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Clinicopathologic Correlates of *MYD88* L265P Mutation and Programmed Cell Death (PD-1) Pathway in Primary Central Nervous System Lymphoma

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Abstract

Primary central nervous system lymphoma (PCNSL) patients have a poorer prognosis than systemic lymphoma. Gain-of-function *MYD88* c.794T>C (p. L265P) mutation and programmed cell death-1 (PD-1) pathway alterations are potential targetable pathways. Our study objective was to determine the clinicopathologic correlates of *MYD88* mutation and PD-1 alterations in PCNSL and the impact of Epstein-Barr virus (EBV) infection. We studied 53 cases including 13 EBV-associated (EBV^{POS}) PCNSL, 49% harbored *MYD88* mutation, none seen in EBV^{POS} PCNSL. *MYD88* protein expression did not correlate with *MYD88* mutation. T-cell and macrophage infiltration was common. All PD-L1 positive tumors were EBV^{POS}. Two PD-L1 positive tumors showed 9p24.1/PD-L1 locus alterations by Fluorescence *In Situ* Hybridization. T cells and macrophages expressed PD-1 and/or PD-L1 in 98% and 83% cases, respectively. *MYD88* mutation or protein expression and PD-1 or PD-L1 expression did not predict outcome. We

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Authorship Contributions

TKS, AEK and NMR designed the study, interpreted data, wrote and revised the manuscript; TKS, SMR and NSG collected the data; AEK and MAT collected and interpreted the immunohistochemistry data; LL, YW and CLV performed and interpreted *MYD88* allele specific PCR, AY performed and interpreted FISH studies; TKS, NMR and LH analyzed the data; NSG, LH, LL, SMR, DSM, JPG, SIP, MAT, AY, CLV provided guidance, intellectual input and contributed to the manuscript.

Conflict of Interest Disclosures:

NSG: Sangamo Biosciences (stock)

SIP: BMS, Rafael Pharma, G1 therapeutics, TEVA (consultant); Gilead, Seattle Genetics (Speaker's Bureau); Takada, Teva, Seattle Genetics, BMS (research funding)

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hypothesize that EBV^{POS} PCNSL has a distinct activation mechanism, independent of genetic alterations.

Keywords

PD-1; Checkpoint blockade; MYD88; Epstein-Barr virus; PCNSL

Introduction:

Primary central nervous system lymphoma (PCNSL) is an aggressive lymphoma with a unique pathobiology, which can be leveraged to develop novel therapeutic approaches. Most cases have diffuse large B-cell lymphoma (DLBCL) histology and a worse prognosis than systemic DLBCL [1]. More than 95% PCNSL cases are of activated B-cell like (ABC) subtype [2]. Epstein-Barr virus (EBV) infection is present in a subset, particularly associated with an immunocompromised state. The impact of EBV infection on cellular pathways and the tumor microenvironment in EBV-associated (EBV^{POS}) lymphomas has been described [3, 4]. Moreover, recent advances have identified key pathogenic abnormalities in PCNSL, including mutations in the B-cell receptor pathway (CD79B) [5], toll-like receptor (TLR) pathway (*MYD88*) [6] and the BCL10-CARD11-MALT1 (BCM) complex (*CARD11*) [7]. These molecular alterations converge on the NF- κ B signaling axis, an anti-apoptotic pathway involved in lymphoma pathogenesis, especially ABC-type DLBCL [8, 9, 10, 11].

MYD88 is an adaptor protein for TLRs and several cytokine-signaling pathways. MYD88 mediates the formation of a protein complex containing IRAK1 and IRAK4 kinases. This results in the activation of IRAK4 kinase and phosphorylation of IRAK1, which further mediates downstream activation of NF- κ B. Gain-of-function mutations in *MYD88* were first described in ABC-type DLBCL by Ngo *et al* and were found to be driver mutations in up to 29% of cases [12]. The most commonly identified mutation results in an amino acid substitution, L265P, altering a residue in the MYD88 Toll/IL-1 receptor (TIR) domain. This *MYD88* c.794T>C (p.L265P) mutation was reported to occur at an even higher frequency (>80%) in lymphoplasmacytic lymphoma (LPL) [13] and recent studies have shown a high frequency of the *MYD88*L265P mutation in sanctuary site DLBCLs, including PCNSL and testicular lymphomas [11].

The role of the tumor microenvironment (TME) in promoting tumor progression has been recognized across several malignancies. Immune checkpoints including programmed death 1 (PD-1) and its ligand, programmed death ligand-1 (PD-L1) play an important role in mediating tumor immune escape [14, 15, 16, 17, 18]. Genetic evaluation of PCNSL cases has revealed copy number abnormalities (CNA) of the 9p24.1/PD-L1 locus [11]. In addition, the presence of PD-L1 protein expression in PCNSL [19, 20] has led to studies incorporating immunotherapeutic strategies for PCNSL with early evidence of clinical activity of nivolumab in relapsed/refractory PCNSL [21].

The presence of *MYD88*L265P mutation has been associated with decreased progression-free survival (PFS) and overall survival (OS) in systemic DLBCL [22], however, the clinical

impact of this mutation in PCNSL is not well described. Moreover, the relationship between MYD88 protein expression and the *MYD88*L265P mutation in PCNSL has not been reported. Similarly the impact of PD-1 pathway markers on systemic DLBCL outcome has been evaluated, however, the corresponding data for PCNSL are sparse [23]. Finally, the frequency of co-occurrence of *MYD88* mutation and PD-1 pathway markers in PCNSL and the impact of EBV infection on these markers has not been reported.

