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ARTICLE

Genomic landscape of Epstein–Barr virus-positive extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue

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Epstein–Barr virus (EBV)-positive extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas) were initially described in solid organ transplant recipients, and, more recently, in other immunodeficiency settings. The overall prevalence of EBV-positive MALT lymphomas has not been established, and little is known with respect to their genomic characteristics. Eight EBV-positive MALT lymphomas were identified, including 1 case found after screening a series of 88 consecutive MALT lymphomas with EBER in situ hybridization (1%). The genomic landscape was assessed in 7 of the 8 cases with a targeted high throughput sequencing panel and array comparative genomic hybridization. Results were compared to published data for MALT lymphomas. Of the 8 cases, 6 occurred post-transplant, 1 in the setting of primary immunodeficiency, and 1 case was age-related. Single pathogenic/likely pathogenic mutations were identified in 4 of 7 cases, including mutations in *IRF8*, *BRAF*, *TNFAIP3*, and *SMARCA4*. Other than *TNFAIP3*, these genes are mutated in <3% of EBV-negative MALT lymphomas. Copy number abnormalities were identified in 6 of 7 cases with a median of 6 gains and 2 losses per case, including 4 cases with gains in regions encompassing several IRF family or interacting genes (*IRF2BP2, IRF2*, and *IRF4*). There was no evidence of trisomies of chromosomes 3 or 18. In summary, EBV-positive MALT lymphomas are rare and, like other MALT lymphomas, are usually genetically non-complex. Conversely, while EBV-negative MALT lymphomas typically show mutational abnormalities in the NF-κB pathway, other than the 1 *TNFAIP3*-mutated case, no other NF-κB pathway mutations were identified in the EBV-positive cases. EBV-positive MALT lymphomas often have either mutations or copy number abnormalities in IRF family or interacting genes, suggesting that this pathway may play a role in these lymphomas.

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INTRODUCTION

Extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas) comprise ~7–8% of B-cell non-Hodgkin lymphomas[1]. Most MALT lymphomas arise in association with chronic inflammation, a result of underlying infection, autoimmune disease, or other unidentified stimuli[1,2]. The most common infectious agent associated with MALT lymphomas is *Helicobacter pylori*, which is present in up to 32% of gastric MALT lymphomas in recent studies[3]. Other organisms, including *Chlamydia psittaci*, *Borrelia burgdorferi*, *Campylobacter jejuni*, and hepatitis C virus, have been implicated in the pathogenesis of MALT lymphomas, with prevalences that vary based on both anatomic site and geographic location[1,2].

MALT lymphomas associated with Epstein–Barr virus (EBV) infection are more rarely reported[4–13]. In contrast to the infectious agents mentioned above, the EBV infection in this subset of MALT lymphomas is present within the neoplastic cells and is clonal. Generally described in small series or single case reports, the actual prevalence of these EBV-positive lymphomas is uncertain. In 2011, we published 4 EBV-positive MALT lymphomas arising in the post-transplant setting[4]. These lymphoproliferations had some features that distinguished them from EBV-negative MALT lymphomas occurring in both immunocompetent individuals as well as in transplant recipients, including a predilection for cutaneous and subcutaneous sites, and plasmacytic differentiation with IgA heavy chain restriction[4]. The EBV-positive post-transplant MALT lymphomas also followed an indolent clinical course, with a subset showing regression with immune reconstitution[4].

Since our initial description, EBV-positive MALT lymphomas have been reported in additional transplant recipients, as well as in patients with a primary immunodeficiency or other iatrogenic immunosuppression, post multiagent chemotherapy, or as a consequence of probable immunosenescence of advanced age[4–13]. These subsequent reports have shown that EBV-positive MALT lymphomas may involve other anatomic locations and express heavy chains other than IgA. However, these additional cases have, in

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Table 1. Clinicopathologic features of EBV-positive MALT lymphomas.

Case no.	Age (years)/ Gender	Immunodeficiency	Site of biopsy	Heavy chain	Treatment	Status at last follow up (years)
1[a]	24/F	SOT	Subcutaneous tissue	IgA	Excision, ROI	ANED (10)
2[a]	44/M	SOT	Subcutaneous tissue	IgA	ROI, antiviral, rituximab	DNED (3.4)
3[a]	12/M	SOT	Orbit	IgA	ROI, antiviral	ANED (16)
4[a]	71/F	SOT	Subcutaneous tissue	IgA	Antiviral, rituximab	ANED (8.8)
5[a]	16/M	PI	Parotid	IgM	Rituximab	DNED (3)
6	63/M	SOT	Parotid	IgA	Excision	ANED (5.5)
7	67/F	Age[b]	Breast	IgA	Excision	ANED (0.5)
8	10/F	SOT	Lip	IgG	CTx, rituximab	AWD (0.9)

EBV Epstein–Barr virus, *MALT* lymphoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, *No.* number, *F* female, *M* male, *SOT* solid organ transplant, *PI* primary immunodeficiency, *ROI* reduction of immunosuppression, *CTx* chemotherapy, *ANED* alive with no evidence of disease, *DNED* died with no evidence of disease, *AWD* alive with disease.
[a]Cases 1–5 were previously reported[4,13].
[b]Patient had no documented immunodeficiency or immunosuppression.

general, shown an indolent clinical course similar to our initial case series[4–7,9,11–13].

It is thought that lymphomagenesis in EBV-negative MALT lymphomas is driven by the synergistic effects of chronic immunological stimulation and genetic abnormalities that result in constitutive activation of the NF-κB pathway[2,15]. In addition to characteristic chromosomal rearrangements involving *MALT1*, *BCL10*, and *FOXP1*, recurrent mutations in other NF-κB pathway genes, including *TNFAIP3* and *TBL1XR1*, are frequently described[1,2,16–40]. Recurrent numerical chromosomal abnormalities, most commonly trisomies of chromosomes 3 and 18, are also seen in EBV-negative MALT lymphomas[1,2]. Although the clinical, histopathologic, and immunophenotypic features of EBV-positive MALT lymphomas are detailed in previous reports, it has not been determined if genetic alterations of the NF-κB pathway or other common MALT lymphoma-associated numerical chromosomal abnormalities play a role in their development. We, therefore, sought to further characterize the genomic features of a series of EBV-positive MALT lymphomas with high throughput sequencing mutation analysis and array comparative genomic hybridization (aCGH) to determine how similar these cases were to EBV-negative MALT lymphomas. In addition, 88 consecutive cases of MALT lymphoma with adequate material were evaluated with EBV-encoded RNA (EBER) in situ hybridization to assess the prevalence of EBV positivity in our MALT lymphoma patient population.

