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EBV-positive diffuse large B-cell lymphoma, not otherwise specified: 2018 update on diagnosis, risk-stratification and management

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Disease overview: Epstein Barr virus-positive (EBV+) diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS) is an entity included in the 2016 WHO classification of lymphoid neoplasms. EBV+ DLBCL, NOS, is an aggressive B-cell lymphoma associated with chronic EBV infection, and a poor prognosis with standard chemotherapeutic approaches.

Diagnosis: The diagnosis is made through a careful pathological evaluation. Detection of EBV-encoded RNA is considered standard for diagnosis; however, a clear cutoff for positivity has not been defined. The differential diagnosis includes plasmablastic lymphoma, DLBCL associated with chronic inflammation, primary effusion lymphoma, HHV8+ DLBCL, NOS, and EBV+ mucocutaneuos ulcer.

Risk-stratification: The International prognostic index (IPI) and the Oyama score can be used for risk-stratification. The Oyama score includes age >70 years and presence of B symptoms. The expression of CD30 is emerging as a potential adverse, and targetable, prognostic factor.

Management: Patients with EBV+ DLBCL, NOS, should be staged and managed following similar guidelines than patients with EBV-negative DLBCL. EBV+ DLBCL, NOS, however, has a worse prognosis than EBV-negative DLBCL in the era of chemoimmunotherapy. There is an opportunity to study and develop targeted therapy in the management of patients with EBV+ DLBCL, NOS.

1 | DISEASE OVERVIEW

Epstein Barr virus-positive (EBV+) diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS), is a clinicopathological entity recognized in the revised 4th edition of the 2016 classification of the World Health Organization (WHO).[1] The seminal report by Oyama and colleagues reported on 22 patients with large cell lymphoma that expressed the EBV-encoded RNA (EBER) in the nuclei of the malignant cells.[2,3] These patients tended to be elderly and had a poor response and short survival with standard combination chemotherapy. In recent years, EBV+ DLBCL, NOS, were noted in younger patients.

EBV infection is common worldwide with a prevalence ranging between 80–95%, depending on the geographical area. In the case of patients with DLBCL, the prevalence of EBV infection is unknown, as no large population-based studies have been performed to date. However, small studies and case series have rendered disparate results with prevalence rates of 5% in Western countries to 10%-15% in Asia and South America.[4–7] The reasons for this difference are unclear but it is likely that virological (e.g., EBV strain) and genetic factors (e.g., HLA types) play a role.

EBV was the first oncogenic virus ever identified, and EBV infection has been associated with a number of malignancies, such as nasopharyngeal carcinoma and Burkitt lymphoma, among others. EBV infection is associated with immunosuppression and chronic antigenic activation, which are key components of the neoplastic process. Patients with EBV+ DLBCL, NOS, usually present with an EBV latency pattern type III, in which all EBV-associated proteins (i.e., latent membrane proteins and nuclear antigens) are expressed.[8] EBV latency

pattern III is associated with a marked immunodeficiency state. Other lymphomas associated with EBV latency pattern III are post-transplant and HIV-associated lymphomas.

Immunosenescence is a process associated with physiological aging characterized by a series of changes in the function of the immune system. T-cell response dysregulation, thymic atrophy, reduced output of new T-cells, development of anergic memory cells, loss of immunosurveillance, and deficiencies in cytokine production as well as limitations in the T-cell receptor repertoire are processes that have been associated with immunosenescence. Such processes might accelerate in the context of chronic infections such as EBV infection. It is likely, however, that other factors might also play a role in the pathogenesis of EBV+ DLBCL, NOS.

Clinically, patients tend to be diagnosed at an older age; hence the former reference to the term "elderly." In addition to common nodal involvement and high International Prognostic Index (IPI) scores, patients tend to have higher rates of extranodal involvement with the gastrointestinal tract, skin and bone marrow being the most commonly affected sites. Also, there is higher proportion of patients with elevated LDH levels, and more advanced clinical stage, as well as worse performance status than patients with EBV-negative DLBCL.

Expectedly, the definition of EBV+ DLBCL, NOS, continues evolving. Recent evidence shows that EBV+ DLBCL, NOS, can be seen in young, immunocompetent individuals.[9–12] These studies have shown similar virological and pathological findings between younger and older patients with EBV+ DLBCL, NOS. It is important to note that there is no clear cutoff for a positive expression of EBER as previously published studies have used rates of positivity ranging from 10% to 50%.[13] This is further complicated by the recent report that small bystander EBV+ lymphocytes in the microenvironment, but not in the large neoplastic cells, appear to convey a distinct poor prognosis.[14]