The present study evaluates the prognostic significance of therapeutically relevant alterations in MYD88 and PD-1 pathways. The primary objective of this study was to examine the clinicopathologic correlates and co-occurrence of *MYD88* mutation and PD-1 pathways in PCNSL and their impact on clinical outcome. The secondary objectives were to determine the relationship between *MYD88* mutation and MYD88 protein expression in tumor cells and to study the differences in prevalence of *MYD88* mutation and PD-1 pathway markers in EBV^{pos} and EBV negative (EBV^{neg}) PCNSL. Expanding knowledge of these pathways can help support clinical trials of agents targeting these pathways in PCNSL and also inform the use of rational combinations and targeted correlative studies.

Materials and Methods:

Patient Selection

After obtaining institutional review board (IRB) approval with waiver of consent, clinical data and archived tumor biopsy specimens from 53 PCNSLs [DLBCL histology including post-transplant lymphoproliferative disease (PTLD) involving the CNS] were analyzed. Patients were eligible for the study if they were at least 18 years of age and diagnosed with PCNSL between 2000 and 2016 at two academic medical centers, Vanderbilt University Medical Center (VUMC) and the University of North Carolina Medical Center (UNCMC) at Chapel Hill. In addition, patients were required to have adequate archived tissue specimens. Archived formalin-fixed paraffin-embedded (FFPE) tissue biopsy material was identified from the files of the Department of Pathology, Microbiology & Immunology (PMI) at VUMC and the Department of Pathology and Laboratory Medicine at UNCMC. Biopsies of secondary CNS involvement in cases of systemic lymphoma were excluded.

Pathologic Review

Studies and analyses were performed at VUMC. Two pathologists (A.E.K and M.A.T) reviewed the cases and reported results are based on consensus findings. Pathology reports and hematoxylin-and-eosin (H&E)-stained sections were reviewed. World Health Organization (WHO) histologic subtype was assigned to each case [24]. If previously documented, cell-of-origin classified by the Hans algorithm (CD10, BCL6, and/or MUM1 immunohistochemistry) [25] and EBV-encoded RNA (EBER) *in situ* hybridization results were recorded. Tumor content on the single tissue block subsequently used for additional studies was estimated to the nearest decile. In tumor-rich areas, tumor density (estimated to the nearest decile) and growth pattern (diffuse, scattered, perivascular) were recorded. Unstained 5- μ m tissue sections on glass slides were prepared for immunohistochemistry and *in situ* hybridization, and 3–5 tissue curls (10–50 μ m) in Eppendorf tubes were prepared for DNA extraction and subsequent polymerase-chain reaction (PCR) experiments.

Immunohistochemistry and *In Situ* Hybridization for EBER

The following immunohistochemical stains were applied to unstained sections of each case after deparaffinization (1-hour incubation, Leica Bond Max IHC stainer, Buffalo Grove, IL): anti-Pax-5 (PA0552, Ready-To-Use, Leica, Buffalo Grove, IL); anti-CD3 (NCL-CD3-PS1, 1:100, Leica, Newcastle, UK); anti-CD68 (PA0191, Ready-To-Use, Leica, Buffalo Grove, IL); anti-PD-L1 (CD274, 13684, 1:100, Cell Signaling, Danvers, MA); anti-PD-1 (PDCD1, HPA035981, 1:75, Sigma-Aldrich Co., St. Louis, MO); and anti-MYD88 (ab133739, 1:500, Abcam, Cambridge, MA). Heat-induced antigen retrieval was performed on the Bond Max with Epitope Retrieval 2 solution for Pax-5, CD3, and PD-1 (20 minutes); CD68 and PD-L1 (5 minutes); and MYD88 (10 minutes). For PD-L1, slides were placed in Protein Block (x0909, Dako, Carpinteria, CA). For EBER *in situ* hybridization, enzyme retrieval was performed using Proteinase K (Ref# S3020, Dako, Santa Clara, CA) for 5 minutes. Slides underwent ISH with the Ready-To-Use EBER probe (ISH5687-A, Leica) for two hours followed by incubation with an anti-fluorescein antibody (AR0222, Leica) for 15 minutes.

The Leica Bond Polymer Refine system was used for visualization of all stains. Slides were dehydrated, cleared, and coverslipped. Positive control tissues included benign tonsil (all immunohistochemical stains), placenta (anti-PD-L1), kidney (anti-MYD88), and EBV-infected tonsil (EBER). Positive RNA control staining for EBER could not be performed due to insufficient material in a majority of cases.

Pax-5 expression was used to confirm tumor content, density, and growth pattern estimated by H&E stain. T-cell content within tumor rich-areas was estimated by CD3 expression. Macrophage content within tumor rich-areas was estimated by CD68 expression. PD-L1 expression on macrophages and tumor cells and PD-1 expression on T cells and tumor cells was recorded as positive or negative. MYD88 expression was graded on a semiquantitative 0-to-3-point scale for each density and intensity of expression as previously described [26, 27]. Low expression was defined as a score of 2–4 and high expression as a score of 5–6. EBER was recorded as positive or negative in tumor cells. These methods are summarized in Supplemental Table S1. Method details for fluorescence *in situ* hybridization for 9p24.1 locus and allele-specific PCR for *MYD88* c.794T>C p.L265P mutation analysis are included in the supplement.