MATERIALS AND METHODS

Case selection and morphologic/immunophenotypic review

This study was approved by the Institutional Review Boards of each institution. Eight cases that met WHO criteria for MALT lymphoma[1] and were EBV-positive based on EBER-in situ hybridization studies were identified in the pathology archives of the University of Pittsburgh Medical Center (UPMC), University of Washington Medical Center, Newcastle upon Tyne Hospitals, Penn State Milton S. Hershey Medical Center, and University of Kentucky College of Dentistry. Five of the 8 cases have been previously reported[4,13]. All available histologic sections, immunohistochemical/in situ hybridization studies, and flow cytometric data were reviewed, and clinical and laboratory data was obtained. In addition, the pathology archives of UPMC were searched for all cases of MALT lymphoma diagnosed between January 2005 and April 2017. One hundred twenty-four MALT lymphomas were identified, and 88 of these cases had adequate formalin-fixed paraffin-embedded (FFPE) tissue blocks available for EBER in situ hybridization.

High throughput sequencing

Seven of the 8 cases of EBV-positive MALT lymphoma had sufficient FFPE tissue material to perform high throughput sequencing mutation analysis. Genomic DNA was extracted from the FFPE tissue and sequencing was performed using a targeted hybrid-capture next-generation sequencing panel comprising 4099 target coding regions within 220 genes recurrently mutated in lymphoma (Cancer Genetics Incorporated [CGI], Rutherford, New Jersey; Supplementary Table 1). Non-synonymous variants and insertions/deletions were recorded using the CGI pipeline followed by manual review (BR, YL, SG). Variants that may represent germline variants with allele frequencies between 40 and 60% were excluded from further analysis. Variants were classified as pathogenic/likely pathogenic or as variants of unknown significance (VUS) based on the in silico prediction and literature review according to Association for Molecular Pathology guidelines[41–46].

Array comparative genomic hybridization

Copy number alterations (CNA) were evaluated in 7 of 8 cases using 400 K SurePrint G3 human aCGH (Agilent Technologies, Santa Clara, CA) as previously described[47,48]. The resulting data was analyzed using Agilent CytoGenomics 5.0 and the aberration detection algorithm ADM-2[47,48]. Further analysis was restricted to copy number (CN) gains and losses >1 Mb in length. Normal CN variants were excluded based on review of the Database of Genomic Variants (http://dgv.tcag.ca)[49].

Immunohistochemical and in situ hybridization studies

EBV in situ hybridization studies were performed using a pre-diluted EBER probe (cat# 760-1209, Ventana Medical Systems, Tucson, AZ), CD30 immunohistochemical stains were performed using a pre-diluted Ber-H2 antibody (cat# 790-2926, Ventana Medical Systems), EBV LMP1 immunohistochemical stains were performed using a 1:100 dilution CS.1-4 antibody (cat# M0897, Dako Agilent, Santa Clara, CA), and EBNA2 immunohistochemical stains were performed using a 1:1000 dilution PE2 clone (cat# ab90543, Abcam, Cambridge, UK). All studies were run on the Ventana Benchmark or Discovery Ultra (Ventana Medical Systems, Tucson, AZ).

RESULTS

Prevalence of EBV positivity in MALT Lymphomas

Among the 88 consecutive cases of MALT lymphoma with available material diagnosed at UPMC over a 12-year period, only 1 case (1%) was EBV positive based on EBER in situ hybridization (Case 2, Table 1). The median age of this cohort of MALT lymphomas was 65.5 years (range 19-92 years).

Clinical features

Eight cases of EBV-positive MALT lymphoma were identified at 5 institutions (Table 1). The median age of the patients was 34 years (range 10–71 years), with a male-to-female ratio of 1:1. Six of the 8 patients were recipients of solid organ transplants, including 4

