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Lymphoma

Epstein-Barr Virus–Positive Diffuse Large B-Cell Lymphoma of the Elderly: What We Know So Far

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ABSTRACT

Epstein-Barr virus–positive (EBV-positive) diffuse large B-cell lymphoma (DLBCL) of the elderly is a newly described lymphoproliferative disorder recently included as a "provisional" entity in the most current WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. The objective of this review is to provide a thorough and current summary of the existing knowledge of this subtype of DLBCL. We will review and discuss the incidence of EBV expression in DLBCL, the pathogenesis behind EBV-driven malignant transformation of B cells, the different EBV latency patterns associated with DLBCL, the distinct pathologic characteristics of EBV-positive DLBCL, the potential predictive and prognostic value of EBV tumoral status in patients with DLBCL, and potential strategies for the treatment of this rare entity, which is characterized by a suboptimal response to therapy and poor survival rate. *The Oncologist* 2011;16:87–96

INTRODUCTION

The classification of lymphoproliferative disorders is an ever-changing field. Multiple classification systems have been used in the past, some based in morphology and some based in clinical features. The current WHO classification is, to date, the most comprehensive classification system, which includes not only morphologic and clinical characteristics but also immunohistochemical, molecular, and genetic aspects of these malignant lymphoid disorders [1]. Epstein-Barr virus–positive diffuse large B-cell lymphoma (EBV-positive DLBCL) of the elderly is among the newer inclusions as a provisional entity in the 2008 WHO Classi-

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fication of Tumors of Hematopoietic and Lymphoid Tissues [2].

EBV-positive DLBCL of the elderly is also known as age-related EBV-positive B-cell lymphoproliferative disorder or senile EBV-associated B-cell lymphoproliferative disorder. Patients with EBV-positive DLBCL of the elderly were initially described by Oyama and colleagues in 2003, in a report of 22 immunocompetent elderly patients [3]. These patients tended to be older than 50 years; however, younger patients have also been reported. Seventy percent of patients presented with extranodal involvement and more than half had advanced disease with poor prognostic International Prognostic Index (IPI) scores. Their clinical course is characterized by a short survival rate of approximately 24 months.

The purpose of this review is to provide a thorough summary of the existing knowledge of this subtype of DLBCL. We will discuss the incidence of EBV expression in DLBCL, the pathogenesis behind EBV-driven malignant transformation of B cells, the different EBV latency patterns associated with DLBCL, the distinct pathologic characteristics of EBV-positive DLBCL, the differential diagnosis of EBV-positive DLBCL, the potential predictive and prognostic value of EBV tumoral status in patients with DLBCL, and potential strategies for the treatment of this rare entity.

The Incidence of EBV in Lymphoproliferative Disorders

Approximately 95% of the general population maintains an asymptomatic yet persistent EBV infection [4]. EBV has been associated with a number of different lymphomas and lymphoproliferative diseases; these include but are not limited to Hodgkin lymphoma, Burkitt lymphoma, post-transplant lymphoproliferative disorders, angioimmunoblastic lymphoma, natural killer (NK)/T-cell lymphoma, human immunodeficiency virus/acquired immunodeficiency syndrome–associated (HIV/AIDS-associated) lymphomas, plasmablastic lymphoma, and DLBCL (Table 1).

Table 1. EBV incidence in different lymphoproliferative disorders

Lymphoma subtype	Percentage of EBV incidence
Hodgkin lymphoma	
developed countries	30%–50%
developing countries	~100%
Burkitt lymphoma	
endemic	100%
sporadic	15%–20%
Post-transplant lymphoproliferative disorder	
<1 year	73%–100%
>5 years	34%–80%
Diffuse large B-cell lymphoma, NOS	9%–15%
Primary CNS lymphoma (non-HIV)	9%–14%
EBV-positive DLBCL of the elderly	**100%**
NK/T-cell lymphoma	100%
Lymphomatoid granulomatosis	100%
HIV/AIDS-related lymphomas	
primary CNS lymphoma	100%
diffuse large B-cell lymphoma	80%
Burkitt lymphoma	30%–50%
primary effusion lymphoma	70%
plasmablastic lymphoma	60%

Abbreviations: AIDS, acquired immunodeficiency syndrome; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; NK, natural killer; NOS, not otherwise specified.

