene in PCNSL (45% of cases; refs.
18, 26, 27, 40). Epigenetic silencing has also been
reported (41). CDKN2A is a well-known tumor suppressor
gene, located on 9p21.3, which encodes a cyclin-dependent
kinase inhibitor (p16) that regulates cell-cycle progression.
Inactivation of CDKN2A has been reported in many mali-
gancies. To our knowledge, the correlation between
CDKN2A inactivation and outcome has been has been
suggested in only 1 small study that included 14 patients
(42). Our multivariate analyses confirm the negative prog-
nostic impact of the CDKN2A genotype on both PFS and OS
in PCNSL. Several other genes are of interest because they
were found to be targeted by homozygous deletions (TOX,
PRDM1, and DOCK5) or by amplification (HDAC9) in
PCNSL. TOX is located on 8q12.1 and encodes a DNA-
binding factor that is required for the development of
many cell lineages in the immune system, such as CD4
(+) T cells, natural killer T cells, and regulatory T cells (43,
44) and may contribute to the observed arrest of B-cell
differentiation in PCNSL. Deletions of TOX in one previous
case of PCNSL (18) and in 4% of childhood acute lymphatic
leukemias have been reported (45). HDAC9, which is a
unique gene located in the 7p21.1 amplified region, encodes
a member of the class IIa histone deacetylase (HDAC)
enzyme family, which is involved in chromatin condensa-
tion and transcriptional repression (46). HDAC9 gain has
been described in squamous cell cervical carcinomas (47),
and higher HDAC9 expression has been associated with
poor prognosis in childhood acute lymphatic leukemias
(48). DOCK5, which maps to 8p21.2-p21.3, encodes the
dedicator of cytokinesis 5 protein and is recurrently deleted
and underexpressed in osteosarcoma (49, 50). All of these
candidate genes should be further analyzed to explore their
potential and specific contributions to PCNSL pathogenesis.
In conclusion, although our study is retrospective and includes a limited number of patients, it expands our knowledge of the molecular genetic heterogeneity of PCNSL. It highlights novel genetic events with potential clinical and biologic relevance, identifies prognostic biomarkers, and supports further investigations in larger prospective studies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Acknowledgments
This work is part of the National Program Cartes d’Identité des Tumeurs [CIT] (http://cit.ligue-cancer.net/ funded and developed by the Ligue Nationale Contre le Cancer (France). This study benefited from the LOC study group network (Réseau National de Centres Experts des Lymphomes Primitifs du SNC, INCa).

Grant Support
A. Gonzalez-Aguilar, B. Boisselier, N. Habbita, M. Rossetto, and A. Bruno are recipients of grants from the ARTC (Association Pour la Recherche Contre les Tumeurs Cérébrales).

Received March 13, 2012; revised June 7, 2012; accepted June 29, 2012; published OnlineFirst July 26, 2012.


Clinical Cancer Research

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Clin Cancer Res  Published OnlineFirst July 26, 2012.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-0845

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