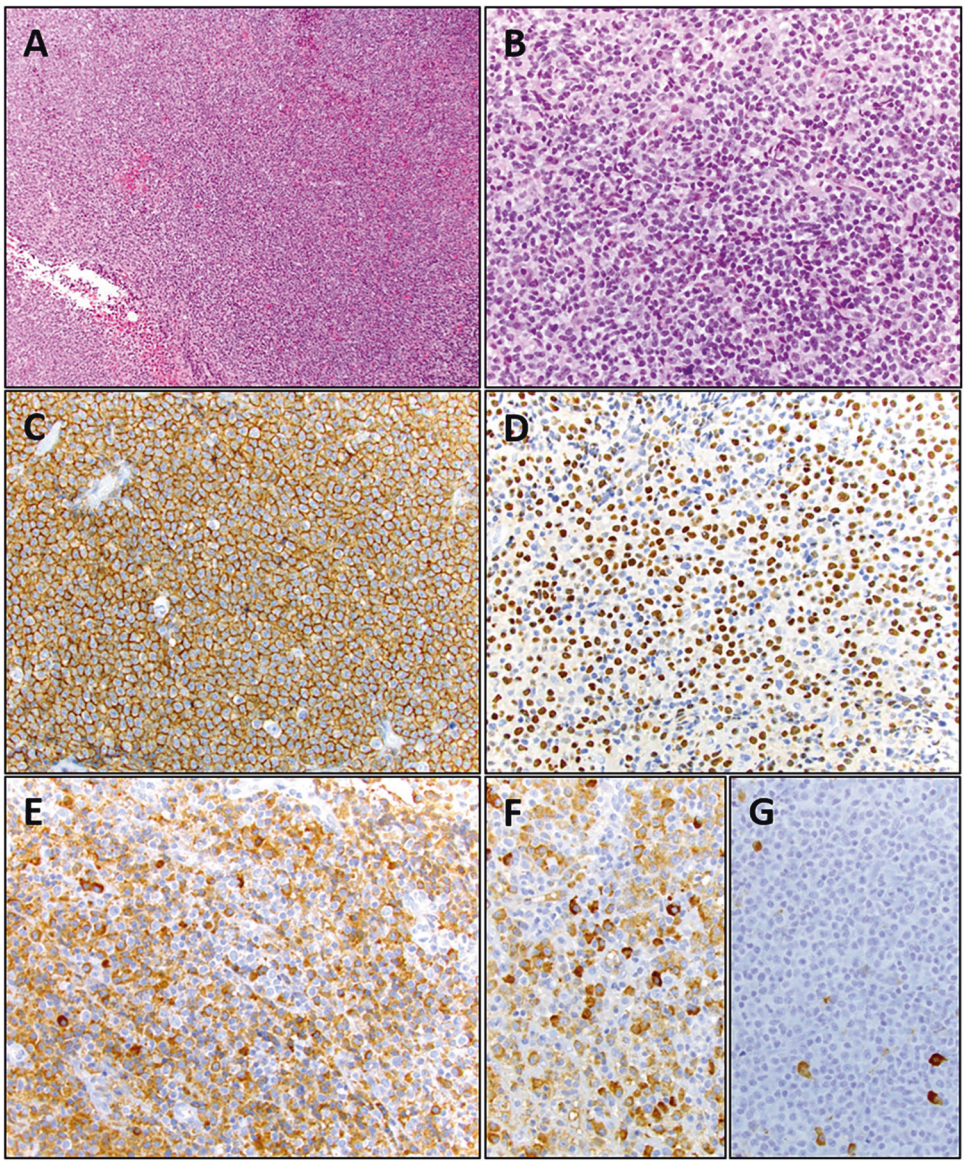

Fig. 1 EBV-positive MALT lymphoma (Case 1). The dense lymphoid infiltrate (**A**) is composed of mostly small lymphoid cells, some of which have a monocytoid appearance with more abundant pale cytoplasm, admixed with many plasma cells and a few large, transformed lymphoid cells (**B**). The lymphoid cells are predominantly CD20-positive B cells (**C**), and both the B cells and plasma cells are diffusely positive for EBER (**D**). Most of the plasma cells express IgA (**E**) and kappa light chain (**F**), with only rare lambda positive cells (**G**). **A**, **B** Hematoxylin-eosin, and **C**–**G** immunohistochemical/in situ hybridization stain with hematoxylin counterstain; **A** original magnification ×200; **B**–**G** original magnification ×400.

heart transplants, 1 kidney transplant, and 1 kidney-pancreas transplant. One patient (Case 5) had a prior diagnosis of ataxia-telangiectasia, and 1 patient (Case 7) did not have any obvious clinical evidence of immunosuppression or immunodeficiency but was 67 years old. Three of the 8 patients presented with subcutaneous tissue masses, 2 cases involved the parotid, and single cases involved the breast, orbital soft tissue, and lip. Case 5, with an EBV-positive MALT lymphoma involving the parotid, also presented with cervical, celiac, and para-aortic lymphadenopathy. The remaining patients had localized disease at presentation. Treatment included excision alone in 2 patients, reduced immunosuppression in 3 patients, antiviral therapy in 3 patients, rituximab in 3 patients, and immunochemotherapy including rituximab in 1 patient. Seven patients achieved a complete response and showed no evidence of disease at a median follow up of 5.5 years (range 0.5–16 years). However, 2 of the 7 patients died of unrelated causes during the follow up period. Patient 8 received immunochemotherapy including rituximab and is currently alive with disease after a follow up of 0.9 years.

Immunophenotypic features

Immunohistochemical stains showed that the lymphoid infiltrates contained many CD20-positive B cells and variable numbers of CD138-positive plasma cells (Fig. 1). Immunoglobulin light chain restriction was demonstrable in all cases, with 7 cases expressing kappa light chain and 1 case expressing lambda. Six cases showed IgA heavy chain restriction, 1 case was IgG positive, and 1 case expressed IgM. EBER in situ hybridization was positive in the vast majority of cells in all cases. Except for case 7, which did not have sufficient material for additional testing, all cases showed rare CD30 positive cells, rare EBV LMP1 positive cells, and were EBNA2 negative. These findings reflect a type II latency pattern.

Genomic features

High throughput sequencing identified variants of any type in 5 of 7 cases. The median number of variants per case was 1 (range 0–7 variants) (Table 2). Single pathogenic/likely pathogenic mutations were detected in 4 of 7 cases, including a nonsense mutation of *TNFAIP3* (p.R183*; c.547C>T), a missense mutation of *BRAF* (p.G469A; c.547C>T), a frameshift mutation of *IRF8* (p.Q401Rfs*52; c.1201delC), and a missense mutation of *SMARCA4* (p.V1016M; c.3046G>A). VUS were identified in 3 cases, including 2 cases that also had pathogenic/likely pathogenic mutations.

aCGH identified CNAs in 6 of 7 cases, with a median of 2 CNAs per case (range 0–49 CNAs). CN gains were more common than losses, with a median of 6 gains per case (range 0–60 CN gains) and a median of 2 losses per case (range 0–9 CN losses). Large CNAs >20 Mb were identified in 4 cases: Case 1 showed both a large CN loss at chromosome 11q14.1-11q23.3 (33.2 Mb) and a CN gain at chromosome 21q11.2-21q22.3 (33.5 Mb); Case 3 harbored a large CN gain at chromosome 21q11.2-21q22.3 (33.6 Mb); Case 5 showed large CN gains at chromosomes 6p25.3-6p21.1 (43.7 Mb) and 19p13.3-19p12 (23.5 Mb); and Case 6 harbored a large CN loss at 14q24.3-14q32.33 (28.7 Mb). Fifteen recurrent regions of CN gain were identified, including gain of 1q42.2-1q42.3 in 4 cases, gains of 6p21.1, 10q24.32-10q24.33, 12q24.23-12q24.31, 15q26.1, and 17q24.2 in 3 cases, and gains of 1p36.11-1p35.3, 4p14, 4q35.1, 8p11.21, 14q32.31-14q32.33, 15q22.2-15q22.31, 16p11.2, 17q23.3-17q24.1, and 21q11.2-21q22.3 in 2 cases each. One recurrent region of CN loss at 1p35.1-1p34.3 was identified in 3 cases. Further analysis of the CNAs showed recurrent gains of regions containing IRF family or interacting genes, including *IRF2BP2* (1q42.3) in 4 cases (Cases 1, 3, 5, and 8), *IRF2* (4q35.1) in 2 cases (Cases 1 and 8), and *IRF4* (6p25.3) in 1 case (Case 5).

