Article

PD-L1 protein expression in most EBV-driven lymphoproliferative disorders is not associated with 9p24.1 amplification

Alan F. Brown[1], Mohamad E. Salama[1], Jay L. Patel[1], Christian N. Paxton[2], Xinjie Xu[1], Sherrie L. Perkins[1], and K. David Li[1,*]

[1]Department of Pathology, University of Utah - ARUP Laboratories, Inc. Salt Lake City, Utah, USA; [2]Institute for Clinical & Experimental Pathology, ARUP Laboratories, Inc. Salt Lake City, Utah, USA.

Abstract: Programmed Cell Death 1 Ligand 1 (PD-L1) protein expression by tumor cells appears to mediate immune evasion and can be upregulated by 9p24.1 amplification. Alternatively, PD-L1 expression has been linked to EBV-driven activation of the AP-1 and MAPK pathways in EBV-positive Hodgkin lymphoma. We sought to evaluate the status of 9p24.1 with respect to PD-L1 expression in EBV-driven lymphoproliferative disorders (EBV+ LPDs).

We studied 36 LPDs (8 EBV+ diffuse large B-cell lymphoma (DLBCL), 10 DLBCL, NOS, and 18 others) using two PD-L1 immunohistochemical (IHC) stains and performed genome-wide copy number variation analysis on a subset of cases. Most cases of EBV+ DLBCL (5/8, 63%) showed PD-L1 expression by IHC and had normal copy number at 9p24.1 (7/8; 88%). One case of EBV+ DLBCL expressed PD-L1 with concurrent 9p24.1 amplification. A subset of EBV-negative DLBCLs showed PD-L1 expression without 9p24.1 amplification. Additionally, EBV+ LPDs displayed significantly lower total genomic aberrations and deletion 6q compared with EBV-negative cases.

PD-L1 expression in most EBV+ DLBCL cases is not due to 9p24.1 amplification. EBV infection appears to coincide with PD-L1 expression, supporting the model for EBV-driven PD-L1 upregulation. PD-L1 expression was seen in some cases lacking both EBV and 9p24.1 amplification, suggesting additional pathway(s) of activation.

Keywords: EBV, diffuse large B-cell lymphoma, lymphoproliferative disorders, PD-L1, immunohistochemistry, microarray

Introduction

EBV-positive diffuse large B-cell lymphoma (EBV+ DLBCL) was originally described in older individuals and recognized in the 2008 WHO Classification as EBV+ DLBCL of the elderly. It is a rare, aggressive, EBV-driven malignancy with a poor prognosis [1, 2]. Initially, the diagnosis of EBV+ DLBCL was restricted to adults over 50 years of age without evidence of immunodeficiency or prior lymphoma [2, 3]. However, this entity will soon be renamed as EBV+ DLBCL in the 2016 WHO revision due to

*Correspondence: K. David Li, Division of Hematopathology, University of Utah -ARUP Laboratories. 500 Chipeta Way, 115-G04, Salt Lake City, UT 84108. Tel: 801-581-5854; Fax: 801-585-3831; (Email) - david.li@hsc.utah.edu

subsequent studies that revealed cases involving a broader age range [4]. Increased awareness of EBV-driven lymphoproliferative disorders (EBV+ LPD) including EBV+ DLBCL has encouraged acceptance of a broader disease spectrum with several etiological explanations for tumoral growth and immune tolerance, including EBV-driven expression of surface programmed cell death-1 ligand (PD-L1) on infected lymphocytes [5].

Briefly, latent EBV utilizes a small number of genes to maintain infection while evading host immunity. Based on different patterns of gene expression, latency can be classified as either type I, II or III with the majority of EBV+ DLBCLs and post-transplant lymphoproliferative disorders (PTLDs) displaying a spectrum of type II and III latency [6]. The oncogenic properties of EBV are thought to be driven primarily by viral latent membrane protein-1 (LMP-1) which expresses multiple cytoplasmic signaling proteins including a CD40-mimicking tail bound to the cytoplasmic membrane that triggers B-cell proliferation via the NF-κB, as well as, JAK2/MAPK, AP-1/Jnk, MEK-ERK, and PI3K-Akt pathways [7–9].

The programmed cell death-1/programmed cell death-1 ligand (PD-1/PD-L1) pathway appears to enact a substantial role in tumor evasion in a growing list of malignancies [10]. PD-L1, also known as CD274 or B7-H1, is an immunomodulatory surface glycoprotein primarily expressed by antigen-presenting cells to regulate cellular immune response [11, 12]. Activation of the PD-1/PD-L1 signal pathway leads to "functional exhaustion" of activated T-cells in peripheral tissues and creates an immunosuppressive microenvironment, allowing tumor cells to escape host immune surveillance and proliferate [11]. Conceptually, treatment with PD-1/PD-L1 blocking antibodies (checkpoint inhibitors) may enhance the endogenous anti-tumor response, leading to improved tumor suppression in conjunction with established chemotherapeutic protocols [10]. In clinical trials using manufactured anti-PD-1/PD-L1 monoclonal antibodies against solid tumors, PD-1 signaling blockade resulted in restoration of immunologic control of the neoplastic cells and long-lasting clinical response [13]. Thus, use of anti-PD-1/PD-L1 (checkpoint inhibitor) therapies represents a promising targeted option in the treatment of other PD-L1 expressing neoplasms including many hematolymphoid malignancies [14].

