Plasmablastic lymphoma (PBL) is a rare and clinically aggressive neoplasm that typically occurs in immunocompromised individuals, including those infected with human immunodeficiency virus (HIV) and solid organ allograft recipients. Most prior studies have focused on delineating the clinicopathological features and genetic attributes of HIV-related PBL, in which MYC deregulation, Epstein-Barr virus (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)-PBL is not well characterized and data on underlying genetic alterations are limited. This led us to perform comprehensive histopathological and immunophenotypic evaluation and targeted sequencing of 18 samples from 11 patients (8 males, 3 females; age range, 12-76 years) with PT-PBL; eight de novo and three preceded by other types of post-transplant lymphoproliferative disorders. Post-transplant PBL displayed morphological and immunophenotypic heterogeneity and some features overlapped those of plasmablastic myeloma. Six (55%) cases were EBV positive and five (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, FANC, 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 the mutational profiles of EBV- and EBV+ cases exhibiting both similarities and differences. Clinical outcomes also varied, with survival ranging from 0-15.9 years after diagnosis. Besides uncovering the biological heterogeneity of PT-PBL, our study highlights similarities and distinctions between PT-PBL and PBL occurring in other settings and reveals potentially targetable oncogenic pathways in subsets of the disease.

Introduction

Plasmablastic lymphoma (PBL) is an uncommon and aggressive B-cell non-Hodgkin lymphoma 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, which may be due to human immunodeficiency virus (HIV) infection, iatrogenic (e.g., after organ transplantation), congenital, or age-related (immune senescence). Prior studies of mostly HIV-associ-
ated PBL have highlighted the prognostic significance of disease stage and Epstein-Barr virus (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.7,11

PBL occurring after solid organ transplantation (PT-PBL) is an uncommon type of post-transplant lymphoproliferative disorder (PTLD), accounting for 6-7% of PTLD and constituting a minor fraction (5-14%) of all PBL.1-4,12,13 Only limited data regarding the pathological and molecular features of PT-PBL have been reported.2,4,12,14

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

Methods

Case selection

We searched our departmental database for cases of PTLD diagnosed over the past 18 years (2002-2019) to select those fulfilling morphological and immunophenotypic features of PBL according to the current World Health Organization classification.1 Other types of PTLD preceding PT-PBL were also identified. Clinical and laboratory data were retrieved from 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 for cytomorphological evaluation and semi-quantitative assessment of the percentage of mature plasma cells. Immunohistochemistry/in situ hybridization was performed to analyze expression of B- and plasma-cell antigens, the cellular microenvironment, a variety of biomarkers and the EBV status/latency profiles (see Online Supplementary Methods).

Immunoglobulin heavy chain (IGH) gene rearrangement analysis

Polymerase chain reaction analysis for immunoglobulin heavy chain (IGH) gene rearrangement was performed on DNA extracted from fresh or FFPE tissue using the BIOMED-2 primers, as described previously.11

Cytogenetic analysis

G-band karyotyping was performed on metaphase preparations obtained after unstimulated overnight culture. Fluorescence in situ hybridization (FISH) was performed on metaphase spreads or FFPE sections using TP53/CEP 17 and MYC/IGH/CEP8 probes (Abbott Molecular, Des Plaines, IL, USA) 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 previously (Online Supplementary Methods). Microsatellite instability (MSI) was also analyzed (Online Supplementary Methods).

Results

Clinical characteristics

We analyzed 18 samples from 11 patients (8 males, 3 females; median age 61 years; range, 12-76 years) with PT-PBL, accounting for 11/177 (6%) of ‘destructive’ B-cell PTLD and 11/98 (11%) of monomorphic B-cell PTLD diagnosed at our institution during the study period. PT-PBL 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 after transplantation (range, 0.6-11.9 years). The intestines were the most common sites of disease (6/11, 55%). Three patients had recurrent PBL and in three patients the PBL was preceded by another type of PTLD. Staging marrow biopsies, performed in seven patients, including three of five with EBV–PBL, showed no evidence of PBL/PTLD. Therapy and outcome data are summarized in Table 1 and details are provided in the Online Supplementary Data. Serum protein electrophoresis revealed low-level monoclonal paraproteins in four of seven (57%) patients with available results; none had lytic bone lesions on imaging. Results of pertinent laboratory tests and imaging studies are listed in Online Supplementary Table S2.