DISCUSSION

EBV-positive MALT lymphomas have become increasingly recognized in recent years[4–13]. However, the true prevalence of these lymphomas has been unclear as most pathologists do not routinely screen for EBV in MALT lymphomas from patients who are not known to be immunosuppressed or immunodeficient. Earlier studies have not always reported the percentage of EBV-positive cells in the MALT lymphomas or have used molecular techniques for EBV detection, which makes it difficult to determine if these MALT lymphomas were in fact EBV-associated or rather if the EBV positivity represented only unrelated latent infection[4]. To address this question, we screened 88 cases of MALT lymphoma diagnosed at UPMC and found a prevalence in our patient population of only 1%. Of the 88 MALT lymphomas screened, 52% were from patients 65 years of age or older, suggesting a low incidence even among those who may have some immune senescence. This would suggest that routine screening for EBV in MALT lymphomas diagnosed in presumably immunocompetent individuals is not warranted.

Five of the 8 EBV-positive MALT lymphomas included in this study have been previously reported[4,13]. The 3 additional cases (Cases 6–8) show similar clinicopathologic features including an association with solid organ transplantation (2 of 3 cases), IgA heavy chain restriction (2 of 3 cases), and an indolent clinical course, with 2 of 3 patients showing no evidence of disease following excision alone. Case 7 was identified in a 67-year-old patient with no known underlying immunodeficiency or immunosuppression, supporting the concept that these EBV-positive lymphoproliferative disorders (LPDs) may also arise in the setting of immune senescence related to aging[10,12]. All of our cases tested had a type II latency pattern, similar to 2/7 EBV-positive marginal zone lymphomas reported by Gong et al.[12].

The development of EBV-negative MALT lymphomas is thought to be driven by the cooperative effects of chronic immunological stimulation and a variety of genetic abnormalities that lead to constitutive activation of the NF-κB pathway[2,15]. Supporting this concept is data from a recent study that identified genetic alterations involving NF-κB pathway genes in 50 of 72 (69%) cases of MALT lymphoma[16]. NF-κB dysregulation and activation is reflected by characteristic chromosomal translocations, such as t(11;18)(q21;q21);*BIRC3-MALT1*, t(14;18)(q32;q21);IGH-*MALT1*, t(1;14)(p22;q32);IGH-*BCL10*, and t(3;14)(p14;q32);IGH-*FOXP1*, as well as deletions and/or inactivating mutations of the *TNFAIP3* gene at 6q23, a negative regulator of the NF-κB pathway (Fig. 2)[1,2,15–36]. Dysregulation and activation of the NF-κB pathway in MALT lymphomas is also mediated by mutations in other genes implicated in the NF-κB pathway such as *BCL10* (4%), *BIRC3* (1%), *CARD11* (2%), *CD79A* (<1%), *CD79B* (1%), *MYD88* (6%), *NFKBIA* (2%), *TBL1XR1* (13%), *TNIP1* (3%), *TRAF3* (3%), and *TRAF6* (2%) (Fig. 2)[16,18,21,22,27,31,32,34,36–40]. In addition to these genetic alterations, varied recurrent numerical chromosomal abnormalities are reported in EBV-negative MALT lymphomas[1,2]. Trisomies of chromosomes 3 or 18 occur in ~25% and 19% of cases, respectively, and recurrent gains of chromosomes 1p, 3p, 3q, 6p, 17q, 18p, 18q, and 19p are reported in ~20% of cases[17,18,20–26,29,50–53].

There is very limited previous data to assess how similar the EBV-positive MALT lymphomas are in terms of their genomic landscape to their EBV-negative counterparts. Previous studies performed on a limited number of cases have shown no evidence of *BCL2*, *BCL6*, *BCL10*, IGH, *MALT1*, or *MYC* gene rearrangements[4,9,14]. Here, we assessed the mutational landscape and looked for CNAs in 7 of our 8 EBV-positive MALT lymphomas. High throughput sequencing identified pathogenic/likely pathogenic mutations in 4 of 7 cases evaluated, including an inactivating mutation of *TNFAIP3* in Case 5[43,54,55]. No additional mutations or CNAs were detected in the *TNFAIP3* gene region in the remaining cases. In addition, no mutations in other genes involved in NF-κB signaling, including *BCL10*, *BIRC3*, *CARD11*, *CD79A*, *CD79B*, *IKBKB*, *MALT1*, *MYD88*, *NFKBIA*, *NFKBIB*, *NFKBIE*, *PTPN13*, or *TBL1XR1*, were detected in our cases; although, it should be noted that some genes in this pathway that are more rarely mutated, such as *MAP3K14* and *TNIP1*, are not covered by the targeted panel used in our study. This is consistent with one prior study that reported no evidence of *MYD88* L265P mutations in 5 EBV-positive MALT lymphomas[12].

Mutations and/or CNAs of IRF family or interacting genes were identified in 5 of 7 EBV-positive MALT lymphomas, including a frameshift mutation in *IRF8* (p.Q401Rfs*52) in Case 2, CN gains in the region of the *IRF2BP2* gene (1q42.3) in 4 cases (Cases 1, 3, 5, and 8), gain of the *IRF2* gene region (4q35.1) in 2 cases (Cases 1 and 8), and gain of the *IRF4* gene region (6p25.3) in Case 5. Although the specific *IRF8* frameshift mutation identified in Case 2 has not been described in EBV-negative MALT lymphomas, rare similar frameshift mutations, as well as missense mutations, have been described in B-cell non-Hodgkin lymphomas, particularly in cases of diffuse large B-cell lymphoma and primary mediastinal large B-cell lymphoma[43,56,57]. IRF family transcription factors are involved in a variety of innate and adaptive immune responses, and it is known that EBV LMP1 and EBNA3C interact with IRF family members during B-cell transformation[58–61]. Although gains of the *IRF4* gene region (6p25.3) are reported in ~11% of EBV-negative MALT lymphomas, mutations or CNAs of other IRF family or interacting genes appear uncommon (Fig. 2)[16,18,20–26,31,36,38]. The IRF alterations identified in our small cohort of cases raise the possibility that dysregulation of the IRF pathway plays a role in the pathogenesis of EBV-positive MALT lymphomas, although further functional studies are warranted to confirm this hypothesis.