Hodgkin Lymphoma

In Hodgkin lymphoma, the incidence of EBV is markedly different depending on the histologic variant. For example, the incidence of EBV is very rare in the lymphocyte-predominant subtype [5]. However, in classic Hodgkin lymphoma the incidence can have a broad range depending on where in the world it is diagnosed. EBV positivity has been observed from 30% to 50% of patients in North America, Brazil, Taiwan, United Arab Emirates, and Western Europe, whereas it is seen in nearly 100% in children of developing countries [6–9]. It is hypothesized that the EBV encoded genes in the Reed-Sternberg cells provide a rescue effect for the cells destined for apoptosis, thereby producing immortalization [10].

Burkitt Lymphoma

The incidence of EBV also demonstrates a significant variability in Burkitt lymphoma based on which variant has been studied. In the endemic variant, primarily seen in Africa, the incidence approaches 100%, whereas EBV positivity is observed in 15%–20% in the sporadic variant and in 30%–40% of the immunodeficiency-related variant [11].

Immunosuppression-Associated Lymphomas

The incidence of EBV is increased in diseases that have significant immunosuppression. In patients with post-transplant lymphoproliferative disease (PTLD) the incidence of EBV ranges from 73% to 100%, being higher in cases that occur within 1 year after transplantation. In the less com-

mon entity of late occurrence PTLD, which is usually diagnosed 5 years after transplantation, EBV is expressed in approximately 34%–80% of these patients [12, 13].

An increased frequency of EBV is also seen in HIV/AIDS-associated lymphomas. In HIV/AIDS-associated primary central nervous system lymphomas, the rate of EBV infection approaches 100% [14], whereas in the immunocompetent population the rate is approximately 9%–14% [15, 16]. In the HIV population, 80% of DLBCL with immunoblastic features, 30%–50% of Burkitt lymphomas and 70% of primary effusion lymphomas are associated with EBV [17, 18]. Moreover, plasmablastic lymphoma also has an association with EBV in 74% of cases [19].

Peripheral T-Cell Lymphomas

EBV has also been associated with a number of T-cell lymphomas. It is found in 100% of NK/T-cell lymphomas and it is required for the diagnosis [20]. Furthermore, it has also been associated with angioimmunoblastic T-cell lymphoma; however, in this entity, EBV infection can be demonstrated in the B cells of the tumor microenvironment [21]. There is also a near 100% EBV association with lymphomatoid granulomatosis [22].

Diffuse Large B-Cell Lymphoma

In patients with DLBCL, the incidence of EBV among patients of Asian or Latin American origin ranges from 9% to 15% [23–25]. However, the incidence is only <5% in Western populations [26, 27]. As its descriptive nomenclature would imply, EBV-positive DLBCL of the elderly is mainly identified in patients older than 50 years. However, younger patients without evidence of immunodeficiency have also been reported [23, 24].

Pathogenesis

A schematic representation of the physiopathology of EBV-positive DLBCL of the elderly is shown in Figure 1.

Epstein-Barr Virus

EBV is a herpes virus with demonstrated B-cell lymphotropism and oncoviral properties. EBV infection starts by its attachment to the CD21 antigen. This initial step prepares the B-lymphocyte for EBV infection. The EBV genome encodes a series of products interacting with or exhibiting homology to a wide variety of antiapoptotic molecules, cytokines, and signal transducers, hence promoting EBV infection, immortalization, and transformation [13, 17]. Elevated titers of EBV antibodies have been found in the serum of some patients with EBV-associated lymphoproliferative disorders and can help in its diagnosis [13, 24]. B-cell differentiation represents a continuum that is initiated when a naive B cell encounters antigen,

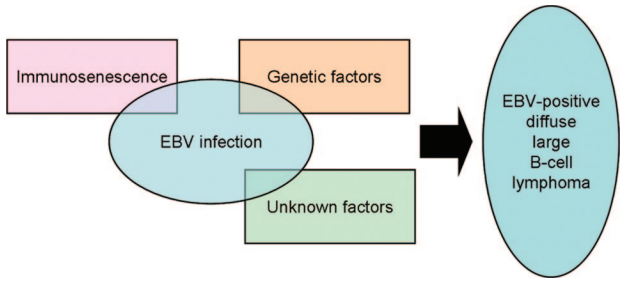

Figure 1. Pathophysiology of EBV-positive DLBCL of the elderly. Abbreviations: DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus.

undergoes a germinal center (GC) reaction, and ends with differentiation into either a memory cell or plasma B cell. Interruption of this process by a transforming event may result in a clonal proliferation where differentiation of the cell is blocked at this stage, resulting in the development of a lymphoid malignancy.