2 | DIAGNOSIS

The WHO recognizes that there is a pathologic spectrum of large cells, either a predominance of large cells with a diffuse pattern as in any DLBCL,[15] or a more common appearance is that of scattered large cells amidst a reactive background with numerous small lymphocytes or histiocytes, reminscent to T-cell/histocyte-rich large B cell lymphoma (THRLBL) and previously denominated as the polymorphous subtype.[13] The large cells may appear as large centroblasts, immunoblasts, sometimes as Hodgkin and Reed-Sternberg (HRS)-like cells.[16] Geographic necrosis is common. Montes-Moreno et al. subdivided the polymorphic subtype into three further subgroups: one called large cell type composed of numerous large cells, another shows that the large cells are HRS-like cells, and another subtype shows only few or no HRS-like cells (Figure 1). However, no prognostic significance has been associated with the morphologic subgroups.[16] The lymphoma cells express B-cell markers such as CD19, CD20, CD22, and CD79. CD30 is expressed in about 40% of cases. Most cases have an activated B-cell phenotype, expressing MUM1/IRF4, and are negative for CD10 and BCL6.[16] Expression of NF-κB and phosphorylated STAT3 are more commonly seen in EBV+ DLBCL, NOS, compared with EBV-negative DLBCL. LMP-1 is expressed in 2/3 of cases while EBNA-2 in 1/3 of cases, hence cases show type II or III latency patterns.[17] Gene expression profiling shows that EBV+ DLBCL, NOS, is molecularly distinct from EBV-negative DLBCL. The gene set enrichment assay demonstrated an enhanced toll-like receptor signaling pathway and the JAK-STAT pathway.[18] Clonal rearrangement of the immunoglobulin gene is seen in approximately 60% of cases.[16]

2.1 | Differential diagnosis

The spectrum of B-cell neoplasms associated with EBV infection has increased exponentially, and before a diagnosis of EBV+ DLBCL, NOS, is rendered, there are several disease processes that have to be taken in consideration, thus in many cases, EBV+ DLBCL, NOS, is a diagnosis of exclusion. The entities to exclude are those with apparent immunosuppression such as patients with post-transplant lymphoproliferative disorders (PTLD),[19] patients with induced iatrogenic immunosuppression such as those receiving methotrexate, tumor necrosis factors inhibitors,[20] or patients with HIV infection. Furthermore, there are specific entities or categories associated with EBV infection such as lymphomatoid granulomatosis characterized by angiocentric lesions involvement of skin, lung or central nervous system[21,22]; fibrin associated DLBCL in patients with chronic inflammation confined to extranodal or intravascular EBV+ proliferations.[23] Classical Hodgkin lymphoma should also be kept in mind because of its variable association with EBV infection. Then, we are left with the entities discussed below.

2.2 | Plasmablastic lymphoma (PBL)

PBL is an aggressive lymphoma with immunoblastic morphology and plasmacytic immunophenotype.[24,25] The lymphoma cells typically are of large size with round-to-oval centrally or eccentrically located nucleus, dispersed chromatin, prominent single nucleolus, and amphophilic cytoplasm with perinuclear hof. Apoptotic cells with accompanying tingible-body macrophages can be seen, imparting a starry-sky pattern at low magnification. Mitotic figures are frequently seen, consistent with a high Ki-67 proliferation index (90–100%). The lymphoma cells mostly express plasmacytic markers such as CD38, MUM1, and CD138. CD79a is uncommonly expressed and CD20 is virtually not expressed in contrast with EBV+ DLBCL, NOS. Frequent (80%) expression of epithelial membrane antigen (EMA) is observed. Most PBLs are positive for EBER (78%) with a predominance of type I latency pattern in contrast with type II and type III in EBV+ DLBCL, NOS. By FISH, *MYC* rearrangement is detected in about 50% of cases; the most common partner is *IGH*.[26] Of note, *MYC* rearrangement was more commonly seen in EBER-positive PBL compared with EBER-negative PBL.

2.3 | DLBCL associated with chronic inflammation

This DLBCL is mostly associated with EBV infection, and arises in patients with a long-standing chronic inflammatory process such as pyothorax, chronic osteomyelitis, metallic implant, or chronic skin