Although *BRAF* mutations are reported in up to 16% of nodal marginal zone lymphomas, this gene is only rarely mutated in EBV-negative MALT lymphoma (Fig. 2)[16,22,36,62]. The activating *BRAF* G469A mutation detected in Case 4 is infrequently reported in non-Hodgkin lymphomas, including a small number of cases of chronic

Table 2. Genomic alterations in EBV-positive MALT lymphomas[a].

Case no.	Pathogenic/likely pathogenic mutations (VAF)			Variants of unknown significance (VAF)			Total no. of CNAs[b]	Recurrent CNAs[c]	
1		None			None		42 gains	Gains:	1p36.12-p35.3, 1q42.2-1q42.3, 4p14, 4q35.1, 6p21.1, 8p11.21, 10q24.32-10q24.33, 12q24.23-12q24.31, 14q32.31-14q32.33, 15q22.2-15q22.31, 15q26.1, 16p11.2, 17q23.3-17q24.1, 17q24.2, 21q11.2-21q22.3
							7 losses	Losses:	1p35.1-1p34.3
2	*IRF8*	p.Q401Rfs*52	(36%)		None		None		NA
3		None			None		2 gains	Gains:	1q42.2-1q42.3, 21q11.2-21q22.3
							0 losses	Losses:	None
4	*BRAF*	p.G469A	(28%)	*NSD2*	p.E1050K	(25%)	0 gains	Gains:	None
							1 loss	Losses:	None
5	*TNFAIP3*	p.R183*	(20%)		None		18 gains	Gains:	1p36.11-1p35.3, 1q42.2-1q42.3, 4p14, 6p25.3-6p21.1, 8p11.21, 10q24.32-10q24.33, 12q24.23-12q24.31, 14q32.31-14q32.33, 15q26.1, 17q24.2
							5 losses	Losses:	1p35.1-1p34.3
6	*SMARCA4*	p.V1016M	(15%)	*APC2*	p.R2098C	(15%)	1 gain	Gains:	None
				BRD4	p.R1237H	(20%)	1 loss	Losses:	None
				CREBBP	p.R1702L	(18%)			
				PLCB4	p.R621H	(21%)			
				TENM4	p.R1300Q	(20%)			
				UBR5	p.R472Q	(15%)			
8		None		*GCC2*	p.E1608G	(27%)	12 gains	Gains:	1q42.2-1q42.3, 4q35.1, 6p21.1, 10q24.32-10q24.33, 12q24.23-12q24.31, 15q22.2-15q22.31, 15q26.1, 16p11.2, 17q23.3-17q24.1, 17q24.2
				NSD2	p.C686R	(15%)	4 losses	Losses:	1p35.1-1p34.3
				POT1	p.R117C	(13%)			

EBV Epstein–Barr virus, *MALT* lymphoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue, *No.* number, *VAF* variant allele frequency, *ND* not done, *CNA* copy number alterations, *NA* not applicable.

[a]Case 7 did not have sufficient FFPE tissue available for genomic analysis.

[b]Supplementary Table 2 provides a list of all identified CNAs in the 7 EBV-positive MALT lymphomas.

[c]CNAs identified in >1 case.

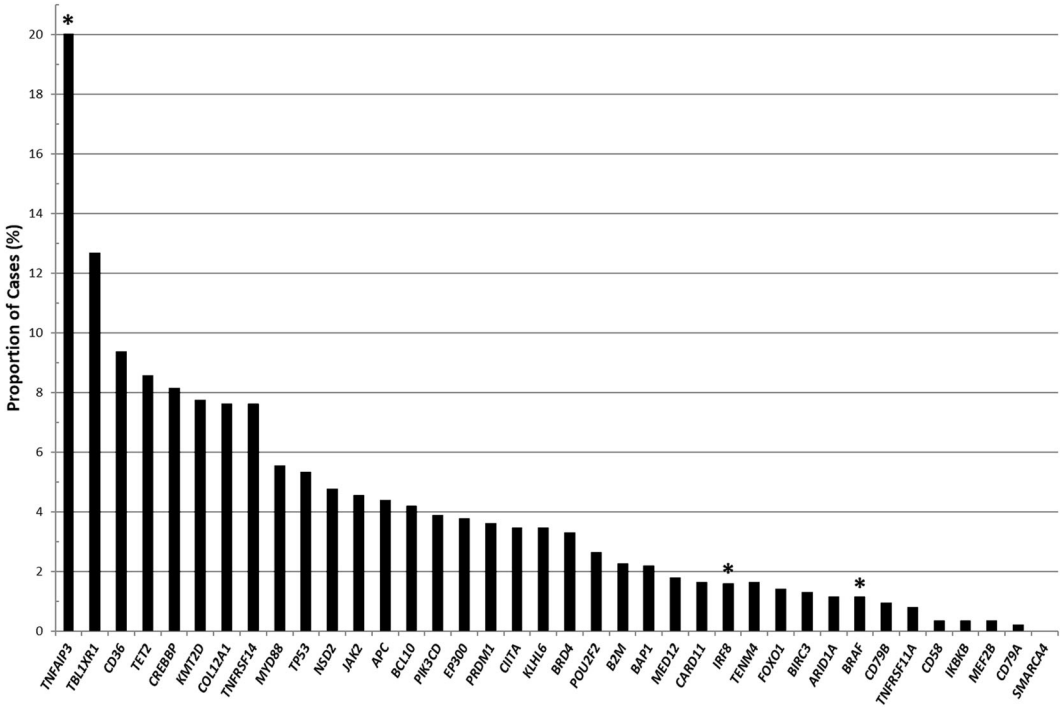

Fig. 2 Mutations reported in MALT lymphomas. The frequencies of the mutations illustrated in this figure are compiled from previous studies of EBV-negative MALT lymphoma[16,18,21,22,27,28,30–36,38–40,68]. Percentages were generated by calculating the total number of cases tested for each gene across the studies as the denominator and the cases with mutations identified in each gene as the numerator. Genes highlighted with an asterisk indicate those with pathogenic/likely pathogenic mutations detected in our series of EBV-positive MALT lymphomas.

lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma and diffuse large B-cell lymphoma[43,62,63]. The significance of this *BRAF* mutation in EBV-positive B-cell LPDs arising in the post-transplant or other immunodeficiency settings is uncertain. However, EBV LMP1 is known to activate the MAP kinase signaling pathway during B-cell transformation, and *BRAF* mutations have been reported in rare EBV-positive and EBV-negative B-cell post-transplant lymphoproliferative disorders[61,62,64].