Disease Variables, Treatment, and Response Assessment

Clinical, laboratory, and outcome data were obtained from the electronic medical record. We collected baseline data for prognostic factors including age, performance status (PS), elevated serum lactate dehydrogenase (LDH), high cerebrospinal fluid (CSF) protein concentration, and tumor involvement of deep brain regions (periventricular regions, basal ganglia, brainstem, and/or cerebellum) [28]. Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) scale. Data regarding the use of high-dose methotrexate (HDMTX) and radiation were collected. Response was assessed based on International Primary CNS Lymphoma Collaborative Group (IPCG) criteria, including brain imaging, eye examination, and CSF cytology [29]. Patient data were collected from the date of diagnosis to the date of last follow-up or death. We included patients diagnosed at least 12 months prior to data collection start date to achieve a stable cohort and minimize attrition

bias. To minimize information bias, the personnel collecting clinical data were initially blinded to pathology results and vice versa.

Statistical Plan

Outcome Definitions—PFS was defined as the time between date of diagnosis and date of progression or last clinical contact. OS was defined as the time between the date of diagnosis and date of death or last clinical contact.

Sample Size Justification—PCNSL is rare, and sample size was limited by specimen availability in this clinicopathologic correlative study. Tissue availability is often limited due to small stereotactic biopsies. Our cohort of 53 patients is relatively large compared with the majority of reported clinicopathologic studies in PCNSL. The sample size allowed inclusion of 2–3 co-variates for multivariable analysis of PFS (failure $n=29$) and OS (failure $n=34$). This was based on the rule of thumb of one covariate per 10–15 failure events (for survival) to provide tighter confidence intervals for our estimates of hazard ratios [30]. The covariate selection was pre-specified, determined by clinical significance and included MSKCC prognostic score[31] and HDMTX treatment.

Analysis Plan—Continuous variables were described using median and range, and categorical variables using number and percentage. Univariate analyses were performed using chi-square or Fisher’s exact tests for categorical variables and Mann-Whitney U tests for continuous variables. PFS and OS (months) were estimated by the Kaplan-Meier method and compared using a log-rank test. A Cox proportional-hazards regression model determined the effect of disease-related variables on PFS and OS. Two-sided 95% confidence intervals (95% CI) were reported for all estimates. A two-sided test with p -value < 0.05 was considered statistically significant. Statistical analyses were performed using Stata/IC 14.2 software.

Results:

Patient, Disease Characteristics, and Treatment

Fifty-three patients were included in our analysis. Median age at diagnosis was 59 years (range 21–84 yrs.). Twenty-seven patients (51%) were male. Four patients (7.5%) succumbed to disease prior to receiving treatment. The patient and disease characteristics of the complete cohort are summarized in Table I. Baseline characteristics and pathologic feature comparison by immune status are included in supplemental tables S2 and S3, respectively. Comparison by *MYD88*L265P mutation status is outlined in table S4.

Cell of Origin and EBER

Data for cell-of-origin d by immunohistochemistry were available for 50 patients, of which 42 showed an ABC-like immunophenotype (84%). EBER-ISH was positive in 25% (13/52) of cases. Ten of the EBV^{POS} cases were associated with human immunodeficiency virus (HIV). The remaining 3 EBV^{POS} cases were associated with PTLD in the CNS. EBV status was unknown for one patient.

Tumor Content, T-Cell and Macrophage Infiltration, and PD-L1/PD-1 Expression

Tumor content of the tissue sections was estimated from H&E and PAX-5 stains and showed 5–100% tumor (only 3 cases had <20% tumor content). Fifteen cases (28%) showed PD-L1 and/or PD-1 expression by lymphoma cells: 8 cases (15%) that were PD-L1 positive and 8 cases (15%) that were PD-1 positive (including one case that was positive for both PD-L1 and PD-1). In 52 cases (98%), T cells (identified by CD3 positivity) expressed PD1, and in 44 cases (83%), tumor-associated macrophages (TAMs) (identified by CD68 positivity) showed PD-L1 expression (Table II). Figure 1 shows representative photomicrographs from a 28-year-old male patient with HIV.

EBER and PD-1/PD-L1 Expression

All 8 cases with tumor cell PD-L1 expression were associated with EBER positivity by ISH. Conversely, 8/12 of EBV^{POS} cases (67%) had tumor cell PD-L1 expression (PD-L1 not tested in one EBV^{POS} case. None of the EBV^{NEG} cases showed tumor cell PD-L1 expression (P=0.001). One of the 13 EBV^{POS} cases (7.7%) showed tumor cell PD-1 expression, while 7/39 EBV^{NEG} cases (18%) showed tumor cell PD-1 expression (P= 0.66).

9p24.1/PD-L1 Locus Abnormalities by FISH

9p24.1/PD-L1 FISH was performed on 48 cases; for the remaining cases, testing was limited by the tissue availability. One case revealed CNA, and another case revealed amplification of the *PD-L1* gene locus. The corresponding IHC slides also showed tumor PD-L1 expression. These two cases were negative for *MYD88*L265P mutation, both were EBV^{POS}, and one also had tumor PD-1 expression.