The mechanism whereby PD-L1 is upregulated in lymphomas is an area of active investigation. It has been observed that B-cell lymphomas with 9p24.1 amplification express increased PD-L1 membrane protein due to increase in gene-to-product expression modulators. The copy number of 9p24.1, a genomic region that includes *CD274* (*PDL1*) and *JAK2*, correlates with surface PD-L1 protein expression due to direct amplification of *CD274*, promoted JAK-STAT signaling, and enhanced AP1-dependent signaling components [11, 15]. Alternatively, a subset of EBV+ LPDs display increased surface PD-L1 expression without 9p24.1 amplification, due to stimulation of both AP1-signaling and JAK-STAT-signaling pathways by EBV-encoded LMP-1 [15–17]. Several other potential routes for PD-L1 expression in hematolymphoid malignancies, including interferon (IFN)-γ, human herpes virus (HHV)-8, and IGH rearrangements are currently under investigation [11, 15, 16, 18, 19]. A comprehensive understanding of PD-L1 expression in hematolymphoid malignancies, as well as, the potential impact of PD-1/PD-L1 checkpoint inhibitor therapies in these entities is developing.

Following recent studies by Chen *et al*. which revealed PD-L1 upregulation via EBV-driven pathway activation in classical Hodgkin lymphoma, we sought to examine the 9p24.1 genetic status and PD-L1 IHC protein expression in a previously uncharacterized group of EBV-driven LPDs, including EBV+ DLBCL [11]. Additionally, we examined genome-wide copy number variation (CNV) by SNP microarray, concentrating on 9p24.1 amplification (containing *CD274* and *JAK2*) to assess the previously observed mutual exclusivity between EBV infection and 9p24.1 amplification as mechanisms for

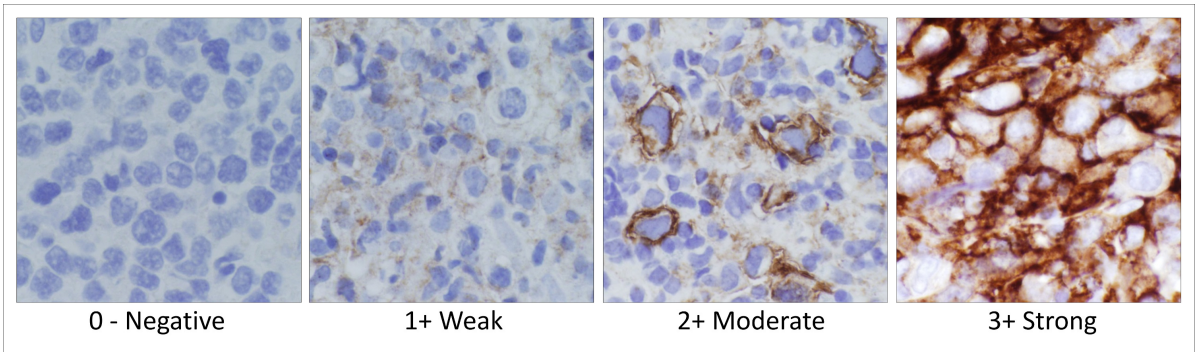

Figure 1: Intensity of linear membranous tumor cell staining by PD-L1 (28-8 pharmDx, Dako) (100x oil magnification).

PD-L1 expression [11, 15, 16]. We hypothesized that EBV infection is a driver of PD-L1 expression in EBV-positive LPDs in the absence of 9p24.1 amplification.

Materials and Methods

Case Selection

The criteria for case inclusion were: (1) morphologically confirmed diagnoses of EBV+ LPDs including EBV+ DLBCL according to the current WHO classification [2, 4] and (2) adequate paraffin-embedded tissue or unstained slides for immunohistochemical (PD-L1) or in situ hybridization (EBV) stains. EBV+ groups were compared against an EBV-negative counterpart when available, for example EBV+ DLBCL cases were reviewed with EBV-negative DLBCL cases. All specimens were reviewed by an experienced hematopathologist prior to inclusion in this study, and if needed, reclassified in accordance with current WHO criteria including recently published updates to ensure accurate final classification. Our case cohort (n=36) was comprised of 35 cases of B-cell LPDs and 1 case of extranodal T/NK lymphoma; including 8 cases of EBV+ DLBCL , 10 cases of EBV-negative DLBCL, not otherwise specified (DLBCL, NOS), and 18 additional cases, predominantly composed of EBV+ LPDs (Table 1). The study proposal was approved by the Institutional Review Board at the University of Utah.

Immunohistochemistry and In situ Hybridization

IHC was performed on archival formalin-fixed paraffin embedded (FFPE) sections (5 μm) of high burden (>50% tumor) tumoral tissue and placed on charged glass slides. Slides were stained for PD-L1 following manufacturer protocol using two rabbit anti-PD-L1 monoclonal antibodies (28-8 pharmDx, predilute, Dako, Carpintera, CA; and, E1L3N XP®, 1:50, Cell Signaling Technology, Danvers, MA) using the Autostainer Link 48 (28-8 pharmDx, Dako) and BenchMark^{TM} Ultra (E1L3N, Ventana Medical Systems, Roche, Tucson, AZ). Two PD-L1 clones (28-8 and E1L3N) were used to assess staining characteristics and inter-stain variability particularly regarding an FDA-approved companion diagnostic stain (28-8).

Linear membranous PD-L1 expression by IHC was independently scored by two experienced hematopathologists for both percentage (%) and intensity (0-absent, 1-weak, 2-moderate, 3-strong) [Figure 1]. Human placental tissue and provided control cell block were used for stain intensity calibration (2+) and quality control for E1L3N and 28-8 stains, respectively. Cases were considered positive for PD-L1 if >5% of tumor cells showed at least 2+ membrane staining [11, 20, 21]. Discordant results were independently scored by a third hematopathologist and reviewed for consensus interpretation.