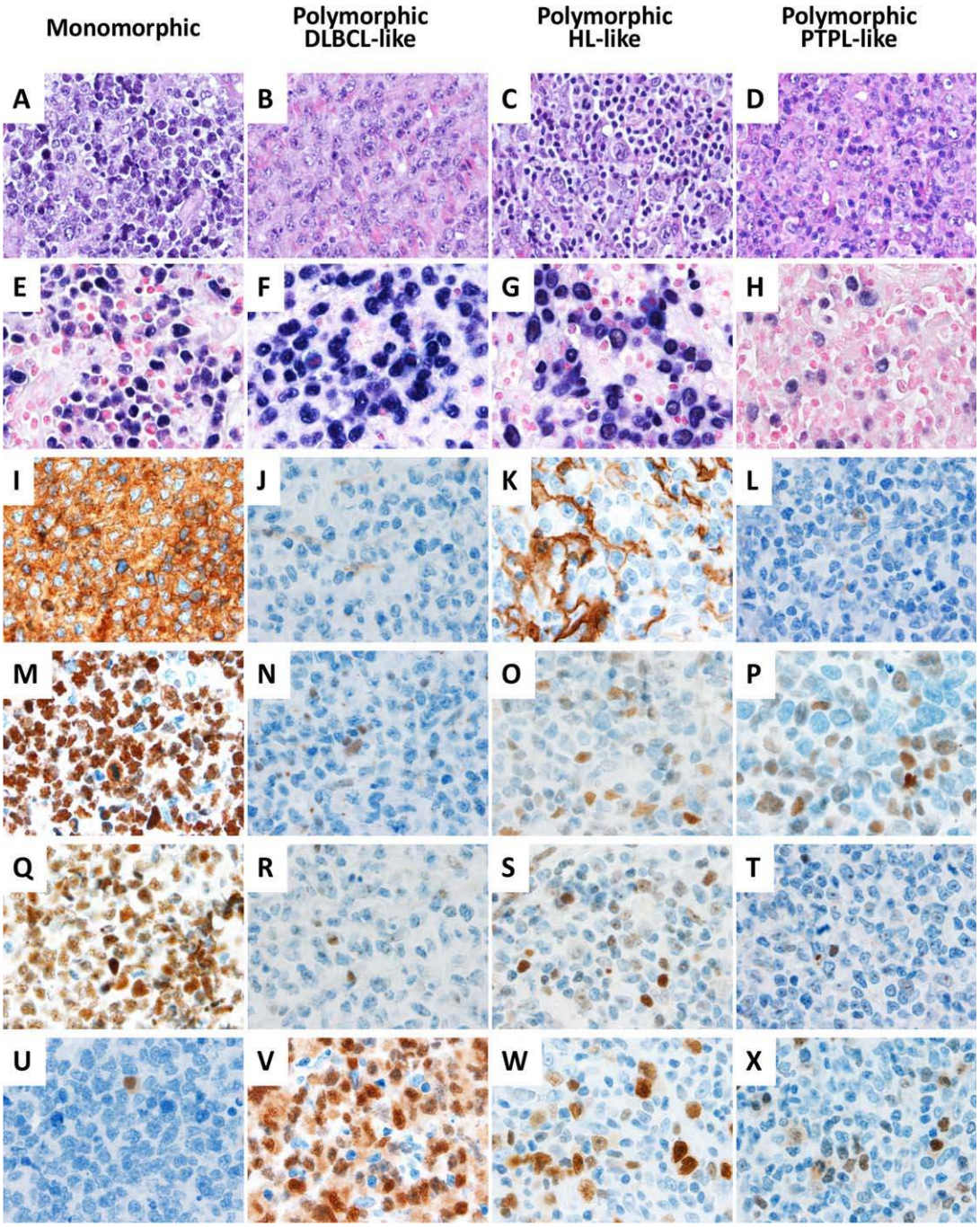

FIGURE 1 EBV-positive diffuse large B-cell lymphoma, not otherwise specified, subtypes according to Montes-Moreno and Chi Y. Ok[12,14,16]. (A-D) The lymph node is completely effaced by large lymphoma cells, with a monomorphic (A) or a T-cell/histiocyte rich large B-cell lymphoma pattern/polymorphic (B) population. Montes-Moreno suggested two additional variants for the polymorphic cases: Hodgkin lymphoma-like (C) and lymphoproliferative disorder -like (D). Hematoxylin and eosin, ×400. E-H. *In situ* hybridization for EBER shows positivity in large lymphoma cells, ×400. I-T: Panel shows that the neoplastic cells are positive for CD10 (I), BCL6 (M) and FOXP1 (Q) in monomorphic subtype, largely negative for CD10 (J), BCL6 (N) and FOXP1 (R) in polymorphic subtypes by immunohistochemistry, ×400. U-X: Panel show expression of different subtypes with MUM1: Negative in monomorphic subtype, and variably positive in the polymorphic subtype, ×400 [Color figure can be viewed at wileyonlinelibrary.com]

ulcers.[27] Morphology is typical for DLBCL. Most lymphoma cells are positive for CD20 and CD79 but can be negative in cases with plasmacytic differentiation. MUM1 and CD138 are positive in such cases. EBER and EBNA-2 are positive in most cases, illustrating a type III latency program.[28,29] Clonal rearrangement of the immunoglobulin gene is seen in most cases. Comparative genomic hybridization on pyothorax-associated lymphoma (PAL) tumor samples demonstrated gain of chromosome 8q24, and *MYC* amplification was found by southern blot technique.[30] Sequencing of the *TP53* gene (exons 5–8) using paraffin-embedded tissue found mutations in 2/3 of cases.[31] By gene