MYD88 Protein Expression and *MYD88* c.794T>C (p.L265P) Mutation Analysis

MYD88 protein expression was seen in 50/52 cases (94%) and varied widely by intensity and density of expression. Thirty-six patients (69%) showed high-level MYD88 expression. *MYD88* mutation test was performed in 33 of these patients and a total of 13/33 (39%) harbored the *MYD88*L265P mutation. There was no correlation between high MYD88 expression and presence of *MYD88*L265P mutation (P= 0.12). *MYD88*L265P mutation results were available for 47 of 53 cases; for remaining cases, testing was limited by exhaustion of the tissue within the available tissue block(s). Forty-nine percent (49%, or 23/47) of cases were positive for the *MYD88*L265P mutation. Figure 2 shows the relationship between MYD88 protein expression score and mutation status. Figure 3 depicts photomicrograph examples of variable MYD88 expression scores.

EBER, Tumor PD-1/PD-L1 Expression and *MYD88* L265P Mutation:

All 12 EBV^{POS} cases lacked *MYD88*L265P mutation, whereas 22 out of 34 (65%) EBV^{NEG} cases harbored *MYD88*L265P mutation (P=0.001). Figures S2 and S3 show the overlap between *MYD88*L265P, EBV status and Tumor PD-1/PD-L1 expression. In our study cohort all tumor PD-L1 positive cases were also EBV^{POS} and lacked *MYD88*L265P mutation.

Treatment Response, Progression-Free Survival, and Overall Survival

Thirty-eight (72%) patients received high-dose methotrexate based therapy. The complete response rate in 42 patients was 29%. At a median follow-up of 10.3 months, median PFS for all patients was 11.47 months (95% CI: 7.77 to 15.16). The median OS was 12.27 months (95% CI: 4.43 to 20.11). The median PFS for HIV-negative and HIV-positive cohorts was 11.23 and 11.47 months, respectively (P=0.86); the corresponding median OS was 12.4 and 3.8 months, respectively (P=0.89). The median PFS for *MYD88*L265P mutation-positive and mutation-negative cases was 8.83 months versus 12.4 months, respectively (P=0.55); the corresponding median OS was 12.27 months versus 12.4 months, respectively (P=0.99). The median PFS for patients with tumor cell PD-L1 expression by IHC was not reached versus 11.47 months in negative cases (P=0.29); the corresponding median OS was 12.76 months versus 12.27 months, respectively (P=0.65). The median PFS for patients with tumor cell PD-1 expression was 12.4 months versus 10.17 months in negative cases (P=0.94); the corresponding median OS for these patients was 12.4 months versus 12.27 months, respectively (P=0.92). The median PFS for patients with PD-L1 expression in macrophages was 11.47 months compared with 8.83 months (P=0.34) for patients lacking PD-L1 expression; the corresponding median OS for these patients was 12.27 months versus 24.77 months, respectively (P=0.49). In multivariate analysis using Cox proportional hazards model with MSKCC class, HD MTX, *MYD88*L265P mutation and tumor cell PD-L1 expression, only higher MSKCC class [HR 4.03 (95% CI 1.42 to 11.44; P=0.008), and lack of HD MTX treatment [HR 4.89 (95% CI 1.73 to 13.83; P=0.002) were predictive of OS. None of these co-variables impacted PFS. In the corresponding models with tumor cell PD-1 and microenvironment PD-L1 expression, MSKCC class and HDMTX treatment remained predictive of OS with no effect on PFS. Figure 4 outlines the impact of *MYD88*L265P mutation, tumor PD-1 and PD-L1 expression on survival. Supplemental figures S5 and S6 show additional survival curves.

Discussion:

This study extends our knowledge of the clinical, pathologic, and immune correlates of PCNSL focusing on *MYD88*L265P mutations and PD-1 checkpoint pathway. We report for the first time how *MYD88* protein expression in PCNSL relates to an underlying *MYD88*L265P mutation. In addition, like prior studies of EBV^{POS} lymphoma, these results support the hypothesis that EBV infection has a distinct effect on tumor microenvironment and oncogenic landscape mediated through viral components rather than underlying genetic mutations.

A majority of the patients in our PCNSL cohort had an ABC-like immunophenotype, as has been previously reported [2]. Previous studies have reported a varying incidence of PD-L1 expression by IHC on PCNSL tumor cells. Berghoff *et al*/reported the presence of PD-L1 expression in 2/20 (10%) EBV^{neg} cases of PCNSL [19]. Other studies have since reported a wide variation of positivity in 4–37% EBV unselected cases [32, 33]. We found no tumor cell PD-L1 expression in our 39 EBV^{neg} cases, whereas 8/12 EBV^{POS} cases (66.7%) showed PD-L1 expression by lymphoma cells. The latter finding is corroborated by several studies of EBV^{POS} lymphoproliferative disorders showing expression of PD-L1 by lymphoma cells

[34, 35, 36]. It has been reported that EBV latent membrane protein 1 (LMP1) enhances PD-L1 promoter activity [35], thereby increasing protein expression. This increase often occurs in the absence of underlying 9p24.1 amplification [35], as seen in our study where only 1 of 8 PD-L1-positive EBV^{POS} cases (12.5%) had 9p24.1 amplification.