EBV and Postgerminal Center DLBCL

Molecular and genetic studies have identified at least two DLBCL profiles derived from GC or post-GC B cells, respectively [28, 29]. EBV encodes multiple microRNAs (miRNAs) from two primary transcripts, BHRF1 and the BARTs. The expression of BHRF1 miRNAs is dependent on the type of viral latency, whereas the BART miRNAs are expressed in cells during all forms of latency [30]. Recently, a dysregulation over BCL6, the master key molecule in GC, directed by three particular EBV-associated miRNAs was demonstrated [31]. It has been proposed that EBV-induced nuclear factor-κB (NF-κB) pathway activation might be required for DLBCL cells to survive [32]; NF-κB has been associated predominantly in post-GC variants of DLBCL, which seems to derive a worse survival rate [25]. Furthermore, genetic factors could also play a role in the development of EBV-associated lymphoma. It has been suggested that a genetically determined immune susceptibility, possibly based on certain HLA types, results in an abnormal response to primary EBV infection in certain parts of Asia [15]. These variations in HLA phenotype may provide a basis for the higher frequency of EBV-positive tumors among Asians.

Immunosenescence

An important mechanism in lymphomagenesis appears to be related to immunosenescence. One component of immunosenescence is a decrease in T-cell response that is thought to be a natural part of aging [33]. The presence of EBV nuclear antigen (EBNA)-2 was observed in 28% of the cases of age-related EBV-associated lymphoproliferative disorder reported by Oyama [24], which is indicative of a type III EBV latency. Interestingly, this incidence is similar to that observed in some

Table 2. EBV-related proteins expressed in various diseases according to the latency patterns

Latency Pattern	EBV-Associated Proteins			Disorder
	EBNA1/LMP2A	LMP1/LMP2B	EBNA2/3A/3B/3C/LP	
I	+	−	−	Burkitt lymphoma
II	+	+	−	Classic Hodgkin lymphoma, PEL, AITL, NK/T-cell lymphoma, nasopharyngeal carcinoma, **EBV-positive DLBCL of the elderly**
III	+	+	+	PTLD, AIDS-related lymphoma, infectious mononucleosis, **EBV-positive DLBCL of the elderly**

Abbreviations: AIDS, acquired immunodeficiency syndrome; AITL, angioimmunoblastic lymphoma; DLBCL, diffuse large B-cell lymphoma; EBNA, EBV nuclear antigen; EBV, Epstein-Barr virus; LMP, latent membrane protein; LP, leader protein; PEL, primary effusion lymphoma; PTLD, post-transplant lymphoproliferative disorder.

case series of HIV-associated lymphomas [34] and post-transplant lymphoproliferative disorders [35]. Immunosenescence is associated with the decline of immune function but this decline is not homogeneous. Some functions such as the cellular immune system are altered, but others are enhanced such as one's innate immunity. Age-related decline of the cellular immunity is a consequence of several factors, including thymic atrophy, reduced output of new T lymphocytes, accumulation of anergic memory cells, deficiencies in the cytokine production, and uncertain antigen presentation. Persistent infection by different herpes viruses and other parasites contribute to the loss of immunosurveillance and premature exhaustion of T cells [36].

Other Factors

EBV-positive DLBCL of the elderly is not related to any known immunodeficiency states. However, Beltran and colleagues described a case of EBV-positive DLBCL of the elderly arising in a human T-lymphotrophic virus (HTLV)-1 carrier [37]. It is known that chronic HTLV-1 infection can cause T-cell dysfunction and B-cell proliferation, inducing a particular state of immunosuppression, favoring lymphomagenesis. It is possible that other unknown immunosuppressive agents or conditions could play a role in the pathogenesis of this disease.