A missense mutation of *SMARCA4*, a catalytic subunit of the SWI/SNF chromatin remodeling complex, was detected in Case 6[65–67]. *SMARCA4* gene alterations are detected in ~7% of solid tumors[65]. Although mutations of *SMARCA4* have not been reported in EBV-negative MALT lymphomas, they are described in ~3% of mature B-cell neoplasms, including a subset of EBV-positive Burkitt lymphomas[16,18,21,22,27,28,30–36,38–40,65,68–70]. While the specific V1016M mutation detected in this case has not been described in B-cell lymphomas, it involves the SNF2 family N-terminal domain, which is involved in histone-DNA interactions[66,67].

Consistent with previous DNA microarray studies of EBV-negative MALT lymphomas, the EBV-positive cases showed a relatively stable karyotype with a low number of CNAs[17,18,23,25,26]. However, unlike EBV-negative MALT lymphomas, the EBV-positive cases did not show trisomies of chromosomes 3 or 18. The recurrent CN gains involving chromosome 1p, 6p, 10q, 12q, 14q, 15q, 16p, 17q, and 21q identified in our cases have also been reported in a variable proportion of EBV-negative MALT lymphomas; however, these CNAs are not specific to MALT lymphomas[17,18,20,21,23–26,29,53,68,71].

Although our data is limited by the rarity of EBV-positive MALT lymphomas, this study indicates that these lymphomas have a genomic profile that in some ways overlaps with that of EBV-negative MALT lymphomas, including a lack of genetic complexity, some overlapping CNAs, and the presence of a *TNFAIP3* mutation in 1 case[1,2,16–36,53,68,71]. However, these EBV-positive cases also demonstrate some differences from EBV-negative MALT lymphomas in that they do not harbor common MALT lymphoma-associated translocations and numerical chromosomal abnormalities, and they lack pathogenic/likely pathogenic mutations in other genes recurrently mutated in EBV-negative MALT lymphomas[1,2,4,9,12,14–18,20–27,29,31,32,34,36–40,50–53]. EBV-positive MALT lymphomas also seem to display a higher proportion of abnormalities involving IRF family or interacting genes (71% of cases evaluated) compared to EBV-negative cases[16,18,20–26,31,36,38]. It should be recognized that the apparent genomic differences between EBV-positive and EBV-negative MALT lymphomas could be due in part to the small size of our study cohort, as well as known differences in the frequency of genetic alterations based on anatomic site and geographic location. However, it is also possible that these differences are related to the presence of EBV. EBV-associated LPDs in immunosuppressed or immunodeficient individuals generally harbor fewer genetic alterations than their EBV-negative counterparts, and it is thought that EBV supports lymphomagenesis in these LPDs, at least in part, through continued activation of NF-κB[61,72–74]. EBV may act in a similar manner in EBV-positive MALT lymphomas, as cases that have responded to immune reconstitution and antiviral therapy support a direct role for EBV in their pathogenesis[4–7,9,11–13].

In conclusion, our study confirms that EBV-positive MALT lymphomas are rare. Although these lymphomas share many features with EBV-negative MALT lymphomas, they also show some differences, including frequent alterations of IRF family or interacting genes and an absence of frequent alterations in the NF-κB pathway. Although further investigations are warranted, this data raises the possibility that IRF family or interacting genes,

in concert with EBV, play a role in the pathogenesis of this rare subset of MALT lymphomas.

DATA AVAILABILITY

All data generated during this study are included in this published article [and its supplementary information files]. For other original data, please contact the corresponding author.