EBV by in-situ hybridization (EBER) was performed on 35 cases using the EBER1 DNP probe

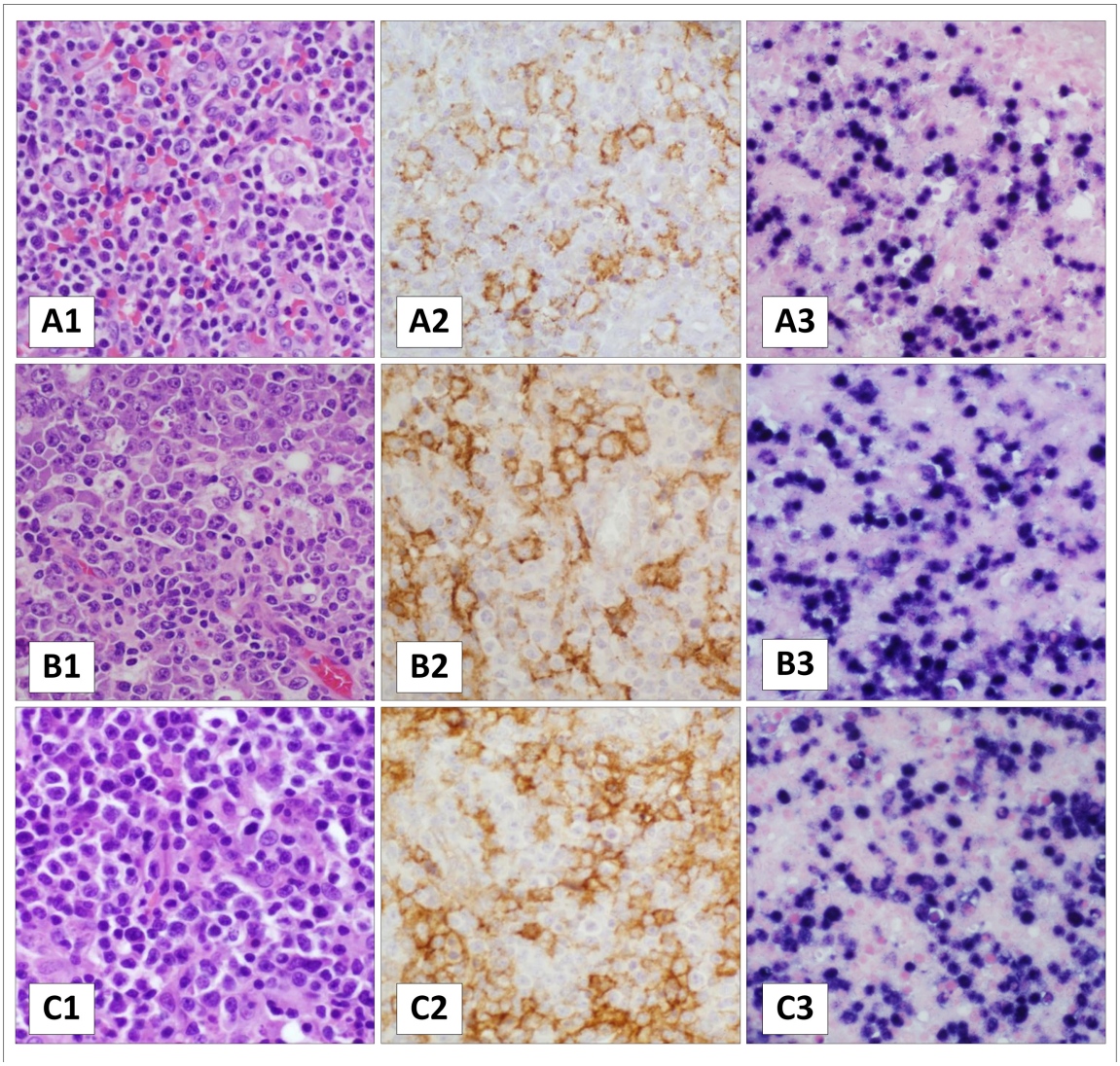

Figure 2: Comparison of H&E (1), PD-L1 IHC (2), and EBER ISH (3) in cases of EBV+ monomorphic, post-transplant lymphoproliferative disorder (A), plasmablastic lymphoma (B), and EBV+ DLBCL (C). Images captured with Olympus DP71 at 40x magnification.

(EBER 1, Ventana Medical Systems, Tucson, AZ) following manufacturer protocol also using a BenchMark™ Ultra autostainer (Ventana). EBER ISH stains were manually scored for percentage of tumor cells staining (%) and were considered positive if any tumor cells expressed strong nuclear staining, with all EBV-positive cases displaying at least 20% tumor cell positivity.

Genomic Microarray

Tissue sections (scrolls, each 5 to 6 μm thick) were cut from FFPE blocks and mounted on glass slides. A single slide from each case was stained with H&E to identify tumor-rich areas and applied as a template for macrodissection of the remaining unstained slides. Genomic DNA from tumor-rich tissue was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) following the

