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Phenogenomic heterogeneity of post-transplant plasmablastic lymphomas

Rebecca J. Leeman-Neill¹, Craig R. Soderquist¹,², Francesca Montanari³, Patricia Raciti⁴; David Park¹, Dejan Radeski⁵, Mahesh M. Mansukhani⁶; Vundavalli V. Murty⁶; Susan Hsiao²; Bachir Alobeid¹; Govind Bhagat¹

Department of Pathology and Cell Biology, ¹Division of Hematopathology, ²Division of Personalized Genomic Medicine, ³Division of Hematology/Oncology, Columbia University Irving Medical Center, NY Presbyterian Hospital, New York, NY, USA

⁴Department of Pathology, Greenwich Hospital, Greenwich, CT, USA

⁵Department of Haematology, Sir Charles Gairdner Hospital, Perth, Australia

⁶Department of Medicine, Division of Cytogenetics, Columbia University Irving Medical Center, NY Presbyterian Hospital, New York, NY, USA

Running Title: Phenogenomic features of post-transplant PBLs

Corresponding authors:
Author: Rebecca Leeman-Neill MD, PhD
Address: Columbia University Medical Center
VC14-238
630 West 168th Street
New York, NY 10032
email: rjl2165@cumc.columbia.edu

Author: Govind Bhagat MD
Address: Columbia University Medical Center
VC14-228
630 West 168th Street
New York, NY 10032
email: gb96@cumc.columbia.edu
ABSTRACT

Plasmablastic lymphoma (PBL) is a rare and clinically aggressive neoplasm that typically occurs in immunocompromised individuals, including those with HIV infection and solid organ allograft recipients. Most prior studies have focused on delineating the clinicopathologic features and genetic attributes of HIV-related PBLs, where MYC deregulation and EBV infection, and more recently, mutations in JAK/STAT, MAP kinase, and NOTCH pathway genes have been implicated in disease pathogenesis. The phenotypic spectrum of post-transplant (PT)-PBLs is not well characterized and data on underlying genetic alterations are limited. Hence, we performed comprehensive histopathologic and immunophenotypic evaluation and targeted sequencing of 18 samples from 11 patients (8 males, 3 females, age range 12-76 years) with PT-PBL; 8 de novo and 3 preceded by other types of PTLDs. PT-PBLs displayed morphologic and immunophenotypic heterogeneity and some features overlapped those of plasmablastic myeloma. Six (55%) cases were EBV+ and 5 (45%) showed MYC rearrangement by fluorescence in situ hybridization. Recurrent mutations in epigenetic regulators (KMT2/MLL family, TET2) and DNA damage repair and response (TP53, mismatch repair genes, FANCA, ATRX), MAP kinase (KRAS, NRAS, HRAS, BRAF), JAK/STAT (STAT3, STAT6, SOCS1), NOTCH (NOTCH1, NOTCH3, SPEN), and immune surveillance (FAS, CD58) pathway genes were observed, with EBV+ and EBV- cases exhibiting similarities and differences in their mutational profiles. Clinical outcomes also varied, with survival ranging from 0-15.9 years post-diagnosis. Besides uncovering the biological heterogeneity of PT-PBL, our study highlights similarities and distinctions between PT-PBLs and PBLs occurring in other settings and reveals potentially targetable oncogenic pathways in disease subsets.
INTRODUCTION

Plasmablastic lymphoma (PBL) is an uncommon and aggressive B-cell non Hodgkin lymphoma (B-NHL) characterized by a proliferation of cells with immunoblastic or plasmablastic morphology, occasionally with a component of mature plasma cells, and an immunophenotype indicative of terminal B-cell differentiation. PBL usually occurs in the context of immune dysregulation: HIV-associated, iatrogenic (e.g. post organ transplantation), congenital, or age-related (immune senescence). Prior studies of mostly HIV-associated PBL highlighted the prognostic significance of disease stage and EBV status and genomic analyses have revealed frequent MYC rearrangements, heterogeneous chromosome/DNA copy number abnormalities, variable transcriptional and microRNA profiles, and recently, recurrent mutations in JAK/STAT, MAPK and NOTCH pathway genes.

PBLs occurring after solid organ transplantation (PT-PBLs) are an uncommon type of post-transplant lymphoproliferative disorder (PTLD), accounting for 6-7% of PTLDs and constituting a minor fraction (5-14%) of all PBLs. Only limited data regarding the pathological and molecular features of PT-PBL have been reported.

