Received: 7 April 2017 | Revised: 12 May 2017 | Accepted: 26 May 2017

DOI: 10.1002/hon.2449

WILEY

ORIGINAL RESEARCH ARTICLE

# Response and survival benefit with chemoimmunotherapy in Epstein-Barr virus-positive diffuse large B-cell lymphoma

**Brady E. Beltran**[1] | **Pilar Quiñones**[2] | **Domingo Morales**[2] | **Jose M. Malaga**[3] | **Julio C. Chavez**[4] | **Eduardo M. Sotomayor**[5] | **Jorge J. Castillo**[6]

[1] Department of Oncology and Radiotherapy, Hospital Nacional Edgardo Rebagliati Martins, Lima, Peru

[2] Department of Pathology, Hospital Nacional Edgardo Rebagliati Martins, Lima, Peru

[3] Department of Hematology, Hospital Alberto Seguin, Arequipa, Peru

[4] Division of Hematology and Oncology, Moffitt Cancer Center, Tampa, FL, USA

[5] Division of Hematology/Oncology, George Washington University Cancer Center, Washington, DC, USA

[6] Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

**Correspondence**
Jorge J Castillo, Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.
Email: jorgej_castillo@dfci.harvard.edu

## Abstract

Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) is a haematologic malignancy with poor prognosis when treated with chemotherapy. We evaluated response and survival benefits of chemoimmunotherapy in EBV-positive DLBCL patients. A total of 117 DLBCL patients were included in our retrospective analysis; 33 were EBV-positive (17 treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone [R-CHOP] and 16 with CHOP), and 84 were EBV-negative (all treated with R-CHOP). The outcomes of interest were complete response (CR) and overall survival (OS) in EBV-positive DLBCL patients (R-CHOP versus CHOP) and in DLBCL patients treated with R-CHOP (EBV-positive vs EBV-negative). There were no differences in the clinical characteristics between EBV-positive and EBV-negative DLBCL patients. Among EBV-positive DLBCL patients, R-CHOP was associated with higher odds of CR (OR 3.14, 95% CI 0.75-13.2; $P$ = .10) and better OS (hazard ratio 0.30, 95% confidence interval [CI] 0.09-0.94; $P$ = .04). There were no differences in CR rate (OR 0.52, 95% CI 0.18-1.56; $P$ = .25) or OS (hazard ratio 0.93, 95% CI 0.32-2.67; $P$ = .89) between EBV-positive and EBV-negative DLBCL patients treated with R-CHOP. Based on our study, the addition of rituximab to CHOP is associated with improved response and survival in EBV-positive DLBCL patients. Epstein-Barr virus status does not seem to affect response or survival in DLBCL patients treated with R-CHOP.

**KEYWORDS**

diffuse large B-cell lymphoma, DLBCL, EBV, Epstein-Barr virus, rituximab

## 1 | INTRODUCTION

Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL), not otherwise specified, is an entity included in the 2016 revision of the World Health Organization (WHO) Classification of Lymphoid Neoplasms [1]. Epstein-Barr virus-positive DLBCL is an entity with a variable prevalence in the world depending geographic aspects with a range between 2% and 15% [2-10]. Several studies have reported poor outcomes with standard regimens [2,7-9].

The addition of the anti-CD20 monoclonal antibody to chemotherapy has positively impacted the response and survival rates of patients with DLBCL [11-13]. Whether the addition of rituximab to chemotherapy improves outcomes in patients with EBV-positive DLBCL is, however, unknown. A few retrospective studies have reported disparate results [9,14]. Finally, the predictive and prognostic value of the EBV status in patients with de novo DLBCL treated with chemoimmunotherapy has not been formally studied.

The main objectives of our study were to compare the response and survival benefit of the addition of rituximab to chemotherapy in patients with EBV-positive DLBCL and to evaluate the effect on response and survival outcomes of EBV status in patients with DLBCL treated with chemoimmunotherapy.