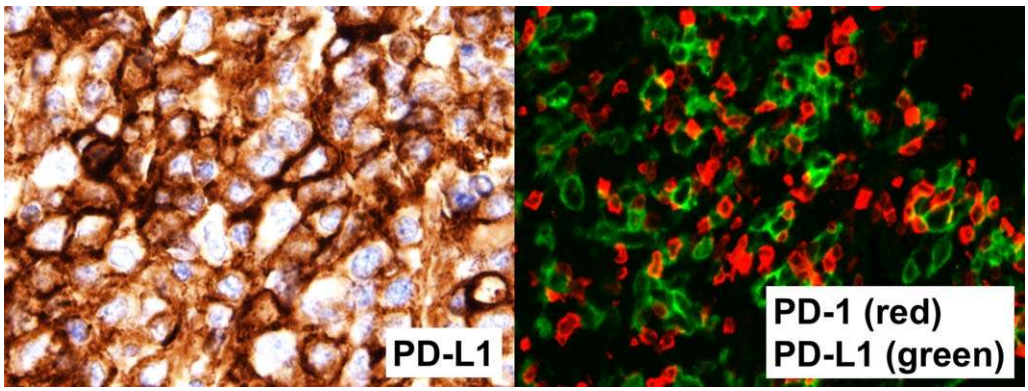

FIGURE 2 Representative positive expression of PD-L1 by lymphoma cells in EBV+ DLBCL, NOS (left), and a representative multiplex immunohistochemistry image showing PD-1 (red) in background T-cells and PD-L1 (green) expression in lymphoma cells in EBV+ DLBCL, NOS (right) [Color figure can be viewed at wileyonlinelibrary.com]

expression profiling, PAL was shown to be molecularly different from nodal DLBCL, with increased expression of activated B-cell-like signature.[32] DLBCL associated with chronic inflammation is an aggressive lymphoma, with 5-year survival rate of 22%.[28] However, a subset of cases carries an excellent prognosis with removal of the undelying chronic lesion.[23,33]

2.4 | Primary effusion lymphoma (PEL)

PEL is a rare B-cell neoplasm mostly affecting immunosuppressed patients who present with a lymphomatous effusion in body cavities, usually without a detectable tumor mass.[34] In cytospin samples, the lymphoma cells show a morphologic range from immunoblastic to markedly irregular or anaplastic features and some cells resemble HRS cells. A prominent Golgi zone adjacent to the nucleus is often present in the lymphoma cells. In tissue sections, the lymphoma cells have round or oval shapes, moderate to large amounts of cytoplasm, and round to variably indented or multilobated nuclei with one or more prominent nucleoli. Mitotic figures are numerous. The lymphoma cells usually express CD45 without expression of pan-B markers (CD19, CD20, CD22, and CD79) or T/NK cell markers. Surface and cytoplasmic immunoglobulins are generally absent. CD30, EMA, CD38, CD138, and HLA-DR are variably positive. Latency-associated nuclear antigen-1 (LANA-1) of HHV8 is typically positive. EBER is positive in about 70% of cases, but LMP1 is negative. Extracavitary PEL shares a similar immunophenotype but with more common expression of B-cell markers. Recurrent cytogenetic abnormalities have not been reported. Comparative genomic hybridization of eight PEL cases showed gain of chromosomes 12 and X in three and two cases, respectively, and amplification within the 1q region in two cases.[35] *BCL-2*, *BCL-6*, and *MYC* genes were not rearranged, and mutations in *MYC*, *HRAS*, *KRAS*, *NRAS*, and *TP53* genes were not found.[36] Clonal rearrangement of the immunoglobulin gene is seen in most cases and can be used for determining lineage. Gene expression profiling showed that PEL is in the differentiation stage of plasmablasts because the gene expression profile showed features of immunoblasts, between EBV-transformed lymphoblastoid cell lines (LCLs) or AIDS immunoblastic lymphoma, and plasma cells from multiple myeloma cell lines.[37]

HHV8-associated lymphoproliferative disorders include a spectrum of disorders of which two may be more relevant for our discussion; one is PEL that has been discussed separately because of its unique features. The other is the so-called germinotropic lymphoproliferative disorder; where large cells appear as plasmablasts and can exhibit kappa or lambda light chain restriction. Tumor cells express a EBV latency pattern type I. In addition to positive HHV8, large cells also express EBER. Large cells lack B lymphoid or plasmacytic markers but can coexpress CD4. In comparison, HHV8 positive DLBLC usually arises in patients with multicentric Castleman disease.[38] It has a spectrum of lesions from scattered plasmablasts in the mantle zones of reactive germinal centers to DLBCL, however these tumors do not exhibit EBV infection, and EBER is negative.