Patterns of EBV Latency in DLBCL

Table 2 shows the different EBV latency patterns and the diseases associated with each of them. It has been shown that there are three different latency patterns in EBV-related malignant disorders and EBV-derived cell lines, recognized as latency patterns I, II, and III. These latency patterns are the result of differential promoter activity and are influenced by host cell factors. More profound immunosuppression is associated with higher latency states [38, 39]. Therefore, in immunocompetent hosts, the virus exists in latency I state and there is no latent membrane 1 (LMP1) expression, which is the essential transforming viral oncoprotein and mimics constitutively active CD40. LMP1 acts via the TNF receptor-activated factor (TRAF) pathway that activates transcription through NF-κB [40, 41]. Latency II and latency III appear in hosts with increasing degrees of immunosuppression, such as organ transplantation and HIV/AIDS patients. A progressive switch from lymphomas with a GC phenotype including Burkitt lymphoma to those with a post-GC or activated B-cell (ABC) phenotype, including immunoblastic and primary effusion lymphomas, has been described [42–44].

EBV-positive DLBCL of the elderly shows an EBV latency type II or III pattern. Its prognosis is inferior as compared with that of age-matched DLBCL without EBV infection. This worse prognosis is independent of IPI score [24]. EBNA1 and LMP2A are the only expressed proteins in type I latency. The most characteristic disease of this type is Burkitt lymphoma. In type II latency, LMP1 and LMP2B are additionally expressed. The most important characteristic example of this type is classical Hodgkin lymphoma. Primary effusion lymphoma, angioimmunoblastic T-cell lymphoma, NK/T-cell lymphoma, and nasopharyngeal carcinoma are other examples of EBV infection with type II latency. In type III latency, all EBV-associated proteins are expressed. Post-transplant lymphoproliferative disorders, AIDS-related immunoblastic lymphoma, and infectious mononucleosis are the characteristic examples of the type III latency [38, 39].

Pathologic Features of EBV-Positive DLBLC of the Elderly

Histopathologic Features

In EBV-positive DLBCL the most common pathologic feature is an effacement of nodal or extranodal structures by