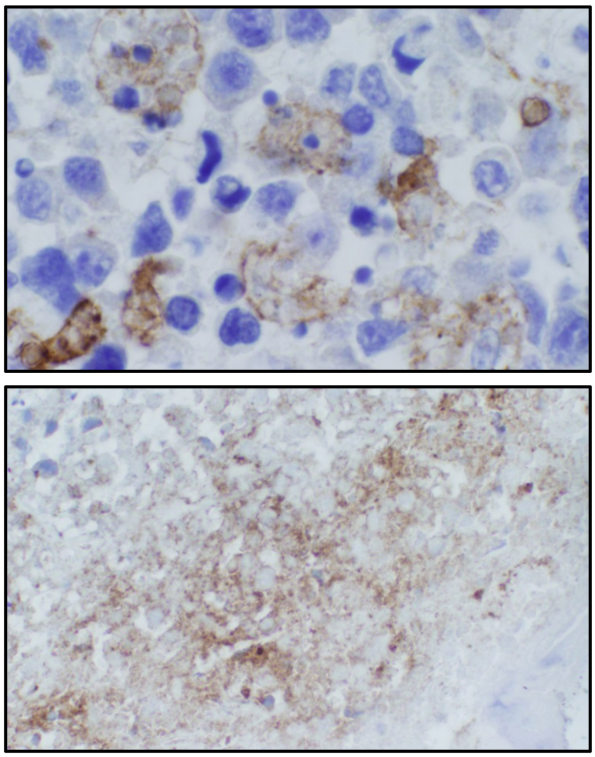

Figure 3: Histiocyte-rich areas (upper panel, 100x oil) and necrotic tissues (lower panel, 50x oil) represented a frequent source of non-tumoral staining in PD-L1 immunohistochemical stains, particularly in high grade neoplasms.

manufacturer's recommended protocol. Following extraction, DNA was quantified using the Quant-iT™ PicoGreen®dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA). The concentration of the DNA stock was adjusted to approximately 12 ng/μl (ranging from 4.3 ng/μl-17.7 ng/μl) using reduced EDTA TE buffer (10 mM Tris-HCl, 0.1 mM disodium EDTA, pH 8) or by vacuum evaporation depending on the starting concentration. In preparation for the assay the DNA stock was plated at 6.6 μl/well (28ng-116.8ng total DNA/well) in MicroAmp Optical 96-well reaction plates (Life Technologies).

Genome-wide copy number variation (CNV) analysis was performed with the OncoScan®FFPE Assay (Affymetrix, Santa Clara, CA, USA) using a modified protocol [22]. This assay contains approximately 220,000 SNP-targeted molecular inversion probes (MIPs) for both genome wide CNV and copy neutral loss of heterozygosity detection at a resolution of 300 kb throughout the genome and 50-100 kb within approximately 900 cancer genes. Data were analyzed using Nexus Copy Number V8.0 (Biodiscovery, Hawthorne, CA, USA) using the Tuscan segmentation algorithm.

A minimum of 20 contiguous probes was required for the determination of a segmental copy number gain or loss. Copy number changes that overlap with common variations are not considered. Regions with amplification were defined as a median log base 2 (log2) ratio of 1.0 or greater. Copy neutral loss of heterozygosity (CN-LOH) was considered as acquired when it is mosaic or larger than 8Mb if terminal or 15Mb if interstitial.

Statistical Methods

For results with objective statistical distribution including microarray results (LOH, CNV length, 6q deletion) the chi-squared test was utilized to test the null hypothesis and aid in the determination of statistical significance (p<0.05).

Results

The majority of EBV+ cases expressed PD-L1 by the 28-8 and E1L3N IHC clones, 91% (21/23) and 71% (13/17), respectively, whereas expression was seen in 53.8% (7/13) and 14.3% (1/7) of EBV-negative cases. Most cases of EBV+ DLBCL (5/8, 63% by E1L3N clone; 5/7, 71% by 28-8 clone) showed PD-L1 expression in tumor cells by IHC and had normal copy number at 9p24.1 (7/8; 88%) (Table 1). Based on the observed individual staining patterns, increased EBER staining appeared to correlate with the degree of PD-L1 expression [Figure 2]. Other EBV+ B-cell lymphoproliferative disorders displayed concordant tumor cell expression of EBER and PD-L1, similar to EBV+ DLBCL. Interestingly, a subset (60% by 28-8 clone) of EBV-negative DLBCL displayed expression