EBV+ mucocutaneous ulcer is an ulcerative process associated with EBV infection presenting usually in the head and neck region as well as in gastrointestinal mucosa.[39] Patients usually have a history of immunosuppression. Histologically, there is a well circumscribed ulcer with necrosis or fibrinoleucocytic infiltrate with underlying cellular infiltrate. The infiltrate is polymorphous with numerous inflammatory cells including small lymphocytes, histiocytes, vascular proliferation admixed with scattered large cells with immunoblastic or HRS appearance. The neoplastic cells express the B-cell markers CD20, CD79a or PAX5, with common expression of CD30, and lack of CD45. EBER is positive in large cells and occasionally in background small lymphocytes. The lesions can regress spontaneously, although may recur in the same site or other cutaneous or mucosal sites.

Further details useful in the differential diagnosis of these entities are shown in Table 1.

3 | RISK STRATIFICATION

Several groups have shown that patients with EBV+ DLBCL have worse prognosis when compared with patients with EBV-negative DLBCL, making EBV per se an adverse prognostic factor. A Japanese study compared the outcomes between 96 patients with EBV-positive

TABLE 1 Differential diagnosis of EBV-positive DLBCL, not otherwise specified

	EBV+ DLBCL, NOS	PBL	DLBCL, chronic inflammation	PEL	HHV8+ DLBCL, NOS	EBV+ mucocutaneous ulcer
Median age, years (range)	71 (50–91)	50 (7–65)	64 (46–82)	Young or middle age	Young adults	>70
Gender: Male to female ratio	1.4	7	12	Male > female	Male > female	Male > female
Clinical features	B-symptoms	B-symptoms; oral cavity mass	Chronic inflammation, pyothorax	Immunosupressed, coexistent Kaposi sarcoma	Multicentric Castleman disease, immunodeficiency	Immunodeficiency, oral or gastrointestinal ulcer
Geographic distribution	Asia > Europe and America	Worldwide	Asia	Worldwide	Worldwide	Worldwide
Disease distribution	Nodal and common extranodal	Oral, gastrointestinal tract	Pleural	Pleural and pericardial cavities	Nodal, spleen	Oral or gastrointestinal mucosa
Stage of disease at diagnosis	III or IV	III or IV	I or II	III or IV	III or IV	IE
Association with HIV infection	-	++	-	+++	+++	++
Pathogenesis	Immune senescence, NF-kB	EBV, HIV, IL10	EBV, IL10, IL6	HHV8 oncogenesis; EBV more limited	HHV8 oncogenesis; IL6 signaling	Underlying immunosuppression
Microscopic features	Polymorphic > monomorphic	Diffuse growth; plasmablastic	Plasmablastic or immunoblastic	Plasmablastic or immunoblastic	Plasmablastic morphology	Ulcer with underlying Hodgkin-like infiltrate
Positive markers	CD20, CD30+/-, CD79, PAX5,	CD138, MUM1/ IRF4, EMA	CD20, CD4	CD45, CD30-/+, CD138+/-, MUM-1	CD20-/+, CD138+/-, IgM-lambda	CD20, PAX5, MUM1, CD30
Negative markers	CD10	CD10, CD20, PAX5	CD10, CD138, ALK	CD10, CD19, CD20, CD138	CD79a, CD138	CD10, BCL6
Proliferation rate (Ki67)	High	>90%	>90%	>90%	>90%	>90% in large cells
Cytoplasmic immunoglobulin	Negative	50–70%	Uncommon	Uncommon	IgA lambda	NA
Association with EBV infection	EBER 100%	EBER 80%	Common	EBER 70%	No	EBER 100%
Latency pattern EBV infection	II > III	I, II	III	I	NA	III, III
HHV8	-	-	-	+++	+++	-
Molecular genetics	Infrequent *MYC*, *BCL2* and *BCL6* rearrangements	*MYC* rearrangements	*TP53* mutations	No *BCL2*, *BCL6*, *MYC* rearrangements; hypermutated immunoglobulins	Unmutated immunoglobulin	*IGH* rearrangements
Gene expression profiling	Toll-like receptor and JAK-STAT		ABC-like signature			ABC-like signature

ABC, activated B-cell; DLBCL, diffuse large B-cell lymphoma; EBER, EBV-encoded RNA; EBV, Epstein-Barr virus; HHV8, human herpesvirus 8; PBL, plasmablastic lymphoma; PEL, primary effusion lymphoma.