## 2 | METHODS

### 2.1 | Case selection

From January 2006 to December 2015, patients with a pathological diagnosis of de novo DLBCL and treated with chemotherapy or chemoimmunotherapy were identified from the medical records at

  

the Hospital Nacional Edgardo Rebagliati Martins in Lima, Peru. Pathological samples were retrieved and reviewed by 2 expert haematopathologists (DM and PQ) and reclassified according to the 2016 WHO Classification of Tumors of the Hematopoietic and Lymphoid Tissues [1]. Patients younger than 18 years or with diagnosis of HIV, primary immunodeficiency, autoimmune disease, or treatment with inmunosuppressants were excluded. The presence of EBV was evaluated by detecting EBV-encoded RNA (EBER) using a fluorescein-labelled peptide nucleic acid probe (Dako) in conjunction with the Dako peptide nucleic acid in situ hybridization detection kit for formalin-fixed paraffin-embedded tissue sections. The presence of EBER in greater than or equal to 20% of malignant cells was considered positive. Patients were treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or rituximab and CHOP (R-CHOP) at standard doses. Both regimens were administered every 3 weeks for up to 6 cycles. The study protocol was reviewed and approved by the Institutional Review Board at the Hospital Nacional Edgardo Rebagliati Martins.

## 2.2 | Data gathering

Clinical data were gathered from the medical records of the selected patients. Clinical parameters were categorized to facilitate analysis and included age, sex, B symptoms, Eastern Cooperative Oncology Group performance status, lactate dehydrogenase (LDH) levels, number of extranodal sites, Ann Arbor clinical stage, International Prognostic Index (IPI) score, response to therapy, and survival outcome. Response assessment was performed based on the 2007 Cheson criteria, whenever possible [15]. Overall survival (OS) was defined as the time in months between the date of DLBCL diagnosis and the date of last follow-up or death of any cause.

## 2.3 | Statistical analysis

Clinicopathological data are presented by using descriptive statistics. The $\chi^2$ test was used to compare categorical variables. Logistic regression was used to evaluate association between clinical factors and complete response (CR), and results are presented as odds ratio (OR) with 95% confidence interval (CI). Survival curves were generated by using the Kaplan-Meier method [16] and compared by using the log-rank test [17]. The Cox proportional-hazard regression method was used to fit univariate survival models [18], and results are reported as hazard ratio (HR) with 95% CI. All reported *P* values are 2-sided and were considered significant if less than .05. Multivariate proportional hazard regression models were not attempted given the small sample size. Calculations and graphics were obtained by using the statistical software STATA version 13.1 (College Station, Texas, USA).

# 3 | RESULTS

## 3.1 | Patients' characteristics

A total of 117 patients with a diagnosis of DLBCL were identified for this study. Of these, 33 (28%) had a diagnosis of EBV-positive DLBCL, 17 were treated with R-CHOP, and 16 with CHOP. The remainder 84 patients (72%) had a diagnosis of EBV-negative DLBCL and were treated with R-CHOP. The patients' characteristics of

**TABLE 1** Selected characteristics of Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) patients treated with R-CHOP or CHOP and EBV-negative DLBCL patients

| | EBV+ DLBCL | | | EBV− DLBCL | |
|---|---|---|---|---|---|
| | CHOP (*n* = 16) | R-CHOP (*n* = 17) | | R-CHOP (*n* = 84) | |
| Characteristic | Number (%) | Number (%) | *P* value[a] | Number (%) | *P* value[b] |
| Age ≥ 60 years | 11 (69%) | 15 (88%) | .17 | 57 (68%) | .10 |
| Poor performance status (ECOG > 1) | 8 (50%) | 6 (35%) | .12 | 27 (32%) | .80 |
| Elevated lactate dehydrogenase levels | 7 (44%) | 7 (41%) | .88 | 46 (55%) | .31 |
| Extranodal sites > 1 | 6 (38%) | 11 (65%) | .12 | 36 (43%) | .10 |
| Advanced stage (III and IV) | 10 (63%) | 6 (35%) | .06 | 35 (42%) | .63 |
| International Prognostic Index score | | | | | |
| Low risk (0-2 factors) | 5 (31%) | 6 (35%) | .06 | 36 (43%) | .56 |
| High risk (3-5 factors) | 11 (69%) | 11 (65%) | | 48 (57%) | |
| Response to treatment | | | | | |
| Complete response | 5 (31%) | 10 (59%) | .02 | 57 (73%) | .49 |
| Partial response | 0 (0%) | 3 (18%) | | 10 (13%) | |
| No response | 11 (69%) | 4 (24%) | | 11 (14%) | |
| Outcome | | | | | |
| Alive | 5 (31%) | 12 (71%) | .009 | 57 (68%) | .48 |
| Dead | 11 (69%) | 5 (29%) | | 27 (32%) | |

CHOP indicates cyclophosphamide, doxorubicin, vincristine, and prednisone; ECOG, Eastern Cooperative Oncology Group; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

[a]Comparison between CHOP and R-CHOP groups in EBV+ DLBCL patients.