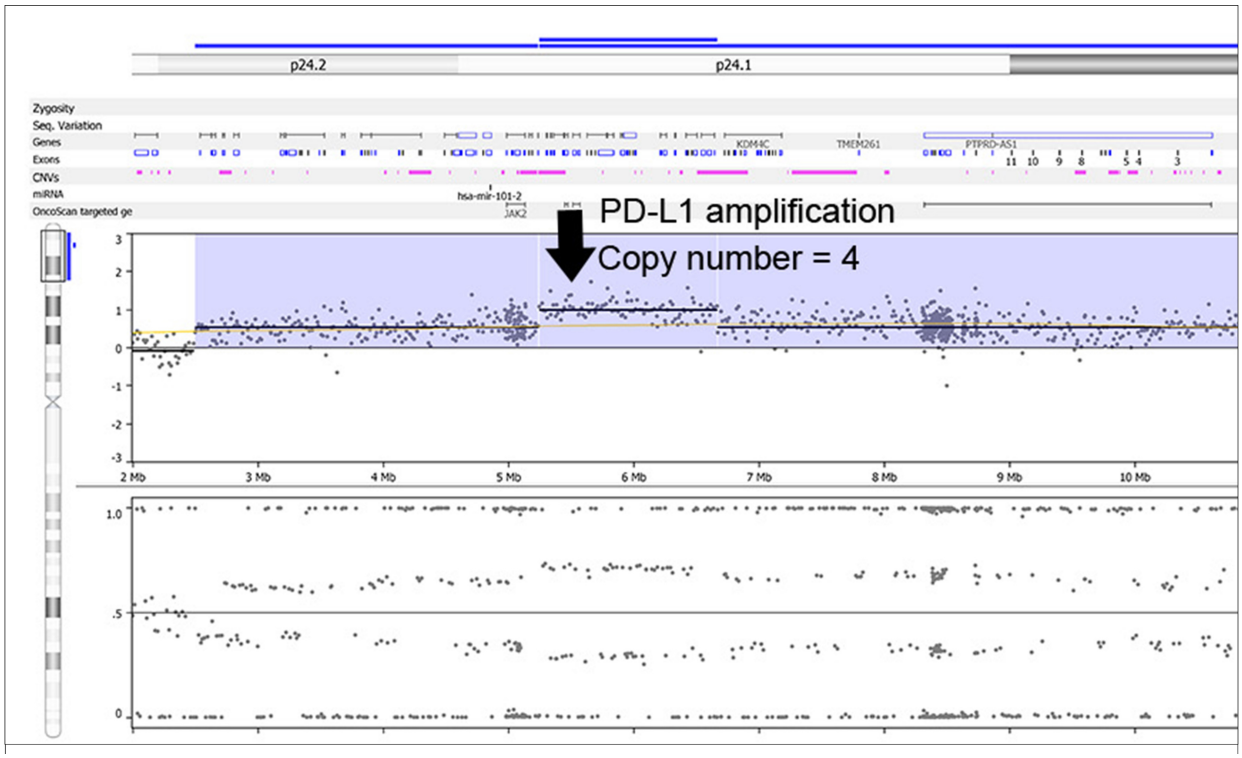

Figure 4: High resolution display of genomic microarray results from one case of EBV+ DLBCL that showed a concurrent 9p24.1 amplification (arrow) involving the *PDL1* gene. Results from chromosome 9 are divided into copy number (top) and B-allele frequency (bottom) plots from genomic microarray. Plots are ordered by genomic position of chromosome 9 along the X axis. The Y axis in the top plot represents the log2 ratio with zero corresponding to a normal copy number. The Y axis in the bottom plot represents B-allele frequency.

of surface PD-L1, suggesting another mechanism of PD-L1 expression. The majority of EBV+ DLBCL that were PD-L1 negative represented small tissue sections predominantly from the gastrointestinal tract, which may have limited thorough representative examination of the overall tumoral environment. We did not appreciate a clear trend for activated B-cell subtype in our EBV+ DLBCL cohort which was possibly limited due to small sample size.

Visual comparison of the two anti-PD-L1 monoclonal antibodies revealed increased sensitivity and slightly reduced background staining with 28-8 clone compared to the E1L3N clone. Only two cases displayed discrepant staining between antibodies. One case of EBV+ mucocutaneous ulcer (MCU) revealed strong PD-L1 staining by 28-8 but absent staining by E1L3N which was thought to be due to poor antigen retrieval or failed reagent delivery due to stark negativity in tumoral cells and microenvironment by E1L3N. The second case, classified as EBV+ DLBCL displayed E1L3N positivity without 28-8 staining (following multiple repeats) which was postulated to be due to antigen degradation in archival FFPE tissue greater than 10 years old. Both IHC clones displayed frequent edge staining artifact, and non-specific staining of intra-tumoral histiocytes and necrotic tissues, which often required review of corresponding H&E and EBV ISH stained slides for accurate PD-L1 interpretation [Figure 3]. Additionally, artifact was inconsistent between samples requiring "customized" review of each case and limiting the ability for automated digital image scoring.