and 107 with EBV-negative DLBCL.[3] Approximately 60% of the EBV+ patients achieved CR after chemotherapy, in contrast with 90% in EBV-negative patients. EBV+ DLBCL patients had worse survival than EBV-negative DLBCL patients with estimated 5-year OS rates of 25% versus 65%, approximately. Similar results were found in a Korean study that evaluated 34 EBV+ patients out of 380 patients with DLBCL.[7] EBV positivity was associated with median OS of 36 months versus not reached in EBV-negative DLBLC patients. A smaller Peruvian study also showed EBV positivity associated with worse prognosis in de novo DLBCL. Out of 74 patients with DLBCL, 11 patients were EBV+. The median OS in EBV+ patients was 7 months compared with 47 months in EBV-negative patients.[40] Of note, only a minority of patients, however, received rituximab as part of their therapy in these studies.

There has been a number of reports on the use of chemoimmunotherapy in patients with EBV+ DLBCL, NOS, with disparate results. In a multicenter consortium study on DLBCL patients treated uniformly with R-CHOP, the response and survival rates of 28 patients with EBV+ DLBCL, NOS, was compared with 695 EBV-negative patients, and showed no statistical differences.[18] Pathologically, there was a higher rate of CD30 expression in EBV+ patients. Similar results were found in a Korean study that evaluated 18 patients with EBV+ DLBCL, NOS, who had similar OS rates to 204 EBV-negative patients with 3-year OS rates of 57% and 60%, respectively.[41] Conversely, a Japanese study showed a median OS of 9 months in 8 patients with EBV+ DLBCL, NOS, treated with R-CHOP while the median OS for EBV-negative patients was not reached.[42] A Spanish study on 47 patients with EBV+ DLBCL mostly treated with R-CHOP-like regimens showed 2-year OS rate of 40%, which appeared lower than patients with EBV-negative DLBCL.[16] A Chinese study also found worse outcomes in patients with EBV+ DLBCL with median OS of 18 months versus median OS that was not reached in EBV-negative patients, although it was not specified the proportion of patients who received chemotherapy and chemoimmunotherapy.[10] A recent Peruvian study showed encouraging results with the use of R-CHOP in 17 patients with EBV+ DLBCL, NOS.[43] The OR and CR rate were 71% and 59%, respectively, and a 5-year OS rate of 54%. These findings were similar to the response and survival rates in EBV-negative DLBCL patients. However, in other more recent Asian studies, the use of R-CHOP was associated with poorer survival outcomes in patients with EBV+ DLBCL, NOS, when compared with EBV-negative DLBCL patients.[44,45] An early study reported EBV+ status was less frequent in DLBCL patients younger than 50 than in older than 50 (7 vs 9%, respectively) and had no impact on PFS and OS when treated with R-CHOP.[46]

In summary, patients with EBV+ DLBCL have a worse prognosis than EBV-negative patients when treated with chemotherapy. The outcomes appear somewhat better, although less favorable, when EBV+ DLBCL patients are treated with chemoimmunotherapy.

3.1 | Other prognostic factors

The IPI score is one of the most commonly used risk stratification tools in DLBCL. In an early report, the IPI score appeared to be of limited prognostic value in patients with EBV+ DLBCL.[3] A prognostic index that consisted on age older than 70 years and presence B symptoms was designed. Patients with zero, one or two factors showed median OS times of 56, 25 and 9 months, respectively. In a smaller study, Beltran and colleagues identified higher IPI and higher Oyama scores to be associated with worse outcome in patients with EBV+ DLBCL.[4] In this study, a notable adverse prognostic factor was lymphopenia defined as an absolute lymphocyte count of $<1.0 \times 10^9$/L.

CD30 expression is not only increased in, but also associated with a worse OS in patients with EBV+ DLBCL.[18] In this study, EBER+/CD30+ DLBCL patients had worse outcome than EBER+/CD30- or EBER-/CD30+ DLBCL patients. Survivin affects cellular apoptosis through indirect mechanisms that lead to inhibition of caspase 9.[47] In DLBCL treated with R-CHOP, high expression of survivin in tumor cells was associated with poor outcomes, especially in the ABC subtype.[48] Serum levels of survivin were high in 12% of DLBCL patients. Higher levels were seen in EBV+ than in EBV-negative DLBCL cases (19 vs. 5%, respectively). High serum survivin levels and were associated with poor outcomes.[44]

4 | MANAGEMENT OF EBV+ DLBCL, NOS

The addition of the anti-CD20 chimeric monoclonal antibody, rituximab, to anthracycline-based chemotherapy has clearly improved survival outcomes in patients with DLBCL in different clinical settings.[49–51] The response to combination chemotherapy appears lower in EBV+ DLBCL than in EBV-negative DLBCL patients. Overall response (OR) rates to cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) range from 30–80%, and complete response (CR) rates from 30% to 50%. More recent data suggest higher rates of response to chemoimmunotherapy, specifically rituximab and CHOP (R-CHOP) with OR rates of 50–90% and CR rates of 30–70%. However, no prospective comparative studies have been performed to date. Response rates to chemotherapy and chemoimmunotherapy in selected studies of patients with EBV+ DLBCL are shown in Table 2. Thus, there is not a standard approach for EBV+ DLBCL and treatment options usually are in concordance with current strategies for de novo DLBCL.