In order to clarify the pathogenetic bases of PT-PBL, we performed morphologic, immunophenotypic and molecular analysis, including targeted genomic sequencing, of a series of PT-PBLs, comprising de novo PBLs, both primary and recurrent tumors, and those preceded by other types of PTLDs.

METHODS

Case selection

We searched our departmental database for cases of PTLD diagnosed over the past 18 years (2002-2019) to select those fulfilling morphologic and immunophenotypic features of PBLs according to the current WHO classification. Other types of PTLDs preceding PT-PBL were
also identified. Clinical and laboratory data were retrieved from the electronic health records. This study was performed according to the principles of the Declaration of Helsinki and a protocol approved by the Institutional Review Board of Columbia University.

**Morphology and immunohistochemistry**

Formalin-fixed, paraffin-embedded (FFPE) tissue sections were stained with hematoxylin and eosin (H&E) for cytomorphologic evaluation and semi quantitative assessment of the percentage of mature plasma cells. Immunohistochemistry (IHC)/in situ hybridization (ISH) was performed to analyze expression of B- and plasma cell antigens, the cellular microenvironment, a variety of biomarkers and EBV status/latency profiles (see Supplemental Methods).

**Immunoglobulin heavy chain (IGH) gene rearrangement analysis**

Polymerase chain reaction (PCR) analysis for IGH gene rearrangement was performed on DNA extracted from fresh or FFPE tissue using the BIOMED-2 primers, as described previously.15

**Cytogenetic analysis**

G-band karyotyping was performed on metaphase preparations after unstimulated overnight cultures. FISH was performed on metaphase spreads or FFPE sections using TP53/CEP 17 and MYC/IGH/CEP8 probes (Abbott Molecular, Des Plaines, IL) using standard methods. Two hundred cells per hybridization were evaluated. For interphase FISH analysis, the cut-off was 1% for IGH/MYC and 4% for TP53/CEP 17 alterations.

**Targeted genomic sequencing**

DNA was extracted from tumors and matched non-tumor tissue for sequencing a panel of 465 cancer-associated genes, as described previously16 (see Supplemental Methods). Microsatellite instability (MSI) analysis was also performed (see Supplemental Methods).

**RESULTS**
Clinical characteristics

Eighteen samples from 11 patients (8 males, 3 females, median age 61 yrs, range 12-76 yrs) with PT-PBLs, accounting for 11/177 (6%) of “destructive” and 11/98 (11%) of monomorphic (M)- B-cell PTLDs diagnosed at our institution during the study period, were analyzed. PT-PBLs occurred in recipients of heart (4/11, 36%), kidney (3/11, 27%), lung (3/11, 27%) and combined liver/kidney (1/11, 9%) allografts at a median of 9.6 years post-transplantation (range 0.6 – 11.9 years). Intestines were the most common disease sites (6/11, 55%). Three patients had recurrent PBLs and in 3 patients the PBL was preceded by another type of PTLD. Staging marrow biopsies, performed in 7 patients, including 3 of 5 with EBV- PBLs, showed no evidence of PBL/PTLD. Therapy and outcome data are summarized in Table 1 and details provided in Supplemental Data. Serum protein electrophoresis detected low-level monoclonal paraproteins in 4/7 (57%) patients with available results and none had lytic bone lesions on imaging. Results of pertinent laboratory tests and imaging studies are listed in Supplemental Table 2.

Morphologic and immunophenotypic features

All PT-PBLs showed diffuse infiltrates of large immunoblastic or plasmablastic cells (Fig. 1). A minor component of small, more mature plasma cells (plasmacytic differentiation), comprising 10- 20% of the neoplastic infiltrate was seen in 5 (45%) cases (Fig 1A, Table 2). Some PBLs had numerous tingible body macrophages, imparting a “starry sky” appearance (Fig 1B) or multinucleated/anaplastic cells (Fig 1C). Foci of necrosis were observed in 6/11 (55%) cases. Detailed immunophenotypes of all cases are listed in Table 2 and flow cytometry results in Supplemental Table 3. Representative cases are illustrated in Fig. 2. All PBLs expressed MUM1/IRF4, 9/11 (82%) were CD138+ and subsets showed B-cell antigen, CD10, CD56, PD-1 or PD-L1 expression. IgG, IgA or IgM was expressed by 5/11 (45%), 2/11 (18%), and 2/11 (18%) cases. All evaluable PBLs were EMA+ and negative for Cyclin D1, CD117, HHV8 and
ALK. Variable CD30 positivity was noted in 7/11 (64%) cases. The Ki-67 proliferation index ranged from 20 to >90% (median 90%). MYC expression ranged from <10% to 90% (median 45%), with 6/10 (60%) cases showing ≥40% MYC expression. Two of the latter expressed BCL2 in ≥50% of cells (“double expressors”). P53 overexpression was observed in 5/10 (50%) cases. The immunoprofiles and/or proportions of cells expressing certain antigens differed in some PBLs on recurrence. A mild to moderate infiltrate of reactive PD-1+ lymphocytes was observed in all 10 cases evaluated and 5/10 (50%) had populations of PD-L1+ macrophages.