REFERENCES

1. Swerdlow S. H., et al. *WHO classification of tumours of haematopoietic and lymphoid tissues*. Revised 4th edn. (International Agency for Research on Cancer, Lyon, 2017).
2. Schreuder, M. I. et al. Novel developments in the pathogenesis and diagnosis of extranodal marginal zone lymphoma. *J. Hematop.* **10**, 91–107 (2017).
3. Mendes, L. S. T., Attygalle, A. D. & Wotherspoon, A. C. Helicobacter pylori infection in gastric extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) lymphoma: a re-evaluation. *Gut* **63**, 1526–1527 (2014). A D. A., A CW.
4. Gibson, S. E. et al. EBV-positive extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue in the posttransplant setting: a distinct type of posttransplant lymphoproliferative disorder? *Am. J. Surg. Pathol.* **35**, 807–815 (2011).
5. Kojima, M. et al. Epstein-Barr virus-related atypical lymphoproliferative disorders in Waldeyer's ring: a clinicopathological study of 9 cases. *Pathobiology* **77**, 218–224 (2010).
6. Ishigaki, S. et al. Methotrexate-associated lymphoproliferative disorder of the stomach presumed to be mucosa-associated lymphoid tissue lymphoma. *Intern. Med.* **57**, 3249–3254 (2018).
7. Nassif, S. & Ozdemirli, M. EBV-positive low-grade marginal zone lymphoma in the breast with massive amyloid deposition arising in a heart transplant patient: a report of an unusual case. *Pediatr. Transpl.* **17**, E141–E145 (2013).
8. Oka, K. et al. Coexistence of primary pulmonary Hodgkin lymphoma and gastric MALT lymphoma associated with Epstein-Barr virus infection: a case report. *Pathol. Int.* **60**, 520–523 (2010).
9. Lum, S. H. et al. Successful curative therapy with rituximab and allogeneic haematopoietic stem cell transplantation for MALT lymphoma associated with STK4-mutated CD4+ lymphocytopenia. *Pediatr. Blood Cancer* **63**, 1657–1659 (2016).
10. de Jong, D. et al. B-cell and classical Hodgkin lymphomas associated with immunodeficiency: 2015 SH/EAHP Workshop Report-Part 2. *Am. J. Clin. Pathol.* **147**, 153–170 (2017).
11. Cassidy, D. P., Vega, F. & Chapman, J. R. Epstein-Barr Virus-positive extranodal marginal zone lymphoma of bronchial-associated lymphoid tissue in the post-transplant setting: an immunodeficiency-related (posttransplant) lymphoproliferative disorder? *Am. J. Clin. Pathol.* **149**, 42–49 (2017).
12. Gong, S. et al. expanding the spectrum of EBV-positive marginal zone lymphomas: a lesion associated with diverse immunodeficiency settings. *Am. J. Surg. Pathol.* **42**, 1306–1316 (2018).
13. Bennett, J. A. & Bayerl, M. G. Epstein-barr virus-associated extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT Lymphoma) arising in the parotid gland of a child with ataxia telangiectasia. *J. Pediatr Hematol. Oncol.* **37**, e114–e117 (2015).
14. Richendollar, B. G., Hsi, E. D. & Cook, J. R. Extramedullary plasmacytoma-like posttransplantation lymphoproliferative disorders: clinical and pathologic features. *Am. J. Clin. Pathol.* **132**, 581–588 (2009).
15. Du, M. Q. MALT lymphoma: a paradigm of NF-kappaB dysregulation. *Semin Cancer Biol* **39**, 49–60 (2016).
16. Cascione, L. et al. Novel insights into the genetics and epigenetics of MALT lymphoma unveiled by next generation sequencing analyses. *Haematologica* **104**, e558–e561 (2019).
17. Braggio, E. et al. Genomic analysis of marginal zone and lymphoplasmacytic lymphomas identified common and disease-specific abnormalities. *Mod. Pathol.* **25**, 651–660 (2012).
18. Ganapathi, K. A. et al. The genetic landscape of dural marginal zone lymphomas. *Oncotarget* **7**, 43052–43061 (2016).
19. Go, H. et al. Thymic extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue: a clinicopathological and genetic analysis of six cases. *Leuk. Lymphoma* **52**, 2276–2283 (2011).
20. Honma, K. et al. TNFAIP3 is the target gene of chromosome band 6q23.3-q24.1 loss in ocular adnexal marginal zone B cell lymphoma. *Genes Chromosomes Cancer* **47**, 1–7 (2008).
21. Johansson, P. et al. Identifying genetic lesions in ocular adnexal extranodal marginal zone lymphomas of the MALT subtype by whole genome, whole exome and targeted sequencing. *Cancers (Basel)* **12**, 986 (2020).
22. Jung, H. et al. The mutational landscape of ocular marginal zone lymphoma identifies frequent alterations in TNFAIP3 followed by mutations in TBL1XR1 and CREBBP. *Oncotarget* **8**, 17038–17049 (2017).
23. Kwee, I. et al. Genomic profiles of MALT lymphomas: variability across anatomical sites. *Haematologica* **96**, 1064–1066 (2011).
24. Matteucci, C. et al. Typical genomic imbalances in primary MALT lymphoma of the orbit. *J. Pathol.* **200**, 656–660 (2003).
25. Kim, W. S. et al. Genome-wide array-based comparative genomic hybridization of ocular marginal zone B cell lymphoma: comparison with pulmonary and nodal marginal zone B cell lymphoma. *Genes Chromosomes Cancer* **46**, 776–783 (2007).
26. Rinaldi, A. et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. *Blood* **117**, 1595–1604 (2011).
27. Zhu, D. et al. Molecular and genomic aberrations in Chlamydophila psittaci negative ocular adnexal marginal zone lymphomas. *Am. J. Hematol.* **88**, 730–735 (2013).
28. Bi, Y. et al. A20 inactivation in ocular adnexal MALT lymphoma. *Haematologica* **97**, 926–930 (2012).
29. Chanudet, E. et al. A20 deletion is associated with copy number gain at the TNFA/B/C locus and occurs preferentially in translocation-negative MALT lymphoma of the ocular adnexa and salivary glands. *J. Pathol.* **217**, 420–430 (2009).
30. Kato, M. et al. Frequent inactivation of A20 in B-cell lymphomas. *Nature* **459**, 712–716 (2009).
31. Hyeon, J. et al. Targeted deep sequencing of gastric marginal zone lymphoma identified alterations of TRAF3 and TNFAIP3 that were mutually exclusive for MALT1 rearrangement. *Mod. Pathol.* **31**, 1418–1428 (2018).
32. Johansson, P. et al. Recurrent mutations in NF-kappaB pathway components, KMT2D, and NOTCH1/2 in ocular adnexal MALT-type marginal zone lymphomas. *Oncotarget* **7**, 62627–62639 (2016).
33. Chanudet, E. et al. A20 is targeted by promoter methylation, deletion and inactivating mutation in MALT lymphoma. *Leukemia* **24**, 483–487 (2010).
34. Moody, S. et al. Novel GPR34 and CCR6 mutation and distinct genetic profiles in MALT lymphomas of different sites. *Haematologica* **103**, 1329–1336 (2018).
35. Novak, U. et al. The NF-{kappa}B negative regulator TNFAIP3 (A20) is inactivated by somatic mutations and genomic deletions in marginal zone lymphomas. *Blood* **113**, 4918–4921 (2009).
36. Vela, V. et al. High throughput sequencing reveals high specificity of TNFAIP3 mutations in ocular adnexal marginal zone B-cell lymphomas. *Hematol. Oncol.* **38**, 284–292 (2020).
37. Venturutti, L. et al. TBL1XR1 mutations drive extranodal lymphoma by inducing a pro-tumorigenic memory fate. *Cell* **182**, 297–316 e27 (2020).
38. Maurus, K. et al. Panel sequencing shows recurrent genetic FAS alterations in primary cutaneous marginal zone lymphoma. *J. Invest. Dermatol.* **138**, 1573–1581 (2018).
39. Liu, F. et al. Mutation analysis of NF-kappaB signal pathway-related genes in ocular MALT lymphoma. *Int. J. Clin. Exp. Pathol.* **5**, 436–441 (2012).
40. Yan, Q. et al. Distinct involvement of NF-kappaB regulators by somatic mutation in ocular adnexal malt lymphoma. *Br. J. Haematol.* **160**, 851–854 (2013).
41. Li, M. M. et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J. Mol. Diagn.* **19**, 4–23 (2017).
42. Tate, J. G. et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res* **47**, D941–D947 (2019).
43. COSMIC: Catalogue of somatic mutations in cancer. https://cancer.sanger.ac.uk/cosmic. (2021).
44. Adzhubei, I. A. et al. A method and server for predicting damaging missense mutations. *Nat. Methods* **7**, 248–249 (2010).
45. Vaser, R., Adusumalli, S., Leng, S. N., Sikic, M. & Ng, P. C. SIFT missense predictions for genomes. *Nat. Protoc.* **11**, 1–9 (2016).
46. Choi, Y. & Chan, A. P. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* **31**, 2745–2747 (2015).
47. Maguire, A. et al. Enhanced DNA repair and genomic stability identify a novel HIV-related diffuse large B-cell lymphoma signature. *Int. J. Cancer* **145**, 3078–3088 (2019).
48. Barrett, M. T. et al. Genomic amplification of 9p24.1 targeting JAK2, PD-L1, and PD-L2 is enriched in high-risk triple negative breast cancer. *Oncotarget* **6**, 26483–26493 (2015).
49. MacDonald, J. R., Ziman, R., Yuen, R. K., Feuk, L. & Scherer, S. W. The Database of Genomic Variants: a curated collection of structural variation in the human genome. *Nucleic Acids Res* **42**, D986–D992 (2014).
50. Zhou, Y. et al. The pattern of genomic gains in salivary gland MALT lymphomas. *Haematologica* **92**, 921–927 (2007).
51. Zhou, Y. et al. Distinct comparative genomic hybridisation profiles in gastric mucosa-associated lymphoid tissue lymphomas with and without t(11;18)(q21;q21). *Br. J. Haematol.* **133**, 35–42 (2006).