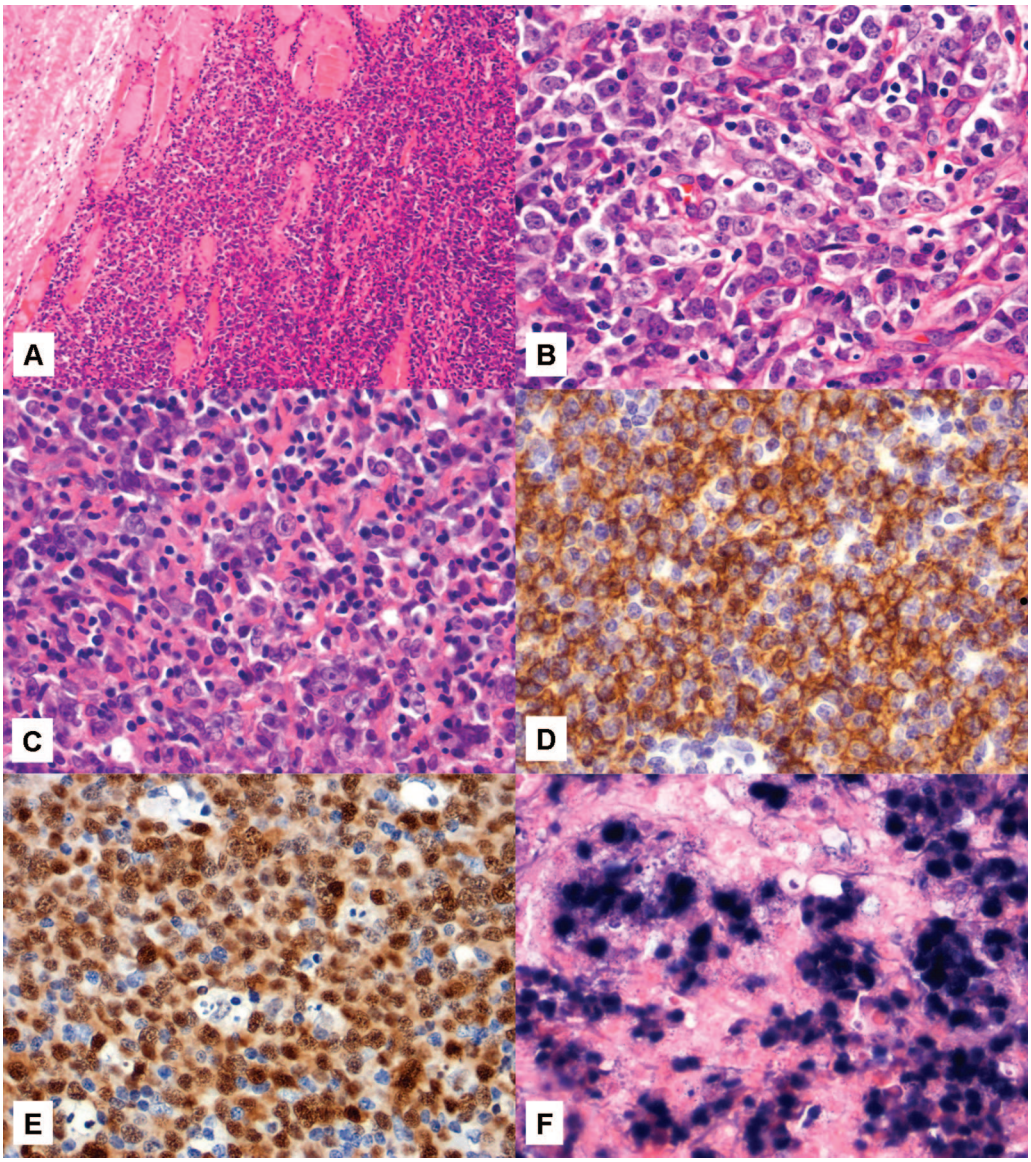

Figure 2. Pathologic characteristics of EBV-positive DLBCL of the elderly. **(A):** EBV-positive DLBCL of the elderly infiltrating soft tissue. Hematoxylin and eosin, ×100. **(B):** Monomorphic pattern displaying large cells with immunoblastic features, characterized by central prominent nucleoli. Hematoxylin and eosin, ×400. **(C):** Polymorphic pattern displaying large cells admixed with abundant reactive inflammatory cells, mainly small lymphocytes. Hematoxylin and eosin, ×400. **(D):** CD20. Positivity for CD20 is consistent with a B-cell lineage. Immunohistochemistry, ×400. **(E):** MUM-1 (nuclear pattern). Most EBV-positive DLBCL of the elderly are positive for MUM-1, consistent with a nongerminal center cell phenotype. Immunohistochemistry, ×400. **(F):** EBV-encoded RNA (EBER) (nuclear pattern). In situ hybridization, ×400.

Abbreviations: DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus.

the malignant lymphocyte (Fig. 2A). Initial descriptions emphasized the presence of two separate variant subtypes, the polymorphous and large-cell subtypes. However, both variants may present with many large cells, including immunoblasts and Reed-Sternberg–like cells [3]. The polymorphous subtype has variable amounts of reactive cells such as small lymphocytes, plasma cells, and histiocytes (Fig. 2B). The large cell subtype refers to the presence of a rather uniform population of large cells with minimal or no reactive component (Fig. 2C). However, most cases commonly show both histologic patterns [45]. Mitoses and apoptosis with tingible body macrophages are common. Necrosis is common and usually extensive. Morphologic features do not appear related to prognosis, as both subtypes behave aggressively.

Immunophenotypic Features

The neoplastic cells are usually positive for the leukocyte common antigen CD45 as well as for B-cell markers CD20,

CD19, CD79a, and PAX-5. Cases with plasmablastic or immunoblastic features show weak or absent CD20 [26]. Light-chain restriction is not usual, except in the cases with plasmablastic differentiation. The GC markers CD10 and BCL6 are usually negative, whereas IRF4/MUM1 (Fig. 2E) is commonly positive, consistent with a post-GC phenotype. EBV-associated latent antigens such as LMP1 and EBNA-2 are positive in 94% and 28% of the cases, respectively. Up to 50% of the cases express CD30 but CD15 is negative. Ki-67 (or MIB-1), a marker of cellular proliferation, usually is expressed in higher than 70% of the malignant cells.