Three PBLs were preceded by other forms of PTLDs; nodal EBV+ M-PTLD (plasmacytoma) (case 5), duodenal EBV+ P-PTLD (case 8), and intestinal EBV- M-PTLD (DLBCL) (case 10).

**EBV status and latency profiles**

The neoplastic cells were EBER+ in 6/11 (55%) PBLs, with 4/6 (67%) showing latency II and 1 case each displaying latency 0/I and latency III profiles. Four EBV+ PBLs and 2 EBV- PBLs occurred in patients seropositive for EBV at the time of transplant. EBV viremia at diagnosis was observed in 4/4 and 2/5 patients with EBV+ and EBV- PBLs with available results (Supplementary Table 2).

**IGH gene rearrangement analysis**

All PT-PBLs and both preceding M-PTLDs showed clonal IGH gene rearrangements. Clonal relatedness was established in all 3 recurrent PBLs and between the PBLs and prior M-PTLDs. The P-PTLD demonstrated oligoclonal products.

**Cytogenetic abnormalities**

Cytogenetic findings are listed in Table 3. All 3 PBLs with informative results showed complex karyotypes. IGH-MYC rearrangements were detected in 5/11 (45%) cases (3 at diagnosis, 2 at recurrence). Multiple copies of MYC due to polyploidy were detected in 2 cases (concurrent with
MYC rearrangement in one case). Three of 6 cases with MYC abnormalities (2 with rearrangements, 1 with gain) showed ≥40% MYC positivity by IHC. PBLs of patients who died of PTLD and those that did not revealed MYC abnormalities in 2/4 (50%) versus 4/7 (57%) cases (p=1.0). Two cases demonstrated IGH rearrangements with unknown partners. Chromosome 17/TP53 abnormalities were detected in 5/8 evaluable cases; subclonal (30-40% of cells) monosomy 17 (1 EBV+, 1 EBV-), multiple copies (polyploidy) of chromosome 17 (2 EBV-), and TP53 deletion (1 EBV+, 1 EBV-). In one case (EBV-), though FISH for TP53 could not be done, targeted genomic sequencing detected loss of TP53.

No MYC or TP53 alterations were observed in the P-PTLD or M-PTLD (DLBCL) preceding PBLs. Insufficient material precluded analysis of the PT-plasmacytoma.

**Targeted genomic sequencing**

Pathogenic and likely pathogenic somatic nonsynonymous variants (SNVs) are enumerated in Table 3 and all, including variants of unknown significance (VUS), are listed in Supplementary Table 1. Excluding samples from 2 patients with high level microsatellite instability (MSI-H), which exhibited up to 194 total SNVs, the PBLs harbored 1-16 pathogenic/likely pathogenic (median 7) and 3-26 total SNVs (median 13) including VUS. MSI status was confirmed by a PCR-based method. All 4 PBL samples classified as MSI-H (cases 6 and 7) demonstrated loss of expression of at least 2 mismatch repair (MMR) proteins (Table 2). After factoring out MSI-H cases, the number of variants was still higher in EBV- than EBV+ PBLs, but the difference was not statistically significant (4.7 mean pathogenic/likely pathogenic and 9.3 total SNVs in EBV+ versus 10.0 mean pathogenic/likely pathogenic and 19.3 total SNVs in EBV- cases, p=0.15 and 0.09). Nor was there a significant difference in the number of variants between patients that died of PBL versus those that did not (4.7 mean pathogenic/likely pathogenic and 10.0 total SNVs vs. 7.3 mean pathogenic/likely pathogenic and 14.0 total SNVs, p=0.51 and 0.6).
Mutations in epigenetic modifier genes were the most frequent in our series, occurring in PBLs from 8/11 (73%) patients, including recurrent mutations in the \textit{KMT2/MLL} family of methyltransferases (\textit{KMT2C}, n=5; \textit{KMT2D}, n=3; and \textit{KMT2A}, n=2), \textit{TET2} (n=3), \textit{ASXL1} (n=2), and \textit{KDM5C} (n=2).