Tumor cells expressed PD-1 in 7/40 (17%) EBV^{neg} cases, which is comparable to previous reports [19]. Other studies have also shown PD-1 expression by lymphoma cells, however, the pathobiologic significance of this expression remains unknown and may reflect the presence of PD-1 surface marker on the lymphoma clone [37, 38]. Almost all cases (98%) showed PD-1 expression by intratumor T-cells. Similarly, a majority of tumor-associated macrophages (83%) were PD-L1 positive. These findings show that a high proportion of cells in the tumor microenvironment express immune checkpoints and point to a “protective” environment conducive to immune evasion. We also noted an overall higher density of macrophage infiltration compared with T-cell infiltration in the tumor microenvironment.

We also studied the relationship between MYD88 protein expression and *MYD88*L265P mutation to determine whether it can be used as a surrogate for the latter. MYD88 protein expression did not positively correlate with *MYD88*L265P mutation. Our findings are consistent with the results seen in systemic DLBCL and testicular DLBCL [26, 27]. MYD88 protein expression was seen in 94% cases while *MYD88*L265P mutation was detected in one-half of cases, similar to previous reports in PCNSL [6, 10, 11, 39, 40, 41, 42, 43]. This pathway is likely active in most cases of PCNSL, regardless of the presence of an underlying activating *MYD88* mutation. None of the EBV^{POS} cases had an underlying *MYD88* mutation, compared with 64.7% of the EBV^{neg} cases. While this has not been widely studied in PCNSL, our study is in agreement with the findings in Chapuy *et al* where *MYD88*L265P was absent in an 8-patient cohort of EBV^{POS} PCNSL cases [11] and more recent data by Gandhi *et al* showing a higher mutation rate in EBV^{neg} cases [44]. Similar findings have been reported for EBV^{POS} DLBCL [45]. Extrapolating from other EBV^{POS} lymphoma studies, most cases involve activation of NF- κ B [46, 47, 48], likely mediated by LMP1 [49], rather than an underlying gene mutation [45].

HIV status, *MYD88*L265P mutation, MYD88 protein expression, tumor cell PD-L1 or PD-1 expression, or tumor microenvironment PD-L1 expression (primarily macrophages) were not predictive of PFS or OS. Very few studies have evaluated the effect of these factors on outcome. In a 42-patient cohort of HIV-negative PCNSL patients, Hattori *et al* reported that *MYD88*L265P mutation was associated with a trend towards a negative impact on survival, highlighting that larger pooled cohorts would be better suited to revealing any significant impact of these biomarkers on patient outcome [42]. Examining immune markers in PCNSL, Cho *et al* reported PD-1 expression (PD-1 \geq 70 cells/HPF) to be associated with worse PFS and OS. This study used a different cut off PD-1 expression and also did not distinguish between tumor cell and tumor microenvironment expression; thus, it is difficult to compare these results with those of our study [50].

Limitations of our study include a relatively small size to examine the effect of all clinically relevant covariates on survival. The inclusion of patients based on tissue availability can introduce a selection bias, but is difficult to avoid in a clinicopathologic study of a relatively

rare tumor. We collected baseline prognostic factors and additional variables such as HIV infection, EBV infection and treatment variables that can potentially affect the outcome to minimize selection bias. An additional limitation of our study is the lack of precise quantitation of PD-1 and PD-L1 expression on cell types. A number of technical factors inherent to the tissue of interest in this study are associated with staining heterogeneity, both among slides (different cases) and within a given slide (single tissue fragment) for each immunohistochemical stain. Technical factors that we have identified include adjacent paucicellular protein-rich brain tissue, partial tumor necrosis (common), edge artefact related to variable penetration of formalin fixation, the archival nature of the material and variable tissue ages, and differences in tissue fixation between the two institutions from which we are drawing cases. Attempts at comparative image analysis on successive tissue sections in this setting are proving challenging.

Strengths of our study include a detailed evaluation of pathways with translational relevance in PCNSL treatment, this is especially important in informing correlative studies with limited tissue availability. Furthermore, the inclusion of immunocompromised patients expands knowledge regarding this cohort that is often excluded from other studies.

