

Phenogenomic heterogeneity of post-transplant plasmablastic lymphomas

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ABSTRACT

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 clinico-pathological 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, *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 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.¹ 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).² Prior studies of mostly HIV-associ-

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ated PBL have highlighted the prognostic significance of disease stage and Epstein-Barr virus (EBV) status^{2,4} and genomic analyses have revealed frequent *MYC* rearrangements, heterogeneous chromosome/DNA copy number abnormalities,^{5,7} variable transcriptional and microRNA profiles^{2,8-10} 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.^{2-4,12,13} 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.¹⁵

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 previously¹⁶ (*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 immunoprofiles 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 EBV 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

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

Patient	1	2	3	4	5	6	7	8	9	10	11
Age, sex	61, M	70, M	76, M	65, M	22, M	12, F	72, M	57, F	64, M	15, M	60, F
Indication for tx	ETOH cirrhosis	NIDCM	CAD	PCKD	Obstructive uropathy	Kawasaki disease	IPF	LAM	Alport syndrome	HLHS	Pulmonary fibrosis
Type of tx	Liver and kidney	Heart	Heart	Kidney	Kidney	Heart	Lung	Lung	Kidney	Heart	Lung
Immuno-suppression at diagnosis	Azathioprine, Tacrolimus, Prednisone	MMF, Cyclosporine, Prednisone	Azathioprine, Cyclosporine, Prednisone	MMF, Cyclosporine, Prednisone	Cyclosporine, Prednisone	Azathioprine, Tacrolimus, Prednisone	Azathioprine, Tacrolimus, Prednisone	Azathioprine, Tacrolimus, Prednisone	Azathioprine, Tacrolimus, Prednisone	Cyclosporine, Prednisone	MMF, Tacrolimus, Prednisone
Site of involvement	Pleural and peritoneal fluid	Small intestine, pulmonary valve	Nasal	Skin, spinal epidural tissue	Skin, LN	Small intestine, pleura	Small intestine, peritoneal fluid	Small intestine	Small intestine, pelvic soft tissue	Small intestine	Liver
Marrow involvement	NA	Neg	Neg	Neg	Neg	Neg	NA	NA	Neg	Neg	NA
Stage	IV	IV	I	IV	IV	IV	IV	IV	IV	IV	I
Time to development of PTLD (years)	0.6	7.0	11.1	1.3	7.8	10.3	11.9	0.9	9.6	10.8	10.7
Therapy	None	R-EPOCH	Radiotherapy	Radiotherapy, Bortezomib-Dexamethasone	R-CP, FCM	CP, GemOx, Surgery	R-EPOCH	R-EPOCH	None	Bortezomib-EPOCH, ASCT	R-EPOCH, Rituximab
Dx to last follow up (years) and status	0.03, dead	0.5, dead	4.1, dead	5.4, dead	0.3, dead	15.9, alive	2.0, dead	0.4, dead	0, dead	6.0, alive	1.4, dead
Cause of death	PTLD/sepsis	Unknown	Cardiac arrest	PTLD	PTLD	NA	PTLD	ICH	Sepsis	NA	Sepsis

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

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 $\geq 40\%$ *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 (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-). 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 poly-

morphic 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 13) 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+ vs. 10.0 mean pathogenic/likely pathogenic and 19.3 total SNV in EBV- cases; $P=0.15$ and $P=0.09$, 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 14.0 total SNV; $P=0.51$ and $P=0.6$, 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 *KMT2/MLL* family of methyltransferases (*KMT2C*, $n=5$; *KMT2D*, $n=3$; and