Morphologic and immunophenotypic features

All PT-PBL showed diffuse infiltrates of large immunoblastic or plasmablastic cells (Figure 1). A minor component of small, more mature plasma cells (plasmacytic differentiation), comprising 10-20% of the neoplastic infiltrate, was seen in five (45%) cases (Figure 1A, Table 2). Some PBL had numerous tingible body macrophages, imparting a ‘starry sky’ appearance (Figure 1B) or multinucleated/anaplastic cells (Figure 1C). Foci of necrosis were observed in six of 11 (55%) cases.

Details of the immunophenotypes of all cases are listed in Table 2 and flow cytometry results in Online Supplementary Table S3. Representative cases are illustrated in Figure 2. All PBL expressed MUM1/IRF4, nine of 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 five (45%), two (18%), and two (18%) of the 11 cases. All evaluable PBL were positive for EMA and negative for Cyclin D1, CD117, HHV8 and ALK. Variable CD30 positivity was noted in seven of the 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 six of ten (60%) cases showing ≥40% MYC expression. Two of the latter cases expressed BCL2 in ≥50% of cells (‘double expressors’). P53 overexpression was observed in five of ten (50%) cases. The immunoprofile and/or proportions of cells expressing certain antigens differed in some PBL on recurrence. A mild to moderate infiltrate of reactive PD-1+ lymphocytes was observed in all ten cases evaluated and five of ten (50%) had PD-L1+ macrophages admixed.

Three PBL were preceded by other forms of PTLD; nodal EBV+ monomorphic PTLD (plasmacytoma) (case 5), duodenal EBV+ P-PTLD (case 8), and intestinal EBV+ monomorphic PTLD (diffuse large B-cell lymphoma, DLBCL) (case 10).

Epstein-Barr virus status and latency profiles

The neoplastic cells were positive for EBER in six of 11 (55%) PBL, with four of these six (67%) showing latency II and one case each displaying latency 0/I and latency III profiles. Four EBV+ PBL and two EBV–PBL occurred in patients
seropositive for EBV at the time of transplantation. EBV viremia at diagnosis was observed in all four EBV+ and two of five patients with EBV- PBL with available results (Online Supplementary Table S2).

**IGH gene rearrangement analysis** All PT-PBL and both preceding monomorphic PTLD showed clonal IGH gene rearrangements. Clonal relatedness was established in all three recurrent PBL and between the PBL and prior monomorphic PTLD. The polymorphic PTLD demonstrated oligoclonal products.

**Cytogenetic abnormalities** Cytogenetic findings are listed in Table 3. All three PBL with informative results showed complex karyotypes. IGH-MYC rearrangements were detected in five of 11 (45%) cases (3 at diagnosis, 2 at recurrence). Multiple copies of MYC due to polyploidy were detected in two cases (concurrent with MYC rearrangement in 1 case). Three of six cases with MYC abnormalities (2 with rearrangements, 1 with gain) showed ≥40% MYC positivity by immunohistochemistry. PBL of patients who died of PTLD, and those who did not, revealed MYC abnormalities in two of four (50%) versus four of seven (57%) cases, respectively (P=1.0). Two cases demonstrated IGH rearrangements with unknown partners. Chromosome 17/TP53 abnormalities were detected in six; subclonal (80-40% of cells) monosomy 17 (1 EBV+, 1 EBV-); multiple copies (polyploidy) of chromosome 17 (2 EBV); and TP53 deletion (1 EBV+, 1 EBV-). Loss of TP53 was detected by targeted genomic sequencing in two cases, including one case with insufficient material for FISH analysis.

No MYC or TP53 alterations were observed in the polymorphic PTLD or monomorphic PTLD (DLBCL) preceding PBL. Insufficient material precluded analysis of the PT-plasmacytoma.