DNA damage response and repair pathway genes were mutated in 7/11 (64%) cases, including 4/5 (80%) EBV- and 3/6 (50%) EBV+ PBLs. Three of 7 (43%) cases, all EBV-, including both MSI-H cases, showed multiple \textit{TP53} mutations. One case with concomitant subclonal monosomy 17 showed 30% P53+ cells, as did 2 with concurrent chromosome 17 gains, suggesting bi/multiallelic inactivation (Tables 2 and 3). Chromosome 17/\textit{TP53} abnormalities were more frequent in PBLs exhibiting plasmacytic differentiation (4/5, 80%) than those lacking it (2/6, 33%), though the difference was not statistically significant (p=0.24). The MSI-H PBLs (Cases 6 and 7) had mutations in the MMR genes, \textit{MLH1} and \textit{PMS2} and \textit{MSH6}, respectively, and they displayed loss of MMR proteins by IHC (Table 2) as well as a high mutational burden. Other recurrently mutated genes included \textit{BRCC3}, \textit{ATRX}, \textit{FANCA}, and \textit{BRIP1}.

Mutations in the mitogen activated protein kinase (MAPK) pathway genes were observed in 6/11 (55%) cases. Eight of 9 mutations, occurring in \textit{KRAS}, \textit{NRAS}, \textit{HRAS}, and \textit{BRAF}, are well-known activating hotspot mutations (\textit{RAS} codons 12, 13, and 61, and \textit{BRAF} codons 469 and 601). Four cases harbored mutations in multiple genes, including one case with concomitant \textit{KRAS} G13D, \textit{NRAS} G12D, and \textit{BRAF} G469V mutations.

Mutations in the \textit{NOTCH} signaling pathway genes were detected in 5/11 (45%) cases. Putative gain- or loss-of-function mutations in genes encoding the \textit{NOTCH} family of proteins were the most frequent. \textit{SPEN}, a negative regulator of \textit{NOTCH} signaling, was mutated in 3 cases. Many of the \textit{NOTCH} pathway mutations were in PBLs with MMR defects and 1 case exhibited mutations in multiple \textit{NOTCH} pathway members at different time points.
Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway genes, including STAT6 (n=3), STAT3 (n=2) and SOCS1 (n=1), were mutated in 4/11 (36%) cases.

Immune surveillance pathway genes were mutated in 4/11 (36%) of cases. FAS was mutated in all 4 cases, all EBV- PBLs, and in the M-PTLD (DLBCL) preceding one PBL. One PBL harbored concurrent FAS and CD58 mutations.

The M-PTLD (plasmacytoma), which harbored a NOTCH1 mutation, acquired a BRIP1 mutation upon transformation. In contrast, some of the mutations observed in the M-PTLD (DLBCL), including a STAT3 variant, were not detected in the clonally related transformed PBL, suggesting divergent evolution from a common ancestor. No variants were identified in the P-PTLD preceding one PBL. The recurrent PBLs showed both acquisition and loss of variants, the recurrent MSI-H PBL (case 6) showing an increasing mutational burden over time.

**DISCUSSION**

Our understanding of PT-PBL pathogenesis is limited and its molecular underpinnings are largely inferred from those reported for HIV-related PBLs. Transcriptional analyses have revealed upregulation of JAK-STAT pathway genes and similarities between PBLs and plasma cell neoplasms (multiple myeloma [MM] and extraosseus plasmacytoma [EOP]) and differences between immunocompetent (IC)-DLBCLs and PBLs, the latter displaying increased expression of MYC and MYB target genes and genes regulating plasma cell differentiation and decreased expression of B-cell receptor and NF-κB signaling pathway genes. However, microRNA expression analysis has suggested two different subclasses of PBL, either resembling Burkitt lymphoma or EOP. Chang et al. documented overlapping chromosome aberrations between PBLs and IC-DLBCLs as well as PBL specific segmental gains at chromosomes 1p and 1q by array comparative genomic hybridization. Whole exome sequencing has uncovered recurrent
gains at 1q21, 6p22 and 11p13, loci that contain several genes (histones, IL6R, MCL1, and CD44) mechanistically linked with B-cell lymphomagenesis.7

MYC deregulation, due to rearrangement, amplification, or activation of certain signaling pathways (e.g. STAT3) is considered important for PBL development.11, 17-19 The frequency of MYC abnormalities (rearrangement and/or gain) in our series (55%) was higher than that reported previously for PT-PBLs (38%), but within the range (36-69%) reported for PBLs arising in other settings.2, 3, 7, 11, 12, 17, 19 In contrast to the study of mostly HIV-related PBLs by Valera et al., MYC rearrangements were more common in EBV- PT-PBLs,19 and in some cases, the rearrangement was detected upon PTLD transformation to PBL or at disease relapse.