5 | FUTURE DIRECTIONS

Novel therapies that will address viral replication, targeting specific pathways in EBV+ DLBCL and improving/modulating the immune response against EBV have been investigated and seem promising in the treatment of this condition.

Typical antiviral therapies are not effective in eradicating EBV from chronically and transformed B-cells. In order for ganciclovir and acyclovir to show antiviral activity, they require proteins of lytic phase to be active, however EBV maintains a latent-phase in B-cell infected cells.[52] Thus, induction of EBV into a lytic phase could lead to an effective exposure to antiviral therapy.[53] Inducers of lytic phase include methylase transferase inhibitors, histone deacetylase inhibitors (HDACs), and proteasome inhibitors among others.[13,53] Arginine butyrate,

TABLE 2 Selected case series on the use of chemo(immuno)therapy in patients with EBV-positive DLBCL

Study	EBER	Regimen	N	OR/CR rate	OS
Oyama, 2007	>50%	CHOP	56	80%/66%	5-year: 25%
Park, 2007	>20%	CHOP	25	72%/NR	5-year: 48%
Beltran, 2011	>20%	R-CHOP	8	NR/66%	3-year: 40%
		CHOP	12	NR/33%	3-year: 40%
Ahn, 2014	>50%	R-CHOP	18	72%/61%	3-year: 57%
Ok, 2014	>10%	R-CHOP	28	89%/NR	5-year: 54%
Sato, 2014	>30%	R-CHOP	8	50%/25%	3-year: 38%
		CHOP	3	33%/33%	3-year: 0%
Lu, 2015	>20%	R-CHOP	35	66%/NR	3-year: ~30%
Song, 2015	NR	R-CHOP	8	63%/50%	3-year: 70%
		CHOP	8	50%/38%	3-year: 25%
Okamoto, 2016	>20%	R-CHOP	13	NR	4-year: 41%
Hong, 2017	>20%	R-CHOP	14	NR	Median: 15 months
Beltran, 2018	>20%	R-CHOP	17	59%/71%	5-year: 54%
		CHOP	16	31%/31%	5-year: 38%

CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; CR, complete response; EBER, EBV-encoded RNA; OR, overall response; OS, overall survival; NR, not reported; R-CHOP, rituximab and CHOP.

which has HDACs properties, and gancyclovir were administered to 15 patients with refractory EBV+ B-cell lymphomas. There were 10 responses with 4 complete responses.[54] Other HDACs, such as panobinostat and belinostat have shown a synergistic effect by sensitizing EBV+ lymphoma cell lines to ganciclovir, however clinical efficacy is yet to be proven.[55]

EBV+ DLBCL has an activated B-cell (ABC) DLBCL profile and is characterized by increased activation of the NF-kB pathway. The proteasome inhibitor bortezomib has induced apoptosis in EBV transformed B-cells and mouse models.[56] However, the addition on bortezomib to chemoimmunotherapy have been of modest benefit in DLBCL.[57,58] Lenalidomide can also target ABC DLBCL by down regulating IRF4 and NF-kB.[59] Early clinical data showed preferential activity in ABC DLBCL.[60] Studies adding lenalidomide to R-CHOP in newly diagnosed DLBCL have shown encouraging results, either concurrently with R-CHOP or as maintenance following R-CHOP.[61,62] Ibrutinib is an inhibitor of the Bruton Tyrosine Kinase (BTK), an important component of the B-cell receptor (BCR) pathway. In a study of 80 patients with refractory/relapsed DLBCL, ibrutinib induced an OR rate in ABC DLBCL and GCB DLBCL of 37% and 5%, respectively.[63] A randomized study of R-CHOP with or without ibrutinib in ABC DLBCL (NCT01855750) and a phase II study evaluating ibrutinib and R-CHOP in EBV+ DLBCL (NCT02670616) are ongoing. The PI3K kinase pathway seems to be critical for upstream signaling of NF-kB in ABC DLBCL cell lines, thus inhibition of the PI3K pathway appears to be another approach for EBV+ DLBCL with ABC signature.[64,65]