52. Takahashi, H. et al. Genome-wide analysis of ocular adnexal lymphoproliferative disorders using high-resolution single nucleotide polymorphism array. *Invest. Ophthalmol. Vis. Sci.* **56**, 4156–4165 (2015).
53. Dierlamm, J. et al. Characteristic pattern of chromosomal gains and losses in marginal zone B cell lymphoma detected by comparative genomic hybridization. *Leukemia* **11**, 747–758 (1997).
54. Compagno, M. et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature* **459**, 717–721 (2009).
55. Das, T., Chen, Z., Hendriks, R. W. & Kool, M. A20/Tumor Necrosis Factor alpha-Induced Protein 3 in immune cells controls development of autoinflammation and autoimmunity: lessons from mouse models. *Front. Immunol.* **9**, 104 (2018).
56. Morin, R. D. et al. Genetic landscapes of relapsed and refractory diffuse large B-cell lymphomas. *Clin Cancer Res.* **22**, 2290–2300 (2016).
57. Mottok, A. et al. Integrative genomic analysis identifies key pathogenic mechanisms in primary mediastinal large B-cell lymphoma. *Blood* **134**, 802–813 (2019).
58. Jefferies, C. A. Regulating IRFs in IFN driven disease. *Front. Immunol.* **10**, 325 (2019).
59. Wang, H. et al. Transcription factors IRF8 and PU.1 are required for follicular B cell development and BCL6-driven germinal center responses. *Proc. Natl. Acad. Sci. USA* **116**, 9511–9520 (2019).
60. Ramalho-Oliveira, R., Oliveira-Vieira, B. & Viola, J. P. B. IRF2BP2: a new player in the regulation of cell homeostasis. *J. Leukoc. Biol.* **106**, 717–723 (2019).
61. Saha, A. & Robertson, E. S. Mechanisms of B-cell oncogenesis induced by Epstein-Barr virus. *J. Virol.* **93**, e00238–19 (2019).
62. Lee, J. W. et al. BRAF mutations in non-Hodgkin's lymphoma. *Br. J. Cancer* **89**, 1958–1960 (2003).
63. Wan, P. T. et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* **116**, 855–867 (2004).
64. Menter, T. et al. Mutational landscape of B-cell post-transplant lymphoproliferative disorders. *Br. J. Haematol.* **178**, 48–56 (2017).
65. Fernando, T. M. et al. Functional characterization of SMARCA4 variants identified by targeted exome-sequencing of 131,668 cancer patients. *Nat. Commun.* **11**, 5551 (2020).
66. Harrod, A., Lane, K. A. & Downs, J. A. The role of the SWI/SNF chromatin remodelling complex in the response to DNA double strand breaks. *DNA Repair.* **93**, 102919 (2020).
67. Brownlee, P. M., Meisenberg, C. & Downs, J. A. The SWI/SNF chromatin remodelling complex: Its role in maintaining genome stability and preventing tumourigenesis. *DNA Repair.* **32**, 127–133 (2015).
68. Cani, A. K. et al. Comprehensive genomic profiling of orbital and ocular adnexal lymphomas identifies frequent alterations in MYD88 and chromatin modifiers: new routes to targeted therapies. *Mod. Pathol.* **29**, 685–697 (2016).
69. Love, C. et al. The genetic landscape of mutations in Burkitt lymphoma. *Nat. Genet.* **44**, 1321–1325 (2012).
70. Kaymaz, Y. et al. Comprehensive transcriptome and mutational profiling of endemic Burkitt lymphoma reveals EBV type-specific differences. *Mol. Cancer Res.* **15**, 563–576 (2017).
71. Cuneo, A. et al. Molecular cytogenetic characterization of marginal zone B-cell lymphoma: correlation with clinicopathologic findings in 14 cases. *Haematologica* **86**, 64–70 (2001).
72. Shannon-Lowe, C., Rickinson, A. B. & Bell, A. I. Epstein-Barr virus-associated lymphomas. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **372**, 20160271 (2017).
73. Capello, D. et al. Genome wide DNA-profiling of HIV-related B-cell lymphomas. *Br. J. Haematol.* **148**, 245–255 (2010).
74. Morscio, J. et al. Gene expression profiling reveals clear differences between EBV-positive and EBV-negative posttransplant lymphoproliferative disorders. *Am. J. Transpl.* **13**, 1305–1316 (2013).

AUTHOR CONTRIBUTIONS

S.E.G. and S.H.S. performed study concept and design; B.R., Y.C.L., A.M., M.T.B., and S.E.G. acquired, analyzed, and interpreted data; L.A.S., C.M.B., M.G.B., and M.H.S. contributed cases and provided clinicopathologic data; SEG and BR wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Boards of each institution with a waiver of consent. It was performed in accordance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

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