Recently, WES and targeted sequencing studies unveiled the mutational landscape, primarily of HIV-related PBLs,7, 11 but until now, the spectrum of genetic alterations underlying PT-PBLs were not explored. While HIV-related PBLs and PT-PBLs appear to exhibit overlapping genomic changes, some differences are evident.

In our series of PT-PBLs, mutations in epigenetic modifiers were among the most frequent alterations (73%). Inactivating mutations of the KMT2/MLL family of histone H3 methyltransferases, which were most common, promote neoplasia via modification of global transcriptional activity.20 These mutations, particularly in KMT2D, are common in M-PTLD (DLBCL) (39%) and IC-DLBCL (30-43%) and occur in 6-10% of MM.21-26 Infrequent KMT2A mutations (6%), but no KMT2D mutations, have been reported in HIV-related PBLs.7 Recurrent loss-of-function mutations were also observed in the methylcytosine dioxygenase TET2 (27%), which have been shown to alter gene transcription via widespread DNA hypermethylation, a process important for PTLD pathogenesis.27 TET2 mutations are frequent in IC-DLBCL and MM22, 23, 25, 26, 28 and occur in 9% of HIV-related PBL.7 In contrast to their common occurrence in EBV+ DLBCL, TET2 mutations were exclusively seen in EBV- PBLs in our cohort.29 Other epigenetic modifiers recurrently mutated in HIV-related PBLs include EP300, which was
mutated in one PT-PBL, as well as TRRAP and HDAC6, which were not in our sequencing panel.7, 11

DNA damage response and repair pathway genetic alterations (mutations and copy number changes) were detected in 82% of our cohort, with chromosome17/TP53 abnormalities being the most common recurrent events (55%). The frequency of TP53 mutations in PT-PBLs (27%) was comparable to that reported in M-PTLD (DLBCL) (36-44%), and similar to the latter, mutations were more common in EBV- cases.21, 30 A lower frequency of TP53 mutations has been observed in HIV-related PBLs (9%).7 Of interest, TP53 mutations are present in up to 23% of IC-DLBCLs, but a high proportion of DLBCLs with plasmablastic/plasmacytoid features (85%) harbor TP53 deletions.24-26, 31 TP53 mutations are uncommon in MM at diagnosis,22, 23, 28 but can be detected at disease progression, concomitant with TP53 deletions, and are associated with poor prognosis.23, 32 MSI, resulting from mutations in DNA MMR genes, was identified in two EBV- PBLs that showed a high mutation burden. MMR defects and MSI are unusual in B-NHL of immunocompetent individuals, but not infrequent in MM33 and immunodeficiency-associated B cell neoplasms, including PTLDs.34

Gain-of-function mutations in MAPK pathway genes also appear to be more frequent in PT-PBLs (55%) compared to HIV-related PBLs (28%).7 Moreover, concurrent mutations of multiple MAPK pathway members, noted in several PT-PBLs, could reflect presence of multiple subclones, as described in MM.36 Different members of the MAPK pathway are mutated in diverse hematological and lymphoid malignancies. However, KRAS and NRAS mutations, which are common in MM,23 are infrequent in IC-DLBCL. Knowles et al. reported NRAS mutation in a post-transplant plasmacytoid immunoblastic lymphoma, which could have represented a PBL.36 Well-known, activating BRAF K601E and codon 469 mutations were observed in our study. The latter and the canonical V600E mutation have also been documented in PBLs arising in other settings.7, 11 Intriguingly, the BRAF V600E mutation was recently reported in an
immunomodulatory therapy-associated EBV+ anaplastic large cell lymphoma. Further studies are required to determine whether this mutation is a recurrent, lineage independent, phenomenon in immune dysregulation-related lymphomas.

Recurrent mutations in members of the NOTCH signaling pathway, which controls B-cell fate determination, were observed in 45% of PT-PBLs, mostly in EBV- cases (80% compared to 17% in EBV+). It is unclear if EBNA2 activates NOTCH signaling in a proportion of EBV+ PBLs. Gain- and loss-of-function alterations in NOTCH pathway genes have been described in a variety of hemato-lymphoid neoplasms, including 24% of HIV-related and some IC-PBLs. The pathogenesis of IC-DLBCLs of the N1 molecular subclass, which harbor NOTCH1 mutations and display a plasmacytic phenotype, is considered to be distinct from DLBCLs of the BN2/Cluster 1 molecular subclass, which have mutations in NOTCH2 and/or the NOTCH regulator, SPEN. However, we observed mutations in both, NOTCH1 and NOTCH2, as well as SPEN, at times concurrently, in PT-PBL. Deregulated activity of the NOTCH signaling pathway has also been implicated in MM pathogenesis, facilitating plasma cell growth and migration, but via different (non-mutational) mechanisms.