In conclusion, our results support and can potentially inform correlative studies in novel drug combination trials in PCNSL, specifically those with checkpoint inhibitors and NF- κ B pathway directed agents like ibrutinib and lenalidomide. For the latter, MYD88 protein expression should be evaluated as a potential predictive marker along with *MYD88* L265P mutational status since a larger proportion of cases show MYD88 protein expression. In these cases, MYD88 may be upregulated by a mechanism other than mutation. Another important consideration for correlative studies is the difference in EBV^{pos} and EBV^{neg} PCNSL. Furthermore, future studies that pool data from several centers for this rare disease and the use of uniform criteria for defining the abnormalities in pathogenic pathways will help to better determine the prognostic significance of these markers while controlling for clinical prognostic markers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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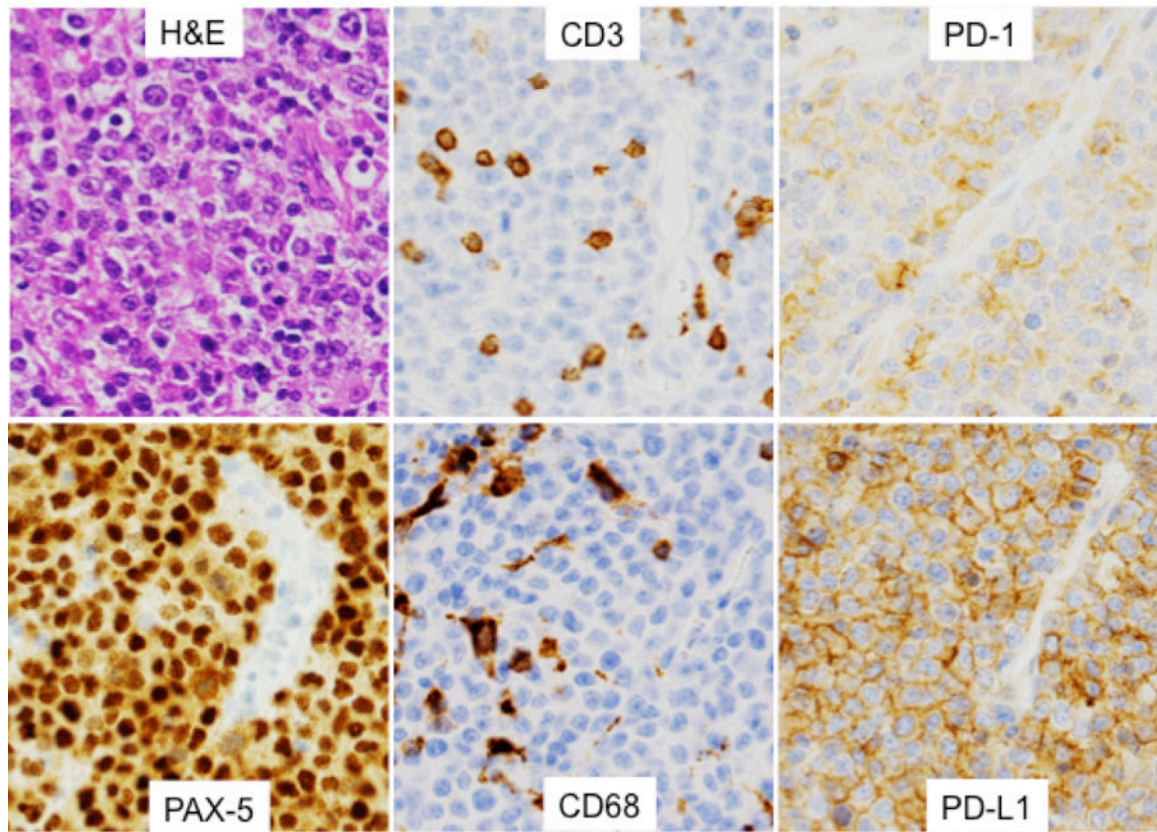


Figure 1. Representative photomicrographs. A) Hematoxylin-and-eosin (H&E)-stained section of a left frontal lobe mass from a 28-year-old male with HIV. The lymphoma cells are large with round to oval nuclei, coarse vesicular chromatin, distinct nucleoli, and scant cytoplasm. B) PAX5 immunostain highlights the lymphoma cells, confirming their B-cell origin. C) CD3 immunostain highlights tumor-associated small T lymphocytes. In this case, T cells represent approximately 15% of the overall specimen cellularity. D) CD68 highlights tumor-associated macrophages, in this case also representing approximately 30% of the cellularity. In this case, the lymphoma cells express both PD-L1 (E) and PD-1 (F). All images were obtained at 400x magnification.

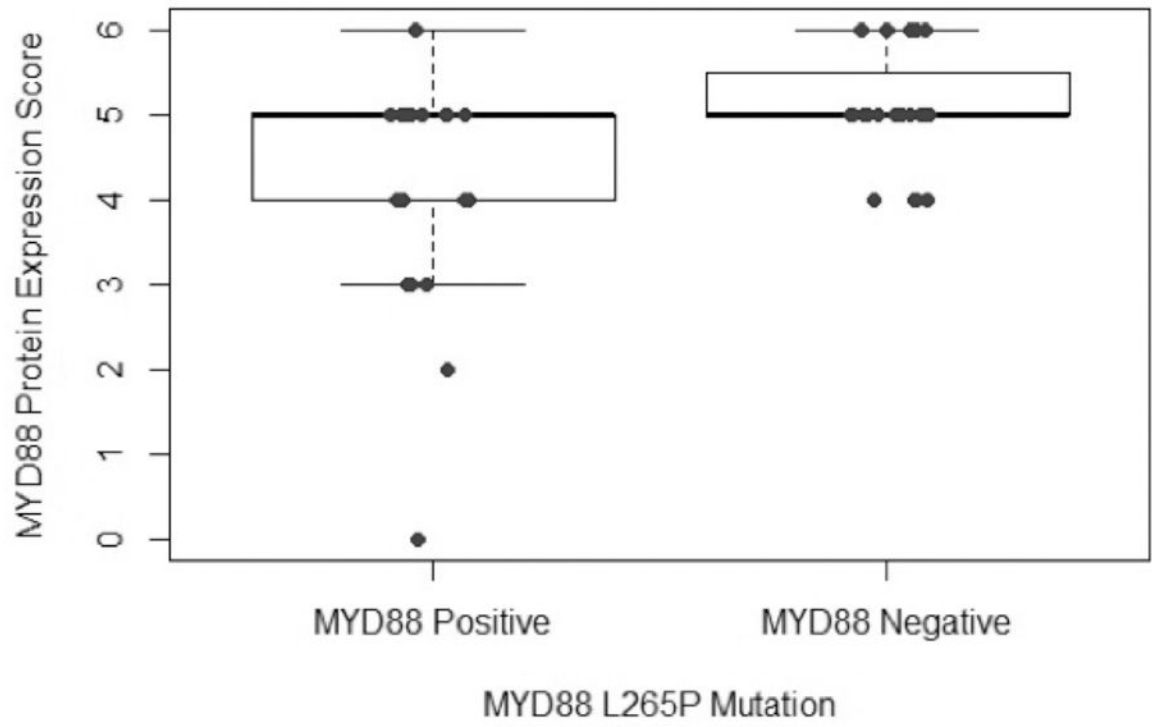


Figure 2:
Plot representing the MYD88 protein expression score (by IHC) vs. MYD88 L265P mutation by PCR