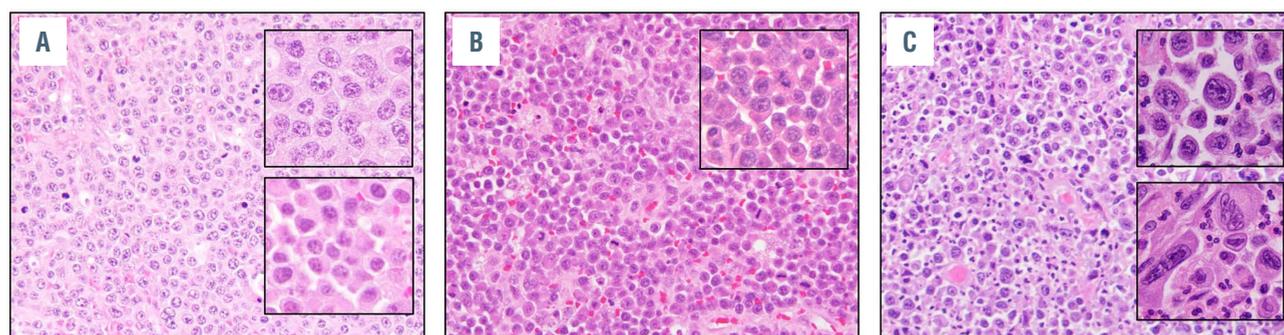


Figure 1. Morphologic spectrum of post-transplant plasmablastic lymphomas. Representative hematoxylin and eosin (H&E)-stained sections of post-transplant plasmablastic lymphomas - from (A) case 4, showing a monotonous infiltrate of plasmablasts, with insets highlighting areas of plasmablastic morphology (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).

KMT2A, $n=2$), *TET2* ($n=3$), *ASXL1* ($n=2$), and *KDM5C* ($n=2$).

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 (43%) cases, all EBV⁻, including both cases with high-level MSI, showed multiple *TP53* mutations. One case with concomitant subclonal monosomy 17 showed 30% P53⁺ cells, as did two with concurrent chromosome 17 gains, suggesting bi/multiallelic inactivation (Tables 2 and 3). Chromosome 17/*TP53* abnormalities were more frequent in PBL exhibiting plasmacytic differentiation (4/5, 80%) than in those lacking it (2/6, 33%), although the difference was not statistically significant ($P=0.24$). PBL with high-level MSI (cases 6 and 7) had mutations in the MMR genes: *MILH1* and *PMS2* or *MSH6*, respectively. Immunohistochemistry showed that these cases also had loss of MMR proteins (Table 2) as well as a high mutational burden. Other recurrently-mutated genes included *BRCC3*, *ATRX*, *FANCA*, and *BRIP1*.

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

Mutations in NOTCH signaling pathway genes were detected in five of 11 (45%) cases. Putative gain- or loss-of-function mutations in genes encoding the NOTCH family of proteins were the most frequent. *SPEN*, 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.

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- κ B signaling pathway genes.^{7,9} However, microRNA expression analysis has suggested two different subclasses of PBL, resembling either Burkitt lymphoma or extra-osseous plasmacytoma.¹⁰ 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.⁵ 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 the development of PBL.^{11,17-19} The frequency of *MYC* abnormalities (rearrangement and/or gain) in our series (55%) was higher than that reported previously for PT-PBL (38%), but within the range (36-69%) reported for PBL arising in other settings.^{2,3,7,11,12,17,19} In contrast to the study of mostly HIV-related PBL by Valera *et al.*, *MYC* rearrangements were more common in EBV⁻ PT-PBL,¹⁹ and in some cases, the rearrangement was detected upon PTLD transformation to PBL or at disease relapse.

Recently, whole exome sequencing and targeted sequencing studies have unveiled the mutational landscape, primarily of HIV-related PBL,^{7,11} but until now the spectrum of genetic alterations underlying PT-PBL has not been explored.

Table 2. Morphologic and immunophenotypic features of post-transplant plasmablastic lymphomas.