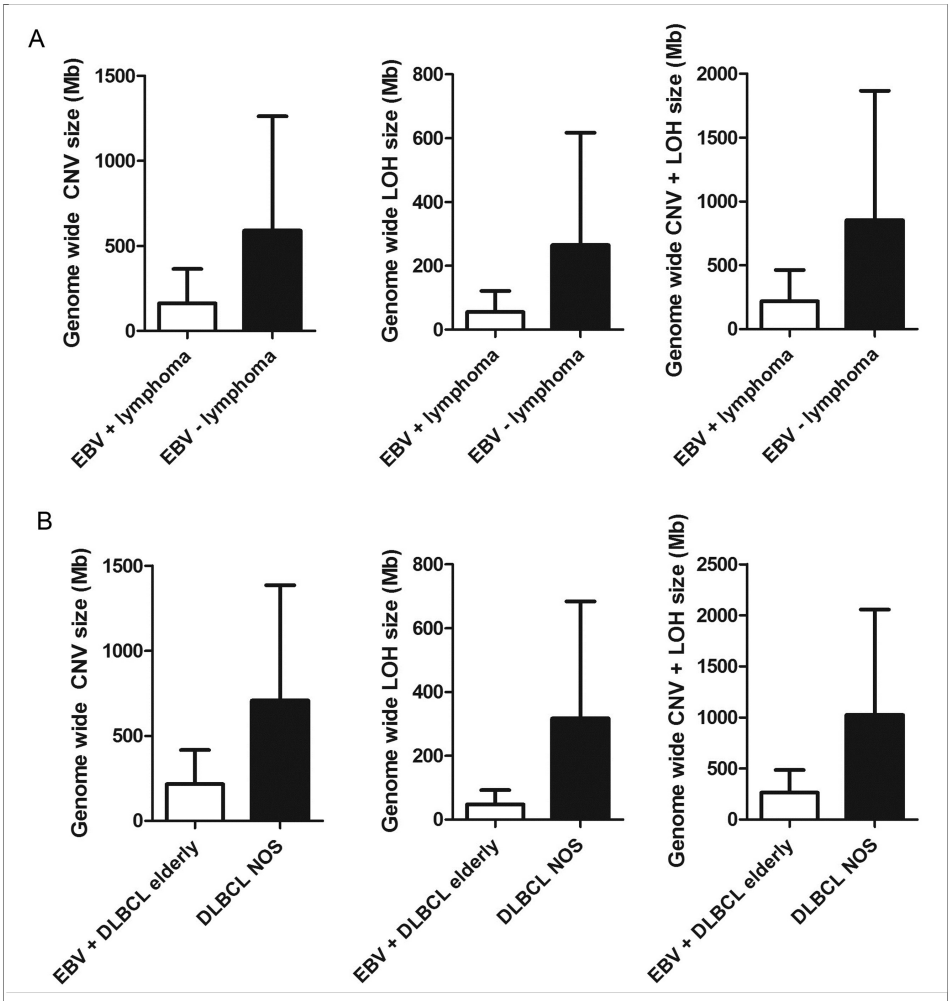

Figure 5: Overall, EBV-positive lymphoproliferative disorders (EBV+ LPDs) (A) exhibited significantly less genomic aberrations compared with EBV-negative LPDs (average total CNV size: 162.2Mb vs. 589.5Mb, p=0.011; average total LOH size: 55.5Mb vs. 264.2Mb, p=0.010; average total CNV+LOH size: 217.7Mb vs. 853.7Mb, p=0.0092). Similarly, EBV-positive DLBCL (EBV+ DLBCL) (B) exhibited significantly less genomic aberrations compared with EBV-negative DLBCL, not otherwise specified (DLBCL, NOS) (average total CNV size: 215.7Mb vs. 707.3Mb, p=0.038; average total LOH size: 47.7Mb vs. 317.1Mb, p=0.029; average total CNV+LOH size: 263.5Mb vs. 1024.4Mb, p=0.032).

Overall, concordance between pathologists following independent interpretation was 89% with 4 cases initially given discordant results due to borderline-low staining in tumor cells (~5%), small biopsy size, rare tumor cells (classical Hodgkin-like) and/or abundant intratumoral histiocytes. Multiple cases displayed frequent histiocytes with PD-L1 expression which required careful (high power) review to differentiate tumor cells from antigen presenting cells. Of note, a particularly difficult interpretation involved a case of DLBCL, NOS with Burkitt-like features and numerous tingible body macrophages.

Genomic microarray was performed on 8 cases of EBV+ DLBCL, 5 EBV- DLBCL, NOS, 9 EBV+ LPDs, and 2 EBV- LPDs. A total of 24 cases were analyzed to maximize usage of the Affymetric OncoScan microarray chip. Microarray results revealed that the majority of EBV-positive lymphoproliferative disor-

ders (16/17) lacked 9p24.1 amplification; however, one case of EBV+ DLBCL exhibited amplification of 9p24.1 region (copy number = 4) involving the *PDL1* gene [Figure 4]. In addition, deletion 6q, which is a common copy number abnormality in lymphoid malignancies, is significantly less frequent in EBV+ LPDs (2/17 in EBV+ LPDs vs. 5/7 in EBV- LPDs, $p<0.01$). EBV+ lymphoma also exhibited significantly less genomic aberrations compared with EBV-negative lymphoma with an average total CNV size: 162.2Mb vs. 589.5Mb, $p=0.011$; average total LOH size: 55.5Mb vs. 264.2Mb, $p=0.010$; average total CNV+LOH size: 217.7Mb vs. 853.7Mb, $p=0.0092$, respectively [Figure 5]. Similarly, EBV+ DLBCL exhibited significantly less genomic aberrations compared with EBV- DLBCL, NOS with an average total CNV size: 215.7Mb vs. 707.3Mb, $p=0.038$; average total LOH size: 47.7Mb vs. 317.1Mb, $p=0.029$; average total CNV+LOH size: 263.5Mb vs. 1024.4Mb, $p=0.032$, respectively. None of the EBV- DLBCL cases displayed *PDL1* locus amplification regardless of PD-L1 expression by IHC stain.

Discussion

Expression of PD-L1 on tumor cells of both solid and hematologic malignancies has been shown to be a poor prognostic factor and a predictive metric for PD-1/PD-L1 checkpoint inhibitor efficacy [13, 23]. Within DLBCLs in general, PD-L1 expression has been associated with activated B-cell (ABC) phenotype, EBV positivity, and rarely 9p24.1 amplification [23]. Targeted immunotherapy of PD-1/PD-L1 has developed as an effective treatment in a growing list of malignancies including some lymphoproliferative disorders.

We reviewed a variety of uncommon EBV-driven lymphoproliferative disorders to assess for PD-L1 expression by immunohistochemical stain and genome wide CNVs by genomic microarray. Based on a prior study by Chen *et al.*, we set a PD-L1 positivity threshold of 5% of the tumor cell population with at least moderate (2+) linear membrane staining intensity; however no formal threshold has been established by consensus perspective [11].

Tumoral PD-L1 expression allows for various mechanisms of T-cell evasion, most importantly

Table 1: PD-L1 Expression by Two IHC Clones in DLBCL and Other EBV-Driven Lymphoproliferative Disorders

Diagnosis	EBER	N	28-8 clone +Tumor[a](%)	N	E1L3N clone +Tumor[a](%)
EBV+ DLBCL	+	7	71%	8	63%
DLBCL, NOS	-	10	60%	5	20%
PTLD, monomorphic, mixed	+	5	100%	3	67%
	-	2	50%	1	0
ILD, mixed	+	5	100%	4	100%
	-	1	0	1	0
Plasmablastic lymphoma	+	4	75%	1	100%
EBV+ mucocutaneous ulcer	+	1	100%	1	0
Extranodal NK/T lymphoma	+	1	100%	-	-
TOTAL	N/A	36	27 (75%)	24	13 (54%)
EBV-POSITIVE	+	23	21 (91%)	17	13 (71%)
EBV-NEGATIVE	-	13	7 (54%)	7	1 (14%)

Abbreviations: PD-L1, programmed death-1 ligand-1 by Immunohistochemistry; EBER, Epstein-Barr virus by probe in-situ hybridization; DLBCL, NOS, diffuse large B-cell lymphoma, not otherwise specified; PTLD, post-transplant lymphoproliferative disorder; ILD, iatrogenic and/or immunodeficiency-related lymphoproliferative disorder; n, number of cases.
[a]: >2+ membrane intensity in at least 5% of tumor cells.

via functional exhaustion and apoptosis of PD-1-expressing intratumoral T-lymphocytes [24]. In vitro studies by Andorsky and colleagues of PD-L1-positive DLBCL cell lines displayed inhibition of T-cell proliferation which likely played a role in antitumor immune suppression, allowing for more aggressive clinical course [25]. Similar to our cohort, factors like age, immunosensence (tolerance), and EBV infection likely led to the increased mortality observed in EBV+ DLBCL occurring in older patients, which appears mitigated in cases involving younger (<50 year of age) individuals [1–3, 26].