[b]Comparison between EBV+ and EBV− DLBCL in R-CHOP patients.

EBV-positive DLBCL patients treated with CHOP and R-CHOP and EBV-negative DLBCL patients treated with R-CHOP are shown in Table 1. There were no differences in age, performance status, LDH levels, number of extranodal sites, stage, and IPI score between EBV-positive patients treated with either CHOP or R-CHOP. Epstein-Barr virus-positive DLBCL patients treated with R-CHOP had higher response rates and a higher proportion of patients alive when compared with EBV-positive DLBCL patients treated with CHOP. There were no differences in age, performance status, LDH levels, number of extranodal sites, stage, IPI score, response rate, and proportion of patients alive between EBV-positive and EBV-negative DLBCL patients treated with R-CHOP. The cause of death was lymphoma progression in all cases.

### 3.2 | Response and survival outcomes in Epstein-Barr virus-positive diffuse large B-cell lymphoma patients

Among patients with EBV-positive DLBCL, the only factor associated with lower odds of CR was advance stage (OR 0.14, 95% CI 0.03-0.65, $P$ = .01). The OR of CR for R-CHOP versus CHOP was 4.14 (95% CI 1.75-13.2; $P$ = .01). About survival, the 5 year OS in EBV-positive patients was 54% (95% CI 35-70%). The 5 year OSs of patients treated with CHOP were 38% (95% CI 15-6%) and 71% (95% CI 39-88%) in patients treated with R-CHOP (Figure 1). Rituximab and CHOP was associated with better OS in patients with EBV-positive DLBCL (HR 0.30, 95% CI 0.09-0.94; $P$ = .04). There was a trend towards worse OS in patients with high/high-intermediate IPI score (HR 2.44; 95% CI 0.83-7.22; $P$ = .10). Treatment with R-CHOP was associated with better OS after adjusting for the IPI score (HR 0.23, 95% CI 0.07-0.74; $P$ = .01). In a stratified analysis, treatment with R-CHOP improved OS in patients with high/high-intermediate risk IPI score (HR 0.09; 95% CI 0.01-0.77; $P$ = .03), but it did not improve outcomes in patients with low/low-intermediate risk IPI score (HR 1.03, 95% CI 0.21-10.0; $P$ = .54).

### 3.3 | Response and survival outcomes in diffuse large B-cell lymphoma patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone

Among patients treated with R-CHOP, the factors associated with lower odds of CR were an elevated LDH level (OR 0.38, 95% CI 0.15-0.97; $P$ = .04) and advance clinical stage (OR 0.39, 95% CI 0.16-0.97; $P$ = .04). Epstein-Barr virus status was not associated with higher or lower odds of CR in DLBCL patients treated with R-CHOP (OR 0.53, 95% CI 0.18-1.56; $P$ = .25). In DLCBL patients treated with R-CHOP, the 5 year OS was 64% (95% CI 52-73%). The 5 year OSs in EBV-positive patients were 71% (95% CI 39-88%) and 63% (95% CI 51-73%) in EBV-negative patients (Figure 2). Epstein-Barr virus status was not associated with better or worse OS (HR 0.93, 95% CI 0.32-2.67; $P$ = .89). A high/high-intermediate IPI score was associated with worse OS (HR 2.49, 95% CI 1.22-5.11; $P$ = .01).

## 4 | DISCUSSION

Herein, we present the results of a retrospective study aimed at comparing the response and survival outcomes of patients with EBV-positive DLBCL treated with chemotherapy and chemoimmunotherapy.