Patient	1	2	3	4	5	6	7	8	9	10	11	
Diagnosis	PBL	PBL	PBL	PBL	M-PTLD (Plasma-cytoma)	PBL	PBL	P-PTLD	PBL	M-PTLD (DLBCL)	PBL	
Mature PC (%)	<5	<5	<5	20	5	5	<5	10	20	20	<5	
Necrosis	No	No	No	No	Yes	No	Yes	Yes	Yes	No	Yes	
Immunophenotype	CD19+, CD20-, PAX5-, CD79a+, CD138+, MUM1+	CD19+, CD20-, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56+, EMA+	CD19-, CD20-, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19-, CD20-, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-/+, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-/+, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-/+, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-/+, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-, PAX5+, CD79a+, CD138+, BCL6-, CD10+, MUM1+, CD56-, EMA+	CD19+, CD20-/+, PAX5+, CD79a+, CD138+, BCL6+, CD10+, MUM1+, CD56-
Ig heavy chain	slg*	IgM	IgG	IgA	-	IgG	IgG	ND	IgG	-	IgA	-
BCL2 IHC	ND	>90%	>90%	<10%	50%	<10%	<10%	>90%	30%	10%	>90%	50%
CMYC IHC	ND	<10%	80%	20%	30%	90%	ND	20%	30%	40%	<10%	70%
P53 IHC	ND	10%	30%	<10%	<10%	90%	40%	<10%	30%	<10%	<10%	<10%
CD30	-	-	<5%	<5%	10%	60%	>90%	20%	-	-	10%	10%
Ki-67	ND	90%	90%	50%	60%	>90%	ND	80%	70%	20%	60%	90%
PD1/PDL1	ND	Mild/-	Mild/-	Rare/-	Mild/-	Rare/-	ND	Mild/20% with macro-phages+	Mild/-	Moderate/ with macro-phages+	Mild/- with macro-phages+	Mild/- with macro-phages+
EBER	-	+	+	+	+	-	-	+	-	-	-	+
Latency type	ND	II	0/I	II	II	NA	NA	III	NA	NA	NA	II
MMR IHC	ND	ND	ND	ND	ND	MLH1-, PMS2-, MSH2+, MSH6+	ND	ND	ND	ND	ND	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; Ig: immunoglobulin; IHC: immunohistochemistry; ND: not determined; NA: not applicable; EBER: Epstein-Barr virus encoded small RNA; MMR: mismatch repair. *slg negative by flow cytometry.