Importantly, PD-L1 protein expression in our cohort of EBV+ DLBCL was not a result of 9p24.1 amplification in most cases. EBV infection coincided with PD-L1 protein expression in EBV+ DLBCL supporting the model for PD-L1 upregulation via EBV-activation of AP-1 and MAPK pathways. Furthermore, Green *et al*. demonstrated that EBV infection in Hodgkin lymphoma and PTLD resulted in PD-L1 expression by latent membrane protein 1 (LMP1) - mediated effects on both the PD-L1 enhancer and promoter [15, 16]. EBV infection appears to modulate neoplasia in EBV+ DLBCL in the absence of commonly seen lymphoid CNVs including deletion 6q. In addition, EBV+ DLBCL exhibited significantly less genomic aberrations compared with EBV- DLBCL. This is consistent with observed lowered cytogenetic complexity, possibly due to EBV-driven (LMP1) NF-κB pathway activation among others [27, 28].

While a trend of increased PD-L1 protein expression was appreciated in EBV+ case, it is important to note that a subset of EBV-negative DLBCL cases revealed positive PD-L1 membrane expression. Previous clinical trials with anti-PD-1 monoclonal antibody therapy (nivolumab), including cases of refractory DLBCL, revealed that tumoral PD-L1 expression was more predictive of treatment response than PD-1 microenvironment expression and even showed efficacy in PD-1 negative cases [29]. Interestingly, several recent studies have observed that very low PD-L1 protein expression in malignant melanoma does not entirely preclude checkpoint inhibitor efficacy, but PD-L1 positive tumors displayed a significantly higher chance of treatment response [30]. The degree of expression seen in some EBV-negative cases would meet the current criteria for PD-L1 positivity in other tumors, and encourages consideration for PD-1/PD-L1 checkpoint inhibitor therapy as a possible option for therapy in primary refractory cases.

Ultimately, confident assessment of PD-L1 status required close manual review of the H&E stain, as well, as EBER to select tumor-rich areas to allow for identification of tumor cell expression as opposed to that of the microenvironment. Proper assessment of PD-L1 IHC expression typically required abundant tissue (> 0.5cm^2) and adequate training for interpretation.

This study encountered several limitations that should be noted, both for proper interpretation and course for future studies. Primarily, the sample sizes of our cohorts were small. Given the heterogeneity and rarity of these diagnoses, therapeutic impact studies evaluating the efficacy of PD-1/PD-L1 checkpoint inhibitors will likely require multi-center recruitment to attain satisfactory power. Further, functional analysis is needed to elucidate the roles of this complex pathway. Secondarily, a consensus threshold for PD-L1 positivity in tumoral B-cells has not been established and should be derived following clinicopathologic correlation of therapeutic efficacy. This is of particular interest in the setting of companion diagnostic immunohistochemistry currently used in several other PD-L1 companion diagnostic algorithms [13, 20, 21, 31]. Thirdly, during our review, we appreciated a high degree of PD-L1 stain variability in tumor cells and the surrounding microenvironment. Thus, we recommend caution when reviewing IHC for PD-L1 due to the potential for strong staining in histiocytes and frequent non-specific staining artifact, which may be particularly impactful when performing companion diagnostic testing. Lastly, additional mechanisms of PD-L1 expression in EBV-negative, 9p24.1 copy number nor-

mal DLBCL should be further evaluated. We did not observe a propensity for PD-L1 expression in non-germinal center phenotype (Hans criteria) DLBCLs, however this may be due to our limited sample size. We did not evaluate for the *PDL1* or *PDL2* translocations to *IGH* that were recently reported by Georgiou *et al*, and may represent a significant source of PD-L1 expression in non-germinal center DLBCL, NOS [19]. With the confines of this study, it is difficult to fully ascribe PD-L1 expression in EBV-positive DLBCL to be EBV-driven given the significant number of EBV-negative DLBCLs with PD-L1 positivity.

Conclusion

There is growing evidence of PD-L1 expression and checkpoint inhibitor efficacy in classical Hodgkin lymphoma suggesting that a broader swath of hematolymphoid neoplasms, particularly with expression of PD-L1, may be amendable to checkpoint inhibitor therapy. In this small cohort, we have shown that most EBV+ DLBCLs express PD-L1 in the absence of 9p24.1 amplification, which may be driven by LMP-1 activation (EBV) of the MAPK and AP-1 signaling cascades. Additionally, the presence of increased PD-L1 protein expression in cases without latent EBV infection or 9p24.1 amplification suggests that additional pathway(s) of PD-L1 expression exist that have not been fully characterized. To this end, broader evaluation of somatic variants and rearrangements, as well as, expanded evaluation of checkpoint inhibitor efficacy is needed to evaluate if the observed PD-L1 expression correlates with therapeutic response. Further investigation is needed to better understand the mechanisms of PD-L1 protein expression and the degree to which PD-L1 expression affects checkpoint inhibitor efficacy in EBV+ LPDs.