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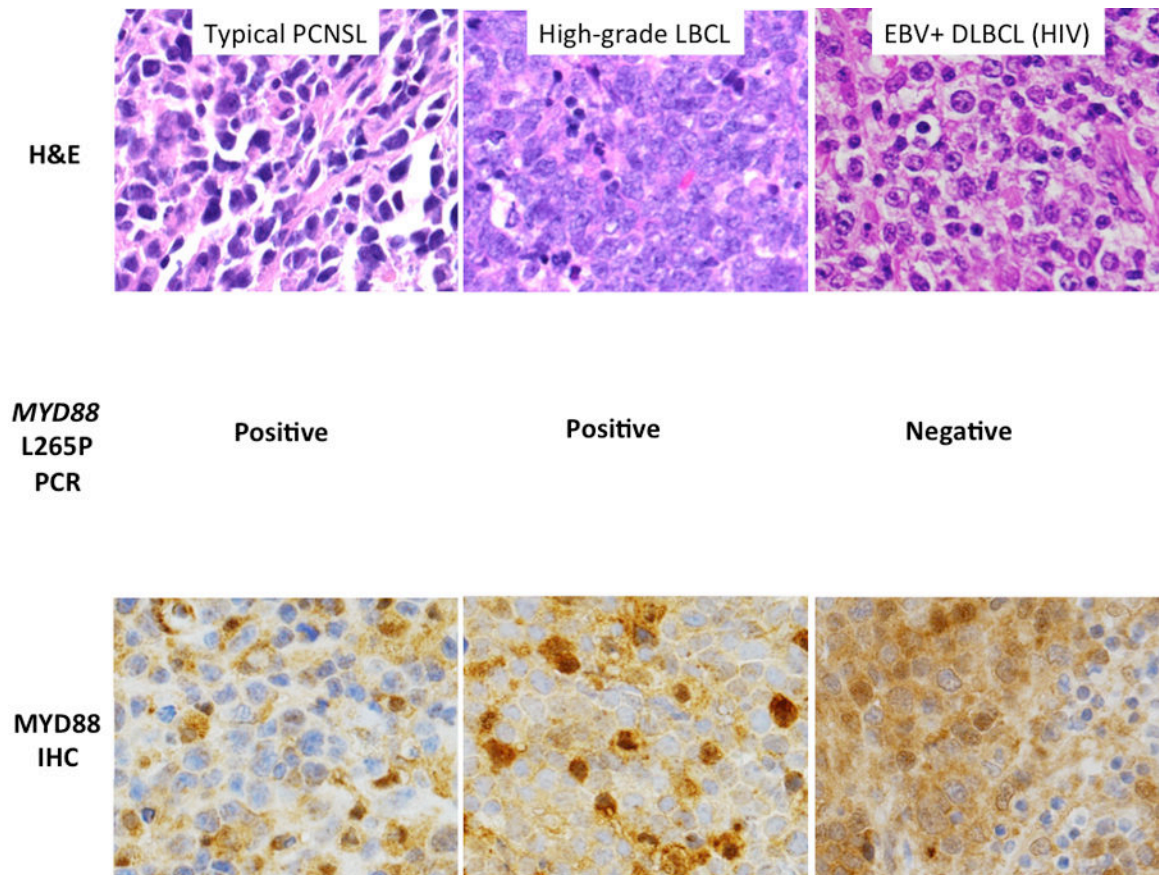


Figure 3: MYD88 immunostain results of representative cases (each photomicrograph 400x magnification): A) Low overall MYD88 expression score of 3 (2/3 for density of tumor-cell expression and 1/3 for level of staining intensity) in a typical case of PCNSL; B) moderate overall expression score (1/3 for density, 3/3 for intensity) in a PCNSL case with a germinal center immunophenotype; and C) high overall expression score (2/3 for density, 3/3/ for intensity) in a case of EBVpos DLBCL isolated to the CNS.