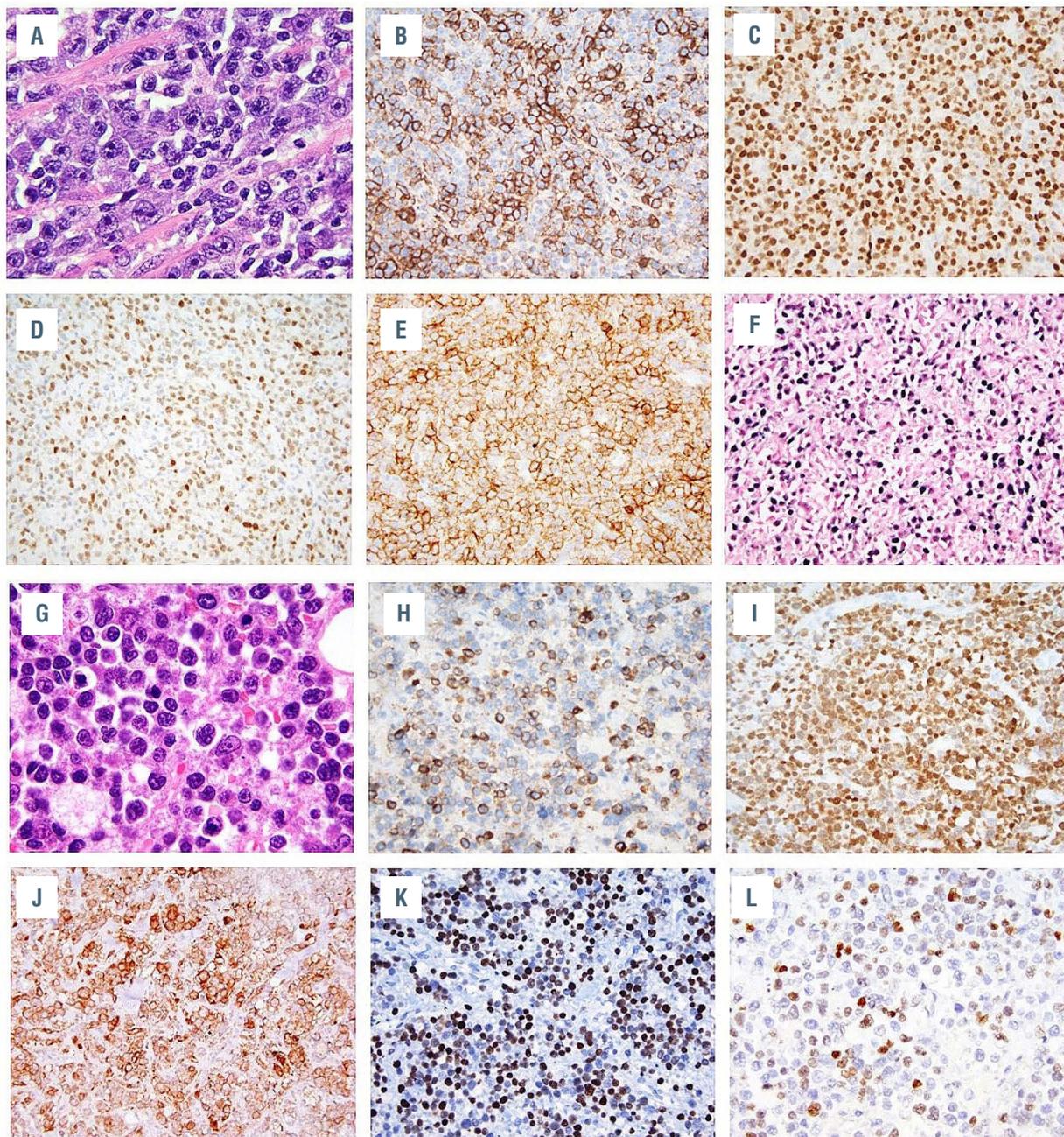


Figure 2. Immunophenotypic features of post-transplant plasmablastic lymphomas. (A) Hematoxylin and eosin (H&E)-stained section of tissue from case 2 showing plasmablasts, which are (B) partially positive for CD138 and (C) diffusely positive for MUM1 and display variable (D) PAX5 and (E) CD56 expression and evidence of (F) EBV infection by *in situ* hybridization for EBER. (G) H&E-stained section of case 9 showing 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.

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 *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*, have also been identified in monomorphic PTL (DLBCL) (39%) and immunocompetent DLBCL (30-43%) and occur in 6-10% of cases of MM.²¹⁻²⁶ Infrequent *KMT2A* mutations (6%), but no *KMT2D* mutations, have been reported in HIV-related PBL.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 in the pathogenesis of PTL.²⁷ *TET2* mutations are frequent in immunocompetent DLBCL and MM^{22,23,25,26,28} and occur in 9% of HIV-related PBL.⁷ In contrast to their common occurrence in EBV⁻ DLBCL, in our cohort *TET2* mutations were exclusively seen in EBV⁺ PBL.²⁹ Other epigenetic modifiers recurrently mutated in HIV-related PBL include *EP300*, which was mutated in one PT-PBL, as well as *TRRAP* and *HDAC6*, which were not in our sequencing panel.^{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/*TP53* abnormalities being the most common recurrent events

Table 3. Genetic alterations in post-transplant plasmablastic lymphomas.