**Targeted genomic sequencing** Pathogenic and likely pathogenic somatic non-synonymous single nucleotide variants (SNV) are listed in Table 3 and all, including variants of unknown significance (VUS), are listed in Online Supplementary Table S1. Excluding samples from two patients with high-level MSI, which exhibited up to 194 total SNV, the PBL harbored one to 16 pathogenic/likely pathogenic (median 7) and three to 26 total SNV (median 15) including VUS. MSI status was confirmed by a polymerase chain reaction-based method. All four PBL samples classified as having high-level MSI (from patients 6 and 7) demonstrated loss of expression of at least two mismatch repair (MMR) proteins (Table 2). After factoring out cases with high-level MSI, the number of variants was still higher in EBV+ than EBV- PBL, but the difference was not statistically significant (4.7 mean pathogenic/likely pathogenic and 9.3 total SNV in EBV+ cases; vs. 2.5 mean pathogenic/likely pathogenic and 14.0 total SNV in EBV- cases; P=0.15 and P=0.6, respectively). There was no a statistically significant difference in the number of variants between patients who died of PBL and those who did not (4.7 mean pathogenic/likely pathogenic and 10.0 total SNV vs. 7.3 mean pathogenic/likely pathogenic and 19.3 total SNV in EBV+ cases; P=0.15 and P=0.09, respectively). In our series, mutations were most frequent in epigenetic modifier genes, occurring in eight of 11 (73%) patients, and included recurrent mutations in the KMT2D/MLL family of methyltransferases (KMT2C, n=5; KMT2D, n=3; and

### Table 1. Clinical features of post-transplant plasmablastic lymphomas.

| Patient | Indication for tx | Type of tx | Immuno-suppression at diagnosis | Site of involvement | Marrow involvement | Time to development of PTLD (years) | Therapy | Dx to last follow up (years) and status | Cause of death |
|---------|-------------------|------------|-------------------------------|---------------------|-------------------|-------------------------------|---------|---------------------------------------|---------------|
| 1       | ETOH cirrhosis    | Liver and kidney | Azathioprine, Tacrolimus, Prednisone | Pleural and peritoneal fluid | NA | 0.6 | None | 0.03, dead | PTLD/sepsis |
| 2       | NIDCM             | Heart     | Cyclosporine, Prednisone      | Small intestine, pulmonary valve | Neg | 0.5 | R-EPOCH | 0.5, dead | Unknown |
| 3       | CAD               | Kidney    | Azathioprine, Cyclosporine, Prednisone | Skin, nasal epithelial tissue | Neg | 4.1 | Radiotherapy | 4.1, dead | Cardiac arrest |
| 4       | PKCD              | Kidney    | Cyclosporine, Prednisone      | Skin, LN | Neg | 5.4 | Radiotherapy, Bortezomib-Dexamethasone | 5.4, dead | PTLD |
| 5       | Obstructive uropathy | Kidney | Azathioprine, Tacrolimus, Prednisone | Small intestine, pleura | Neg | 0.3 | R-CP, PUM | 0.3, dead | PTLD |
| 6       | Kawasaki disease | Heart     | Azathioprine, Tacrolimus, Prednisone | Small intestine, peritoneal fluid | NA | 15.9 | CP, GemOx, Surgery | 15.9, alive | PTLD |
| 7       | IPF               | Kidney    | Azathioprine, Tacrolimus, Prednisone | Small intestine, pleura | NA | 2.0 | R-EPOCH | 2.0, dead | PTLD |
| 8       | LAM               | Lung      | Azathioprine, Tacrolimus, Prednisone | Small intestine, pelvic soft tissue | NA | 0.4 | R-EPOCH | 0.4, dead | PTLD |
| 9       | Alport            | Kidney    | Azathioprine, Tacrolimus, Prednisone | Small intestine, pleura | Neg | 0 | R-EPOCH, ASCT | 0, dead | ICH |
| 10      | HLHS              | Heart     | Cyclosporine, Prednisone      | Liver | Neg | 6.0 | Rituximab | 6.0, alive | Sepsis |
| 11      | Pulmonary fibrosis| Lung      | MMF, Tacrolimus, Prednisone | Liver | NA | 1.4 | None | 1.4, dead | Sepsis |

M: male; F: female; tx: transplant; dx: diagnosis; ETOH: alcohol-related; NIDCM: non-inferiority dilated cardiomyopathy; CAD: coronary artery disease; PKCD: polycystic kidney disease; IPF: idiopathic pulmonary fibrosis; LAM: lymphangioleiomyomatosis; HLHS: hypoplastic left heart syndrome; MFM: mycophenolate mofetil; LN: lymph node; PTLD: post-transplant lymphoproliferative disorder; R-EPOCH: rituximab, etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone; R-CP: rituximab, cyclophosphamide, prednisone; PUM: fludarabine, cyclophosphamide, mycophenolate; CP: cyclophosphamide, prednisone; GemOx: gemcitabine, oxaliplatin; ASCT: autologous stem cell transplant; Dxs: diagnosis; NA: not analyzed; ICH: intracranial hemorrhage.
**Discussion**