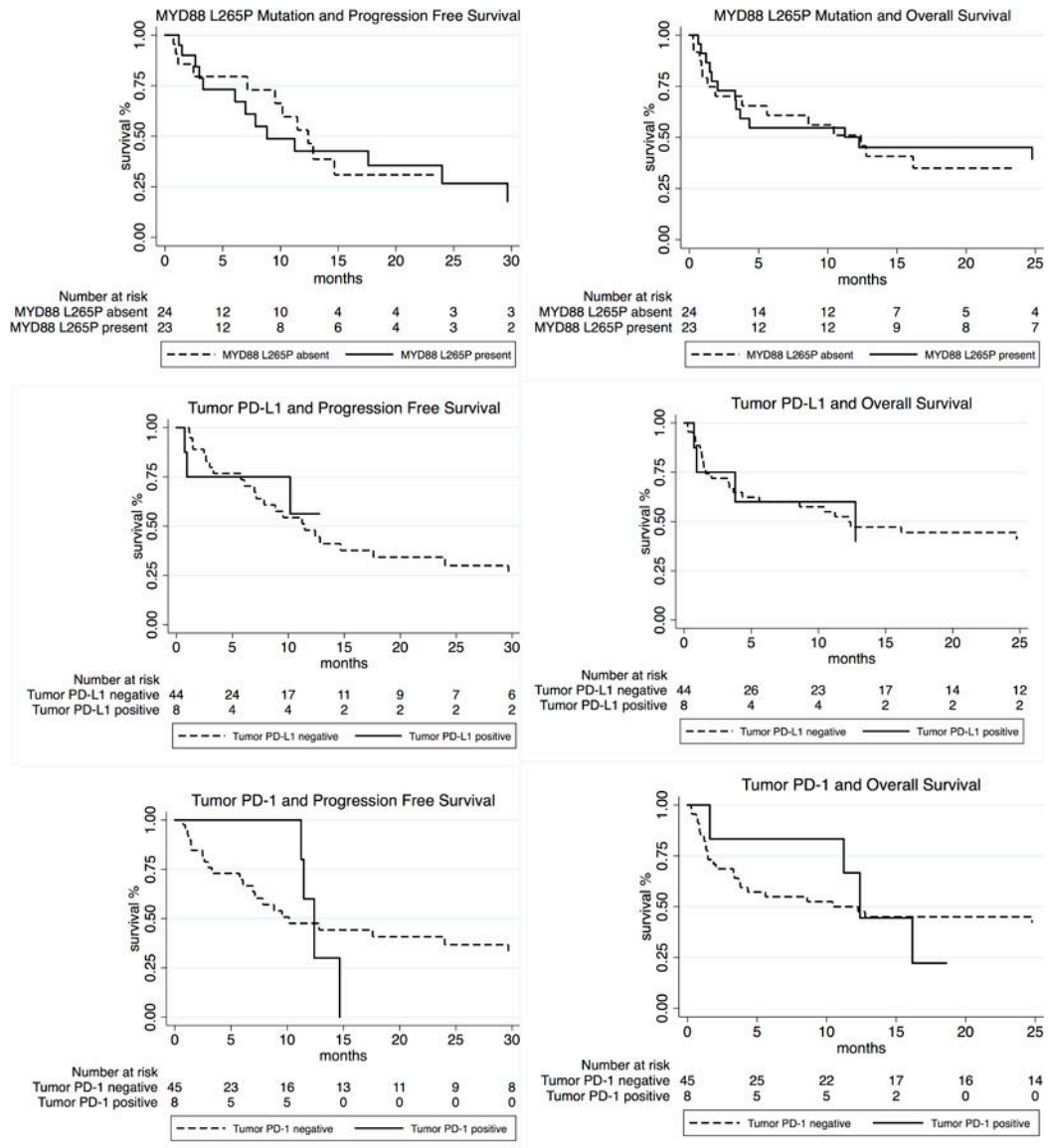


Figure 4: Survival curves outlining the impact of MYD88 L265P mutation, tumor cell PD-L1 and PD-1 expression on PFS and OS.

Table I:

Patient Characteristics

	N*	All Patients [#]
Median age, years (range)	53	59 (21–84)
Male	53	51% (27)
ECOG performance status 2	53	58% (31)
MSKCC Class	53	34% (18)
1		25% (13)
2		41% (22)
3		
LDH elevated	46	50% (23)
Median ALC at Diagnosis (range)	50	110–4080
Elevated CSF Protein	35	80% (28)
Deep Brain Involvement	53	55% (29)
Multiple Foci of Involvement	53	47% (25)
CSF involvement	51	12% (6)
Ocular Involvement	53	8% (4)
HIV positive	53	23% (12)
Post-organ transplant	53	6% (3)
High-dose methotrexate	53	72% (38)
Whole-brain radiation therapy	53	42% (22)

* N is number of non-missing values.

[#] Numbers after proportions are frequencies unless otherwise specified

HIV: human immunodeficiency virus; ECOG: Eastern Cooperative Oncology Group; MSKCC: Memorial Sloan Kettering Cancer Center; LDH: lactate dehydrogenase; ALC: absolute lymphocyte count; CSF: cerebrospinal fluid

Table II:

Pathologic Characteristics

	N*	All Patients [#]
EBER-ISH positive	52	25% (13)
Tumor content (PAX-5) 20% >20%	51	6% (3) 94% (48)
T cell infiltration (CD3) Low (<5%) Moderate (5–20%) High (>20%)	53	30% (16) 32% (17) 38% (20)
Macrophage infiltration (CD68) Low (<5%) Moderate (5–20%) High (>20%)	52	2% (1) 44% (23) 54% (28)
Tumor PD-L1 expression	52	15% (8)
Tumor PD-1 expression	53	15% (8)
Tumor PD-L1 and PD-1 expression	53	28% (15)
PD-L1 expression on macrophages	53	83% (44)
PD-1 on T cells	53	98% (52)

* N is number of non-missing values.

[#] Numbers after proportions are frequencies unless otherwise specified

HIV: human immunodeficiency virus, EBER-ISH: Epstein-Barr virus-encoded ribonucleic acid in situ hybridization