Case	EBV+									EBV-																	
	3			8			4			2		11	5		6			7		1		10			9		
	PBL	P-PTLD	PBL	PBL	PBL	PBL	PBL	M-PTLD	PBL	PBL	PBL	M-PTLD	PBL	PBL	PBL	PBL	M-PTLD	PBL	PBL	PBL	PBL	PBL	PBL	PBL			
Diagnosis	FFPE	FFPE	FFPE	FFPE	FFPE	Fresh	Fresh	FFPE	FFPE	Fresh	Fresh	FFPE	FFPE	Fresh	Fresh	FFPE	FFPE	Fresh	Fresh	FFPE	FFPE	Fresh	Fresh	FFPE	FFPE		
Tissue type	no									yes																	
Clonally related	no									yes																	
Pathogenic/probably pathogenic SNV	7	0	1	5	5	3	10	1	2	26	37	49	25	7	13	8	16	7	63	72	145	25	12	12	8	10	6
VUS	12	0	2	2	2	7	3	0	2	89	109	194	50	19	25	16	26	13	89	109	194	50	19	25	16	26	13
Total SNV	19	0	3	7	7	10	13	1	4	26	37	49	25	7	13	8	16	7	63	72	145	25	12	12	8	10	6
Epigenetic modifiers	KMT2C (MLL3)																										
	KMT2D (MLL2)																										
	KMT2A (MLL1)																										
	TET2																										
	ASXL1																										
	DNMT3A																										
	EP300																										
	KDM5C																										
	CTCF																										
MAPK pathway	KRAS																										
	NRAS																										
	HRAS																										
	BRAF																										
	MAP2K1																										
	NF1																										
	ARAF																										
JAK/STAT pathway	STAT3																										
	STAT6																										
	SOCS1																										
NOTCH pathway	NOTCH1																										
	NOTCH2																										
	NOTCH3																										
	NOTCH4																										
	SPEN																										
Immune surveillance pathway	FAS																										
	CD58																										
DNA damage repair	TP53																										
	FANCA																										
	ATRX																										
	BRIP1																										
	BRCC3																										
Microsatellite instability	MLH1																										
	PMS2																										
	MSH6																										
Cell cycle	MCM7																										
	CCND3																										
Cytokine signaling/NF-kB	TNFAIP3																										
	CARD11																										
	FYN																										
	MYD88																										
	IRF1																										
	IRF8																										
RTK/PI3K signaling	NF2																										
	MTOR																										
G protein signaling	GNB1																										
	GNA13																										
Ribosomal proteins	RPL5																										
	RPL10																										
RNA processing	PRPF8																										
	KLHL6																										
Transcription Factors	WT1																										
	FOXA1																										
Other	PAX5																										
	FBXW7																										
	GSK3B																										
	MYOD1																										
	UBR5																										
	PHOX2B																										
Copy number	Loss of TP53																										
	Gain of 7																										
	Partial loss of 9p																										
	Partial loss of 6p																										
Cytogenetics	Karyotype	CK	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	CMYC (FISH)	N	N	N	N	+8	R	N	NR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	TP53 (FISH)	NR	N	-17	N	L	N	N	NR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MSI status		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Missense R=Rearrangement
 Nonsense G=Gain
 Frameshift L=Loss
 Noncoding/splice site N=Normal
 M = Multiple mutations CK= Complex Karyotype
 Crosshatch=likely pathogenic

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.

(55%). The frequency of *TP53* mutations in PT-PBL (27%) was comparable to that reported in monomorphic PTL (DLBCL) (36-44%) and, as in the latter, mutations, were more common in EBV⁻ cases.^{21,30} A lower frequency of *TP53* mutations has been observed in HIV-related PBL (9%).⁷ 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,^{22,23,28} 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⁻ 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 MM³³ and immunodeficiency-associated B cell neoplasms, including PTL.³⁴

Gain-of-function mutations in MAPK pathway genes also appear to be more frequent in PT-PBL (55%) compared to HIV-related PBL (28%).⁷ Moreover, concurrent mutations of multiple MAPK pathway members, noted in several PT-PBL, could reflect the presence of multiple subclones, as described in MM.³⁵ Different members of the MAPK pathway are mutated in diverse hematologic and lymphoid malignancies. However, *KRAS* and *NRAS* mutations, which are common in MM,²⁵ 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.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 PBL arising in other settings.^{7,11} Intriguingly, the *BRAF* V600E mutation was recently reported in an immunomodulatory therapy-associated EBV⁺ anaplastic large cell lymphoma.³⁷ 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,³⁸ 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.³⁹ 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 PBL and some immunocompetent PBL.^{7,1} 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*.^{24,26} 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-mutational) mechanisms.⁴⁰