Our understanding of PT-PBL pathogenesis is limited and its molecular underpinnings are largely inferred from those reported for HIV-related PBL. Transcriptional analyses have revealed upregulation of JAK-STAT pathway genes and similarities between PBL and plasma cell neoplasms (multiple myeloma [MM] and extra-osseous plasmacytoma) and differences between immunocompetent DLBCL and PBL, 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, resembling either Burkitt lymphoma or extra-osseous plasmacytoma. Chang et al. documented overlapping chromosomal aberrations between PBL and immunocompetent DLBCL 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 11p15, loci that contain several genes encoding the NOTCH family of proteins were the most frequent. A negative regulator of NOTCH signaling, was mutated in three cases. Many of the NOTCH pathway mutations were in PBL with MMR defects and one case exhibited mutations in multiple 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 four of 11 (36%) cases.

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

The monomorphic PTLD (plasmacytoma), which harbored a NOTCH1 mutation, acquired a BRIP1 mutation upon transformation. In contrast, some of the mutations observed in the monomorphic 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 polymorphic PTLD preceding one PBL. The recurrent PBL showed both acquisition and loss of variants; the case with recurrent high-level MSI PBL (case 6) showed an increasing mutational burden over time.

**Figure 1. Morphologic spectrum of post-transplant plasmablastic lymphomas.** Representative hematoxylin and eosin (H&E)-stained sections of post-transplant plasmablastic lymphomas - from (A) case 4, showing a monotonous infiltrate of plasmablasts, with insets highlighting areas of plasmablastic morphology (top) and focal areas of plasmacytic differentiation (bottom), (B) case 5, showing numerous tingible body macrophages that impart a “starry sky” appearance, and (C) case 7, showing pleomorphic morphology, with insets displaying areas of plasmablastic morphology (top) and anaplastic-appearing multinucleated cells (bottom).

DNA damage response and repair pathway genes were mutated in seven of 11 (64%) cases, including four of five (80%) EBV– and three of six (50%) EBV+ PBL. Three of seven cases (43%) cases, all EBV–, including both cases with high-level EBV– and three of six (50%) EBV+ PBL. Three of seven mutated in seven of 11 (64%) cases, including four of five (80%) and three of six (50%) EBV+ PBL. Three of seven cases (43%) cases, all EBV–, including both cases with high-level EBV– and three of six (50%) EBV+ PBL. Three of seven cases (43%) cases, all EBV–, including both cases with high-level EBV– and three of six (50%) EBV+ PBL. Three of seven cases (43%) cases, all EBV–, including both cases with high-level EBV– and three of six (50%) EBV+ PBL. Three of seven cases (43%) and three of six (50%) EBV+ PBL. Three of seven cases (43%) cases, all EBV–, including both cases with high-level EBV– and three of six (50%) EBV+ PBL. Three of seven cases (43%) cases, all EBV–, including both cases with high-level EBV– and three of six (50%) EBV+ PBL. Three of seven cases (43%) cases, all EBV–, including both cases with high-level EBV– and three of six (50%) EBV+ PBL.
Table 2. Morphologic and immunophenotypic features of post-transplant plasmablastic lymphomas.