JAK/STAT signaling, either constitutive activation consequent to mutations, downstream effect of cytokine signaling, or due to EBV infection, contributes to the pathogenesis of several types of lymphoid neoplasms. Recurrent alterations in constituents of the JAK/STAT pathway, as observed in 36% of our cases and a higher proportion (62%) of HIV-related PBLs, are known or predicted to enhance signaling. The STAT3 D661Y mutations, also detected in 8% of HIV-related PBLs and STAT6 E372K mutations occur in the SH2 and DNA binding domains, respectively, resulting in nuclear localization and activation of the transcription factors. Recurrent STAT3 mutations were noted exclusively in EBV+ HIV-related PBLs, but mutations in several JAK/STAT pathway members, including STAT3, were observed in both EBV+ and EBV- PT-PBLs. Multiple concomitant mutations in SOCS1, a negative regulator of JAK family...
proteins, present in one PT-PBL, have not been functionally characterized, but are predicted to inactivate SOCS1. Abrogation of SOCS1 and SOCS3 function by epigenetic silencing or mutations has been described in other immunodeficiency-associated NHLs, including M-PTLD (DLBCL) and P-PTLD.

Mutations in immune surveillance-associated genes also occurred in PT-PBLs. FAS, a member of the TNF receptor superfamily and an important mediator of T-cell cytotoxicity, was recurrently mutated in our series. FAS mutations have not been previously reported in PTLDs or PBLs, but have been documented in MM and are common in IC-DLBCL, particularly in Cluster 1 that frequently also harbors NOTCH pathway mutations, and, as in our series, are almost exclusively seen in EBV- cases. In addition to FAS mutation, one PBL had a frameshift mutation in CD58, another immune surveillance-related protein, which activates natural killer cells and is commonly mutated in IC-DLBCL.

Prior studies have reported variants in PRDM1, an inducer of terminal B-cell differentiation and regulator of MYC, in 20-50% of PBL. However, many of the variants were not expected to be deleterious. PRDM1 variants were detected in 4% of HIV-related PBLs. None of the PT-PBLs in our cohort had pathogenic PRDM1 mutations, only a single VUS (Q586H) was observed.

Similar to our findings in PT-PBLs, differences in the frequency or spectrum of genomic abnormalities between EBV+ and EBV- tumors has also been delineated in other types of B-cell PTLDs. A lower mutation burden has been noted in EBV+ compared to EBV- M-PTLDs (DLBCL), which has been ascribed to the inherent oncogenic activity of the virus, and hence, a reduced requirement for proto-oncogene or tumor suppressor gene alterations.

EBV+ cases constituted 55% of our PT-PBLs, a frequency not significantly different from previous studies of PT-PBLs (67-79%) and intermediate between that reported for HIV-related
PBLs (75-90%) and IC-PBLs (33-50%).\textsuperscript{2,4,7,12} Data regarding the EBV latency program in PBLs have been conflicting. Ambrosio et al. reported a noncanonical EBV latency program i.e. partial expression of proteins characteristic of type II latency with simultaneous expression of lytic phase proteins in HIV+ and HIV- cases.\textsuperscript{10} Similarly, gene expression studies of HIV-related PBL showed much higher expression of the EBV lytic genes (BALF4 and BALF5) than canonical latency program genes in most cases.\textsuperscript{7} Castillo et al., however, described latency I or III in most HIV-related EBV+ PBLs, latency I in IC-PBL, and predominantly latency III in PT-PBL.\textsuperscript{3} The vast majority of our EBV+ cases displayed a latency II profile, similar to the observations of Morscio et al.,\textsuperscript{2} but different from those of Zimmerman et al., who reported mostly latency 0/I in PT-PBL.\textsuperscript{14}