Molecular Features

In situ hybridization (ISH) for EBV-encoded RNA (EBER) is positive in the majority of neoplastic cells (Fig. 2F). Double-labeling studies demonstrate that the large majority of the infected cells are B cells and only rare T cells are EBER-positive [24]. Molecular studies can demonstrate monoclonal heavy- and light-chain immunoglobulin gene rearrangements, consistent with a B-cell origin. Similarly, analysis of EBV terminal repeat domains demonstrates clonality.

Differential Diagnosis

Reactive Interfollicular Expansion, Including Infectious Mononucleosis

Although infectious mononucleosis is rare in the elderly, it does occur. Similar to other reactive processes, it results in pathologic changes of marked interfollicular expansion with a spectrum of cells ranging from small to intermediate and large cells with immunoblastic features, plasma cells, and histiocytes. This process may show abundant EBV-positive cells, and the affected cells may be both small and large. Similarly, there are frequent residual and hyperplastic GCs. The spectrum of processes with this pattern includes infectious mononucleosis, chronic active EBV infection, and drug reaction. In chronic active EBV infection, the abnormal cells are T cells. Among drugs, phenytoin is well known, but methotrexate in patients with rheumatoid arthritis can also result in similar pathologic features.

Other EBV-Associated DLBCL Variants

Certain categories of DLBCL that are also EBV-positive are excluded from the diagnosis of EBV-positive DLBCL of the elderly. They include lymphomatoid granulomatosis, plasmablastic lymphoma, primary effusion lymphoma, and DLBCL associated with chronic inflammation. Similarly, the presence of EBV-positive DLBCL in younger patients is excluded on the basis of the higher likelihood that these patients may have an underlying or undetected immunodeficiency. The neoplastic cells of post-transplant lymphoproliferative disease also express type III EBV latency and EBER is found to be positive [39]. The clinical history is the most important criterion for the differentiation between EBV-positive DLBCL and post-transplant lymphoproliferative disease [27].

Classic Hodgkin Lymphoma

Classic Hodgkin lymphoma is characterized by an abundant reactive background, which includes small lymphocytes, plasma cells, eosinophils, and histiocytes. The neoplastic cells usually represent approximately 1% of the cellular infiltrate; however, there are occasional cases of nodular sclerosis Hodgkin lymphoma displaying nodules with sheets of large neoplastic cells that mimic DLBCL. Features that raise the suspicion for Hodgkin lymphoma include the presence of sclerosis that tends to surround cellular nodules, the presence of retraction around neoplastic cells, and the large cells marking with CD30 (strong reactivity, membrane, and Golgi pattern), PAX-5 (faint), and usually CD15. Necrosis can be focal but it is more frequent and more extensive in EBV-positive DLBCL of the elderly.

Classic Hodgkin lymphoma can be associated with EBV [46]. The nodular sclerosis subtype shows a 10%–40% association with EBER and LMP1 positivity, whereas the mixed cellularity subtype can be positive in up to 75% of cases. Reed-Sternberg or Hodgkin cells usually express EBER and LMP1 positivity.

Methods of Detection of EBV in DLBCL

Immunohistochemistry (ICH) and ISH are the most important and most frequently used methods for the detection of EBV in DLBCL. CD20, PAX-5, and CD79a are the essential surface markers to determine the B-cell origin of the lymphoma. CD15 and CD30 are the other markers used to differentiate from Hodgkin lymphoma. CD30 has been detected in some of the cases with EBV-related DLBCL and it has been characterized as an activation marker. Ki-67 is another important marker in this entity, and positivity is seen in generally higher than 70% of cells. Viral markers, LMPs, and EBNAs are detected by IHC [24, 27, 47, 48].

ISH studies have demonstrated preferential localization of EBV outside the GC (i.e., in interfollicular or extrafollicular locations). This may account for the absence of EBV in association with follicular lymphoma or lymphocyte-predominant Hodgkin lymphoma, which are neoplasms deriving from a GC origin [39].

EBER ISH is the most important test and it has the high-

est diagnostic sensitivity in the diagnosis of EBV-positive DLBCL. EBER expression can range from 10% to almost all of the tumor cells. There is no morphologic or immunohistochemical pattern that is specific for this entity.