| Patient | Diagnosis | Mature PC (%) | Necrosis | Immunophenotype | Ig heavy chain | BCL2 IHC | P53 IHC | Ki-67 | PD1/PDL1 | EBER | Latency type | MMR IHC |
|---------|-----------|---------------|----------|----------------|--------------|----------|---------|-------|----------|------|--------------|---------|
| 1       | PBL       | <5            | No       | CD19+, CD19+   | sIg-*        | ND       | ND      | ND    | Mild/-   | -    | ND           | ND      |
| 2       | PBL       | 5             | No       | CD19+, CD19+   | IgM          | >90%     | 10%     | 90%   | Mild/-   | -    | II          | ND      |
| 3       | PBL       | 20            | No       | CD19+, CD19+   | IgG          | >90%     | 30%     | 70%   | Mild/-   | -    | 0/1         | ND      |
| 4       | M-PTLD (Plasmacytoma) | 5            | Yes      | CD19+          | IgA          | 50%      | <10%    | 50%   | Mild/-   | +    | II          | ND      |
| 5       | PBL       | <5            | No       | CD19+          | IgA          | 50%      | <10%    | <10%  | Mild/-   | +    | II          | ND      |
| 6       | PBL       | 10            | No       | CD19+          | IgA          | 70%      | >90%    | 20%   | Mild/-   | +    | II          | ND      |
| 7       | PBL       | 20            | Yes      | CD19+          | IgA          | 40%      | 50%     | 30%   | Mild/-   | +    | II          | ND      |
| 8       | P-PTLD    | 20            | Yes      | CD19+          | IgA          | 30%      | 10%     | 70%   | Mild/-   | +    | II          | ND      |
| 9       | PBL       | 20            | Yes      | CD19+          | IgA          | 30%      | 20%     | 60%   | Mild/-   | +    | II          | ND      |
| 10      | M-PTLD (DLBCL) | 20          | Yes      | CD19+          | IgA          | 30%      | 20%     | 30%   | Mild/-   | +    | II          | ND      |
| 11      | PBL       | <5            | Yes      | CD19+          | IgA          | 30%      | 20%     | 10%   | Mild/-   | +    | II          | ND      |

PBL: plasmablastic lymphoma; M-PTLD: monomorphic post-transplant lymphoproliferative disorder; P-PTLD: polymorphic post-transplant lymphoproliferative disorder; DLBCL: diffuse large B-cell lymphoma; PC: plasma cells; GI: immunoglobulin; EBER: Epstein-Barr virus encoded small RNA; MMR: mismatch repair; * sIg negative by flow cytometry.
While HIV-related PBL and PT-PBL appear to exhibit overlapping genomic changes, some differences are evident.

In our series of PT-PBL, mutations in epigenetic modifiers were among the most frequent alterations (73%). Inactivating mutations of the \textit{KMT2/MLL} family of histone H3 methyltransferases, which were most common, promote neoplasia via modification of global transcriptional activity.\textsuperscript{20} These mutations, particularly in \textit{KMT2D}, have also been identified in monomorphic PTLD (DLBCL) (39%) and immunocompetent DLBCL (30-43%) and occur in 6-10% of cases of MM.\textsuperscript{21-26} Infrequent \textit{KMT2A} mutations (6%), but no \textit{KMT2D} mutations, have been reported in HIV-related PBL.\textsuperscript{7} Recurrent loss-of-function mutations were also observed in the methylcytosine dioxygenase \textit{TET2} (27%), which have been shown to alter gene transcription via widespread DNA hypermethylation, a process important in the pathogenesis of PTLD.\textsuperscript{27} \textit{TET2} mutations are frequent in immunocompetent DLBCL and MM\textsuperscript{22,23,24,25,28} and occur in 9% of HIV-related PBL.\textsuperscript{7} In contrast to their common occurrence in EBV\textsuperscript{+} DLBCL, in our cohort \textit{TET2} mutations were exclusively seen in EBV\textsuperscript{−} PBL.\textsuperscript{29} Other epigenetic modifiers recurrently mutated in HIV-related PBL include \textit{EP300}, which was mutated in one PT-PBL, as well as \textit{TRRAP} and \textit{HDAC6}, which were not in our sequencing panel.\textsuperscript{7,11}

Alterations (mutations and copy number changes) in DNA damage response and repair pathway genes were detected in 82% of our cohort, with chromosome 17/\textit{TP53} abnormalities being the most common recurrent events.
### Table 3. Genetic alterations in post-transplant plasmablastic lymphomas.

| Case | FBV-/ | PBL | PTLD | FFPE | PTLD | FFPE | FFPE | FFPE | FFPE | FFPE | FFPE | FBV-/ |
|------|--------|-----|------|------|------|------|------|------|------|------|------|------|
| 3    | 1      | 1   | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| 4    | 2      | 2   | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    |
| 6    | 7      | 7   | 7    | 7    | 7    | 7    | 7    | 7    | 7    | 7    | 7    | 7    |
| 9    | 10     | 10  | 10   | 10   | 10   | 10   | 10   | 10   | 10   | 10   | 10   | 10   |