PBLs in our series exhibited morphologic and immunophenotypic heterogeneity, in line with prior observations.\textsuperscript{2,51} Almost half, including EBV+ and EBV- cases, displayed minor foci of plasmacytic differentiation, concordant with the findings of other investigators,\textsuperscript{3,51} and this feature was more common in PBLs with chromosome 17/TP53 abnormalities. As reported for other B-cell PTLDs,\textsuperscript{48} the majority (64%) of PT-PBLs showed evidence of germinal center transit. Although the B-cell program is characteristically downregulated in PBLs,\textsuperscript{2,3} a proportion of cases express B-cell antigens.\textsuperscript{3,52,53} Partial CD20 expression was observed in 27% of PT-PBLs, which is similar to that reported for other types of PBLs (23%).\textsuperscript{53} Over half the PT-PBLs showed variable PAX5 and/or CD79a positivity. Expression of CD79a has been documented in 45% of HIV-related and 68% of PT-PBLs \textsuperscript{3} and PAX5 in 23-26% of mostly HIV-related PBLs.\textsuperscript{52,53} In contrast to the findings of Montes-Moreno et al., who noted more frequent CD20 and/or PAX5 expression in EBV- PBLs, a higher proportion EBV+ PT-PBLs (60%) were PAX5+.\textsuperscript{53} The two EBV- PAX5+ and CD79a+ PBLs were MSI-H, otherwise, no differences in functional groups of mutations were apparent between cases expressing or lacking B-cell antigens. Since all PTLDs preceding PBLs received rituximab, it is unclear if anti-CD20 antibody therapy was
responsible for CD20 negativity of the PBLs and/or promoted plasmablastic differentiation. Expression of CD56 and CD10, observed in a subset of PT-PBLs, has been reported more frequently in HIV-related and PT-PBLs.\textsuperscript{2,3,53} Moreover, variability in the Ki-67 proliferation indices of our PT-PBLs is in accord with the findings of Morscio et al. (25% to 100% Ki-67 labeling in PT-PBLs).\textsuperscript{2}

PD-L1 expression, tumor infiltration by PD1+ T cells, and upregulation of genes related to immune escape, have been observed in EBV+ PBLs\textsuperscript{7,8,11} and PTLDs.\textsuperscript{54} PD-L1 expression by tumor cells was observed in subsets of EBV+ and EBV- PT-PBLs, the latter also harboring mutations in immune evasion related genes (\textit{FAS} and \textit{CD58}). PD-1 expression by tumor cells noted in 1 case has been previously reported in 5% of PBLs.\textsuperscript{52}

Thorough clinicopathologic correlation is essential for resolving the differential diagnosis of PBL, which includes other neoplasms with plasmablastic features e.g. large B-cell lymphoma arising from HHV-8–associated multicentric Castleman disease, primary effusion lymphoma, and ALK+ large B-cell lymphoma. Negative staining for HHV8 and ALK excluded these possibilities. Distinguishing between PBL and plasmablastic MM, however, can be difficult and a multimodality approach is required for correct diagnosis. Serum paraprotein analysis is not helpful as monoclonal proteins can be observed in some PBL patients,\textsuperscript{14} as was the case in our series. Importantly, none of the PT-PBLs with bone marrow biopsies showed morphologic or immunophenotypic evidence of marrow involvement, all lacked bone (lytic) lesions on imaging as well as myeloma related laboratory abnormalities, including hypercalcemia, and the vast majority occurred at mucosal sites, findings arguing against MM.\textsuperscript{55} Furthermore, although some of the genetic abnormalities of PT-PBLs overlapped with MM, they were detected in both EBV+ and EBV- PT-PBLs and in HIV-related PBLs,\textsuperscript{7,11} and the overall complement of alterations differed from that of MM.
PT-PBLs usually occur late after transplantation (median 96 months, range 2-360 months)\(^2, 14\) and are more frequent in males and in recipients of heart and kidney allografts,\(^2, 3, 12, 14\) which was also true in our series. However, in contrast to a predominance of skin and lymph node involvement described previously,\(^2, 3\) we noted a high frequency of intestinal disease (55%), with primary skin involvement observed in only 18% of patients. In addition to the oral cavity, the gastrointestinal tract is also a frequent site for HIV-related PBLs.\(^3\)

Age, stage, and nodal involvement impact prognosis of PBLs, which is typically poor,\(^12\) although long survival, as observed for some of our PT-PBL patients, has been reported previously.\(^2\) In contrast to the study of Zimmermann et al., we did not observe a worse prognosis of EBV- PT-PBLs or PBLs harboring \textit{MYC} and/or \textit{IGH} rearrangements.\(^14\) We also could not determine any correlation between patient outcome and particular functional groups of mutations or the mutational burden, though most had stage IV disease. Most patients in our cohort received lymphoma-directed therapies using conventional chemotherapy and/or radiotherapy, however two, including one still alive, also received bortezomib, a proteasome inhibitor frequently used to treat MM, which in combination with lymphoma regimens has been shown to be effective in treating PBL.\(^3\)