Epstein-Barr virus-positive DLBCL is an entity included in the 2016 WHO Classification of Lymphomas, which has been associated with poor outcomes with standard chemotherapy regimens [19]. Complete response rates in EBV-positive DLBCL patients with CHOP and CHOP-like regimens have ranged between 30% and 60%, with overall response rates between 50% and 80%. Also, the 5 year OS in patients with EBV-positive DLBCL had previously been reported as ranging between 0% and 50%, depending on the series [7-9,14]. More recently, the use of chemoimmunotherapy has been associated with improvement in patients with EBV-positive DLBCL, manifested by CR rates ranging between 30% and 60%, overall response rates between 60%

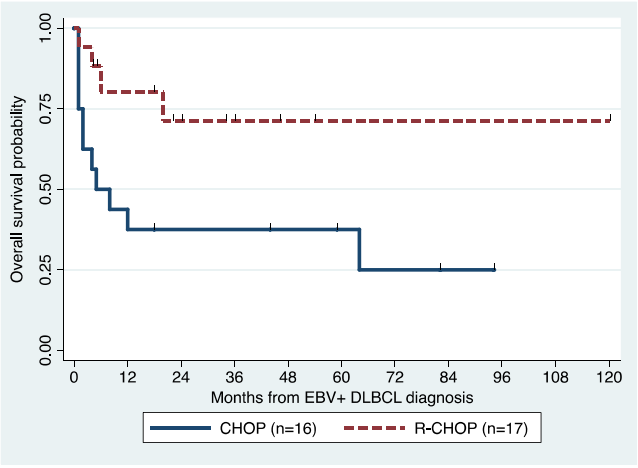


**FIGURE 1** Estimated survival curves in patients with Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) according to therapy received

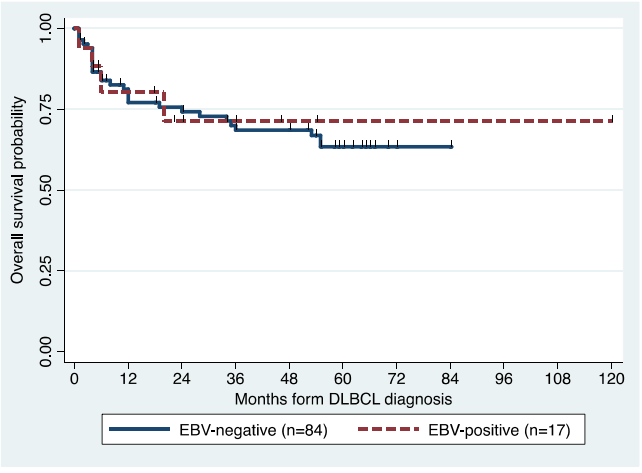


**FIGURE 2** Estimated survival curves in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) according to Epstein-Barr virus (EBV) status

and 90%, and 5 year OS rates ranging between 30% and 70% [2,6,9,14,20,21]. It is difficult, however, to draw solid conclusions from the available data, as there is heterogeneity between the published studies about age and EBER positivity cutoff.

The 2008 WHO classification included the provisional entity of EBV-positive DLBCL of older people in which age older than 50 years was a criterion. Recent studies have challenged the age cutoff of 50 years for the diagnosis of EBV-positive DLBCL. First, EBV-positive DLBCL has been diagnosed in younger, otherwise immunocompetent patients [22-25], and second, the virological, clinical, and pathological characteristics of such patients are similar to older patients with EBV-positive DLBCL [21,26]. These arguments support the inclusion of younger patients in ours and other studies on EBV-positive DLBCL patients, as long as an underlying immunodeficiency is ruled out. Our study showed no clinical differences between younger and older patients with EBV-positive DLBCL. A second point of controversy is the cutoff for EBER positivity. In previous studies, positive expression has ranged between 10% and 50%. It is likely that response and survival outcomes within the same cohort would vary depending on the cutoff point. Additional studies are needed to define the cutoff for positive expression of EBER in EBV-positive DLBCL. Our study suggests 20% as a potential clinically relevant cutoff that can be used for reproducibility.