**Epigenetic modifiers**
- KMT2C (MLL2)
- KMT2D (MLL2)
- KMT2A (MLL2)
- TET2
- ASXL1
- DNMT3A
- EP300
- KDM5C
- CYFIP

**MAPK pathway**
- KRAS
- NRAS
- HRAS
- BRAF
- MAP2K4
- NF1
- ARAF

**JAK/STAT pathway**
- STAT5
- STAT3
- SOCS5

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

**DNA damage repair**
- TP53
- FANCA
- ATRX
- BRF1
- BRCA1

**Microsatellite instability**
- MLH1
- PMS2
- MSH6

**Cell cycle**
- CCNE
- CCND1

**Cytokine signaling/NF-kB**
- TNFAIP3
- CARD11
- PIK3CA
- MYD88
- IRAK1
- ITPKB

**RTK/PI3K signaling**
- NF1
- MTOR

**G protein signaling**
- GNA12
- GNA13

**Ribosomal proteins**
- RPL5
- RPL12

**RNA processing**
- PRPF8
- KHDRB1

**Transcription factors**
- WT1
- FOXA3

**Other**
- PAQR7
- FBXW7
- GSK3B
- MYCIN
- UBR4
- PRKCD

**Copy number**
- Loss of TP53
- Gain of 1p
- Partial loss of 1p
- Partial loss of 16p

**Cytogenetics**
- CNV (FFPE)
- FTPS (FFPE)
- MSI-H

**MSI status**
- N

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PBL: plasmablastic lymphoma; M-PTLD: monomorphic post-transplant lymphoproliferative disorder; FFPE: formalin-fixed, paraffin-embedded; VUS: variant of unknown significance; SNV: single nucleotide variant; FISH: fluorescence in situ hybridization; MSI: microsatellite instability; MSI-H: high-level microsatellite instability; NR: no result.
The frequency of TP53 mutations in PT-PBL (27%) was comparable to that reported in monomorphic PTLD (DLBCL) (86-44%) and, as in the latter, mutations were more common in EBV- cases. A lower frequency of TP53 mutations has been observed in HIV-related PBL (9%). Of interest, TP53 mutations are present in up to 23% of immunocompetent DLBCL; however, a high proportion of DLBCL with plasmablastic/plasmacytoid features (85%) harbor TP53 deletions. 24,26,31 TP53 mutations are uncommon in MM at diagnosis, 22,23,25  but can be detected at disease progression, concomitant with TP53 deletions, and are associated with poor prognosis. 23,24 MSI, resulting from mutations in DNA MMR genes, was identified in two EBV- PBL that showed a high mutation burden. MMR defects and MSI are unusual in B-cell non-Hodgkin lymphomas of immunocompetent individuals, but not infrequent in MM 26 and immunodeficiency-associated B cell neoplasms, including PTLD. 28

Gain-of-function mutations in MAPK pathway genes also appear to be more frequent in PT-PBL (55%) compared to HIV-related PBL (20%). Moreover, concurrent mutations of multiple MAPK pathway members, noted in several PT-PBL, could reflect the presence of multiple subclones, as described in MM. 31 Different members of the MAPK pathway are mutated in diverse hematologic and lymphoid malignancies. However, KRA S and NRAS mutations, which are common in MM, 23 are infrequent in immunocompetent DLBCL. Knowles et al. reported an NRAS mutation in a post-transplant plasmacytoid immunoblastic lymphoma, which could have represented a PBL. 30 Well-known, activating BRAF V600E and codon 469 mutations were observed in our study. The latter and the canonical V600E mutation have also been documented in PBL arising in other settings. 31 Intriguingly, the BRAF V600E mutation was recently reported in an immunomodulatory therapy-associated EBV-anaplastic large cell lymphoma. 30 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, 32 were observed in 45% of PT-PBL, mostly in EBV- cases (80% vs. 17% in EBV+ cases); it is unclear whether EBNA2 activates NOTCH signaling in a proportion of EBV- PBL. 29 Gain- and loss-of-function alterations in NOTCH pathway genes have been described in a variety of hematologic malignancies, including 24% of HIV-related PBL and some immunocompetent PBL. 33 The pathogenesis of immunocompetent DLBCL of the N1 molecular subclass, which harbor NOTCH1 mutations and display a plasmacytic phenotype, 26 is considered to be distinct from DLBCL of the BN2/Cluster 1 molecular subclass that have mutations in NOTCH2 and/or the NOTCH regulator, SPEN. 34,35 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 the pathogenesis of MM, facilitating plasma cell growth and migration, but via different (non-mutation- al) mechanisms. 36