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.⁴¹ 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 PBL⁷ and *STAT6* E372K mutations, occur in the SH2 and DNA binding domains, respectively, resulting in nuclear localization and activation of the transcription factors.^{42,43} Recurrent *STAT3* mutations were noted exclusively in EBV⁺ HIV-related PBL,¹¹ 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*.⁴⁴ Abrogation of *SOCS1* and *SOCS3* function by epigenetic silencing or mutations has been described in other immunodeficiency-associated non-Hodgkin lymphomas, including monomorphic PTL (DLBCL) and polymorphic PTL.⁴⁵

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 PTL or PBL, but have been documented in MM.⁴⁶ They are also common in immunocompetent DLBCL, particularly in Cluster 1, which frequently also harbor NOTCH pathway mutations,²⁴ and, as in our series, are almost exclusively seen in EBV⁻ cases.²⁹ In addition to *FAS* mutation, one PBL had a frameshift mutation in *CD58*, another immune surveillance-related protein required for activation of natural killer cells, which is commonly mutated in immunocompetent DLBCL.⁴⁷

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.⁷ 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 PTL.^{21,48,50} A lower mutation burden has been noted in EBV⁺ compared to EBV⁻ monomorphic PTL (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 (33-50%).^{2,47,12} 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.¹⁰ 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.⁷ Castillo *et al.*, however, described latency I or III in most HIV-related EBV⁺ PBL, latency I in immunocompetent PBL, and predominantly latency III in PT-PBL.³ The vast majority of the EBV⁺ cases in our study displayed a latency II profile, similar to the observations of Morscio *et al.*,² but different from those of Zimmerman *et al.*, who reported mostly latency 0/I in PT-PBL.¹⁴

In our series, PBL exhibited morphologic and immunophenotypic heterogeneity, in line with prior obser-

vations.^{2,51} Almost half of the cases, including both EBV⁺ and EBV⁻ cases, displayed minor foci of plasmacytic differentiation, concordant with the findings of other investigators.^{3,51} This feature was more common in PBL with chromosome 17/TP53 abnormalities. As reported for other B-cell PTL, the majority (64%) of PT-PBL showed evidence of germinal center transit. Although the B-cell program is characteristically downregulated in PBL,^{2,3} a proportion of cases express B-cell antigens.^{3,52,53} Partial CD20 expression was observed in 27% of PT-PBL, a frequency similar to that reported for other types of PBL (23%).⁵³ 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-PBL³ and PAX5 in 23-26% of mostly HIV-related PBL.^{52,53} In contrast to the findings of Montes-Moreno *et al.*, who noted more frequent CD20 and/or PAX5 expression in EBV⁻ PBL, a larger proportion of EBV⁺ PT-PBL (60%) was PAX5⁺.⁵³ 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 PTL 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.^{2,3,53} Moreover, variability in the Ki-67 proliferation indices of our PT-PBL is in line with the findings of Morscio *et al.* (25-100% Ki-67 labeling in PT-PBL).²

PD-L1 expression, tumor infiltration by PD1⁺ T cells, and upregulation of genes related to immune escape, have been observed in EBV⁺ PBL^{7,8,11} and PTL.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 (*FAS* and *CD58*). PD-1 expression by tumor cells noted in one case has been previously reported in 5% of PBL.⁵²

Though 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,¹⁴ 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.⁵⁵ 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.^{7,11} 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)^{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 PBL.³

Age, stage, and nodal involvement influence the prognosis of PBL, 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 Zimmermann *et al.*, we did not observe that patients with EBV⁻ PT-PBL or PBL harboring *MYC* and/or *IGH* rearrangements had a worse prognosis.¹⁴ 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.³

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 PTL, 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, MM supervised molecular analyses, VM performed cytogenetic analyses, SH performed molecular analyses and analyzed data, 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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