Our study shows higher rates of CR with R-CHOP versus CHOP in patients with EBV-positive DLBCL. Epstein-Barr virus-positive DLBCL patients treated with R-CHOP had 4-fold higher odds of obtaining CR. This is also one of the largest studies including over 30 patients with EBV-positive DLBCL showing an improved survival associated with R-CHOP therapy over CHOP. Rituximab and CHOP was associated with a third of the all-cause mortality in EBV-positive DLBCL patients when compared with CHOP. Rituximab had shown to improve response and survival in patients with DLBCL [11-13], and it would have been intuitive that rituximab would also improve outcomes in EBV-positive DLBCL. However, no prospective study has been done to confirm such assumption. Given the rarity of the disease, at least in Western countries, a prospective study of this nature would be difficult to perform.

The role of EBV status in DLBCL patients treated with R-CHOP remains undefined. Our study showed that EBV status, defined as positive EBER expression in at least 20% of malignant cells, is neither predictive of CR nor prognostic of survival in DLBCL patients treated with R-CHOP. Although our results are encouraging for patients with EBV-positive DLBCL who will be more likely treated with R-CHOP, one could consider our findings preliminary, and larger studies are needed to further define the predictive and/or prognostic role, if any, of EBV status in DLBCL patients.

In a previous study, we showed that EBV-positive DLBCL patients are more likely to have a nongerminal centre profile based on the Hans method [2], which has been associated with worse response and survival rates than germinal centre DLBCL [27], although the prognostic value of the Hans method has been challenged [28]. The limited data on the presence of BCL2, BCL6, and MYC gene rearrangements and/or immunohistochemical expression in EBV-positive DLBCL suggest that EBV-positive DLBCL does not typically have a double-hit biology [29].

Our study has a number of limitations. One is related to the small number of patients included in this study. The other is the retrospective nature of the study. However, we have included consecutive patients diagnosed following a uniform approach and treated with standardized and widely accepted and used therapy regimens. Finally, our study is applicable to a Peruvian population and might or might not be representative of response and survival rates in other ethnicities. We believe the diagnostic criteria used for the definition of EBV-positive DLBCL in this study could serve as a platform to initiate multi-institutional prospective studies in patients with this rare lymphoma.

In conclusion, our study shows a response and survival benefit of the addition of rituximab to chemotherapy in patients with EBV-positive DLBCL. Furthermore, there were similar response and survival outcomes with R-CHOP in EBV-positive and EBV-negative DLBCLs. This supports that the management of EBV-positive DLBCL should mimic the widely accepted treatment of de novo DLBCL per current guidelines. Additional studies are warranted to confirm our findings in other populations.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose associated with the present work.

## ACKNOWLEDGEMENTS

There was no specific funding for this study.

## REFERENCES

1. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127:2375-2390.
2. Beltran BE, Castillo JJ, Morales D, et al. EBV-positive diffuse large B-cell lymphoma of the elderly: a case series from Peru. *Am J Hematol*. 2011;86:663-667.
3. Hoeller S, Tzankov A, Pileri SA, Went P, Dirnhofer S. Epstein-Barr virus-positive diffuse large B-cell lymphoma in elderly patients is rare in Western populations. *Hum Pathol*. 2010;41:352-357.
4. Hofscheier A, Ponciano A, Bonzheim I, et al. Geographic variation in the prevalence of Epstein-Barr virus-positive diffuse large B-cell lymphoma of the elderly: a comparative analysis of a Mexican and a German population. *Mod Pathol*. 2011;24:1046-1054.
5. Montes-Moreno S, Odqvist L, Diaz-Perez JA, et al. EBV-positive diffuse large B-cell lymphoma of the elderly is an aggressive post-germinal center B-cell neoplasm characterized by prominent nuclear factor-kB activation. *Mod Pathol*. 2012;25:968-982.
6. Ok CY, Li L, Xu-Monette ZY, et al. Prevalence and clinical implications of Epstein-Barr virus infection in de novo diffuse large B-cell lymphoma in Western countries. *Clin Cancer Res*. 2014;20:2338-2349.
7. Oyama T, Yamamoto K, Asano N, et al. Age-related EBV-associated B-cell lymphoproliferative disorders constitute a distinct clinicopathologic group: a study of 96 patients. *Clin Cancer Res*. 2007;13:5124-5132.
8. Park S, Lee J, Ko YH, et al. The impact of Epstein-Barr virus status on clinical outcome in diffuse large B-cell lymphoma. *Blood*. 2007;110:972-978.
9. Sato A, Nakamura N, Kojima M, et al. Clinical outcome of Epstein-Barr virus-positive diffuse large B-cell lymphoma of the elderly in the rituximab era. *Cancer Sci*. 2014;105:1170-1175.
10. Uner A, Akyurek N, Saglam A, et al. The presence of Epstein-Barr virus (EBV) in diffuse large B-cell lymphomas (DLBCLs) in Turkey: special

emphasis on 'EBV-positive DLBCL of the elderly'. *APMIS*. 2011;119:309-316.