Although EBER ISH is the diagnostic test for these cases, it is not uniformly recommended that routine EBV testing be done in all cases of DLBCL in patients older than 50 years. That recommendation may be justified only after prospective clinical studies are performed to understand the prognostic and/or predictive role of EBV in DLBCL [49].

EBV and Its Association with DLBCL Treatment Response and Survival

EBV as a Prognostic Factor in DLBCL

Few published studies support a possible prognostic relationship between EBV tumoral status and DLBCL. Oyama and colleagues had reported EBV-positive B-cell lymphomas occurring commonly in the elderly population with the presumption that immunologic deterioration was a predisposing factor [24]. This Japanese study evaluated 1,792 patients with DLBCL and compared 96 patients with EBV-positive DLBCL with 107 EBV-negative DLBCL. In the survival analysis, EBV-positive DLBCL patients, evaluated by the presence of EBV-encoded RNA (EBER) within the tumoral cells, had a shorter survival rate than EBV-negative DLBCL patients with a median overall survival (OS) of 24 months versus not reached ($p < .0001$). In a subset analysis, EBV tumoral status was an independent prognostic factor in patients with high and low IPI scores ($p = .0001$ and .0004, respectively). A Korean study by Park and colleagues also evaluated the expression of EBER within the tumoral cells of patients with DLBCL treated with chemotherapy and chemoimmunotherapy [25]. EBER-positive DLBCL cases were associated with a worse OS (36 months versus not reached; $p = .026$) and a worse progression-free survival (13 versus 36 months; $p = .018$). In subset analyses, EBV positivity was associated with a worse OS and progression-free survival in patients with high IPI scores and a non-GC phenotype. In a smaller study, Morales and colleagues evaluated the prognostic significance of EBER expression in a Peruvian population with DLBCL treated with chemotherapy alone, without rituximab. EBV-positive DLBCL patients had a significantly shorter OS (7 versus 47 months; $p = .001$) [23]. In the multivariate analysis, EBER expression and IPI scores were determined to be independent prognostic indicators. Gibson and colleague described the characteristics of five Caucasian patients with EBV-positive DLBCL [26]. Although the investigators did not perform a formal survival analysis, a poor outcome was observed in the EBV-positive DLBCL patients. Similarly, Hoeller and colleagues recently published data on eight European patients with EBV-positive DLBCL [27]. Again, no formal survival analysis was done but patients with EBV latency types II and III had poor survival rate. Finally, Paydas and colleagues evaluated the effect of six biologic parameters, one of which was EBV latent membrane protein-1 (LMP-1), in patients with DLBCL. They demonstrated that LMP-1 expression was deemed the most important poor prognostic indicator associated with the shortest survival rate (21 months) when compared to other molecular markers such as survivin, VEGF-A, and VEGF-C [50].

One study, however, did not show an association between EBV tumoral status and survival in patients with DLBCL. Kuze and colleagues studied 114 patients with DLBCL, from which 13 patients expressed EBER. When the 58 patients who were EBV-negative were compared with the 12 patients who had EBV-positive DLBCL, there was no statistical difference in survival [48].

These data, however, have several caveats: (1) most of the studies are largely limited to Asian or Hispanic populations in which the incidence of EBV-positive DLBCL seems higher than that in the Caucasian population [26, 27]; and (2) the majority of patients received chemotherapy without the addition of rituximab; rituximab has been shown to increase survival in patients with DLBCL in several randomized controlled trials [51–53], rendering these patients as suboptimally treated.

In summary, the role of EBV in the prognosis of DLBCL has not been completely elucidated but the available data implicate EBV positivity as a potential predictor of worse survival rate in patients with DLBCL.