Acknowledgements

The authors thank Sheryl Tripp for her significant contribution managing, validating and performing PD-L1 protein immunohistochemistry. The authors claim no conflict of interests. The study is supported by William L. Roberts Memorial Grant from University of Utah Department of Pathology.
Received: January 23, 2017 **Accepted**: April 26, 2017
Published: May 16, 2017

References

1. Park S, Lee J, Ko YH, et al. The impact of Epstein-Barr virus status on clinical outcome in diffuse large B-cell lymphoma. Blood 2007; 110(3): 972-978.
2. Swerdlow SH, Campo E, Harris NL, Jaffe SA, Pileri SA, Stein H, Thiele J, Vardiman JW (Eds). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (4th ed.). IARC Press, Lyon 2008: 296-305.
3. Dojcinov SD, Venkataraman G, Pittaluga S, et al. Age-related EBV-associated lymphoproliferative disorders in the Western population: a spectrum of reactive lymphoid hyperplasia and lymphoma. Blood 2011; 117(18): 4726-4735.
4. Swerdlow, SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016; 127(20): 2375-2390.
5. Ok CY, Li L, and Young KH. EBV-driven B-cell lymphoproliferative disorders: from biology, classification and differential diagnosis to clinical management. Experimental & Molecular Medicine 2015; 47(1): e132.
6. Liu, F, Asano, N, Tatematsu A, et al. Plasmablastic lymphoma of the elderly: a clinicopathological comparison with age-related Epstein-Barr virus?associated B cell lymphoproliferative disorder. Histopathology 2012; 61(6): 1183-1197.
7. Huen DS, Henderson SA, Croom-Carter D, Rowe M. The Epstein-Barr virus latent membrane protein-1 (LMP1) mediates activation of NF-kappa B and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. Oncogene 1995; 10(3): 549-560.
8. Dawson CW, Tramountanis G, Eliopoulos AG, Young LS. Epstein-Barr virus latent membrane protein 1 (LMP1) activates the phosphatidylinositol 3-kinase/Akt pathway to promote cell survival and induce actin filament remodeling. J Biol Chem 2003; 278(6): 3694-3704.

9. Roberts ML, Cooper NR. Activation of a ras- MAPK-dependent pathway by Epstein-Barr virus latent membrane protein 1 is essential for cellular transformation. Virology 1998; 240(1): 93-99.
10. Zhang J, Gao J, Li Yanyan, Nie J, Dai L, Fang J. Circulating PD-L1 in NSCLC patients and the correlation between the level of PD-L1 expression and the clinical characteristics. Thoracic Cancer 2015; 6: 534-538.
11. Chen BJ, Chapuy B, Ouyang J, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clinical Cancer Research 2013; 19(13): 3462-3473.
12. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26: 677-704.
13. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443-2454.
14. Armand, P. Immune checkpoint blockade in hematologic malignancies. Blood 2015; 125(22): 3393-3400.
15. Green MR, Monti S, Rodig SJ, et al. Integrative analysis reveals selective 9p24. 1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood 2010; 116(17), 3268-3277.
16. Green MR, Rodig S, Juszczynski P, et al. Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy. Clinical Cancer Research 2012; 18(6): 1611-1618.
17. Fang, W., Zhang, J., Hong, S et al. EBV-driven LMP1 and IFN-gamma up-regulate PD-L1 in nasopharyngeal carcinoma: Implications for oncotargeted therapy. Oncotarget 2014; 5(23): 12189-12202.
18. Stack G, Stacey MA, Humphreys IR. Herpesvirus exploitation of host immune inhibitory pathways. Viruses 2012; 4(8):1182-1201.
19. Georgiou, K., Chen, L., Berglund, M, et al. Genetic basis of PD-L1 overexpression in diffuse large B-cell lymphomas. Blood 2016; 127(24): 3026-3034.
20. Phillips T, Simmons P, Inzunza HD, et al. Development of an automated PD-L1 immunohistochemistry (IHC) assay for non-small cell lung cancer. Appl Immunohistochem Mol Morphol 2015; 23: 541-549.
21. Dako PD-L1 IHC 28-8 pharmDx, Melanoma Interpretation Manual (US Version). Carpintera, CA: Dako Agilent Technologies; 2016.
22. Paxton CN, Rowe LR, South ST. Streamlining the OncoScan®Array Procedure for Use in a Clinical Laboratory. J Assoc Genetic Technologists 2014; 41(2): 61-65.
23. Kiyasu, J., Miyoshi, H., Hirata, A., et al. Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. Blood 2015; 126(19): 2193-2201.
24. Dong H, Strome SE, Salomao DR, et al. Tumor associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8(8): 793-800.
25. Andorsky DJ, Yamada RE, Said J et al. Programmed death ligand 1 is expressed by non-Hodgkin Lymphomas and inhibits the activity of tumor-associated T cells. Clinical Cancer Research 2011; 17(13): 4232-4244.
26. Nicolae A, Pittaluga S, Abdullah S, et al. EBV positive large B cell lymphomas in young patients: a nodal lymphoma with evidence for a tolerogenic immune environment. Blood 2015; 126(7): 863-872.
27. Ok CY, Papathomas TG, Medeiros LJ, Young KH. EBV+ diffuse large B-cell lymphoma of the elderly. Blood 2013; 122(3): 328-340.
28. Montes-Moreno S, Odqvist L, Diaz-Perez JA, et al. EBV+ diffuse large B-cell lymphoma of the elderly is an aggressive post-germinal center B-cell neoplasm characterized by prominent nuclear factor-kB activation. Mod Pathol 2012; 25(7): 968-982.
29. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 2015; 372(4): 311-319.
30. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015; 372(4): 320-330.
31. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small cell lung cancer. N Engl J Med 2015; 372: 2018-2028.