JAK/STAT signaling, due to constitutive activation consequent to mutations, downstream effects of cytokine signaling, or EBV infection, contributes to the pathogenesis of several types of lymphoid neoplasms. 34 Recurrent alterations in constituents of the JAK/STAT pathway, as observed in 36% of our cases and a higher proportion (62%) of HIV-related PBL, are known or predicted to enhance signaling. The STAT3 D661Y mutation, also detected in 8% of HIV-related PBL 2 and STAT6 E327K mutations, occur in the SH2 and DNA binding domains, respectively, resulting in nuclear localization and activation of the transcription factors. 34,35 Recurrent STAT3 mutations were noted exclusively in EBV- HIV-related PBL, 31 but mutations in several JAK/STAT pathway members, including STAT3, were observed in both EBV- and EBV PT-PBL. 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. 33 Abrogation of SOCS1 and SOCS3 function by epigenetic silencing or mutations has been described in other immunodeficiency-associated non-Hodgkin lymphomas, including monomorphic PTLD (DLBCL) and polymorphic PTLD. 36

Mutations in immune surveillance-associated genes also occurred in PT-PBL. FAS, a member of the tumor necrosis factor receptor superfamily and an important mediator of T-cell cytotoxicity, was recurrently mutated in our series. FAS mutations have not been previously reported in PTLD or PBL, but have been documented in MM. 38 They are also common in immunocompetent DLBCL, particularly in EBV- cases. FAS is a receptor for the FASL, an important mediator of T-cell-dependent, Fas-dependent apoptosis. 39 Gain-of-function mutations in FASL, a negative regulator of JAK family proteins, present in one PT-PBL, have not been functionally characterized, but are predicted to inactivate SOCS1. 33 Abrogation of SOCS1 and SOCS3 function by epigenetic silencing or mutations has been described in other immunodeficiency-associated non-Hodgkin lymphomas, including monomorphic PTLD (DLBCL) and polymorphic PTLD. 36

Prior studies have reported variants in PRDM1, an inducer of terminal B-cell differentiation and regulator of MYC, in 20-50% of PBL. 11,17 However, many of the variants were not expected to be deleterious. 11,17 PRDM1 variants were detected in 4% of HIV-related PBL. 33 None of the PT-PBL in our cohort had pathogenic PRDM1 mutations and only a single VUS (Q586H) was observed.

Similar to our findings in PT-PBL, differences in the frequency or spectrum of genomic abnormalities between EBV- and EBV+ tumors have also been delineated in other types of B-cell PTLD. 20,60 A lower mutation burden has been noted in EBV- compared to EBV+ monomorphic PTLD (DLBCL); this 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-PBL, a frequency not significantly different from previous studies of PT-PBL (67-79%) and intermediate between that reported for HIV-related PBL (75-90%) and immunocompetent PBL (53-50%). 34,35 Data regarding the EBV latency program in PBL have been conflicting. Ambrosio et al. reported a non-canonical 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. 35 Similarly, gene expression studies of HIV-related PBL have shown a much higher expression of the EBV lytic genes (BALF4 and BALF5) than canonical latency program genes, in most cases. 34 Castillo et al., however, described latency I or II in most HIV-related EBV+ PBL, latency I in immunocompetent PBL, and predominantly latency III in PT-PBL. 35 The vast majority of the EBV- cases in our study displayed a latency II profile, similar to the observations of Morisco et al., 36 but different from those of Zimmerman et al., who reported mostly latency 0/I in PT-PBL. 36