EBV as a Predictive Factor of Treatment Response in DLBCL

Few studies have shown that EBV-positive DLBCL patients have a worse response to chemotherapy than their EBV-negative counterparts. Oyama and colleagues evaluated response to chemotherapy in 96 and 107 patients with EBV-positive and EBV-negative DLBCL, respectively [24]. When evaluating only the patients treated with anthracycline-containing regimens (62 of which who were EBV-positive and 104 of which who were EBV-negative), overall response rates of 80% and 99% were observed, respectively, favoring the EBV-negative DLBCL patients ($p < .0001$). Furthermore, the complete response (CR) rates were 66% and 91%, respectively. However, only one patient was treated with chemotherapy in combination with rituximab in this series. Park and colleagues showed similar results [25]. Among 232 patients with DLBCL assessable for response, 207 were EBV-negative and 25 were EBV-positive. EBV-negative and EBV-positive DLBCL patients had overall response rates of 92% and 72%, respectively

($p = 0.006$); however, no CR data were reported. Additionally, less than 20% of the patients studied received rituximab as part of their initial therapy. A smaller study by Yoshino and colleagues found that 4 of 50 patients with primary gastric DLBCL were EBV-positive [54]. These patients had a poor response to radiochemotherapy with two patients experiencing progressive disease, one partial response and one CR with subsequent systemic relapse.

On the basis of these scant data, EBV-positive DLBCL patients seem to have worse response to chemotherapy than EBV-negative DLBCL. However, the response to rituximab-containing regimens has not been appropriately evaluated to date, and further studies are needed to elucidate the role of rituximab and EBV-directed therapy in EBV-positive DLBCL.

Therapeutic Approaches for EBV-Positive DLBCL

Currently, there is no uniformly accepted treatment for EBV-positive DLBCL beyond the current standard therapy for DLBCL. Most of the published treatment experiences are reports that have evaluated chemotherapy alone with suboptimal results [23, 25, 48]. The standard treatment for DLBCL is the combination of rituximab, a chimeric anti-CD20 monoclonal antibody, and CHOP (R-CHOP). R-CHOP has shown to prolong survival in patients with DLBCL at early and advanced stages in a series of randomized controlled trails [51–53]. It is likely that R-CHOP will also provide a survival benefit in EBV-positive DLBCL. However, there have been scant data published on the use of rituximab-based regimens in patients with this condition. For example, Park and colleagues reported that only 2 of 34 cases (6%) were treated with R-CHOP [25]. Furthermore, Gibson and colleagues reported that only 1 of 4 cases (25%) treated with R-CHOP obtained a CR and that the patient relapsed shortly afterward [26]. Ideally, prospective studies are needed to clarify whether rituximab can overcome the adverse prognostic significance of EBV positivity in DLBCL. This is particularly important since rituximab has been shown to overcome other adverse prognostic influences in DLBCL, such as BCL-2 expression [55]. However, given its aggressive nature, and the commonly seen poor outcomes of EBV-positive DLBCL, it is likely that other novel therapies may be needed.

EBV-positive lymphomas can be amenable to immunotherapy with EBV-specific cytotoxic T-cell lymphocytes, which has achieved success on preventing and treating EBV-associated lymphomas in patients undergoing solid organ transplantation [56]. Unfortunately, this approach has not been used in immunocompetent patients with DLBCL. Other potential approaches have included arginine butyrate, which has histone deacetylating properties, in combination with ganciclovir. This regimen was used in 15 patients with EBV-positive lymphoid malignancies with encouraging results [57]. However, most of the patients had histologies other than DLBCL or had an underlying immunosuppressive condition.

Preclinically, EBV proliferations have shown association with a variety of molecular pathways, opening potential avenues of research and novel treatment ideas. The combination of valproic acid, with its histone deacetylating properties, and ganciclovir have shown efficacy on depleting EBV-transformed cells when compared with chemotherapy alone [58]. Bortezomib, a proteasome inhibitor approved for the treatment of plasma cell myeloma, blocks NF-κB, which has been associated with EBV-associated B-cell transformation [59]. Bortezomib in combination with other molecularly targeted agents or chemotherapy may prove to be of value in the treatment of EBV-positive DLBCL. A series of mammalian targets of rapamycin inhibitors may also be of value on treating EBV-positive DLBCL [60, 61].

Finally, there are several ongoing clinical trials focused on treating relapsed/refractory EBV-positive lymphomas in immunocompetent patients with EBV-specific cytotoxic T-cell lymphocytes (NCT00058617, NCT00779337), the combination of ganciclovir or valganciclovir and arginine butyrate (NCT00917826), and the combination of bortezomib and ganciclovir (NCT00093704) [62]. Results from these trials are eagerly expected, as they could be of paramount importance in the treatment of EBV-positive DLBCL of the elderly.

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