11. Feugier P, Van Hoof A, Sebban C, et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol*. 2005;23:4117-4126.
12. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *J Clin Oncol*. 2006;24:3121-3127.
13. Pfreundschuh M, Kuhnt E, Trumper L, et al. CHOP-like chemotherapy with or without rituximab in young patients with good-prognosis diffuse large-B-cell lymphoma: 6-year results of an open-label randomised study of the MabThera International Trial (MInT) Group. *Lancet Oncol*. 2011;12:1013-1022.
14. Song CG, Huang JJ, Li YJ, et al. Epstein-Barr virus-positive diffuse large B-cell lymphoma in the elderly: a matched case-control analysis. *PLoS One*. 2015;10: e0133973
15. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol*. 2007;25:579-586.
16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
17. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep*. 1966;50:163-170.
18. Cox D. Regression models and life tables (with discussion). *J R Statist Soc B*. 1972;34:187-220.
19. Castillo JJ, Beltran BE, Miranda RN, Young KH, Chavez JC, Sotomayor EM. EBV-positive diffuse large B-cell lymphoma of the elderly: 2016 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2016;91:529-537.
20. Ahn JS, Yang DH, Duk Choi Y, et al. Clinical outcome of elderly patients with Epstein-Barr virus positive diffuse large B-cell lymphoma treated with a combination of rituximab and CHOP chemotherapy. *Am J Hematol*. 2013;88:774-779.
21. Lu TX, Liang JH, Miao Y, et al. Epstein-Barr virus positive diffuse large B-cell lymphoma predict poor outcome, regardless of the age. *Sci Rep*. 2015;5:12,168
22. Beltran BE, Morales D, Quinones P, Medeiros LJ, Miranda RN, Castillo JJ. EBV-positive diffuse large b-cell lymphoma in young immunocompetent individuals. *Clin Lymphoma Myeloma Leuk*. 2011;11:512-516.
23. Cohen M, Narbaitz M, Metrebian F, De Matteo E, Preciado MV, Chabay PA. Epstein-Barr virus-positive diffuse large B-cell lymphoma association is not only restricted to elderly patients. *Int J Cancer*. 2014;135:2816-2824.
24. Hong JY, Yoon DH, Suh C, et al. EBV-positive diffuse large B-cell lymphoma in young adults: is this a distinct disease entity? *Ann Oncol*. 2015;26:548-555.
25. Nicolae A, Pittaluga S, Abdullah S, et al. EBV-positive large B-cell lymphomas in young patients: a nodal lymphoma with evidence for a tolerogenic immune environment. *Blood*. 2015;126:863-872.
26. Ok CY, Ye Q, Li L, et al. Age cutoff for Epstein-Barr virus-positive diffuse large B-cell lymphoma—is it necessary? *Oncotarget*. 2015;6:13,933-13,945.
27. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103:275-282.
28. Castillo JJ, Beltran BE, Song MK, et al. The Hans algorithm is not prognostic in patients with diffuse large B-cell lymphoma treated with R-CHOP. *Leuk Res*. 2012;36:413-417.
29. Johrens K, Trappe RU, Lenze D, et al. Age and cellular composition influence overall survival in a collective of non-immunocompromised patients with EBV-positive diffuse large B-cell lymphoma from a German lymphoma center. *Leuk Lymphoma*. 2016;57:2791-2803.

**How to cite this article:** Beltran BE, Quiñones P, Morales D, et al. Response and survival benefit with chemoimmunotherapy in Epstein-Barr virus-positive diffuse large B-cell lymphoma. *Hematological Oncology*. 2017;1-6. https://doi.org/10.1002/hon.2449