In our series, PBL exhibited morphologic and immunophenotypic heterogeneity, in line with prior obser-
vations.\textsuperscript{2,21} Almost half of the cases, including both EBV- and EBV+ cases, displayed minor foci of plasmacytic differentiation, concordant with the findings of other investigators.\textsuperscript{21} This feature was more common in PBL with chromosome 17/TP53 abnormalities. As reported for other B-cell PTLD,\textsuperscript{40} the majority (64%) of PT-PBL showed evidence of germinal center transit. Although the B-cell program is characteristically downregulated in PBL,\textsuperscript{23} a proportion of cases express B-cell antigens.\textsuperscript{3,22,23,50} Partial CD20 expression was observed in 27% of PT-PBL, a frequency similar to that reported for other types of PBL (23%).\textsuperscript{50} Over half of the PT-PBL showed variable PAX5 and/or CD79a positivity. Expression of CD79a has been documented in 45% of HIV-related and 68% of PT-PBL,\textsuperscript{5} and PAX5 in 23-26% of mostly HIV-related PBL.\textsuperscript{3,50} In contrast to the findings of Montes-Moreno \textit{et al.,} who noted more frequent CD20 and/or PAX5 expression in EBV+ PBL, a larger proportion of EBV+ PT-PBL (60%) was PAX5+.\textsuperscript{5} The two EBV PAX5+ and CD79a+ PBL had high-level MSI; otherwise, no differences in functional groups of mutations were apparent between cases expressing or lacking B-cell antigens. Since all patients with PTLD preceding PBL received rituximab, it is unclear whether the anti-CD20 antibody therapy was responsible for CD20 negativity of the PBL and/or promoted plasmablastic differentiation. Expression of CD56 and CD10, observed in a subset of PT-PBL, has been reported more frequently in HIV-related and PT-PBL.\textsuperscript{3,20,50} Moreover, variability in the Ki-67 proliferation indices of our PT-PBL is in line with the findings of Moscio \textit{et al.} (25-100% Ki-67 labeling in PTLB).\textsuperscript{5}

PD-L1 expression, tumor infiltration by PD1+ T cells, and upregulation of genes related to immune escape, have been observed in EBV+ PBL\textsuperscript{7,11} and PTLD.\textsuperscript{54} PD-L1 expression by tumor cells was observed in subsets of EBV+ and EBV- PT-PBL; the latter also harboring mutations in immune evasion-related genes (\textit{FA5} and \textit{CD58}). PD-1 expression by tumor cells noted in one case has been previously reported in 5% of PBL.\textsuperscript{52}

Thorough clinicopathological 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 HHV8-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 multimodal approach is required to make a correct diagnosis. Serum paraprotein analysis is not helpful as monoclonal proteins can be observed in some PBL patients,\textsuperscript{44} as was the case in our series. Importantly, none of the PT-PBL 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 that do not support a diagnosis of MM.\textsuperscript{39} Furthermore, although some of the genetic abnormalities observed in PT-PBL overlapped with those of MM, they were detected in both EBV- and EBV+ PT-PBL and in HIV-related PBL.\textsuperscript{3,51} Furthermore, the overall complement of alterations differed from that of MM. PT-PBL usually occur late after transplantation (median 96 months; range, 2-360 months)\textsuperscript{21,22} and are more frequent in males and in recipients of heart and kidney allografts,\textsuperscript{2,12,14} which was also true in our series. However, in contrast to a predominance of skin and lymph node involvement described previously,\textsuperscript{3,22} 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 PBL.\textsuperscript{3}

Age, stage, and nodal involvement influence the prognosis of PBL, which is typically poor,\textsuperscript{22} although long survival, as observed for some of our PT-PBL patients, has been reported previously.\textsuperscript{22} In contrast to Zimmermann \textit{et al.}, we did not observe that patients with EBV+ PT-PBL or PBL harboring MYC and/or ICH rearrangements had a worse prognosis.\textsuperscript{32}

We could not determine any correlation between patients’ outcome and particular functional groups of mutations or mutational burden, although most had stage IV disease. Most patients in our cohort received lymphoma-directed therapies using conventional chemotherapy and/or radiotherapy. However two patients, including one who is 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.\textsuperscript{33}

In summary, our study is the first to investigate 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 PBL as well as subtypes of immunocompetent 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.

**Disclosures**

No conflicts of interest to disclose.

**Contributions**

RL and GB led the project, analyzed data and wrote the manuscript. PR performed research and analyzed data. CS performed research, analyzed data and critically reviewed the manuscript. DR and FM performed research and obtained clinical data. BA and DP contributed to interpreting the data and critically reviewed the manuscript.

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