EBV blocks the expression of the highly immunogenic proteins during latency and expresses lytic proteins that impairs antigen processing by infected cells and by producing viral cytokines that impairs the immune system.[66] Efficacy of adoptive T-cell therapy using specific EBV cytotoxic T-cells (CTLs) was evaluated in patients with EBV-related PTLD in 1995.[67,68] The most common method for development of specific EBV CTLs is to establish LCLs by in vitro EBV infection.[69] The infusion of EBV CTLs was effective as treatment, in cases of PTLD, and as a prophylaxis, in patients undergoing transplantation.[70,71] Although effective, the time to manufacture CTLs (up to 6–12 weeks) may be too long for a patient with an aggressive lymphoma. To generate CTLs in a rapid manner, donor-derived EBNA1-specific T-cells were developed by a faster method using cytokine secreting system with selection of interferon-gamma secreting EBNA1-specific cells. The process to generate these EBV-CTLs takes approximately 3 days. In a study of 10 patients with EBV+ refractory PTLD after allogeneic HSCT, the administration of EBNA1-specific EBV-CTLs was able to restore T-cell immunity against EBV and induced a response rate of 70%.[72] In immunocompetent patients, generation of CTLs using this methodology did not yield the same efficacy with a response rate of 30% in patients with EBV+ Hodgkin lymphoma.[73] In an effort to improve immunogenicity and expand the EBV antigen profile potentially targeted by CTLs, dendritic cells transduced by adenovirus and EBV transformed LCL as antigen-presenting cells were used to activate and expand LMP-1/2 specific T-cells. In this trial of 50 patients with EBV+ lymphomas, the response rate was 64%.[74]

A potential biomarker with therapeutic implications is the expression of program death ligand 1 (PD-L1) in EBV+ DLBCL. Upregulation of PD-L1 is a mechanism of immune evasion on several cancers by inactivating anti-tumor T-cells responses.[75] Increased serum soluble PD-L1 was associated with poor prognosis in DLBCL patients treated with R-CHOP.[76] PD-L1 expression was positive in 100% of patients with EBV+ DLBCL as opposed to DBLCL, NOS (11%).[77] Expression PD-

L1 in tumor cells and microenvironment (mPD-L1) was studied in a larger study (n = 1557); of those 114 (9%) had positive EBER expression. The expression of PD-L1 and mPD-L1 was significantly associated with EBV+ status. Additionally, PD-L1 and mPD-L1 expression was noted in 16 and 41% of patients with EBV+ DLBCL.[78,79] A study restricted to younger EBV+ DLBCL patients showed increased PD-L1 in tumor cells as well as nonmalignant histiocytes.[11] Preclinical data showed that PD-1 blockade was associated with restoration of proliferation and activation of T-cells in PD-L1+ EBV+ DLBCL in greater degree than PD-L1+ EBV-negative DLBCL.[80] Thus, targeting the PD-L1/PD-1 pathway represents a potential therapeutic approach for EBV+ DLBCL. There are ongoing studies using durvalumab (NCT03212807), a PD-L1 inhibitor, and nivolumab (NCT02973113), a PD-1 inhibitor, in combination with EBV CTLs in refractory EBV+ lymphomas.

Chimeric antigen receptor (CAR) T-cells directed against tumor-associated markers, such as CD19, are undergoing clinical development in leukemia and lymphoma.[81,82] There is preclinical evidence of efficacy of LMP1-directed CAR T-cell in nasopharyngeal carcinoma, a malignancy associated with EBV infection.[83] Thus, immunotherapy seems to offer an interesting and effective alternative for patients with EBV-related lymphomas, in particular EBV-positive DLBCL of the elderly given its success and tolerability.

6 | CONCLUSION

In summary, EBV+ DLBCL, NOS, is an uncommon aggressive lymphoma subtype associated with a worse prognosis in the era of chemo-immunotherapy. Current studies have shown that EBV impacts the outcome of individuals with different ethnic background. Patients from Asia, Latin America and East Europe seem to have relatively poor survival, whereas North American patients showed no survival difference from EBV-negative DLBCL patients. This preliminary observation suggests that future clinical and biological analyses on EBV+ DLBCL, NOS, should be stratified per ethnic background. The incidence of EBV-positive DLBCL is likely underestimated as EBER testing is not routinely performed in pathological samples. Furthermore, an accepted cutoff for EBER positivity has not been defined. Particular signaling pathways such as CD30, NF-kB, BCR, and PD-1 appear closely related to mechanistic dysregulation in EBV+ DLBCL, NOS, patients. Due to the rarity of EBV+ DLBCL, the development of multi-institutional prospective studies is warranted.

CONFLICT OF INTERESTS

The authors have no conflict of interest to disclose.

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