In summary, our study is the first to describe the genetic landscape of PT-PBL, revealing recurrent mutations in epigenetic modifiers and DNA damage response and repair, MAPK, JAK/STAT, NOTCH, and immune surveillance pathway genes. The observed genomic alterations overlap those reported for HIV-related PBLs as well as subtypes of IC-DLBCL and MM. Our findings reiterate the phenotypic heterogeneity of this rare type of PTLD, provide novel insights into PT-PBL biology and identify pathways amenable to targeted therapies.
REFERENCES


Table 1: Clinical features of PT-PBLs

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<td>PCKD</td>
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<td>Heart</td>
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<td>Small intestine and pulmonary valve</td>
<td>Skin, spinal epidural tissue</td>
<td>Skin, LN</td>
<td>Small intestine, pleura</td>
<td>Small intestine, peritoneal fluid</td>
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<td>Small intestine</td>
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**Abbreviations**: tx transplant; dx diagnosis; NIDCM non-ischemic dilated cardiomyopathy; CAD coronary heart disease; PCKD polycystic kidney disease; IPF idiopathic pulmonary fibrosis; LAM lymphangioleiomyomatosis; HLHS hypoplastic left heart syndrome; LN lymph node; MMF mycophenolate mofetil; R-EPOCH rituximab, etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone; R-CP rituximab cyclophosphamide, prednisone, FCM fludarabine, cyclophosphamide, mytoxantrone, CP cyclophosphamide, prednisone, GemOx gemcitabine, oxalipatin; ASCT autologous stem cell transplant; NA Not analyzed
Table 2: Morphologic and immunophenotypic features of PT-PBLs

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ND: Not determined
NA: Not applicable
PC: Plasma cells
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### Table 3. Genetic alterations in PT-PBLs

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### MAPK pathway
- ERK5
- HRAS
- Hras
- B-Raf
- MAP2K1
- N F 1
- ARAF

### JAK/STAT pathway
- STAT3
- STAT6
- SOCS1

### NOTCH pathway
- NOTCH1
- NOTCH2
- NOTCH3
- NOTCH4
- SPEN

### Immune surveillance pathway
- FAS
- CD58

### DNA damage repair
- TP53
- TIA1
- ATMD
- BRCA1
- BRCD

### Microsatellite instability
- MLH1
- MSH2
- MSH6

### Cell cycle
- MCM7
- CCND3

### Cytokine signaling/NF-k B
- TNFAIP3
- CARD11
- MYD88
- IRF1
- IRF8

### RTK/PI3K signaling
- NF2
- MTOR
- G protein signaling
- GNB1

### Ribosomal proteins
- RPL5
- RPL10

### RNA processing
- PRPF8
- KLHL6

### Transcription factors
- WT1
- FOXA1

### Other
- PAX5
- FBXW7
- GSK3B
- MYOD1
- UBR5
- PHOX2B

### Copy number
- Gain of 7
- Loss of TP53
- Partial deletion of 7

### Cytogenetics
- ES
- ES (FISH)
- TP53 (FISH)

### MSI status
- N=Normal
- M=Multiple mutations
- CK=Complex Karyotype

### Copynumber
- Loss of TP53
- Gain of 7

### Gene
- TP53
- ES
- FISH
- N=Normal
- M=Multiple mutations
LEGEND TO FIGURES

Fig. 1. Morphologic spectrum of PT-PBLs. Representative H&E-stained sections of PT-PBLs from A) Case 4 shows a monotonous infiltrate of plasmablasts, with insets highlighting areas of plasmablastic morphology (top) and focal areas of plasmacytic differentiation (bottom) B) Case 5 shows numerous tingible body macrophages in PBL that impart a “starry sky” appearance and C) Case 7 shows pleomorphic morphology, insets show areas of plasmablastic morphology (top) and areas showing anaplastic-appearing multinucleated cells (bottom).

Fig. 2. Immunophenotypic features of post-transplant plasmablastic lymphomas. (A) H&E stained section of case 2 displays plasmablasts, which are (B) partially positive for CD138 and (C) diffusely positive for MUM1 and display variable (D) PAX5 and (E) CD56 expression and evidence of (F) EBV infection by in situ hybridization for EBER. (G) H&E stained section of case 9 displays plasmablasts, which are (H) partially positive for CD79a, (I) diffusely positive for MUM1 and display (J) aberrant CD10 expression, (K) P53 overexpression, and (L) moderate (30%) MYC expression.